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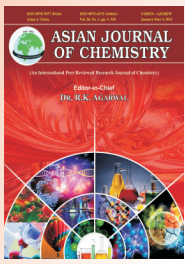

Simultaneous Determination of Three Active Ingredients in *Schisandra chinensis* (Turcz.) Baill by HPLC

WEIMING CHENG[1], JIE GAO[2] and SHAN ZHONG[3,*]

[1]Zhejiang Institute for Food and Drug Control, No 86 Lane 1 Jichang Road Jianggan Dist. 310004, Hangzhou Zhejiang Province, P.R. China
[2]Grade 2011, Zhejiang University of TCM, No. 548, Binwen Road, Binjiang Dist. 310053, Hangzhou Zhejiang Province, P.R. China
[3]State Key Laboratory of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University (Guangzhou Medical University), No. 151 YanJiang Road Yuexiu Dist. 510120, Guangzhou, Guangdong Province, P.R. China

*Corresponding author: Tel: +86 18664535213; E-mail: zhong-shan2000@hotmail.com

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In present study, an HPLC method was setup for the simultaneous determination of 3 active ingredients in *Schisandra chinensis* (Turcz.) Baill. HPLC conditions included Kromasil-C_{18} column (250 × 4.6 mm, 5 µm) and the mobile phase was a mixture of MeOH-CH_3CN-H_2O (5:1:4). The flow rate was 1 mL/min, the column temperature was at 30 °C and the UV detection wavelength was 246 nm. The 3 active ingredients had good linear relationships *i.e.*, schizandrin in the range of 0.10-6.0 µg (R^2 = 0.999 8), deoxyschizandrin in the range of 0.15-9.0 µg (R^2 = 0.999 2) and schizandrin B in the range of 0.05-3.0 µg (R^2 = 0.999 7). Their average recoveries were 99.3 % (RSD = 1.3 %), 97.1 % (RSD = 2 %) and 95.2 % (RSD = 2 %), respectively. This method was steady with high precision and good repeatability and could be used for the determination of the 3 active ingredients and quality control of *S. chinensis*.

Keywords: *Schisandra chinensis*, Schizandrin, Deoxyschizandrin, Schizandrin B, HPLC.

INTRODUCTION

Schisandra chinensis (Turcz.) Baill, also known as "Beiwuweizi" in China, belongs to the Schisandraceae family, with the dry mature fruits as the medicament portions. *S. chinensis* mainly distributes in east Asia, southeast Asia and southern parts of north America. It has been used in clinic for a long period in the treatment of insomnia[1], cough and asthma[2], hyperglycemia[3] and hepatitis[4], *etc*. Modern pharmacological researches have also confirmed such bioactivities of *S. chinensis* as immunological enhancement, anti-tumor, cardiovascular enhancement, hypoxia endurance improvement, *etc.*,[5-10].

As the main medicinal ingredients of *S. chinensis*[11], lignan exhibits various interesting bioactivities, such as anti-tumor, anti-virus, hepatoprotection and anti-aging. Among the lignin, schizandrin, deoxyschizandrin and schizandrin B are 3 main components, possessing the above bioactivities. In this study, a steady HPLC method, with high precision and good repeatability, was set up for the simultaneous determination of the above 3 active components inside *S. chinensis*, aiming to provide a rapid, simple and precise method for the quality control of *S. chinensis*.

EXPERIMENTAL

Agilent 1100 series HPLC system (Angilent Technologies, USA) was equipped with quaternary pump (G1311A), automatic sampler (G1313A), UV variable-wavelength detector (1314A-UV) and column oven (CTO-10ASVP); Pa2251 electronic analytical balance from Sartorius Group, Germany; Tw20 constant temperature bath box from Julabo Labortechnik GmbH Company, Germany.

Standards of schizandrin, deoxyschizandrin and schizandrin B were supplied by National Institute for Food and Drug Control (NIFDC, Beijing, China) with the batch number as 110857-200507, 110764-200408, 110765-200508, respectively. Three batches of *S. chinensis* were purchased from Haozhou Market of Meteria Medica (Batch No: 20100425, 20100827 and 20110911), the voucher specimens were identified by Pharmacogonist Yiguo Sun and kept under certain conditions for future identification. Methanol and acetonitrile were of HPLC grade and other reagents used were of analytical grade. Deionized water was prepared using a Millipore water purification system.

HPLC conditions: The separation of the 3 ingredients was carried out on a Kromasil-C_{18} (250 mm × 4.6 mm, 5 µm).

The solvents used for HPLC separation were MeOH-CH_3CN-H_2O (5:1:4) and the analysis was monitored at 246 nm with the column temperature of 30 °C and the injection volume was 20 µL.

Preparation of standard solution: Certain amounts of the 3 standard compounds were dissolved with methanol into volumetric flasks, respectively. Then measured 1 mL schizandrin, 1 mL deoxyschizandrin and 1.5 mL schizandrin B standard solution, respectively, into a 50 mL volumetric flask and diluted to the concentrations of 100.3, 150.4 and 51.3 µg/mL.

Preparation of sample solution: 10 g dried *S. chinensis* was finely ground into powder (80 mesh), about 0.5 g sample was accurately weighted, then added into a 50 mL conical flask. 20 mL MeOH was added into the flask and performed ultrasonic extraction in a constant temperature bath for 0.5 h. The extract solution was then cooled to room temperature and diluted to the volume. After centrifuged at 12 000 rpm for 10 min, the supernatant was passed through a 0.22 µm membrane filter and the filtrate was ready for the chromatographic analysis.

RESULTS AND DISCUSSION

Linear regression analysis for each component was performed by the external standard method. The above 3-compound solution was accurately injected 1, 5, 10, 20, 30 and 60 µL, respectively. The linearity of each compound was calculated by plotting the peak area (Y) *vs*. concentration (X). (Table-1). All the 3 components showed good linearities in wide concentration ranges.

TABLE-1
LINEAR REGRESSION EQUATION AND LINEAR RANGES

Components	Regression equation	R^2	Linear range (µg)
Schizandrin	Y=11.22X+1.76	0.999 8	0.10~6.0
Deoxyschizandrin	Y=8.31X+2.55	0.999 2	0.15~9.0
Schizandrin B	Y=7.09X+2.11	0.999 7	0.05~3.0

Precision: The standard mixture solution of the 3 components was injected into HPLC six times continuously and the area of each peak was used for the calculation of precision (Fig. 1). The results showed that relative stand deviation (RSD) of peak area of each standard was 1.3, 1.4 and 2.1 %, respectively.

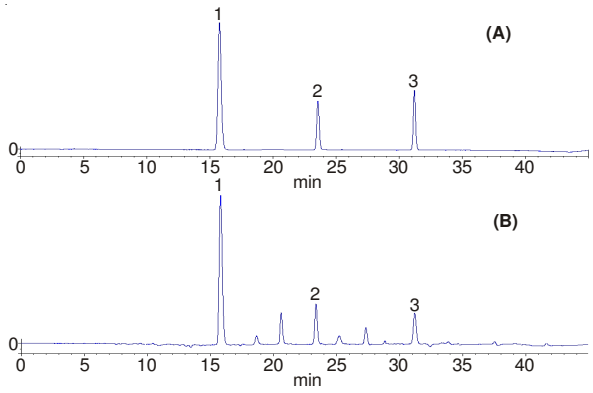

Fig. 1. Typical HPLC chromatograms of the 3 ingredients mixture (a) and *S. chinensis* (b) 1: schizandrin; 2: deoxyschizandrin; 3: schizandrin B

Stability: As for the stability test, the same sample solution was analyzed at designated time points in 24 h. The results showed that RSDs of peak areas were 0.9, 1.3 and 1.5 % and found to be stable for the experiment.

Repeatability: Repeatability was carried out using six samples solution after the same treatment procedure. The results showed that RSD of each peak area was 1.1, 0.8 and 1.2 %, respectively.

Recovery test: The sample with known targeted contents was spiked with certain amounts of the 3 standards. Then the spiked sample was processed in accordance with the established method for the HPLC detection. The average recoveries for schizandrin, deoxyschizandrin and schizandrin B determined were 95.2-99.3 % (Table-2).

Application of the HPLC method for the quantitation studies: The experiment determined 3 samples of different batches with the above method. The contents of the 3 components were shown in Table-3.

TABLE -2
RECOVERY RESULTS OF THE 3 ACTIVE INGREDIENTS

Component	Contents in samples (mg)	Added (mg)	Found (mg)	Recovery (%)	Mean (%)	RSD (%)
Schizandrin	0.7156	0.6933	1.4034	99.2	99.3	1.3
	0.7033	0.6933	1.3807	97.7		
	0.6905	0.6933	1.3762	98.9		
	0.7191	0.6933	1.4117	99.9		
	0.6832	0.6933	1.3869	101.5		
	0.7200	0.6933	1.4036	98.6		
Deoxyschizandrin	0.1112	0.1034	0.2106	96.1	97.1	2.0
	0.1093	0.1034	0.2075	95.0		
	0.1073	0.1034	0.2089	98.3		
	0.1117	0.1034	0.2141	99.0		
	0.1062	0.1034	0.2087	99.2		
	0.1119	0.1034	0.2101	95.0		
Schizandrin B	0.0940	0.0858	0.1765	96.1	95.2	2.0
	0.0924	0.0858	0.1737	94.8		
	0.0907	0.0858	0.1742	97.3		
	0.0945	0.0858	0.1773	96.6		
	0.0897	0.0858	0.1688	92.1		
	0.0946	0.0858	0.1755	94.3		

TABLE-3
CONTENTS DETERMINATION OF THE 3 ACTIVE INGREDIENTS IN 3 BATCHES

Batch	Schizandrin (mg/g)	Deoxyschizandrin (mg/g)	Schizandrin B (mg/g)
20100425	0.92	0.33	0.16
20100827	1.17	0.20	0.11
20110911	1.34	0.21	0.17

Optimization of HPLC separation conditions: In order to get a separation with better resolution of targeted components with shorter analytical time, we compared with three different column temperature: 20, 30 and 40 °C, then we found that the higher temperature, the shorter retention time with almost the same resolution, so we chosen 30 °C finally for protecting the lifespan of the column.

Six chromatographic columns of the same specification were compared in the experiment *i.e.*, Zorbax SB-C_{18}, Kromasil-C_{18}, Eclipse-C_{18}, Symmetry Shield RP_{18}, Hypersil-C_{18} and Extend-C_{18}, according to the effect of separation, Kromasil-C_{18} (250 mm × 4.6 mm, 5 μm) was used for the further research of the methodology.

Besides, reflux extraction and ultrasonic extraction were investigated in the experiments. Considering the advantages of high efficiency, ultrasonic extraction was chosen as the way to prepare the sample solutions. In the meantime, we compared the efficiency of different elution solvents, namely methanol and chloroform, the results indicated that the 2 solvents almost had the same efficiency. Considering the toxicity and safety, methanol was finally selected as the extract solvent.

Conclusion

From the content determination of 3 batches *S. chinensis*, it could be found that the batch harvested in August and September had higher contents of the 3 target compounds, which was consistent with the literature and the conventional harvest experience, indicating that better bioactivities might be obtained. In traditional medical use, only the dried fruits of *S. chinensis* was applied into the clinic. Our current research revealed that the certain lignans also existed in the leaves and stems and the further research was being carried out for the difference comparison among the fruits, leaves and stems.

In short, 3 active components were quantitatively determined in *S. chinensis*. On the whole, this developed method was simple, accurate for the determination of schizandrin, deoxyschizandrin and schizandrin B and reliable for the quality control and further efficacy study of *S. chinensis* in clinic.

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