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ORIGINAL ARTICLES

LC-MS determination and pharmacokinetic study of salidroside in rat plasma after oral administration of suspensions of traditional Chinese medicine *Erzhi Wan* and Fructus *Ligustri lucidi*

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Abstract: A simple, rapid and sensitive liquid chromatography-mass spectrometry (LC-MS) method was developed for the determination of salidroside in rat plasma and study of its pharmacokinetics after oral administration of suspension of *Erzhi Wan* and Fructus *Ligustri lucidi* into Wistar rats. Plasma sample of 200 μL was extracted with acetic ether-isopropanol (2 : 1) and the extraction was performed on a Kromasil C_{18} column (150 mm × 4.6 mm, 5 μm) with the mobile phase of methanol-water (41 : 59, v/v) within a run time of 6.0 min. The analyte was monitored with positive electrospray ionization (ESI) by selected ion monitoring (SIM) mode. The target ions were m/z 323.05 for salidroside and m/z 411.05 for internal standard (IS) geniposide. A good linear relationship was obtained over the range of 5.0 - 500.0 ng/mL and the lower limit of quantification was 5.0 ng/mL. The validated method was successfully applied to the pharmacokinetic study of salidroside in rat plasma after oral administration of suspension of *Erzhi Wan* and Fructus *Ligustri lucidi*.

Keywords: liquid chromatography-mass spectrometry (LC-MS); pharmacokinetics; salidroside; *Erzhi Wan*; Fructus *Ligustri lucidi*

1 Introduction

Erzhi Wan, listed in Chinese Pharmacopoeia (2010 Edition) [1], composed of Fructus *Ligustri lucidi* and Herba *Ecliptae*, is a well-known traditional Chinese medicinal (TCM) formulation, which was first recorded by Wang Ang in his 1682 AD publication *Yifang Jijie* (Collection of Medical Formulas) [2]. According to Wang Ang, *Erzhi Wan* is a nourishing formula, which can "supplement the lower back and knees, strengthen the sinews and bones, strengthen the kidneys, and blacken hair" [2]. The pair of herbs, Fructus *Ligustri lucidi* and Herba *Ecliptae*, as basic composition units of TCM formulations, have special clinical significance and are increasingly mentioned in modern Chinese medical literature [3,4]. It is reported that they have some kinds of synergetic effects. Fructus *Ligustri lucidi*, recorded in Chinese Pharmacopoeia (2010 Edition) [1], has been used as traditional medicine for thousands of years. Recent pharmacological studies have shown that Fructus *Ligustri lucidi* has many strong activities, such as anti-fatigue, anti-oxidant, anti-tumor, anti-aging, and adaptogenic ones [5,6]. Salidroside (Figure 1a) is one of the active ingredients in Fructus *Ligustri lucidi* and *Erzhi Wan*, with enhancing immunity, anti-aging and some other important pharmacological effects [7,8]. Therefore, it is significant to study the pharmacokinetics of salidroside in *Erzhi Wan* and Fructus *Ligustri lucidi* for the clinical use of them.

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Many analytical methods for the determination of salidroside in *Rhodiola* species have been reported and the pharmacokinetics of pure salidroside in rat plasma has also been studied [9-13]. However, there are few literature reports on the determination of salidroside in Fructus *Ligustri lucidi*. Besides, due to the interaction of TCM ingredients, it is necessary to explore whether some other ingredients in Fructus *Ligustri lucidi* or Herba *Ecliptae* affect the pharmacokinetic behavior of salidroside. As far as we know, there is no published report on the pharmacokinetic studies of the compound in rat plasma after administration of *Erzhi Wan* and Fructus *Ligustrum lucidum*. In our study, a liquid chromatography-electrospray ionisation-tandem mass spectrometry (LC-ESI-MS) method was developed and validated for the determination of salidroside in rat plasma and for the pharmacokinetic study of the compound after oral administration of *Erzhi Wan* and Fructus *Ligustrum lucidum*.

2 Experimental

2.1 Materials, chemicals and reagents

Fructus *Ligustri lucidi* and Herba *Ecliptae* were purchased from Tongrentang (Shenyang, China) and identified by Professor Qi-Shi Sun (Department of Pharmacognosy, Shenyang Pharmaceutical University, Shenyang, China). Salidroside was purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Geniposide (Figure 1b) was isolated from *G. jasminoides* in our laboratory. Its structure was fully char-

acterized based on chemical and spectroscopic analysis (UV, IR, NMR, and MS), and the purity was determined as more than 98% by HPLC analysis. Methanol (HPLC grade) was purchased from Fisher Scientific (Pittsburgh, PA, USA). All other reagents were of analytical grade and obtained from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Distilled water, prepared from demineralized water, was used throughout the experiments.

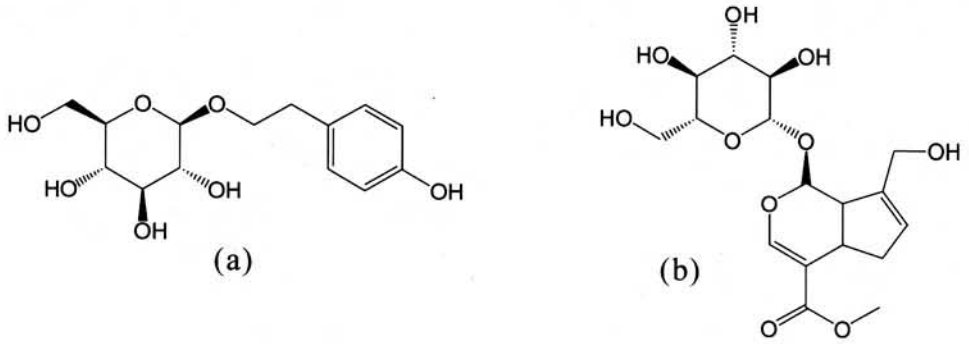

Figure 1 Chemical structures of salidroside (a) and IS (b)

2.2 Animals

Male pathogen-free Wistar rats (220 − 270 g) were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University. Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University and the protocol was approved by the Animal Ethics Committee of this institution. The rats were fed with a standard laboratory diet and water for at least 3 days before the experiments.

2.3 LC-MS instruments and analytical conditions

The assay was performed on Shimadzu (Japan) LC-MS 2010A system. Liquid chromatographic separation was achieved on a Kromasil C_{18} column (150 mm × 4.6 mm, 5 μm) and preceded by a C_{18} guard column (4.0 mm × 2.0 mm, Phenomenex, Torrance, CA, USA). The column and autosampler tray temperature were kept constant at 25 ℃ and 4 ℃, respectively. The mobile phase was methanol-water (41 : 59, v/v). The flow rate was set at 0.8 mL/min with 25% of the eluent split into the inlet of the mass spectrometer and the injection volume was set at 20 μL. Time of the analysis was 6 min.

The electrospray ionization (ESI) was performed using nitrogen to assist nebulization (the flow rate was set at 1.5 L/min). The analytes were ionized by an ESI source in positive ion mode under the following source conditions: Nebulizing gas 1.5 L/min; drying gas 2.0 L/min; CDL temperature 250 ℃; heat block temperature 200 ℃; detector voltage 1.75 kV; the other parameters were fixed as for the tuning file. Analysis was carried out in selected ion monitoring at the $[M + Na]^+$, m/z 323.05 for salidroside and $[M + Na]^+$ m/z 411.05 for IS. The full-scan mass spectra of salidroside and IS after injection in mobile phase are shown in Figure 2.

2.4 Preparation of *Erzhi Wan* suspension and Fructus *Ligustri lucidi* suspension

Erzhi Wan suspension was made according to preparation of *Erzhi Wan* stated in Chinese Pharmacopoeia (2010 Edition) [1]. Fructus *Ligustri lucidi* suspension was made to have the same concentration of Fructus *Ligustri lucidi* as *Erzhi Wan* suspension. The dosage form of suspension was chosen for its convenience in gastric perfusion.

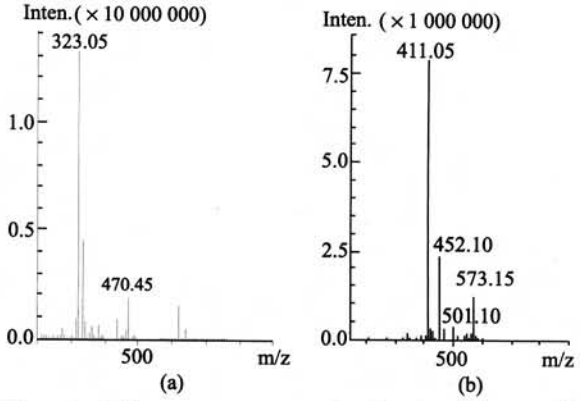

Figure 2 Full scan mass spectra of salidroside (a) and IS (b)

Erzhi Wan suspension was prepared as follows: about 100.0 g Fructus *Ligustri lucidi* was crushed to powder, and then was sifted through a 100 mesh sieve. 30.0 g of Herba *Ecliptae* was decocted twice, 1 h each time, with suitable amounts of water. After that, the decoction was filtered and concentrated. The concentrate was mixed with 30.0 g fine powder and added with appropriate amounts of water to 100 mL, giving a *Ligustri lucidi* concentration of 0.3 g/mL (expressed as the weight of herbal material in water). Shake well before use.

Fructus *Ligustri lucidi* suspension was prepared as follows: 30.0 g of Fructus *Ligustri lucidi* powder (prepared above) was suspended with appropriate amounts of water to 100 mL, giving a *Ligustri lucidi* concentration of 0.3 g/mL (expressed as the weight of herbal material in water). Shake well before use.

2.5 Preparation of plasma samples

The plasma (200 μL) was spiked with 50 μL of IS (40 ng/mL), 50 μL of methanol, and 1 mL of acetic ether-isopropanol (2 : 1). The mixture was vortex-mixed for 3 min,

and then centrifuged at 15 000 rpm for 3 min. The supernatant was transferred into a clean test tube and evaporated to dryness in a thermostatic controller at 30 ℃ under a slight stream of nitrogen. The residue was reconstituted in 100 μL mobile phase, with vortex mixing for 2 min, and repeatedly centrifuged at 15 000 rpm for 5 min. An aliquot of 20 μL was injected into the LC-MS system for analysis.

2.6 Method validation

2.6.1 Selectivity

Selectivity was assessed by comparing chromatograms of six different batches of blank rat plasma with those of corresponding standard plasma samples spiked with salidroside, IS, and a plasma sample obtained after drug administration.

2.6.2 Linearity and lower limit of quantification (LLOQ)

Calibration curves were prepared by assaying standard plasma samples at six concentrations of salidroside ranging from 5.0 to 500.0 ng/mL. The linearity was determined by plotting the peak area ratio (y) of salidroside to IS versus the nominal concentration (x) of salidroside. The calibration curve was constructed by weighted ($1/x^2$) least squares linear regression. The LLOQ was estimated in accordance with the base line noise, considering a signal-to-noise ratio of 10 : 1.

2.6.3 Precision and accuracy

The intra-day precision and accuracy were determined by analyzing six QC replicates at concentrations of 10.0, 100.0 and 400.0 ng/mL for salidroside on one day ($n = 5$), and inter-day precision and accuracy by repeated analysis on three consecutive days ($n = 5$ series per day). Accuracy was defined as the relative error (RE %) while the precision was defined as the relative standard deviation (RSD %).

2.6.4 Extraction recovery

Recovery of the extraction procedure was evaluated at three concentrations (10.0, 100.0 and 400.0 ng/mL) for salidroside and at 40.0 ng/mL for the IS. It was determined by comparing the mean peak areas ($n = 6$, at each concentration) obtained from plasma samples spiked before extraction with those from plasma samples spiked after extraction.

2.6.5 Stability

The stability of processing (3 freeze-thaw cycles), sample storage (at room temperature for 4 h, at −20 ℃ for 7 d), and post-treatment (in the reconstituted extract at 4 ℃ kept from light and in the auto-sampler tray for 8 h) was assessed by analyzing replicates ($n = 3$) of QC samples. The resulting concentrations were compared with their theoretical concentrations, and the percentage of deviation was calculated. Samples were to be concluded stable if the relative error was within ±15%.

2.6.6 Pharmacokinetic application

Wistar rats were divided into two groups randomly ($n = 6$) and were fasted 12 h before dosing and with free access to water during the experiment. The proposed method was applied to the pharmacokinetic studies after oral administration of 2 mL suspension of *Erzhi Wan* (group one) and 2 mL suspension of *Ligustrum lucidum* (group two). Blood samples from the orbital veins of the rats (0.5 mL) were withdrawn into the heparinized tubes at 0 (before dosing), 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 5, 9, and 12 h and immediately centrifuged at 15 000 rpm for 5 min. The plasma samples were stored at −20 ℃ until analysis. The plasma concentrations of salidroside at different time points are expressed as mean ± SD, and the mean concentration versus time curve was plotted. All the data were calculated by DAS 2.1 statistical software (Pharmacology Institute of China).

3 Results and discussion

3.1 Method validation

3.1.1 Specificity

No endogenous interference was observed at retention times of salidroside (3.6 min) and IS (5.2 min) because of the high selectivity of SIM mode. Typical chromatograms of blank plasma, spiked plasma sample and subject sample are shown in Figure 3.

3.1.2 Matrix effect

Three replicate analyses of samples (10.0, 100.0 and 400.0 ng/mL for salidroside) in plasma and in methanol were analyzed respectively. Area ratios were between 90% and 110%, showing that matrix effects could be ignored.

3.1.3 Linearity and LLOQ

The representative regression equation was $y = 1.01 \times 10^{-3} x + 2.26 \times 10^{-2}$ over the range of 5 − 500 ng/mL with a correlation coefficient of 0.9981. The LLOQ in plasma was 5 ng/mL with the RSD and RE within ±20%.

3.1.4 Precision and accuracy

The intra-day and inter-day precision and accuracy are presented in Table 1. The precision and accuracy of the developed method conformed to the criteria for the analysis of biological samples according to the guidance of FDA, where the RSD determined at each concentration level is required not to exceed ±15% and RE within ±15% of the actual value.

3.1.5 Extraction recovery and stability

The recoveries of salidroside at three concentration levels (10, 100 and 400 ng/mL) were 71.4%, 69.5% and 68.1%, respectively, and the absolute recovery of IS was 78.5%. The extraction efficiency was stable, indicating that the sample preparations were consistent, precise, and reproducible at different concentration levels.

The stability results are shown in Table 2, which indicated that salidroside had no significant degradation under the conditions previously described.

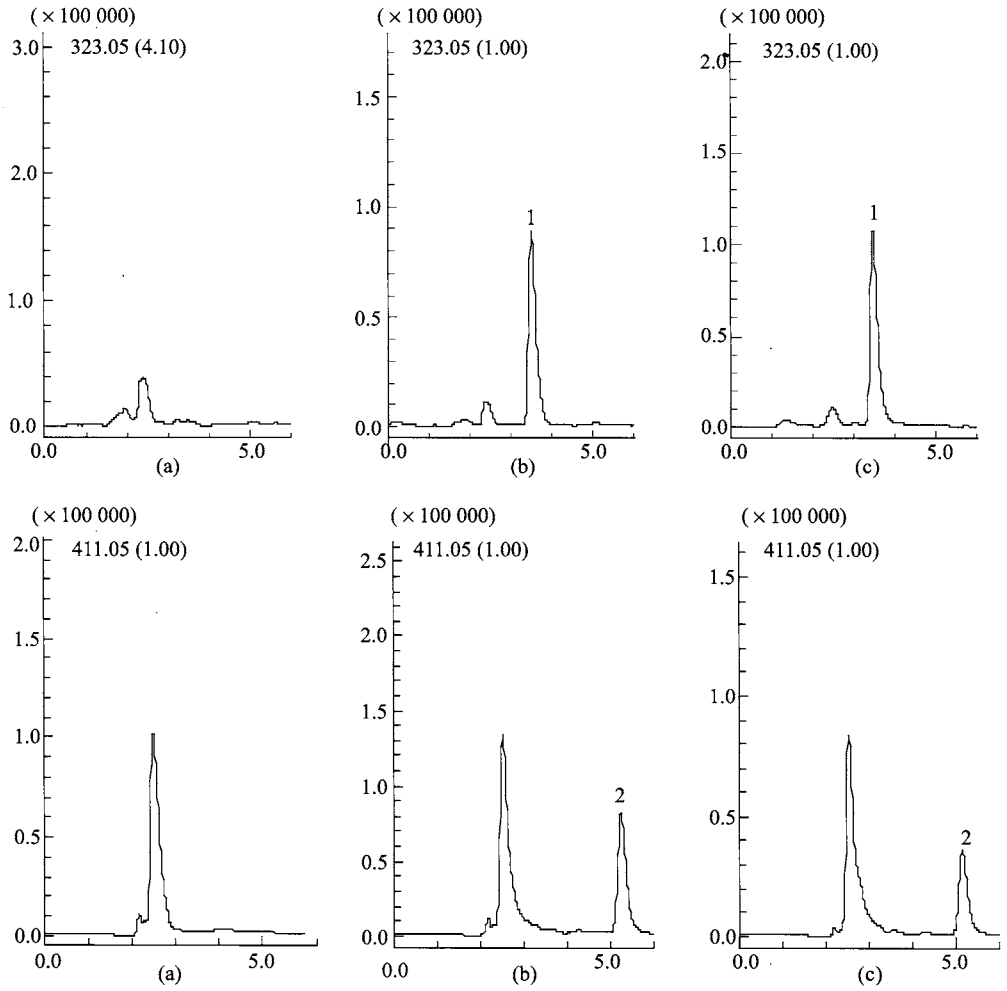

Figure 3 SIM chromatograms of a blank rat plasma (a), a blank rat plasma spiked with salidroside (400 ng/mL) and IS (b), and a rat plasma sample 30 min after oral administration of *Erzhi Wan* suspension (c). 1, salidroside; 2, IS.

Table 1 Precision and accuracy of salidroside in rat plasma

Spiked concentration (ng/mL)	Inter-day precision ($n=5$)			Intra-day precision ($n=15$)		
	Measured concentration (mean ± SD, ng/mL)	RE (%)	RSD (%)	Measured concentration (mean ± SD, ng/mL)	RE (%)	RSD (%)
10	10.14 ± 0.79	1.0	8.0	10.14 ± 0.75	1.0	8.0
100	98.50 ± 5.30	−1.5	5.4	98.50 ± 5.30	−1.5	5.4
400	392.70 ± 14.20	−1.9	3.7	392.70 ± 55.50	−1.9	14.2

Table 2 Stability of salidroside in rat plasma

Spiked concentration (ng/mL)	Short-term stability		Freeze and thaw stability		Post-preparative stability (24 h at room temperature)		Stability for 15 d at −20 ℃	
	Mean ± SD	RSD(%)	Mean ± SD	RSD(%)	Mean ± SD	RSD(%)	Mean ± SD	RSD(%)
10	10.8 ± 0.5	4.1	10.3 ± 0.9	8.7	9.7 ± 0.6	5.5	10.9 ± 0.3	2.2
100	97.8 ± 2.6	2.7	109.6 ± 6.0	5.4	98.0 ± 2.9	2.9	110.3 ± 2.9	2.6
400	378.0 ± 8.3	2.2	409.1 ± 8.1	2.0	375.1 ± 11.7	3.1	435.3 ± 31.8	7.3

3.2 Application in pharmacokinetic studies

The developed and validated LC-MS method was successfully used to monitor the plasma concentrations of salidroside in rats after oral administration of suspensions of *Erzhi Wan* and *Ligustri lucidi* at a dose of 2.4 g (expressed as the weight of *Ligustri lucidi* in water, containing 6.0 mg of salidroside) per kg body weight. The mean plasma concentration-time profile is illustrated in Figure 4. The pharmacokinetic parameters are listed in Table 3.

In this paper, the pharmacokinetic parameters of salidroside were, to a certain degree, in accordance with previous reports [10-13]. Meantime, we found that the pharmacokinetic parameters of salidroside after oral administration of

suspension of *Erzhi Wan* were different from those after oral administration of suspension of Fructus *Ligustri lucidi*.

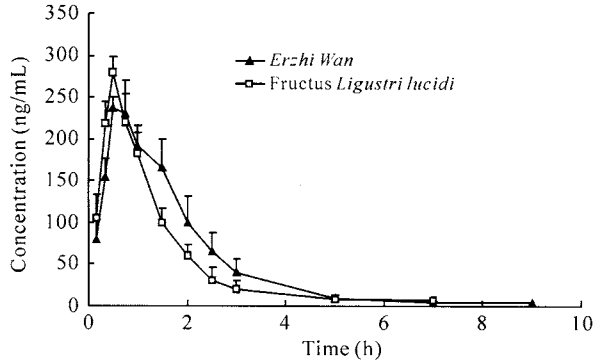

Figure 4 Mean plasma concentrations *vs.* time profile in six rats after oral administration of *Erzhi Wan* (▲) and Fructus *Ligustri lucidi* (□) (at a dose containing salidroside 6.0 mg/kg) ($n = 6$).

It was demonstrated that the absorption of salidroside after oral administration of suspension of *Erzhi Wan* was slower than that of Fructus *Ligustri lucidi*, with their T_{max} 0.63 and 0.54 h, respectively. The $t_{1/2}$ value after administration of *Erzhi Wan* was 1.02 h, while that after administration of Fructus *Ligustri lucidi* was 0.75 h. AUC_{0-t} of salidroside after administration of *Erzhi Wan* was much higher than that after administration of Fructus *Ligustri lucidi*, while its C_{max} was significantly lower ($P < 0.05$). These differences could be due to the following causes: (1) other constituents in *Erzhi Wan*, such as the saponins and wedelolactone from Herba *Ecliptae*, facilitated the dissolution of salidroside from Fructus *Ligustri lucidi*; (2) some ingredients in *Erzhi Wan* affected the absorption and metabolism of salidroside, thus increasing its bioavailability; (3) there are some other kinds of synergetic effects.

Table 3 The pharmacokinetic parameters of salidroside in rat plasma after oral administration of *Erzhi Wan* and Fructus *ligustri lucidi* (at a dose containing salidroside 6.0 mg/kg) ($n = 6$, mean ± SD)

Parameter	Administration of *Erzhi Wan*	Administration of Fructus *ligustri lucidi*
$t_{1/2}$ (h)	1.02 ± 0.40	0.75 ± 1.40
T_{max} (h)	0.63 ± 0.14	0.54 ± 0.10
C_{max} (ng/mL)	247.40 ± 18.80	282.30 ± 15.90
AUC_{0-t} [ng/(h·mL)]	435.00 ± 69.00	361.40 ± 35.60
$AUC_{0-\infty}$ [ng/(h·mL)]	461.30 ± 70.80	370.30 ± 41.70

4 Conclusion

A rapid and reliable LC-MS method has been developed for the determination of salidroside in rat plasma after oral administration of *Erzhi Wan* and Fructus *Ligustri lucidi*. The method is simple and ensures accurate, specific and precise determination of salidroside in rat plasma. This is the first report of a pharmacokinetic study of salidroside after administration of *Erzhi Wan* and Fructus *Ligustri lucidi*. The pharmacokinetic results may be useful for further studies of the clinical applications of *Erzhi Wan* and Fructus *Ligustri lucidi*.

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