A high-performance liquid chromatography (HPLC) method for determination of chlorogenic acid and emodin in Yinhuang Jiangzhi Tea

Jie Shen¹, Xiang-Hui Xu²*
¹Department of Pharmacy, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai 200071, China
²Department of Pharmacy, Shanghai Construction Group Hospital, Shanghai 200083, China

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ABSTRACT

Objective: To establish a high-performance liquid chromatography (HPLC) method for the determination of chlorogenic acid and emodin in Yinhuang Jiangzhi tea. Methods: The contents of chlorogenic acid and emodin in Yinhuang Jiangzhi tea were determined with Diamonsil C18 column by eluting with acetonitrile: 0.4% phosphoric acid in water (13:87) and methanol: 0.1% phosphoric acid in water (80:20), with detection at 327 nm and 254 nm respectively. The flow rate was 1.0 mL/min, and the column temperature was 30 °C. Results: The correlation between injection volume and peak area of chlorogenic acid and emodin was Y=17.955X-0.2848 (R=0.9999, 0.041-0.41 μg) and Y=33.975X+186.36 (R=0.9995, 0.0395-0.395 μg), respectively. The mean recovery of chlorogenic acid and emodin was 100.38% (RSD=2.34%, n=9) and 101.21% (RSD=2.59%, n=9), respectively. Conclusions: The established HPLC method is relatively simple, accurate, and specific, and can be used for the quality control of Yinhuang Jiangzhi tea.

1. Introduction

Yinhuang Jiangzhi tea is a new herbal preparation in traditional Chinese medicine (TCM) developed by our hospital (Shanghai Pharmaceutical Approval No. z05191129 (HUYAOZHIZI)) and consists of four types of medicinal herbs including rhubarb, honeysuckle, semen cassiae and Longjing green tea. It can be used for the treatment of hyperlipidemia (i.e., elevation of serum cholesterol and triglycerides), obesity, constipation, among others. This preparation is developed based on a proven recipe compiled by our hospital’s well-known veteran TCM practitioner Pi’an Shen, and during its application in clinical practice for over 30 years, Yinhuang Jiangzhi tea has shown beneficial effects in hyperlipidemia. To effectively control the quality of this TCM preparation for its guaranteed clinical efficacy, we quantitatively analyzed this TCM preparation and established a method to measure the contents of its two major components, chlorogenic acid and emodin[1-4].

2. Instrument and materials

The main instruments used in this study included an HPLC system (Agilent 1200, Agilent Technologies Co., Ltd, USA), a B5500-MT ultrasonic generator (Sino-American Joint Venture Shanghai Branson Ultrasonic Co., Ltd, China; 80 W, 40 KHz), and a BSA2245-CW electronic balance (Sartorius, Germany). Reference standards for chlorogenic acid (110753-200413) and emodin (110756-200110) were purchased from the National Institute for Food and Drug Control of China. Herbal medicine Jinyinhua (Flos Lonicerae; honeysuckle) identified as the dry flower buds of Lonicera japonica Thunb and rhubarb identified as the dried root and rhizome of the Polygonaceae plant Rheum palmatum L. were both purchased from Shanghai Huayu Pharmaceutical Co., Ltd. Yinhuang Jiangzhi tea and blank control were made in our hospital’s preparation room. The water used in this study was purified water, methanol was HPLC-grade, and other reagents were of analytical grade.

*Corresponding author: Xiang-Hui Xu, Department of Pharmacy, Shanghai Construction Group Hospital, Shanghai 200083, China.
E-mail: Samyaoyaoa@163.com

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2.1 Determination of chlorogenic acid content

2.1.1 Chromatographic conditions
The chromatographic separation was performed on a C18 column (Diamonsil 4.6 mm 250 mm, 5 μm) using acetonitrile: 0.4% phosphoric acid (13.87 v/v) as the mobile phase with detection at a wavelength of 254 nm. The flow rate of mobile phase was 1.0 mL/min, column temperature was 30 °C, and injection volume was 10 μL.

2.1.2 Preparation of sample solutions
An electronic balance was used to accurately measure 0.4 g of sample, which was then dissolved in 50 mL of 50% methanol in a volumetric flask. The solution was weighed and ultrasonicated for 30 min (80 W, 40 kHz). After cooling, the sample solution was weighed again and the weight loss was compensated by adding 50% methanol. The solution was filtered after mixing and then 1 mL of solution was transferred to a 5 mL volumetric flask using a transfer pipette. Subsequently, 50% methanol was added to the flask to the mark. After mixing thoroughly, the solution was subjected to microporous membrane filtration (0.45 μm) to obtain the final solution for chromatography. Chlorogenic acid reference solution (0.041 μg/mL) was prepared using chlorogenic acid and 50% methanol solution. Jinyinhua negative control was prepared according to the formula and preparation method of the herbal preparation. The negative control solution was prepared according to the preparation method of the sample solution.

2.2 Determination of emodin content

2.2.1 Chromatographic conditions
The chromatographic separation was performed on a C18 column (Diamonsil 4.6 mm 250 mm, 5 μm) using acetonitrile: 0.1% phosphoric acid (80:20 v/v) as the mobile phase with detection at a wavelength of 254 nm. The flow rate of mobile phase was 1.0 mL/min, column temperature was 30 °C, and injection volume was 10 μL.

2.2.2 Preparation of sample solution
Yinhuang Jiangzhi tea was pulverized into powder (filtered with No. 4 mesh). An electronic balance was used to accurately measure 0.2 g of sample, which was then transferred to a volumetric flask and dissolved in 25 mL of methanol. The solution was weighed and heat refluxed for 1 h. After cooling, the solution was weighed again and the weight loss was compensated by adding methanol solution. After agitation and microporous membrane filtration (0.45 μm), the final solution was obtained. Emodin reference solution (0.0395 mg/mL) was prepared using emodin and methanol. The negative control (the herbal preparation sample without rhubarb) was prepared according to the formula and preparation method of herbal preparation and the negative control solution was prepared according to the preparation method of sample solution.

3. Results

3.1 Chlorogenic acid content

3.1.1 System suitability
The comparison of the chromatographs of the sample solution, negative solution, and chlorogenic acid reference solution indicated that under the chromatographic conditions stated in Section 2.1.1, the chromatographic peak of chlorogenic acid was observed without any interference and the peak was well-shaped (Figure 1).

![Figure 1. HPL Chromatogram of Chlorogenic Acid in Yinhuang Jiangzhi tea](image)

(A: Chlorogenic acid reference, B: Sample, C: Negative reference; 1 – Retention time of chlorogenic acid)

3.1.2 Examination of linear correlation
Firstly, 10.25 mg of chlorogenic acid reference was accurately measured and transferred to a 25 mL volumetric flask. After addition of 50% methanol until the mark on the flask and thorough mixing, a chlorogenic acid solution (0.41 mg/mL) was obtained. Next, 1 mL of chlorogenic acid solution was transferred to a 10 mL volumetric flask containing 50% methanol until the 10 mL mark and then shaken for homogenous mixing to obtain the 0.041 mg/mL chlorogenic acid solution. Finally, 1.0 μL, 2.0 μL, 3.0 μL, 5.0 μL, and 10.0 μL of 0.041 mg/mL chlorogenic acid solution were accurately pipetted and injected into the HPLC system. A standard curve was generated with the x-axis as the chlorogenic acid content and the y-axis as the peak area value. Within 0.041-0.41 μg, the amount of chlorogenic acid injected had a satisfactory linear correlation with the peak area, and the regression equation was $Y=17.95X-0.2848$ ($r=0.9999$).

3.1.3 Precision evaluation
The same chlorogenic acid reference solution (0.041 mg/mL) was repeatedly injected and evaluated six times with an injection volume.
of 10 μL each time. The average chlorogenic acid peak area was calculated to be 2729.217 and the relative standard deviation (RSD) value was 0.35% (n=6), suggesting a high precision of the tests.

3.1.4 Repeatability evaluation
The test was conducted under the same chromatographic conditions. The sample solution to be tested was prepared six times using the same method described in Section “2.1.2”. Then, 10 μL of each of the obtained sample solutions and reference control solution were injected and tested. The peak area values were measured and the chlorogenic acid content was calculated to be 7.02 mg/g with an RSD of 2.17%, suggesting a satisfactory repeatability of the tests.

3.1.5 Stability evaluation
The same sample solution was injected at 0, 2, 4, 8, 10, and 12 h after preparation. The average peak area value was calculated to be 987.20 with an RSD of 0.35% (n=6), suggesting that the sample solution remained stable for 12 h.

3.1.6 Recovery test
Nine samples, each containing 0.2 g of sample powder with known component contents (Batch No. 20120203), were added to 8.0 mL, 10.0 mL, and 12.0 mL of chlorogenic acid reference solution (mass concentration 0.152 mg/mL). The sample solution was prepared using the method described in Section “2.1.2”. The final samples to be tested were injected for peak area measurement. The average recovery rate was calculated to be 100.38%, with an RSD of 2.34%. The results indicate high reliability of the proposed test method (see Table 1).

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample’s chlorogenic acid content (mg)</th>
<th>Additional sample (mg)</th>
<th>Content (mg)</th>
<th>Recovery (%)</th>
<th>Average recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
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<td>1.216</td>
<td>2.7538</td>
<td>100.53</td>
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<td></td>
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<td>1.5034</td>
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<td>2.7404</td>
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<td>1.216</td>
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<td>96.42</td>
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<td></td>
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<tr>
<td>4</td>
<td>1.5201</td>
<td>1.520</td>
<td>3.0024</td>
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<td>3.0231</td>
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<td>100.38</td>
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<td>1.520</td>
<td>3.0394</td>
<td>101.10</td>
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<td></td>
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<tr>
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<td>1.824</td>
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<tr>
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<td>3.3562</td>
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<td>9</td>
<td>1.4951</td>
<td>1.824</td>
<td>3.3598</td>
<td>102.23</td>
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<td></td>
</tr>
</tbody>
</table>

3.1.7 Sample measurement
The measurement was performed under the same chromatographic conditions. Three batches of samples obtained by using the method described in Section “2.1.2” were used to prepare sample solutions for measurement. Ten microliters of each sample solution and reference solution were injected and the peak area was measured. The chlorogenic acid content was calculated by using an external standard method and the results are presented in Table 3.

3.2 Emodin content
3.2.1 System suitability
The comparison of the chromatographs of the sample solution, negative solution, and emodin reference solution indicated that under the chromatographic conditions stated in Section 2.1.1, the chromatographic peak of emodin was observed without any interference and showed a satisfactory shape (Figure 2).

3.2.2 Examination of linear correlation
Firstly, 9.875 mg of emodin reference sample was accurately measured and transferred to a 25 mL volumetric flask. After addition of methanol until the mark on the flask and thorough mixing, an emodin solution (0.395 mg/mL) was obtained. Next, 10 mL of emodin solution was transferred to a 100 mL volumetric flask, containing methanol until the 100 mL mark and then shaken for homogenous mixing to obtain the 0.0395 mg/mL emodin solution. Finally, 1.0 μL, 2.0 μL, 3.0 μL, 5.0 μL, and 10.0 μL of 0.0395 mg/mL emodin solution were accurately pipetted and injected into the HPLC system. After linear regression analysis, a standard curve was generated with the emodin content as the x-axis and the peak area as the y-axis. Within 0.0395-0.395 μg, the emodin injection amount showed a significant linear correlation with the peak area, and the regression equation was Y=33.975X+186.36 (r=0.9995).

3.2.3 Precision evaluation
The same emodin reference solution (0.0395 mg/mL) was repeatedly injected and evaluated six times with an injection volume...
of 10 μL each time. The average emodin peak area was calculated to be 701.5134 and the RSD value was 0.93 % (n=6), suggesting high precision of the tests.

### 3.2.4 Repeatability evaluation
The test was conducted under the same chromatographic conditions. The sample solution to be tested was prepared six times using the same method described in Section “2.2.2”. Then, 10 μL of each of the obtained sample solutions and reference control solution were injected and tested. The peak area values were measured and the emodin content was calculated to be 0.310 mg/g with an RSD of 2.04%, suggesting a satisfactory repeatability of the tests.

### 3.2.5 Stability evaluation
The same sample solution was injected at 0, 4, 8, and 10 h after preparation. The average peak area value was calculated to be 845.42 with an RSD of 1.97 % (n=6), suggesting that the sample solution remained stable for 10 h.

### 3.2.6 Recovery test

#### Table 2
Results of emodin sample recovery test.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample’s emodin content (mg)</th>
<th>Additional sample (mg)</th>
<th>Content (mg)</th>
<th>Recovery (%)</th>
<th>Average recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2290</td>
<td>0.1975</td>
<td>0.4335</td>
<td>103.55</td>
<td>101.21</td>
<td>2.59</td>
</tr>
<tr>
<td>2</td>
<td>0.2305</td>
<td>0.1975</td>
<td>0.4278</td>
<td>99.88</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>0.2312</td>
<td>0.1975</td>
<td>0.4212</td>
<td>96.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.2294</td>
<td>0.2370</td>
<td>0.4615</td>
<td>101.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.2303</td>
<td>0.2370</td>
<td>0.4745</td>
<td>97.56</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.2308</td>
<td>0.2765</td>
<td>0.5164</td>
<td>104.15</td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>0.2310</td>
<td>0.2765</td>
<td>0.5142</td>
<td>102.11</td>
<td></td>
<td></td>
</tr>
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</table>

Nine samples, each containing 1.0 g of sample powder with known component contents (Batch No. 20120203), were added to 5.0 mL, 6.0 mL, and 7.0 mL of emodin reference solution (mass concentration 0.0395 mg/mL). The sample solution was prepared using the method described in Section “2.2.2.” The final samples to be tested were injected for peak area measurement and the chromatographic measurement conditions were the same as above. The average recovery rate was calculated to be 101.21%, with an RSD of 2.59%. The results indicate high reliability of the proposed test method (see Table 2).

### 3.2.7 Sample measurement
The measurement was performed under the same chromatographic conditions. Three batches of samples obtained using the method described in Section “2.1.2” were used to prepare sample solutions for measurement. Ten microliters of each sample solution and reference solution were injected and the peak area was measured. The content of chlorogenic acid was calculated by using the external standard method and the results are presented in Table 3.

#### Table 3
Measurement results of the samples.

<table>
<thead>
<tr>
<th>Sample batch No.</th>
<th>Chlorogenic acid</th>
<th>Emodin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mass concentration (mg/g)</td>
<td>Average mass concentration</td>
</tr>
<tr>
<td>120203</td>
<td>7.47</td>
<td>7.57</td>
</tr>
<tr>
<td></td>
<td>7.64</td>
<td>7.61</td>
</tr>
<tr>
<td></td>
<td>6.73</td>
<td>6.96</td>
</tr>
<tr>
<td>120410</td>
<td>7.02</td>
<td>7.21</td>
</tr>
<tr>
<td></td>
<td>7.27</td>
<td>2.18</td>
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</table>

### 4. Discussion
In recent years, quality issues concerning TCM herbal preparations have received increasing attention from society[5-8]. Among TCM preparations produced using herbal components, those prepared at hospitals have met with issues regarding quality. Treatment efficacy, which is an important parameter in treatment with TCM, will be unavoidably affected by quality decline of herbal components. Currently, quality standards of herbal medicine prepared in hospitals are remarkably lower than that in the market. Only few quantitative indicators (e.g. content determination) were used to evaluate hospital TCM preparations. Considering the quality decline of herbal components that are currently commercially available, it is necessary...
to enhance the quality standards of hospital TCM preparations and develop more relevant quantitative indicators for quality control, thus ensuring high-quality medicine to be delivered to patients.

In this study, we investigated the content determination method for the two active ingredients (chlorogenic acid and emodin) in Yinhuang Jiangzhi tea. Chlorogenic acid can inhibit lipooxygenase activity during prostaglandin metabolism. Pharmacological effects of emodin include anti-inflammatory antibacterial, immune regulation, trypsin activity inhibition, antioxidant, free radical scavenging, among others. According to relevant literature[9,10], honeysuckles and rhubarbs from different regions vary greatly in chlorogenic acid and emodin contents. As a result, when these materials are used for production, the quality of the resulting TCM preparations will vary inevitably. Hence, it is necessary to develop relevant quantitative indicators and standards. In this study, content measurement of the three sample batches demonstrated that sample batch 120203 and sample batch120410 varied in emodin content to a certain degree, which might be attributed to the quality of herbal components used for production.

References


[9] Liang S. Comparative study on the main active ingredient content of Lonicerae Flos from different producing areas. Chin J Ethnomed Ethnopharm 2014; 23(9): 4-5.