Simultaneous Quantification of Five Lignans from *Schisandra chinensis* in Various Tissues of Rats

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In this research study, a rapid, sensitive, and specific high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (HPLC–ESI–MS/MS) method was established and validated, in regard to the simultaneous quantification of five sedative and hypnotic lignans (schisandrin, schisandrol B, schisantherin A, deoxyschisandrin, and schisandrin B) in various tissues of rats (including heart, liver, spleen, lung, and kidney). The purpose of the study was to clarify the tissue distribution of the total lignans extract of *Schisandra chinensis* (*SC*). Then, the analytes were separated on a MERCK Purospher STAR LP C_{18} column (250 mm × 4.6 mm, 5 μm), with a mobile phase consisting of 0.05% (v/v) formic acid acetonitrile, and 0.05% (v/v) formic acid water, and a flow rate of 1 mL/min. All of the calibration curves of the five components showed good linearity ($r > 0.9950$), with ranges of 4.8 to 1920 ng/mL for analytes. The intra-day and inter-day precisions (relative standard deviation [RSD] %) were within 13.76% for all of the analytes. The average recoveries of the five analytes were greater than 85.23%, and the mean value of the matrix effect ranged from 82.3% to 93.4%. The five analytes were confirmed to be stable during the storage, preparation, and analytic procedures. The major target tissues of the total lignans extract of the *SC* in the rats were the livers and kidneys.

Keywords: *Schisandra chinensis*, lignans, tissue distribution, HPLC–ESI–MS/MS

Introduction

Schisandra chinensis is one of the most well-known and officially documented traditional Chinese medicine (TCM) sedatives and tonics in the Chinese Pharmacopoeia. It is derived from the dried fruit of *S. chinensis* (Turcz.) Baill and possesses astringency for sweating, seminal emission, diarrhea, and tranquillizing of the mind. It has been used in clinical practice in China for thousands of years for the treatment of jaundice, spontaneous diaphoresis, and nocturnal diaphoresis, as well as other liver diseases [1]. The preparations of this species have recently become more prevalent due to its known antihepatotoxic effects in Western countries.

The major bioactive compounds of *S. chinensis* (*SC*) are lignans with a dibenzo-cyclooctadiene skeleton, including schisandrin, schisandrol B, schisantherin A, deoxyschisandrin, schisandrin B and C, gomisin J and N, and so on [2–8], which have been reported to show antihepatotoxic, antiasthmatic, anti-human immunodeficiency virus (HIV), and anti-tumor effects, as well as platelet-activating factor antagonistic and central nervous system (CNS)-protecting activities [9–11]. Diphenyl diester bifendate (DDB) is an important synthetic intermediate of schisandrin C, which has been shown to have the ability to significantly decrease the level of glutamic pyruvic transaminase (GPT) and glutamic oxalic transaminase (GOT) and increase the concentrations of hepatic microsomes (for example, CYP450), as well as have the function of liver detoxication. It has been used to protect the liver from poison damage, such as carbon tetrachloride, and anticancer drugs, along with anti-tuberculotic and other chemicals currently used for the treatment of chronic hepatitis in China [12].

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Therefore, in this study, a simple method was developed using macroporous resin to obtain the total lignans extract from the extract of *S. chinensis*, which was collected using an 85% ethanol solution. The following were the identified major bioactive lignans: schisandrin A (SinA), schisandrin B (SinB), schisandrin C (SinC), schisandrol A (SolA), schisandrol B (SolB), and schisantherin A (SthA) [13].

A number of studies which focused on the pharmacokinetics of *Schisandra* lignans have been performed in the previous several decades [14–20]. However, the examinations of the tissue distribution of rats have been correspondingly deficient and have mainly focused on the single lignans of *SC*, rather than the total. The previous studies have reported that considerable concentrations of *Schisandra* lignans could be detected in the hearts, livers, spleens, lungs, and kidneys of rats, and the use of acupuncture could significantly promote the histological distribution, which demonstrated that the lignans of *SC* were widely distributed in the rat tissues.

In view of the abovementioned beneficial effects, it was concluded that detailed in vivo tissue distribution studies of the total lignans extract of *SC* were required. In this study, five lignans were selected as the representative markers. These included schisandrin, schisandrol B, schisantherin A, deoxyschisandrin, and schisandrin B (Figure 1). In this study, a simple, sensitive, and reliable high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (HPLC–ESI–MS/MS) method was developed for the simultaneous determination of the five active *Schisandra* lignans in the biological samples. Using this method, the main target organs of the five lignans of *SC* in the rats were explored. Then, the method was validated in terms of selectivity, sensitivity, accuracy, precision, and recovery. This was the first report in which the tissue was the main distribution

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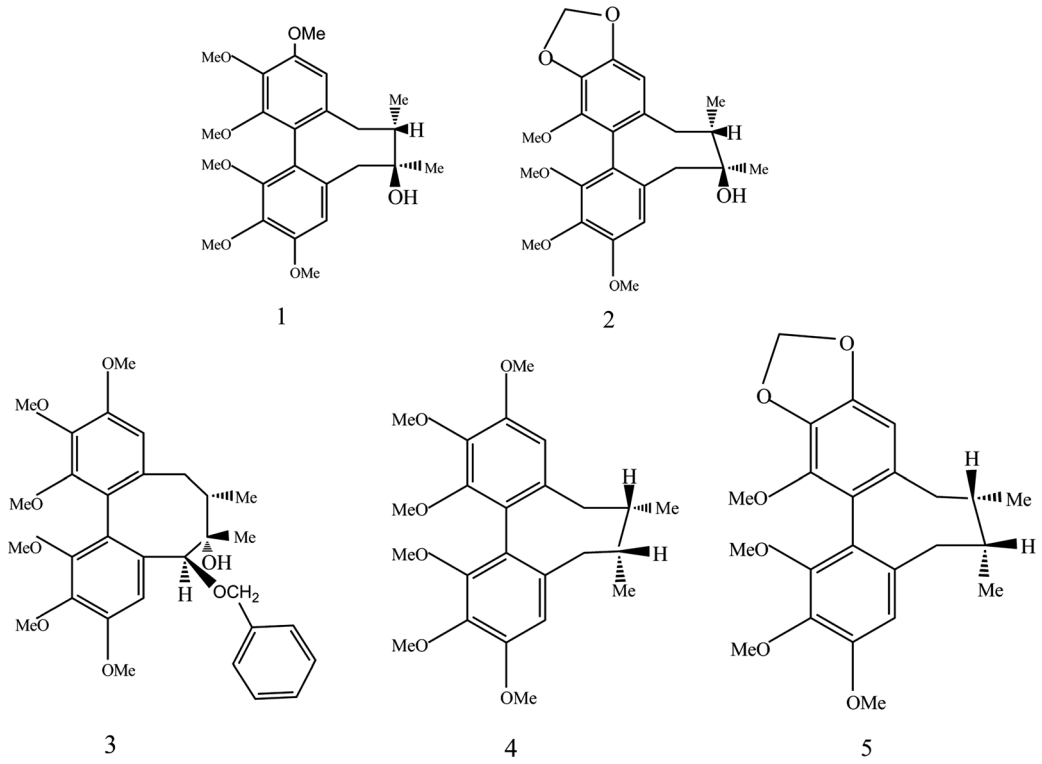

Figure 1. Chemical formulas of lignan components identified in the total fraction obtained from *SC*: 1, schisandrin; 2, schisandrol B; 3, schisantherin; 4. deoxyschisandrin; and 5, schisandrin B

organ used to simultaneously analyze the schisandrin, schisandrol B, schisantherin A, deoxyschisandrin, and schisandrin B in the rat tissues following the oral administration of the total lignans extract of *SC*. Therefore, the present study provided a rational explanation of why the total lignans extract of *SC* could potentially be beneficial for the treatment of hepatic diseases and may be helpful for the future research and development of drugs for liver diseases using the total lignans extract of *SC*.

Experimental

Materials and Reagents. In this research study, the acetonitrile for the HPLC was purchased from Merck (Darmstadt, Germany), and the water for the HPLC was prepared using a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA). The formic acid was purchased from the Nanjing Reagent Factory (Nanjing, China). The schisandrin, schisandrol B, schisantherin A, deoxyschisandrin, and schisandrin B (purity >98%) were purchased from the National Institute for the Control of Pharmaceuticals (Nanjing, China), and the testosterone (purity >99%) was obtained from Dr. Ehrenstorfer GmbH (Germany). The total lignans extract of *SC* was obtained from this study's laboratory and was pre-determined to contain schisandrin (19.84%), schisandrol B (5.91%), schisantherin A (0.99%), deoxyschisandrin (3.69%), and schisandrin B (8.03%). Their chemical structures are detailed in Figure 1.

Animals. The complete experimental protocol was carried out under the guidelines of the use of living animals in scientific investigations. All of the studies were conducted in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China. All of the protocols regarding living animals used in this study were from the Experimental Animal Center of Nanjing University of Chinese Medicine, license no. SYXK (Su) 2014-0001. The animals were healthy male adult Sprague-Dawley rats, weighing between 220 and 250 g, and approximately 3 months of age. They were maintained under a 12 h light/dark cycle at a controlled temperature (25 °C and 55% to 60% relative humidity), with free access to food and tap water until the day of the experiment. The 15 healthy rats were divided into three groups of five.

Instrumentation and Conditions. The assay was carried out using an Agilent 1200 series HPLC system equipped with an online degasser, a binary solvent delivery system, and a thermostatically controlled column apartment. Also, a G2710BA single quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA), which was equipped with an electrospray source, was connected to the liquid chromatography (LC) system. The chromatographic separation was achieved using a MERCK Purospher STAR LP C_{18} column (250 mm × 4.6 mm, 5 μm), and the column was maintained at 30 °C. The mobile phase consisted of 0.05% (v/v) formic acid in water (A) and 0.05% (v/v) formic acid in acetonitrile (B), using the following gradient program: 0 to 7 min, 55% B; 7 to 25 min, from 55% to 80% B; and 25 to 30 min, from 80% to 85% B. The flow rate was 1.0 mL/min, and the injection volume was 10 μL. ESI–MS conditions were as follows: drying gas (N_2) flowing rate, 10.0 L/min; drying gas temperature, 350 °C; pressure of the nebulizer, 35 psi; capillary, 3500 V; and fragmentor, 80 V. A select-ion-monitoring (SIM) in the positive ion mode was used. Then, all of the analyte ions were recorded as follows: *m/z* 432 → 415 $[M–H_2O+H]^+$ for the schisandrin, *m/z* 416 → 399 $[M–H_2O+H]^+$ for the schisandrol B, *m/z* 414 → 400 $[M–CH_3+H]^+$ for the schisantherin A, *m/z* 416 → 402 $[M–CH_3+H]^+$ for the deoxyschisandrin, *m/z* 400 → 386 $[M–CH_3+H]^+$ for the schisandrin B, and *m/z* 288 → 289 $[M+H]^+$ for the testosterone. All of the operations, as well as the acquirement and analysis of the data, were controlled by Chemstation software (Agilent Technologies, USA).

Preparation of the Total Lignans Extract of *SC*. Fructus *S. chinensis* is the desiccative ripe fruit which comes from the *S. chinensis* (Turcz.) Baill belonging to the botanical family Magnoliaceae. It is mainly planted in Jilin, Liaoning, and Heilongjiang provinces. The plant materials used in this study

were purchased from the Chinese Medicine Pieces Co. Ltd. of Tongling, Fengyuan, in Anhui Province, batch number: 120405. The dry *SC* was crushed into pieces before using. The dried fruits of *SC* were crushed into pieces before using and boiled twice in 85% ethanol under a reflux condenser, for 2 h each time. After merging the filtrate, and condensing to certain concentration under reduced pressure, the impurities were removed from the extract on a macroporous resin and, finally, the powder was obtained containing the total lignans extract of *SC*. The powder was dissolved in 0.5% sodium carboxymethyl cellulose, at a concentration of 0.5 g/mL, and then administered to the rats.

Preparation of the Calibration Standards, Quality Control, and Internal Standard Samples. A stock solution of the five analytes was prepared by dissolving certain amounts of schisandrin, schisandrol B, schisantherin A, deoxyschisandrin, and schisandrin B in methanol (purity methanol, 100%). These were separately diluted with methanol to obtain the following target concentrations: 57.54 μg/mL for the schisandrin, 12.22 μg/mL for the schisandrol B, 121.68 μg/mL for the schisantherin A, 59.70 μg/mL for the deoxyschisandrin, and 62.70 μg/mL for the schisandrin B. The stock solution of the five analytes was then diluted with methanol to obtain a series of working standard samples, as follows: 0.0288, 0.0576, 0.1151, 0.2302, 0.4604, 1.1510, 4.6040, 11.5080, and 28.7700 μg/mL for schisandrin; 0.0061, 0.0122, 0.0244, 0.0488, 0.0976, 0.2440, 0.9760, 2.4400, and 6.1000 μg/mL for schisandrol B; 0.0609, 0.1217, 0.2434, 0.4868, 0.9736, 2.4360, 9.7440, 24.3600, and 60.9000 μg/mL for schisantherin A; 0.0299, 0.0597, 0.1194, 0.2388, 0.4776, 1.1194, 4.7760, 11.9400, and 29.8500 μg/mL for deoxyschisandrin; and 0.0314, 0.0627, 0.1254, 0.2508, 0.5016, 1.2540, 5.0160, 12.5400, and 31.3500 μg/mL for schisandrin B. In addition, a working standard solution of the internal standard (IS) was also prepared by diluting the IS stock solution (2.83 mg/mL) to a final concentration of 28.3 μg/mL using methanol. All of the solutions were maintained at 4 °C and were brought to room temperature before use.

The calibration curve samples for the rat hearts, livers, spleens, lungs, and kidneys were prepared by a 20 μL spiking of one of the abovementioned working standard solutions into 100 μL of blank rat tissue, as follows: 0.0048, 0.0096, 0.0192, 0.0384, 0.0768, 0.1920, 0.7680, 1.9200, and 4.8000 μg/mL for schisandrin; 0.0010, 0.0020, 0.0040, 0.0080, 0.0160, 0.0400, 0.1600, 0.4000, and 1.0000 μg/mL for schisandrol B; 0.0102, 0.0203, 0.0406, 0.0812, 0.0325, 0.0650, 0.2560, 0.6496, and 1.6240 μg/mL for schisantherin A; 0.0050, 0.0100, 0.0200, 0.0400, 0.0800, 0.2000, 0.8000, 2.0000, and 5.0000 μg/mL for deoxyschisandrin; and 0.0052, 0.0104, 0.0208, 0.0416, 0.0832, 0.2080, 0.8320, 2.0800, and 5.2000 μg/mL for schisandrin B. The quality control (QC) samples of the rat livers were also obtained using the above process, as follows: 0.0384, 0.1920, and 1.9200 μg/mL for schisandrin; 0.0080, 0.0400, and 0.4000 μg/mL for schisandrol B; 0.0812, 0.0650, and 0.6496 μg/mL for schisantherin A; 0.0400, 0.2000, and 2.0000 μg/mL for deoxyschisandrin; and 0.0416, 0.2080, and 2.0800 μg/mL for schisandrin B.

Biological Sample Preparation. In this study, 20 μL of IS working solution was added to 100 μL of tissue sample, and then precipitated with 200 μL of acetonitrile. The mixture was vortexed for 2 min and centrifuged at 12,000*g* for 5 min. A 10 μL aliquot of the supernatant was then injected into the liquid chromatography–mass spectrometry (LC–MS) for analysis.

Method Validation

Assay Specificity. The specificity of the method was investigated by preparing and analyzing the five replicates of the drug-free rat biological samples. Each blank sample was tested for endogenous interference using a proposed extraction procedure and LC–MS conditions.

Linearity and the Lower Limit of Quantitation (LLOQ). The calibration curves for each analyte in the different tissue samples were evaluated by plotting the peak area of each analyte versus the peak area of the IS against the tissue sample concentrations with a weighed factor ($1/C^2$). The linearity of each analyte was obtained using eight calibration standards in five replicates.

Extraction Recovery and Matrix Effect. The extraction recovery was evaluated at three QC concentrations, and the internal standard testosterone, at a concentration of 28.3 μg/mL. Each set of samples was prepared in replicates of five at each concentration level. The recovery was calculated by comparing the mean peak areas of the analytes added into the blank liver homogenates and extracted with those of the regularly pretreated QC samples at the corresponding concentrations. Meanwhile, the matrix effect (ME) was measured by comparing the peak responses of the post-extracted liver homogenates spiked with the analytes and the IS, with those of the standard solution containing equivalent amounts of the analytes prepared in the mobile phase.

Sample Stability. The stabilities of the five lignans were assessed by comparing the mean concentration of the stored QC samples. The measured concentrations for the five tested lignans at each QC level were determined at room temperature (25 °C) for 24 h and at −20 °C for at least 2 weeks, following three freeze and thaw cycles.

Precision and Accuracy. In this research study, the intra-day and inter-day precision and accuracy were investigated by quantifying five sets of QC samples at three concentration levels, on three consecutive days, respectively. Then, the precision was expressed by the relative standard deviation (RSD %), and the accuracy was evaluated by (mean concentration − nominal concentration) / (nominal concentration) × 100%.

Tissue Distribution Study. The rats were randomly divided into two groups of 15 (five rats were used in the distribution phase, five were used in the equilibrium phase, and the other five rats were used in the elimination phase), which corresponded to 2, 3.5, and 6 h. All of the rats were given a 5.0 g/kg dose of the ethanol extract of *SC* (equivalently 0.222 g/kg the total lignans extract of *SC*, which contains 44.03 mg/kg of schisandrin, 13.11 mg/kg of schisandrol B, 2.19 mg/kg of schisantherin A, 8.18 mg/kg of deoxyschisandrin, and 17.83 mg/kg of schisandrin B) by oral administration. Rats were sacrificed at each time point, and then, the samples from a number of tissues (heart, liver, spleen, lung, and kidneys) were quickly dissected. The tissues were rinsed well with ice cold saline, blotted dry, and weighed. Then, the samples were homogenized with a physiological saline solution (0.9%, w/v) to prepare the 0.5 g/mL homogenates. Blank tissue homogenates were prepared by the same method as mentioned above, using rats without prior drug exposure. The tissue homogenates were kept frozen at −20 °C until the analysis was performed.

Results and Discussion

The Results of Method Validation

Assay Specificity. The chromatogram of a blank plasma sample was compared with the spiked rat tissue containing the five lignan analytes (Figure 2). The retention times of the IS, schisandrin, schisandrol B, schisantherin A, deoxyschisandrin, and schisandrin B were found to be 7.0, 7.6, 8.8, 12.68, 18.0, and 21.0 min, respectively. There were no endogenous interferences observed at the retention times of the five analytes and IS in any of the blank rat tissue samples in this study.

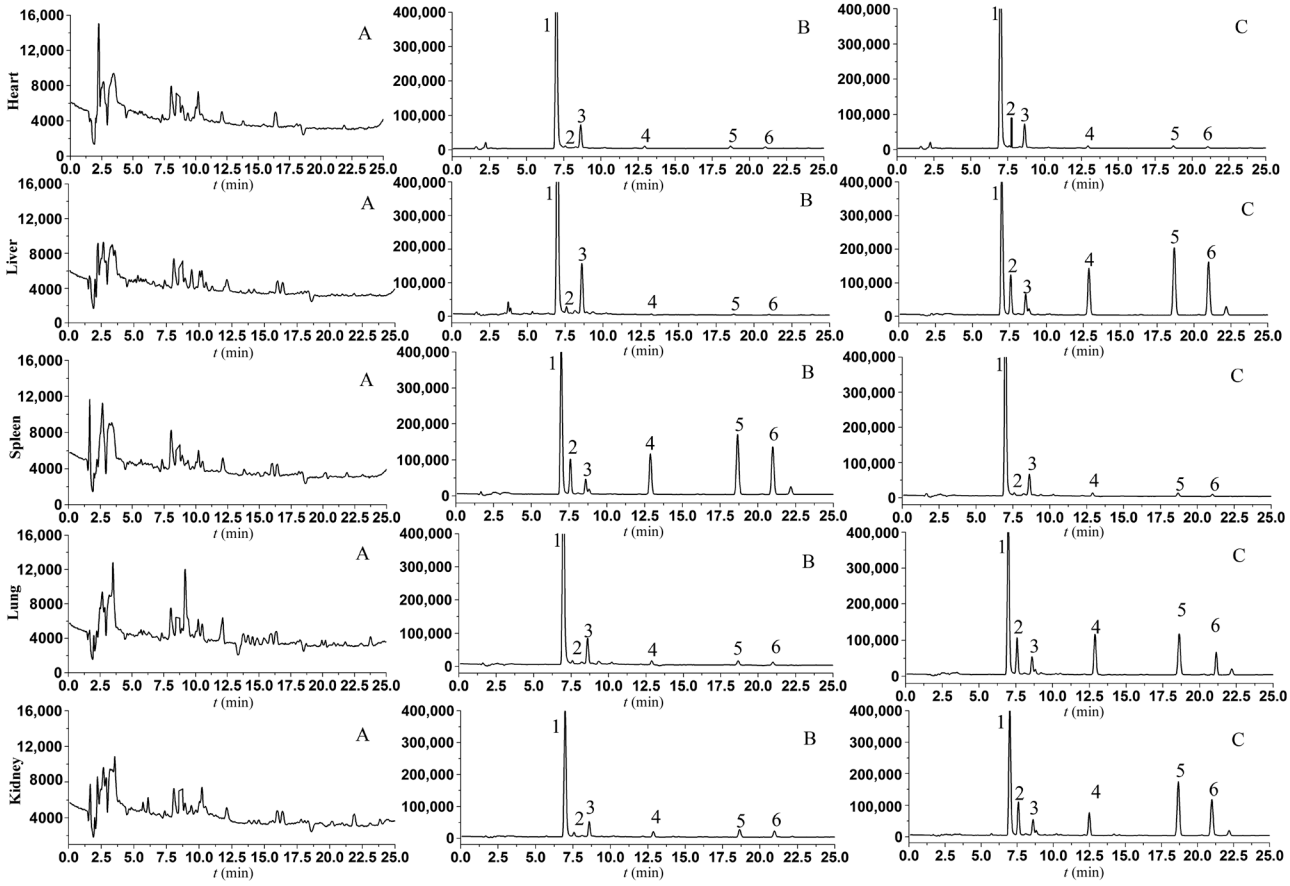

Figure 2. Representative MS chromatograms of the specific tests of the rat tissues: A, blank tissue sample; B, blank tissue spiked with mixed standard and internal standard; C, tissue samples after intragastric administration, i.g. of the total lignans extract of *SC* to the rats after 3.5 hours spiked with internal standard: 1, testosterone; 2, schisandrin; 3, schisandrol B; 4, schisantherin; 5, deoxyschisandrin; and 6, schisandrin B

LLOQ. In this study, the calibration curve required a correlation coefficient (r) of 0.9950 or better. The results were fitted into a linear regression analysis. The calibration curves, along with the LLOQ of the analytes, are detailed in Table 1.

Extraction Recovery and Matrix Effect. Under the given conditions, the extraction recoveries of the five analytes in the rat liver homogenates were found to range from 85.23% to 97.62% at three concentration levels, and the average extraction recovery of IS was 88.7%. These findings indicated that the recoveries of the five analytes and IS were acceptable. The mean value of matrix effect for all of the analytes was determined to range from 82.3% to 93.4% over the entire QC concentration range and 87.8% for IS. These results demonstrated that no coeluting substance significantly influenced the ionization of the

Table 1. Linear, range regression, equations, correlation coefficients, and LLOQ of the five studied lignans in the various rat tissues (mean ± SD, n = 5)

Tissue samples	Component	Linear equation	Linearity range (μg/mL)	r
Heart	Schisandrin	$y = 0.1706x + 0.0070$	0.004795–1.918	0.9972
	Schisandrol B	$y = 0.1142x + 0.0008$	0.001018–0.4073	0.9990
	Schisantherin A	$y = 0.3555x + 0.0156$	0.01014–4.056	0.9975
	Deoxyschisandrin	$y = 0.4702x + 0.0048$	0.004975–1.990	0.9980
	Schisandrin B	$y = 0.2997x + 0.0062$	0.005225–2.090	0.9958
Liver	Schisandrin	$y = 0.2390x + 0.0049$	0.004795–1.918	0.9975
	Schisandrol B	$y = 0.1749x + 0.0006$	0.001018–0.4073	0.9979
	Schisantherin A	$y = 0.8039x - 0.0003$	0.01014–4.056	0.9983
	Deoxyschisandrin	$y = 0.4354x + 0.0083$	0.004975–1.990	0.9977
	Schisandrin B	$y = 0.4037x - 0.0026$	0.005225–2.090	0.9952
Spleen	Schisandrin	$y = 0.2190x + 0.0046$	0.004795–1.918	0.9975
	Schisandrol B	$y = 0.1512x + 0.0006$	0.001018–0.4073	0.9979
	Schisantherin A	$y = 0.6443x - 0.0099$	0.01014–4.056	0.9970
	Deoxyschisandrin	$y = 0.4969x + 0.0012$	0.004975–1.990	0.9977
	Schisandrin B	$y = 0.3677x + 0.0018$	0.005225–2.090	0.9973
Lung	Schisandrin	$y = 0.2187x + 0.0040$	0.004795–1.918	0.9995
	Schisandrol B	$y = 0.1522x + 0.0004$	0.001018–0.4073	0.9999
	Schisantherin A	$y = 0.1334x + 0.0121$	0.01014–4.056	0.9987
	Deoxyschisandrin	$y = 0.4484x + 0.0084$	0.004975–1.990	0.9997
	Schisandrin B	$y = 0.2996x + 0.0094$	0.005225–2.090	0.9992
Kidney	Schisandrin	$y = 0.2180x + 0.0079$	0.004795–1.918	0.9962
	Schisandrol B	$y = 0.1513x + 0.0013$	0.001018–0.4073	0.9984
	Schisantherin A	$y = 0.1535x + 0.0061$	0.01014–4.056	0.9970
	Deoxyschisandrin	$y = 0.5369x + 0.0014$	0.004975–1.990	0.9985
	Schisandrin B	$y = 0.3112x + 0.0063$	0.005225–2.090	0.9970

analytes and IS. Therefore, the analytical method presented in this study was considered to be reliable. The results are summarized in Table 2.

Sample Stability. The measured concentrations for the five tested lignans at each QC level were determined to have deviated within 15%, which indicated that they were stable at room temperature (25 °C) for 24 h and at −20 °C for at least 2 weeks, following three freeze and thaw cycles. The results of the stability studies are listed in Table 3.

Precision and Accuracy. In this study, the required precision did not exceed 15%, and the accuracy was within ±15% of the actual value, with the exception of ±20% at LLOQ. The precision ranged from 1.97% to 14.76% for the homogenates of the rat tissue, which indicated that the method presented had a satisfactory accuracy, precision, and reproducibility, as detailed in Table 4.

Tissue Distribution Analysis. The representative MS chromatograms of the specific testing and the concentrations of schisandrin, schisandrol B, schisantherin, deoxyschisandrin, and schisandrin B in the rat tissues following the oral administrations of the total lignans extract of *SC* are respectively shown in Figure 2 and Figure 3.

Within 2 h of the absorption phase, considerable concentrations of *Schisandra* lignans were detected in all of the analyzed tissues. These results confirmed that the five bioactive lignans in the gradients of the total lignans extract of *SC* underwent a rapid and wide distribution to the tissues. The highest tissue level of the *Schisandra* lignans appeared in the tissues of the livers and kidneys, followed by the hearts and lungs, and the lowest distributions were found in the spleens. For example, the tissue contents of schisandrin in the hearts, livers, spleens, lungs, and kidney were 7.60, 16.92, 3.05, 5.59, and 8.21 μg/g, respectively,

Table 2. Summary of the extraction recovery and matrix effects of the five lignans and IS in the rat liver homogenates (n = 5)

Compound	Nominal concentration (μg/mL)	Extraction recovery mean (%)	RSD (%)	Matrix effect mean (%)	RSD (%)
Schisandrin	0.01918	85.57 ± 9.83	11.49	84.5	11.73
	0.07672	97.62 ± 11.31	11.59	89.0	13.22
	0.7672	95.85 ± 10.76	11.23	97.0	8.31
Schisandrol B	0.004073	85.89 ± 11.70	13.62	92.8	10.84
	0.01629	86.04 ± 8.47	9.85	84.1	12.61
	0.1629	96.81 ± 5.02	5.19	82.3	9.33
Schisantherin A	0.04056	87.36 ± 6.04	6.92	88.3	12.21
	0.1622	86.09 ± 11.78	13.69	92.7	7.24
	1.622	96.88 ± 9.97	10.29	90.4	10.52
Deoxyschisandrin	0.01990	85.26 ± 6.36	7.46	87.8	11.61
	0.07960	86.02 ± 3.30	3.84	89.4	13.43
	0.7960	94.34 ± 10.66	11.30	93.4	7.44
Schisandrin B	0.02090	85.23 ± 9.50	11.15	90.6	12.82
	0.08360	88.97 ± 2.67	3.00	84.4	10.73
	0.8360	90.82 ± 13.08	14.40	90.7	9.34
Testosterone	0.2830	88.70 ± 8.51	9.60	87.8	10.91

Table 3. Stability of analyses of the rat liver homogenates (mean ± SD)

Compound	Nominal concentrations (μg/mL)	Room temperature/24 h		−20 °C, 2 weeks		Three freeze and thaws cycles	
		Measured (μg/mL)	RSD (%)	Measured (μg/mL)	RSD (%)	Measured (μg/mL)	RSD (%)
Schisandrin	0.01918	0.0149 ± 0.00	10.80	0.0147 ± 0.00	12.49	0.0138 ± 0.00	6.34
	0.07672	0.0642 ± 0.01	10.54	0.0645 ± 0.00	6.27	0.0642 ± 0.00	5.34
	0.7672	0.6656 ± 0.03	4.91	0.6551 ± 0.01	6.41	0.6718 ± 0.03	4.24
Schisandrol B	0.004073	0.0029 ± 0.00	11.57	0.0032 ± 0.00	13.45	0.0029 ± 0.00	12.45
	0.01629	0.0130 ± 0.00	10.76	0.0142 ± 0.00	11.80	0.0133 ± 0.00	7.41
	0.1629	0.1418 ± 0.01	6.57	0.1408 ± 0.01	6.43	0.1467 ± 0.01	4.37
Schisantherin A	0.04056	0.0321 ± 0.00	7.10	0.0311 ± 0.00	6.94	0.0303 ± 0.00	6.90
	0.1622	0.1226 ± 0.01	7.17	0.1222 ± 0.01	6.54	0.1227 ± 0.01	6.26
	1.622	1.4494 ± 0.10	6.83	1.4544 ± 0.08	5.22	1.4887 ± 0.10	7.04
Deoxyschisandrin	0.01990	0.0192 ± 0.00	9.35	0.0176 ± 0.00	13.54	0.0169 ± 0.00	11.65
	0.07960	0.0731 ± 0.01	11.88	0.0719 ± 0.01	7.90	0.0668 ± 0.00	1.94
	0.7960	0.7244 ± 0.07	9.82	0.6958 ± 0.05	6.76	0.6891 ± 0.02	2.75
Schisandrin B	0.02090	0.0199 ± 0.00	8.31	0.0197 ± 0.00	5.86	0.0188 ± 0.00	7.73
	0.08360	0.0791 ± 0.01	9.01	0.0788 ± 0.00	2.79	0.0778 ± 0.00	5.53
	0.8360	0.6760 ± 0.06	9.61	0.6685 ± 0.04	5.60	0.6495 ± 0.06	8.02

Table 4. Precision and accuracy of the five lignans in the rat liver homogenates (n = 51)

Compound	Nominal concentrations (μg/mL)	Intra-day		Inter-day	
		Measured (μg/mL)	RSD (%)	Measured (μg/mL)	RSD (%)
	0.01918	0.0150 ± 0.00	10.55	0.0134 ± 0.00	11.05
Schisandrin	0.07672	0.0641 ± 0.00	8.22	0.0617 ± 0.01	13.67
	0.7672	0.6554 ± 0.05	8.33	0.6590 ± 0.02	3.63
	0.004073	0.0028 ± 0.00	13.67	0.0028 ± 0.00	6.67
Schisandrol B	0.01629	0.0134 ± 0.00	13.22	0.0111 ± 0.00	9.59
	0.1629	0.1233 ± 0.01	10.79	0.1056 ± 0.00	4.38
	0.04056	0.0325 ± 0.03	13.55	0.0337 ± 0.00	10.43
Schisantherin A	0.1622	0.1320 ± 0.02	14.12	0.1536 ± 0.02	9.87
	1.622	1.4524 ± 0.11	7.86	1.3259 ± 0.03	1.97
	0.01990	0.0174 ± 0.00	13.82	0.0156 ± 0.00	14.76
Deoxyschisandrin	0.07960	0.0713 ± 0.00	5.59	0.0735 ± 0.00	5.14
	0.7960	0.6896 ± 0.06	9.27	0.7592 ± 0.05	7.03
	0.02090	0.0186 ± 0.00	7.83	0.0161 ± 0.00	6.47
Schisandrin B	0.08360	0.0745 ± 0.00	3.61	0.0727 ± 0.00	3.48
	0.8360	0.698 ± 0.10	14.01	0.6386 ± 0.02	3.25

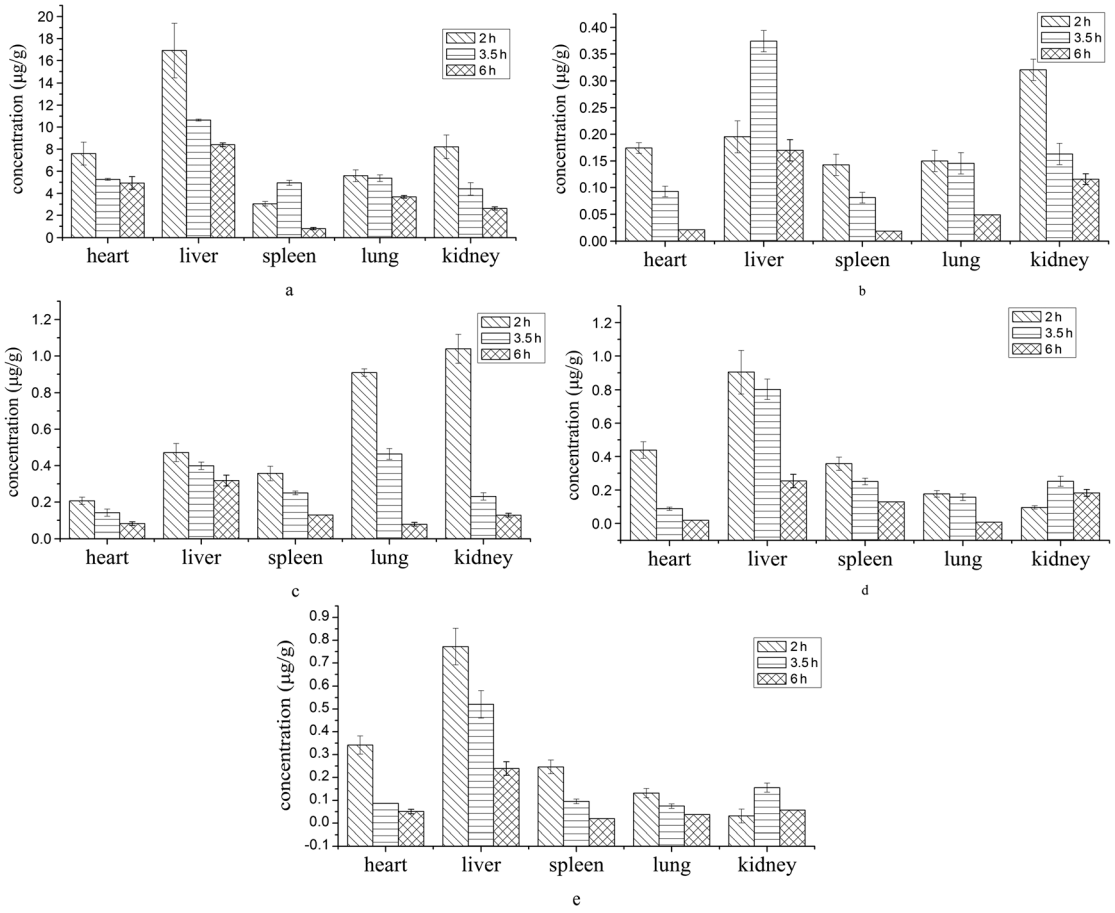

Figure 3. Concentrations of the five lignans in rat tissues at different time points after the oral administrations of the total lignans extract of *SC*: a, schisandrin; b, schisandrol B; c, schisantherin; d, deoxyschisandrin; and e, schisandrin B

which indicated that the *Schisandra* lignans may be absorbed or metabolized primarily through the liver and kidneys. As previously mentioned, these results verified the traditional viewpoint that *SC* in the heart, lungs, and kidneys possesses CNS-protecting activities and boosts the protective role of the liver and kidneys [21–23].

Then, concentrations of the five lignans were found to be obviously decreased 3.5 h after the administrations of the total lignans extract of *SC* when compared with the absorption phase in the four tissues, with the exception that the amount of the five lignans in the liver was almost the same as in the distribution phase, and the highest concentrations occurred in the liver tissues. For instance, the tissue concentrations of schisandrin in the tissues of the hearts, spleens, lungs, and kidneys became decreased by 23.64%, 38.81%, 7.22%, and 47.20%, respectively. However, the decreases were only 4.76% in the liver tissues. These results suggested that the peak times of the five lignans were delayed in the livers.

Although the tissue concentrations of the five lignans were found to significantly decrease at six hours after the administrations in all of organ tissues, particularly in spleens and lungs, some ingredients could not be detected during the elimination phase. The concentrations of the five lignans in the liver were still higher than those in the other tissues, and maintained to a certain content, which demonstrated that the retention time of the five lignans in the rats' liver tissues was longer and therefore displayed lasting medicinal effects. This could have been associated with the effects of the total lignans extract of *SC*, which in turn led to significant liver protectional improvements. These findings were found to be consistent with this study's preliminary pharmacodynamic experiment results.

As we have already resolved in the previous study [24], the separation was measured in 11 lignans of *SC*, which are deoxyschizandrin, schisandrin, schizandrin C, schisantherin, schisantherin B, schizandrin, schizandrol B, schisanhenol, gomisin G, gomisin J, and angeloylogomisin H. Therefore, we choose the high content and important five lignans as the object of analysis in this study. Despite this, recent studies have analyzed some lignans in rat biological samples after the oral administration of extracts or monomer. However, the metabolites of *SC* have not been research thoroughly, even though metabolite identification is an important part of drug discovery and development. Understanding drug metabolism can help to explain and predict a variety of events related to the efficacy and the toxicity of the herb drugs.

Recently, high-performance liquid chromatography–mass spectrometry (HPLC–MS) has become a routine and efficient tool for detecting and identifying drug metabolites [25–32]. With the development of various data acquisition methods, LC–MS, especially for high-resolution mass spectrometry (HRMS), has exhibited excellent performances for metabolite detection because of its high-throughput, high-speed, and high detection sensitivity [33, 34]. HRMS system provides the acquisition of full-scan MS or MS/MS spectra and product-ion spectral data sets for the metabolites by means of information dependent acquisition (IDA), enabling the collection of MS spectra for the identification of both target and non-target compounds. In previous reports, some minor metabolites from the full-scan mass chromatograms using HPLC–time-of-flight mass spectrometry (TOF/MS) have been likely to be overwhelmed by interferences from the background or the matrix and their product-ion mass spectra acquisitions have not been

triggered. Therefore, we will further study the metabolism or tissue distribution of the other lignans in the future.

Conclusions

In this research study, a sensitive and selective HPLC–ESI–MS method was developed for the first time and used for a tissue distribution study in rats after oral administrations of the total lignans extract of *SC*. The major target tissues of the total lignans extract of *SC* in the rats were the livers and kidneys, which may provide a rational explanation as to why the total lignans extract of *SC* could be beneficial to the treatment of hepatic diseases, and the mechanism about that is being studied in our laboratory. The present in vivo tissue distribution study of the total lignans extract of *SC* in rats will potentially provide helpful information for the development of suitable new hepatic drugs based on the total lignans extract of *SC*.

Conflict of Interest

The authors declare that they have no conflict interests.

Ethical Approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed. All of the protocols on living animals used in this article were from the Experimental Animal Center of Nanjing University of Chinese Medicine, license no. SYXK (Su) 2014-0001.

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