Determination of Nevadensin in Malan Ganhan capsules by Capillary Electrophoresis

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Abstract. The article analyzed nevadensin content in Malan Ganhan capsules by capillary electrophoresis (CE) technology. The electrolyte solution was 30 mmol borax solution with 16 kV voltage and 335 nm UV wavelength. The content of nevadensin in Malan Ganhan capsules was 0.991 mg/g (RSD=3.43%) (n=6). The recovery of nevadensin in Malan Ganhan capsules sample was in the range of 81.7%-100.2% (n=5). The method is simple and practical.

1. Introduction

Malan Ganhan capsules are consisted of indian kalimeris herb, helleborus thibetanus franch, fiewflower lysionotus herb, perilla, aleppo avens, fineleaf schizonepeta herb and cortex caesalpiniae radicis. It has the effect of relieving, diffusing the lung and suppressing cough. It used for the treatment of headache, chills, fever, nose blowing and cough. Nevadensin has various biological effects such as mycobacterium tuberculosi, anti-inflammatory, lowering blood pressure and eliminating free radicals [1]. The RP-HPLC method was developed by Wang et al [2] for the determining Nevidensin in Ocimum basilicum L.. The Microsorb C₁₈ column (5μm, 4.6mmX250mm) was applied with a mixture of methanol and 1% acetic acid(86:14) as mobile phase at the flow rate of 0.5ml/min. The detection wavelength was 335nm. Qiu [3] identified Paris saponin I and Paris saponin II by TLC and determined the content of nevadensin in Yanlian compound tablets by HPLC. The Dimensions C₁₈ column (250mmx4.6mm, 5μm) was applied with a solvent system of methanol: water(including 1% acetic acid) (75:25). The flow rate was set at 1.0ml/min, and the wavelength of detector was set at 330nm. The optimizing extraction of Ganqing Granules was studied by Tian et al [4] using the L₀(3⁴) orthogonal experiment. With gallic, nevadensin, hygroscopicity, fluidity and formability as the indexes, the dry granulating process was estimated. The results indicated that the optimizing extraction procedures were as follows: adding 7 -fold water, refluxing extraction for 2 times (1 hour per time); concentrating of reduced pressure to the relative density of around 1.09 under the temperature of 75°C- 80°C; the hygroscopicity, fluidity and formability of granules were in accord with manufacture requirements. The structure - activity relationship of eleven pure and natural flavonoids scavenging DPPH were investigated by Chen et al [5] by the spectrophotometry for the detection of DPPH. The results indicated that the eleven flavonoids may be effective for scavenging
DPPH. 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 [6] 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. Capillary electrophoresis was proven to be an effective technology in drug analysis [7-10] by many facts. In this paper, the nevadensin content in Malan Ganhan capsules was determined by CE.

2. Experimental section

2.1 Instruments and Reagents
Experimental instrument: CL1030 high performance capillary electrophoresis instrument (Cailu Beijing Scientific Instrument company); HW2000 chromatography workstation (Nanjing Qianpu Software); Capillary column (44 cm effective length, 52 cm overall length, 75 μm inner diameter) from Hebei Yongnian Ruifeng Chromatographic Devices company).

Nnevadensin (Chinese Drugs and Biological Products); Malan Ganhan capsules (Yunnan Baiyao Pharmaceutical limited company, Batch number: ZFA1737); The analytical grade reagents utilized in experiment; Double distilled water was adopted.

2.2 Experimental Methods
First, 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 sample injection, the processing method was the same as above.

The experiment was performed at 16 kV voltage and 27°C temperature. The UV collection wavelength was 335 nm. The sample injection time was 10s (7.5 cm height difference).

2.3 Sample Preparation
Malan Ganhan capsules sample solution: Malan Ganhan capsules was accurately weighed 1.033 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 Malan Ganhan capsules 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 tested at 16 kV voltage. The UV collection wavelength was 335 nm.

Based on past experiment experience and the sample investigation, the buffer solution selected 30mmol/L sodium borate 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. Under the above conditions, the running process was repeated three times and the results averaged for each standard solution. The chromatogram of nevadensin standard solution was showed in Figure 1. The standard curve was drawn by experimental data. The linear regression equation of nevadensin (y: peak area, μV·s; x: density, mg/ml) and the linear range was as follows: y= 636.8+487451X (r=0.997), 0.00742-0.475 mg/ml.
3.2.2 Precision test. A nevadensin standard solution was running for six times under the above conditions, the RSD of nevadensin migration time and peak area was 1.33% and 3.81%, respectively.

3.2.3 Determination of sample content. Under the above conditions, Malan Ganhan capsules sample solution was run. Separation chromatogram of the Malan Ganhan capsules sample solution was showed in Figure 2. Measured nevadensin content in Malan Ganhan capsules was 0.991 mg/g (RSD=3.43%)(n=6).

3.2.4 Recovery. After determination for five times, the recovery of nevadensin in Malan Ganhan capsules sample was in the range of 81.7% - 100.2% (n=5). The average recovery was 91.6%.
4. Conclusion
This paper detected nevadensin content in Malan Ganhan capsules using capillary electrophoresis. Measured nevadensin content in Malan Ganhan capsules was 0.991 mg/g (RSD=3.43%) (n=6)

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