DOI: 10.21276/sjmps.2016.2.8.6

Saudi Journal of Medical and Pharmaceutical Sciences
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2413-4929 (Print)
ISSN 2413-4910 (Online)

Research Article

Determination of three active compounds of methanolic extract of *Schisandra chinensis* by RP-HPLC

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Abstract: To establish a method for determination of contents of schizandrol A, deoxyschisandrin and schisandrin B in *Schisandra chinensis* (Turcz.)Baill.. An RP-HPLC method was developed. A Kromasil C_{18} column (250 mm×4.6 mm, 5 μm) was used, with the mobile phase of acetonitrile-water by gradient elution, the flow rate was 1.0 mL·min^{-1}and the UV absorbance was monitored at 218 nm. The linear range of schizandrol A,deoxyschisandrin,schisandrin B were 19.92~398.4 μg·mL^{-1}, 6.24~124.8 μg·mL^{-1}, 12.5~ 250 μg·mL^{-1}, the mean recovery were 99.5%, 98.6%, 98.5%;. The method is suitable for the quality control of *Schisandra chinensis* (Turcz.)Baill.
Keywords: schizandrol A ; deoxyschisandrin; schisandrin B; RP-HPLC

INTRODUCTION

Fructus Schisandrae chinensis, originated from the dried ripe fruits of *Schisandra chinensis* (Turcz.) Baill. was ranked as high-grade herbal drug in ancient medical book ' Shen Nong Bencao Classics '. lignans were the most abundant and active components derivative isolated from the fruit of *Schisandra chinensis*, which has demonstrated wide range of pharmacological activities including; hepatoprotective and neuroprotective effects, antioxidant, anti-inflammatory, immunomodulating effects and so on[1-10]. In the past few years, chromatographic techniques has been successfully applied to determine the contents of bioactive components in herbal medicines[11, 12].

In our study, *Schisandra chinensis* were extracted by water, 75% ethanol and methanol respectively in order to screening of active fraction. The result of the sedative and hypnotic experiments indicated that the methanol extract showed the highest pharmacological activities than that of the other extracts. The results showed that the methanol extract of *Schisandra chinensis* can shorten sleep latency significantly, increase sleeping time. Herein, we developed methods for a systematic identification of active fractions of *Schisandra chinensis*.In this study, a simple, reliable and sensitive analysis method by high-performance liquid chromatography coupled with diode array detection was developed for quantitative determination of the three active compounds in Fructus Schisandrae chinensis.

MATERIALS AND METHODS

Instrumentation

HPLC analyses were performed using a Shimadzu RP-HPLC system (Shimadzu Corporation, Japan) consisting of an SCL-10AVP system controller, LC-10ATvp infusion pumps, SPD-10Avp UV detector, ANASTAR chromatography workstation. Mettler Analytical Balance AE240(Mettler-Toledo Instruments Co. , Ltd.)

Chemical reagents

Schizandrol A (lot number: 0857-200304), deoxyschisandrin (lot number: 0764-200107), schisandrin B (lot number: 0765-200205). Standard reference were all obtained from National Institute for the Control of Pharmaceutical and Biological Products, China. HPLC-grade acetonitrile were obtained from Fisher Scientific (Pittsburgh, PA), ultra pure water, *Schisandra chinensis* was collected from Heilongjiang, China.

Preparation of Sample solution

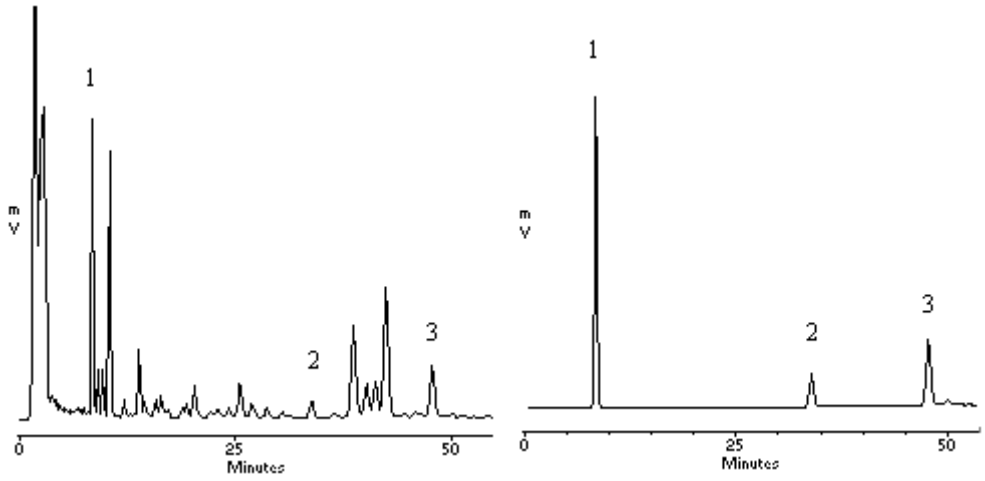
About 1 g of the sample was accurately weighed and ultrasonic extracted by 50 ml menthol for 30 min, make up the weight loss, shake, and the supernatant filtered (0.45μm), made for the test solution.

Preparation of mixed standard solution

Reference substance schizandrol A, deoxyschisandrin and schisandrin B were accurately weighed and dissolved in 50 ml of methanol as a stock solution. The final concentration of the standard solution were 199.2, 62.4 and 125μg/ml, respectively

Chromatographic Conditions

Column was Kromasil C18 (5 μm, 4.6 mm × 250 mm); A binary gradient elution system consisting of acetonitrile (A) and water (B) was used with the following gradient program: 0-55 min, 70% B-55%B; detection wavelength was set at 218 nm and the flow rate was 1.0 mL/min. The injection volume was 20 μl and the column temperature was maintained at 30ºC.



A **B**

Fig-1:HPLC chromatograms of *Schisandra chinensis* fruits (A), reference substances (B)
1- schizandrol A 2- deoxyschisandrin 3- schisandrin B

RESULTS AND DISCUSSION

Linearity

The calibration curve were obtained by plotting the concentration(μg/ml) as the x-axis versus peak areas on Y-axis and regression equations were computed. Six concentrations of the four Standards were analyzed. Calibration curves of all analytes showed good linearity over a wide concentration range. The test results were given in Table-1.

Table-1: Result of regression analysis on calibration curves

Compd	Regression equation	r^2	Linear range (μg/mL)
schizandrol A	Y_1=5.587 ×10^6X+1.212×10^6	0.9991	19.92~398.4
deoxyschisandrin	Y_2=3.756 ×10^7 X+2.693 ×10^6	0.9996	6.24~124.8
schisandrin B	Y_3=1.777 ×10^7 X-5.550 ×10^5	0.9994	12.5~ 250

Precision

The stock solutions of reference substances were measured for five times under the same analytical conditions in a day. the relative standard deviation (RSD) values of retention time were all lower than 0.3%, while the RSD values of peak area were 0.80%, 0.47% and 0.62%, respectively.The results showed that the assay had a good intra-day precision.

Stability

For the stability testing, sample solution was stored at ambient temperature (25°C) for 24h. The same real sample was re-analyzed after 12 and 24h time intervals and compared against fresh sample. The RSD values of the stability was less than 2.0%. It is illustrated that the solution was found to be stable within 24h during assay determination.

Accuracy

The accuracy of the method was determined by means of recovery experiments. Particular amount of the authentic standards were added into the pre-analyzed sample solution at three levels (80%, 100% and 120%, three replicates of each concentration).The mixed samples were analyzed using the developed HPLC method mentioned above. The average recoveries of schizandrol A, deoxyschisandrin and schisandrin B were 99.5%,98.6% and 98.5% respectively,and RSD s of the above three compounds were 1.7%(n=9),1.9%(n=9) and 1.6 %(n=9) respectively.It was confirmed from recovery results that this developed method is highly accurate for the determination of these components.

Sample analysis

The validated HPLC method was applied to the simultaneous determination of three active components in herbs. The contents of schizandrol A, deoxyschisandrin and schisandrin B were calculated by external standard method (Table-2).

Table-2 Determination results of samples (w%, *n*=3)

No	schizandrol A	deoxyschisandrin	schisandrin B
1	0.41	0.09	0.18
2	0.48	0.11	0.20
3	0.52	0.13	0.26
4	0.61	0.15	0.29
5	0.49	0.12	0.22

CONCLUSION

Under above optimized chromatographic conditions, 3 chromatographic peak were well separated. The convenient and high specific method could be used to identify and evaluate the quality of *Schisandra chinensis*. The developed method also lays a solid foundation for further comprehensive study on the pharmacodynamic-pharmacokinetic effects of *Schisandra chinensis*.

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