Determination of Ferulic Acid in *Angelica Sinensis* after Solid-Liquid Separation using Ionic Liquid as the Solid Extractant

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Abstract. At present paper, an ionic liquid (N-butyl pyridinium hexafluorophosphate, [BPy]PF\(_6\)) that is liquid at room temperature and solid above 75°C was synthesized and the contents of ferulic acid (FA) at *Angelica sinensis* was determined after solid-liquid separation using [BPy]PF\(_6\) as the solid extractant by spectrophotometry. The results show that FA is extracted completely by [BPy]PF\(_6\) at pH 3.34 and [BPy]PF\(_6\) at FA can be stripped by 0.02 mol/L NaOH. The content of FA in commercially available *Angelicae sinensis* was 0.057%. The working curve, linear range, detection limit and recovery rate of FA were Y = 0.0732X + 0.054 \(\left(R^2 = 0.9995\right)\), 5.150×10\(^{-3}\)–3.508 mmol/L, 2.870×10\(^{-4}\) mmol/L and 90.5–109.5%, respectively.

1 Introduction

*Angelica sinensis* is the root of umbelliferae *Angelica sinensis* (Oliv.) diels and for gynecology of traditional Chinese medicine. Ferulic acid (FA) is active ingredient in *Angelica sinensis* and the structure of FA is shown in Fig. 1. FA has a calming effect and can increase coronary blood flow, protection of ischemic myocardium. Due to \(\alpha\)-receptor blocking effects, FA can inhibit aortic smooth muscle contraction and counter pressor effect of chlorine methyl amine, phenylephrine and \(\beta\)-adrenergic etc. Sodium FA benefits to improve the myocardial oxygen supply and demand balance but also has the effect of anti-platelet aggregation. FA can be a mild activation of lymphocytes and promote proliferation of lymphocytes. FA has some anti early pregnant effects. A variety method confirmed that FA is an antioxidant. Hydroxyl groups on the benzene ring (Fig. 1) are active groups, can eliminate free radicals, inhibit oxidation reaction and free radical reactions, combine with membrane phospholipids, protect the organization from damage of antagonists such as membrane lipid, and generate effect of anti-atherosclerosis. FA that can be absorbed by human body and be excreted in the urine is a very safe drug [1, 2]. Because contain a lot of effective constituents in *Angelica sinensis*, determination of FA content by separate UV spectrophotometry can produce serious disturbance and large deviation. Therefore, FA was determined by UV Spectrophotometry after separation [3], or separated and determined directly by high performance liquid chromatography (HPLC) [4], thin-layer chromatography (TLC) [5] and high performance capillary electrophoresis (HPCE) [6]. However, these methods use petroleum ether, methanol and other flammable, explosive volatile organic solvents in the separation process, and cause environmental damage. Therefore green extraction, determination and regulation of FA content in *Angelica sinensis* are important tasks for the health of human body and environmental protection.

Figure 1. Structural formula of FA.

Ionic liquids (ILs) are generally considered as green solvents due to their high thermal stability, very low flammability and negligible vapor pressure. In particular, their highly tunable nature, this positions ILs as the only true designer solvents [7-9]. These characteristics make them to behave very outstanding properties when they are used as solvents compared to conventional molecular liquids. So far ILs has attracted much attention as novel solvents [10-14]. Many researchers have reported solvent extraction behavior of metal ions with water immiscible IL. Hydrophobic ILs with imidazolium cation is frequently used as solvents or diluents in solvent extraction systems [15-19].
Temperature controlled solid-liquid extraction (TC-SLE) is considered a branch of extraction chemistry [20,21]. In this technique, organic phases such as naphthalene, biphenyl, paraffin waxes and N-butylpyridinium hexafluorophosphate ([BPy]PF₆) that are solids at room temperature are employed as extraction solvents when the temperature is higher than their melting points. Water insoluble complexes are readily extracted into the molten organic phases and distribution equilibrium is rapidly achieved at the higher temperature. Phase separation can be easily obtained by cooling the extraction system to room temperature. Previously, we reported the determination of iron [21], copper [22] after TC-SLE and separation of rare earth elements [23,24] by TC-SLE using [BPy]PF₆ as a solid solvent. At present, there are no reports on the separation of organic compounds by TC-SLE. In this paper, the contents of FA at Angelica sinensis was determined by spectrophotometry after TC-SLE using [BPy]PF₆ that is solid at room temperature and liquid above 75°C as the solid extractant. The results were satisfactory.

2 Experimental

2.1 Regents and Apparatus

FA reserve solution was dissolved in ethanol with a certain mass of 99.9% FA, and diluted to 5×10⁻² mol/L solution with water. Dilute the reserve solution to the desired concentration when using. Angelica sinensis purchased from Quanzhou Xinmanxing Dayaofang. Other chemicals used were of analytical or guaranteed reagent-grade and used without further purification. Distilled water was used throughout unless otherwise specified.

An Avatar 360 FT-IR (Nicolet, Waltham, MA, USA) was used to record the IR spectrum. STA 409 PC integrated thermal analyzer (Netzsch, Germany) was employed to note TG-DSC diagram. AV-1800 spectrophotometer (Mapada, Shanghai, China) was used for the determination of FA. A pHS-3C meter (Jinpeng Analytical Instruments Ltd., Shanghai, China) was employed to monitor the pH values at room temperature. A WRS-1B digital melting point apparatus (Precision & Scientific Instrument Co., Ltd., Shanghai, China) was used to determine the melting point of [BPy]PF₆. TC-SLE was carried out using the device described in our previous report [23]. In the device, a Model CSS501 thermostat (Zhongbao thermostat Co., Ltd., Chongqing, China) was used to control the temperature of extraction vessel with a water jacket and a Model DF-101B magnetic stirrer (Rongkai Industry & Trade Ltd., Luoyang, China) was employed to vigorously stir the solution.

2.2 Synthesis and Characterization of [BPy] PF₆

The two steps of the synthesis and the structure of [BPy]PF₆ are shown in Fig. 2. The density of was determined to be 1.2501 g/ml with specific gravity bottle method [25]. The melting point of [BPy] PF₆ is 73.8 °C-75.5 °C measured by WRS-1B digital melting point apparatus. The infrared spectrum (IR) of [BPy]PF₆ (Fig. 3) showed the following bands: 3434.22 cm⁻¹ corresponds to the -O-H stretching vibration of a small amount of water absorbed in [BPy]PF₆, 3146.12 cm⁻¹ and 3106.19 cm⁻¹ are the C-H ring stretching vibrations; 2972.59 cm⁻¹ is the asymmetric stretching peak of C-H from -CH₃; 2882.80 cm⁻¹ is the symmetric stretching peak of C-H from -CH₂; 1638.34 cm⁻¹ and 1489.30 cm⁻¹ are the stretching vibration of C=N; 1505.15 cm⁻¹ and 1470.65 cm⁻¹ are characteristic peaks of the pyridine ring; 1440.32 cm⁻¹ (med.) is the bending asymmetry vibration peak of -CH₂; 1173.46 cm⁻¹ is the in-plane bending vibration peak of the ring C-H bonds; 782.57 cm⁻¹ is the C=C bending vibration peak of the pyridine ring; 685.18 cm⁻¹ is the bending (deformation) vibration peak of C-H on the ring and 833.86 cm⁻¹ and 556.80 cm⁻¹ are characteristic absorption peaks of PF₆. Fig. 4 is the TG-DSC diagram of [BPy]PF₆. It can be seen from Fig. 4 that the extrapolation starting temperature of [BPy]PF₆ thermal decomposition is 323.1 °C, the maximum weight loss rate corresponding to the decomposition temperature is 361.8 °C. The reaction end temperature is 386.4 °C. The whole process was weighed down by 94.5%, and the residual mass was 5.5% for the decomposition of ILs. The thermal decomposition temperature is about 330°C. Before the decomposition of [BPy]PF₆ there is little weight loss, indicating that the thermal stability of [BPy] PF₆ is better.

![Figure 2. Two-step synthesis of [BPy]PF₆.](image)

![Figure 3. IR of [BPy] PF₆.](image)
2.3 TC-SLE Procedures of Angelica Sinensis FA

0.8 g [BPy] PF₆ (organic phase) and 10 mL aqueous phase containing a certain amount of FA and buffer solution in TC-SLE device. The solution was cooled to room temperature after stirring at (80 ± 0.1) °C for 10 min in a magnetic stirrer. Water acidity was determined by pH meter after consolidating organic phase and separating automatically two phases. The content of FA in the aqueous phase was measured at 321 nm by spectrophotometer. FA extracted into [BPy] PF₆ was determined after 10 min back-extraction by 10 ml 0.02 mol/L NaOH solution. The extraction rate of FA (%E) is the ratio of FA extracted into [BPy] PF₆ and FA content in the initial aqueous phase.

2.4 Preparation of Angelica Sinensis Sample

Weigh accurately about 10.0000 g drying Angelica sinensis into a mortar, pulverizing, heating dissolution in a beaker, hot filtration. The filtrate after cooling is transferred to a 100 mL volumetric flask and fixed capacity for use.

3 Results and Discussion

The dosage experiment of [BPy] PF₆ showed that 0.8 g [BPy] PF₆ could completely extract 2.5 mol/L 10⁻² mol/L FA in a volume of water phase 10 mL. The 0.8 g [BPy] PF₆ was used for follow-up experiment.

3.1. Effect of pH on TC-SLE of FA in Angelica Sinensis

Fig. 5 is the influence of pH value on the extraction of FA by [BPy] PF₆. From the diagram, pH value has a great influence on the extraction of FA. The extraction first increases with the increase of pH value, and the extraction rate decreases with pH > 4. In this experiment, pH = 3.34 is selected as the best extractive acidity.

3.2 Back-extraction of FA

FA extracted in [BPy] PF₆ can be back extracted with NaOH solution, as shown by Fig. 6. It is visible from Fig. 6, and 0.02 mol/L NaOH can completely strip the FA in [BPy] PF₆.

3.3 Determination of FA

According to the above test method, the regression equation of FA working curve is \( Y = 0.0732X + 0.054 \) (correlation coefficient \( R^2 = 0.9995 \)), FA accords with beer's law in 5.150×10⁻³ - 3.508 mmol/L range. The limits of detection (LOD) based on signal-to-noise ratio of 3 (S/N=3) were obtained to be 2.870×10⁻⁴ mmol/L for FA. After the TC-SLE separation of FA, the results of UV spectrophotometry at 321 nm were shown in Table 1. From Table 1, the content of FA in Angelica sinensis was similar to that in Sichuan Angelica sinensis reported by literature [26].

Table 1. Determination of content of FA in Angelica sinensis.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Measured concentration (mmol/L)</th>
<th>Average value of concentration (mmol/L)</th>
<th>RSD (%)</th>
<th>The mass fraction of FA in Angelica sinensis (%)</th>
<th>Value of literature [26] (%)</th>
<th>Relative error (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>2.947</td>
<td>2.937</td>
<td>4.77</td>
<td>0.057</td>
<td>Gansu Angelica sinensis 0.139</td>
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<tr>
<td>2</td>
<td>3.073</td>
<td></td>
<td></td>
<td></td>
<td>Yunnan Angelica sinensis 0.076</td>
<td>-25.0</td>
</tr>
<tr>
<td>3</td>
<td>2.785</td>
<td>2.937</td>
<td>4.77</td>
<td>0.057</td>
<td>Sichuan Angelica sinensis 0.059</td>
<td>-3.39</td>
</tr>
<tr>
<td>4</td>
<td>2.797</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.081</td>
<td></td>
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</table>
Table 2 is an experiment on the recovery of FA in *Angelica sinensis* according to the ratio of 0.5:1, 1:1 and 1.5 to 1. From table 2, the recovery rate of FA is 90.5 - 109.5%.

**Table 2.** The recovery of the determination of FA in *Angelica sinensis*.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>FA content in sample (mmol/L)</th>
<th>Amount of the standard added (mmol/L)</th>
<th>Measurement (mmol/L)</th>
<th>Percent recovery (%)</th>
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<tr>
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<td>4.257</td>
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<td></td>
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<td>3.963</td>
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<td>2.971</td>
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<tr>
<td></td>
<td></td>
<td>4.586</td>
<td>7.608</td>
<td>100.9</td>
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</table>

**Conclusion**

A TC-SLE system with an eco-friendly IL as a solid extraction solvent has been developed for the separation and determination of FA in *Angelica sinensis*. The IL phase becomes solid when the temperature decreases to room temperature. After solid-liquid separation, a quantitative extraction of FA is performed. Furthermore the determination of FA in *Angelica sinensis* can be achieved based on a fixed pH at room temperature. The unique properties of the IL allow a highly efficient and selective separation and determination of FA. The proposed TC-SLE with IL provides an alternative route for the separation and determination of FA in different matrices.

**References**


