Rapid Fingerprint Analysis of *Radix Scutellariae* by UFLC–DAD

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To better control the quality of *Radix Scutellariae* (RS), a fast fingerprint method based on ultra-fast liquid chromatography coupled with diode array detection (UFLC–DAD) was developed. In this study, the analysis time of ~85 min when using conventional high-performance liquid chromatography was shortened to ~40 min on UFLC. The UFLC method was validated in terms of stability (<5.80% relative standard deviation (RSD)), precision (<3.48% RSD) and reproducibility (<3.56% RSD). Finally, eight batches of RS were analyzed by UFLC–DAD, and their similarities were systematically processed with professional analytical software, Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A), which was recommended by State Food and Drug Administration of China. The developed UFLC method is efficient, reproducible, stable, precise, and can be used as a more efficient approach for the quality monitoring and assessment of RS.

Introduction

Traditional Chinese medicine (TCM), which has had 5,000 years of application, is widely used in Asian and African countries (1–3). Because of its low toxicity and good therapeutic performance, herbal medicine has attracted considerable attention throughout the world in recent years. The curative effects of TCM are principally based on the synergic effects of multiple ingredients, and the quality and authenticity of TCM cannot be evaluated by only one or two active pharmacological compounds in herbal medicines (4, 5). Therefore, establishing an efficient method to identify the species and control the quality of TCM faces many challenges. With the development of analytical techniques, chromatographic fingerprinting, which can provide systemic characterization of herbal products, has been accepted by many countries and organizations (1, 6–8). Several chromatographic methods have been widely used for fingerprinting, such as thin-layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) (2, 9, 10). However, these methods usually suffer from lengthy analysis times, requiring approximately one or more hours for a single run. In recent years, ultra-fast liquid chromatography (UFLC), which employs submicrometer porous particles as the stationary phase, has been successfully applied in the rapid profiling of TCMs (11, 12). UFLC has been demonstrated to be a powerful technique in chromatographic fingerprinting, with the advantages of short analysis time, high resolution and good separation performance (13).

Experimental

Reagents and chemicals

Baicalein and baicalin were purchased from Aladdin (Shanghai, China). RS samples from different regions in China were purchased from local drug stores and labeled Sample 1–8. HPLC-grade methanol was obtained from Fisher Scientific (Piscataway, 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, an Ultimate 3000 diode array detector and Chromeleon software. The chromatographic conditions of conventional HPLC and UFLC are shown in Table I.

Preparation of standard and sample solution

The stock solutions of baicalein and baicalin were separately prepared in methanol at a concentration of 1 mg/mL. They were stored at 4 °C before use.

All dried RS samples were crushed with a grinder. One gram of powder was immersed in 15 mL methanol and ultrasonically extracted for 4 h, then kept statically for 1 h. The mixture was centrifuged at 10,000 rpm and the upper solution was filtered through a membrane with pore size of 0.45 μm. The filtrate was collected for the subsequent chromatographic analysis.

Method validation of UFLC

Sample 2 was used in the method validation of UFLC. The precision of the method was determined by replicate injections of...
the same extractant of Sample 2 for five times in one day. The repeatability was determined by analyzing five independently extracted RS samples. The stability of the sample was determined with the same Sample 2 extractant for 0, 2, 4, 8, 16, 24 and 48 h.

Data analysis
The data analysis was performed with 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 system was used to calculate the correlation coefficients of the chromatographic profiles of eight batches of RS samples and to generate the simulative mean chromatogram (SMC). The similarities of different chromatographic fingerprints were compared with the SMC.

Results and Discussions
Comparison of conventional HPLC and UFLC for fingerprinting RS
The chromatograms of conventional HPLC and UFLC were compared and shown in Figure 1. A complete fingerprint of RS was obtained in \(\approx 85\) min using conventional HPLC, with baicalein eluting at 68 min and baicalin eluting at 55 min. When UFLC was used, the analysis time was shortened to \(\approx 40\) min, with baicalein eluting at 23 min and baicalin eluting at 10 min. Additionally, more peaks were found when using UFLC, indicating that this method provides better separation efficiency.

In addition, although a sample of only 3 \(\mu\)L was injected for UFLC analysis and a sample of 10 \(\mu\)L was used for HPLC, stronger signals were obtained when using UFLC, demonstrating that UPLC could afford better sensitivity.

Validation of UFLC method
The UFLC method was evaluated in terms of stability, precision and reproducibility. The results are shown in Table II. The relative standard deviation (RSD) values of relative retention time (RRT), which was the ratio of the retention time of an individual peak to that of the reference peak; and relative peak area (RPA), which was the ratio of the peak area of an individual peak to that of the reference peak, were used to evaluate the method. Peak 4 was assigned as the reference peak because it was a stronger peak and had a moderate retention time in the RS chromatograms. During the 48-h stability test, the RSDs of RRT and RPA were less than 4.60 and 5.80%, respectively. The precision was less than 0.30% for RRT and 3.48% for RPA. Five

![Figure 1. Chromatograms of the sample separated on conventional HPLC and UFLC (detection wavelength: 280 nm): conventional HPLC (peaks: 1, baicalin; 2, baicalein) (A); UFLC (peaks: 4, baicalin; 6, baicalein) (B).](https://academic.oup.com/chromsci/article-abstract/51/10/939/343753)
independently extracted RS samples were analyzed, and the RSDs of RRT and RPA were less than 0.82 and 3.56%, respectively. The UFLC–diode array detection (DAD) method was stable, precise and reproducible in conducting an RS chromatographic fingerprint.

**UFLC fingerprint analysis of RS from different regions**

Eight different production batches were tested and eight common fingerprint peaks were found. Using the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A), the RRT and RPA of eight common fingerprint peaks were calculated and shown in Tables III and IV with their average and RSD values. The RSD values of RRT were less than 2.5%, whereas the RSD values of RPA were much larger. This phenomenon indicated that the contents of the common compounds varied in RS from different regions.

The SMC and the eight chromatographic fingerprints from different batches are compared in Figure 2. The result of similarity analysis is listed in Table V. The results indicated that,

**Table III**

<table>
<thead>
<tr>
<th>Peak number</th>
<th>RRT</th>
<th>Average</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RRT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>0.135</td>
<td>0.135</td>
<td>0.134</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.432</td>
<td>0.434</td>
<td>0.431</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.615</td>
<td>0.615</td>
<td>0.612</td>
</tr>
<tr>
<td>Sample 4</td>
<td>0.432</td>
<td>0.426</td>
<td>0.423</td>
</tr>
<tr>
<td>Sample 5</td>
<td>0.615</td>
<td>0.614</td>
<td>0.612</td>
</tr>
<tr>
<td>Sample 6</td>
<td>0.432</td>
<td>0.423</td>
<td>0.422</td>
</tr>
<tr>
<td>Sample 7</td>
<td>0.615</td>
<td>0.611</td>
<td>0.610</td>
</tr>
<tr>
<td>Sample 8</td>
<td>0.432</td>
<td>0.421</td>
<td>0.422</td>
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</table>

**Table IV**

<table>
<thead>
<tr>
<th>Peak number</th>
<th>RPA</th>
<th>Average</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>0.117</td>
<td>0.127</td>
<td>0.187</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.135</td>
<td>0.127</td>
<td>0.189</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.159</td>
<td>0.238</td>
<td>0.284</td>
</tr>
<tr>
<td>Sample 4</td>
<td>0.117</td>
<td>0.127</td>
<td>0.187</td>
</tr>
<tr>
<td>Sample 5</td>
<td>0.135</td>
<td>0.127</td>
<td>0.189</td>
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<td>0.127</td>
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</tr>
<tr>
<td>Sample 8</td>
<td>0.135</td>
<td>0.127</td>
<td>0.189</td>
</tr>
</tbody>
</table>

Figure 2. UFLC–DAD fingerprints of eight batches of RS at 280 nm. S1–S8 represent eight batches of RS samples labeled Samples 1 to 8; R: the reference chromatogram of RS generated from Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A).
except for Sample 4, the similarities for the other samples are higher than 0.94. Because Samples 2, 3 and 4 were all grown in Hebei, this indicates that RS samples from the same producing area may be quite different in similarity.

Conclusions
A UFLC–DAD chromatographic method was successfully established for the quality control of RS. Compared with conventional HPLC, UFLC was prominent with a shorter analysis time, higher resolution and better separation performance. The method was well validated, and satisfactory stability, precision and reproducibility were obtained. Eight batches of RS samples from different cultivating regions were evaluated. Furthermore, the UFLC method developed in this study will provide an important reference to establish a fast quality control method for related TCM preparations.

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References