Research on flavonoids contents in *Fructus sophorae* with capillary zone electrophoresis

**Qiang Shi**¹², **Lu-zhi Sui**³ and **Yuan-qi Lu**¹

¹Analysis and Testing Centre, Dezhou University, University West Road 566, Dezhou, PR China
²Pharmaceutical Research and Development Centre, Dezhou University, University West Road 566, Dezhou, PR China
³Dezhou Environmental Monitoring Centre, Jinhua Street 326, Dezhou, PR China

**Abstract**: Genistin, genistein, kaempferol, quercetin and rutin, five kinds of flavonoids in *Fructus sophorae*, have been analyzed by capillary zone electrophoresis with internal standard calibration. Buffer pH and concentration, applied voltage, β-cyclodextrin and ethanol concentration were optimized and the optimum conditions are: 20 mmol/L borax (pH 9.5) with 8 mmol/L β-cyclodextrin and 5% (v/v) ethanol and at a voltage of 25 kV. The contents of five flavonoids in *Fructus Sophorae* grown in different area of Dezhou, Shandong Province of China were determined by the developed method and with satisfactory results. The distributions of the studied flavonoids were also investigated.

**Keywords**: β-cyclodextrin (β-CD); Capillary Zone Electrophoresis (CZE); flavonoids; genistin; genistein; kaempferol; quercetin; rutin.

**INTRODUCTION**

The flavonoids, which have a large family of over 4000 compounds and possess the capacity of radical absorbance, have many medical effects and are active constituents of many Chinese herbal medicines (Merken and Beecher, 2000). So, identification and determination of flavonoids occupied a very important place in the efficacy and therapeutic reproducibility for Chinese herbal medicine and their medicinal preparation.

Many methods (Maleš and Medić-Šarić, 2001; Sutthanut et al., 2007; Bian et al., 2005; Chen et al., 2001; Zeng et al., 1990; Fiamigos et al., 2004; Qu et al., 2001; Bo et al., 2002; Chen et al., 2002; Cao et al., 2004; Chen et al., 2000; Jiang et al., 2004; Lu et al., 2004; Lu et al., 2005; Lu et al., 2008; Tian, 2002; Pan et al., 2004; He et al., 2012; Liu et al., 2011) have been used in flavonoids analysis. Of all the reported methods, high performance capillary electrophoresis (HPCE) has the advantages of rapidity, high resolution, high efficiency, low cost and has won the acceptance of many analysts from all over the world in recent years.

*Fructus sophorae*, the fruit of Leguminosea plant *Sophora japonica* L., is a traditional Chinese herbal medicine, which has the function of anti-inflammatory, anti-cancer, cooling the blood and staunching (Tian, 2002) and has long been used for osteoporosis, cancer and other illness since ancient time (Ma and Lou, 2006). Genistin, genistein, kaempferol, quercetin and rutin are five flavonoids in *Fructus sophorae* (Jiang and Xiao, 1986). Accurate determination of the five flavonoids is very important for its quality control and its medicinal preparation development.

So far, flavonoids in *Fructus sophorae* have been analyzed by high-performance liquid chromatography (HPLC) (Bian et al., 2005; Pan et al., 2004; He et al., 2012; Liu et al., 2011) and high-performance capillary electrophoresis (Chen et al., 2002). Nevertheless, the analysis time is relatively long (Bian et al., 2005; Pan et al., 2004; He et al., 2012; Liu et al., 2011) and only a few of the above analytes have been analyzed (Chen et al., 2002). In this paper, a capillary electrophoretic method with internal standard calibration has been developed for determination of genistin, genistein, kaempferol, quercetin and rutin in *Fructus sophorae*. The method was applied to analyze the five flavonoids in *Fructus sophorae* of different growth area in Dezhou, Shandong Province, China. The method has the advantages of accuracy, rapidity and low cost and was very important for the quality control of *Fructus sophorae* and its medicinal preparations. As the producing area are very important for the chemical constituents variation and curative effect of Chinese herbal medicine, the results was helpful for the medicinal resource development of *Fructus sophorae* in Dezhou, Shandong Province, China.

**MATERIALS AND METHODS**

**Chemicals and reagents**

All chemicals, unless otherwise stated, were of analytical reagent grade. All flavonoids and 4-methylumbelliferone (internal standard) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Genistin stock solution of 1.0 mg/mL was prepared in 80% (v/v) ethanol solutions. Other standard solutions of 1.0 mg/mL were prepared in ethanol. Buffer solutions were prepared from borax (concentration range: 5-25 mmol/L), β-cyclodextrin (concentration range: 0-10 mmol/L) and ethanol (concentration range: 0-10%, v/v) by dissolving them in

*Corresponding author: e-mail: luyuanqi2002@hotmail.com*
Research on flavonoids contents in Fructus sophorae with capillary zone electrophoresis

ultrapure water. The final pH values were adjusted with 0.1mol/L sodium hydroxide and 0.1mol/L sodium dihydrogen phosphate. All buffer solutions were filtered through a 0.45µm membrane filter and sonicated for 10 min before use.

Electrophoretic conditions
The analysis were carried out in a P/ACE MDQ HPCE system (Beckman Coulter Inc., Fullerton, CA, USA) equipped with a diode array detector operated at 254 nm. A 60.2 cm×50 µm I. D. fuse-silica capillary (Yong Nian Optical Fiber Factory, Hebei Province, China) was utilized with an effective length of 50 cm, and its temperature was maintained at 25°C. Data were collected and analyzed using the Karat 7.0 software from Beckman Coulter running on a IBM Pentium IV-1GHz computer. The working buffer of capillary electrophoresis was 20 mmol/L borax (pH 9.5), with 8 mmol/L β-cyclodextrin and 5% (v/v) ethanol. Buffer pH was adjusted with a pHS-3C pH meter (Leici Instrumentation Factory, Shanghai, China). Electrophoretic procedures, including capillary flushing, quantification and quantitation are the same as reported (Lu et al., 2008).

Sample preparation
Fructus Sophorae samples were collected from Pingyuan (37.16N, 116.44E), Linyi (37.21N, 116.86E), Ningjin (37.64N, 116.80E), Lingxian (37.34N, 116.58E), Qingyun (37.37N, 117.37E), Yucheng (36.95N, 116.66E), Leling (37.74N, 117.22E), Xiajin (36.95N, 116.00E), Wucheng (37.21N, 116.08E), Qihe (37.79N, 116.76E) and Decheng district (37.45N, 116.29E), 11 different areas of Dezhou, Shandong Province in later October, 2007. Afterwards they were washed with ultrapure water and then air dried in the sun for several days after which they were ground in a mill. The samples were sieved to obtain particles of 0.25 mm in diameter. They were dried for 6 hours at 60°C before use.

Thereafter, 0.1000 g of the powder was extracted with 7 mL of 80% (v/v) ethanol by ultrasonication at room temperature for 20 min, then centrifuged at 3000 rpm for 10 min. The resulted supernatant solution was moved into a volumetric flask of 25 mL. The extraction process was repeated three times and the extracts were combined and diluted to 25 mL. Afterwards, 5 mL of the above sample extracts was transferred to a 10 mL volumetric flask and diluted to mark after 0.8 mL internal standard stock solution was added before analysis.

Method verification
The linearity of the five analytes in standard solutions was investigated. The calibration graphs were plotted by concentration (x,µg/mL) against peak area ratio (y, analyte/internal standard). The detection limits were acquired based on three times noise. The reproducibility is estimated by making eight replicate injection of a standard mixture solution under the selected optimum conditions.

Accurate amount of the five flavonoids were added to a Fructus sophorae sample (from Leling city) to do recovery experiments and the recovery value were achieved by the corresponding calibration curve on the same conditions.

RESULTS
Linearity, repeatability and detection limits
The linearity, detection limits and repeatability of the method were shown in table 1 and table 2. The calibration plots of the five analytes were linear in the concentration ranges of 1-220 µg/mL, 0.5-100 µg/mL, 0.5-100 µg/mL, 1-200 µg/mL and 1-220 µg/mL for genistin, genistein, kaempferol, quercetin and rutin, respectively. The variation in migration time and peak area were in the range of 0.39-0.85% and 1.91-3.23%, respectively. The detection limits of the five analytes were in the 0.11-0.32µg/mL range.

Fig. 1: Effects on migration time of the six compounds by buffer pH (a), and concentration (b): 1. genistin; 2. genistein; 3. 4-methylumbelliferone; 4. kaempferol; 5. quercetin; 6. rutin (The meaning of 1, 2, 3, 4, 5, 6 were the same in the figures of the whole paper).
Recovery
The recoveries of the five flavonoids were shown in table 3 and were in the 93.6-105.2% range, which were satisfactory.

DISCUSSION

Effect of buffer pH and buffer concentration
Structures of the six analytes suggest that their pKa should be in the pH 7.81-9.93 range (Morin et al., 1997; Liang et al., 1997; McGhie and Markham, 1994; Wolfbeis et al., 1984). Borax buffer was employed as the running buffer and the effects of buffer pH on the separation was investigated in the pH 7.5-10.0 range. As shown in fig. 1 (a), the migration time of the six compounds increase with the increase of the buffer pH. The six compounds can be well separated when the buffer pH is higher than 9.0. However, higher pH value results in longer analysis time and the analytes are more susceptible to oxidation. In consideration of the analysis time and good separation, pH 9.5 was chosen as a compromise.

Keep the buffer pH at 9.5 and other conditions the same as the pH optimization the influence of buffer concentration was investigated in the 5–25 mmol/L concentration range. As indicated in fig. 1 (b), the migration time and the resolution of the six compounds increase with increasing buffer concentration, the six compounds can be well separated when buffer concentration was higher than 15 mmol/L. The results also showed that the capillary current increase with the increase of the buffer concentration, which will increase the Joule heating effect and in the long run sacrifice the detection limits. In consideration of resolution, analysis time and detection limits, 20 mmol/L was selected as the optimum buffer concentration.

Effect of applied voltage
The effect of applied voltage on the separation was examined in the range of 19–27 kV. The results showed that with the increase of applied voltage, the migration time of the six compounds decreased, which results in shorter analysis time and an improvement of the efficiency. However, the baseline noise increased apparently when the applied voltage exceeded 25 kV, which can make the detection limits deteriorate due to the pronounced Joule heating caused by the applied voltage increase. So, 25 kV was selected as the optimum applied voltage.

Effect of β-CD concentration
Incorporation of β-CD into the running buffer can form some inclusion-complex between hydrophobic analytes and β-CD thus improving the separation (Hoffstetter-Kuhn et al., 1991). In an attempt to attain improvement in the separation of the six compounds, the effect of β-cyclodextrin (β-CD) concentration was examined in the 0–10 mmol/L concentration range. The results were shown in fig. 2, the migration time of rutin didn’t change significantly, while those of other compounds decreased with the increase of the β-CD concentration. Simultaneously, optimum separation can be achieved when the β-CD concentration was at 8 mmol/L, so 8 mmol/L β-CD was adopted in the further experiments.

Fig. 2: The effects of β-CD concentration on migration time of the analytes.

Effect of ethanol
Organic solvent can be added to the buffer solution to reduce the electro-osmotic flow (EOF) thus increasing the time window and improving separation. Different organic modifiers including MeOH, EtOH, n-PrOH and n-BuOH were tested. Based on experiments, EtOH was chosen as the organic modifier. Setting the other conditions as above, 0-10% (V/V) EtOH was used as organic modifier to improve the separation. The results were shown in fig. 3, the migration time of the six compounds increased with the increase of the ethanol concentration. And optimum separation was achieved when ethanol concentration was 5% (v/v). Hereby, 5% (v/v) ethanol was adopted as organic modifier in the following experiments. Under the above optimized conditions, good separation of the six compounds was achieved in 10 min, fig. 4 shows a typical electropherogram of the six compounds.

Fig. 3: Effects of ethanol concentration.
**Table 1:** The regression equations and detection limits\(^a\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Regression equation(^b)</th>
<th>Correlation Coefficient</th>
<th>Linear range ((\mu g/mL))</th>
<th>Detection limit(^c) ((\mu g/mL))</th>
</tr>
</thead>
<tbody>
<tr>
<td>genistin</td>
<td>Y=0.0113X-0.00450</td>
<td>0.9998</td>
<td>1~220</td>
<td>0.27</td>
</tr>
<tr>
<td>genistein</td>
<td>Y=0.03037+0.00061</td>
<td>0.9999</td>
<td>0.5~100</td>
<td>0.11</td>
</tr>
<tr>
<td>kaempferol</td>
<td>Y=0.02249X-0.00557</td>
<td>0.9998</td>
<td>0.5~100</td>
<td>0.14</td>
</tr>
<tr>
<td>quercetin</td>
<td>Y=0.0123X-0.00468</td>
<td>0.9999</td>
<td>1~200</td>
<td>0.25</td>
</tr>
<tr>
<td>rutin</td>
<td>Y=0.0087X-0.00083</td>
<td>0.9995</td>
<td>1~220</td>
<td>0.32</td>
</tr>
</tbody>
</table>

\(^a\) CE conditions are the same as in fig. 4

\(^b\) In the regression equation, the X value is the concentration of analytes \((\mu g/mL)\), the y value is the peak area ratio (analyte/ internal standard)

\(^c\) The detection limit is evaluated on the basis of a signal-to-noise of 3.

**Table 2:** Reproducibility of the peak area and migration time of the compounds \((n=8)\).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration ((\mu g/mL))</th>
<th>Migration time (\text{min})</th>
<th>Peak area (\mu A U \cdot \text{sec})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>R.S.D. (%)</td>
<td>Mean</td>
</tr>
<tr>
<td>genistin</td>
<td>50</td>
<td>7.015</td>
<td>0.59</td>
</tr>
<tr>
<td>genistein</td>
<td>50</td>
<td>7.507</td>
<td>0.39</td>
</tr>
<tr>
<td>4-methylumbelliferone</td>
<td>80</td>
<td>8.016</td>
<td>0.65</td>
</tr>
<tr>
<td>kaempferol</td>
<td>50</td>
<td>8.503</td>
<td>0.54</td>
</tr>
<tr>
<td>quercetin</td>
<td>50</td>
<td>9.329</td>
<td>0.85</td>
</tr>
<tr>
<td>rutin</td>
<td>50</td>
<td>9.618</td>
<td>0.76</td>
</tr>
</tbody>
</table>

**Table 3:** The recoveries in this method with *Fructus sophorae* grown in Leling City \((n=5)\).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Original amount ((mg/g))</th>
<th>Added amount ((mg/g))</th>
<th>Found ((mg/g))</th>
<th>Recovery ((%))</th>
<th>R.S.D. ((%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>genistin</td>
<td>89.68</td>
<td>10.0</td>
<td>99.04</td>
<td>93.6</td>
<td>3.1</td>
</tr>
<tr>
<td>genistein</td>
<td>1.21</td>
<td>10.0</td>
<td>10.89</td>
<td>96.8</td>
<td>3.5</td>
</tr>
<tr>
<td>kaempferol</td>
<td>7.92</td>
<td>10.0</td>
<td>18.13</td>
<td>102.1</td>
<td>4.2</td>
</tr>
<tr>
<td>quercetin</td>
<td>N.F.</td>
<td>10.0</td>
<td>10.52</td>
<td>105.2</td>
<td>3.7</td>
</tr>
<tr>
<td>rutin</td>
<td>9.55</td>
<td>10.0</td>
<td>19.47</td>
<td>99.2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**Table 4:** The contents of the five analytes in *Fructus Sophorae* in different areas of Dezhou \((mg/g, n=5)\)

<table>
<thead>
<tr>
<th>Growth area</th>
<th>genistin ((%))</th>
<th>genistein ((%))</th>
<th>kaempferol ((%))</th>
<th>quercetin ((%))</th>
<th>rutin ((%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pingyuan County</td>
<td>70.93 (2.35%)</td>
<td>0.53 (3.40%)</td>
<td>2.15 (3.25%)</td>
<td>2.42 (3.72%)</td>
<td>9.23 (2.18%)</td>
</tr>
<tr>
<td>Linyi County</td>
<td>65.52 (3.17%)</td>
<td>0.52 (2.96%)</td>
<td>6.81 (2.36%)</td>
<td>1.06 (2.79%)</td>
<td>9.55 (3.12%)</td>
</tr>
<tr>
<td>Ningjin County</td>
<td>78.94 (3.14%)</td>
<td>0.48 (4.43%)</td>
<td>5.33 (3.17%)</td>
<td>1.08 (3.05)</td>
<td>9.05 (3.40%)</td>
</tr>
<tr>
<td>Lingxian County</td>
<td>55.73 (1.45%)</td>
<td>0.37 (3.91%)</td>
<td>4.88 (2.93%)</td>
<td>1.08 (3.48%)</td>
<td>11.49 (1.89%)</td>
</tr>
<tr>
<td>Qingyun County</td>
<td>54.48 (2.82%)</td>
<td>0.54 (2.43%)</td>
<td>7.98 (2.04%)</td>
<td>0.74 (4.2%)</td>
<td>14.12 (2.77%)</td>
</tr>
<tr>
<td>Yucheng County</td>
<td>53.98 (3.46%)</td>
<td>0.42 (3.38%)</td>
<td>2.60 (3.61%)</td>
<td>1.65 (2.60%)</td>
<td>16.32 (2.61%)</td>
</tr>
<tr>
<td>Leling County</td>
<td>89.68 (1.84%)</td>
<td>1.21 (2.79%)</td>
<td>7.92 (2.76%)</td>
<td>N. F(^b)</td>
<td>9.55 (2.52%)</td>
</tr>
<tr>
<td>Xiaijin County</td>
<td>80.38 (2.13%)</td>
<td>0.42 (4.32%)</td>
<td>9.32 (1.81%)</td>
<td>N. F(^b)</td>
<td>5.23 (3.36%)</td>
</tr>
<tr>
<td>Wucheng County</td>
<td>80.49 (2.59%)</td>
<td>0.51 (3.25%)</td>
<td>2.85 (3.49%)</td>
<td>1.42 (2.80%)</td>
<td>9.67 (3.07%)</td>
</tr>
<tr>
<td>Qihe County</td>
<td>86.20 (1.98%)</td>
<td>1.35 (2.29%)</td>
<td>5.24 (3.23%)</td>
<td>1.13 (3.06%)</td>
<td>13.32 (2.22%)</td>
</tr>
<tr>
<td>Decheng District</td>
<td>42.04 (4.02%)</td>
<td>0.42 (3.75%)</td>
<td>8.79 (2.18%)</td>
<td>0.58 (3.83%)</td>
<td>16.89 (1.74%)</td>
</tr>
</tbody>
</table>

\(^1\) The data in the parentheses denote RSD, \(^2\)NF = not found
Fig. 4: Electropherogram of a mixture of five flavonoids and the internal standard. Conditions: Borax, 20 mmol/L; pH 9.5; ethanol, 5% (v/v); β-cyclodextrin, 8 mmol/L; applied voltage, 25kV; temperature 25°C; UV detection wavelength 254nm.

Sample analysis

The developed method was applied to determine genistin, genistein, kaempferol, quercetin and rutin in *Fructus sophorae* grown in different areas of Dezhou, Shandong Province, China. The analysis results were shown in Table 4. As can see from Table 4, the contents of genistin and kaempferol are higher than what Pan et al. (genistin, 17.1 mg/g) (Pan et al., 2004) and He et al. (genistin, 4.48-7.09 mg/g and kaempferol, 0.09-0.22 mg/g) (He et al., 2012) have reported. The contents of rutin and quercetin are comparable to what Liu et al. (rutin, 8.7 mg/g) (Liu et al., 2011), Chen et al. (rutin, 27.5-29.6 mg/g) (Chen et al., 2002) and Bian et al. (quercetin, 0.2-4.2 mg/g) (Bian et al., 2005) have reported. The contents of genistein are lower than the results of He et al., (2.36-3.05 mg/g) (He et al., 2012).

Acknowledgements

This work was jointly supported by the Key Technologies R&D Programme of Shandong Province (2010GSF10615).

References


Lu YQ, Wu CH and Yuan ZB (2005). Determination of...


