Research Article

A Simple and Convenient Method for Simultaneous Determination of Schizandrol A, Schizandrol B, Schisandrin A, γ -Schisandrin, and Schisandrin C

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A simple, rapid, and specific HPLC method was established for simultaneous determination of five major lignans (Schizandrol A, Schizandrol B, Schisandrin A, γ -Schisandrin, and Schisandrin C) in *Schisandra chinensis*. The five lignans can be separated completely on Kromasil C₁₈ column (250 nm $\times$ 4.6 nm) and then detected at 254 nm using methanol (mobile phase A) and water (mobile phase B) with gradient elution as the mobile phase at 1.0 mL/min flow rate. The column temperature was 30°C. The method was validated in terms of linearity, precision, stability, repeatability, and recovery. Results showed that the method is accurate and reproducible.

1. Introduction

The dried ripe fruit of *Schisandra chinensis* (*S. chinensis*) has been used for many years in traditional Chinese medicine. Modern pharmacological studies show that *S. chinensis* has many biological activities, such as for liver protection [1], hypnogenesis, sedation [2], antioxidative stress [3], vasodilation, anti-inflammation [4], anticancer, immunity enhancement, and anticholesterol disease [5]. In addition, this species is approved for common food and health food utilization because of its efficiency and safety. *S. chinensis* is one of the most widely used and studied herbs in Asia; this herb is added in common food, health food, and Chinese multiherb remedies in China. Therefore, establishing a simple and convenient method for quantitative analysis of the bioactive compounds of *S. chinensis* is rather important and necessary. The fruit of *S. chinensis* has been proven to contain a variety of pharmacologically active lignans, particularly those with a dibenzocyclooctadiene skeleton. Most of the pharmacological properties of *S. chinensis* have been attributed to these compounds. Among

the bioactive compounds, Schizandrol A, Schizandrol B, Schisandrin A, γ -Schisandrin, and Schisandrin C are the most effective.

Owing to the importance of lignans, some methods for lignan detection have been developed [6, 7]. Liu et al. established a method of detecting nine lignans in 48 min by HPLC-DAD-MS combined with Chemometrics [8]. Kim et al. established a method to detect three lignans by online TLC-DART-MS [9]. Zhang et al. developed a matrix solid-phase dispersion extraction combined with high-performance liquid chromatography for determination of five lignans from the *Schisandra chinensis* [10]. Given that most methods require expensive equipment such as mass spectrometry (MS), it only can be used by many researchers equipped with MS [11–13].

Therefore, some simple method which does not need expensive equipment is needed for the researchers.

In the present study, Schizandrol A, Schizandrol B, Schisandrin A, γ -Schisandrin, and Schisandrin C were detected by a simple and easy method based on HPLC-DAD. The novel and simple method can be utilized in the study and research of the *S. chinensis*, especially for the undeveloped

and developing areas. And because of the short detection time, it is also useful to the researchers who need to detect their contents often.

2. Experiment

2.1. Reagents. HPLC grade methanol was used in the experiments. Ultrapure water was obtained using the Milli-Q system (Millipore, Bedford, MA, USA) and used in the entire test. Standards for quantitative analysis were purchased from the National Institution for Food and Drug Control.

2.2. Instrumentation and Analytical Conditions. HPLC system 1200 series (Agilent Technologies, USA) equipped with Chemstation B.03.02 software (Agilent Technologies, USA), which were comprised of a quaternary solvent delivery pump, an online vacuum degasser, an autosampler, a thermostated compartment, and diode array detector (DAD), were used for chromatographic analysis. All separation processes were performed using a Kromasil C₁₈ column (250 mm × 4.6 mm i.d. with 5.0 μm particle size).

Mobile phase A was methanol and phase B was water. The linear gradient condition (30% B for 0 min to 8 min; 30% to 20% B for 8 min to 12 min; 20% to 10% B for 12 min to 20 min; 10% B for 20 min to 30 min) was applied for the separation process. The five lignans can be separated completely by employing this process. The flow rate was 1.0 mL/min, and the column temperature was 30°C, which was maintained for the entire experiment. The eluate was monitored at 254 nm, and the injection volume was 20 μL. The peak identification was based on the retention time and DAD spectrum against the standard presented in the chromatogram.

2.3. Preparation of the Standard Solution. Quantification was based on the external standard method. The stock solutions of each analyte were prepared by dissolving these standards in methanol. The solutions were separately and accurately prepared as follows: *Schizandrol A* (0.0032 g), *Schizandrol B* (0.0042 g), *Schisandrin A* (0.0046 g), *γ-Schisandrin* (0.0032 g), and *Schisandrin C* (0.0040 g) were placed in centrifugal tubes, in which 2, 2, 2, 2, and 2.5 mL of methanol were then added, respectively. After 10 min ultrasonication, the final concentrations were 1600 μg/mL *Schizandrol A*, 2100 μg/mL *Schizandrol B*, 2300 μg/mL *Schisandrin A*, 1600 μg/mL *γ-Schisandrin*, and 1600 μg/mL *Schisandrin C*. All of the solutions were stored at −4°C.

The working standard solutions were prepared using the stock solutions. The appropriate stock solutions and methanol were mixed well. Finally, the working standard solutions (800, 262.5, 287.5, 200, and 200 μg/mL) of *Schizandrol A*, *Schizandrol B*, *Schisandrin A*, *γ-Schisandrin*, and *Schisandrin C* were prepared. Subsequently, the mixed standard work solutions (1.25 μg/mL to 300 μg/mL) of *Schizandrol A*, *Schizandrol B*, *Schisandrin A*, *γ-Schisandrin*, and *Schisandrin C* were prepared by using the working standard solutions. These preparations are presented in Table 1. All of the solutions were stored at −4°C.

TABLE 1: Concentration of a series of standard mixed solution.

Concentration	1	2	3	4	5	6	7	8
Schizandrol A (μg/mL)	320.0	240.0	160.0	80.0	40	20	10	5.0
Schizandrol B (μg/mL)	105.0	78.8	52.5	26.3	13.1	6.6	3.3	1.6
Schisandrin A (μg/mL)	115.0	86.3	57.5	28.8	14.4	7.2	3.6	1.8
γ-Schisandrin (μg/mL)	80.0	60.0	40.0	20.0	10.0	5.0	2.5	1.3
Schisandrin C (μg/mL)	80.0	60.0	40.0	20.0	10.0	5.0	2.5	1.3

2.4. Sample Extraction Procedure. Dry (without mildew) *S. chinensis* were pulverized using medicinal herb grinders. The powdered sample was weighed accurately and soaked in 80% ethanol for 12 h (1:12 material-to-solvent ratio). The powdered sample was refluxed using 80% ethanol for 2 h; this process was repeated three times at 90°C. The extracts were combined before being filtered by the filter and then diluted with 80% ethanol until the final volume of 0.25 g herbs/mL was reached. The diluted solution was filtered through a syringe filter (0.45 μm) and then stored at −4°C before injection.

3. Results and Discussion

3.1. Wavelength Optimization. The five bioactive compounds were scanned between 190 and 400 nm using the UV detector. The maximum absorbance values of *Schizandrol A*, *Schizandrol B*, *Schisandrin A*, *γ-Schisandrin*, and *Schisandrin C* were 217, 204, 207, 214, and 219 nm, respectively. Given the serious end absorption near 210 nm, the detection wavelength was set at 254 nm, in which all of the compounds have apparent absorption band. The results are shown in Figure 1.

3.2. Detector Optimization. The DAD detector can detect not only one or few but all wavelengths. Therefore, analyzing the characteristic absorption and retention time of the analyte was easier. A general analysis can be made using the characteristic absorption and retention time. In this way, the error rate is remarkably reduced and conveniently used in the assessment of each chromatographic peak of the corresponding materials.

3.3. Mobile Phase Optimization. Various organic solvents in different ratios resulted in different peak separations. The composition and ratios of the mobile phase were optimized using different organic solvents, which include methanol, acetonitrile, and water in different concentrations. In the present study, the mobile phase composition was selected as mobile phase for the simultaneous analysis because of better resolution and shorter analysis time than other phases. Typical HPLC-PDA chromatograms of the standard samples are presented in Figures 2 and 3.

FIGURE 1: UV scanning spectra of *Schizandrol A*, *Schizandrol B*, *Schisandrin A*, γ -*Schisandrin*, and *Schisandrin C*.

3.4. Preparation of the Standard Curve. Under optimized chromatographic condition, the mixed working standards with different concentrations were injected (three replicates), and then the peak areas were recorded. Average values for the three repetitions were presented as the final results. The peak area and the corresponding concentration are presented in Table 1. The stand curve is established by linear regression analysis of the concentration and the peak area. The standard curve of each analyte was found to be linear (Table 2).

3.5. Precision. Precision was measured by intra- and interday variabilities. The mixed working standard solutions of low, medium, and high concentrations were analyzed to calculate the relative standard deviation (RSD). The intraday precision was determined by five-time repeated analysis of same samples. Average values were used in RSD calculation. The RSDs of the low-concentration solutions were 0.47%, 0.77%, 1.72%, 1.62%, and 0.56% ($n = 5$). The RSDs of the medium-concentration solutions were 0.16%, 0.17%, 0.11%, 0.11%, and 0.10% ($n = 5$). Finally, the RSDs of the high-concentration

TABLE 2: Regression equations, R^2 , and linear range of five standards.

	Regression equation	R^2	Linear range ($\mu\text{g/mL}$)
Schizandrol A	$A = 45.351C + 13.088$	$R^2 = 0.9994$	5.000–320.000
Schizandrol B	$A = 33.692C + 11.552$	$R^2 = 0.9999$	1.641–105.000
Schisandrin A	$A = 42.53C + 21.926$	$R^2 = 0.9999$	1.800–115.000
γ -Schisandrin	$A = 32.742C + 6.967$	$R^2 = 0.9999$	1.250–80.000
Schisandrin C	$A = 33.786C + 7.159$	$R^2 = 0.9999$	1.250–80.000

FIGURE 2: Chromatograms of Schizandrol A, Schizandrol B, Schisandrin A, γ -Schisandrin, and Schisandrin C under optimal chromatographic condition. (a) Schizandrol A, (b) Schizandrol B, (c) Schisandrin A, (d) γ -Schisandrin, and (e) Schisandrin C. FIGURE 3: Chromatograms of alcohol extracts of *S. chinensis* under optimal chromatographic condition.

TABLE 3: Peak area and RSD of intraday precision of low concentration.

	A_1	A_2	A_3	A_4	A_5	RSD (%)
Schizandrol A	239.7	240.4	238	238.3	237.9	0.47
Schizandrol B	59	59.4	58.3	58.7	58.4	0.77
Schisandrin A	88.6	87.6	84.7	86.1	86.4	1.72
γ -Schisandrin	44.4	43.9	43.4	42.9	42.7	1.62
Schisandrin C	44.6	44.5	44.2	44.5	44.9	0.56

TABLE 4: Peak area and RSD of intraday precision of median concentration.

	A_1	A_2	A_3	A_4	A_5	RSD (%)
Schizandrol A	3697.4	3704.6	3710.5	3705.8	3712.7	0.16
Schizandrol B	911.5	913.2	914.9	914	915.4	0.17
Schisandrin A	1270.1	1272.2	1273.3	1273.9	1272.5	0.11
γ -Schisandrin	672.2	672.6	674	673.5	673.6	0.11
Schisandrin C	693.1	694.2	694.2	694.6	695	0.10

solutions were 0.15%, 0.16%, 0.09%, 0.13%, and 0.13% ($n = 5$). The results are presented in Tables 3–5.

Interday precision was determined by repeated analysis of similar samples with intraday variabilities (i.e., continuously

for three days, once every day). The RSDs of the low-concentration solutions were 0.50%, 0.70%, 0.68%, 0.34%, and 0.67%. The RSDs of the medium-concentration solutions were 0.58%, 0.69%, 0.41%, 0.50%, and 0.63%. Finally, the

TABLE 5: Peak area and RSD of intraday precision of high concentration.

	A_1	A_2	A_3	A_4	A_5	RSD (%)
Schizandrol A	14726.3	14727.9	14736.1	14760.9	14775.2	0.15
Schizandrol B	3549.5	3546.8	3551.3	3557.2	3560.7	0.16
Schisandrin A	4911.3	4913.1	4914.4	4918.7	4921.5	0.09
γ -Schisandrin	2624.7	2626.9	2627.4	2630.1	2633.6	0.13
Schisandrin C	2708.6	2710.3	2711.2	2717.8	2713.2	0.13

TABLE 6: Peak area and RSD of interday precision of low concentration.

	A_1	A_2	A_3	RSD (%)
Schizandrol A	239.7	241.5	242	0.5
Schizandrol B	59.0	59.8	59.6	0.7
Schisandrin A	88.6	87.9	89.1	0.7
γ -Schisandrin	44.4	44.7	44.5	0.3
Schisandrin C	44.6	44.9	44.3	0.7

TABLE 7: Peak area and RSD of interday precision of median concentration.

	A_1	A_2	A_3	RSD (%)
Schizandrol A	3697.4	3732.5	3736.2	0.58
Schizandrol B	911.5	920.6	923.7	0.69
Schisandrin A	1270.1	1280.2	1277.5	0.41
γ -Schisandrin	672.2	677.5	678.5	0.50
Schisandrin C	693.1	699.9	701.4	0.63

TABLE 8: Peak area and RSD of interday precision of high concentration.

	A_1	A_2	A_3	RSD (%)
Schizandrol A	14726.3	14768.4	14787.9	0.21
Schizandrol B	3549.5	3560.2	3571.4	0.31
Schisandrin A	4911.3	4922.4	4936.8	0.26
γ -Schisandrin	2624.7	2634.7	2640.1	0.30
Schisandrin C	2708.6	2714.5	2723.2	0.27

RSDs of the high-concentration solutions were 0.21%, 0.31%, 0.26%, 0.30%, and 0.27%. Data show that the current method was stable for 24 h and 72 h. The results are presented in Tables 6–8.

3.6. Stability. To evaluate the stability of the method, the mixed working standard solutions of different concentrations were analyzed at 0, 3, 6, 9, 15, 21, and 24 h at room temperature through the developed method. The peak area was recorded, and RSD was calculated. The RSDs were 0.30%, 0.31%, 0.30%, 0.37%, and 0.48% ($n = 6$), which indicated that the established method was stable in a day. The results are shown in Table 9.

3.7. Repeatability. Six portions were accurately weighed from the same *S. chinensis* sample; the *Schizandrol A*, *Schizandrol B*, *Schisandrin A*, γ -*Schisandrin*, and *Schisandrin C* contents

were detected through the developed method; the RSDs of the samples were 0.09%, 0.76%, 1.19%, 0.34%, and 0.11%, respectively. Analyte contents were 4.46 mg/g, 2.80 mg/g, 0.70 mg/g, 2.53 mg/g, and 0.26 mg/g, respectively. The results are presented in Table 10.

3.8. Recoveries. In the recovery test, nine portions from the same sample were accurately weighed. In these portions, the amounts of *Schizandrol A*, *Schizandrol B*, *Schisandrin A*, γ -*Schisandrin*, and *Schisandrin C* were known. After homogeneously dividing these portions into three groups, 50%, 100%, and 150% of *Schizandrol A*, *Schizandrol B*, *Schisandrin A*, γ -*Schisandrin*, and *Schisandrin C* were added separately. The resultant samples were extracted and analyzed using the developed method. The experiments were repeated three times for each level. The peak area was recorded, and then the recovery percent was calculated, which is the ratio of measured and added amounts. As shown in Tables 11–13, the average recovery rate was 98.1% and the average RSD is 1.92%, which indicated that the developed method is reliable and stable.

3.9. Application. By using the developed method, the analyte contents of the herbs from Heilongjiang, Liaoning, Hebei, Shanxi, Henan, and Hubei province in China were measured. Marked differences were observed in the analyte content of these samples. The *S. chinensis* from Hebei has the highest amount of *Schizandrol A*, *Schizandrol B*, and γ -*Schisandrin*. By comparison, the *S. chinensis* from Shanxi has the highest amount of *Schisandrin A* and *Schisandrin C*. The analyte contents of the herbs from Liaoning and Hebei were similar, in which *Schizandrol A*, *Schizandrol B*, and γ -*Schisandrin* were relatively high. The analyte contents were similar among the herbs from Shanxi, Hubei, and Henan, in which *Schizandrol A* content was high, whereas the amounts of the other lignans were low. The results are shown in Table 14.

4. Conclusion

Given that *Schizandrol A*, *Schizandrol B*, *Schisandrin A*, γ -*Schisandrin*, and *Schisandrin C* comprise the majority of the lignans, establishing a convenient and reliable analytical method to detect these lignans is important for the researchers. In this study, a simple method was developed for simultaneous detection of the lignans. Halstead et al. established a method to detect *Schizandrol A*, *Schizandrol B*, *Schisandrin A*, and γ -*Schisandrin*, in which each analyte can be detected within 28 min to 58 min [14].

Compared with other simple methods that do not need expensive equipment, the method developed in the present study has shorter detection time and can detect more analytes. Lee and Kim established a method to detect nine kinds of lignan, in which the detection time for *Schizandrol A*, *Schizandrol B*, *Schisandrin A*, γ -*Schisandrin*, and *Schisandrin C* is 39 min. Compared with previous method, the detection time of the current method is shorter [15]. Tan et al. established a rapid detection method to determine the *Schisandrin* and *Schisantherin A*. It just costed 10 mins. Although the

TABLE 9: Peak area and RSD of stability.

	A_{0h}	A_{3h}	A_{6h}	A_{9h}	A_{15h}	A_{21h}	A_{24h}	RSD (%)
Schizandrol A	3704.6	3712.7	3704.1	3720.3	3708.2	3732.5	3742.6	0.30
Schizandrol B	913.2	915.4	914	918.2	914.9	920.6	923	0.31
Schisandrin A	1272.2	1272.5	1270.8	1276.2	1270.1	1280.2	1278.9	0.30
γ -Schisandrin	672.6	673.6	676.3	679	677.4	677.5	679.2	0.37
Schisandrin C	694.2	695	698.9	703.1	700.2	699.9	702.1	0.48

TABLE 10: Peak area and RSD of repeatability.

	A_1	A_2	A_3	A_4	A_5	A_6	RSD (%)
Schizandrol A	5058.0	5068.6	5071.2	5068.9	5063	5066.4	0.09
Schizandrol B	2388.6	2352.7	2353.3	2359.3	2392.8	2378.4	0.76
Schisandrin A	753.4	772	773.1	773.7	777.5	778.5	1.19
γ -Schisandrin	2061.1	2080.2	2077.9	2078.9	2077.9	2076.6	0.34
Schisandrin C	223.8	223.8	223.9	223.7	223.2	223.7	0.11

TABLE 11: Peak area and RSD of recovery (50%).

	1	2	3	Quantity found	Quantity added	Recovery	RSD (%)
Schizandrol ($\mu\text{g/mL}$)	56.8	57.2	57.3	57.10	57.50	99.30	0.46
Schizandrol B ($\mu\text{g/mL}$)	34.6	34.3	33.9	34.27	34.90	98.19	1.02
Schisandrin A ($\mu\text{g/mL}$)	9.5	9.6	9.1	9.40	9.80	95.92	2.81
γ -Schisandrin ($\mu\text{g/mL}$)	29.8	30.3	30.9	30.33	31.50	96.30	1.82
Schisandrin C ($\mu\text{g/mL}$)	3.2	3.3	3.3	3.27	3.40	96.08	1.77

TABLE 12: Peak area and RSD of recovery (100%).

	1	2	3	Quantity found	Quantity added	Recovery	RSD (%)
Schizandrol ($\mu\text{g/mL}$)	113.8	113.6	115.2	114.20	115.0	99.30	0.76
Schizandrol B ($\mu\text{g/mL}$)	70.1	68.7	67.3	68.70	69.8	98.42	2.04
Schisandrin A ($\mu\text{g/mL}$)	19.1	18.7	20.3	19.37	19.6	98.81	4.30
γ -Schisandrin ($\mu\text{g/mL}$)	62.5	60.9	61.7	61.70	63.0	97.94	1.30
Schisandrin C ($\mu\text{g/mL}$)	6.5	6.9	6.3	6.57	6.8	96.57	4.65

TABLE 13: Peak area and RSD of recovery (150%).

	1	2	3	Quantity found	Quantity added	Recovery	RSD (%)
Schizandrol ($\mu\text{g/mL}$)	173.6	172.9	174.2	173.57	172.50	100.62	0.37
Schizandrol B ($\mu\text{g/mL}$)	102.8	103.4	104.1	103.43	104.70	98.79	0.63
Schisandrin A ($\mu\text{g/mL}$)	28.6	27.9	29.3	28.60	29.40	97.28	2.45
γ -Schisandrin ($\mu\text{g/mL}$)	92.9	93.8	94.6	93.77	94.50	99.22	0.91
Schisandrin C ($\mu\text{g/mL}$)	10.4	10.1	9.7	10.07	10.20	98.69	3.49

TABLE 14: Content of Schizandrol A, Schizandrol B, Schisandrin A, Schisandrin B, and Schisandrin C of *S. chinensis* from six provinces.

	Heilongjiang	Liaoning	Hebei	Shanxi	Henan	Hubei
Water content (%)	8.21	10.74	8.02	8.40	9.80	6.98
Schizandrol (mg/g)	2.11	5.10	5.50	0.08	0.01	0.03
Schizandrol B (mg/g)	1.57	3.28	3.40	0.37	0.16	0.06
Schisandrin A (mg/g)	3.02	0.80	1.25	4.85	4.06	4.07
γ -Schisandrin (mg/g)	1.06	2.49	2.62	0.19	—	0.06
Schisandrin C (mg/g)	0.47	0.55	0.48	0.75	0.64	0.60
Total content (mg/g)	8.24	12.21	13.24	6.24	4.87	4.84

analytes were fewer than the essay, the detection time was rather short [16]. Moreover, the developed method exhibited high linearity, repeatability, intraday and interday assay precision, accuracy, and reliability. This method provides a simple, rapid, and sensitive technique for determination of *Schizandrol A*, *Schizandrol B*, *Schisandrin A*, γ -*Schisandrin*, and *Schisandrin C* by using HPLC-PDA at a single wavelength (i.e., 254 nm).

Competing Interests

The authors declare that they have no competing interests.

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