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Determination of Bergenin in Pulmonaria officinalis by High Performance Capillary Electrophoresis

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Abstract This paper presents the determination of bergenin content of Pulmonaria officinalis by high performance capillary electrophoresis (HPCE) method. The borax solution as buffer solution was chosen, and its concentration was 20 mmol/L at a constant voltage of 20kV and injecting time of 10s. Linearity was kept in the concent ration range of 0.085~1.35mg/mL of bergenin with correlation coefficient of 0.998. Measured bergenin content in Pulmonaria officinalis was 0.231 mg/g (RSD = 3.44%) (n = 6). The recovery was in the range of 97% - 116% (n=6). This method is specific, simple and rapid and accurate, which is suitable for the detection of the content of bergenin in Pulmonaria officinalis.

1. Introduction

Pulmonaria officinalis is consists of bitter apricot seed, ephedra herb, hairyvein agrimonia herb and bud, japanese ardisia herb, root, snakegourd seed, cape jasmine fruit, natural indigo, clam shell(calcined), etc. It has the effect of hemostasis, reduce phlegm, guiding qi downward, Dingchuan, Antiperspirant and antipyretics. It is used for the treatment of spitting blood, hemoptysis, bloody sputum, cough, asthma, shortness of breath and excess injury lung [1]. Bergenin is the main effective component of japanese ardisia herb. Liao [1] developed a quantitative method for determination bergenin in bergenin grains using HPLC, the determination is carried out on Agilent column C18 (5μm, 4.6 mm×250 mm) and mobile phase consisting of methanol – water(17:83) with the flow rate of 1.0mL/min. The wavelength was set at 275nm. Bergenin is the active ingredient of saxifragaceae plants, such as purple bergenia herb, featherleaf rodgersflower, astilbe, et al. It is white loose needle crystal or crystalline powder, micro gas, bitter, In case of light and heat discoloration. It is soluble in methanol and slightly soluble in water, acetone or ethanol. The research showed bergenin has the effect of relieve cough, immune enhancement, promoting the recovery of diseased tissue, antiviral HIV, anticoagulant and liver protection [2]. Chen et al [3] developed aquantitative method for determination of bergenin, 11-o-galloyl bergenin and 11-o-(4-hydroxyl benzoyl) bergenin in Cissus pteroclada Hayata with HPLC, the expermient is studied in gradient separations with a column SinoChrom ODS-BP C18 (5μm, 4.6mm × 200mm) and solvent consisted of MeCN-0.1% formic acid. The flow rate is 0.8mL/min. The wavelength was set at 275 nm. Li et al [4] established HPLC method for determining of bergenin in An'erning Keli. The content of bergenin in An'erning Keli was obtained by HPLC on a Boston C18 column (250 mm×4.6 mm, 5μm) with the column temperature of 25°C. The mobile phase consisted of methol and water (17:83, V/V) with the flow rate of 1.0 mL/min. The detection wavelength was 430 nm. Luo et al [5] developed a quantitative method for determination bergenin and resveratrol simultaneously in Cissus pteroclada Hayata using HPLC, the determination is carried out in gradient separations with a column SinoChrom ODS-BP C18 (5μm, 4.6 mm×200 mm) and mobile phase consisting of water-acetonitrile with the flow rate of 1.0mL/min. The wavelength was set at 318nm. Chen et al [6] established an method for determination bergenin and quercitroside simultaneously in Ardisiae Japonicae Herba Dispensing Granules. An HPLC was performed on Diamonsil C18 column (4.6mm ×250 mm, 5μm) at 30°C with the mobile phase of acetonitrile (A)-0.1% phosphoric acid (B) for gradient elution at a flow rate of 1.0 mL/min. Liang et al [7]

established a method for simultaneous determination of bergeninum, chlorogenic acid, baicalin, wogonoside, scutellarin, baicalein, wogonin and apigenin in Qinggan sanjie formula. The HPLC method was applied. The determination was investigated on Phenomenex Gemini C18 (150 mm×3 mm, 3μm) with mobile phase consisting of 0.2% formic acid-0.2% formic acid methanol (gradient elution) with the flow rate of 0.4 ml/min. The column temperature was 20°C and the detection wavelength at 270 nm. He et al [8] established a RP-HPLC method for the determining the content of bergeninum in compound bergeninum tablets. The column used is Diamonsil C18(5μm, 4.6mm ×250mm) and the temperature of 30°C. The mobile phase is acetonitrile-water(15:85). The flow rate was 1.0 mL/min and the detection wavelength at 272nm. The bergenin was extracted from Suogudan via ultrasonic assisted alcohol method, and was determined using ultraviolet spectrophotometry by Wang et al [9]. The results showed that the optimal extraction conditions were as follows: alcohol concentration 50%, extraction temperature 60°C, ratio of solid to liquid 1:14, extraction time 50 min. Because Bergenin having wide range of biological activities, eight aza-bergenin derivatives were synthesized by Mannich reaction and Mitsunobu reaction, and their structures were characterized by 1H NMR, 13C NMR and HRMS by Mao et al [10]. Their antitumor activities were investigated in vitro against human tumor cell line A549 by the MTT assay. The simple method based on matrix solid-phase dispersion with molecularly imprinted polymers as the sorbent was explored by Zhu et al [11] for selective extraction of bergenin from Ardisia japonica. The MIPs were synthesized using bergenin as a template molecule, methacrylic acid as a functional monomer, and ethylene glycol dimethacrylate as a crosslinking agent. The polymers have been characterized by scanning electron microscopy and Fouriertransform infrared spectrometry. The maximum extraction yield of bergenin was studied. The optimized extraction conditions as: 2/1 as the ratio of MIPs to the sample; 8 min as the dispersion time; 10% aqueous methanol as a washing solvent and methanol-acetic acid (99: 1, V/V) as an elution solvent. The extract obtained was discussed by HPLC. In this paper, the bergenin content in Pulmonaria officinalis 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, 52 cm overall length, 44 cm effective length) from Hebei Yongnian Ruifeng Chromatographic Devices Co., Ltd.).
bergenin (Chinese Drugs and Biological Products); Pulmonaria officinalis (Jilin huangzihua 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 8 min. After three times running, capillary was cleaned again using the above method.
Measurements were carded out at 20 kV voltage and experimental temperature at 20°C. UV detection wavelength was 275 nm. Injection time was 10s (7.5 cm height difference).

2.3 Sample Preparation

Pulmonaria officinalis sample solution: Pulmonaria officinalis powder was accurately weighed 3.004 g, added 30 mL water with 30% methanol, extracted time of 2h at 100°C, filtered, washed and set the volume to 50 mL that was the Pulmonaria officinalis sample solution.
Bergenin standard solution: Bergenin was accurately weighed 5.4 mg, added 4 mL water with 30% methanol.

3. Results and Discussion

3.1 Selection electrophoresis conditions

Based on past experiment experience, we chose 20 mmol/L borax solution as a running buffer solution.

According to the literature, bergenin maximum absorption wavelength was at 275 nm, so we chose the 275 nm detection wavelength.

3.2 Quantitative analysis

3.2.1 Standard curve

First, bergenin standard solution that the concentration were 1.350, 0.675, 0.338, 0.169, 0.085 mg/mL was prepared. Each standard solution was run for three times under the above electrophoresis conditions and the results averaged. The chromatogram of bergenin 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 bergenin (peak area: y μV•s, density: x mg/mL) and the linear range was as follows: Y=-6404.58+129218.88X (r=0.998), 0.085~1.350 mg/mL.

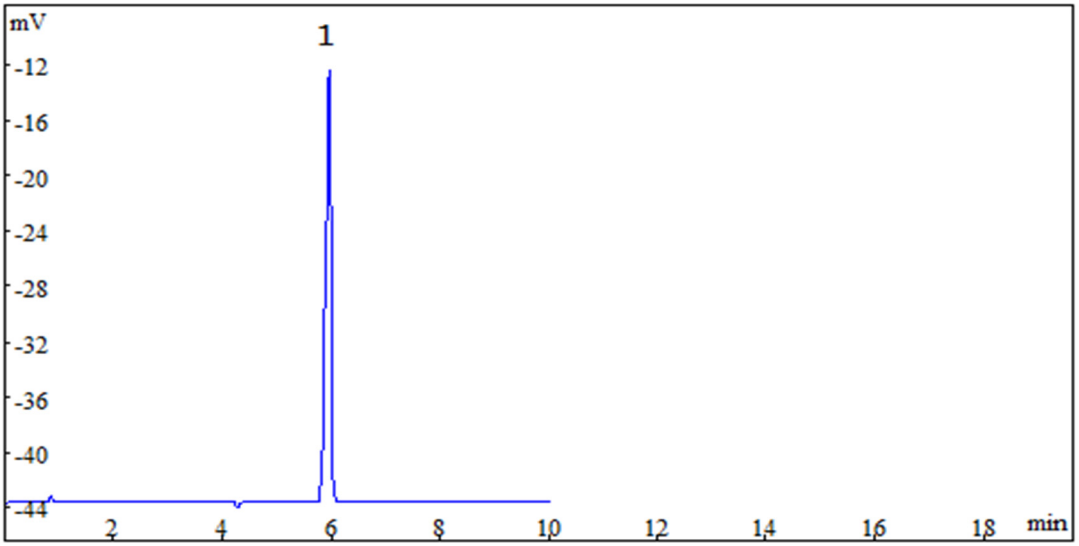

Fig.1 Electrophorogram of bergenin standard solution 1- bergenin

3.2.2 Precision test

A bergenin standard solution precisely drew and continuously injected for sixt times under electrophoretic separation conditions, the RSD of bergenin peak area were 3.92%, indicating good precision.

3.2.3 Determination of sample content

Under selected electrophoresis conditions, Pulmonaria officinalis sample solution was run. Separation chromatogram of the Pulmonaria officinalis sample solution was showed in Figure 2. Measured bergenin content in Pulmonaria officinalis was 0.231 mg/g (RSD = 3.44%) (n = 6).

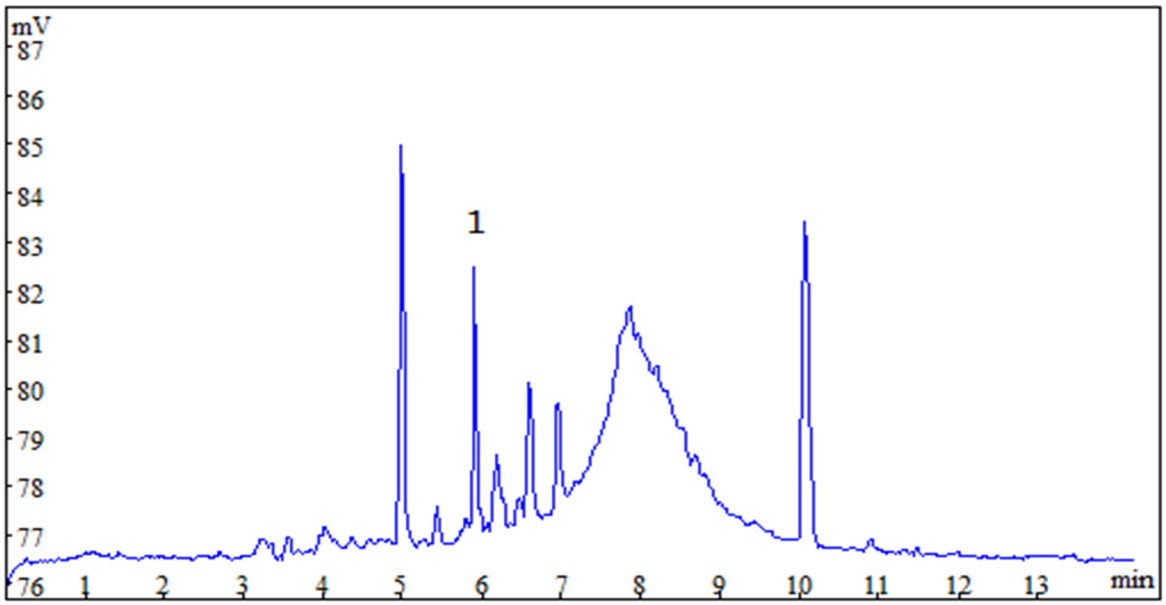

Fig.2 Electrophorogram of Pulmonaria officinalis sample solution
1- bergenin

3.2.4 Recovery

After determination for six times, the recovery of bergenin in Pulmonaria officinalis sample was in the range of 97% - 116% (n=6).

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