Fingerprint Analysis and Multi-ingredient Determination Using a Single Reference Standard for Saposhnikoviae Radix

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A method employing high performance liquid chromatography-diode array detection (HPLC-DAD) has been developed to evaluate the quality of Saposhnikoviae Radix (SR) comprehensively through fingerprint analysis and multi-ingredient determination. In the fingerprint analysis, 12 out of 15 common peaks were identified with high performance liquid chromatography-tandem mass spectrometric (HPLC-MS/MS) and related references. To discriminate the 15 batches of samples collected from different origins, similarity analysis and principal component analysis (PCA) were performed. Meanwhile, the simultaneous quantification of four chromones was achieved by firstly applying a method of using a single standard to determine multiple components (SSDMC) with conversion factor. The method has the advantage of feasibility, economy and simplicity compared with traditional external standard method (ESM). The method possessed desirable linearity ($R^2 \geq 0.9997$), precision (RSD <4.0%), accuracy (97.4 – 106.6%) and robustness. This study indicated that the method of fingerprint analysis integrated with multi-ingredient determination using the HPLC technique was reliable for the overall quality evaluation of SR.

Keywords Saposhnikoviae Radix, conversion factor, fingerprint, multi-ingredient determination

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Introduction

Saposhnikoviae Radix (SR), the dried root of Saposhnikovia divaricata (Turcz.) Schischk., is a popular traditional herbal medicine that has been used for thousands of years in China, Japan, and Korea.1–3 As an important member of traditional Chinese medicine (TCM), it is widely applied for treating headaches, febrility, vertigo and arthralgia. It is also the major component of a number of traditional Chinese prescriptions, such as Yu-Ping-Feng-San and Tong-Xie-Yao-Fang.4,5 Phytochemical studies have revealed that SR contains various bioactive ingredients, such as chromones, coumarins, and polyacetylenes,6–10 of which chromones are most relevant to pharmacological efficacy11,12 as they have exhibited effectiveness as analgesic,2 anti-inflammatory,13 anti-proliferative and antioxidant compounds.14

Although so many beneficial effects have been shown, there are few published reports about the comprehensive quality evaluation of SR. Therefore, there is an urgent need to develop a reliable and simple method for qualitative and quantitative analysis of the components in SR, which is helpful for controlling the quality of this herbal medicine comprehensively. Chromatographic fingerprinting, as a comprehensive quantifiable identification method, has been internationally accepted as a feasible means for species authentication and quality evaluation of TCM.15–18 The identity, consistency and authenticity of samples can be determined by comparison of their chromatographic fingerprints using stoichiometry, such as similarity analysis, principal component analysis (PCA) and cluster analysis.19,20 For more comprehensive quality control of TCM, simultaneous quantification of multiple ingredients also is essential.16,17,21 The content of a few marker compounds might relatively accurately reflect the quality of the herbal medicine. However, insufficient chemical reference standards often makes the multi-ingredient determination by traditional external standard method (ESM) impossible. As an alternative method, single standard to determine multiple components (SSDMC), which only needs a single reference standard for multi-component determination, has the advantage of feasibility, economy and simplicity compared with ESM, and has been included in pharmacopeias.22–24 It can reduce the cost and difficulty of simultaneous determination of multiple components, especially when the component is of low content level or unstable and hard to be purified from the plant.25,26

In this study, high performance liquid chromatography-diode array detection (HPLC-DAD) fingerprint of SR was constructed based on the chromatogram of 15 batches of samples. Twelve components were identified by high performance liquid chromatography-tandem mass spectrometric (HPLC-MS/MS) and related references. A similarity analysis and PCA were performed with the aim of determining differentiation of origin and identification of authenticity according to the characteristic of common peaks in fingerprint chromatograms. For further assessment of SR, the same chromatographic condition was applied to quantitative analysis. The four chromones prim-O-glucosylcimifugin (GC), cimifugin (C), 4’-O-β-glucosyl-5-O-
methylvisamminol (GV) and sec-O-glucosylhamaudol (GH), which have definite pharmacological activity and are abundant in SR, were simultaneously determined using the SSDMC method with GV selected as the internal standard. The method was validated and employed for quantitative analysis of SR from various regions. Compared with the reported literature, the method established in this work was more simple, more economical, and can be more widely used. The aim of the present study was to offer a reliable and simple HPLC-DAD method both for fingerprint analysis to distinguish origins and to determine authenticity and quantification of active multiple ingredients in SR. The combination of qualification and quantification would offer a more comprehensive method for quality evaluation of SR.

Experimental

Chemicals, reagents and materials
GC, C, GV and GH were obtained from the National Institute for Control of Biological and Pharmaceutical Products (Shenyang, China). Psoralen, xanthotoxin and bergapten were provided by Chengdu MUST Bio-technology Co., Ltd. (Chengdu, China). The purity (≥98%) of these reference standards was assumed as provided by the suppliers.

Methanol and acetonitrile of chromatographic grade was obtained from Fisher Scientific (99.9%, USA). High purity water was obtained from Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China).

The samples of SR were collected from Inner Mongolia (SR 1 – 3), Heilongjiang (SR 4 – 6), Liaoning (SR 7, 8), Jilin (SR 9 – 11), Hebei (SR 12, 13), Anhui (SR 14) and Gansu (SR 15). All samples were identified by Professor Jiaying (School of Traditional Chinese Medicine, Shenyang Pharmaceutical University, Shenyang, China). Voucher specimens were deposited with Shenyang Pharmaceutical University, Shenyang, China. The samples were dried, pulverized and sieved through an 80-mesh (0.180-mm) stainless-steel sieve before extraction. SR-13 was used for method development studies.

Preparation of sample solutions
An amount of 0.5 g of the powdered drug was placed in a 50 mL glass conical flask, 15.0 mL of 85% methanol solution was added, then the solution was treated in an ultrasonic bath for 45 min. The solution was passed through a filter (0.22 μm) and the successive filtrate was used as the test solution.

Preparation of standard solutions
Stock solutions of GC, C, GV and GH were prepared by dissolving accurately weighed standards with methanol to yield the concentrations of 257.4, 55.83, 249.4 and 40.60 μg mL⁻¹, respectively, and stored in a 25-mL brown volumetric flask at 4°C. The stock solutions were diluted to the desired concentration with methanol prior to use.

Instruments
The fingerprint analyses were performed by an Agilent 1260 HPLC System, comprised of a quaternary solvent delivery system, an on-line degasser, an autosampler, a column temperature controller and a diode-array detector (DAD) coupled with a chemstation (LC 3D Systems Rev. B, 04, 03 [32]) (Agilent Technologies, Palo Alto, CA). Samples were primarily separated on a ZORBAX SB-C18 column (4.6 × 150 mm, 3.5 μm, Agilent), with a guard column (4.6 × 10 mm, 5 μm, Agilent) at a temperature of 25°C, injection volume of 10 μL, and using water (solvent A) and acetonitrile (solvent B) as mobile phases at a flow rate of 0.8 mL min⁻¹. The eluted gradient was as follows: 13 – 15% solvent B at 0 – 6 min; 15% solvent B at 6 – 9 min; 15 – 18% solvent B at 9 – 11 min; 18 – 21% solvent B at 11 – 17 min; 21 – 25% solvent B at 17 – 18 min; 25 – 27% solvent B at 18 – 25 min; 27 – 35% solvent B at 25 – 27 min; 35 – 42% solvent B at 27 – 37 min; 42 – 50% solvent B at 37 – 38 min; 50 – 74% solvent B at 38 – 60 min. The wavelength of the DAD detector was set at 254 nm and the on-line UV spectra were acquired in the range of 200 – 400 nm.

The above HPLC system with elution program to 32 min was used for the determination of four chromones.

The chromatographic procedure detailed above for fingerprint analysis was applied to HPLC-MS/MS analysis. It was performed on a Shimadzu (Shimadzu 20A, Japan) high performance liquid chromatography system, consisting of a vacuum degasser, a binary pump, an auto sampler, and a diode array detector system, and connected to an API 4000 Q-trap mass spectrometer (AB Sciex, Foster City, CA), which was equipped with an electrospary ionization (ESI) source. Analyst 1.6 software was used for data acquisition and processing. Nitrogen was used as the nebulizer. Source parameters were set as follows: curtain gas: 30 psi; ion source: 5500 V; temperature: 500°C; nebulizer gas (Gas 1): 50 psi; auxiliary gas (Gas 2): 50 psi.

Enhanced mass spectrum (EMS) scan was set with a declustering potential (DP) of 40 V and an entrance potential (EP) of 10 V. All MS1 spectra were collected by enhanced product ions (EPI) mode, which was triggered by EMS according to the information depend acquisition (IDA) mode with threshold set at 80000 counts per second (cps). The EPI scan rate was 4000 Da/s and the scan range was 50 – 600 Da. Collision energy (CE) was set at 30 eV, while collision energy spread (CES) was fixed at 15 eV under positive mode. Dynamic exclusion was enabled.

Identification of chemical compounds
The chromatographic peaks were identified with the chemical reference standards or tentatively identified by comparison precursor ion (m/z) values, product ion (m/z) values and UV spectra with that in the literature.

Data analysis of fingerprint
The professional software “Similarity Evaluation System for Chromatographic Fingerprint of TCM (Ver. 2009A, Beijing, China)” was used to analyze the similarity of the entire chromatographic profiles among tested samples. PCA was carried out based on the relative peak area of 15 characteristic peaks of all the 15 batches of samples, using SIMCA-P 13.0 Software.

Quantification with SSDMC
Calculation of conversion factors and relative retention time. As reported, the conversion factor of the reference standard X (Fx) was the ratio of responses in a unit concentration between GV (AX/CX) and analyte (AX/CX). Here A represents the response value and C represents the concentration value of a linear point. The relative retention time of the analyte (RRT,:) was calculated as the ratio of retention time of the analyte (tR) and GV (tGV).

The Fx and RRT: of each analyte was obtained as the mean values calculated from the triplet of seven gradient concentrations, and the three independent calibration standard solutions were prepared as indicated in Preparation of standard
Validation of analytical method

The developed SSDMC quantitative method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), precision (repeatability, different days, different operators, different instruments and columns), accuracy, ruggedness and robustness as guided in the United States Pharmacopeia (USP).

The results obtained by the SSDMC method were compared with those obtained by traditional ESM using paired t-test by SPSS.

Results and Discussion

Optimization of extraction

Considering the coextraction of various compounds with different polarity, methanol and ethanol were tested as the extraction solvents; the latter formed a leading peak, so methanol was chosen as the preliminary extraction solvent. Compared with soaking and reflux, ultrasound at room temperature was selected as the extraction method for the content of four markers and the convenience and celerity of the method. Then the proportion of methanol (100, 85, 70, 55, 40, and 25%, v/v), ratio of solid to liquid (1:20, 1:30, 1:40), extraction time (30, 45, and 60 min), extraction cycle (1 cycle, 2 cycles, 3 cycles) were examined by comparison of the extraction yield of the four markers in SR by the one-variable-at-a-time procedure. The results showed that the most suitable extraction method was ultrasound at room temperature with 30 fold of 85% methanol for 45 min.

Optimization of chromatographic conditions

Optimization of parameters in HPLC was achieved by investigating the influence of the chromatographic column, mobile phase, and detection wavelength.

Considering the polarity of the glycoside compounds and the applicable conditions of columns, three chromatographic columns covering different column length, manufacturers, packings and particle sizes were compared: Agilent ZORBAX SB-C18 (4.6 × 150 mm, 3.5 μm), Agilent Eclipse XDB-C18 (4.6 × 250 mm, 5 μm) and GL Inertsil ODS-EP (4.6 × 150 mm, 5 μm). Of all the chromatographic columns, Agilent ZORBAX SB-C18 was found to produce better separation considering the retention time and peak tailing factors of the four markers (Fig. 1).

Various compositions of mobile phase were investigated with the sample solution. Water–acetonitrile gradient elution provided good resolution and sharp peaks. Mobile phase modifiers, such as acetic acid and phosphoric acid, were added and it was found that the advent of newly emerged peaks would influence the quantitative nature of chromones but could not remarkably enrich the fingerprint information. Consequently, acetonitrile and water with no acid modifier were chosen to produce the desired separation.

A wavelength of 254 nm was selected as the detection wavelength for multi-ingredient determination and fingerprint analysis for stronger absorption.

Identification of chemical compounds in fingerprint chromatograms

The HPLC fingerprint of SR was obtained according to the method developed above (Fig. 2). Of the total 15 common peaks, 12 were identified by the hyphenated chromatographic techniques HPLC-MS/MS. The components are: GC (1), C (2), GV (3), 5-O-methylvisamminol (6), GH (7), psoralen (8), xanthotoxin (9), hamaudol (10), divaricatol (11), bergapten (12), 3′-O-acetylhamaudol (13), and 3′-O-angeloyhamaudol (15). Among them, compounds 1, 3, 7, 8, 9 and 12 were unambiguously verified with the chemical reference standards. The other five were tentatively identified by comparing precursor ion (m/z) values, product ion (m/z) values and UV spectra with related research due to unavailability of chemical reference standards. Information for the 12 components defined are displayed in Table 1.

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Similarity analysis

Chromatograms of the 15 SR samples collected from different regions were analyzed to generate a reference chromatogram by...
using the mean chromatogram as common mode (Fig. 2). Fifteen peaks were assigned as characteristic peaks for taking up about 97.7% of the total peak areas. The values of similarity between each chromatogram of these 15 samples and the simulated mean chromatogram were in the range of 0.858 – 0.991. The similarities of samples from northeastern and northern China (SR 1 – 11, 13) as well as Gansu (SR 15) were higher than 0.92, which suggested good consistency and stability of quality. The similarities of SR 12 from Anhui were 0.858 and values for SR 14 from Hebei which was purchased from Yunnan, were 0.886. The results were different compared with northeast and north area samples. Variation of the similarity might be due to a number of factors, such as different origins, production processes and storage conditions. As we know, Yunfangfeng (a type of SR root) dominates the SR market in Yunnan, so this situation could lead to relatively hoarding of SR. It is possible that due to prolonged storage time some ingredients had changed, thus, sample 12 manufactured in Hebei was found to be separated.

![Figure 2](image)

**Figure 2**  Liquid chromatographic fingerprints of 15 batches of SR and simulative mean chromatogram of SR. Twelve components identified: 1. prim-O-glucosylcimifugin, 2. cimifugin, 3. 4′-O-β-glucosyl-5-O-methylvisamminol, 6. 5-O-methylvisamminol, 7. sec-O-glucosylhamaudol, 8. psoralen, 9. xanthotoxin, 10. hamaudol, 11. divaricatol, 12. bergapten, 13. 3′-O-acetyhamaudol, 15. 3′-O-angeloyhamaudol.

**Table 1**  Retention time, MS/MS fragment ions, maximum absorption wavelength and identification of peaks in fingerprint chromatograms

<table>
<thead>
<tr>
<th>Peak</th>
<th>$t_R$/min</th>
<th>Precursor ion [M+H]$^+$ m/z</th>
<th>Product ion m/z</th>
<th>$\lambda_{max}$/nm</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.05</td>
<td>469.1</td>
<td>307.1, 289.0, 259.1</td>
<td>214, 300</td>
<td>prim-O-glucosylcimifugin</td>
</tr>
<tr>
<td>2</td>
<td>16.37</td>
<td>307.0</td>
<td>289.1, 259.2</td>
<td>214, 298</td>
<td>cimifugin</td>
</tr>
<tr>
<td>3</td>
<td>18.19</td>
<td>452.7</td>
<td>291.0, 273.1, 243.2</td>
<td>214, 232, 294</td>
<td>4′-O-β-glucosyl-5-O-methylvisamminol</td>
</tr>
<tr>
<td>4</td>
<td>21.47</td>
<td>323.1</td>
<td>272.9, 243.1</td>
<td>252, 294</td>
<td>5-O-methylvisamminol</td>
</tr>
<tr>
<td>5</td>
<td>26.33</td>
<td>439.1</td>
<td>277.0, 259.1, 205.2</td>
<td>252, 298</td>
<td>sec-O-glucosylhamaudol</td>
</tr>
<tr>
<td>6</td>
<td>31.89</td>
<td>291.1</td>
<td>272.9, 243.1</td>
<td>252, 294</td>
<td>psoralen</td>
</tr>
<tr>
<td>7</td>
<td>33.17</td>
<td>217.1</td>
<td>202.1, 185.0, 174.1</td>
<td>214, 248, 302</td>
<td>xanthotoxin</td>
</tr>
<tr>
<td>8</td>
<td>35.37</td>
<td>277.1</td>
<td>259.2, 205.2</td>
<td>258, 302</td>
<td>hamaudol</td>
</tr>
<tr>
<td>9</td>
<td>36.18</td>
<td>335.0</td>
<td>275.0, 233.0</td>
<td>258, 300</td>
<td>divaricatol</td>
</tr>
<tr>
<td>10</td>
<td>36.95</td>
<td>217.1</td>
<td>202.1, 174.1</td>
<td>222, 266, 312</td>
<td>bergapten</td>
</tr>
<tr>
<td>11</td>
<td>45.91</td>
<td>319.0</td>
<td>259.1, 231.1, 205.2</td>
<td>258, 300</td>
<td>3′-O-acetyhamaudol</td>
</tr>
<tr>
<td>12</td>
<td>57.66</td>
<td>359.0</td>
<td>259.1, 241.1, 205.2</td>
<td>250, 300</td>
<td>3′-O-angeloyhamaudol</td>
</tr>
</tbody>
</table>
PCA

To provide a more visual comparison of the chromatograms of samples from different origins, PCA was introduced. It is well known that the therapeutic actions of herbal medicines are based on different concentration levels, and the results exhibited a good linear relationship with each selected at three levels, such as ratio of components in mobile phase (±1%), time program of mobile phase (initial section ± 1 min), wavelength of UV visible detector (± 2 nm), flow rate (± 0.1 mL min⁻¹), injection volume (1, 5, 10 μL) and column temperature (± 2 °C). The investigation was carried out with one linear data point closest to the concentration of tested samples. The values of conversion factors at each level of six factors showed that all the factors had little influence on the concentration (R² ≥ 0.9997) over the concentration range. The LOD ranged from 0.0759 to 1.302 μg mL⁻¹, and the LOQ ranged from 0.2530 to 0.4340 μg mL⁻¹ (Table 2).

Precision and accuracy

Repeatability was assessed at three different sample concentration levels. The RSDs were in the range of 0.6 - 1.8%. For intermediate precision, including different days, different operators, and different equipment and columns, the RSDs were in the range of 1.0 - 1.8%, 1.3 - 2.5% and 1.6 - 2.9%, respectively. The results obtained by the two methods showed no remarkable differences (P ≥ 0.391). Accuracy was measured by spiking known amounts of the four analytes at three different concentration levels. The accuracy of each analyte was 97.4 - 106.6% (RSDs = 0.1 - 1.1%). The accuracy between the two methods showed no remarkable differences (P = 0.265).

These results showed that the precision and accuracy of the SSDMC method would suffice for the routine quality control requirements of Chinese herbal medicines.

Robustness

The SSDMC method was validated to be feasible in a single laboratory as mentioned above. For wider application of the SSDMC method, the stability of the conversion factor was investigated by robustness tests, including the environmental parameters and the operational parameters.

For the environmental parameters, two instruments (Agilent 1260 and Agilent 1100) and three columns (Agilent ZORBAX SB-C18 (4.6 × 150 mm, 3.5 μm), Agilent Eclipse XDB-C18 (4.6 × 250 mm, 5 μm) and GL Inertsil ODS-EP (4.6 × 150 mm, 5 μm)) were tested with a set of linearity data. All these columns can separate the 15 components on the baseline. The statistical results showed that the conversion factors vary with different equipment and columns, and the RSDs were below 1.0% except for GH (6.2%) because of the low content level in the samples (1.015 - 40.60 μg mL⁻¹). The results indicate good ruggedness of the F. As for relative retention time, different equipment had little influence with the maximum RSD of 1.1%, but changed significantly among different columns, with the maximum RSD of 13.2%. It was speculated that different types of columns contain different packing that would affect the retention behavior of various substances in varying degrees. When the SSDMC method is applied, there is a need to compare the affinity between column packing and the four chromones, which could affect the RRT and even the elution order.

For the operational parameters, all parameters were validated with each selected at three levels, such as ratio of components in mobile phase (±1%), flow rate (± 0.1 mL min⁻¹), injection volume (1, 5, 10 μL) and column temperature (± 2 °C). The investigation was carried out without any single data point closest to the concentration of tested samples. The values of conversion factors at each level of six factors showed that all the factors had little influence on the

### Table 2: Regression equations, correlation coefficients, linearity ranges, LOD, LOQ, F and RRT for the SSDMC

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Regression equation</th>
<th>R²</th>
<th>Linearity range/μg mL⁻¹</th>
<th>LOD/μg mL⁻¹</th>
<th>LOQ/μg mL⁻¹</th>
<th>F</th>
<th>RRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC</td>
<td>Y = 27.27X – 19.85</td>
<td>0.9999</td>
<td>6.435 - 257.4</td>
<td>0.1302</td>
<td>0.4340</td>
<td>1.043 ± 0.021</td>
<td>0.56 ± 0.01</td>
</tr>
<tr>
<td>C²</td>
<td>Y = 26.17X – 29.98</td>
<td>0.9999</td>
<td>1.396 - 55.83</td>
<td>0.07591</td>
<td>0.2530</td>
<td>0.7496 ± 0.0137</td>
<td>0.89 ± 0.01</td>
</tr>
<tr>
<td>GV</td>
<td>Y = 36.41X – 9.37</td>
<td>0.9999</td>
<td>6.235 – 249.4</td>
<td>0.0938</td>
<td>0.3126</td>
<td>1.000 ± 0.000</td>
<td>1.00 ± 0.00</td>
</tr>
<tr>
<td>GH</td>
<td>Y = 30.28X – 13.46</td>
<td>0.9997</td>
<td>1.015 – 40.60</td>
<td>0.1104</td>
<td>0.3679</td>
<td>0.938 ± 0.046</td>
<td>1.44 ± 0.00</td>
</tr>
</tbody>
</table>

a. prim-O-glucosylcinifugin, b. cinifugin, c. 4'-O-β-glucosyl-5-O-methylvisamminol, d. sec-O-glucosylhamaudol.
value of conversion factors with the RSD below 1.1%, except for the factor of wavelength, with the maximum RSD of 13.1%, which is most closely connected to the signal. This was consistent with the literature.26 It can be concluded that the six operational parameters can be adjusted in a narrow range to acquire satisfactory system suitability except for the factor of wavelength.

The method validation results indicated that the developed SSDMC method was efficient, accurate and sensitive for simultaneous determination of multiple ingredients in SR by carefully controlling the wavelength of the detector.

Application of the SSDMC method

The developed SSDMC method was applied to determine the contents of GC, C, GV and GH in 15 batches of samples collected from different origins. The results were compared with those obtained from traditional ESM using paired t-test by SPSS. It could be seen that no significant differences were found in the quantitative results with \( P = 0.082 \) (Table 3).

<table>
<thead>
<tr>
<th>No.</th>
<th>GC(^{C})</th>
<th>C(^{C})</th>
<th>GV(^{C})</th>
<th>GH(^{C})</th>
<th>Total</th>
<th>GC(^{E})</th>
<th>C(^{E})</th>
<th>GV(^{E})</th>
<th>GH(^{E})</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.30</td>
<td>0.09</td>
<td>0.15</td>
<td>0.05</td>
<td>0.59</td>
<td>0.30</td>
<td>0.09</td>
<td>0.15</td>
<td>0.05</td>
<td>0.59</td>
</tr>
<tr>
<td>2</td>
<td>0.13</td>
<td>0.07</td>
<td>0.17</td>
<td>0.01</td>
<td>0.39</td>
<td>0.13</td>
<td>0.07</td>
<td>0.17</td>
<td>0.01</td>
<td>0.38</td>
</tr>
<tr>
<td>3</td>
<td>0.13</td>
<td>0.12</td>
<td>0.22</td>
<td>0.03</td>
<td>0.50</td>
<td>0.13</td>
<td>0.12</td>
<td>0.22</td>
<td>0.03</td>
<td>0.50</td>
</tr>
<tr>
<td>4</td>
<td>0.18</td>
<td>0.09</td>
<td>0.17</td>
<td>0.02</td>
<td>0.46</td>
<td>0.18</td>
<td>0.09</td>
<td>0.17</td>
<td>0.02</td>
<td>0.46</td>
</tr>
<tr>
<td>5</td>
<td>0.20</td>
<td>0.06</td>
<td>0.26</td>
<td>0.03</td>
<td>0.55</td>
<td>0.20</td>
<td>0.06</td>
<td>0.26</td>
<td>0.03</td>
<td>0.55</td>
</tr>
<tr>
<td>6</td>
<td>0.24</td>
<td>0.03</td>
<td>0.25</td>
<td>0.02</td>
<td>0.54</td>
<td>0.25</td>
<td>0.03</td>
<td>0.25</td>
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\( P = 0.082 \) --- \( P = 0.334 \)

a. prim-O-glucosylcimifugin, b. cimifugin, c. 4’-O-β-glucosyl-5-O- methylvisamminol, d. sec-O-glucosylhamaudol, e. The results of precision tests analyzed by the SSDMC and the ESM are the same.

Conclusions

A method integrating fingerprint analysis and multi-ingredient determination for quality assessment and control of SR based on HPLC-DAD was proposed for the first time. Fifteen batches of SR from different sources were assessed and distinguished by chromatographic fingerprint analysis in combination with similarity analysis and PCA. Additionally, the SSDMC method was first developed to quantify the main active components in SR. The established method has been proven to be reliable and simple, indicating that the combination of chromatographic fingerprint analysis and simultaneous determination of the four bioactive chromones is recommendable for the comprehensive quality assessment of SR.

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