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ORIGINAL ARTICLE

Comparative Study of the Pharmacokinetic Parameters for Salidroside in Normal and Estrogen-Deficient Female Rats after Oral Administration of an Aqueous Extract of *Fructus Ligustri Lucidi* using a Validated Ultra-Performance Liquid Chromatography Mass Spectrometry/Mass Spectrometry Method

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Submitted: 29-Jun-2019 Revised: 01-Oct-2019 Accepted: 17-Feb-2020 Published: 20-Oct-2020

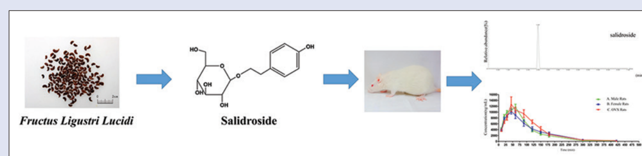
ABSTRACT

Background: Salidroside, one of the main active ingredients in *Fructus Ligustri Lucidi* (FLL), is well demonstrated to exert anti-osteoporotic effect. However, the plasma pharmacokinetic profile of salidroside in FLL in estrogen-deficient rats remains unknown. **Objective:** The objective was to develop a sensitive, rapid, and accurate ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) method for the determination of the pharmacokinetics profile of salidroside after oral administration of FLL aqueous extract in normal and ovariectomized (OVX) rats. **Materials and Methods:** OVX and normal rats were orally administrated with FLL at a bolus of 7 g/kg. Plasma samples were precipitated by methanol, and the supernatant was chromatographed by a Waters BEH C_{18} column with a gradient elution of ammonium acetate and acetonitrile. Quantification was carried out on the electrospray ionization, positive multiple reaction monitoring modes. **Results:** The lower limit of detection was 50 ng/mL, and the dynamic linear range was 50–30,000 ng/mL with a value of $R^2 > 0.99$. The intra- and inter-day precisions were lower than 14.67%, and accuracy was in the range of 99.29%–103.37%. The recovery of salidroside ranged from 88.90% to 101.78%, with the matrix effect ranging from 85.53% to 100.45%. The $t_{1/2}$, $MRT_{0-\infty}$, and apparent volume of distribution for salidroside increased in OVX rats. **Conclusion:** A sensitive, accurate, and rapid method was successfully established and validated for the determination of plasma characteristics of salidroside in Sprague–Dawley (SD) rats. The results suggest that ovariectomy could interfere with salidroside metabolism in SD rats.

Key words: *Fructus Ligustri Lucidi*, ovariectomized rats, plasma characteristic, salidroside, ultra-performance liquid chromatography mass spectrometry/mass spectrometry

SUMMARY

- Salidroside, one of the main active ingredients in *Fructus Ligustri Lucidi* (FLL), has demonstrated an anti-osteoporotic effect. This study was undertaken to determine the pharmacokinetics profile of salidroside after oral administration of FLL aqueous extract in normal and ovariectomized rats. Our results indicated that ovariectomy could interfere with salidroside metabolism in Sprague–Dawley (SD) rats. Our study also provides a sensitive, accurate, and rapid method for the determination of plasma characteristics of salidroside in SD rats by ultra-performance liquid chromatography mass spectrometry/mass spectrometry.



Abbreviations used: AUC_{0-t}: Area under the drug-time curve from zero to the last measurable plasma concentration point; CL: Clearance; BUCM: Beijing University of Chinese Medicine; C_{max}: Peak concentration; ESI-MS/MS: Electrospray ionization-tandem mass spectrometry; FLL: *Fructus Ligustri Lucidi*; FSH: Follicle-stimulating hormone; GnRH: Gonadotropin-releasing hormone; LH: Luteinizing hormone; LLOQ: Lower limit of quantification; MRM: Multiple reaction monitoring; MRT: Mean residence time; OVX: Ovariectomized; PDA: Photodiode array detection; PG: Progesterone; QC: Quality control; RSD: Relative standard deviation; SD: Sprague–Dawley; SPE: Solid-phase extraction; $t_{1/2}$: Elimination half time; TCM: Traditional Chinese medicine; T_{max}: Peak concentration time; UPLC-ESI-MS/MS: UPLC-electrospray ionization tandem-mass spectrometry/mass spectrometry; UPLC-MS/MS: Ultra-performance liquid chromatography-mass spectrometry/mass spectrometry; V_d: Apparent volume of distribution.

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DOI: 10.4103/pm.pm_278_19

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Cite this article as: Chen B, Tang J, Niu M, Zhu R, Li L, Wang L, *et al*. Comparative study of the pharmacokinetic parameters for salidroside in normal and estrogen-deficient female rats after oral administration of an aqueous extract of *Fructus Ligustri Lucidi* using a validated ultra-performance liquid chromatography mass spectrometry/mass spectrometry method. Phcog Mag 2020;16:471-8.

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INTRODUCTION

Osteoporosis is a silent disease characterized by decreased bone mass and altered bone microarchitecture, which predicts an increased risk of bone fragility and fracture.[1] With the aged population increasing all over the world, estrogen deficiency-induced postmenopausal osteoporotic fractures affect one in three women over age 50 and has become a major public health issue.[2,3] Estrogen-deficient female (ovariectomy) rats is one of the frequently used osteoporotic animal models to evaluate the efficacy and mechanisms of herbs against osteoporosis.[4-6] *Fructus Ligustri Lucidi* (FLL), named NvZhenZi in Chinese, the dried mature fruit of *Ligustrum lucidum Ait*. (*Oleaceae*),[7] has been clinically used to treat osteoporosis for more than 1000 years in traditional Chinese medicine (TCM). Accumulating evidence suggested that FLL exerts an anti-osteoporotic effect through the regulation of calcium metabolism,[8,9] oxidative stress,[7] lipid metabolism,[10] and estrogen receptor expression.[4]

Salidroside (2-[4-hydroxyphenyl] ethyl β–D-glucopyranoside), one of the main ingredients in FLL aqueous extract,[7] has been demonstrated to attenuate bone loss and improve trabecular bone microarchitecture through the regulation of calcium homeostasis,[11] inhibition of oxidative stress,[12] and promotion of angiogenesis and osteogenesis[13] in estrogen-deficient female rats and diabetic animals. Moreover, accumulating evidence demonstrated that the bioavailability of salidroside in FLL extracts has been improved in rats with migraine or insomnia.[4,14,15] Furthermore, sex could also dramatically affect pharmacokinetics and bioavailability of the drug, which is associated with drug efficacy as well as side effects.[16-18] This prompted us to hypothesize that the plasma characteristics of salidroside may be altered in estrogen-deficient rats.

Various analytical methods, such as liquid chromatography coupled with ultraviolet,[19] electrospray ionization-tandem mass spectrometry (ESI-MS/MS),[20,21] photodiode array detection,[22,23] and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS),[24,25] have been developed for the determination of the pharmacokinetic profile of salidroside in biological fluids after administration of *Rhodiola rosea* L. extracts or FLL ethanol extract. However, these methods were characterized by low sensitivity or relative complexity and are time-consuming. Recently, ultra-performance liquid chromatography MS/MS (UPLC–MS/MS) method[26-28] has been developed with the features of 10-fold improvement in analysis speed, 5-fold improvement in resolution, high sensitivity,[29] and low matrix effect[30] in comparison with HPLC–MS/MS method. Regarding biological sample preparation, methods, such as radioiodine-labeling,[31] liquid–liquid extraction,[19-25,32] and solid-phase extraction (SPE),[24] have been developed. However, radiation may produce a potential health risk for the investigators, while SPE combined with MS/MS may increase the experimental expense.[21] Moreover, the metabolic properties of salidroside in estrogen-deficient models after intragastric gavage administration of FLL aqueous extract remain unclear so far. Therefore, in the current study, we made an attempt to investigate the plasma characteristics of salidroside in ovariectomized (OVX) rats after oral administration of FLL aqueous extract using a one-step protein precipitation and UPLC–MS/MS method. The results demonstrated that UPLC–MS/MS is a simple and specific tool for the determination of salidroside in rat plasma. The $t_{1/2}$ for salidroside in OVX rats was significantly higher than those of female normal rats. The $MRT_{0-\infty}$ and apparent volume of distribution (V_d) for salidroside in OVX rats were higher than those of male littermates.

MATERIALS AND METHODS

Chemicals and reagents

Salidroside (purity >98%) was purchased from Chengdu PuFeiDe Biotech Co., Ltd (Sichuan, China) and paracetamol (purity >99%) was obtained from Dr. Ehrenstorfer GmbH (Germany) which served as internal standard [IS; Figure 1]. An ELISA kit for determination of estrogen levels was obtained from Cusabio Biotech Co Ltd. HPLC-grade acetonitrile and methanol were bought from Merck Inc., Kenilworth, NJ, USA. HPLC-grade ammonium acetate was obtained from Fisher Scientific (FairLawn, NJ, USA). Ultra-pure water was prepared by a Milli-Q system (Bedford, MS, USA).

Preparation of *Fructus Ligustri Lucidi* aqueous extract

FLL was purchased from Beijing TongRenTang pharmacy and authenticated by Zexin Ma from the Chinese Medicine and Medica Museum, Beijing University of Chinese Medicine (BUCM), China. FLL aqueous extract was prepared as previously described.[7] The salidroside concentrations in FLL aqueous extracts were determined as follows: 100 μL of FLL aqueous extract was spiked with 300 μL of methanol to precipitate protein. After centrifugation for 15 min (12,000 rpm) at 4°C, the supernatants were dried under nitrogen at 40°C. One hundred microliters of methanol was added to dissolve the residues. Then, the samples were processed for UPLC-MS/MS analyses. The preparation yielded 0.776 mg/g of salidroside in FLL aqueous extract.

Animal experiments

The animal protocol was approved by the animal care committee of BUCM, Beijing, China (No. BUCM-4-20171012-4014). Moreover, all experiments were performed in accordance with the Guidelines for Animal Experimentation of BUCM. Every possible means and effort were provided to eliminate the potential stress, pain, and mortality of the surgical rats.

Ten male and 17 female SD rats (10 weeks old; body weight, 200 ± 10 g) were purchased from Beijing SiBeiFu Animal Technology Co. Ltd (license no. SCXK [Jing] 2015-0015, Beijing, China). The rats were housed in a clean level breeding room (certification number SCXK [Jing] 2016-0038) at the temperature of 22°C ± 2°C, humidity of 55% ± 5%, with a 12-h light/dark cycle, and allowed free access to tap water and food.

After 1 week of acclimation, estrogen-deficient female rats were prepared as previously described.[7,33] Briefly, ovaries were bilaterally removed from seven anesthetized female rats. Ten female and ten male rats were set as the normal controls.

Determination of estrogen levels in the rats

After 1 month of normal feeding, the drug-free plasma of the rats in the three groups was collected. The estrogen levels in normal female, male, and OVX rats were analyzed by a rat-estrogen ELISA kit (CSB-E07279r).

Ultra-performance liquid chromatography mass spectrometry/mass spectrometry analysis

An UPLC-ESI-MS/MS system was used, consisting of a Waters ACQUITY™ UPLC (Waters; Milford, MA, USA) and ACQUITY TQD (triple quadrupole mass spectrometer) equipped with an ESI source, to analyze plasma profile. MassLynx™ 4.1 software (Waters Corporation; Milford, MA, USA) was used for data acquisition and analysis.

UPLC separation was conducted on a Waters BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm; Waters) with a gradient of 5 M ammonium acetate solution (a) and acetonitrile (b) (0–2 min [5%–45%]; 2–5 min, [45%–86%]; 5–6 min, [86%–100%]; and 7 min, 5%) at a flow rate of 0.2 mL/min. The autosampler was maintained at 4°C. The injection volume was 5 μL.

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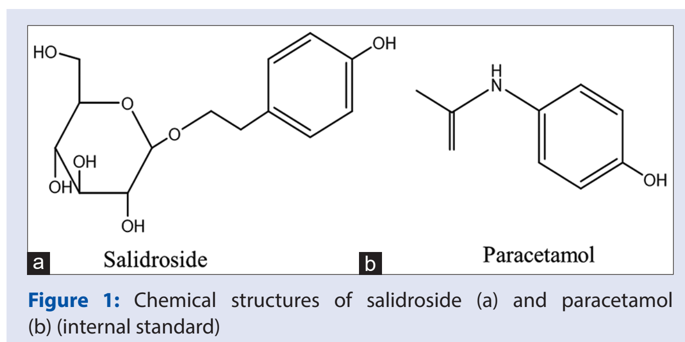

Figure 1: Chemical structures of salidroside (a) and paracetamol (b) (internal standard)

Quantification was carried out on the positive multiple reaction monitoring (MRM) mode with the *m/z* 318.09→121.16 for salidroside; *m/z* 152.03→110.09 for IS [Figure 2]. The operation conditions were optimized as follows: the cone voltage was at 10 kV. The source temperature was at 120°C, with nitrogen as the nebulizing gas. Desolvation temperature was set at 500°C. Argon was used as collision gas with a flow rate of 0.10 mL/min.

Sample preparations

Standard stock solutions of salidroside (300 μg/mL) and IS (300 ng/mL) were prepared in methanol. The working standard solutions of salidroside and IS were made by a serial dilution of the standard stock solutions with methanol, and all the solutions were stored at 4°C until use.

Calibration samples were prepared by spiking blank rat plasma with serial dilutions of salidroside and IS solutions (10 μL). Calibration plots were constructed within the range of 50–30,000 ng/mL for salidroside in rat plasma. Quality control (QC) samples were prepared in parallel with the calibration samples to afford three concentration levels (102.5, 1025, and 10,250 ng/mL).

For the calibration standard and QC samples, a 95-μL aliquot of plasma was spiked with 5 μL of standard solution and 10 μL of IS. For the analysis, a 100-μL plasma was spiked with 10 μL of IS, and 300 μL of methanol was added to precipitate the protein. After vortexing for 30 s and centrifugation at 12,000 rpm for 15 min at 4°C, the supernatants were dried under nitrogen at 40°C. One hundred microliters of methanol was then added to dissolve the residues. After centrifugation for 15 min, 12,000 rpm at 4°C, the clear supernatants were collected, and 5 μL of the solution was injected into the UPLC-MS/MS system.

Method validation

Specificity

To investigate the specificity of the method and eliminate the potential interference from endogenous substances,[34] six different batches of blank rat plasma samples were spiked with analytes and IS. The plasma samples were analyzed to compare the autobiographic profiles regarding retention times and MRM responses.

Linearity and sensitivity

The calibration standards of salidroside were prepared by spiking blank plasma with a certain amount of working solutions to yield desired concentration levels. Six calibration curves for salidroside were individually prepared at concentration ranges between 50 and 30,000 ng/mL on 6 consecutive days. The calibration curves were quantitated in the form of $y = A + B x$, where y represents the peak area ratio of the analytes to IS and x represents the nominal concentration. The weighted ($1/x^2$) least-square linear regression was conducted to get the slope, intercept, and correlation coefficient (R^2)[25] which was required to be above 0.99. The relative standard deviation (RSD) was required to be between ± 15%. The lower limit of quantification (LLOQ) was defined as the lowest concentration at which a signal-to-noise ratio (S/N) was ≥10[32] and a RSD within 20%.

Precision and accuracy

The precision and accuracy of the assay was performed as previously described.[21] Briefly, the predicted concentration (obtained from the calibration curve) was compared to the actual concentration of salidroside spiked into the blank plasma samples at three concentration levels (102.5, 1025, and 10,250 ng/mL) with six replicates on 6 consecutive days. The intra- and inter-day accuracy were calculated as the observed concentration/spiked concentration × 100%, which was required to be within 100% ±15%. The intra- and inter-day precisions were evaluated as RSD, which should not exceed 15%.

Extraction recovery and matrix effect

The extraction recovery and matrix effect were obtained at the same concentration level in the determination of the precision and accuracy parameters. The extraction recovery was corresponded to the ratio between the peak area from the extracted QC samples and the peak area from the extracted blank plasma that was spiked with the standard solutions and IS.[34] The matrix effect was determined by comparing peak areas of the spiked samples (n = 6, at each level) with those of standard solution at the corresponding concentration levels (102.5, 1025, and 10,250 ng/mL).

Stability

The stability of salidroside in rat plasma at low and high concentrations was evaluated by mimicking freshly prepared QC samples that were stored under the following conditions with six replicates: (i) freeze and thaw stability: the QC samples were three-repeat thawed at room temperature for 2 h and then frozen at −20°C for 24 h;[22] (ii) short- and long-term stability: the QC samples were maintained at room temperature for 6 h and −20°C for 4 weeks; (iii) postpreparative stability: the QC samples were kept in an autosampler at 4°C for 24 h; and (iv) stability of stock solutions: the stock solutions of three concentration levels for salidroside were evaluated after storage for 4 h at room temperature and 4 weeks at 4°C.

Pharmacokinetic profile of salidroside in rat plasma samples

One month after OVX, all rats were fasted for 12 h and followed by intragastric gavage administration with the FLL aqueous extract at a bolus of 7 g/kg. The blood was collected from the orbital vein of rats by capillary after dosing at the following time points: 0, 10, 20, 30, 45, and 60 min, and 1.5, 2, 2.5, 3, 5, 7, 10, and 24 h. Then, the blood was centrifuged at 3500 rpm/min for 10 min, and the plasma was then stored at −80°C until use.

The following parameters were used to evaluate pharmacokinetic characteristics: C_{max}, T_{max}, $AUC_{0\text{-}t}$, $t_{1/2}$, CL, V_d, and MRT.

Statistical analysis

The parameters of drug absorption, distribution, and elimination were calculated by noncompartmental analysis (NCA) using the PKSolver software package,[35] a program for pharmacokinetic data analysis in Microsoft Excel. Data were expressed as mean ± standard deviation. The differences between groups were analyzed by one-way ANOVA test when normality (and homogeneity) was satisfied using SPSS software (Version 20) (IBM Corporation; ArmonK, NY, USA). Otherwise, nonparametric and Dunnett's T3 tests were used. $P < 0.05$ was set for statistical significance.

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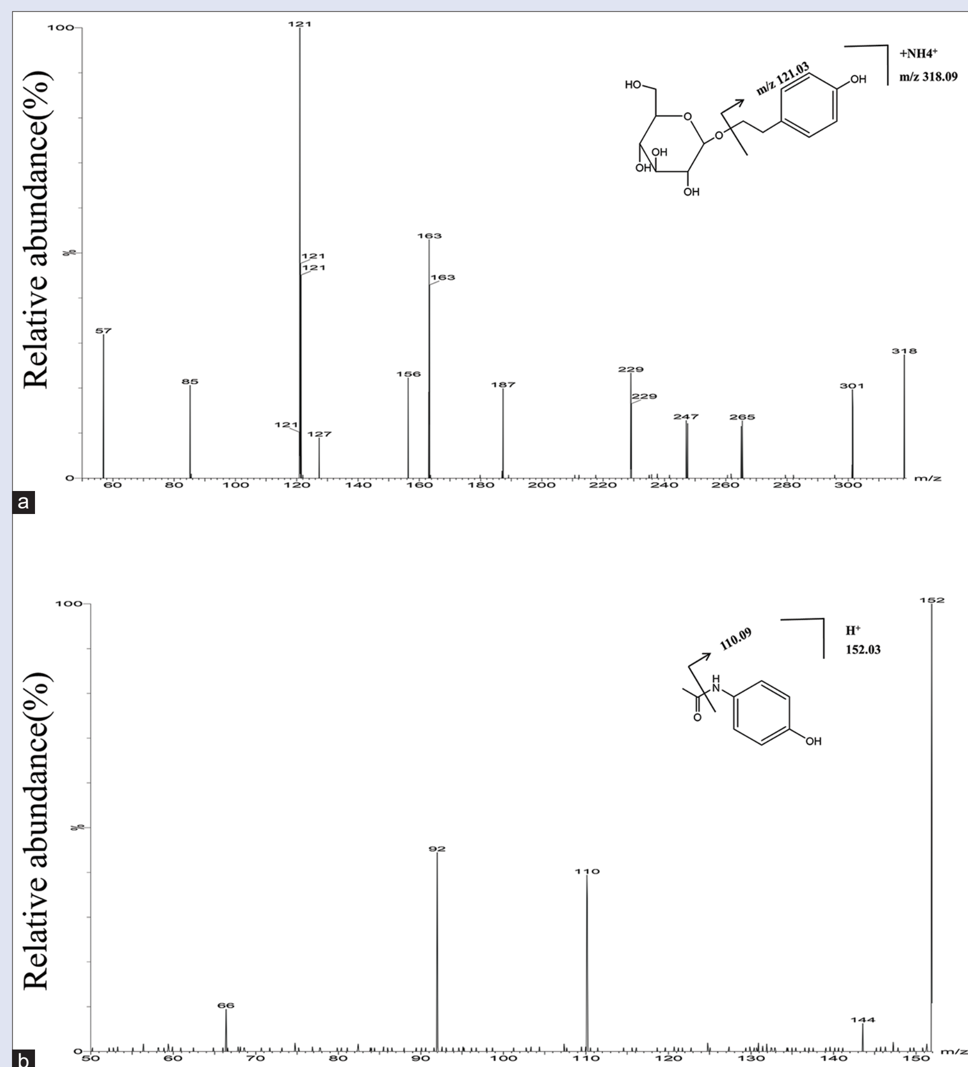

Figure 2: MS^2 spectra of salidroside ([M + NH4]+, *m/z* 318.09) (a) and paracetamol ([M + H]+, *m/z* 110.09) (b)

RESULTS AND DISCUSSION

Estrogen levels in different groups of rats

The estrogen levels in male normal, female normal, and OVX rats were 45.74 ± 7.35, 87.46 ± 3.68, and 50.49 ± 9.08 pg/ml, respectively, indicating that the estrogen levels in OVX rats were statistically significantly reduced when compared to those of the normal female controls ($P < 0.05$). The results suggest that OVX rat models were successfully established.

Method development

An UPLC-MS/MS method was established to assess the rat plasma autobiographic profiles of salidroside in FLL aqueous extract. Moreover, in order to obtain symmetric peak shapes and appropriate retention times, we evaluated different constituents of mobile phases, including methanol-water, laetrile-ammonium acetate, and acetonitrile-5 M ammonium acetate. Based on the preliminary data, the mobile phase composed of acetonitrile-5 M ammonium acetate produced an acceptable peak shape and a short retention time and was thus employed for the gradient ablution. Moreover, we found that signal intensity of full-scan mass spectra of salidroside in positive-ion mode was stronger than that in negative-ion mode. Under the full-scan positive-ion mode, salidroside was an ammonia adduct ion ($[M+NH^4]^+$) of *m/z* 318.09, whereas IS was a hydrogen adduct ion ($[M+H]^+$) of *m/z* 152.03. Therefore, salidroside was analyzed in the positive-ion mode. Paracetamol was employed as the IS due to its structure, autobiographic and mass spectrographic behavior, and stability, which were similar to those of salidroside [Figure 2].[22,25]

The MRM mode was employed to obtain high signal intensity and low noise background of multiple analytes at the same time with the transitions.

As for the sample preparation, three volumes of methanol were adopted for the protein precipitation owing to fast processing procedure and high extraction rate over the liquid–liquid extraction of ethyl acetate, chloroform, and isopropanol.

Method validation

Specificity

The mass spectrograms of the blank rat plasma that either spiked with salidroside (1000 ng/ml) or IS (300 ng/ml) and the rat plasma samples that obtained 30 min after oral administration of the FLL aqueous extract were shown in Figure 3. The retention time was 2.49 min for salidroside, whereas 2.55 min for IS. There were no observable endogenous peaks in the blank rat plasma which could interfere with the analytes or IS,

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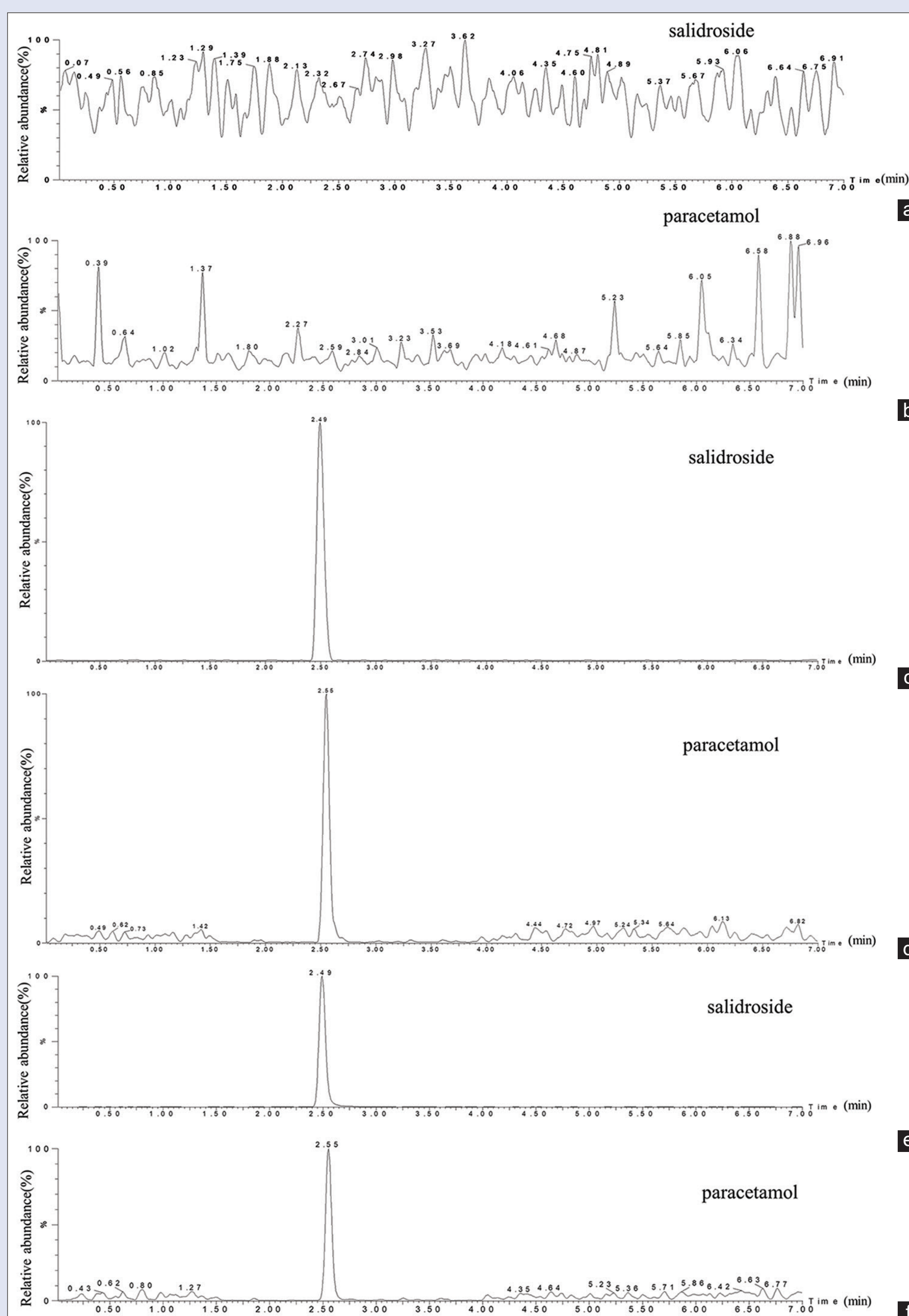

Figure 3: Multiple reaction monitoring chromatograms of salidroside and paracetamol (IS) in (a and b) blank rat plasma sample; (c and d) blank rat plasma sample spiked with salidroside (1000 ng/mL) and IS (300 ng/mL); and (e and f) rat samples were collected at 30 min after oral administration of an aqueous extract of *Fructus Ligustri Lucidi* (7 g/kg) with IS (300 ng/mL). IS: Internal standard

suggesting a high specificity between the analytes and IS at the indicated retention time.

Linearity and sensitivity

The calibration curves showed a good linearity in the concentration range of 50–30,000 ng/mL for salidroside by examining calibration samples (daily prepared for 6 consecutive days) in triplicates at eight different concentration levels. The typical regression equation is $y = 0.001x - 0.003$, where y is the peak area ratio of salidroside to IS and x is the concentration of salidroside in plasma. The correlation coefficient (R^2) was ≥0.999, and the deviation of all calibration concentrations was within ±15%.

The LLOQ for salidroside in plasma was defined as 50 ng/mL based on S/N = 10, and the lower limit of detection was estimated to be about 10 ng/mL based on S/N = 3. The obtained data indicate that 50 ng/mL satisfied the requirements of the pharmacokinetic studies and was therefore selected as the lowest concentration in the calibration curves.

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Precision and accuracy

The intra-day accuracy of salidroside ranged from 99.73% to 103.88% with a precision of 10.52%–14.66%. Moreover, the inter-day accuracy of salidroside ranged from 99.29% to 103.37% with a precision of 10.42%–13.13%. The results were conformed to Food and Drug Administration guidance requirements for the analysis of biological samples, where RSD determined at each concentration level does not exceed ±15% of the actual value. The results suggest that the developed method is accurate and precise for the determination of salidroside in rat plasma samples.

Extraction recovery and matrix effect

The recovery of salidroside ranged from 88.90% to 101.78% in three QC levels with a RSD of <9.96%. These results were within acceptable limit, suggesting that the method has a satisfactory extraction recovery.

The matrix effects for salidroside at low, middle, and high concentration levels were 100.45% ± 11.75%, 88.29% ± 6.61%, and 85.53% ± 5.00%, respectively, with a RSD ranging from 5.80% to 11.70%. There is no co-eluting matrix effect during the ionization of salidroside and IS, indicating no signal suppression or enhancement during the study.

Stability

As shown in Table 1, salidroside standard solutions were stable at room temperature for 4 h and at 4°C for 30 days. QC samples were stable at room temperature for 6 h, at −20°C for 4 weeks, and at 4°C for 24 h in an autosampler, as well as after three freeze-thaw cycles, indicating that salidroside was stable during the preparation and analysis.

Plasma characteristic study

The validated method was successfully used to study the pharmacokinetic characteristics in male, female, and OVX rats after oral administration of an aqueous extract of FLL. The mean concentration–time profiles of salidroside in rat plasma are depicted in Figure 4.

As illustrated in Figure 4 and Table 2, the peak concentration time (T_{max}) for salidroside was lower in normal male (47 min) and female (45.5 min) rats than that in OVX ones (68.57 min), but did not reach a statistical difference. However, the elimination half time ($t_{1/2}$) in OVX rats was 311.78 min, thus much higher than that in female (140 min) normal controls ($P < 0.05$), indicating that ovariectomy slows down the elimination of salidroside in female rats. In addition, the mean residence time ($MRT_{0-\infty}$) in the OVX rats was statistically significantly higher than that in male littermates ($P < 0.05$). However, there was no significant difference in $MRT_{0-\infty}$ between normal male and female rats, indicating that sex differences may not affect the elimination of salidroside *in vivo*.

In addition, the apparent volume of distribution (V_d) of salidroside in the OVX rats was higher than that in male and non-OVX female littermates. Our results are in line with a previous investigation that ovariectomy affects pharmacokinetic parameters of isoflavone bioavailability and various ingredients of *Eucommiae cortex* extracts in mice[36] and rats.[37]

Since ovariectomy has been well validated to mainly cause the estrogen deficiency in animals and those appear most of clinical features in patients with postmenopausal osteoporosis,[38,39] the alterations of plasma profiles of salidroside in OVX rats could, therefore, be attributed to the evidence that estrogen interferes with absorption and metabolism through the regulation of hepatic enzymes.[40,41] In addition, Goulding *et al*. demonstrated that estrogen-mediated induction of growth factors

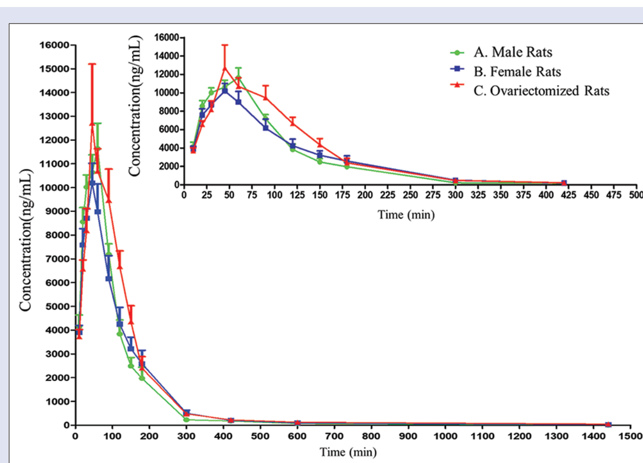

Figure 4: Mean plasma concentration–time profiles of salidroside in rat plasma samples of male, female, and OVX rats after oral administration of *Fructus Ligustri Lucidi* aqueous extract (7 g/kg). The upright panel magnifies the plasma profiles of salidroside in male, female, and OVX rats from 0 to 500 min. OVX: Ovariectomized

Table 1: Stability of salidroside in rat plasma samples under various storage conditions (*n*=6)

Experimental condition	Found			
	Nominal (ng/mL)	Mean±SD (ng/mL)	RSD (%)	Accuracy (%)
Standard solution 4 h at RT	102.5	103.80±2.18	2.10	101.27
	1025	1068.40±133.02	12.45	104.23
	10250	10812.34±842.58	7.79	105.49
Standard solution 4 weeks at 4°C	102.5	102.23±4.11	4.02	99.74
	1025	1064.32±141.74	13.32	103.84
	10250	10767.13±899.28	8.35	105.05
QC samples 6 h at RT	102.5	102.56±10.74	10.47	100.06
	1025	1100.65±112.96	10.26	107.38
	10250	10972.87±1286.20	11.72	107.05
QC samples 4 weeks at−20°C	102.5	102.23±4.42	4.32	99.74
	1025	1055.28±105.47	9.99	102.95
	10250	10746.35±1132.55	10.54	104.84
QC samples autosampler 24 h at 4°C	102.5	100.93±6.85	6.79	98.46
	1025	1091.84±78.20	7.16	106.52
	10250	10812.61±861.40	7.97	105.49
QC samples 3 freeze-thaw cycles	102.5	100.60±2.99	2.97	98.15
	1025	1021.34±59.27	5.80	99.64
	10250	10744.07±1159.13	10.79	104.82

SD: Standard deviation; RSD: Relative SD; RT: Room temperature; QC: Quality control

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Table 2: Pharmacokinetic plasma parameters of salidroside in rats

Parameters	Oral administration of FLL aqueous extract (7 g/kg)		
	Male rats (*n*=10)	Female normal rats (*n*=10)	Ovariectomized rats (*n*=7)
	Salidroside (mean±SD)		
C_{max} (ng/ml)	12813.85±2847.37	11353.87±2611.66	14429.95±5538.03
T_{max} (min)	47±15.49	45.5±13.01	68.57±20.96
$t_{1/2}$ (min)	140.00±49.41	134.96±44.28	311.78±172.40*
$AUC_{0-\infty}$ (ng/ml·min)	1355790±361354.9	131432±345270.6	1616301±255073.4
$MRT_{0-\infty}$ (min)	107.72±15.17	144.17±46.98*	159.10±38.09#
V_d (mL/ng)	0.15±0.04	0.11±0.01	0.25±0.11*,#
CL (mL/ng·min)	0.00061±0.00014	0.00062±0.00015	0.00049±7.63E-05

**P*<0.05: Compared with the normal female rats; #*P*<0.05: Compared with the male rats. C_{max}: Peak concentration; T_{max}: Peak concentration time; $AUC_{0-\infty}$: Area under the drug-time curve from zero to the last measurable plasma concentration point; $t_{1/2}$: Elimination half time; CL: Clearance; V_d: Apparent volume of distribution; MRT: Mean residence time; FLL: *Fructus Ligustri Lucidi*; SD: Standard deviation

from uterine tissue was not involved in the bone-preserving actions in OVX rats.[42] However, further investigations are still needed to study the plasma pharmacokinetics of salidroside in OVX rats exposed to exogenous estrogen. Moreover, additional studies using different phases of the estrus cycle of normal female rats will contribute to elucidating the role of estrogen in determining the plasma profile of salidroside.

While analyzing the plasma characteristics of salidroside in OVX rats, we also need to consider the contribution of other sex hormones in this process. Ovariectomy not only provokes a decrease in estrogen, testosterone, and progesterone (PG), but also triggers an alteration in the levels of follicle-stimulating hormone (FSH),[43] luteinizing hormone (LH),[44] and gonadotropin-releasing hormone (GnRH) release.[45-48] Given that estrogen supplementation could effectively prevent most of the effects of ovariectomy in animals models,[49-51] we still cannot rule out the involvement of LH, FSH, GnRH, and PG in the regulations of pharmacokinetics of salidroside in OVX rats.

One limitation of the current study is that the non-operated rats instead of sham-operated animals were used as the controls. Historically, sham-operated rats which experience the same manipulations as the OVX rats are ideal controls for the study. However, Kruger and Morel[52] and Noorafshan *et al*.[53] demonstrated that there are no obvious differences in the blood and stereological parameters between sham and un-manipulated rats. In addition, the results of the current study are not confirmed in human clinical trials.

Together, ovariectomy appears to slow down the rate of the clearance rate of salidroside and increase time remaining and volume distributed around the body, which indicates that ovariectomy could influence the metabolism of salidroside after oral administration of FLL aqueous extracts. Further investigations are needed to study the effect of the serum from OVX rats after oral administration of FLL aqueous extract on the regulation of osteoblastogenesis and osteoclastogenesis.

CONCLUSION

A simple, sensitive, and precise UPLC-MS/MS method has been developed for the determination of plasma profile of salidroside in OVX rats after oral administration of FLL aqueous extract. This method could be used for further investigation of tissue distribution and plasma protein-binding capacity of salidroside in OVX rats. The results may suggest that ovariectomy could interfere with the plasma profile of salidroside in SD rats.

Acknowledgements

We thank Dr. Yuelin Song at Modern Research Center for TCM at BUCM for his assistance with data analysis and Dr. Yongqian Zhang at Life Sciences School at Beijing Institute of Technology for his contribution to the establishment of the analytic methods.

Financial support and sponsorship

Financial supports came from Beijing Municipal Natural Science Foundation (Grant No. 7172126), National Natural Science Foundation of China (Grant No. 81874373), and the Program for Innovative Research Team in University, China (Grant No. IRT_17R11).

Conflicts of interest

There are no conflicts of interest.

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