Determination of Schisandrin A and Schisandrin B in Traditional Chinese Medicine Preparation Huganpian Tablet by RP-HPLC

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A reverse phase (RP)-HPLC method for separation and determination of Schisandrin A and Schisandrin B was presented, using a C18 Bondclone column, with methanol–water (v/v = 68:32) as mobile phase at a flow-rate of 1.00 mL·min−1, and UV detection at 220 nm. The tested parameters included mobile phase composition and UV detection wavelength. Good linearities were observed within concentration ranges of Schisandrin A 0.008–4.8 mg·L−1 (r = 0.9996), and Schisandrin B 0.005–3.1 mg·L−1 (r = 0.9994), respectively. The limit of detection (LOD) (S/N = 3) were 0.005 mg·L−1 Schisandrin A and 0.002 mg·L−1 Schisandrin B, respectively. The method was applied to determine the 2 compounds in a traditional Chinese medicine preparation for treatment of hepatic diseases, Huganpian tablet. To eliminate matrix effect, Oasis hydrophilic lipophilic balance (HLB) solid-phase extraction (SPE) was used to purify the ultra-sonically extracted solution of the drug sample. Combined with the HLB SPE purification procedure, the HPLC method gave satisfactory results for quantitation of Schisandrin A and Schisandrin B in 3 types of Huganpian tablet samples, with spiking recoveries ca. 98% (relative standard deviation (R.S.D.) ≤ 3.5%) (n = 5).

Key words Schisandrin A; Schisandrin B; HPLC; Huganpian tablet; hydrophilic lipophilic balance (HLB) solid-phase extraction (SPE)

Introduction

Schisandra chinensis (Turcz.) BAIL. is often referred to as an example of medicinal plant used in traditional Chinese medicine (TCM). The diversity of biomedical and pharmacological effects of Schisandra chinensis was reviewed by authors.1–3)

The active ingredients of Schisandra chinensis include lignans, in which dominated are Schizandrin and analogs, such as Schisandrin A, Schisandrin B (Fig. 1), Neoschizandrin, Schizandrol, etc. Those compounds are representative of natural dibenzocyclooctadiene lignans existing in many species of the Schisandraceae family, most of which exhibit various pharmacological functions such as sedation, hypnotic activity, anticonvulsant and neuroprotective effects, liver protection, etc. Among these functions, the most remarkable is the protective effect against liver injury in humans.4)

Thus, Schisandra chinensis is often used as an indispensable material in those TCM formula or medicine preparations for treatment of liver diseases. Huganpian tablet, as one of those TCM preparations, is made from Chaihu (Bupleurum chinense), Yinchen (Artemisia capillaris THUNB), BanLanGen (Isatis tinctoria), Wuweizi (Schisandra chinensis), Zhudanfen (Pulvis felli suis), Ludou (Vigna radiata (L.) Wilczek), etc., and used to treat chronic hepatitis, early hepatocirrhosis, and other liver diseases. The commercial pharmaceutical product was registered in 2000 and included firstly in Chinese Pharmacopeia 2005 (ChP 2005). As described in ChP 2005, the quality evaluation of Huganpian Tablets is based on TLC qualitative inspection of Schisandrin B and HPLC quantitation of Schizandrol.5) However, in fact, Schisandrin A and Schisandrin B occur predominately as the main active ingredients of the drug. Thus, these 2 compounds should be considered as the more reasonable quality markers, or at least as complementary quality markers of the drug.

Chromatographic methods are most commonly used for qualitative identification or quantitation of those natural dibenzocyclooctadiene lignans in raw herbal materials, and medicine or biological matrix samples.6–19) In this work, a HPLC method was developed for separation and quantitation of Schisandrin A and Schisandrin B, with methanol-water as mobile phase, and UV detection. The present method was used to determine Schisandrin A and Schisandrin B in Huganpian Tablets sample. With both of ultra-sonicate extraction and hydrophilic lipophilic balance (HLB) solid-phase extraction (SPE) purification, satisfactory results were obtained.

Experimental

Chemicals and Instrumentation The standards of Schisandrin A and Schisandrin B were purchased from the National Institutes for Food and Drug Control of China (Beijing, China). Chromatographic grade methanol was commercially available from Shield Company (Tianjin, China), and other reagents were all of analytical grade. Water was obtained from a MillQ apparatus (Millipore, Milford, MA, U.S.A.).

Stock solutions of Schisandrin A and Schisandrin B (herein...
the concentration = 480 and 420 µg·mL⁻¹ for Schisandrin A and Schisandrin B, respectively) was prepared with methanol as the solvent. When necessary, a working solution was obtained by diluting the stock solution with methanol or the HPLC mobile phase solution.

The chromatograph system used was a Waters 510 HPLC with a 486 Tunable Absorbance Detector and a Rheodyne 7725i manual injector (U.S.A.), and a Bondelone C18 column (150 × 3.9 mm, 10 µm, Phenomenex, U.S.A.) was used for separation with a C18 guard cartridge (4 × 8 mm, Phenomenex).

The Oasis® HLB SPE cartridge was commercially available from Waters Corporation (Milford, MA, U.S.A.). A SK3300LH ultrasonic generator (Shanghai, China) was used to degas the mobile phase, and extract ingredients of the drug sample. An IKA MS1 minishaker (German) was used for pretreatment of the samples.

Chromatography Procedure
The HPLC system was conditioned with mobile phase until the triplicate injections of standard solution showed identities of retention time and peak area. Then the data of chromatographic injection could be recorded and used, all injections were in triplicate.

Separation Conditions of Schisandrin A and Schisandrin B
The mobile phase was methanol–water (v/v = 68 : 32) at flow rate of 1.00 mL·min⁻¹, the column temperature was ambient temperature, and UV detected at 220 nm. The injector loop volume was 20 µL.

The mobile phase solution was degassed by ultra-sonication, and all sample solutions and mobile phase solutions were filtered with a 0.45 µm membrane prior to use.

Pretreatment of the Pharmaceutical Sample
In this paper, the tested Huganpian tablet (sugarcoating tablet) included 3 types of samples from different pharmaceutical companies. Theses samples all were commercially available.
Sample I: KUIHUA® Hugan Pian, produced in Heilongjiang, China.
Sample II: COCOKING® Hugan Pian, produced in Shanghai, China.
Sample III: XINFENGYAOYE® Hugan Pian, produced in Guandong, China.

The listed component in the attached dug instruction of the 3 types of Huganpian tablet samples are same and as follows: Chaihu (Bupleurum chinense), Yinchen (Artemisiacapillaris THUN), BanLanGen (Isatis tinctoria), Wuweizi (Schisandra chinensis), Zhudanfen (Pulvis fellis suis), Ludou (Vigna radiata (LINN.) WILCZEK). However no detail (such as percentage of the components) was shown.

The 3 types of Huganpian tablet have the similar appearance.

The sugar-coat of the tablets was thoroughly removed, and the tablet cores were then grinded to fine powder of 35 mesh (500 µm). Then, 0.25 g of the grinded powder was put into a 50 mL conical flask with a stopper, in which 20.0 mL methanol–water (v/v = 68 : 32) was then added, shaken for 5 min, and ultra-sonicately extracted for 15 min. The obtained solution was filtered with a 0.45 µm membrane, and then a aliquot of 2.00 mL was introduced to an Oasis HLB cartridge (3 mL, 60 mg) pre-activated with 5% methanol (v/v) (3 mL) and washed with water (5 mL), followed by sequential eluting with 5 mL 5% (v/v) methanol solution, and 5 mL 2% acetic acid–5% methanol solution (v/v), 5 mL 2% ammonia–5% methanol solution (v/v), 3 mL methanol, respectively. The final methanol elute was collected and evaporated to dryness with nitrogen gas, and reconstituted with 2.00 mL mobile phase, followed by a 0.45 µm membrane filtering for chromatography analysis.

Optimization of Separation Conditions for Schisandrin A and Schisandrin B
As Schisandrin A and Schisandrin B have no remarkable acid or basic properties, thus a buffer is not necessary in the HPLC mobile phase for separation of these 2 compounds. Methanol–water solution was experimentally shown to be suitable, and a proper ratio (v/v = 68 : 32) of methanol and water could be confirmed by simple procedure, which gave good peak shape and sufficient chromatographic resolution (Fig. 2).

UV Detection Wavelength for Schisandrin A and Schisandrin B
The UV absorption of Schisandrin A and Schisandrin B is reported in a wide range between 210 to 250 nm. In this work, wavelengths between 210–250 nm were compared for UV detection of Schisandrin A and Schisandrin B. The results showed that the detection sensitivity decreased with the wavelength increasing; 210 nm gave
highest detection sensitivity. However, in the followed real sample analysis, other influence need to be taken into account. When relative short wavelength such as 210 nm was used as detection wavelength, the matrix effect of the sample’s extract increased and interfered with chromatogram profile (Fig. 3). Thus, in view of more sensitivity and less matrix interference, 220 nm was finally chosen for the following chromatography.

**Linearity and Sensitivity of the Method for Schisandrin A and Schisandrin B** Under the chosen conditions, the typical chromatogram of Schisandrin A and Schisandrin B standard mixture is shown in Fig. 2. Good linearity between peak area and Schisandrin A or Schisandrin B concentration were observed in concentration ranges of 0.008–4.8 mg·L⁻¹ Schisandrin A and 0.005–3.1 mg·L⁻¹ Schisandrin B, respectively. The regression equations were shown as Table 1, where \( A \) is the peak area (\( \mu \)V·s) and \( C \) is the concentration (mg·L⁻¹) of Schisandrin A or Schisandrin B, and the limit of detections (LOD) was assigned as \( S/N = 3 \).

**Application of the Method for Quantitation of Schisandrin A and Schisandrin B in Huganpian Tablet Samples** The present HPLC method was utilized to determine Schisandrin A and Schisandrin B in 3 types of Huganpian tablet sample.

Ultra-sonication and Soxhlet extraction methods are commonly used for extraction of Schisandrin A and Schisandrin B. In this work, ultra-sonication was used, with the mobile phase (methanol–water \( v/v = 68:32 \)) as extract solvent. However, the ultra-sonically extracted solution contained many other compounds, which resulted weak resolution of the chromatography peaks (Fig. 3), and hindered quantitation of Schisandrin A and Schisandrin B. Thus, a purifying procedure was developed for ultra-sonication extraction solution. An Oasis HLB SPE cartridge was used to purify the extract. The details of the purifying procedure are described in “Pretreatment of the Pharmaceutical Sample.” When the ultra-sonically extracted solution was treated with an Oasis HLB SPE cartridge, prior to chromatographic injection, the chromatography peak resolution was significantly improved (Fig. 4a).

Based on the HLB SPE purifying procedure, the ultra-sonicate extraction conditions were optimized, including ultra-sonication time and methanol percentage in extract solvent. The Sample I (KUIHUA® Huganpian tablet) was here used to investigate the optimization. It was indicated that the extraction rate of Schisandrin A and Schisandrin B was almost independent of ultra-sonication time between 15–60 min, and that 68% methanol produced an adequate extraction rate of Schisandrin A and Schisandrin B.

As per the procedure in “Pretreatment of the Pharmaceutical Sample” and the chosen conditions, and the HLB SPE cartridge purifying steps above, Schisandrin A and Schisandrin B in 3 types of Huganpian tablet samples was ultra-sonicately extracted, HPLC separated and determined, respectively. A typical chromatogram of the Huganpian tablet samples was shown as Fig. 4a, and the measured results were shown in Table 2.

**Robustness of the Method for Pharmaceutical Sample Assay** To validate the present method, the spiked recoveries of Schisandrin A and Schisandrin B were investigated. Both of 120 µg Schisandrin A and 105 µg Schisandrin B were spiked to an aliquot of 0.12 g grinded tablet (herein KUIHUA® Huganpian tablet was selected for the test) core powder, and then extracted by ultra-sonication and purified as described in “Pretreatment of the Pharmaceutical Sample.” A resulting typical chromatogram was shown in Fig. 4b.

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**Table 1. Sensitivity and Linearity of the Method**

<table>
<thead>
<tr>
<th>Linear range (mg·L⁻¹)</th>
<th>Regression equation</th>
<th>( r )</th>
<th>LOD (mg·L⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>Schisandrin A 0.008–4.8</td>
<td>( A = 2.09 \times 10^3 + 9.08 \times 10^2 C ) 0.9996</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Schisandrin B 0.005–3.1</td>
<td>( A = 2.60 \times 10^3 + 1.19 \times 10^2 C ) 0.9994</td>
<td>0.002</td>
<td></td>
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</tbody>
</table>

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**Table 2. The Determination Results (Average (R.S.D.%), \( n = 5 \)) of Schisandrin A and Schisandrin B in 3 Types of Huganpian Tablet Samples**

<table>
<thead>
<tr>
<th>Sample I</th>
<th>Sample II</th>
<th>Sample III</th>
</tr>
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<tbody>
<tr>
<td>Schisandrin A (µg·g⁻¹) 410 (2.8%)</td>
<td>397 (3.0%)</td>
<td>401 (2.6%)</td>
</tr>
<tr>
<td>Schisandrin B (µg·g⁻¹) 815 (3.3%)</td>
<td>802 (3.4%)</td>
<td>810 (3.0%)</td>
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</table>
Comparing the chromatogram of the KUIHUA® Huganpian tablet sample (Fig. 4a) and the spiked one (Fig. 4b) with aspects of retention time, peak shape and peak width, the identification of Schisandrin A or Schisandrin B in Huganpian Tablet sample by UV detection was proven correct. The spiking recoveries of Schisandrin A and Schisandrin B were ca. 98%, with relative standard deviation (R.S.D.) less than 3.5% ($n = 5$).

**Conclusion**

The present HPLC method was developed for separation and quantitation of Schisandrin A and Schisandrin B, and used to determine these 2 compounds in 3 types of Huganpian tablet. With ultra-sonicate extraction and HLB SPE purification, satisfactory results were obtained.

**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

**References**