Determination of Betaine in Jujube by Capillary Electrophoresis

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Abstract. This paper presents the determination of betaine content in jujube 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℃. 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 jujube was 85.91 mg/g (RSD = 16.6%) (n = 6). The recovery of betaine in jujube sample was in the range of 86.2% - 116.6% (n=3). This method is specific, simple and rapid and accurate, which is suitable for the detection of the content of betaine in jujube.

1. Introduction
Jujube is Zizyphus jujuba Mill. Fruit. It contains rich nutrients Sugar, fat, vitamin, organic acids, amino acids and a variety of trace elements, etc. It also has saponins, alkaloids, flavonoids, and triterpene compound medicinal ingredients. It has the effects of decocted paste for strengthening middle, tranquilizing the mind by nourishing the heart [1]. Pu et al [2] established a method for determination of cAMP (Adenosine cyclic 3, 5-monophosphate) in Ziziphus jujube and analysis the content of cAMP in different cultivars of Ziziphus jujube. Stationary phase was C18 column (5μm, 4.6 mm×250 mm), mobile phase was composed of methanol-0.02 mol/L KH2PO4 (18:82), detection wavelength at 260 nm, and flow rate was 1.0 mL/min. Zhou et al [3] established a method for the content determining of oleanolic acid and ursolic acid in Zizyphus Jujuba of Xinjiang. The content of oleanolic acid and ursolic acid was detected by RP-HPLC. The separation was performed on a Kromasil C18 column (250 mm×4.6 mm, 5 μm). The mobile phase was methanol-0.03 mol/L sodium dihydrogen phosphate (adjusted to pH 3.0 with phosphoric acid) (90:10); the flow rate was 0.8 mL/min; the detective wavelength was set at 210 nm. The column temperature was 25°C. The contents of betulic acid, oleanic acid, ursolic acid, cyclic AMP and cyclic GMP in Jujubae Fructus was determined by Gao et al [4] using HPLC. The chromatographic conditions were performed on with Shim-pack VP-ODS C18 column (150 mm×4.6 mm, 5μm) with the mobile phase consisted of methanol-water with 0.2% phosphoric acid ( 90:10), and the detection wavelength at 210 nm for betulic acid, oleanic acid and ursolic acid, and of methanol-0.05 mol/L KH2PO4 solution ( 10:90) at 254 nm for cyclic AMP and cyclic GMP. In this research, the contents of betulic acid, oleanic acid, ursolic acid, cyclic AMP and cyclic GMP in different Jujubae Fructus cultivars were tested and analyzed by HPLC. Wei et al [5] pointed out the optimization of sample preparation conditions
including reaction temperature, anthrone concentration and amount, color development time and cooling time for the determination of soluble sugar in red jujube fruits using a combination of single factor and orthogonal array designs. The results showed that 1 min reaction at room temperature in the presence of 3 mL of 25 mg/mL anthrone reagent followed by 25 min of cooling was found to be optimal. The optimized analytical method was characteristics of simple operation, rapidity and high sensitivity. Taking red jujube as raw materials, in the traditional water extraction, alcohol sinking method extracting jujube polysaccharide on the process of water extraction process of adding papain, papain and its hydrolysis conditions of polysaccharide extraction yield, the influence of the optimal extracting technological conditions was determined by Zhang et al [6]. It used the red jujube polysaccharide extraction yield as index, liquid ratio, enzyme amount, extraction temperature, extraction time as factors, designs orthogonal experiment. Experimental results showed that red jujube polysaccharide in the best condition for material liquid is 1:7, plus enzyme was 4.0 mg/mL, extraction temperature was 60°C, the extraction time was 3 h, jujube polysaccharide can be extracted as the basis of optimization of process. A method was explored by Chen et al [7] using ICP-AES for the simultaneous determination on the content of trace heavy metals including Na, Mg, Al, K, etc in 3 spieces of jujube of Xin Jiang district. Zhao et al [8] determined metal elements in Zizyphus Jujuba by flame atomic absorption spectrometry (FAAS). The Zizyphus Jujuba powder was digested with HNO₃ by microwave digestion before determination, eight metal elements in Zizyphus Jujuba were determined by FAAS. The results of the experiment indicated that the contents of the eight metal elements were rich in Zizyphus Jujuba, among which are comparatively high Na, K, Ca, Mg, Fe, Zn, Cu and Mn in Zizyphus Jujuba. The work conditions, accuracy and precision of the method were investigated. Flame atomic absorption spectrometry was used to determine the metallic elements of Fe, K, Ca, Mg, Cu in jujube juice, and the Mn, Zn, Sn, Pb, Cr was detected using graphite furnace atomic absorption spectrometry by Yan et al [9]. The analytical parameters including lighting system, heating procedures and the concentration of standard samples were studied. Based on the optimum experiment conditions, the linear equations and correlation coefficients were obtained. Jialexi et al [10] investigated the extraction process of trace selenium in red dates. The experiment adopted Box-Behnken experimental design of the response surface method to choose experimental factors and standards, then optimizing 3 factors: the ratio of material to liquid, the ratio of digestive fluid and the digestion time which influence the extraction rate of red rates of selenium. In this paper, the betaine content in jujube 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); Jujube (Weifang market); 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
Jujube sample solution: Jujube powder was accurately weighed 1.110 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 jujube 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.

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, jujube sample solution was run. Separation chromatogram of the jujube sample solution was showed in Figure 2. Measured betaine content in jujube was 85.91 mg/g (RSD = 16.6%) (n = 6).
3.5. Recovery
After determination for three times, the recovery of betaine in jujube sample was in the range of 86.2% - 116.6% (n=3).

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