Rapid Fingerprint Analysis of Ligusticum Chuanxiong by UFLC–DAD

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In the present study, a rapid fingerprint analysis of Ligusticum chuanxiong (L. chuanxiong) was developed based on ultra-fast liquid chromatography coupled with diode array detection (UFLC–DAD). The analysis time was shortened from approximately 75 min on conventional high-performance liquid chromatography (HPLC) to 40 min on UFLC. This method was validated in terms of stability ([<4.40% relative standard deviation (RSD)], precision (<2.82% RSD) and repeatability (<4.26% RSD). Six batches of L. chuanxiong from different sources were analyzed by UFLC–DAD, and the results were systematically processed using professional analytical software that was recommended by the State Food and Drug Administration of China. The similarities of these six batches of samples were evaluated. Compared to conventional HPLC, the UFLC–DAD method was fast and sensitive and consumed less solvent, and is widely applicable for quality monitoring of traditional Chinese medicine.

Introduction

Traditional Chinese medicine (TCM) is well known for its complex components. It contains hundreds and thousands of substances. The components can vary, depending on growing soil, climate, harvest conditions and storage time (1). Moreover, different percentages of individual components or different components can be found between the same TCM species. The therapeutic effects of TCM are complicated and may be based on the synergic effects of multiple compounds (2, 3). Therefore, it is crucial to develop an efficient method to assess fingerprint, a useful identification method for the comprehensive control of the quality of TCMs, which has been accepted by many countries and organizations (4–6).

Chromatographic technologies, such as thin-layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography (HPLC), have been used to fingerprint TCMs. Among them, TLC is a traditional method, fast and easy to operate, but with poor resolution. GC is suitable to volatile compounds. HPLC is the most popular fingerprint method with high precision, sensitivity and reproducibility. However, conventional HPLC suffers from the shortcomings of long analysis time, low or medium resolution and large solvent consumption. Hence, fast separation with high resolution was desired in the quality control of TCM, especially when dealing with large amounts of samples. Recently, ultra-performance liquid chromatography (UPLC) or ultra-fast liquid chromatography (UFLC) have been gaining popularity in the rapid profiling of TCMs due to their faster analysis and superior separation performance (7, 8) compared to conventional HPLC.

Ligusticum chuanxiong (L. chuanxiong) is the dried rhizome of L. chuanxiong herb (Umbelliferae), which has widely been used in TCM for more than two thousand years for the treatment of angina pectoris, cardiac arrhythmias, hypertension and stroke (9). The primary components in L. chuanxiong include essential oils (e.g., ligustilide), phenolic acids (e.g., ferulic acid) and alkaloids (e.g., ligustrazine) (9, 10). The fingerprint of L. chuanxiong has been widely studied with chromatographic methods, most of which are based on HPLC (11–13). However, these methods were very time-consuming. In this study, UFLC coupled with diode array detection (DAD) was developed for the rapid identification and quality evaluation of L. chuanxiong.

Experimental

Reagents and chemicals

Ferulic acid and ligustrazine were purchased from Aladdin (Shanghai, China). Six batches of L. chuanxiong samples were purchased from local drugstores. HPLC-grade methanol was obtained from Fisher Scientific (Fair Lawn, NJ). All other chemicals were of analytical grade.

Instrument and analytical conditions

All assays were performed on a Dionex (Sunnyvale, CA) Ultimate 3000 UHPLC+ system equipped with two Ultimate 3000 RS pumps, an Ultimate 3000 RS autosampler, an Ultimate 3000 RS column compartment and an Ultimate 3000 DAD. Chameleon software was used for data collection and handling. A VP-ODS (+ 0.6 × 150 mm, 5 μm, Shimadzu) column was used for conventional HPLC analysis. A Zorbax RRHD Eclipse XDB-C18 column (2.1 × 100 mm, 1.8 μm, Agilent) was used for UFLC analysis. The mobile phase consisted of methanol (A) and 1% acetic acid (B). The gradient conditions for the conventional HPLC were: 0–15 min 23% A, 25 min 30% A, 40 min 35% A, 60 min 40% A, 75 min 80% A and 75.1–85 min 90% A. The flow rate was 1 mL/min and the injection volume was 10 μL. The UFLC conditions were: 0–2 min 20% A, 10 min 30% A, 30 min 50% A and 40 min 70% A. The flow rate was 0.4 mL/min and the injection volume was 3.0 μL. The column temperature was 35°C.
Preparation of standard sample solution and extraction of L. chuanxiong
The stock solutions of ferulic acid and ligustrazine were prepared separately in methanol at concentrations of 1 mg/mL. They were stored at 4°C before use. The dried L. chuanxiong samples were crushed with a grinder. One gram of powder was immersed in 15 mL methanol and ultrasonically extracted for 4 h, and then kept statically for 1 h. The mixture was centrifuged at 10,000 rpm and the supernatant solution was filtered through a membrane with a pore size of 0.45 μm. The filtrate was collected for HPLC analysis.

UFLC method validation
The analytical precision was determined by injecting the extractant of the same L. chuanxiong for five times in one day. The repeatability was determined by analyzing five independently extracted L. chuanxiong samples from the same batch. The sample stability test was evaluated with the same sample extract for 0, 2, 4, 8, 16 and 24 h. The similarity evaluation of L. chuanxiong was performed on six batches of samples from different regions.

Data analysis
The data analysis was handled by the professional software Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A), which was recommended by the State Food and Drug Administration (SFDA) of China. This software was used to calculate the correlation coefficients of the chromatographic profiles of six batches of L. chuanxiong samples, and to generate the simulative mean chromatogram (SMC). The similarities of different chromatographic fingerprints were compared with the SMC.

Results and Discussion
Comparison of conventional HPLC and UFLC fingerprints
The chromatograms of the conventional HPLC and UFLC are compared in Figure 1. For conventional HPLC, a complete

![Figure 1. Chromatograms of L.chuanxiong (Sample 1) on conventional HPLC and UFLC at 280 nm: HPLC (A); UFLC (B). Standard compounds: 1, ligustrazine; 2, ferulic acid.](image)

Table I
Analytical Results of Stability, Precision and Repeatability of Common Peaks in L. chuanxiong

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Stability</th>
<th>Precision</th>
<th>Reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSD of RRT (%)</td>
<td>RSD of RPA (%)</td>
<td>RSD of RRT (%)</td>
</tr>
<tr>
<td>1</td>
<td>0.88</td>
<td>4.40</td>
<td>0.34</td>
</tr>
<tr>
<td>2</td>
<td>0.87</td>
<td>4.11</td>
<td>0.85</td>
</tr>
<tr>
<td>3</td>
<td>0.74</td>
<td>2.49</td>
<td>0.22</td>
</tr>
<tr>
<td>4</td>
<td>0.20</td>
<td>0.32</td>
<td>0.18</td>
</tr>
<tr>
<td>5</td>
<td>0.09</td>
<td>0.63</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>0.26</td>
<td>4.36</td>
<td>0.06</td>
</tr>
<tr>
<td>7 (S)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>0.49</td>
<td>2.66</td>
<td>0.45</td>
</tr>
<tr>
<td>9</td>
<td>0.49</td>
<td>2.56</td>
<td>0.47</td>
</tr>
<tr>
<td>10</td>
<td>0.49</td>
<td>3.88</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Table II
RRT of Common Peaks in Six Batches of L. Chuanxiong Samples

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>RRT</th>
<th>Average</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>2</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>3</td>
<td>0.46</td>
<td>0.46</td>
<td>0.46</td>
</tr>
<tr>
<td>4</td>
<td>0.52</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>5</td>
<td>0.72</td>
<td>0.72</td>
<td>0.72</td>
</tr>
<tr>
<td>6</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>7 (S)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>1.04</td>
<td>1.04</td>
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<tr>
<td>9</td>
<td>1.25</td>
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</tr>
<tr>
<td>10</td>
<td>1.25</td>
<td>1.25</td>
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</table>

Table III
RPA of Common Peaks in Six Batches of L. Chuanxiong Samples

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>RPA</th>
<th>Average</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>2</td>
<td>0.12</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>3</td>
<td>0.08</td>
<td>0.13</td>
<td>0.32</td>
</tr>
<tr>
<td>4</td>
<td>0.01</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>5</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>6</td>
<td>0.30</td>
<td>0.33</td>
<td>0.32</td>
</tr>
<tr>
<td>7 (S)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>0.14</td>
<td>0.22</td>
<td>0.18</td>
</tr>
<tr>
<td>9</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>10</td>
<td>0.05</td>
<td>0.05</td>
<td>0.12</td>
</tr>
</tbody>
</table>
fingerprint of *L. chuanxiong* was obtained in 75 min at a flow rate of 1.0 mL/min; with UFLC, the analysis time was shortened to 40 min at a flow rate of 0.4 mL/min. Obviously, UFLC provided fast separation even at a low flow rate, indicating low cost. In addition, although less sample was used for UFLC analysis (3.0 μL for UFLC versus 10 μL for HPLC), stronger and more analytical signals were obtained. These results indicate that UFLC had superior sensitivity and resolution to the conventional HPLC.

**Validation of UFLC method**

The UFLC method was validated in terms of stability, precision and repeatability. The results are shown in Table I. During the 24 h stability test, the relative standard deviation (RSD) values of relative retention time (RRT), which was the ratio of retention time of the individual peak to that of the reference peak, and relative peak area (RPA), which was the ratio of the peak area of the individual peak to that of the reference peak, were less than 0.88 and 4.40%, respectively. The precision was less than 0.85% for RRT and 4.26% for RPA. Five independently extracted *L. chuanxiong* samples were analyzed, and the RSDs of RRT and RPA were less than 0.26 and 2.82%, respectively. These results indicate that the UFLC–DAD method was stable and precise.

**UFLC fingerprint analysis of *L. chuanxiong* from different regions**

The UFLC fingerprint chromatograms of *L. chuanxiong* from six different batches were compared and 10 common fingerprint peaks were found. Peak 7 was assigned as the reference peak because it was the strongest and had a moderate retention time. Peak 1 and 2 were determined to be ligustrazine and ferulic acid, respectively. Using the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A), the RRT and RPA of 10 common fingerprint peaks were calculated, and the results are shown in Tables II and III with their average and RSD values, respectively. The RSD values of RRT were less than 2.9%, and the RSD values of RPA were relatively larger. These data indicated that the common compounds were almost the same, but the contents varied in *L. chuanxiong* from different regions.

The SMC and the six chromatographic fingerprints from different batches are shown in Figure 2. The results of the similarity analysis are listed in Table IV. The similarity results of the samples are more than 0.97, which indicates that *L. chuanxiong* from different regions were acceptably similar.

**Conclusion**

A UFLC–DAD method for the profiling of *L. chuanxiong* has been established and validated. Compared with conventional HPLC, UFLC provides a shorter analysis time, higher resolution and better separation performance. As a feasible and reliable method, UFLC–DAD showed good stability, precision and reproducibility. Six batches of *L. chuanxiong* samples from different cultivating regions were evaluated; this was helpful to improve the quality control of *L. chuanxiong*. Furthermore, the UFLC method developed in this study provided an important reference to establish a fast quality control method for other related TCM preparations.
Acknowledgments
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