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Determination of eight lignans in *Schisandra chinensis* and *Schisandra sphenanthera*

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Abstract

A high performance liquid chromatography method for the determination of eight lignans contents in *Schisandra chinensis* and *Schisandra sphenanthera* was developed. The chromatographic column was Agilent ZORBAX 300SB-C18 column (4.6 mm × 250 mm，5 μm). The mobile phase was methanol-water, a gradient elution was conducted and the detection wavelength was at 230 nm. The results showed that the recovery rate of eight lignans was 92.2-102.9% and RSD was 1.5-4.2%. The established content determination method was simple, sensitive, accurate and stable, and can be used to control the quality of *S. chinensis* and *S. sphenanthera*.

Introduction

Schisandra chinensis is a traditional Chinese medicine, of which fruits of *S. chinensis* (Turcz.) Baill and *Schisandra sphenanthera* Rehd. et Wils have been recorded as "*S. chinensis* Fructus" and "*S. sphenanthera* Fructus" in the Chinese pharmacopoeia, with functions such as astringing the lungs, intestines and sweat, arresting the seminal emission, soothing the nerves, anti-diarrhea, and coagulation. Schisandrol A and schisantherin contents are respectively used for the quality control of *S. chinensis* Fructus and *S. sphenanthera* Fructus in the Chinese Pharmacopoeia (The State Pharmacopoeia Commission of PR China, 2010), but the selected indexes are too simple and short of a specificity, so that they can not really reflect their inherent quality. A large number of chemical and pharmacological studies have shown that the main active constituents of *S. chinensis* include schizandrol A, schisandrol B, schisantherin, schisandrin A, schisandrin B, etc, those of *S. sphenantherae* include schisantherin, schisandrin A, anwulignan and so on, and these components have some important pharmacological effects (Chen et al., 1997).

There have been many reports on the content determination of lignan components in *S. chinensis* and *S. sphenanthera*. Most of them focus on several main lignans, such as schisandol A, schisandrol B, schisantherin, schisandrin A and schisandrin B. Fewer reports on the lignans with lower contents but have some important pharmacological activities (such as schisanhenol and anwulignan).

In this study, a HPLC method (Huang et al., 2006; Li et al., 2011) was used to simultaneously separate and determine the content of eight active lignan components (schisandrol A, schisan-drol B, schisantherin, schisanhenol, anwulignan, schi-sandrin A, schisandrin B and schisandrin C) in *S. chinensis* and *S. Sphenanthera*. Based on the analysis on the results of content determination, a quality standard was established, in order to provide a more comprehensive, scientific and

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efficient method for the evaluation and quality control of *S. chinensis* and *S. Sphenanthera* medicinal materials.

Materials and Methods

Instruments and reagents

LC-2010A liquid chromatograph, including LC solution working station, automatic sample injector and ultra-violet detector etc (Shimadzu Company, Japan); SHIM-PACK VP-ODS C18 chromatographic column (4.6 mm × 150 mm, 5 μm, Shimadzu Company, Japan), AL204 Electronic balance (Mettler-Toledo Instruments Co. Ltd, Switzerland); DHG-9245A Drier (Shanghai Hengke Science and Technology Co. Ltd, China).

Methanol and ethanol were of HPLC grade and purchased from Guoyao Biotechnology Co. (China). Water was purified using a Milli-Q system (Millipore, USA). All the reagents were of analytical grade.

Reference substances

Schisandrol A (batch number: 110857-200910), schisantherin (batch number: 111529-200302) and wuanlinan (batch number: 111844-201002) were purchased from National Institutes for Food and Drug Control. Schisandrol B, schizandrin A, schizandrin B, schizandrin C and schisanhenol were made in our laboratory. Their chemical structures all were verified by ^{1}H-NMR and ^{13}C-NMR, and the detection by high-performance liquid chromatography showed that they all had a single peak, with a purity greater than 98%.

Experimental materials

Fifteen batches of *S. chinensis* and 5 batches of *S. Sphenanthera* medicinal materials were collected from different places. All of them were identified by Prof. Zhang Lihua, College of Pharmacy, Beihua University. Samples of the medicinal materials were dried in nature and crushed (40 mesh), and then stored in a cool and dry place for later use. Types and sources of the samples are shown in Table I.

Table II

Gradient elution conditions

Time (min)	A (%)	B (%)
0-15	60-75	40-25
16-20	75	25
21-30	75-90	25-10
31-40	90-100	10-0
41-45	100	0
46-55	100-60	0-40

Chromatographic conditions

Chromtographic column: Agilent ZORBAX 300SB-C18 Chromtographic column (4.6 mm × 250 mm and 5 μm); Shimadzu GVP guard column (4.6 mm); flow phase: methanol (A), water (B), gradient elution (gradient conditions are shown in Table II); column temperature: 27°C; flow velocity: 0.8 mL•min^{-1}; detection wavelength: 230 nm.

Preparation of the reference substance solutions

Schisandrol A (10 mg), schisandrol B (10 mg), schisantherin (8 mg), schisanhenol (8 mg), anwulignan (8 mg), schisandrin A (10 mg), schisandrin B (10 mg) and schisandrin C (8 mg) were precisely weighed and dissolved in appropriate amount of methanol in 10 mL constant volume bottles, respectively, to obtain 1.0, 1.0, 0.8, 0.8, 0.8, 1.0, 1.0 and 0.8 mg/mL stock solutions of

Table I

Types and sources of the medicinal materials

No.	Types	Sources	No.	Types	Sources
1	FS	Anguo, Hebei	11	FS	Tonghua, Jilin
2	FS	Shangzhi, Henlongjiang	12	FS	Wangqing, Jilin
3	FS	Mudanjiang, Henlongjiang	13	FS	Changchun, Jilin
4	FS	Hulin, Henlongjiang	14	FS	JIFDC
5	FS	Dandong, Liaoning	15	FS	JIFDC
6	FS	Benxi, Liaoning	16	KL	Hengshan, Hunan
7	FS	Tieling, Liaoning	17	KL	Pingwu, Sichuan
8	FS	Dunhua, Jilin	18	KL	Songxian, Henan
9	FS	Zuojia, Jilin	19	KL	Yangcheng, Shanxi
10	FS	Jiaohe, Jilin	20	KL	Haozhou, Anhui

FS: *Fructus schisandrae* (*S. chinensis* Fructus); KL: *Kadsura longepedunculata* (*S. Sphenanthera* Fructus); JIFDC: Jilin Institute for Food and Drug Control

the reference substances. 1.0 mL of the reference substance stock solutions were placed in 10 mL volumetric flasks, respectively, and methanol was added to the flasks to dilute the solution at the scale, for the preparation of mixed reference solution.

Preparation of test solutions

10 g dried sample powder weighed precisely was dissolved in 300 mL methanol. The ultrasound extraction was conducted for 20 min. The solution was shaken and then left to stand for 10 min, and filtered. The residue was dissolved in 150 mL methanol, and the solution was extracted with ultrasound extraction for 20 min 2 times. Filtrates from three extraction were mixed and the mixed solution was dried with decompression evaporation. The residue was dissolved in methanol and set to the constant volume of 100 mL, and the solution was filtered with a 0.45 μm film membrane to obtain the sample solution.

Results

Analysis on the linearity

1.0 mL of each reference stock solution was precisely weighed, respectively. The solutions were prepared into solutions of series mass concentrations by multiple dilution method. In line with the chromatographic conditions described in section 2.4, 10 μL of the prepared solutions were injected and the peak areas were recorded. The peak area y was taken as the ordinate and the mass concentration of reference substances x (μg) was taken as the abscissa to obtain regression equations, linear range and correlation coefficients of each component for the analysis on the linear regression. As shown in Table III, the results showed that each component exhibited a good linearity in the experimental range.

Limit of quantitation (LOQ) and limit of detection (LOD)

1.0 mL of each reference stock solution were precisely weighed, respectively. The solutions were prepared into solutions of series mass concentrations by multiple dilution method. In line with the chromatographic conditions described in section 2.4, 10 μL of the prepared solutions were injected and the peak areas were recorded. SNR (signal to noise ratio) of 10:1 was used as the limit of quantitation and SNR of 3:1 as the limit of detection. It was estimated that the LOQ of schisandrol A, schisandrol B, schisantherin, schisanhenol, anwulignan, schisandrin A, schisandrin B and schisandrin C in schisandra was 25, 25, 20, 10, 40, and 12.5, 25 and 20 μg/mL, respectively; the LOD of them was 5, 5, 8, 2, 8, and 2.5, 5 and 4 μg/mL, respectively.

Precision test

10 μL of each of the mixed reference solution were injected in line with the chromatographic conditions described in section 2.1 and continuously measured 6 times. Peak area RSD values of schisandrol A, schisandrol B, schisantherin, schisanhenol, anwulignan, schisandrin A, schisandrin B and schisandrin C in schisandra were 1.43%, 1.89%, 2.39%, 2.41%, 2.87%, 3.49%, 3.58% and 3.9%, respectively, indicating that the precision of this method used for the content determination of these eight lignans should be good.

Reproducibility test

In order to make sure that the results could more accurately reflect methodological parameters, samples with higher contents of 8 lignans (No. 20) were chosen for the analysis, and the reproducibility, stability and recovery of the method were investigated. The medicinal material powders (No. 20) were simultaneously prepared into 5 copies of the test sample solution according to the method described in section 2.6, and 10

Table III

Regression equations, linearity and correlation coefficients

	Regression equation	Linear range (μg/mL)	R
Schisandrol A	y=2192.5x + 586.4	25-500	0.9997
Schisandrol B	y=2288.5x + 300.3	25-500	0.9997
Schisantherin	y=2642.2x + 336.8	20-400	0.9996
Schisanhenol	y=1142.9x + 108.5	20-400	0.9994
Anwulignan	y=2032.8x + 85.29	20-400	0.9995
Schisandrin A	y=2473.6x + 35.86	25-500	0.9995
Schisandrin B	y=1278.9x + 123.3	25-500	0.9996
Schisandrin C	y=2298.5x + 578.5	20-400	0.9997

μL of samples were injected in line with the chromatographic conditions described in section 2.4, to measure peak area RSD values of the eight lignans, respectively. Peak area RSD (relative standard deviation) values of the eight lignans were 1.47%, 2.31%, 2.47%, 6.86%, 2.82%, 2.12%, 3.86% and 4.55%, respectively, showing a good reproducibility of this method although a poor reproducibility of the method could be seen in the content determination of schisanhenol and schisandrin C due to their lower contents in the test samples.

Stability test

The medicinal material powders (No. 20) were prepared into the test sample solutions according to the method described in section 2.6. The test sample solutions were injected and measured at 1, 2, 4, 8, 16 and 24 hours respectively, relative peak areas of the chromatographic peaks were recorded, and RSD values were calculated based on the relative peak areas measured at different time points. The results showed that contents of the eight lignans were stable within 24 hours and RSD values of them were 1.6, 2.9, 2.9, 5.7, 2.9, 1.8 and 4.2%, respectively.

Recovery test

A sample recovery method was used in this test. 9 copies of 0.1 g medicinal material powders containing the above 8 lignans (No. 20) were precisely weighed. They were added to the reference solutions according to the high, medium and low concentrations, respectively, and three copies of them were added to the solution at each concentration in parallel, which were prepared into sample solutions according to the method described in section 2.6. 10 μL of the sample solutions were injected in line with the chromatographic conditions described on section 2.4 to calculate the recovery and the results are shown in Table IV. The results showed that recoveries of the eight lignans at the high, medium and low concentrations was within 92.2-102.9%, and RSD values of their recoveries were within 1.5-4.2%.

Sample determination

Test solutions of *S. chinensis* and *S. sphenanthera* samples (Table I) were prepared in line with the method described in section 2.6. 10 μL (n = 3) of the sample solutions were injected in line with the chromatographic conditions. The chromatograhic peak

Table IV

Recovery test results（n = 6）

	Blank (μg)	Addition (μg)	Detection (μg)	Recovery (%)	RSD (%)
Schisandrol A	780.2	1000	1764.6	98.0	2.0
		500	1259.1	97.3	2.3
		250	1018.5	98.5	2.5
Schisandrol B	425.5	1000	1431.5	101.4	2.3
		500	928.9	100.8	2.7
		250	686.1	102.5	3.1
Schisantherin	840.5	800	1619.5	97.5	3.3
		400	1193.4	94.4	2.4
		200	998.5	95.0	2.7
Schisanhenol	20.2	80	98.8	93.1	3.2
		40	59.3	95.6	2.8
		20	38.8	93.2	4.2
Anwulignan	1080.6	800	1878.4	99.8	1.5
		400	1466.6	98.7	1.7
		200	1268.7	98.9	1.9
Schisandrin A	1170.3	1000	2152.7	98.5	2.0
		500	1642.2	97.6	2.8
		250	1435.5	101.3	2.6
Schisandrin B	670.9	1000	1673.6	100.4	1.8
		500	1185.0	102.1	2.2
		250	940.4	102.9	2.4
Schisandrin C	30.1	80	108.1	93.5	2.6
		40	69.0	96.3	2.9
		20	47.8	92.2	3.9

Table V

Determined contents of eight lignans in the samples (mg/g, *n* = 3)

No.	A	B	C	D	E	F	G	H	T
1	2.9	0.8	1.1	+[a]	-[b]	0.6	4.0	0.3	9.8
2	6.0	4.1	2.5	0.4	-[b]	1.1	3.8	0.6	18.6
3	3.5	2.9	1.5	0.3	-[b]	0.4	2.6	0.2	11.4
4	4.7	4.6	1.6	0.5	-[b]	1.0	3.8	0.9	17.0
5	6.9	4.0	2.2	0.6	-[b]	1.4	3.2	0.7	18.9
6	4.5	3.4	1.1	0.4	-[b]	0.9	3.6	0.6	14.4
7	3.8	1.3	0.5	+[a]	-[b]	0.3	2.2	+[a]	8.1
8	5.7	2.4	1.5	0.7	-[b]	1.9	3.0	0.6	15.8
9	6.6	2.5	1.6	0.7	-[b]	0.7	3.2	0.4	15.8
10	4.6	1.7	1.1	0.7	-[b]	0.7	2.4	0.3	11.6
11	7.2	2.9	1.9	0.8	-[b]	0.9	3.8	0.5	18.0
12	6.0	3.3	2.0	0.9	-[b]	1.3	4.0	1.4	18.9
13	4.0	1.4	0.9	0.5	-[b]	0.8	1.7	0.4	9.8
14	5.9	3.8	2.0	0.4	-[b]	1.1	4.3	1.3	18.7
15	5.2	1.8	1.2	0.4	-[b]	0.6	1.4	0.7	11.2
16	+[a]	0.5	0.5	0.7	1.2	6.4	-[b]	-[b]	9.3
17	+[a]	+[a]	2.6	0.2	1.6	2.5	+[a]	0.3	7.1
18	+[a]	-[b]	3.5	0.5	2.4	4.6	+[a]	0.4	11.4
19	-[b]	-[b]	3.8	0.4	2.9	4.8	+[a]	+[a]	11.9
20	0.8	0.4	0.8	+[a]	1.1	1.2	0.7	+[a]	5.0

A: schisandrol A, B: schisandrol B, C: schisantherin, D: schisanhenol, E: anwulignan, F: schisandrin A, G: schizandrin B, H: schizandrin B, T: total contents of 8 Lignans. +[a]: < LOQ, -[b]: < LOD.

areas were recorded, and based on the regression equations, schisandrol A, schisandrol B, schisantherin, schisanhenol, anwulignan, schisandrin A, schisandrin B and schisandrin C contents in the schisandra samples were calculated. The results are shown in Table V. Representative chromatogram of the reference substances, *S. chinensis* and *S. Sphenanthera* are shown in Figure 1.

A: mixed reference substances, B: *S. chinensis* (No. 5), C: *S. Sphenanthera* (No. 20). 1: schisandrol A, 2: schisandrol B, 3: schisantherin, 4: schisanhenol, 5: anwulignan, 6: schisandrin A, 7: schisandrin B, 8: schisandrin C.

Discussion

Many studies have indicated that although the main ingredients of *S. chinensis* Fructus and *S. sphenanthera* Fructus are lignans (Opletal et al., 2004; Lu and Chen, 2009; Slanina et al., 1997), their kinds and contents are markedly different (Yuan et al., 2011). In this study, a simple, rapid and sensitive HPLC method has been developed for the simultaneous quantitative analysis of eight lignans in *S. chinensis* and *S. sphenanthera*, and the content of eight active lignan components in 15 batch of *S. chinensis* and 5 batch of *S. Sphenanthera* were determined in this method. The results of samples determination showed that types of lignans contained in schisandra from different origins were the same, namely, they all contained schisandrol A, schisandrol B, schisantherin, schisanhenol, anwulignan, schisandrin A, schisandrin B and schisandrin C, and no anwulignan was detected in 15 batches of schisandra. There was a great difference in the lignan constituents content of schisandra from different origins, suggesting that although lignan constituents contained in schisandra from different rigins were similar, their quality should be quite different, and appropriate methods should be established to control the quality of them.

The type and content of 5 batches of *S. chinensis* were quite different, showing a great difference in the quality of *S. Sphenanthera* from different origins; the comparison of them showed that 5 batches of the samples all contained schisantherin, anwulignan and schisandrin A, and their contents were higher compared with the others (accounting for 61.92-96.88% of total content of above 8 lignans), suggesting that the 3 lignans may be the common components in *S. chinensis* from different origins, based on which the quality control of *S. Sphenanthera* can be achieved. Anwulignan is a lignan of

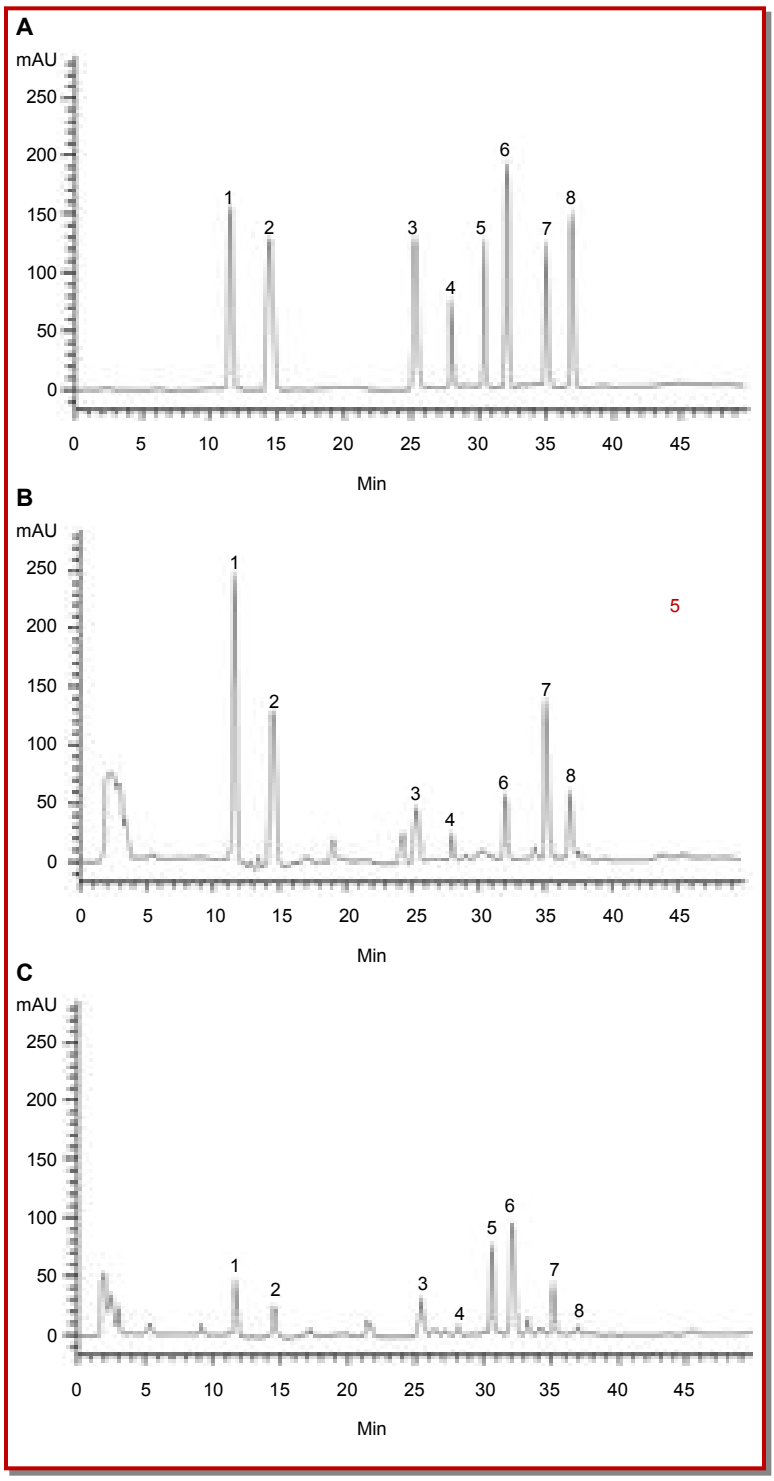

Figure 1: Chromatograms of reference substances, *Fructus schisandrae* and *Kadsura longepedunculata*

dibenzylbutanes, the other 7 lignans are all dibenzocyclooctenes lignans, and there are significant differences in the ultraviolet absorption spectra between the two types of lignans. Therefore, we need to select the appropriate detection wavelength to ensure that all lignans has a strong absorption. In this study, we investigated the absorption of eight lignans in 280 nm, 230 nm, 254 nm and 300 nm, respectively. The result showed that there was almost no absorption of the eight lignans at 300 nm wavelength; the absorption of anwulignan was strong and that of the other 7 lignan was weaker at 280 nm wavelength; at 254 nm wavelength, the situation was just opposite to that at 280 nm wavelength, showing an extremely weak

wavelength was chosen as the detection wavelength in this experiment.

Conclusion

A simple, rapid and sensitive HPLC method has been developed for the simultaneous quantitative analysis of eight lignans in *S. chinensis* and *S. sphenanthera*. It was evident that this approach was a useful and rapid technique for identification of main lignans.

Conflict of Interest

The authors have no competing interests to disclose.

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