SHORT COMMUNICATION

Simultaneous quantitative determination of eight index constituents and compatibility changes in Longchai Decoction by UPLC–Q-TOF-MS

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Received 24 March 2013; revised 28 May 2013; accepted 12 June 2013

KEY WORDS
UPLC–Q-TOF-MS; Longchai Decoction; Quantitative determination; Compatibility

Abstract The goal of this research was to develop a simple, rapid and sensitive method for simultaneous quantitative determination of salidroside, gardenoside, liquiritin, baicalin, wogonoside, wogonin, saikosaponin A and saikosaponin D in Longchai Decoction by ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC–Q-TOF-MS), in order to control the quality of Longchai Decoction and to analyze the changes of chemical components before and after the compatibility of the component herb drugs. The chromatographic separation was performed on the Waters ACQUITY BEH C18 column (2.1 mm × 100 mm, 1.8 μm) using the mixture of acetonitrile and 0.1% (v/v) methanoic acid as mobile phase with a gradient elution program at the flow rate of 0.3 mL/min and the column temperature of 30 °C. The eight components of the standards achieved baseline separation. Regression analysis revealed a linear relationship (r² > 0.9998) between the contents and the peak areas of the mixed standard substances. The average recovery rates were between 99.72% and 102.13% with RSD values were less than 2.82% (n = 5). The obtained results indicated that the content of index components were higher in co-decoction compared to mixed decoction. This method with a good resolution and high precision can be used for the quality control of Longchai Decoction.

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http://dx.doi.org/10.1016/j.apsb.2013.06.006
1. Introduction

Composite formula of Chinese medicine (CFCM) contains a large number of chemical compositions which are the material basis for the clinical efficacy. For further mining the compatibility and mechanisms of action, the efficacy of CFCM is supposed to be clarified. So the research on effective substance basis is momentous for the modern study of CFCM. In recent years, LC–MS\(^1\)–\(^4\) is widely used in component analysis and identification, which provides an important method for the study of CFCM.

Longchai Decoction used for the treatment of chronic hepatitis B comes from the commonly used decoctions of Jiangsu Province Hospital of Traditional Chinese Medicine. It is mainly composed of eight Chinese herbal medicines, such as Herba Solanii Nigri, Radix Bupleuri, Radix Scutellariae, Radix et Rhizoma Glycyrrhizae, Herba Sedi, Herba Hedyotis Diffusae, Fructus Ligustri Lucidi, Fructus Gardenide. It can act against the pathogenesis of hepatitis B and is capable of reducing the clinical symptoms of damp-heat and toxin by heat-clearing and detoxicating, and further regulating spleen and stomach\(^5\)–\(^6\). Because of its significant clinical curative effect, it is important to systematically improve the quality evaluation of Longchai Decoction. This experiment aims to establish the comprehensive method of quality evaluation for the simultaneous quantitative determination of index constituents and overall quality control. Salidroside (from Fructus Gardenide), which has obvious protective effects on the liver\(^7\), can significantly reduce the rise of ALT and NO in serum caused by liver damage and decrease the content of MDA and TG. Baicalin, wogonoside and wogonin (from Radix Scutellariae) can eliminate free radical and exert an antioxidant activity in the different systems of the body. They also have liver protective and chologagogic effect by significantly reducing ALT activity of acute hepatic injury caused by carbon tetrachloride\(^8\). Saikosaponin A and saikosaponin D (from Radix Bupleuri) can protect live cells by inhibiting cholinesterase\(^9\) and play an important role in the repair of acute hepatic injury caused by carbon tetrachloride, \(\alpha\)-galactosamine, lipopolysaccharide and BCG\(^10\). Both gardenoside (from Fructus Gardenide) and liquiritin (from Radix et Rhizoma Glycyrrhizae) have the ability of protecting liver and reducing the enzyme activity. The eight index components are the main efficacy components in Longchai Decoction; they all have varying degrees of liver protective effects. The contents of the eight chemical compositions in Longchai Decoction are higher than other chemical compositions. So it is easy to accurate qualitative and quantitative analyses for the eight chemical compositions. UPLC–Q-TOF-MS was adopted for qualitative analysis and quantitative analysis of eight index components, including salidroside, gardenoside, liquiritin, baicalin, wogonoside, wogonin, saikosaponin A and saikosaponin D (Fig. 1) in the study, providing the reference for quality control and drug research and development of Longchai Decoction. Moreover, the UPLC coupled with quadrupole time-of-flight mass spectrometry (Q-TOF-MS) could provide higher sensitivity and selectivity, better resolution, narrower peaks and shorter retention time. The analysis of chemical compositions in compatibility provides the basis for elucidating the scientific connotation of traditional decoction theory for CFCM.

2. Materials and methods

2.1. Chemicals and reagents

The herbal pieces of Longchai Decoction collected from medicinal materials company (Bozhou City, Anhui Province) was identified by Prof. Xunhong Liu, Nanjing University of Chinese Medicine. All the samples of the herbal pieces were kept in the Laboratory of Chinese Medicine Identification. The reference standards of gardenoside, liquiritin, baicalin, wogonin, saikosaponin A and saikosaponin D were purchased from the National Institute for the Control of Pharmaceutical and Biological Products. The standard of salidroside was available from Shanghai Forever Biotech Co., Ltd. (China).

![Figure 1](image.png)  
**Figure 1** The chemical structures of eight reference substances.
wogonoside was obtained from Sichuan Weikeqi Biological Technology Co., Ltd. (China). The purity of all standards was above 98.0%. Acetonitrile and methanol were purchased from Merck (Germany) and Jiangsu Hanbon Science and Technology Co., Ltd. (China). Methanoic acid was purchased from Nanjing Chemical Reagent Co., Ltd. (China). All reagents used in the study were of HPLC grade. Ultra-pure water was prepared by using a ultra-pure water purification system (Nanjing EPED Co., Ltd. China).

2.2. Chromatographic conditions

Chromatographic analysis was performed on a Waters Acquity UPLC system, equipped with a binary pump solvent management system, an online degasser and an autosampler. All separations were carried out on an ACQUITY UPLC™ BEH C18 column (2.1 mm × 100 mm, 1.8 μm) at 30 °C. The mixture of (A) acetonitrile and (B) 0.1% (v/v) methanoic acid aqueous solution was chosen as the mobile phase using a gradient program: 0–4 min, 5–9% A; 4–6 min, 9–13% A; 6–12 min, 13–22% A; 12–15 min, 22–28% A; 15–19 min, 28–40% A; 19–23 min, 40–60% A; 23–24 min, 60–5% A and stayed at 5% A for 5 min. The flow rate was 0.3 mL/min and the injection volume was 2 μL.

2.3. Mass spectrum conditions

The mass spectrometry was performed on a quadrupole orthogonal acceleration time-of-flight (TOF) tandem mass spectrometer. The nebulizer gas was set at 550 L/h and the temperature at 350 °C under both negative and positive ion modes. The cone gas was set at a flow rate of 50 L/h, and the source temperature was set at 110 °C. The capillary voltage was set at 10 kV and the cone voltage at 15 V. The TOF data were collected between m/z 50 and m/z 1000 with a low collision energy of 5 V for quantitative analysis and the MS/MS experiments were performed using high collision energy of 45 V for fragment ion information. All analyses were acquired using an independent reference spray via the Lock Spray interference to ensure accuracy and reproducibility. The molecular masses of the precursor ion and the product ions were accurately determined using leucine-enkephalin (m/z 554.2615) as a reference compound11–13. The accurate mass and composition for the molecular ions or quasi-molecular ions in the mass spectrum were characterized based on their retention behavior and MS information compared to related standards.

2.4. Standard solution

The standard stock solutions containing salidroside at 95.20 μg/mL, liquiritin at 196.0 μg/mL, baicalin at 182.0 μg/mL, wogonoside at 200.8 μg/mL, wogonin at 177.2 μg/mL, saikosaponin A at 159.2 μg/mL and saikosaponin D at 197.6 μg/mL were prepared in 70% (v/v) methanol in water. The stock solutions were appropriately diluted to prepare a series of standard working solutions, and then stored away from light at 4 °C. The solutions were brought to room temperature and filtered through 0.22 μm membrane filters before UPLC–Q-TOF-MS analysis.

2.5. Sample solution

0.32 g of dried sample ground powder (50 mesh sieve) of two different kinds of decoctions (co-decoction: water extract of mixed eight constituent herbs of Longchai Decoction; mixed decoction: mixed water extract of each individual herbs of Longchai Decoction) was accurately weighed and respectively dissolved in 50 mL 70% (v/v) methanol in water in 100 mL conical flasks with stoppers, then sonicated (40 kHz, 500 W) at room temperature for 30 min. The same solvent was added to compensate for the lost weight during the extraction. The two types of liquid were filtered through 0.22 μm filters before injected into the UPLC–Q-TOF-MS system for analysis.

3. Results and discussion

3.1. Method optimization

3.1.1. Optimization of UPLC conditions

A series of preliminary experiments were carried out using different mobile phases including acetonitrile/water, methanol/water and acetonitrile/methanoic acid14–18. The best separation of these eight index compositions was obtained using acetonitrile and 0.1% (v/v) methanoic acid aqueous solution by gradient elution. Under the selected UPLC conditions, the resolution among these ingredients was more than 1.5.

3.1.2. Selection of MS conditions

MS spectra were studied in both positive and negative modes. The negative mode was employed in our paper as it had better sensitivity of ion response than the positive mode, making it easier to confirm the molecular ions or quasi-molecular ions in identification of each peak. The chemical structures of eight components were characterized based on their retention behavior and MS information compared to related standards.

3.1.3. Optimization of extraction procedure

In order to achieve a complete and efficient extraction of active components in Longchai Decoction, the extraction methods, different solvents and extract time were investigated. The previous researches23–25 showed that ultrasonic extraction was more convenient and effective than refluxing extraction, thus we also chose ultrasonic extraction. Using sonication, various solvents including different concentrations of aqueous methanol and aqueous ethanol were screened. It was found that 70% (v/v) methanol in water was the most efficient extraction solvent. In addition, it was demonstrated that the components was extracted completely within 30 min, thus the extraction time was fixed to 30 min. Finally, the sample solutions were prepared by ultrasonic extraction with 70% (v/v) methanol in water for 30 min.

3.2. Method validation

3.2.1. Calibration curves, LOD and LOQ

The calibration curves were constructed by plotting the peak area versus the concentration of each analyte with a series of concentrations of standard solutions (n=6). The linearity for the investigated compounds is shown in Table 2.

A series of diluted standard solutions were prepared to determine the limits of detection (LOD) and limits of quantification (LOQ) when the signal-to-noise ratios (S/N) of analytes were about 3 and 10, respectively (Table 2).
3.2.2. Precision, repeatability and stability

Intra- and inter-day variations were determined for evaluating the precision of the developed method. For intra-day precision, the mixed standards solutions were analyzed for five replicates within one day; for inter-day test, the solutions were examined in duplicates for consecutive three days. Precisions were expressed by relative standard deviation (RSD). Repeatability was determined by using five samples from the same batch of co-decoction. They were treated with the above sample preparation procedure and analyzed with the established UPLC method, and then variations (%RSD) were calculated. Stabilities of these eight compounds were tested at five time points (0, 4, 10, 16 and 24 h). The results are shown in Table 3. The RSD values of the precision, repeatability and stability of the target components were all less than 3.19%.

3.2.3. Recovery test

A known amount of the eight standards were added to the co-decoction, then the mixture was extracted and analyzed by the above method (n=5). The quantification of each marker was accomplished according to the corresponding standard curve. The results in Table 4 show that the recovery of these eight constituents ranges 99.72–102.13% and that the RSD values are less than 2.82%.

3.3. Qualitative analysis

These eight index components including salidroside, gardenoside, liquiritin, baicalin, wogonoside, wogonin, saikosaponin A and saikosaponin D have been identified according to the data analysis.
including retention time \((t_R)\), the maximum UV absorption wavelength \((\lambda_{\text{max}})\), the molecular ion peaks and the ESI-MS data (Table 1).

The total ion chromatograms of co-decoction and mixed decoction were analyzed and compared in both positive and negative ion modes (Fig. 2). The variation trend of the chemical compositions was almost the same. In co-decoction, the peak area at \(t_R 5.30\) min increased the most significantly, and others at \(t_R 2.56, 7.90, 11.74, 14.33, 18.86, 19.69\) and \(21.43\) min increased to different degrees.

3.4. Quantitative analysis

The method described above was subsequently used for the simultaneous determination of the eight constituents in both co-decoction and mixed-decoction. As shown in Table 5, the contents of eight index components are higher in traditional decoction (co-decoction) than in physically mixed decoction, which may be caused by the solubilization during co-decocting process of the medicinal ingredients in Longchai Decoction. These changes may also be part of the material basis of the efficacy of Longchai Decoction.

4. Conclusions

An accurate, stable and feasible UPLC–Q-TOF-MS method is developed and validated for rapid qualitative and quantitative analyses of eight index components in Longchai Decoction. The method established in this research provides a reference for the quality control in drug research and development of Longchai Decoction.
Decoction. It preliminarily analyzes the changes of the chemical composition before and after compatibility, confirming that the differences in the chemical compositions between co-decoction and mixed decoction were very obvious. The content of the 8 index components increases in co-decoction compared with physically mixed decoction. The obtained result using the described method can provide experimental data based on the chemical material basis to explore the mechanism of Longchai Decoction.

Acknowledgments

This project was supported by the Science and Technology Foundation Construction Plan of Jiangsu Province (No. BM2009903) and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (No. ysxx-2010).

Table 5 Contents of the eight index components in both traditional decoction and mixed dividual decoctions (n=3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Average content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Traditional decoction</td>
</tr>
<tr>
<td>Salidroside</td>
<td>5.5</td>
</tr>
<tr>
<td>Gardenoside</td>
<td>4.2</td>
</tr>
<tr>
<td>Liquiritin</td>
<td>1.6</td>
</tr>
<tr>
<td>Baicalin</td>
<td>6.9</td>
</tr>
<tr>
<td>Wogonoside</td>
<td>6.5</td>
</tr>
<tr>
<td>Wogonin</td>
<td>3.1</td>
</tr>
<tr>
<td>Saikosaponin A</td>
<td>0.8</td>
</tr>
<tr>
<td>Saikosaponin D</td>
<td>0.7</td>
</tr>
</tbody>
</table>

References


