Optimization of baicalin water extraction process from Scutellaria baicalensis (a traditional Chinese medicine) by using orthogonal test and HPLC

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A B S T R A C T

In this study, we optimized the baicalin water extraction process from Scutellaria baicalensis Georgi, Lamiaceae (a traditional Chinese medicine). Orthogonal test design (L9(3)4) was used to analyze the optimization of water extraction process of baicalin from S. baicalensis. The effect of solid–liquid ratio, extraction time and soaking time on the yield of baicalin were investigated and optimized by orthogonal test. High-performance liquid chromatography was employed for the determination of extraction yield of baicalin. Analysis of variance was carried out to study the effects of the above three factors. The results showed that solid–liquid ratio plays a significant role in attaining maximum extraction yields of baicalin. However, the other two factors had some effect (not statistically significant) on the extraction yield of baicalin. Conclusively, the optimum experimental conditions such as the solid–liquid ratio (1:12), extraction time (30 min) and soaking time (1 h) for the water extraction of baicalin were proposed which can provide the maximum extraction yield of baicalin. In addition, the score based on the content of baicalin and total solid residues yield were used as evaluation indexes for baicalin uterus suppositories evaluation.

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Introduction

Baicalin (baicalein 7-O-β-D-glucuronic acid) (1) known as huangqin in Chinese traditional medicine, is the principal flavonoid derivative found in the roots of Scutellaria baicalensis Georgi, Lamiaceae (Scutellariae radix) (Lu et al., 2011). Baicalin have excellent biological actions and become more and more popular in pharmaceutical and food industries, cosmetics, and displays anti-inflammatory (Meng et al., 2009), anticancer activity, effective against bacterial infections and oxidative stress–related diseases (Izui et al., 2016). Previously, different extraction methods such as heat reflux extraction (HRE), ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE) were reported to isolate and purify baicalin (Wang et al., 2010). However, little is known about the optimization of baicalin water extraction process. Endometritis is an inflammatory condition of the uterus common in cows after parturition. It is mostly caused by bacteria and leads to severe economic losses such as culling, treatment cost, reproductive inefficiency, milk discard, and increased risk of antibiotic residues in food products. Antibiotics such as cephalosporins, penicillin or a combination of ampicillin with oxytetracycline or cloxacillin are commonly used for the treatment of endometritis (Klugman et al., 1997). While, plant-derived antibacterial (baicalin) could be a promising alternative to avoid antibacterial resistance. Baicalin has shown synergistic effects with oxytetracycline and tetracycline (Novy et al., 2011) and potent against inflammation. Therefore, traditional Chinese medicine, i.e. baicalin uterus suppositories, will be an effective therapy against endometritis. Based on these considerations, we optimized the water extraction process of baicalin uterus suppositories to ensure effective treatment of endometritis and to reduce the risk of antibacterial resistance and drug residues in food, especially in milk. Many previous studies used orthogonal test (OT) for the optimization of analytical extraction procedures. It can save time and lesson the number of assays (Zhao et al., 2011). Therefore, in the present study, OT (a chemometric method) was used to optimize the baicalin water extraction process through high performance liquid chromatography (HPLC), and to evaluate the factors which mainly influence the quantitative yield.

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of baicalin. The effects of extraction time, solid–liquid ratio and soaking time on baicalin yield were studied by orthogonal test L9(3)4. Baicalin uterus suppositories were evaluated based on baicalin content and total solid residues yield values. In addition, this work will provide a basis for further studies to have a check on the quality control of baicalin uterus suppositories preparation.

![Baicalin Structure](image)

of baicalin. The effects of extraction time, solid–liquid ratio and soaking time on baicalin yield were studied by orthogonal test L9(3)4. Baicalin uterus suppositories were evaluated based on baicalin content and total solid residues yield values. In addition, this work will provide a basis for further studies to have a check on the quality control of baicalin uterus suppositories preparation.

Materials and methods

Samples and chemicals reagents

Baicalin standard (batch number: Z0271504) were obtained from Chinese veterinary medicine supervision center and the roots of Scutellaria baicalensis Georgi, Lamiaceae (batch number: 15062501) were bought from Runhe Chinese medicine processing plant Ltd. (Harbin, China). Methanol and phosphoric acid (HPLC grade) were obtained from Kermel Chemical Reagent Company (Tianjin, China). Deionized water provided by Wahaha Company Ltd. (Hangzhou, China).

Baicalin extraction protocol and sample preparation

The roots of S. baicalensis crushed by a grinder (AK-1000A, Wening, China) into powder form of particle size less than 150 μm and soaked in water for 1 h. It is then heated in water at 100 °C and concentrated to a density of 1.05–1.15 g/cm3. The extract is placed in a vacuum chamber for drying at 50 °C for 5 h. The extract was ground into powder and 0.1 g of powder dissolved in 25 ml methanol. The solution was ultrasonicated (KQ3200 Kunshan Ultrasonic Instrument Co., Ltd., China) for 30 min and the precipitate is removed by centrifugation. Finally, the solution was analyzed by HPLC.

Orthogonal test design

Orthogonal test design was to include three factors and three levels to optimize the water extraction process of baicalin including the solid–liquid ratio, soaking time, and extraction time. Table 1 shows orthogonal test L9(3)4 parameters. Each treatment was performed in triplicates.

HPLC identification and quantification

A High Performance Liquid Chromatography (HPLC) Acquity (Waters Alliance, USA) equipped with a 2998-UV detector (Waters, USA) was used. The system was computer controlled installed with Empower2 software employed for the analysis of data. The chromatographic column (Venusil XBP C18 (L) (5 μm, 250 mm × 4.6 mm), Bonna-Agela Technologies, China) was used for the separation of baicalin. An isocratic flow rate of 1 ml/min maintained through the column at an injection volume of 10 μl. The experiments were performed at a detection wavelength of 280 nm and at a column temperature of 25 °C. Mobile phase consisted of methanol–water–phosphoric acid (ratio 47:53:0.2 (v/v/v)).

Analysis of data

The experiments were performed three times unless otherwise mentioned. Analysis of data was performed by SPSS (version 17.0, Chicago, USA) software using multivariate analysis of variance (MANOVA). The value of p was chosen <0.05.

Results

Validation of the extraction method

In order to optimize the proposed extraction method, the level of linearity, precision, stability, reproducibility and recovery of experiments were performed. The results showed that the extraction process was fully validated in terms of the above parameters. The chromatograms obtained for baicalin standard substance and S. baicalensis are shown in Fig. 1. Baicalin peak appears at a retention time of approximately 12 min.

Linear range and standard curve determination

Baicalin standard substance containing baicalin content 95.9% was precisely weighed (8.0 mg) and dissolved in 10 ml methanol in a brown volumetric flask to obtain stock solution (contain baicalin 767.20 μg/ml). The stock solution was then diluted to the required concentrations (15.34, 38.36, 153.4, 306.88, 767.20 μg/ml) to draw the standard curve under the chromatographic conditions as stated above. Linear regression was applied to calculate the standard curve. The coefficients of determination (R2) value (0.9999) revealed a good linearity over the selected range for baicalin (15.34–767.20 μg/ml) as shown in Fig. 2.

Precision and stability experiments

The precision of the method was determined for the same standard substance six times. The coefficient of variation (%RSD) value (0.54%) showed (Table 2) that the method is precise. The stability of the optimized extraction process was checked at 0, 2, 4, 8, 12 and 24 h using the same standard substance kept at room temperature. In order to determine the stability, same chromatographic condition was employed as mentioned in “Materials and methods” section. The %RSD value (0.56%) displayed in Table 2 indicates that the samples were in good stability within 24 h.

Reproducibility and recovery of baicalin

The reproducibility of the method was determined for S. baicalensis (same batch number). The process was repeated six times and 0.96% RSD value showed that the repeatability of the method was good (Table 2). The recovery of baicalin content was calculated for the same six samples (repeated six times) shown in Table 3. The recovery of baicalin obtained was 0.3069 mg after adding baicalin (0.2612 mg). The recovery rate was calculated by the following formula.

\[
\text{Recovery rate} = \frac{\text{detected value} - \text{added value}}{\text{original value}} \times 100
\]
Fig. 1. HPLC chromatograms of baicalin. (A) Standard solution of baicalin and (B) sample of Scutellaria baikalensis extract (whereas n = 3).

Fig. 2. Standard curve of baicalin. Horizontal axis represents concentration of baicalin in µg/ml and vertical axis represents peak area (µAU (absorption unit) × S (time in sec)).

![](image)

**Table 2**

<table>
<thead>
<tr>
<th>Precision</th>
<th>Stability</th>
<th>Reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak area (µAU × S)</td>
<td>4,729,420 ± 255</td>
<td>4,734,606 ± 265</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.54</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Note: Results indicated mean ± SD, where n = 6.

The recovery of the sample was 99.3% and the RSD was 1.55%, which indicated that the recovery rate of the method was good and the method was feasible (Table 3).

**Effect of solid–liquid ratio, extraction time and soaking time on the yield of baicalin**

In this study, we investigated the effect of solid–liquid ratio, extraction time and soaking time on the extraction yield of baicalin. Fig. 3B displays the effect of solid–liquid ratio on baicalin content. Solid–liquid ratio was fixed at 1:6, 1:8, 1:10, 1:12 and 1:14. It has been noted that at 1:10–1:14, the extraction yield of baicalin increased and reached a maximum value (32.7 mg/g) when solid/liquid ratio was 1:12. The yield of baicalin decreased after solid–liquid ratio exceeded 1:12. The results showed that the appropriate solid–liquid ratio was in between 1:10 and 1:14, therefore, the solid–liquid ratio (1:10, 1:12 and 1:14) was included in the orthogonal test. The effect of extraction time and soaking time on extraction yield of baicalin from *S. baikalensis* are shown in Fig. 3A and C. All other extraction conditions (e.g. soaking time and solid–liquid ratio) were fixed at 1 h and 1:12 for investigation of extraction time effect on baicalin yield. The results revealed that the maximum extraction yield of baicalin was obtained at 60 min (Fig. 3A). Similarly, the effect of soaking time on the extraction yield of baicalin is shown in Fig. 3C. The results showed that the appropriate soaking time was between 30 and 90 min, therefore, the soaking times (30, 60 and 90 min) were included in the orthogonal test.

**Optimization of the extraction parameters**

The orthogonal test was used to optimize the operating conditions for the extraction of baicalin which is a scientific and systematic method. Orthogonal test has many advantages such as to reduce the testing treatments and to analyze scientific results. In the present study, all the selected factors were evaluated using an orthogonal L9(3)^4 test design. Orthogonal test results, K and R values are displayed in Table 4, and analysis of variance is shown in Table 5. It has been noted that the mean extraction yields of baicalin was good at A1 (extraction time; 30 min), B2 (solid–liquid ratio; 12) and C1 (60 min). In addition, analysis of variance showed that solid–liquid ratio was the most important factor in extraction process, and other factors (soaking time and extraction time) have non-significant interactions with extraction yield of baicalin. It can be seen from Table 4 that the influence level of extracting conditions on baicalin yield varies in order; B > C > A. Total solid yield was calculated by the following formula.

\[
\text{Total solid yield (\%)} = \left( \frac{M - m}{W} \right) \times 100\%
\]

where *M* is the weight (g) of the total solid residues after extraction, *m* is the weight (g) of evaporating dish; and *W* is the weight of the sample (root of *S. baikalensis*). The content of baicalin and total solid residues yield were used as evaluation indexes for baicalin.
Table 3
Result of recovery of baicalin in medicinal materials.

<table>
<thead>
<tr>
<th>No</th>
<th>Original (mg)</th>
<th>Added (mg)</th>
<th>Detected (mg)</th>
<th>Recovery (%)</th>
<th>Mean value (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3069</td>
<td>0.2612</td>
<td>0.5638</td>
<td>98.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.3069</td>
<td>0.2612</td>
<td>0.5682</td>
<td>100.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.3069</td>
<td>0.2612</td>
<td>0.5678</td>
<td>99.89</td>
<td>99.30</td>
<td>1.55</td>
</tr>
<tr>
<td>4</td>
<td>0.3069</td>
<td>0.2612</td>
<td>0.5598</td>
<td>96.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.3069</td>
<td>0.2612</td>
<td>0.5714</td>
<td>101.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.3069</td>
<td>0.2612</td>
<td>0.5667</td>
<td>99.46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Fig. 3.](image)

Fig. 3. The effect of (A) extraction time, (B) solid–liquid ratio, and (C) soaking time on baicalin content (whereas n = 3).

Table 4
Results of L9(3⁴) orthogonal test.

<table>
<thead>
<tr>
<th>Level</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Total baicalin (mg)</th>
<th>Total solid residues yield (%)</th>
<th>Overall score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>57.35</td>
<td>33.91</td>
<td>97.52</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>55.45</td>
<td>34.70</td>
<td>87.03</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>47.65</td>
<td>35.57</td>
<td>96.55</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>40.9</td>
<td>31.70</td>
<td>77.66</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>52.1</td>
<td>34.17</td>
<td>93.16</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>52.45</td>
<td>36.43</td>
<td>90.02</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>35.35</td>
<td>29.57</td>
<td>67.15</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>55.4</td>
<td>36.00</td>
<td>96.84</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>57.35</td>
<td>36.70</td>
<td>99.78</td>
</tr>
</tbody>
</table>

K₁ = 281.10  K₂ = 254.84  K₃ = 263.77  R = 17.23

Overall score = \{100 \times \left( \frac{\text{baicalin content}}{\text{maximum baicalin content}} \right) \times 0.7 \} + \{100 \times \left( \frac{\text{total solid residues yield}}{\text{maximum total solid residues yield}} \right) \times 0.3 \}

whereas 0.3 is the weight coefficient of solid yield rate and 0.7 is the weight coefficient of baicalin content.
Table 5
Analysis of variance.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Sum of squares</th>
<th>Freedom</th>
<th>Mean square error</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>727,009</td>
<td>2</td>
<td>363.505</td>
<td>0.214</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>33,100</td>
<td>2</td>
<td>16.550</td>
<td>19.209</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>64,410</td>
<td>2</td>
<td>32.205</td>
<td>8.468</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>126,724</td>
<td>2</td>
<td>63.362</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: F(0.1) = 9, F(0.05) = 19.

x p < 0.05

Table 6
Optimum scale-up test.

<table>
<thead>
<tr>
<th>R1</th>
<th>R2</th>
<th>R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scutellaria baicalensis Georgi (g)</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Baicalin content (mg/g)</td>
<td>62.32</td>
<td>60.29</td>
</tr>
<tr>
<td>Total solid yield (%)</td>
<td>32.53</td>
<td>33.40</td>
</tr>
</tbody>
</table>

Optimization of process amplification

In order to investigate the stability of the extraction process, the optimum conditions according to the above results were chosen (extraction time, 30 min; solid–liquid ratio, 1:12 and soaking time, 60 min) and the yield of baicalin and total solid residues yield were calculated. The results (Table 6) are stable and feasible at 50 times more of the sample tested under the above optimum extraction conditions which confirmed that the optimized extraction process is stable and precise.

Discussion

The optimization for accurate, sensitive and reliable inexpensive methods is very important in the development of highly accurate methods (Elerath and Pecht, 2009). Our data revealed that the method is validated in terms of the level of linearity, stability, precision, recovery and reproducibility of experiments. The level of linearity is checked by linear regression model as stated in previous reports. Previous reports demonstrated that extraction time and extraction temperature are the major factors in the optimization of an extraction process. Additionally, several factors were taken into consideration while optimizing an extraction method. Among these factors, to choose a good, easily available and cheap extraction solvent is a fundamental factor (Ekberg et al., 2008). Some researcher used ethanol for extraction of flavonoids from Scutellariae radix (Song and Meng, 2010). In this study, we chose water as an extraction solvent because of its easy availability and cheap in price than other solvents. In our study, the effects of three experimental factors such as extraction time, solid–liquid ratio and soaking time on baicalin yield from the root of S. baicalensis were studied by orthogonal test L9 (3)4 design. Orthogonal test is a scientific method allowed to select the best experimental conditions for water extraction process of the substance (Kong et al., 2013). Previous reports also demonstrated that orthogonal test is a concise chemometric test with good analysis of results and reduce testing treatment amounts. A study reported the isolation and purification of baicalin along with wogonin and oroxylin A from the medicinal plant S. baicalensis and obtained good peak resolution for the target compounds (Li et al., 2014). Similarly, we also obtained good peak resolution for baicalin. The chromatogram for baicalin as standard substance and S. baicalensis extract appears at a retention time of 12 min. Furthermore, we verified the stability of the optimized extraction process by choosing the optimum conditions such as extraction time, 30 min; solid–liquid ratio, 1:12 and soaking time, 60 min which confirmed that the optimized extraction process is stable and precise.

Conclusion

In summary, orthogonal test L9 (3)4 results revealed the optimum extracting conditions for baicalin isolation including solid–liquid ratio (1:12), extraction time (30 min) and soaking time (1 h). The results showed that the extraction process was reliable, stable, inexpensive and feasible. Total solid residues yield and baicalin content were used as evaluation indexes for the uterus suppositories preparation process to ensure the standard and efficacy of baicalin uterus suppositories and to have a check-up on the quality of industrial production and application.

Ethical statement

All the experiments protocols were performed in accordance with guidelines and approved protocols of Northeast Agricultural University.

Authors contribution

JL designed and supervised experiments; HN and ZW performed experiments; ZL provided help during experiments, and IM contributed to the writing of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgment

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References


