Quantitative analysis of lignans in Ginseng Schisandra Granules by HPLC

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Abstract: A simple and rapid analytical method for the quantitative determination of two lignans of Ginseng Schisandra Granules was developed. The determination was carried out on a Kromasil C$_{18}$ column (250 mm×4.6 mm, 5 μm) with mobile phase of acetonitrile-water (52:48). UV detection was performed at 218 nm, flow rate was 0.8 ml/min. The linear range of schizandrol A and schizandrol B were 16.03–160.3 μg/mL, 5.26–52.6 μg/mL, the mean recovery were 98.9%, 98.1%. The method is suitable for the quality control of Ginseng Schisandra Granules.

1. Introduction
Ginseng Schisandra Granules is a typical TCM prescription consisting of two Chinese materia materials: Panax ginseng and Schisandra chinensis. It has exact clinical efficacy in the treatment of neurasthenia. Lignans were active constituents extracted and isolated from Schisandra chinensis, which has shown wide range of pharmacological activities including: hepatoprotective and neuroprotective effects, anti-inflammatory, immunomodulating effects, suppress the generation of superoxide and improve learning and memory abilities [1-10]. The concentration of lignans in raw material herbs are variable, which is associated with cultivating location, harvest season, age of the plant and so on. Controlling its content is very important for the quality control of the finished product. Therefore, two lignans(schizandrol A and schizandrol B) were chosen as representative quantity-control indicators of Ginseng Schisandra Granules.

In the past few years, liquid chromatography analytical techniques has been successfully applied to simultaneously determine the contents of bioactive components in herbal medicines [11-13]. Although the application of Ginseng Schisandra Granules has been growing steadily in recent years, we have not found any scientific reports assessing the quality of these granules. Therefore, a quantitative method for evaluating the quality consistency of Ginseng Schisandra Granules should be developed. In this study, a simple, rapid and sensitive analysis method by high-performance liquid chromatography coupled with diode array detection was developed for quantitative determination of schizandrol A and schizandrol B in Ginseng Schisandra Granules.

2. Materials and methods

2.1. Instrumentation
The 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.).

2.2. Chemical reagents
Schizandrol A (lot number: 110857-201211), Schizandrol B (lot number: 110543-200708). Standard reference were all obtained from National Institute for the Control of Pharmaceutical and Biological Products, China. Acetonitrile were HPLC-grade and purchased from Fisher Scientific (Pittsburgh, PA), ultra pure water, Ginseng Schisandra Granules were commercial products (batch number: 20140901, 20140903, 20141002).

2.3. Preparation of Sample solution
About 1 g of the sample was accurately weighed and dissolved in 5 ml menthol. Then 5 mL of the extract of sample was adsorbed for 30 min with a column of AB-8 macroporous resin (R15 mm × H100 mm) and the resin was washed with 100 mL of 10% ethanol, then the hesperidin was eluted from the macroporous resin with 100 mL of 95% ethanol. The eluate was collected, evaporated to dryness. The residue was again dissolved in 10 ml menthol.

2.4. Preparation of mixed standard solution
Reference substance schizandrol A and schizandrol B were accurately weighed and dissolved in 10 mL of methanol as a stock solution. The final concentration of the standard solution were 160.3 and 52.6 μg/mL, respectively.

2.5. Chromatographic Conditions
Column was Kromasil C18 (5 μm, 4.6 mm × 250 mm); The mobile phase consisted of acetonitrile-water (52:48) with flow rate of 0.8 mL/min. The injection volume was 10 μl and the column temperature was maintained at 30°C. Figure 1 depicts the representative chromatogram obtained with the present method.

![Figure 1. HPLC chromatograms of Schisandra chinensis fruits (A), reference substances (B)](image)

3. Results and discussion

3.1. Linearity
The calibration curve were constructed by plotting the peak areas (Y-axis) versus the corresponding concentration (x-axis, μg/mL) and regression equations were also computed. Six concentrations of the two 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 | $Y_1=27388.9X+2517.6$ | 0.9998 | 16.03~160.3          |
| schizandrol B | $Y_2=30778.6X+2591.5$ | 0.9999 | 5.26~52.6            |

3.2. **Precision**
The intraday variability were used for the evaluation of the precision. The stock solutions of two reference substances were measured for five times based on the above method in a day. the relative standard deviation (RSD) values of retention time of schizandrol A and schizandrol B were 0.51% and 0.83%, while the RSD values of peak area were 0.72% and 0.95%, respectively. The results indicated clearly that the assay had a good intra-day precision.

3.3. **Stability**
The stability experiment was determined for the sample solution at the time interval of 0, 2, 4, 6, 8, 12, and 24 h at room temperature, which was analyzed using the established method. The RSD values of the stability was less than 2.0%, demonstrating that the sample solutions were stable within 24 h at room temperature.

3.4. **Accuracy**
The accuracy of the developed method was determined by recovery experiments. Particular amount of the two standards were added into the pre-analyzed sample at three different concentrations (80%, 100% and 120%, three replicates of each concentration). The mixed samples were extracted and analyzed by the developed method mentioned above. The average recoveries of schizandrol A and schizandrol B were 98.9% and 98.1% respectively, and RSD were 1.5%(n=9) and 1.8%(n=9) respectively. It was illustrated that the extraction method was of high accuracy for the determination of the two components.

3.5. **Sample analysis**
The newly established quantitative method was applied to evaluate the quality of 5 batch of Ginseng Schisandra Granules. The amounts of schizandrol A and schizandrol B were calculated using calibration curve method. The results of the assay are shown in Table 2.

Table 2. Determination results of samples (mg/g, n=3)

| No | schizandrol A | schizandrol B |
|----|---------------|---------------|
| 1  | 1.12          | 0.35          |
| 2  | 1.16          | 0.39          |
| 3  | 1.08          | 0.33          |
| 4  | 1.11          | 0.34          |
| 5  | 1.15          | 0.37          |

4. **Conclusion**
The aim of this study was to develop a simple and rapid method for determination of schizandrol A and schizandrol B in Ginseng Schisandra Granules. The method was designed to be specific, sensitive, accurate and reproducible. The sensitivity and simplicity of the method makes it suitable for routine analysis and quality evaluation of pharmaceutical formulations. The developed method give scientific support for the clinical use and quality control of Ginseng Schisandra Granules.
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