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Determination of Betaine in Forsythia Suspensa by High Performance Capillary Electrophoresis

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Abstract. This paper presents the determination of betaine content of Forsythia suspensa by high performance capillary electrophoresis (HPCE) method. The borax solution was chosen as buffer solution, and its concentration was 40 mmol with capillary column (75μm×52/60cm) 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·ml-1 of betaine with correlation coefficient of 0.999. The recovery was in the range of 97%-117% (n=5), The content of betaine was 281.5 mg·g-1and RSD value of 9.6% (n=6) in Forsythia suspensa. This method has the advantage of rapid, accurate and good repeatability in separation and determination of betaine in Forsythia suspensa.

1. Introduction

Forsythia suspensa is one of the traditional Chinese medicine. Its pharmacological effects is very extensive. It is the main raw material of many Chinese medicine, having antibacterial, strengthen the heart, conducive to pee and alleviate vomiting action in clinical treatment. The extraction solution of Forsythia suspensa has antibacterial action outside the body, inhibiting staphylococcus, E. Coli, salmonella typhi, diphtheria, shigella and streptococcus positive negative bacterium [1, 2]. ZHAO Shao-hua et al [3] established a high performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD) method for the simultaneous determination of oleanolic acid and ursolic acid in the leaves of Forsythia suspensa. The chromatographic separation was executed a Waters Symmetry C_{18} column (4.6 mm× 250 mm, 5μm) with a mobile phase composed of methanol and 0.4% acetic acid (93:7, V/V) at a flow rate of 0.4 mL/min. Huang Jiu-lin et al [4] determined the content of forsythia and total flavonoids in different parts of Forsythia suspensa, studied their antioxidant ability, and analysed the relations of their content with antioxidant ability. Liang Jun et al [5] established an high performance liquid chromatography and fluorescence detection (HPLC-FLD) method for simultaneous determination of five components such as (+)-pinoresinol-β-D-glucoside, (+)-epipinoresinol-β-D-gluceside, phillyrin, phillygenin and forsythoside in different parts of Forsythia suspensa. The separation was performed on a reversed phase C_{18} column (4.6 mm× 250 mm, 5μm) by a gradient elution program with a mobile phase gradient consisting of methanol and water at the flow rate of 1.0 mL/min. To obtain optimal process conditions for the extraction of forsythin from leaves of Forsythia suspensa, the effects of ethanol concentration, material-to-liquid ratio, extraction

temperature, extraction time on forsythin yield were investigated using single factor method by Ji Li-li [6]. Xia Hui et al [7] established an HPLC method for the simultaneous determination of phillyrin and forsythoside A in Forsythia suspensa. The separation was carried out in an Agilent reversed-phase Zorbax SB C_{18} column (4.6 mm× 250 mm, 5μm). The mobile phase was contained acetonitrile and 0.2% formic acid water solution in a gradient elution mode. In this paper, the betaine content in Forsythia suspensa 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); Forsythia suspensa (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

Forsythia suspensa sample solution: Forsythia suspensa powder was accurately weighed 1.4448 g and 6.0040 g, added 30 mL water with 30% and 50% methanol, cold soak time of 12 h, filtered, washed and set the volume to 50 mL that were the Forsythia suspensa sample solution 1 and 2.

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), 0.011-1.450 mg/mL.

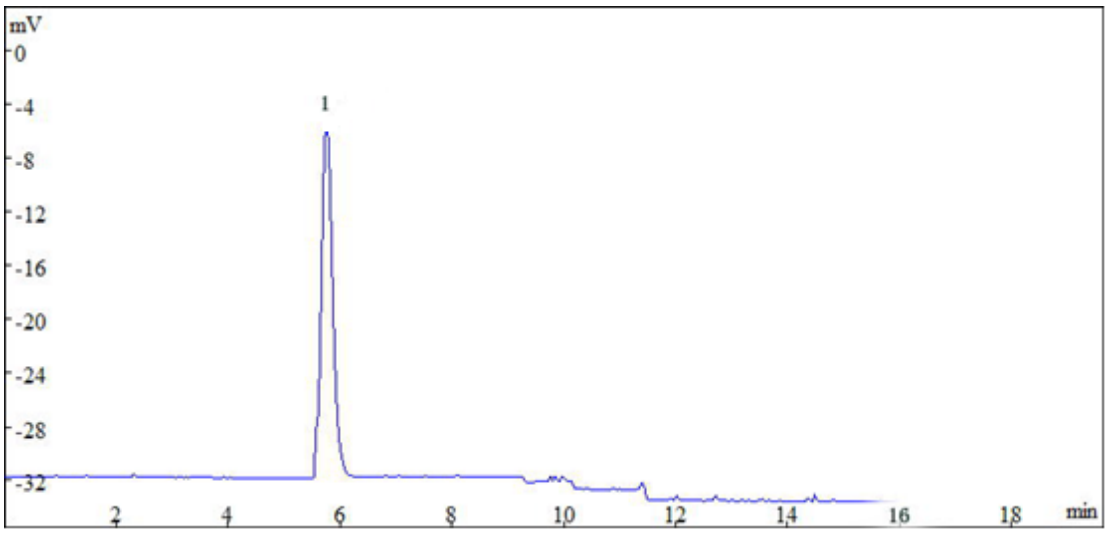

Fig.1 Electrophorogram of betaine standard solution
1-betaine

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, Forsythia suspensa sample solution were run. Separation chromatogram of the Forsythia suspensa sample solution was showed in Figure 2. Measured betaine content in Forsythia suspensa sample 1 and sample 2 were 281.5 mg/g (RSD = 9.6%) (n = 6) and 274.1 mg/g (RSD = 4.2%) (n = 6).

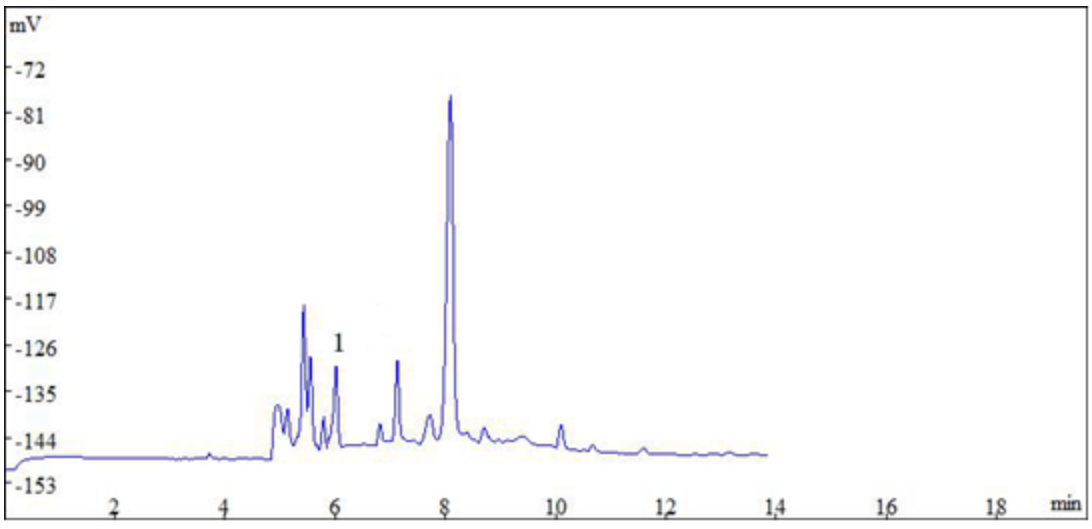

Fig.2 Electrophorogram of Forsythia suspensa sample solution
1-betaine

3.5. Recovery

After determination for four times, the recovery of betaine in Forsythia suspensasample 1 was in the range of 97% - 117% (n=6).

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References
[1] Yang Xue-yan, Liu Cheng-lun, Guizhou Agriculture Science, 2012, 9, 233-237
[2] Zhou Yan, Zhang Tao, Zheng Qin-qin, Yang Cheng-lin, Fan Hao-yue, Current Biotechnology, 2014, 4(4), 292-295
[3] ZHAO Shao-hua, LIU Min-yan, WANG Yu-feng, XU Hong-hui, ZHANG Yong-feng, WANG Meng, Food Science, 2012, 33(2), 224-226
[4] Huang Jiu-lin, Wei Chun-yan, Li Qing-hua, Journal of Agri. Sci., 2012, 40(9), 5209-5210
[5] Lian Jun, Zhang Yu, Xia Yong-gang, Wang Bing-you, Kuang Hai-xue, ACTA Chinese Medicine and Pharmacology, 2015, 43(2), 18-22
[6] Ji Li-li, Forest By-product and speciality in China, 2014, 1, 11-13
[7] Xia Hui, Liu Feng-qun, Zhou Yan-ping, Zhang Shi-long, Zhou Xu, Han Jin, Pharm J Chin PLA, 2014, 30(1), 60-62