Determination of Nevadensin in Fewflower Lysionotus Herb by Capillary Electrophoresis

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Abstract. This paper investigated the determination of nevadensin content in Fewflower Lysionotus Herb by high performance capillary electrophoresis (HPCE) method. The borax solution of 30 mmol concentration containing was chosen as buffer solution. The experiment was performed at a constant voltage of 16 kV and UV detection wavelength of 335 nm. The content of nevadensin content in Fewflower Lysionotus Herb was 2.818 mg/g (RSD=3.22%) (n=6). The recovery of nevadensin in Fewflower Lysionotus Herb sample was in the range of 88.1%-105.8% (n=5). This method is suitable for the detection of the content of nevadensin in Fewflower Lysionotus Herb.

1. Introduction

Fewflower Lysionotus Herb is whole herbs of Lysionotus Pauciflorus of gesneriaceae plant. It has a long history of medication as traditional Chinese medicine. It has the functions of clearing lung and phlegm, cooling blood and hemostasis, clearing dampnes and eliminating indigestion and activating meridians to stop pain. It is used for the treatment of lymphoid tuberculosis, chronic bronchitis, Spitting blood, bruises, cough with lung heat, bacillary dysentery, plot rickets, rheumatic pain, etc. Nevadensin has various biological effects such as mycobacterium tuberculosis, anti-inflammatory, lowering blood pressure and eliminating free radicals [1]. For exploring the extraction process of nevadensin from Fewflower Lysionotus, the single factor experiment and artificial neural network genetic algorithm technology was utilized by Zhang et al [2] to optimize the concentration of ethanol, extraction time, extraction times and solid-liquid ratio. The better extraction of nevadensin was obtained as follows: ethanol concentration 84%, extracting time 2.8h, solid-liquid ratio 1:17, and extraction 2 times, respectively. An HPLC-CL linkage method was based on the strong sensitive chemiluminescence of the CH₃CN-H₂O₂ and Cu(NH₄)²⁺ system in alkaline medium was established by Wu et al [3]. The experiment was investigated on a Hypersil ODS column with a mixture of CH₃CN and HCONH(CH₃)₂ and 0.1 mol/L H₂C₂O₄ (27:6:67, v/v/v) as mobile phase. Zhang et al [4] determined eleven trace metal elements (Cd, Cu, Ni, Pb, Mn, Hg, Zn, As, Fe, Cr and Ca) contents in Fewflower Lysionotus Herb. The Fewflower Lysionotus Herb samples were digested with a mixture of HNO₃ and HClO₄, trace metal elements were measured with ICP-AES method. Zhang et
al [5] explored a comparative experiment for nevadensin content in four species gesneriaceae plants from Guizhou. The experiment was carried out on Agilent ZORBA SB-C18 column (4.6mm×150mm, 5μm) with a mixture of methanol and 0.5% acetic acid (65:35) as mobile phase at the flow rate of 1.0mL/min. The detection wavelength was set at 335nm. The root, stem, leaf and flower of genuine medicinal materials in Yunnan province were investigated by Chen et al [6] using FT-IR. The different part of the same Fewflower Lysionotus Herb presented similar absorption peak. Because they locate in different part, contain chemical ingredients and different contents, their absorbed varied IR peaks indicated different intensity. The obtained IR spectra present the root, stem, leaf and flower of Fewflower Lysionotus Herb lack of peaks. According to the shape and range of absorption peak raised by the vibration of qualitative analysis and functional group, the chemical ingredients of Fewflower Lysionotus Herb can be obtained. There are alkaloid, flavonoid, flavonoid aglycones and β-sitostero in Fewflower Lysionotus Herb. Gou et al [7] studied the antioxidant activity in vitro of total polyphenol from Fewflower Lysionotus Herb. The single factor was employed to analyze the effects of extraction time, ultrasonic power, extraction temperature, extraction times, and ethanol concentration liquid-material ratio on the extraction rate of total polyphenol. The antioxidant activity in vitro of total polyphenol from Fewflower Lysionotus Herb was assessed by reducing power, hydroxyl and DPPH free radical scavenging effect. The experiment indicated that the optimum condition for extracting the polysaccharides assisted by ultrasonic wave was extraction time of 32min and ultrasonic power of 100% and extraction temperature of 25°C and 3 extraction times and ethanol concentration liquid-material ratio of 20:1. Total polyphenol from Fewflower Lysionotus Herb was an effective and natural antioxidant and free radical Scavenger. The influence of the three kinds of B vitamins, VB1, VB2 and VB3 on the interaction between BSA and lysionotin in physiological buffer (pH=7.4) was studied by Zhang et al [8] using various spectroscopic methods including UV-vis absorption, fluorescence and circular dichroism spectroscopy. The binding parameters such as the Stern-Volmer dynamic quenching constant, binding constant, the number of binding sites and binding distance were obtained, and the influence mode of the vitamins were developed. In recent years, capillary electrophoresis has been widely used in drug analysis [9-11]. In this paper, the nevadensin content in Fewflower Lysionotus Herb 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.).

Nevadensin (Chinese Drugs and Biological Products); Fewflower Lysionotus Herb (purchase in Anhui Haozhou pharmacy); 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 5 min. After three times running, capillary was cleaned again using the above method.

Measurements were carried out at 16 kV voltage and experimental temperature at 27°C. UV detection wavelength was 335 nm. Injection time was 10s (7.5 cm height difference).

2.3 Sample Preparation

Fewflower Lysionotus Herb sample solution: Fewflower Lysionotus Herb was accurately weighed 1.2655 g, added 40 mL water containing 80% methanol, extracted time of 24h at 27°C, filtered,
washed and set the volume to 50 mL that was the Fewflower Lysionotus Herb sample solution.

Nevadensin standard solution: Nevadensin was accurately weighed 0.0038 g, added 4 mL water containing 80% methanol.

3. Results and Discussion

3.1 Selection electrophoresis conditions
The experiment was carried out at 16 kV voltage. UV detection wavelength was 335 nm.

Based on past experiment experience, 30 mmol/L borax solution was chosen as electrolyte solution.

3.2 Quantitative analysis

3.2.1 Standard curve. First, nevadensin standard solution was prepared and its concentrations were 0.475, 0.2375, 0.1187, 0.0594, 0.02969, 0.01484, 0.00742 mg/mL. Each standard solution was run for three times under the above electrophoresis conditions and the results averaged. The chromatogram of nevadensin 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 nevadensin (peak area: y μV•s, density: x mg/mL) and the linear range was as follows: y = 636.8+487451X (r=0.997), 0.00742-0.475 mg/mL.

![Fig.1 Electrophorogram of nevadensin standard solution](image)

3.2.2 Precision test. A nevadensin standard solution precisely drew and continuously injected for six times under electrophoretic separation conditions, the RSD of nevadensin migration time and peak area were 1.33% and 3.81%, indicating good precision.

3.2.3 Determination of sample content. Under selected electrophoresis conditions, Fewflower Lysionotus Herb sample solution was run. Separation chromatogram of the Fewflower Lysionotus Herb sample solution was showed in Figure 2. Measured nevadensin content in Fewflower Lysionotus Herb was 2.818 mg/g (RSD=3.22%)(n=6).
3.2.4 Recovery. After determination for five times, the recovery of nevadensin in Fewflower Lysionotus Herb sample was in the range of 88.1%-105.8% (n=5). The average recovery was 94.2%.

4. Conclusion
This paper investigated the determination of nevadensin content in Fewflower Lysionotus Herb by high performance capillary electrophoresis method. Measured nevadensin content in Fewflower Lysionotus Herb was 2.818 mg/g (RSD=3.22%) (n=6).

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