Simultaneous determination of geniposidic acid, chlorogenic acid and geniposide in eucommia by HPLC

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Abstract: A high performance liquid chromatography (HPLC) method was established for simultaneous determination of geniposidic acid, chlorogenic acid and geniposide in eucommia. Detection at 240 nm with a reversed-phase column, CH₃OH volume fraction, acidic additive and pH value of mobile phase were studied for their effects on the separability of the compounds. The most suitable separation was obtained with isocratic gradient elution system using CH₃OH-H₂O-H₃PO₄ (12.00 : 87.96 - 0.04, volume ratio) at a flow-rate of 1.0 mL/min. Under the experimental conditions, the capacity factors of three compounds are in 3-13. The sample is separated rightly. The analysis time is 30 min and the retention time of geniposidic acid, chlorogenic acid and geniposide are 6.7 min, 10.5 min and 21 min, respectively.

Key words: eucommia; HPLC; geniposidic acid; chlorogenic acid; geniposide
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1 INTRODUCTION

Eucommia (named duzhong or tuchung in China) is considered as a high-quality anti-hypertensive natural drug without side-effects. Present researches show that the therapeutic effects of eucommia are attributed to the broad existence of biologically active compounds of iridoids, phenylpropanoids, lignin and their glycosides in the barks and leaves. Up-to-date researches in Japan expose that geniposidic acid (GPA) shows intense hypertensive activity in clinical. So Japanese Health and Welfare Ministry authorized it as a food additive for specific health use to prevent hypertensive. And Geniposide (GP) has cancer inhibitory activity, chlorogenic acid (CGA) shows anti-oxidation and antimutagenicity effects.

Hereofore, paper chromatography, capillary electrophoresis and HPLC are the common methods for the analysis of these components. There are some problems in these methods such as limited sensitivity, only determine one or two compounds in plants selectively, simultaneous determination using complex gradient step elution systems, which induce column unsteadiness and need long analysis time. In this experiment, three compounds GPA, CGA and GP from eucommia are separated successfully and analyzed simultaneously by HPLC with isocratic gradient elution. Moreover with the capacity factor as a guideline, the effects of CH₃OH volume fraction, acidic additive and pH value of mobile phase were also investigated in detail. This method can be used in the determination of eucommia products (such as drugs, cortex, leaves, decoction and tincture) for its precision, rapidness and stabilization.

2 EXPERIMENTAL

2.1 Reagents and raw material

GPA and GP, generous gifts from Dr Deyama in Japan; CGA was purchased from national institute for the control of pharmaceutical and biological products in China; CHCl₃, CH₃OH, C₆H₅OH, HCOOH, CH₁COOH, and H₃PO₄, were of analytical grade. Barks of the eucommia from Zhangjiajie in Hunan Province; leaves were collected in autumn.
2.2 HPLC system
Biotronik BT8100 apparatus was equipped with BT8200UV-vis detector, C-R6A chromatopac recorder and ODS-C18 reversed-phase column (inside diameter 4.6 mm, length 200.00 mm, stuffing diameter 10 μm). CH₃OH-Η₂O-H₃PO₄ (12.00:87.96:0.04) was used as mobile phase at a flow-rate of 1.0 mL/min. Sample solution was filtered through a micro-bore membrane which aperture is 0.45 um, then injected 6 μL solution into HPLC. Qualitative analysis is made by retention time and quantitative analysis by peak area.

2.3 Standard solutions handling
Stock solutions of GPA, CGA, GP was prepared by dissolving standard substances GPA, CGA and GP in CH₃OH to the concentration of 1.36 mg/mL, 1.32 mg/mL and 1.42 mg/mL, respectively. The stock solutions were further diluted with CH₃OH to provide working standards solutions which were stored at --10 °C and protected from light.

2.4 Extraction of eucommia sample
2.00 g sample was set in Soxhlet extractor and extracted by adding 60 mL CHCl₃ to discard gutta-percha compounds. The residue was evaporated to remove CHCl₃ and extracted again with 60 mL CH₃OH. The CH₃OH solution was evaporated to dry under low pressure and the residue was reconstituted with CH₃OH in 25 mL flask. Extraction solution can be stable for at least 8 h at room temperature when protected from light.

3 RESULTS AND DISCUSSION

3.1 Detection wavelength
The standard solutions of GPA, CGA and GP were scanned at a UV-vis spectrophotometer to make absorption curves. GPA and CGA have strong absorptive peaks at λ = 240 nm, CGA have two peaks at λ = 238, 335 nm. For a simultaneous determination of GPA, CGA and GP by HPLC detector, λ = 240 nm was chosen as the detection wavelength.

3.2 Mobile phase
3.2.1 Effect of CH₃OH volume fraction
Because of the carboxyl in the molecular structure of GPA, it has delicate preservation with a nonpolarity ODS-C18 reversed-phase column. Different volume fractions of CH₃OH in mobile phase were experimented to study their effects on the capacity factor k' of GPA, CGA and GP. The results are listed in Table 1, where φ(CH₂OH) is the volume fraction of CH₂OH in mobile phase.

<table>
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<tr>
<th>φ(CH₂OH)/%</th>
<th>Capacity factor k'</th>
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<tbody>
<tr>
<td></td>
<td>GPA</td>
</tr>
<tr>
<td>8</td>
<td>4.96</td>
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<tr>
<td>12</td>
<td>2.73</td>
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<tr>
<td>16</td>
<td>1.68</td>
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<td>20</td>
<td>1.00</td>
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<td>24</td>
<td>0.15</td>
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<td>28</td>
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It can be seen that with the increase of CH₂OH volume fraction, the scouring ability is enhanced and the retention time of GPA, CGA and GP become shorter, especially for GPA, which can hardly be preserved. The smaller the capacity factors become, the more obvious a baseline drift becomes. The sharpness of peaks is not significantly changed by adding CH₃OH. Under this condition, the eucommia sample that includes many other compounds cannot be separated rightly because of the interaction of these components and resulting in the overlapness of the apices in chromatogram.

On the contrary, if the volume fraction of CH₂OH is too low, the GP peak is significantly broadened and the analysis time becomes longer. To shorten the analysis time and avoid the baseline fluctuation, 12% CH₂OH was used elementarily.

3.2.2 Effect of acidic additive
When aqueous solution of 12% CH₂OH is chosen, the analysis time is a little longer (42 min) and the peaks of GPA, CGA are dragged peaks, which affects the accuracy of the analysis. To avoid these problems, some acidic additives were added in mobile phase. The presence of these acids can change the ionic strength of the mobile phase, also cause easily deprotonation and result in lower polarity of GPA and CGA, so the peak profile and selectivity of retention are modified. Different volume fraction of HCOOH, CH₃COOH, and H₃PO₄ are added to the mobile phase in our experiments. The results are listed in Table 2, where φ is the volume fraction of acidic additive in mobile phase.

Comparison Table 2 with Table 1, it is found that the separating selectivity of the mobile phase varies with the kind of acid, which affects the retention property of solutes on column. CH₃COOH can shorten the retention time of GPA, CGA, CA. Low concentration of HCOOH and H₃PO₄, en-