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Analytical Methods

Determination of six lignans in *Schisandra chinensis* (Turcz.) Baill. Fruits and related Chinese multiherb remedies by HPLC

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ABSTRACT

A simple, rapid and specific HPLC method was established for simultaneous determination of six major lignans in *Schisandra chinensis* and related Chinese multiherb remedies (CMRs) containing this herb. The six lignans were successfully separated on a C_{18} column by gradient elution using acetonitrile and water as the mobile phase, and detection wavelength was set at 225 nm. The method was validated through the following performance criteria: linearity, precision, repeatability, stability, accuracy, limit of detection (LOD) and quantification (LOQ). This assay was successfully used for determination of six lignans in 10 raw herbs collected from different regions in China and five Chinese multiherb remedies. Significant variations were demonstrated in the contents of six compounds in these samples. This HPLC method established is suitable for routine quantitative analysis of *S. chinensis* and multiherb remedies containing this herb.

1. Introduction

Schisandra chinensis (Turcz.) Baill., Wu Wei Zi in Chinese, is widely used in Chinese prescriptions as a sedative and tonic to treat various diseases and could also be used as flavouring agent of foods. (State Pharmacopoeia, 2005). The major active components of *S. chinensis* are lignans, which have a dibenzocyclooctadiene skeleton and high absorption of UV radiation. These lignans have been found to have activities that could prevent breast, colon, prostate and thyroid cancer, hepatotoxicity and heart disease, etc. (Chang et al., 2005; Hancke, Burgos, & Ahumada, 1999; Ip & Ko, 1996; Li, Xu, Zhang, Liu, & Tan, 2005; Ohsugi et al., 1999). And there are many Chinese multiherb remedies (CMRs) containing *S. chinensis*, which plays an important part in therapeutics. However, the amount and distribution of lignans are extremely varied depending on the different plant origins and harvest season. Thus, a rapid HPLC method for simultaneous determination of major lignans, especially the six lignans in *S. chinensis*, is required for quality control of this medicinal herb and its related CMRs.

In term of quantitative analysis of *S. chinensis*, several analytical methods have been reported for the determinations of lignans, including capillary electrophoresis (Chen, Li, Gao, Hu & Chen, 2005), capillary electrochromatography (Kvasnickova et al., 2001), high-performance liquid chromatographic techniques (Halstead, Lee, Khoo, Hennell, & Bensoussan, 2007; Jian, Wang, Lu, Sun, & Gong, 2007; Opletal, Sovova, & Bartlova, 2004; Sladkovsky, Solich, & Opletal, 2001), gas chromatography coupled with mass spectrometry (Li, Cui, Song, Liang, & Chau, 2003; Zhu, Cao, & Fan, 2007) and high-performance liquid chromatography coupled with mass spectrometry (He, Lian, & Lin, 1997; Huang, Song, Liu, & Liu, 2007). More recently, Wang et al. developed a method of five lignans in Fructus schisandrae by pressurised capillary electrochromatography (Wang et al., 2007). However, the preparation and pre-equilibration of the capillary was laborious and time-consuming. The HPLC method, which has good sensitivity, less interference and lower limits of detection, is very convenient and sensitive for determination of these components with obvious UV absorption in *S. chinensis*.

The aim of this study was to establish a reliable HPLC method for simultaneous determination of the six major lignans in *S. chinensis*, schisandrin (1), schisandrol B (2), schisantherin A (3), deoxyschisandrin (4), γ-schisandrin (5) and schisandrin C (6), was established. Optimisation of the extraction solvent and HPLC method were followed by a comprehensive validation study, which covered linearity, precision, repeatability, stability, accuracy, limits of detection and quantification. We expected that this HPLC method would be helpful for the quality control of *S. chinensis* and its related CMRs.

2. Experimental procedures

2.1. Apparatus

HPLC was performed on an Agilent 1100 series HPLC system (Agilent, Waldbronn, Germany) consisting of quaternary pump,

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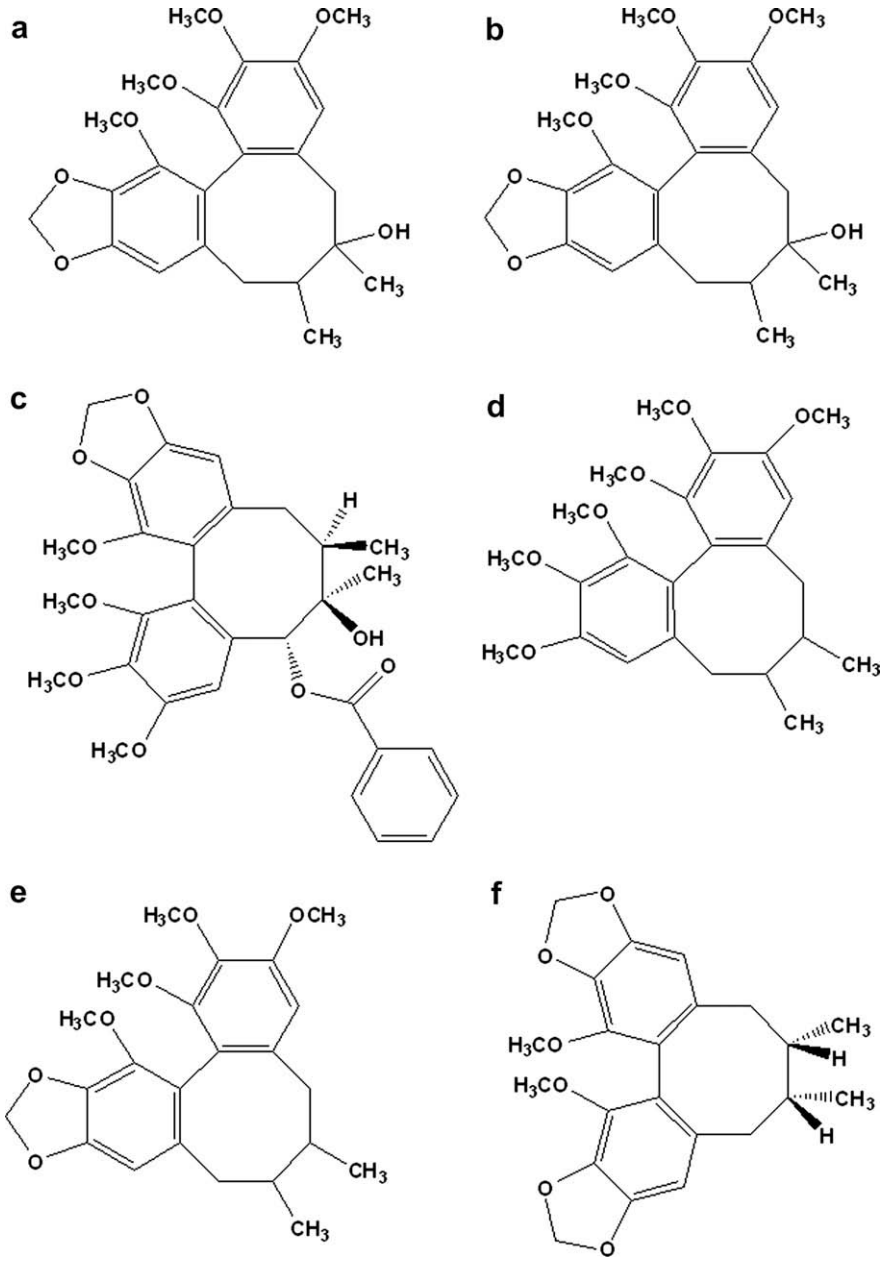

Fig. 1. Chemical structures of six lignans 1. schisandrin; 2. schisandrol B; 3. schisantherin A; 4. deoxyschisandrin; 5. γ-schisandrin and 6. schisandrin C.

on-line degasser, well-plate autosampler, thermostatic column compartment and diode-array detector (DAD). An Agilent Zorbax SB-C_{18} column (3.0 × 100 mm, 3.5 μm) was maintained at 30 °C. Detection wavelength was set at 225 nm. The mobile phase consisted of acetonitrile (A) and water (B) at a flow-rate of 0.8 mL min^{-1}. A gradient programme was as follows: 0–4 min, 40–45%B; 4–12 min, 45–50%B; 12–16 min, 50–68%B; 16–20 min, 68–75%B; 20–25 min, 75–95%B, with a hold time of 15 min; injection volume: 5 μL.

A versatile plant pulveriser (Tianjin, China) was used to power the medicines into powder. An SB 3200 ultrasonic generator (50 kHz, 120 W) from Shanghai Branson Ultrasonics Co. Ltd. (Shanghai, China) was used to extract lignans from samples.

2.2. Standards and reagents

Standards of schisandrin, schisantherin A, deoxyschisandrin, γ-schisandrin were purchased from the National Institute for the Control of Pharmaceutical & Biological Products (NICPB, Beijing, China). Schisandrol B and schisandrin C were isolated from *S. chinensis* by these authors. Their structures were fully characterised by nuclear magnetic resonance (NMR) spectroscopy and MS (Fig. 1), and their purities were shown to be over 98.5%.

Acetonitrile (Fisher, USA) was of HPLC grade and ultrapure water was prepared by Milli-Q System (Millipore, Bedford, MA, USA). All other reagents were of analytical grade.

2.3. Samples

The herbs were purchased raw from Shanghai Huayu Medicine Co. Ltd. The five kinds of CMRs containing *S. chinensis* were purchased from Leiyunshang pharmaceutical stores (Shanghai, China). The CMRs and their compositions, manufacturer, batch number and sample weight for analysis were listed in Table 1.

Table 1
The medicinal herbs or CMRs and their compositions, manufacturer, batch number.

No.	Raw herbs and Chinese multiherb remedies (components)	Regions collected or manufacturer	Batch No.
1[a]	*Schisandra chinensis* (Turcz.) Baill	Anguo, Hebei Province	070315
2[a]	*Schisandra chinensis* (Turcz.) Baill	Tieli, Heilongjiang Province	070110
3[a]	*Schisandra chinensis* (Turcz.) Baill	Xian, Shanxi Province	070720
4[a]	*Schisandra chinensis* (Turcz.) Baill	Yangquan, Shanxi Province	061222
5[a]	*Schisandra chinensis* (Turcz.) Baill	Changchun, Jilin Province	060809
6[a]	*Schisandra chinensis* (Turcz.) Baill	Shijiazhuang, Hebei Province	061010
7[a]	*Schisandra chinensis* (Turcz.) Baill	Shenyang, Liaoning Province	070208
8[a]	*Schisandra chinensis* (Turcz.) Baill	Fushun, Liaoning Province	070628
9[a]	*Schisandra chinensis* (Turcz.) Baill	Datong, Shanxi Province	070102
10[a]	*Schisandra chinensis* (Turcz.) Baill	Siping, Jilin Province	070518
11[b]	Hugan tablet (Radix Bupleuri, Herba Artemisiae Scopariae, Radix Isatidis, Fructus Schisandrae, Pulvis Fellis, Semen Phaseoli Radiati)	Heilongjiang Sunflower Pharmaceutical Co. Ltd.	060422
12[b]	Jiangtang pills (Ginseng radix rubric, Astragalus mongholicus, Solomonseal rhizome, Poria cocos, Atrctylodis macrocephalae, Radix puerariae, Fructus Schisandrae, Coptis chinensis, Radix et rhizoma rhei, Radix glycyrrhizae)	Liaoning Traditional Chinese Medicinal College Pharmaceutical Co. Ltd.	060201
13[b]	Compound Schisandra Syrup (Fructus Schisandrae)	Shanghai Meiyou Pharmaceutical Company	070506
14[b]	Shenqi Wuweizi tablet (Fructus Schisandrae, Radix codonopsitis, Astragalus mongholicus, zizyphi spinosi semen	Gansu Duyiwei Biomedical Pharmaceutical Co. Ltd.	060226
15[b]	Shengmaiyin (Panax ginseng, Ophiopogonis tuber, Fructus schizandrae)	Hubei Huquan Pharmaceutical Co. Ltd.	070301

[a] *Schisandra chinensis* (Turcz.) Baill medicinal herbs.
[b] Chinese multiherb remedies containing *Schisandra chinensis*.

2.4. Standard solutions

Standards of the six lignans dissolved in methanol were prepared from stock standard solutions containing 2950 μg ml^{-1} schisandrin, 1445 μg ml^{-1} schisandrol B, 788 μg ml^{-1} schisantherrin A, 1098 μg ml^{-1} deoxyschisandrin, 2015 μg ml^{-1} γ-schisandrin and 715 μg ml^{-1} chisandrin C. The stock solutions were serially diluted, mixed and used for preparation of standard solutions, which were stored at 4 °C. Calibration curves were established based on seven concentrations with the ranges of 2.95–590 μg ml^{-1} for schisandrin, 1.445–289 μg ml^{-1} for schisandrol B, 0.788–157.6 μg ml^{-1} for schisantherin A, 1.098–219.6 μg ml^{-1} for deoxyschisandrin, 2.015–403 μg ml^{-1} for γ-schisandrin and 0.715–143 μg ml^{-1} for schisandrin C by diluting these stocking solutions in series.

2.5. Preparation of sample solutions

The dried *S. chinensis* and its related CMRs were milled to an homogeneous powder, sieved through a No. 100 mesh, and further dried at 60 °C for 5 h. Accurately weighed powder samples (0.5 g for *S. chinensis*, 1 g or 1 mL for its related CMRs) were extracted by sonication in 50 mL methanol for 30 min. The sample solutions were then made up the loss weight with methanol after extraction and filtered through 0.45-μm nylon filters into amber sample vials for HPLC analysis.

3. Results and discussion

3.1. Optimisation of chromatographic conditions

Scanning from 200 to 400 and 225 nm was selected as detection wavelength for acquiring chromatograms. In order to achieve better chromatographic separation, various linear gradients of acetonitrile–water were investigated at a flow-rate of 0.8 mL min^{-1}. Finally, the gradient programme described above was chosen as it allowed the six major peaks to be clearly separated. Typical chromatograms of standards and samples were shown in Fig. 2.

3.2. Optimisation of sample preparation

In order to achieve the optimal extraction conditions, variables involved in the extraction procedure such as extraction solvents, extraction time were investigated.

In this experiment, four solvents were investigated to optimise the optimal solvent for extraction of lignans. The solvents used were water, 50% aqueous methanol, methanol and ethanol. The results indicated that deoxyschisandrin, γ-schisandrin and schisandrin C could hardly be detected in the sample extracted by pure water, whilst total peak areas of the analytes of interest reached the highest values when pure methanol was employed as extraction solvent. Thus, methanol was the most efficient solvent for the extraction of these lignans. Extraction time had a close relationship with extraction efficiency. In the assay, extraction efficiency in samples was compared by sonication with methanol for 15, 30 and 45 min, respectively. The results indicated that the highest extraction efficiency was obtained by sonication for 30 min in pure methanol.

3.3. Method validation

3.3.1. Calibration curves, limits of detection and quantification

The calibration curve of the individual standards was constructed using seven concentrations ($n = 3$), by plotting peak areas against the concentration of analytes. Good linearity ($r > 0.9997$) was observed in calibration curves over the concentration ranges investigated. Limits of detection of six lignans varied from 0.01927 to 0.07880 μg ml^{-1}, and limits of quantification ranged from 0.05780 to 0.1576 μg ml^{-1}. The results are summarised in Table 2.

3.3.2. Precision

The quality control samples at low, medium and high were analysed in a set of five on a single assay day to determine intra-day precision, and analysed in duplicate on each of three consecutive days for inter-day variation. RSDs of the intra-day and inter-day measurement variations were all less than 2% for six components.

3.3.3. Repeatability

In order to test the repeatability, six sample solutions of *S. chinensis* in Jilin Province were prepared. The contents of six lignans were 5.511, 3.095, 1.127, 0.6214, 4.503 and 0.7191 mg/g, and the RSDs were 1.03%, 0.98%, 1.53%, 0.77%, 0.79% and 0.89%, respectively. Thus repeatability was very good.

3.3.4. Stability

For stability test, the same sample solution was analysed every 24 h over 6 days at the room temperature. The RSD of contents of

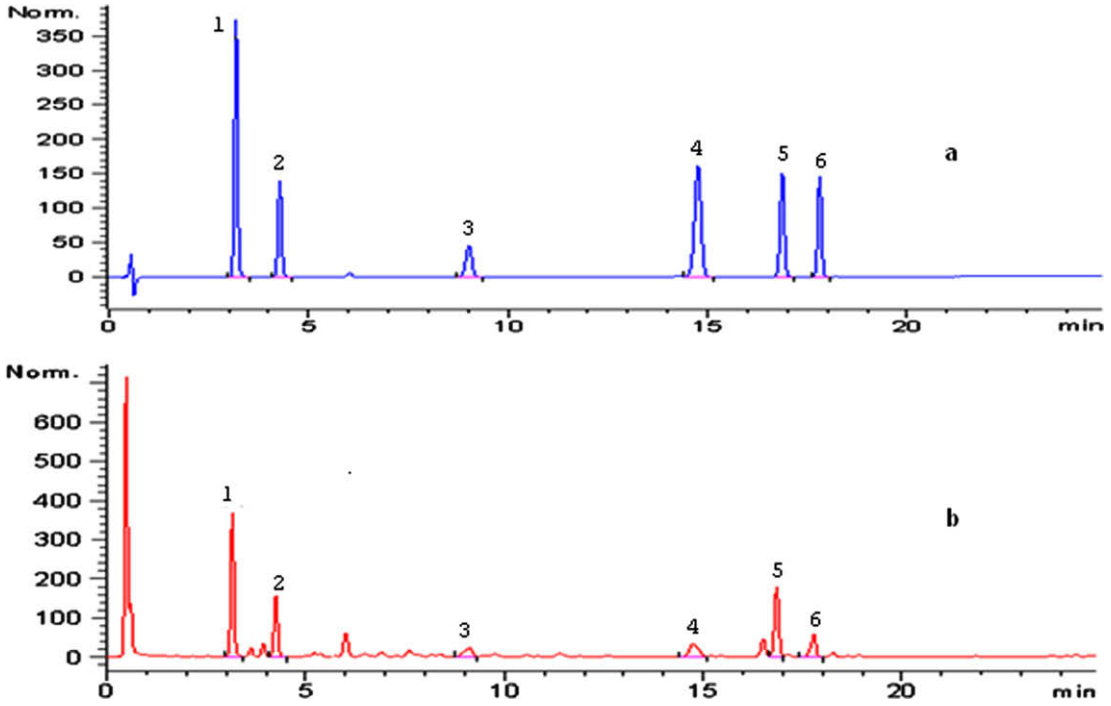

Fig. 2. Chromatograms of standards and sample solutions 1. schisandrin; 2. schisandrol B; 3. schisantherin A; 4. deoxyschisandrin; 5. γ-schisandrin and 6. schisandrin C.

Table 2
Regression equations, LODs and LOQs for six standards.

No.	Linear regression	Linear range (μg ml^{-1})	r	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
1	$y = 28.61x + 15.02$	2.950–590.0	0.9999	0.02360	0.09440
2	$y = 24.9x + 4.332$	1.445–289.0	0.9999	0.01927	0.05780
3	$y = 25.29x + 1.820$	0.7780–157.6	0.9999	0.07880	0.1576
4	$y = 67.16x + 10.21$	1.098–219.6	0.9998	0.07320	0.1464
5	$y = 22.47x - 5.735$	2.015–403.0	0.9999	0.01679	0.05038
6	$y = 53.81x + 8.872$	0.7150–143.0	0.9999	0.01430	0.05720

1. Schisandrin, 2. schisandrol B, 3. schisantherin A, 4. deoxyschisandrin, 5. γ-schisandrin and 6. schisandrin C.

the six lignans in the same sample (Jilin Province) ranged between 0.37% and 2.03%, which indicated that the sample was stable over 6 days under the experimental conditions.

3.3.5. Recovery

In order to evaluate the accuracy of this method, recovery was performed by adding standard solutions at low, medium and high levels (50%, 100% and 150%) to 0.2 g *S. chinensis* samples (Jilin Province) with known content of six components (that same as repeatability). The samples ($n = 9$) were then extracted according to the procedure described above and analysed. The recovery of each component was calculated as the percentage of the net amount of each compound obtained after extraction from that had been added prior to the extraction. The recovery results were summarised in Table 3. It was indicated that the extraction method was efficient enough for determination of the six lignans in *S. chinensis*.

3.4. Sample analysis

The established HPLC method was applied to determination in triplicate of six lignans in *S. chinensis* and its related CMRs. The contents ($n = 3$) of the six lignans in different samples are listed in Table 4. The total content of the six lignans was up to

Table 3
Recovery experiment of analytical method for six components.

No.	Original (mg)	Spiked (mg)	Found (mg)	Mean recovery (%)	RSD (%) ($n = 3$)
1	1.102	0.4917	1.613	103.8	2.01
		0.9833	2.053	96.7	1.67
		1.475	2.567	99.3	0.93
2	0.619	0.3613	0.9752	98.6	1.55
		0.7225	1.321	97.2	1.03
		1.084	1.728	102.3	2.04
3	0.2254	0.1970	0.4297	103.7	0.88
		0.3940	0.6261	101.7	1.64
		0.5910	0.8318	102.6	0.92
4	0.1243	0.1373	0.2620	100.3	0.89
		0.2745	0.4051	102.3	2.11
		0.4118	0.5356	99.9	1.45
5	0.9007	0.5038	1.392	97.6	1.98
		1.008	1.8971	98.9	0.85
		1.511	2.463	103.4	0.73
6	0.1438	0.1788	0.3186	97.8	1.44
		0.3575	0.4970	98.8	1.86
		0.5363	0.6704	98.2	2.24

1. Schisandrin, 2. schisandrol B, 3. schisantherin A, 4. deoxyschisandrin, 5. γ-schisandrin and 6. schisandrin C.

Table 4
The measurement results of lignans in *Schisandra chinensis* and CMRs containing *Schisandra chinensis* (mg g^{-1}).

Samples	SD	SB	SA	DS	γS	SC
1[a]	0.1093	0.06750	4.189	1.791	0.09000	0.1883
2[a]	4.160	2.138	0.8846	0.6022	3.235	0.4333
3[a]	0.1349	0.1245	2.261	1.174	0.5037	0.1282
4[a]	0.1849	0.1411	6.136	2.251	0.1869	0.2518
5[a]	5.532	3.111	1.133	0.6216	4.523	0.7236
6[a]	2.532	1.675	0.8843	0.6431	2.087	0.2988
7[a]	3.275	1.468	0.8327	0.5877	2.896	0.3391
8[a]	2.119	0.8763	1.437	0.6855	1.945	0.2815
9[a]	0.2294	0.1879	5.914	2.133	0.2653	0.3016
10[a]	3.017	1.295	2.858	1.784	1.892	0.3575
11[b]	0.1554	0.06110	0.09060	0.01490	0.07290	0.008300
12[b]	0.4444	0.2063	0.08000	0.05020	0.3525	0.03680
13[b]	1.529	0.3774	0.2385	0.1122	ND	ND
14[b]	0.2510	0.02980	0.9611	0.5269	0.08010	0.1090
15[b]	0.06231	0.008236	ND	ND	ND	ND

SD: schisandrin, SB: schisandrol B, SA: schisantherin A, DS: deoxyschisandrin, γS: γ-schisandrin and SC: schisandrin C.
ND: not detected.
[a] *Schisandra chinensis* (Turcz.) Baill medicinal herbs.
[b] Chinese multiherb remedies containing *S. chinensis*.

15.6442 mg g^{-1} found in *S. chinensis* of Jilin Province. However, the contents of the six lignans in raw herbs collected from different origins varied dramatically. Among the six lignans, the content of schisantherin A was highest, 6.1356 mg g^{-1} found in the raw herb of Shanxi Province, whilst schisandrin was highest in other provinces and up to 5.5317 mg g^{-1} found in Jilin Province. Of all the raw herbs analysed, the total content of the six lignans in *S. chinensis* of Jilin Province was the highest, followed by Heilongjiang, Liaoning, Shanxi, Hebei Province. Contents of the six lignans in different regions varied from geographical source, harvest, and storage et al. In the CMRs, Small amounts of γ-schisandrin and schisandrin C were detected in Hugan tablet, Shenqi Wu Wei Zi tablet and Jiangtang pills, whilst little was found in Compound Schisandra Syrup and Shengmaiyin due to low content and low water-solubility of the two components. It appears that the expected increase in concentration of active components in CMRs, compared to those in raw herbs, has not been observed. The low concentration of lignans in these CMRs may be due to the inefficient extraction process, poor herb quality, decomposition of compounds during the extraction procedure, or the excessive dilution in the preparation of the final product.

4. Conclusions

This HPLC method established has been applied successfully to simultaneous determination of six lignans in 10 raw herbs from different regions in China and five CMRs. Additionally, the method was validated for good linearity, limits of detection and quantification, precision, repeatability, stability and accuracy. Therefore, this simple, rapid, low-cost and reliable HPLC method is suitable for routine quantitative analysis and quality control of *S. chinensis* and its related CMRs containing bioactive multi-components.

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References

Chang, G. T., Kang, S. K., Kim, J. H., Chung, K. H., Chang, Y. C., & Kim, C. H. (2005). Inhibitory effect of the Korean herbal medicine, Dae-Jo-Whan, on platelet-activating factor-induced platelet aggregation. *Journal of Ethnopharmacology, 102*, 430–439.

Chen, A. J., Li, C. H., Gao, W. H., Hu, Z. D., & Chen, X. G. (2005). Separation and determination of active components in (*Schisandra chinensis*) Baill and its medicinal preparations by non-aqueous capillary electrophoresis. *Biomedical Chromatography, 19*, 481–487.

Halstead, C. W., Lee, S., Khoo, C. S., Hennell, J. R., & Bensoussan, A. (2007). Validation of a method for the simultaneous determination of four Schisandra lignans in the raw herb and commercial dried aqueous extracts of *Schisandra chinensis* (Wu Wei Zi) by RP-LC with DAD. *Journal of Pharmaceutical and Biomedical Analysis, 45*, 30–37.

Hancke, J. L., Burgos, R. A., & Ahumada, F. (1999). *Schisandra chinensis* (Turcz.) Baill. *Fitoterapia, 70*, 451–471.

He, X. G., Lian, L. Z., & Lin, L. Z. (1997). Analysis of lignan constituents from *Schisandra chinensis* by liquid chromatography-electrospray mass spectrometry. *Journal of Chromatography A, 757*, 81–87.

Huang, X., Song, F. R., Liu, Z. Q., & Liu, S. Y. (2007). Studies on lignan constituents from (*Schisandra chinensis*) (Turcz.) Baill fruits using high-performance liquid chromatography/electrospray ionization multiple-stage tandem mass spectrometry. *Journal of Mass Spectrometry, 42*, 1148–1161.

Ip, S. P., & Ko, K. M. (1996). The crucial antioxidant action of schisandrin B in protecting against carbon tetrachloride hepatotoxicity in mice. A comparative study with butylated hydroxytoluene. *Biochemical Pharmacology, 52*, 1687–1693.

Jian, G., Wang, Y. W., Lu, X. C., Sun, X. H., & Gong, F. J. (2007). Determination of atractylenolide II in rat plasma by reversed-phase high-performance liquid chromatography. *Biomedical Chromatography, 21*, 299–303.

Kvasnickova, L., Glatz, Z., Sterbova, H., Kahle, Vladislav, Slanina, J., & Musil, P. (2001). Application of capillary electrochromatography using macroporous polyacrylamide columns for the analysis of lignans from seeds of *Schisandra chinensis*. *Journal of Chromatography A, 916*, 265–271.

Li, X. N., Cui, H., Song, Y. Q., Liang, Y. Z., & Chau, F. T. (2003). Analysis of volatile fractions of (*Schisandra chinensis*) (Turcz.) Baill using GC-MS and chemometric resolution. *Phytochemical Analysis, 14*, 23–33.

Li, Y., Xu, C., Zhang, Q., Liu, J. Y., & Tan, R. X. (2005). In vitro anti-Helicobacter pylori action of 30 Chinese herbal medicines used to treat ulcer diseases. *Journal of Ethnopharmacology, 98*, 329–333.

Ohsugi, M., Fan, W., Hase, K., Xiong, Q., Tezuka, Y., Komatsu, K., et al. (1999). Active-oxygen scavenging activity of traditional nourishing-tonic herbal medicines and active constituents of *Rhodiola sacra*. *Journal of Ethnopharmacology, 67*, 111–119.

Opletal, L., Sovova, H., & Bartlova, M. (2004). Dibenzo[a,c]cyclooctadiene lignans of the genus *Schisandra*: Importance, isolation and determination. *Journal of Chromatography B, 812*, 357–371.

Pharmacopoeia of the People's Republic of China (2005). Beijing: Peoples Medicinal Publishing House (Vol. I, pp. 44–45).

Sladkovsky, R., Solich, P., & Opletal, L. (2001). Simultaneous determination of quercetin, kaempferol and (E)-cinnamic acid in vegetative organs of *Schisandra chinensis* Baill by HPLC. *Journal of Pharmaceutical and Biomedical Analysis, 24*, 1049–1054.

Wang, J. J., Chen, Y., Lin, M., Fan, G. R., Zhao, W. C., Wu, Y. T., et al. (2007). Development of a quality evaluation method for *Fructus schisandrae* by pressurized capillary electrochromatography. *Journal of Separation Science, 30*, 381–390.

Zhu, M., Cao, Y., & Fan, G. R. (2007). Microwave-assisted extraction and fingerprint studies of (*Schisandra chinensis*) (Turcz.) by high performance liquid chromatography and gas chromatography. *Journal of Separation Science, 30*, 67–73.