Objective: To develop an HPLC-DAD method for simultaneous determination of chlorogenic acid, caffeic acid, 1, 5-dicaffeoylquinic acid, rutin, spinacetin and quercetin in *Inula* flower dispensing granule.

Methods: Chromatographic separation was performed on a thermo hypersil BDS C18 column (4.6 mm x 250 mm, 3 μm) with a 0.5% citric acid-acetonitrile gradient at the flow rate of 1 ml/min. The column temperature was maintained at 35°C, and detection wavelength was set at 360 nm.

Results: Chlorogenic acid, caffeic acid, 1, 5-dicaffeoylquinic acid, rutin, spinacetin and quercetin were linear in the ranges of 0.101-2.020 μg (r=0.9998), 0.052-1.040 μg (r=0.9997), 0.210-4.200 μg (r=0.9997), 0.142-2.840 μg (r=0.9998), 0.045-0.900 μg (r=0.9996) and 0.050-1.000 (μg (r=0.9997), respectively. The average recoveries were 98.37%, 98.57%, 99.11%, 98.93%, 98.81% and 99.26%, respectively, and the RSDs were 0.82%, 0.91%, 0.86%, 1.19%, 0.93% and 0.86%, respectively.

Conclusion: The HPLC-DAD method was accurate, convenient and reproducible for quality control of the multi-component in *Inula* flower dispensing granule.

Keywords: *Inula* flowers dispensing granule, Composition, HPLC-DAD, Chlorogenic acid, Caffeic acid, Quercetin.
Methods

Trials for ascertaining chromatographic conditions and system suitability: Using a Thermo Hypersil BDS C18 (4.6 mm × 250 mm, 3 μm) chromatographic column, and mobile phase of 0.5% citric acid aqueous solution (A)-acetonitrile (B), a gradient elution procedure was carried out at a flow rate of 1.0 ml/min, column temperature of 35°C and detection wavelength of 360 nm. The injection volume of the mixture of standards was 10 μL.

Preparation of standard solutions: The following amounts of standards were accurately and separately weighed: 10.122 mg chlorogenic acid, 5.201 mg caffeic acid, 10.503 mg 1, 5-dicaffeoylquinic acid, 10.140 mg rutin, 4.510 mg spinach protein and 5.021 mg dermatoside. Each standard was put in a separate 10 ml volumetric flask and dissolved in 70% methanol to achieve standard stock solutions containing 1.012 mg/ml chlorogenic acid, 0.520 mg/ml caffeic acid, 1.050 mg/ml 1, 5-dicaffeoylquinic acid, 1.014 mg/ml rutin, 0.451 mg/ml spinach protein, and 0.502 mg/ml dermatoside. A mixture of the stock solutions was obtained by mixing 1 ml of stock solution of chlorogenic acid, 1.0 ml caffeic acid, 2.0 ml 1, 5-dicaffeoylquinic acid, 1.4 ml rutin, 1.0 ml spinach protein, and 1.0 ml dermatoside in a 10 ml volumetric flask, and making up the volume to mark with 70% methanol. This resulted in final concentrations of 0.101 mg/ml chlorogenic acid, 0.052 mg/ml caffeic acid, 0.210 mg/ml 1, 5-dicaffeoylquinic acid, 0.142 mg/ml rutin, 0.045 mg/ml spinach protein, and 0.050 mg/ml dermatoside.

Preparation of the test sample solution: The Inula granule samples were ground, and 0.5 g of each sample was accurately weighed and put into a stopped conical flask to which 30 ml of 70% methanol was added. The flask was then weighed, and the sample was extracted for 60 min by ultrasonic (power 150 W, frequency 40 kHz), after which the flask was re-heated on cooling. After making up for losses in 70% methanol, the flask was shaken and the contents were filtered through a 0.22 μm microporous membrane. The filtrate was used as the test sample solution.

Investigation of linear relationship: Under the outlined chromatographic conditions, 1, 2, 4, 8, 16, and 20 μL of the mixed standard solution were accurately withdrawn for analysis. A standard curve was drawn with an abscissa of amount of standard compound (μg), and an ordinate of the range of peaks, so as to get regression equations and correlation coefficients.

Results

Chromatographic conditions and system suitability

The gradient elution procedure is summarized in Table 1.

Under the above chromatographic conditions, the resolutions between chromatographic peaks of chlorogenic acid, caffeic acid, 1, 5-dicaffeoylquinic acid, rutin, spinach, quercetin and their adjacent chromatographic peaks were all greater than 1.5, and the number of theoretical plates was above 9000. The DAD spectra showed that the peaks of chlorogenic acid, caffeic acid, 1, 5-dicaffeoylquinic acid, rutin, spinach and quercetin in the sample spectrograms were all uni-component peaks.

Table 1. Gradient elution conditions.

<table>
<thead>
<tr>
<th>T (min)</th>
<th>A (%)</th>
<th>B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>88</td>
<td>12</td>
</tr>
<tr>
<td>10</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>35</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>45</td>
<td>35</td>
<td>65</td>
</tr>
</tbody>
</table>
Table 2. Regression equations, correlation coefficients and linear range of six components (n=6).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Linear equation</th>
<th>R</th>
<th>Linear range/μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>y=43121x+1105.2</td>
<td>0.9998</td>
<td>0.101-2.020</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>y=30712x+2822.3</td>
<td>0.9997</td>
<td>0.052-1.040</td>
</tr>
<tr>
<td>1, 5-dicaffeoylquinic acid</td>
<td>y=50520x+1731.1</td>
<td>0.9997</td>
<td>0.210-4.200</td>
</tr>
<tr>
<td>Rutin</td>
<td>y=20832x+2033.2</td>
<td>0.9998</td>
<td>0.142-2.840</td>
</tr>
<tr>
<td>Spinacetin</td>
<td>y=23543x+1221.5</td>
<td>0.9996</td>
<td>0.045-0.900</td>
</tr>
<tr>
<td>Quercetin</td>
<td>y=42038x+983.6</td>
<td>0.9997</td>
<td>0.050-1.000</td>
</tr>
</tbody>
</table>

Table 3. Contents of six active constituents of Inula flower dispensing granule.

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Concentration (mg•g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chlorogenic acid</td>
</tr>
<tr>
<td>0907043</td>
<td>4.51</td>
</tr>
<tr>
<td>0912235</td>
<td>4.46</td>
</tr>
<tr>
<td>0927182</td>
<td>4.43</td>
</tr>
<tr>
<td>1509021 S</td>
<td>4.27</td>
</tr>
<tr>
<td>1203010 S</td>
<td>4.19</td>
</tr>
<tr>
<td>1308003 S</td>
<td>4.20</td>
</tr>
<tr>
<td>1305012 S</td>
<td>4.15</td>
</tr>
</tbody>
</table>

Linearity test

The regression equations and correlation coefficients obtained in the linearity tests are shown in Table 2. The regression equations and correlation coefficients indicate that the HPLC-DAD method has a very good degree of linearity.

Precision test

The RSD values of chlorogenic acid, caffeic acid, 1, 5-dicaffeoylquinic acid, rutin, spinacetin, and quercetin were 1.21%, 1.03%, 0.93%, 1.06%, 1.34%, and 0.85%, respectively. This demonstrates that the HPLC-DAD method used has very high precision.

Repeatability test

In the repeatability test, the RSD values of chlorogenic acid, caffeic acid, 1, 5-dicaffeoylquinic acid, rutin, spinacetin, and quercetin were 1.32%, 1.06%, 1.21%, 1.36%, 0.91%, and 1.40%, respectively. These RSD values indicate that the HPLC-DAD method has very high degree of repeatability.

Stability test

In the 24 h stability test, the RSD values of chlorogenic acid, caffeic acid, 1, 5-dicaffeoylquinic acid, rutin, spinacetin, and quercetin were 1.30%, 0.78%, 1.19%, 1.05%, 1.28%, and 0.94%, respectively. These results show that the tested solutions remained stable within 24 h at room temperature.

Load recovery

The mean recovery of the six components ranged from 97.22 to 99.48%, and all RSD values were less than 2.0%, indicating that the method had a good accuracy.

Composition of Inula granule

The concentrations of the six components of Inula granule are shown in Table 3.

Discussion

In the selection of wavelength for use in the analysis, the six components were scanned at 200-400 nm with a Diode Array Detector (DAD), and it was found that chlorogenic acid, caffeic acid and 1, 5-dicaffeooylquinic acid absorbed maximally at 327 nm, and rutin absorbed maximally at 254 nm. Spinacetin absorbed maximally at 210 nm and 360 nm, while quercetin had maximum absorption at 260 and 370 nm. In view of factors like chromatographic peak area and baseline stability, 360 nm was chosen as the detection wavelength. During the preparation of samples, various solvents were first separately investigated for their suitability e.g. methanol, water, and methanol (60%, 70%, and 80%). The results revealed that 70% methanol had the best effect on the extraction of the six components. In addition, preliminary trials showed that 60 min of ultrasound resulted in complete extraction of sample. Hence the ultrasonic duration was set at 60 min. In addition, trials with gradient elution using various combinations of solvents revealed that with acetonitrile -0.05% phosphoric acid, all the six components in the test samples were separated at baseline, each with a well-defined and sharp chromatographic peak.
The results of analysis of the constituents of *Inula* granule indicated that chlorogenic acid, caffeic acid, 1, 5-dicaffeoylquinic acid, rutin, spinacetin and quercetin were present in all the batches studied. There were only minor differences in composition between the batches from different companies, which are an indication of stability in quality in the various batches. The order of abundance of the components was 1, 5-dicaffeoyl quinic acid > rutin > chlorogenic acid > caffeic acid > spinach quercetin.

*Inula* dispensing granule is a common Chinese patent medicine used in clinics, but its quality control has so remained sketchy. In this study, HPLC-DAD was developed and used successfully for the first time to determine the contents of six active components of the dispensing granule. This technique, which has very high accuracy, is easy to operate, and is considered a reference method for quality control.

**References**


*Correspondence to*

Peijun Cai
Suzhou University
PR China