IOP Conf. Series: Materials Science and Engineering **274** (2017) 012074 doi:10.1088/1757-899X/274/1/012074

Determination of Betaine in Lycium Barbarum L. by High Performance Capillary Electrophoresis

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Abstract. This paper presents the determination of betaine content in Lycium barbarum L. 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 20℃. Linearity was kept in the concent ration range of 0.0113~1.45mg of betaine with correlation coefficient of 0.9. The recovery was in the range of 97.95%~126% (n=4). The sample content of betaine was 29.3mg/g and RSD 6.4% (n=6). This method is specific, simple and rapid and accurate, which is suitable for the detection of the content of betaine in Lycium barbarum L.

1. Introduction

As sources of natural antioxidants, a lot of attention has been paid to plants. Lycium barbarum L. is one of the most significant traditional Chinese medicinal plant species. Most of its functions were reported as immunity improvement, anti-oxidation, anti-radiation, anticancer, enhancing hemopoiesis, anti-aging, and enhancing sex. The presence of various functional components like polysaccharides, flavonoids, and carotenoids in Lycium barbarum L. [1, 2]. MA Baolon et al [3] established the determination method of spermine and spermidine in Lycium barbarum L. leaves. Analytica1 condition is approached and put forward optimal condition on HPLC for chloride derivatives of spermine and sperm idine. Yi Zhibiao et al [4] established the method of determining Scopoletin in Lycium barbarum L. by HPLC. A method was executed on a C_{18} column using the methanol-0.1% acetic acid as the mobile phase. Quercetin was used as standard to establish a new spectrofluormetric method for determination of total flavonoids in Lycium barbarum L. by FENG Junxia et al [5]. TAN Liang et al [6] established a high performance liquid chromatography method for simultaneous determination of protocatechuic aldehyde, catechin, epicatechin, caffeic acid and ferulic acid in Lycium barbarum L. from diferent habitats, different species in same habitats, and different wild and artificial cultivation in same habitats. A method for determination of lycopene in Lycium barbarum L. using HPLC was developed by Chen Xiang-ming et al [7]. Lycopene was separated on an ultimate C_{18} column (250 × 4.6mm, 5μm) with acetic ether and acetonitrile as mobile phase, and the detection wavelength was set at 475nm. In this paper, the betaine content in Lycium barbarum L. 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); Lycium barbarum L. (Weifang 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 $mol \cdot L^{-1}$ hydrochloric acid solution, double-distilled water, 1 $mol \cdot L^{-1}$ sodium hydroxide solution, double-distilled water, buffer solution, each for 5 min. After three times running, capillary was cleaned again using the above method.

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

2.3. Sample Preparation

Lycium barbarum L. sample solution: Lycium barbarum L. powder was accurately weighed 5.342 g, added 30 mL water with 30% methanol, cold soak time of 12 h, filtered, washed and set the volume to 50 mL that was the Lycium barbarum L. sample solution.

Betaine standard solution: betaine was accurately weighed 5.8 mg, added 4 mL water.

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 μV•s, density: x mg/mL) and the linear range was as follows: y=745.7+8370.3x (r=0.99), 0.0113-1.450 mg/mL.

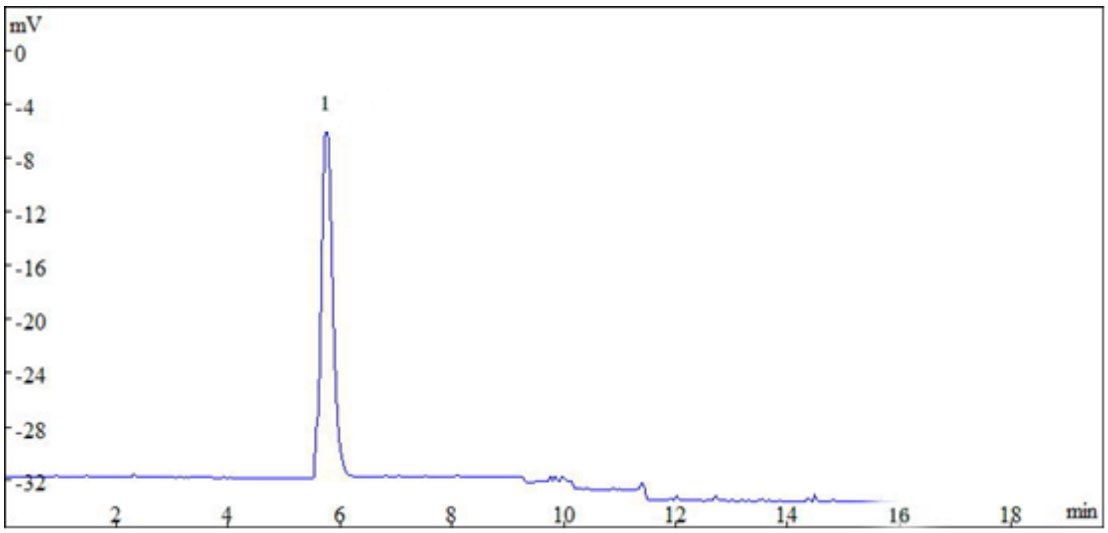

Fig.1 Electrophorogram of betaine standard solution
1-betaine

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, Lycium barbarum L. sample solution was run. Separation chromatogram of the Lycium barbarum L. sample solution was showed in Figure 2. Measured betaine content in Lycium barbarum L. was 29.3 mg/g (RSD = 6.4%) (n = 6).

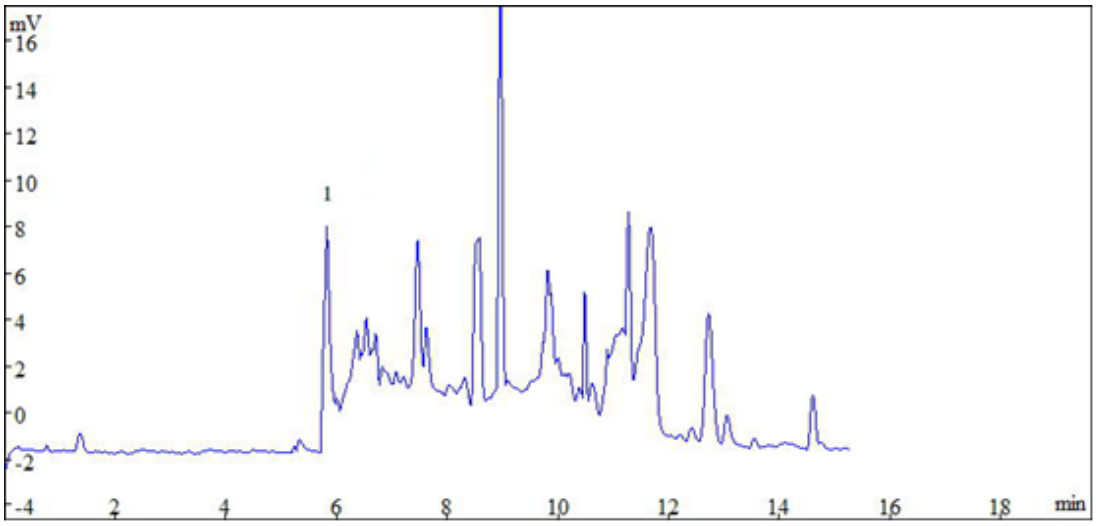

Fig.2 Electrophorogram of Lycium barbarum L. sample solution
1-betaine

3.5. Recovery

After determination for four times, the recovery of betaine in Lycium barbarum L. sample was in the range of 97.9% - 126% (n=4).

Acknowledgments
This study were supported by the Natural Science Foundation of Shandong Province (No. ZR2015BL029 and ZR2010BL025), Open Project of State Key Laboratory of Supramolecular Structure and Materials (No. sklssm201323) (Jilin University), State Key Laboratory of Inorganic Synthesis and Preparative Chemistry (No. 2011-13) (Jilin University).

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