

Determination of Betaine in Lycii Cortex by Capillary Electrophoresis

Xuwei Peng, Haixing Liu*

Chemistry & Chemical and Environmental Engineering College, Weifang University, Weifang 261061, P.R. China

*Corresponding author e-mail: liuhx@wfu.edu.cn

Abstract. This paper presents the determination of betaine content in Lycii Cortex by high performance capillary electrophoresis (HPCE) method. The borax solution was chosen as buffer solution, and its concentration was 40 mmol at a constant voltage of 20kV and injecting pressure time of 10s at 14°C. Linearity was kept in the concentration range of 0.0113~1.45mg of betaine with correlation coefficient of 0.9. The content of betaine in Lycii Cortex was 61.9 mg/g (RSD = 13.4%) (n = 7). The recovery was in the range of 86.6% - 118.1% (n=4). This method is specific, simple and rapid and accurate, which is suitable for the detection of the content of betaine in Lycii Cortex.

1. Introduction

Lycii Cortex is *Lycium chinense* Mill. root bark, wide distribution and commonly used Chinese medicine. It has the effect of cooling blood, nourishing fever and removing heat. It is suitable for treatment of night sweat due to insufficiency of the refined materials in the viscera, lungs cough and fever, cough blood, internal heat and quench thirst. It has the function of lowering blood sugar, lowering blood pressure, reducing blood lipids fat levels, clearing heat, antibacterial and antiviral [1]. Liu et al [2] established an HPLC method for the determination of total scopoletin content in wild and cultivated Lycii Cortex from different areas. The chromatographic column was C18, the mobile phase was acetonitrile-1% acetic acid (15:85), the flow rate was 0.8 mL/min, the column temperature was at 30°C, the detective wavelength was at 345 nm. Liu et al [3] compared the content change of betaine in wild and cultivated Lycii Cortex from different areas. The content of betaine in Lycii Cortex was studied by HPLC-ELSD. Zhao et al [4] determined and compared the contents of kukoamine A /B and ferulic acid in Lycii Cortex from three different sources (including wild, cultivation and commercially available). The high performance liquid chromatography-diode array detector (HPLC-DAD) was used for determining the contents of kukoamine A /B and ferulic acid in Lycii Cortex. The separation was performed on the Synergi 4u Hydro - RP 80A column (4.6 mm×250 mm, 4 μm) with the mobile phase of acetonitrile-0.5% trifluoroacetic acid at a constant flow rate of 1.0 mL/min by using gradient elution, the column temperature was 30°C and the detection wavelength was 278 nm. The method for determination of scopoletin in Lycii Cortex using HPLC was developed by Zhang et al [5]. Scopoletin was separated on a ZORBAX C18 chromatographic column with 40% methanol (including 0.2% phosphoric acid) as mobile phase, detection wavelength was 345nm. A HPLC method was used to the determination of 18 amino acids in Lycii Cortex [6]. The sample of Lycii Cortex was derived with 10-



ethyl-acridine-2-sulfonyl chloride as procolumn derivation reagent. Akasil-C18 chromatographic column was utilized as stationary phase with mixtures of acetonitrile solution with different mixing ratios as mobile phase in gradient elution, and determined with fluorescence detector. Li et al [7] studied a method for the determining of Kukoamine A in Suxiao Yatongning tinct. The HPLC method was performed on the Agilent HC-C18 (4.6 mm×250 mm, 5 μm) column, the mobile phase consisted of acetonitrile and 0.1% trifluoroacetic acid, gradient elution flow rate was 1.0 mL/min, the column temperature was 35°C, the UV detector was 278 nm. Liu et al [8] investigated the content of microelement of wild and cultivated Lycii Cortex radicis. Collecting wild and cultivated Lycii Cortex radicis in different areas and applying microwave digestion to determine the content of microelements Fe, Mn, Ca, Mg, K, Zn with the method of FAAS in the samples. Liu et al [9] investigated the content of heavy metals in wild and cultivated Lycii Cortex. The sample was prepared via microwave digestion. Using atomic absorption spectroscopy to determine the content of Pb, Cd, As, Hg, Cu in wild and cultivated Lycii Cortex. Sheng et al [10] established an HPLC method for determining of vanillic acid in Lycii Cortex. The separation was performed on an uncoated fused silica capillary column (75μm, 60cm), the running voltage was 14kV, the buffer was 20 mmol/L borax solution, injection time was 5s, and the detection wavelength was 260nm. In this paper, the betaine content in Lycii Cortex was determined by High Performance Capillary Electrophoresis.

2. Experimental section

2.1. Instruments and Reagents

Experimental instruments: CL-1030-type high performance capillary electrophoresis (Beijing Cailu Scientific Instrument Co., Ltd.); HW2000-type chromatography workstation (Nanjing Qianpu Software Ltd.); Capillary (75 μm inner diameter, 60 cm overall length, 52 cm effective length) from Hebei Yongnian Ruifeng Chromatographic Devices Co., Ltd.).

Betaine (Chinese Drugs and Biological Products); Lycii Cortex (Jilin yinhe pharmaceutical Co., Ltd.); Other reagents used in the experiments were all analytical grade; Double-distilled water was used.

2.2. Experimental Methods

Before the start of the experiment, capillary was successively washed with 1 mol·L⁻¹ hydrochloric acid solution, double-distilled water, 1 mol·L⁻¹ sodium hydroxide solution, double-distilled water, buffer solution, each for 8 min. After three times running, capillary was cleaned again using the above method.

Measurements were carded out at 20 kV voltage and 14 °C experimental temperature. UV detection wavelength was 195 nm. Injection time was 10s (7.5 cm height difference).

2.3. Sample Preparation

Lycii Cortex sample solution: Lycii Cortex powder was accurately weighed 1.125 g, added 30 mL water with 25% methanol, cold soak time of 24 h, filtered, washed and set the volume to 50 mL that was the Lycii Cortex sample solution.

Betaine standard solution: Betaine was accurately weighed 5.8 mg, added 4 mL water with 25% ethanol.

3. Results and Discussion

3.1. Selection electrophoresis conditions

Based on past experiment experience, we chose 40 mmol/L borax solution as a running buffer solution. According to the literature, Betaine maximum absorption wavelength was at 195 nm, so we chose the 195 nm detection wavelength.

3.2. Standard curve

First, betaine standard solution that the concentration were 1.45, 0.72, 0.36, 0.18, 0.091, 0.045, 0.023, 0.011 mg/mL was prepared. Each standard solution was run for three times under the above electrophoresis conditions and the results averaged. The chromatogram of betaine standard solution was showed in Figure 1. Taking concentration as the abscissa and peak area as the ordinate, the standard curve was drew. Linear regression equation of betaine (peak area: $y \mu\text{V}\cdot\text{s}$, density: $x \text{ mg/mL}$) and the linear range was as follows: $y=745.7+8370.3x$ ($r=0.99$), 0.0113-1.450 mg/mL.

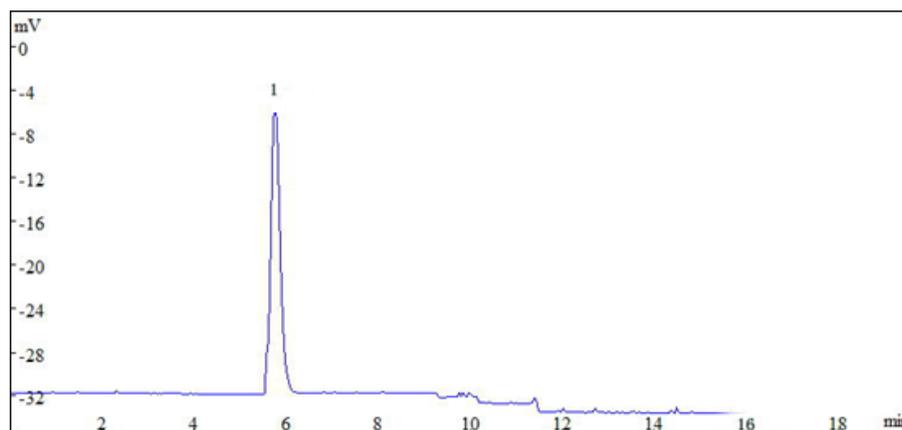


Fig.1 Electrophorogram of betaine standard solution
1-betaine

3.3. Precision test

Betaine standard solution precisely drew and continuously injected for six times under electrophoretic separation conditions, the RSD of betaine migration time and peak area was 3.5% and 4.6% ($n=6$), indicating good precision.

3.4. Determination of sample content

Under selected electrophoresis conditions, Lycii Cortex sample solution was run. Separation chromatogram of the Lycii Cortex sample solution was showed in Figure 2. Measured betaine content in Lycii Cortex was 61.9 mg/g (RSD = 13.4%) ($n = 7$).

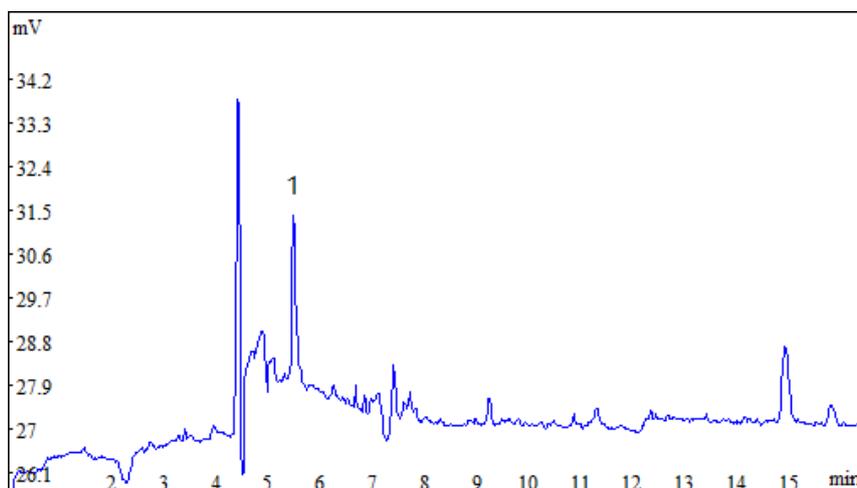


Fig.2 Electrophorogram of Lycii Cortex sample solution
1-betaine

3.5. Recovery

After determination for three times, the recovery of betaine in Lycii Cortex sample was in the range of 86.6% - 118.1% (n=4).

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