Micelle-Mediated Extraction of Dibenzocyclooctadiene Lignans from *Schisandra chinensis* with Analysis by High-Performance Liquid Chromatography

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Micelle-mediated extraction offers a convenient alternative to conventional extraction systems. A new method based on micelle-mediated extraction was developed for the separation and determination of dibenzocyclooctadiene lignans from *Schisandra chinensis* by high-performance liquid chromatography with photodiode array detection. Various experimental conditions using the micelle-mediated method were investigated to evaluate the extraction process. Ethylene glycol monoalkyl ether (Genapol X-080), a non-ionic surfactant oligoethylene glycol monoalkyl ether, was chosen as the extract solvent. The chromatographic separation was accomplished on a Shiseido Capcell Pak C18 analytical column (250 × 4.6 mm i.d., 5 μm particle diameter), detected by ultraviolet absorption at 254 nm. The isocratic elution was achieved with a mobile phase composed of water–acetonitrile–formic acid (70:30:0.1) at a flow rate of 0.6 mL/min. The method was optimized and fully validated against dibenzocyclooctadiene lignans (schizandrin, gomisin A and gomisin N). With 15% Genapol X-080, a liquid to solid ratio of 100:1 (mL/g) and ultrasonic-assisted extraction for 60 min, the extraction percentage of total dibenzocyclooctadiene lignans reached the highest value. The non-ionic surfactant Genapol X-080 solution is an effective alternative for the extraction of bioactive lignans from *S. chinensis*.

Introduction

The plant of *Schisandra chinensis* (Schisandraceae), which is widely distributed in the regions of the Russian Far East, Korea, Japan and northeastern China, is an important medicinal plant used for anti-aging, sedative and tonic agents in traditional Chinese medicine (1). The lignans of dibenzocyclooctadiene type are major constituents; a volatile oil with mono- and sesquiterpenes, an oil, organic acids and small amounts of additional compounds are also present. Dibenzocyclooctadiene lignans provide many pharmacological effects such as antitumour (2–4), anti-inflammatory (5), antioxidative (6–8), hepatoprotective (9–10) and neuroprotective activities (11–13). A previous study reported that dibenzocyclooctadiene lignans isolated from *S. chinensis* significantly attenuated the neurotoxicity induced by L-glutamate in primary cultures of rat cortical cells (14). The extraction of active compounds from medicinal plants most prevalently involves organic sources and alcohol–water mixtures. Unfortunately, these methods are usually time consuming and a large amount of hazardous and volatile solvents is required. Recently, alternative solvent systems like surfactant–water solutions and ionic liquids have been suggested, primarily driven by consumer acceptance and environmental aspects (15).

Surfactants form micellar systems in aqueous solutions. Micelle-mediated extraction offers a convenient alternative to the conventional extraction systems (16–17). Normally, the micelle-mediated extraction process can be divided into two parts: the first step is to solubilize the target compounds from the plant into the aqueous surfactant solution; the second step is to preconcentrate target compounds based on phase separation by the cloud-point methodology. This study skipped the second step because cloud-point extraction is not effective to extract dibenzocyclooctadiene lignans (data not shown). The high cloud point can lead to incompatibility with temperature-sensitive target compounds. The extracted solution can be directly analyzed by high-performance liquid chromatography (HPLC) without further cleanup and evaporation steps. This methodology offers the advantages of safety, low cost and low toxicity compared with classical organic solvents (18).

Ethylene glycol monoalkyl ether (Genapol X-080), non-ionic surfactant oligoethylene glycol monoalkyl ether, was chosen as the extract solvent. Several research groups have successfully used Triton X-100 (19) or Genapol X-080 in extraction procedures (20). Because of high ultraviolet (UV) absorbance, Triton X-100 showed broad peaks in the HPLC chromatogram (20). However, Genapol X-080 does not absorb above 210 nm, and thus, will not interfere with the determination of lignans. Therefore, Genapol X-080 was chosen as the surfactant in this experiment. Many studies have used Genapol X-080 as the surfactant for the extraction of natural products (20–26). These publications indicate the potential of Genapol X-080 for the extraction of bioactive constituents from natural products, namely coumarins, polyphenols, isoflavonoids, anthaquinones, diterpenoids and saponins; however, it has not yet been applied to the extraction of lignans.

Ionic liquid-based extractions of dibenzocyclooctadiene lignans from *S. chinensis* were recently reported (27–29). However, surfactant–water mixtures have not yet been applied for the extraction of dibenzocyclooctadiene lignans from *S. chinensis*. In this paper, the application potential of the micelle-mediated method has been evaluated by employing Genapol X-080 for the extraction of three major active lignans, schizandrin, gomisin A and gomisin N, from *S. chinensis*. The extraction using organic solvent, a standard method recommended in the Korean Pharmacopoeia (30) was also compared.
Experimental

Materials

The three compounds (schizandrin, gomisin A and gomisin N) were isolated from S. chinensis and identified by comparison of their spectral data with those reported in the literature (14, 31). The purity of these compounds was determined to be more than 98% by normalization of the peak areas detected by HPLC–UV absorbance detection (UVD) analysis. The sample of S. chinensis fruits was purchased from Kyungdong Traditional Herbal Market (Seoul, Korea). HPLC grade solvents (acetonitrile, water and methanol) and reagents were obtained from BDH Chemicals (Poole, UK). Genapol X-080 and formic acid (analytical grade) were purchased from Sigma (St. Louis, MO). Triple deionized water (Millipore, Milford, MA) was used for all preparations.

Chromatographic conditions

The HPLC system consisted of a chromatographic pump (P680, Dionex, Germany), an injector (7725i, Rheodyne, Bristol, CT) equipped with a photodiode array detector (UVD 340U, Dionex, Germany). The output signal of the detector was recorded by a DionexChromelon Chromatography Data System. Chromatographic separation was achieved on a Shiseido Capcell Pak RP18 MG (5 μm, 4.6 × 250 mm i.d.). The mobile phases were composed of acetonitrile, water and formic acid (70:30:0.1) at a flow rate of 0.6 mL/min, and monitored at 254 nm.

Preparation of standard solution

A stock standard solution of schizandrin, gomisin A and gomisin N was prepared in methanol at a concentration of 0.1 mg/mL. The appropriate amount of every standard solution was mixed and diluted with methanol as indicated.

Validation procedure

For the calibration curves, limit of detection (LOD) and limit of quantization (LOQ), a stock solution, which contained the three analytes, was prepared and diluted to a series of appropriate concentrations for the construction of calibration curves. Five concentrations of the mixed standard solution were injected in triplicate. The calibration curves of the lignans were constructed by plotting peak areas versus the concentration of each analyte. The lowest concentration of the working solution was diluted with methanol to yield a series of appropriate concentrations, and the LOD and LOQ under the chromatographic conditions were separately determined at signal-to-noise ratios (S/Ns) of 3 and 10, respectively. The results are shown in Table I.

The measurement of intra-day and inter-day variability was utilized to determine the precision of this newly developed method. The intra-day variation was determined by an analysis at the three concentrations of the mixed standard solution three times within one day. For the inter-day variability test, the solution was examined in triplicate for three sequential days (1, 3 and 5 days). The relative standard deviation (RSD) was used as a measure of precision. The results are given in Table II.

A recovery test was used to evaluate the accuracy of the method. The diluted sample solution (50.0 mg/mL) spiked with the mixture of standard samples (0.5 mg/mL for each standard) at the ratios of 2:1, 1:1 and 1:2, respectively. The resultant samples were analyzed by using the proposed method. The mean recovery was calculated in three assays for the standard. The results are given in Table II.

Table I

<table>
<thead>
<tr>
<th>Compound</th>
<th>Calibration curve*</th>
<th>( r^2 )</th>
<th>Test range [µg/mL]</th>
<th>LOD (ng/mL)</th>
<th>LOQ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizandrin</td>
<td>( y = 0.520 x + 0.3782 ) 0.9966</td>
<td>2.5–40.0</td>
<td>85.86</td>
<td>260.19</td>
<td></td>
</tr>
<tr>
<td>Gomisin A</td>
<td>( y = 0.453 x + 0.3295 ) 0.9969</td>
<td>2.5–40.0</td>
<td>69.92</td>
<td>211.88</td>
<td></td>
</tr>
<tr>
<td>Gomisin N</td>
<td>( y = 0.458 x + 0.2678 ) 0.9998</td>
<td>2.5–40.0</td>
<td>164.56</td>
<td>496.67</td>
<td></td>
</tr>
</tbody>
</table>

*\( y = \) peak area, \( x = \) amount (µg/mL).

Table II

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inter-day (n = 3)</th>
<th>Intra-day (n = 3)</th>
<th>Sample to standard</th>
<th>Spiked amount (µg/mL)</th>
<th>Accuracy (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount</td>
<td>RSD (%)</td>
<td>Amount</td>
<td>RSD (%)</td>
<td></td>
</tr>
<tr>
<td>Schizandrin</td>
<td>0.102</td>
<td>0.102</td>
<td>0.221</td>
<td>1:2</td>
<td>0.196</td>
</tr>
<tr>
<td></td>
<td>0.050</td>
<td>0.049</td>
<td>0.397</td>
<td>1:1</td>
<td>0.195</td>
</tr>
<tr>
<td></td>
<td>0.022</td>
<td>0.022</td>
<td>1.245</td>
<td>1:1</td>
<td>0.193</td>
</tr>
<tr>
<td>Gomisin A</td>
<td>0.102</td>
<td>0.088</td>
<td>0.673</td>
<td>1:2</td>
<td>0.141</td>
</tr>
<tr>
<td></td>
<td>0.050</td>
<td>0.042</td>
<td>0.805</td>
<td>1:1</td>
<td>0.111</td>
</tr>
<tr>
<td></td>
<td>0.021</td>
<td>0.017</td>
<td>2.395</td>
<td>1:1</td>
<td>0.081</td>
</tr>
<tr>
<td>Gomisin N</td>
<td>0.101</td>
<td>0.106</td>
<td>0.886</td>
<td>1:2</td>
<td>0.151</td>
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<tr>
<td></td>
<td>0.050</td>
<td>0.042</td>
<td>0.173</td>
<td>1:1</td>
<td>0.126</td>
</tr>
<tr>
<td></td>
<td>0.022</td>
<td>0.018</td>
<td>1.125</td>
<td>1:1</td>
<td>0.101</td>
</tr>
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</table>

Results

Analysis of lignans by HPLC–UVD

The chromatographic conditions were optimized to obtain chromatograms that showed good resolution of adjacent peaks.
Reversed-phase columns have been usefully applied to analyze the components of natural resources. The preferred chromatographic conditions were obtained by using a Shiseido Capcell Pak RP18 MG (5 μm, 4.6 x 250 mm i.d.). The isocratic elution system consisted of acetonitrile and 0.1% formic acid and was employed for the simultaneous determination of these three active lignans of *S. chinensis* (Figure 1). Various mixtures of water and acetonitrile in combination with formic acid were tested as a mobile phase. Acid is known to achieve better separation for phenolic compounds by depressing the tailing of the peaks (32–33). Under the current chromatographic conditions, the addition of 0.1% formic acid in water increased the resolution of the peaks. The wavelength for detection was set at 254 nm, at which the three compounds showed the maximum absorption as measured by UVD. The presence of schizandrin, gomisin A and gomisin N in this herb was verified by comparing each retention time and UV spectrum with those of each standard compound and by spiking with authentic standards. As a result, the optimal mobile phase consisting of water–acetonitrile–formic acid (70:30:0.1) was subsequently employed for the analysis of *S. chinensis*, which led to satisfactory resolution and peak shape at 254 nm (Figure 2B).

**Calibration and validation of the HPLC methods**

According to the International Conference on Harmonization (ICH), the linearity of calibration curves should be evaluated by at least five concentrations of each compound. In the present study, the linearity of schizandrin, gomisin A and gomisin N was calculated by five concentrations of each compound; the regression equation was calculated in the form of $y = ax + b$, where $y$ and $x$ are the values of peak area and concentration of each compound, respectively. The $r^2$ values of the regression coefficients ($r^2$) and results of regression analyses are listed in Table I. Calibration curves were linear in a relatively wide range of concentrations (0.0025–0.04 mg/mL) with high correlation coefficient values ($r^2 > 0.9995$) between peak area ($y$) and concentration of each compound ($x; \mu g/mL$).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$R_1$</th>
<th>$R_3$</th>
<th>$R_3$</th>
<th>$R_4$</th>
<th>$R_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizandrin</td>
<td>OH</td>
<td>OCH$_3$</td>
<td>OCH$_3$</td>
<td>OCH$_3$</td>
<td>OCH$_3$</td>
</tr>
<tr>
<td>Gomisin A</td>
<td>OH</td>
<td>–OCH$_2$O–</td>
<td>OCH$_3$</td>
<td>OCH$_3$</td>
<td></td>
</tr>
<tr>
<td>Gomisin N</td>
<td>H</td>
<td>–OCH$_2$O–</td>
<td>OCH$_3$</td>
<td>OCH$_3$</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** Structures of schizandrin, gomisin A and gomisin N.

The LOD and LOQ were measured based on the method recommended by ICH [LOD = $3.3 \delta /S$, LOQ = $10\delta /S$, where $\delta$ is the standard deviation (SD) of the response and $S$ is the slope of the calibration curve]. The LODs of schizandrin, gomisin A and gomisin N were 85.9, 69.9 and 164.6 ng/mL, respectively. The LOQs of schizandrin, gomisin A and gomisin N were 206.19, 211.88 and 498.67 ng/mL, respectively, which showed the high sensitivity under this chromatographic condition (Table I).

A precision test was conducted by studying the intra-day and inter-day variability for schizandrin, gomisin A and gomisin N. The intra-day variability was assayed at three concentrations on the same day and inter-day variability was assayed at three concentrations on three days. As listed in Table II, the RSD of intra-day and inter-day variability was less than 2.9%, which demonstrated the precision of this method.

The accuracy of the method in this study was determined by the method of standard addition. The dilute sample solution was spiked with the mixture of standard samples of schizandrin, gomisin A and gomisin N at the ratios of 2:1, 1:1 and 1:2, respectively. The resultant samples were analyzed by using the proposed method. For comparison, an unspiked sample was concurrently prepared and simultaneously analyzed. As listed in Table II, the mean recovery of each compound was 96.9–103.2% with RSD values less than 1.8%.

**Effect of the surfactant concentration on the extraction efficiency of dibenzocyclooctadiene lignans**

Figure 3A shows that the extraction of dibenzocyclooctadiene lignans from the fruits of *S. chinensis* increased slightly in the surfactant concentration range from 5 to 10%. The extraction efficiency of each lignin was almost similar when the surfactant concentration increased from 10 to 20%. Considering the extraction efficiency, 15% of Genapol X-080 was chosen as the optimum concentration of surfactant for further studies.

**Effect of liquid–solid ratios on the extraction efficiency of dibenzocyclooctadiene lignans**

The liquid–solid ratio is the ratio between the volumes of solvent to amount of crude material. It is one of the factors influencing the extraction efficiency of dibenzocyclooctadiene lignans (Figure 3B). The experimental results show that a liquid–solid ratio of 100:1 (mL/g) was sufficient for each compound to reach the highest efficiency, so this was employed in the following experiments.

**Effect of extraction time on the extraction efficiency of dibenzocyclooctadiene lignans**

The effect of extraction time on the extraction efficiency of dibenzocyclooctadiene lignans was studied by varying the extraction time from 10 to 90 min (Figure 3C). The results indicated that the amounts of dibenzocyclooctadiene lignans were almost similar between 10 and 90 min. The amounts of dibenzocyclooctadiene lignans reached the highest value for each compound when extracted for 60 min. Therefore, in the following experiments, 60 min was selected to extract the dibenzocyclooctadiene lignans.
Comparison of extraction methods

To evaluate the performances of micelle-mediated extraction and organic solvent extraction, a comparison was applied. All experiments were performed in triplicate. The results are shown in Table III. There was little difference in extraction yields obtained by each method. The extraction yield of schizandrin in micelle-mediated extraction was one and a half times more than in organic solvent extraction. Unlike with conventional organic solvent extraction, the green solvent Genapol X-080 was used and only a small amount of organic solvent, methanol (1 mL), was used in the proposed method. Although the extraction time of the proposed method (60 min) was longer than that of organic solvent extraction (40 min), micelle-mediated extraction should be a fairly satisfactory method, considering the expenditures of sample amount, extraction solvent and yield.

Table III
Comparison of Extraction Methods

<table>
<thead>
<tr>
<th></th>
<th>Micelle-mediated extraction</th>
<th>Organic solvent extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction yield of schizandrin (mg/g)</td>
<td>9.80 ± 1.33</td>
<td>6.48 ± 0.05</td>
</tr>
<tr>
<td>Extraction yield of gomisin A (mg/g)</td>
<td>1.20 ± 0.25</td>
<td>1.18 ± 0.01</td>
</tr>
<tr>
<td>Extraction yield of gomisin N (mg/g)</td>
<td>2.62 ± 0.42</td>
<td>2.19 ± 0.02</td>
</tr>
<tr>
<td>Sample amount (mg)</td>
<td>200</td>
<td>500</td>
</tr>
<tr>
<td>Solvent type</td>
<td>Genapol X-080</td>
<td>Methanol</td>
</tr>
<tr>
<td>Volume of used methanol (mL)</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Extraction time (min)</td>
<td>60</td>
<td>40</td>
</tr>
</tbody>
</table>
extraction (37–38). They also consume large amounts of volatile and hazardous organic solvents, and require long extraction times and recovery energy. During the last few years, environmentally friendly techniques become more attractive, following the development of green chemistry (28). Micelle-mediated extraction offers a convenient alternative to conventional extraction systems (16–17). Micellar extraction is used for the extraction of phyto compounds from natural products (20–26). A micellar extraction method was developed for the determination of five anthraquinone derivatives in Chang-Qing tea by using Genapol X-080 (21). Isoflavone genistein from soybeans was micelle-mediated extracted by using Genapol X-080 (22). A micelle-mediated extraction method of tanshinones, the primary abietane-type diterpenes from Salvia miltiorrhiza, has also been optimized (26).

The application potential of the micelle-mediated method has been evaluated by employing Genapol X-080 for the extraction of three major active lignans, schizandrin, gomisin A and gomisin N, from S. chinensis. The major finding of the present study is that the non-ionic surfactant Genapol X-080 is an effective alternative for the extraction of bioactive lignans from S. chinensis. With 15% Genapol X-080, a liquid–solid ratio of 100:1 (mL/g) and ultrasonic-assisted extraction for 60 min, the extraction percentage of total dibenzocyclooctadiene lignans reached the highest value. Instead of conventional organic solvent extraction, the green solvent Genapol X-080 and only a small amount of organic solvent, methanol (1 mL), were used in the proposed method. Extraction yields of all three compounds in micelle-mediated extraction were higher than those in organic solvent extraction while using a small sample amount.

Conclusion

The results indicated that the non-ionic surfactant Genapol X-080 is an effective alternative for the extraction of bioactive lignans from S. chinensis. Genapol X-080 shows higher extraction efficiency than commonly used organic solvents. Micelle-mediated extraction is a powerful method for the extraction of active constituents from herbal medicines; it is also a rapid method, because the extracts can be directly injected into HPLC without removal of the surfactant.

Acknowledgments

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