Simultaneous determination of four active components in rat plasma by ultra-high performance liquid chromatography tandem-mass spectrometry/mass spectrometry and its application to a pharmacokinetic study after oral administration of *Callicarpa nudiflora* extract

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ABSTRACT

Background: *Callicarpa nudiflora* has been commonly used as a Chinese folk medicine for resolving toxin, dispersing edema and hemostasis; however, its pharmacokinetic (PK) behavior remains unknown. In our present study, a simple and sensitive ultra-high performance liquid chromatography tandem mass spectrometry method was firstly developed on simultaneous determination and PK study of four active components (luteoloside, dracocephaloside, juncein and nudifloside) following the oral administration of *C. nudiflora* extract to investigate their PK profiles. **Materials and Methods:** Chromatographic separation was achieved on a Phenomenex® Kinetex C_{18} column (50 mm × 2.1 mm, 1.7 μm) with gradient elution using a mobile phase consisted of acetonitrile (A) and 0.05‰ formic acid in water (B). The quantitation was carried out by multiple reaction monitoring using electrospray ionization in the negative ion mode. **Results:** Calibration curves offered satisfactory linearity, with correlation coefficients >0.99 for all compounds within the concentration range. The low limits of quantification were 1.03 ng/mL for luteoloside, 1.16 ng/mL for dracocephaloside, 0.82 ng/mL for juncein and 0.88 ng/mL for nudifloside, respectively. The intra- and inter-day precisions (relative standard deviation) were within 7.4% and the accuracies (relative error) ranged from −7.4% to 7.9%. **Conclusion:** This method was successfully applied to the PK studies of luteoloside, dracocephaloside, juncein and nudifloside in rat plasma after oral administration of *C. nudiflora* extract, four analytes exhibited quick absorption with peak concentrations occurring at around 25 min and eliminated rapidly.

Key words: *Callicarpa nudiflora,* determination, pharmacokinetics, rat plasma, ultra-high performance liquid chromatography tandem mass spectrometry

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INTRODUCTION

Callicarpa nudiflora, a member of the verbenaceae family, is distributed in Guangdong, Guangxi and Hainan provinces of mainland China.[1] It has been commonly used as a Chinese folk medicine for resolving toxin, dispersing edema and hemostasis.[2] Modern pharmacological studies indicate that *C. nudiflora* and its preparation *C. nudiflora* tablets exhibit a variety of pharmacological effects, such as anti-inflammatory,[3] hemostatic,[4] antibacterial[5] and cytotoxicity activities.[6] Phytochemical research revealed that *C. nudiflora* contained many constituents, like flavonoids,[7] terpenoids,[8] volatile oils[9] and so on. However, most studies have only focused on its chemical and pharmacological research, its pharmacokinetic (PK) behavior was unknown until recently, therefore, it is essential to investigate the PKs of its active components to supply research information

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for clinical use. Existing studies showed that flavonoids in *C. nudiflora* had the anti-inflammatory and hemostasis activities.[10] Luteoloside, dracocephaloside, juncein, three abundant flavonoid glycosides present in *C. nudiflora*, were found to possess anti-inflammatory[11] and antibacterial effects.[12,13] Nudifloside, belongs to the terpenoids, was reported to display the cytotoxicity against a K562 cell line with IC_{50} values of 20.7 μg/mL.[14] The above studies suggested that luteoloside, dracocephaloside, juncein and nudifloside could be found to contribute to the pharmacological efficacy of *C. nudiflora* and chosen as markers for quantification.

Recently, several studies have been developed concerning the quantification of luteoloside *in vivo*.[15,16] However, there is no report to determine dracocephaloside, juncein and nudifloside up to now, not to mention the simultaneous PKs studies of luteoloside, dracocephaloside, juncein and nudifloside after oral administration of the *C. nudiflora* extract. Since the therapeutic effects of Chinese folk medicine are based on the complex interactions of multiple ingredients, it is of paramount importance to develop an analytical method to allow four compounds to be quantified simultaneously in rat plasma.

In the present study, a simple and sensitive ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method was firstly developed for the simultaneous determination of luteoloside, dracocephaloside, juncein and nudifloside in rat plasma after oral administration of the *C. nudiflora* extract. The structures of the four compounds are shown in Figure 1. It was expected that the results obtained would provide some references to evaluate the clinical application of *C. nudiflora*.

MATERIALS AND METHODS

Materials and reagents

The reference standards of luteoloside, dracocephaloside, juncein and nudifloside were isolated and purified in our laboratory and their structures were unequivocally confirmed by comparing ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR and MS data with those reported previously.[7,14] The purity (>98%) of all compounds was checked by HPLC-ultraviolet methods. Liquiritin (internal standard, purity >99%, lot number [111610–200604]) were purchased from the National Institute for Food and Drug Control (Beijing, China). Acetonitrile and methanol (HPLC grade) were purchased from Sigma-Aldrich Inc. (Taufkirchen, Germany). Formic acid, acetic acid, and ammonium acetate (MS grade) were purchased from Fluka BioChemika (Buchs, Switzerland). Deionized water was generated from a Milli-Q-system (Millipore, Milford, MA, USA). All other reagents were of analytical grade.

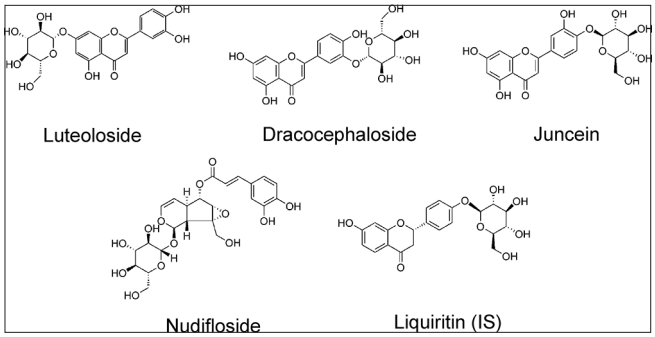

Figure 1: The structures of four active components in *Callicarpa nudiflora* and an internal standard (at column width)

The herb materials of *C. nudiflora* were collected from Wuzhishan, Hainan, China in August 2012 and identified by Prof. Guiping Yuan at Jiangxi Provincial Institute for Drug and Food Control, China.

Instrumentation and analytical conditions

Chromatographic separation was performed on an Agilent 1290 Series UHPLC system (Agilent Technologies, USA) equipped with a binary pump (G4220A), an autosampler (G4226A) and a column compartment (G1316C). A Phenomenex® Kinetex C_{18} column (50 mm × 2.1 mm, 1.7 μm, Phenomenex, CA, USA) was employed to separate all analytes and the internal standard. The mobile phase was comprised of acetonitrile (A) and 0.05‰ formic acid (B) using a linear gradient elution of 10–43% A at 0–7.0 min, 43–95% A at 7.0–7.1 min, 95% A at 7.1–9.0 min, the re-equilibration time was 2.0 min. The flow rate was set at 0.45 mL/min, the column oven temperature (TEM) was maintained at 40°C, the injection volume was 1 μL. A 10 s needle wash with 1:1 (v/v) acetonitrile/water was used to reduce carryover for each sample. The TEM in the sample compartment of the autosampler was set at 4°C.

The API 4000 triple-stage quadrupole mass spectrometer (AB Sciex, USA) equipped with a Turboionspray™ interface was used as the mass spectrometric detection. Analyst® Software (version 1.5.2, Applied Biosystems, CA, USA) was used to control the LC–MS/MS system as well for data acquisition and processing. All analytes, including the IS, were detected using electrospray ionization (ESI) in the negative ionization mode and quantified by multiple reaction monitoring (MRM) mode. The parameters in the source were set as follows: Curtain gas, 25 psi; ion source gas 1 (GS 1), 60 psi; ion source gas 2 (GS 2), 55 psi; source

TEM, 500°C; capillary voltage, −4500 V; entrance potential, −10 V; collision cell exit potential, −13 V. The dwell time of each MRM transition was 80 ms. Nitrogen was used in all cases. The precursor-to-product ion pairs, declustering potential (DP), collision energy (CE) and retention time (t_R) for each analyte are listed in Table 1.

Preparation of standard solutions, calibration standards and quality control samples

Stock solutions of all analytes were separately prepared by dissolving accurately weighed amounts in methanol. All stock solutions were mixed and diluted with methanol to prepare a final mixed standard solution containing 10,300 ng/ml of luteoloside, 11,600 ng/ml of dracocephaloside, 8250 ng/ml of juncein and 8750 ng/ml of nudifloside, respectively. This mixed standard solution was then serial diluted in methanol to yield a series of working solutions. Standard stock solution of liquiritin (IS) was prepared at 100 ug/mL in methanol and was successively diluted to result in a final concentration of 250 ng/mL. All solutions were stored at 4°C and kept away from light.

The working solutions were used to spike the blank rat plasma with appropriate volumes, providing finally eight calibration standards containing a mixture of all analytes with the concentrations ranging from 2.06 to 1030 ng/mL for luteoloside, 2.32–1160 ng/mL for dracocephaloside, 1.65–825 ng/mL for juncein and 1.75–875 ng/mL for nudifloside, respectively.

Quality control (QC) plasma samples were prepared containing luteoloside (4.12, 103.00, 824.00 ng/mL), dracocephaloside (4.64, 116.00, 928.00 ng/mL), juncein (3.30, 82.50, 660.00 ng/mL) and nudifloside (3.50, 87.50, 700.00 ng/mL) in the same manner as the calibration standards. The spiked samples were processed according to the procedures for plasma samples as described below.

Preparation of sample solutions

All frozen standards and plasma samples were thawed gradually to room TEM and thoroughly vortexed prior to analysis. To a 100 aliquot of rat plasma, 10 μL of the IS solution (250 ng/mL of liquiritin), 50 μL of 0.25 mol/L hydrochloric acid and 10 μL of methanol (volume of the corresponding working solution for the calibration curve and the QC samples) was added. After vortexing for 1 min, 300 μL of acetonitrile was added, and the mixture was vortexed for an additional 2 min for protein precipitation. Following centrifugation for 10 min at 13,000 rpm, the whole supernatant was pipetted into a 1.5-mL microcentrifuge tube and evaporated to dryness under a gentle stream of nitrogen gas at 40°C for approximately 10 min. The dried residues were reconstituted in 100 μL of 50% of acetonitrile and centrifuged at 13,000 rpm for 10 min. Finally, the supernatants (85 μL) were transferred into glass vials prior to injection into the UHPLC-ESI-MS/MS for analysis.

Table 1: Optimized MRM parameters for four analytes and IS

Analytes	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (eV)	t_R (min)
Luteoloside	447.1	285.0	−80	−32	5.77
Dracocephaloside	447.1	285.0	−80	−32	6.74
Juncein	447.1	285.0	−80	−32	6.46
Nudifloside	523.4	160.9	−84	−48	5.70
Liquiritin (IS)	417.2	254.7	−53	−35	5.83

MRM: Multiple reaction monitoring; DP: Declustering potential; CE: Collision energy t_R: Retention time; IS: Internal standard

Preparation of *Callicarpa nudiflora* extract

Dried whole plants (1 kg) were extracted three times (2 h/each) with 80% alcohol (10 L/each). The extracting solution was combined and evaporated under reduced pressure to yield *C. nudiflora* extract (180 g). To calculate the administered dose, the contents of luteoloside, dracocephaloside, juncein and nudifloside in the extract were measured quantitatively to be 1.08 mg/g, 0.92 mg/g, 1.34 mg/g and 1.42 mg/g, respectively, through an external standard method using the same chromatography conditions as described above.

Method validation of determination

The validation of this method was performed in accordance with the Food and Drug Administration Bioanalytical Method Validation publication.[17] The specificity of the method was assessed by comparatively analyzing the chromatograms of blank plasma samples of six individual rats, blank plasma spiked with the analytes and IS, and rat plasma after oral administration of the *C. nudiflora* extract. The calibration curves were constructed by plotting peak area ratios (y) of each analyte to the IS versus nominal concentrations (x) of least square linear regression analysis with a weighting factor of $1/x^2$. The calibration curves were prepared daily prior to sample analysis. The low limits of quantification (LLOQ) was defined as the lowest concentration that can be quantitatively determined with precision not exceed 20% and accuracy within ± 20%. Intra-day precisions and accuracies were tested on the same day of six replicates at three QC levels. Inter-day precisions and accuracies were determined at the same concentration levels, but in 3 consecutive days. The precision was calculated using the relative standard deviation (RSD). RSD = (standard deviation [SD]/calculated concentration) ×100. The accuracy was calculated using the relative error (RE). RE = ([calculated concentration − nominal concentration]/

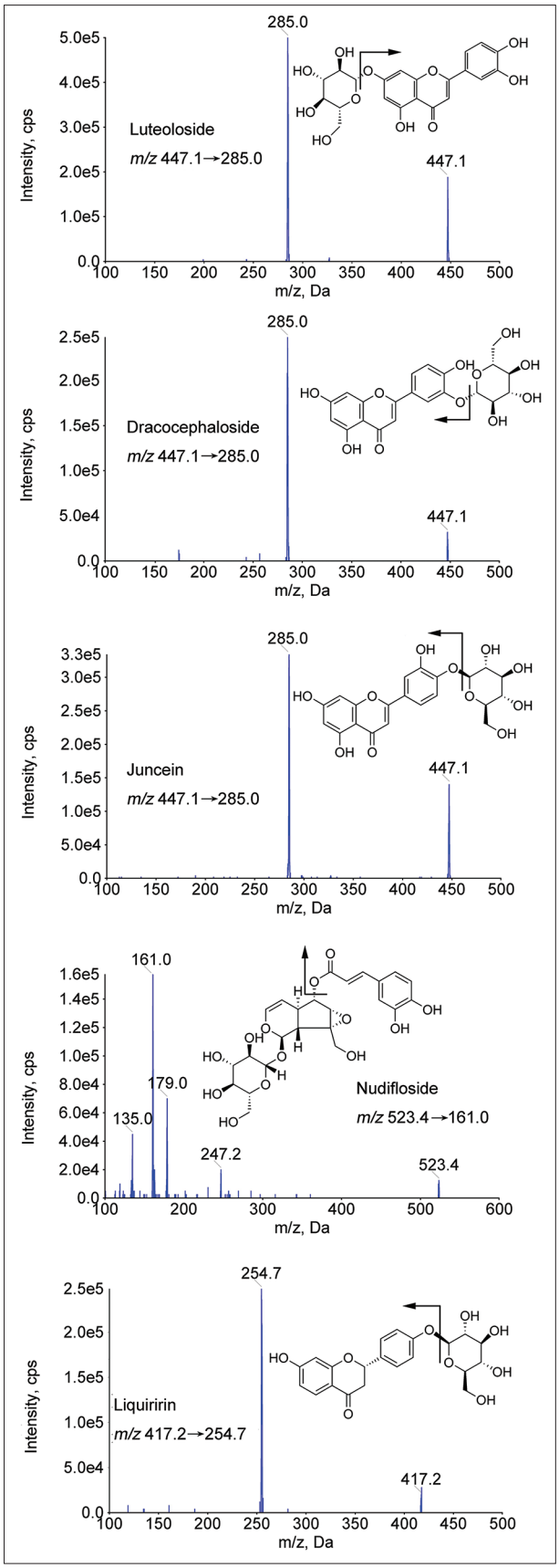

Figure 2: Mass spectrometry (MS)/MS spectra of four analytes and their proposed fragmentation pathway (at column width)

nominal concentration) × 100. The RSD at each QC level is required to below 15%, and the RE should be within ± 15%. The extraction recoveries were determined by calculating the ratios of the peak areas of samples added before extraction against those of samples added after extraction at three QC levels. This procedure was repeated for six replicates. The matrix effect was determined by calculating the ratios of the peak areas at three QC levels dissolved with blank matrix extract against those dissolved with 50% of acetonitrile. The stability of each analyte in plasma samples was studied at three QC levels in three different storage conditions: Three freeze–thaw cycles; 8 h after being prepared at room TEM; long-term stability at −20°C for 14 days. The sample was considered to be stable when the RE was within ± 15%.

Animals

Sprague-Dawley (SD) male rats, weighing 200 ± 20 g, were purchased from Hunan Silaikejing Laboratory Animal Co. Ltd. Animals were allowed to adapt to the controlled environmental conditions (TEM 22°C ± 2°C; humidity 55% ± 10%; a 12 h/12 h light/dark cycle) with free access to standard laboratory food and water. The study was approved by the Animal Ethics Committee on the Use of Animals of Jiangxi Institute for Drug and Food Control (Nanchang, China) and all animal experiments were performed according to the guidelines of this institution.

Pharmacokinetic analysis

Six SD rats were fasted for 12 h and had free access to water before dosing. The *C. nudiflora* extract was dissolved with 0.5% carboxymethylcellulose-Na solution. Six SD rats were given orally with 5 g/kg *C. nudiflora* extract. Blood samples of 0.4 mL were collected in heparinized centrifuge tubes from the eye veins of rats at 5, 10, 20, 30, 45, 60, 90, 120, 180, 300, 420 min after administration. Meanwhile, plasma samples were immediately centrifuged at 3000 rpm for 5 min at 4°C, plasma samples were then removed into 1.5-ml tubes and stored at −20°C until analysis.

The PK parameters were determined by the Drug and Statistics (DAS 3.0) software (Bontz Inc., Beijing, China). The PK parameters, such as area under the concentration-time curve from time zero to the last measured concentration (area under the curve [AUC_{0-t}]), AUC extrapolated to infinity ($AUC_{0-\infty}$) and terminal elimination half-life ($t_{1/2z}$) were calculated by a noncompartmental analysis method. Peak concentration (C_{max}) and the time of C_{max} were measured directly from the concentration–time curve. All results were expressed as the arithmetic mean ± SD.

Table 2: The regression equations, correlation coefficients, linear ranges and LLOQs for the determination of four analytes in rat plasma

Analytes	*y*=ax+b	R^2	Linear range (ng/mL)	LLOQ (ng/mL)
Luteoloside	$y=1.09\times10^{-2}x+1.46\times10^{-2}$	0.9980	2.06-1030	1.03
Dracocephaloside	$y=2.44\times10^{-2}x+7.25\times10^{-4}$	0.9983	2.32-1160	1.16
Juncein	$y=1.79\times10^{-2}x+9.65\times10^{-3}$	0.9985	1.65-825	0.82
Nudifloside	$y=1.54\times10^{-2}x+3.13\times10^{-4}$	0.9971	1.75-875	0.88

LLOQ: Low limits of quantification

Table 3: Intra-day and inter-day precisions and accuracies of four analytes in rat plasma (*n*=6)

Analytes	QC (ng/mL)	Intra-day precision			Inter-day precision		
		Measured concentration (mean±SD)	RSD (%)	RE (%)	Measured concentration (mean±SD)	RSD (%)	RE (%)
Luteoloside	2.06	2.02±0.09	4.5	−1.9	2.00±0.13	6.5	−2.9
	206	217.43±3.56	1.6	5.6	218.70±4.61	2.1	6.2
	824	803.83±12.21	1.5	−2.5	848.47±46.45	5.5	3.0
Dracocephaloside	2.32	2.30±0.06	2.6	−0.9	2.40±0.05	2.1	3.5
	232	236.50±4.30	1.8	1.9	242.42±13.89	5.7	4.6
	928	937.67±14.28	1.5	1.0	918.53±10.86	1.2	−1.0
Juncein	1.65	1.78±0.04	2.3	7.9	1.58±0.08	5.1	−4.2
	165	170.49±2.04	1.2	3.3	156.17±11.52	7.4	−5.4
	660	687.12±9.54	1.4	4.1	683.79±8.33	1.2	3.6
Nudifloside	1.75	1.77±0.12	6.8	1.1	1.85±0.07	3.8	5.7
	175	183.38±6.28	3.4	4.8	169.16±8.79	5.2	−3.3
	700	648.50±12.44	1.9	−7.4	651.3±46.69	7.2	−7.0

SD: Standard deviation; RSD: Relative standard deviation; RE: Relative error

Table 4: Recoveries and matrix effect of four analytes in rat plasma (*n*=6)

Analytes	Spiked concentration (ng/mL)	Extraction recovery (%)		Matrix effect (%)	
		Mean	RSD	Mean	RSD
Luteoloside	2.06	82.1	4.3	102.4	2.7
	206	80.8	7.8	98.6	3.4
	206	82.3	3.9	94.6	2.6
Dracocephaloside	2.32	85.4	2.6	93.9	5.4
	232	84.2	4.7	103.8	3.6
	928	85.6	4.3	96.2	1.5
Juncein	1.65	91.6	2.0	92.3	4.3
	165	80.1	4.9	98.3	3.3
	660	84.1	5.7	102.5	6.9
Nudifloside	1.75	80.5	3.4	94.2	3.7
	175	83.8	4.6	98.5	6.1
	700	88.0	8.2	92.9	5.3

RSD: Relative standard deviation

RESULTS AND DISCUSSION

Optimization for ultra-high performance liquid chromatography tandem mass spectrometry parameters

All MS parameters were optimized via direct infusion of the reference standard into the mass spectrometer separately using a syringe pump (Harvard Apparatus, Holliston, MA, USA) at a flow rate of 5 μL/min. The parent ions were selected by Q1 scans, and the transition ions were selected by product ion scans. The ionization mode was optimized in both the positive and the negative ion modes, we found the response of all analytes in negative mode were higher than that in positive mode. Therefore, ESI in negative ion mode was selected for the ionization in our research. Then the DP and the CE was optimized to get maximum sensitivity for the $[M-H]^-$ ions and to find the maximum response for the MS/MS fragment ion. The product ion scan spectrum of the analytes and IS are shown in Figure 2.

Compared to conventional HPLC, UHPLC we choosed allowed the simultaneous analysis of four analytes and IS with a shorter chromatographic run time, it could also improve both efficiency and resolution. Next, since the efficiency of the ionization process and good chromatographic behavior were highly dependent on the mobile phase composition, different solvent mixtures (methanol–water, acetonitrile–water, acetonitrile–methanol–water) and mobile phase additives were tested. The results showed that acetonitrile–water generated the highest peak response, but all mobile phases mentioned above displayed peak-tailing chromatographic peak shapes. In order to overcome the peak-tailing effect to

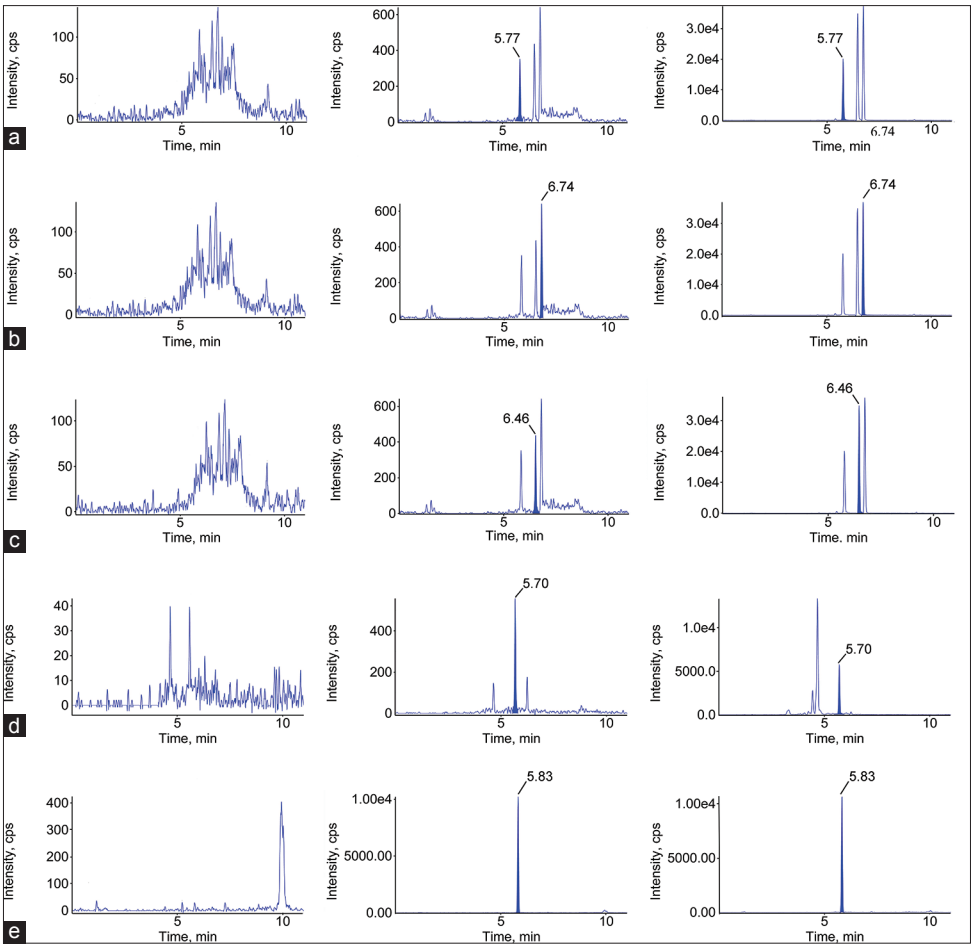

Figure 3: Representative multiple reaction monitoring chromatograms of luteoloside (a), dracocephaloside (b), juncein (c), nudifloside (d) and IS (e) in the rat plasma. (1) Blank plasma, (2) blank plasma spiked with the analytes at low limits of quantification and (3) rat plasma samples at 20 min after oral administration of the *Callicarpa nudiflora* extract (at full page width)

improve the resolution for analytes and IS, the addition of different electrolytes including formic acid, acetic acid, and ammonium acetate to the water phase was tested. We found the addition of formic acid in an acetonitrile–water gradient could improve the peak shape of all analytes but reduced the MS sensitivity of the most analytes. Eventually, a low (0.05‰) formic acid concentration was found to be a good compromise between MS response and general analyte peak shape. Besides, it was no easy to separate the luteoloside, dracocephaloside and juncein as isomers owning to their similar polarity, therefore, we compared different types of chromatographic columns (Phenomenex® Kinetex C_{18} column [50 mm × 2.1 mm, 1.7 μm], Agilent Eclipse XDB-C18 [3.0 mm × 100 mm, 1.8 μm], Waters ACQUITY UPLC® BEH C_{18} column [50 mm × 2.1 mm, 1.7 μm]) by gradient elution to produce satisfactory separation for isomers within a shorter analysis time and found Phenomenex® Kinetex C_{18} column (50 mm × 2.1 mm, 1.7 μm) was most suitable. Other chromatographic conditions, including the flow rate of the mobile phase and column TEM were optimized as well.

Preparation of the plasma samples

Protein precipitation, with the advantages of a faster sample preparation and lower costs, was found to be effective to deproteinization due to the low probability of losses in preparation of the plasma samples. When 300 μL acetonitrile was used as the plasma protein precipitating reagent, the recoveries of four analytes from rat plasma were satisfied. Evaporation of the supernatant and reconstitution of the residues in 50% of acetonitrile caused an increase in baseline noise. Additionally, the acidification of plasma with hydrochloric acid (0.25 mol/L) before protein precipitation could further enhance the response of the four analytes, the reason may be that the acidification could promote the release of the analytes from plasma protein.

Method validation results

Specificity

The chromatograms of blank plasma, spiked plasma, and oral administered rat plasma are shown in Figure 3. No

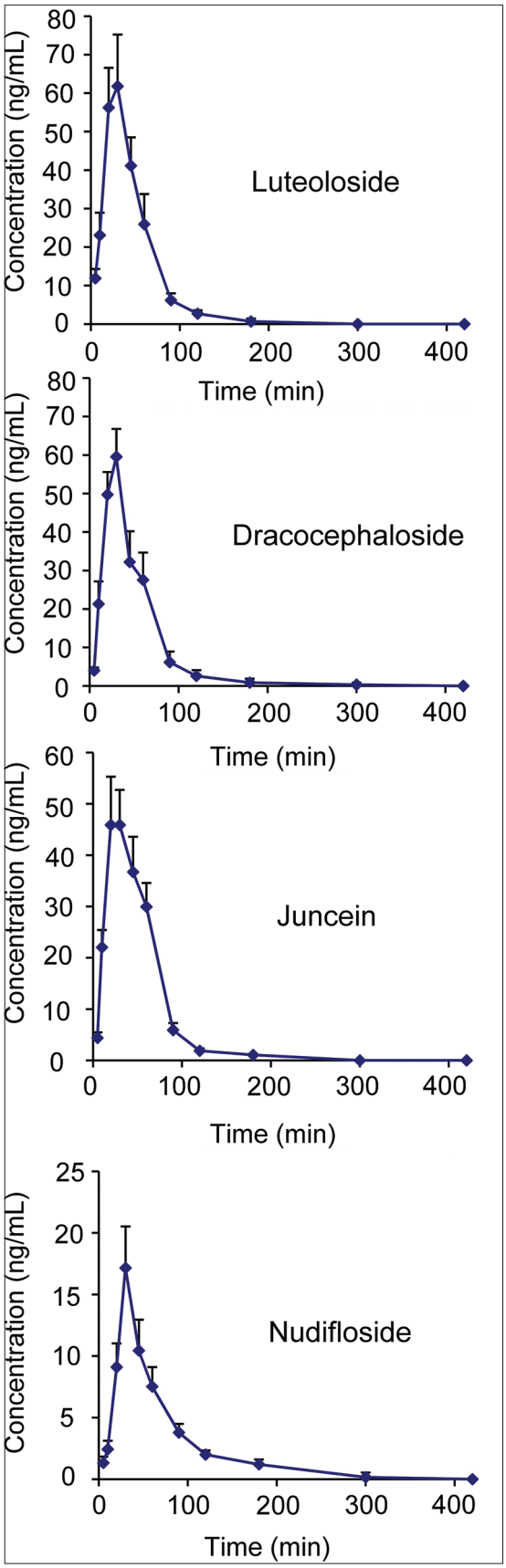

Figure 4: Plasma concentration-time curves of four active components after oral administration of *Callicarpa nudiflora* extract. Data are expressed as mean ± standard deviation (n = 6) (at column width)

endogenous interference was observed at the t_Rs of the analytes and IS, the peaks of the analytes and IS were detected with excellent resolution and exhibited good peak shape under the chromatographic conditions.

Linearity and low limits of quantification

The linear ranges, typical regression equations and LLOQs of four analytes are shown in Table 2. The calibration curves for the four analytes were found to be linear with the regression coefficients (R^2) all higher than 0.99. The chromatograms of four analytes at LLOQ level are given in Figure 3. The method is sensitive for the quantitative evaluation of four analytes as the precisions and the accuracies at the LLOQ were <15%.

Precision and accuracy

Table 3 summarizes the intra-day and inter-day precisions and accuracies of four analytes. The intra- and inter-day precisions (RSD) were within 7.4% and the accuracies (RE) ranged from −7.4% to 7.9%, they were all within ± 15% for all the QC levels, which indicates that our method was accurate, reliable, and reproducible.

Recovery and matrix effect

The mean extraction recoveries of four analytes at three QC levels were found to be in the range 80.1–91.6% with RSD <8.2%, which indicates that the protein precipitation method was acceptable. The matrix effects ranged from 92.3% to 103.8% with RSD <6.9%, which demonstrates that no significant matrix effect existed on the ionization of four analytes [Table 4].

Stability

The results shown in Table 5 demonstrate the good stability of four analytes in plasma samples during three freeze–thaw cycles (RE in the range −7.7–6.3%, RSD <7.3%), at 8 h at room TEM (RE in the range −5.6–7.0%, RSD <7.0%) and at a TEM of −20°C for 14 days (RE in the range −4.7–6.1%, RSD <8.4%). The method is proved to be applicable for routine analysis.

Pharmacokinetic study

This validated UHPLC-MS/MS method was sensitively applied to the PK studies of luteoloside, dracocephaloside, juncein and nudifloside in rat plasma after oral administration of the *C. nudiflora* extract. Figure 4 depicts the mean plasma concentration–time profiles of the four analytes in rats. Their main PK parameters were summarized in Table 6. It was observed four analytes exhibited quick absorption with C_{max} occurring at around 25 min and were eliminated rapidly thereafter, these behaviors may be attributed to the relatively high polar of the compounds. Three flavonoid glycosides (luteoloside, dracocephaloside and juncein) exhibited consistent plasma concentration–time and PK

Table 5: Stability of four analytes in rat plasma at different storage conditions (*n*=6)

Analytes	QC (ng/mL)	Three freeze-thaw cycles (%)		Room temperature for 8 h (%)		Storage at−20°C to 14 days (%)	
		Accuracy RE	RSD	Accuracy RE	RSD	Accuracy RE	RSD
Luteoloside	2.06	3.4	4.7	−1.9	1.5	−2.9	3.5
	206	−6.0	4.1	−3.1	4.6	3.6	6.4
	824	−2.8	7.3	−2.3	6.0	−2.9	5.1
Dracocephaloside	2.32	2.6	3.4	2.6	6.3	−2.2	4.0
	232	−5.2	6.1	2.1	3.6	2.9	5.1
	928	1.1	4.0	1.9	2.0	0.6	7.6
Juncein	1.65	5.5	5.8	6.7	6.3	3.0	1.8
	165	−7.7	7.2	−5.6	5.7	−4.7	6.4
	660	5.6	2.3	−2.7	3.6	−3.2	4.7
Nudifloside	1.75	6.3	3.2	3.4	5.0	6.1	7.0
	175	3.0	5.9	7.0	7.0	4.8	5.9
	700	1.7	4.3	2.8	5.6	2.1	8.4

RSD: Relative standard deviation; RE: Relative error; QC: Quality control

Table 6: Pharmacokinetic parameters of the four analytes after the oral administration of *Callicarpa nudiflora* extract (*n*=6)

Analytes	AUC_{0-t} (ng/mL min)	$AUC_{0-\infty}$ (ng/mL* min)	C_{max} (ng/mL)	$t_{1/2z}$ (min)	T_{max} (min)
Luteoloside	3134.65±413.45	3134.68±413.46	67.55±5.67	25.39±9.05	26.67±5.16
Dracocephaloside	2944.69±571.63	2944.72±571.64	59.55±7.24	26.04±2.02	30.00±0.00
Juncein	2801.90±304.44	2801.94±304.44	52.10±3.07	26.49±0.74	23.33±5.16
Nudifloside	987.50±232.30	1002.13±266.55	17.16±3.36	42.58±39.55	30.00±0.00

AUC_{0-t}: Area under the concentration-time curve from time zero to the last measured concentration; $AUC_{0-\infty}$: Area under the curve extrapolated to infinity; $t_{1/2z}$: Terminal elimination half-life; C_{max}: Peak concentration T_{max}: Time of C_{max}

parameters *in vivo* as isomers; the trend in concentration–time profiles of luteoloside was basically consistent with the literature report.[15] Although the administered doses of nudifloside were higher than the flavonoid glycosides, the AUC_{0-t} of nudifloside was lower than those of the flavonoid glycosides, which demonstrates that the bioavailability of nudifloside was lower than that of the flavonoid glycosides.

CONCLUSIONS

A simple and sensitive UHPLC–MS/MS method was firstly developed and validated for the simultaneous determination of luteoloside, dracocephaloside, juncein and nudifloside in rat plasma. This method has been successfully applied in a PK study of four compounds after oral administration of the *C. nudiflora* extract. The validated method and the PK parameters in this paper would be helpful to provide certain references to clinical application of *C. nudiflora*.

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