Determination of Arctiin in Lingyang Ganmao tablets by Capillary Electrophoresis

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Abstract. This paper investigated the determination of arctiin content in Lingyang Ganmao tablets by high performance capillary electrophoresis (HPCE) method. The borax solution of 37.5 mmol concentration containing 12.5% methanol was chosen as buffer solution. The experiment was performed at a constant voltage of 16 kV and UV detection wavelength of 277 nm. The content of arctiin in Lingyang Ganmao tablets was 12.81 mg/g (RSD=3.5%) (n=6). The recovery was in the range of 81.3%-113.4% (n=5). This method is suitable for the detection of the content of arctiin in Lingyang Ganmao tablets.

1. Introduction

Lingyang Ganmao tablet is composed of antelope horn, great burdock achene, fermented soybean, honeysuckle flower, fineleaf schizonepeta herb, weeping forsythiae capsule, common lophatherum herb, platycodon root, liquorice root, peppermint. It has the function of heat-clearing and detoxifying. It is used for treatment of influenza, colds and coughs, dizziness fever, sore throat, etc. The electronic tongue system with virtual instrument technology was explored by Shi et al [1] and used to distinguish and analyze 4 kinds of Chinese patent medicines for cold symptom. The respond signal of electronic tongue was first preprocessed using the featurepoint extraction (FPE) method and discrete wavelet transform (DWT) method, respectively. On the base of clustered property and classification results of sample points, the DWT applied “db4”as mother wavelet and decomposed 8 levels was chosen as a recommended feature extraction method. The principal component analysis, cluster analysis and back propagation neural network were then applied for distinguish and identify the 4 kinds of Chinese patent medicines. Lin et al [2] evaluated the quality situation and problems of Lingyang Ganmao Tablets by testing and analyzing samples collected from drug manufacture and market all over China in 2013. The legal test with exploratory research was combined. The Lonicera confusa DC was inspected by HPLC-ELSD. The contents of honeysuckle, liquorice, forsythia, burdock were determined by HPLC. The NIR spectrum database was established as the foundation for instant test. The statistical analysis was complied for the results of legal test and exploratory research. Lin et al [3] established the GC fingerprint of Lingyang Ganmao tablets. The analysis was carried out on DB-5ms capillary column. Flame ionization detector was used to temperature of 250 ℃ (temperature programming). The temperature of injector was selected 240 ℃. The nitrogen was selected as carrier gas with flow rate of 1.0 mL/min; the sample size was 1 μL by split sampling with split ratio of 20:1. Using peppermint ketone as reference, Similarity Evaluation Software for Chromatographic...
**Fingerprint of Traditional Chinese Medicine** *(2012 edition)* was applied for the similarity analysis of 10 batches of Lingyang Ganmao tablets. Du et al [4] established a method for determining content of total lignanoids in arctium tomentosum Mill seeds. In accordance with total lignanoids and arctiin in the same peak absorption, arctiin was used as reference substance and absorption value of the samples at 280 nm was determined. Li et al [5] established a method for separation of high purity arctigenin in Arctii Fructus with combining acid hydrolysis by high-speed counter-current chromatography (HSCCC). The crude extract of Arctii Fructus was obtained with alcohol extraction and acid hydrolysis, followed using chloroform extraction. The separation condition was obtained. The solvent system was chosen with petroleum ether-ethyl acetate-methyl-water (2:5:3:4, v/v) where the upper phase was applied for the stationary phase and the lower phase as the mobile phase and the flow rate of 10.0 mL/min. The rotary speed was 850 rpm and the detection wavelength was set at 280 nm. Cao et al [6] determined the contents of matairesinoside, matairesinol, arctiin, and arctigenin in different parts of Forsythia suspensa by HPLC. The solvent system was composed of acetonitrile-water with the flow rate was of 1 mL/min at gradient elution. The detection wavelength was set at 220 nm and the column temperature was 30 °C. Gu et al [7] established the method of determining forsythiaside, arctin, lonicera macranthoides saponin B and dipsacus kawabata saponin B in Shuanghuanglian oral liquid. The UPLC-MS/MS method was used with BEH C18 (100 mm × 2.1 mm, 1.7 μm). The mobile phase was consisted of acetonitrile-0.1%formic acid solution in gradient elution mode. The flow rate was set at 0.4 mL/min. Electrospray ionization source and multi-reaction ion monitoring was adopted. In this paper, the arctiin content in Lingyang Ganmao tablets 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.).

Arctiin (Chinese Drugs and Biological Products); Lingyang Ganmao tablets (Beijing Tongrentang Pharmaceutical limited company, Batch number: 16120228); 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 21°C. UV detection wavelength was 277 nm. Injection time was 10s (7.5 cm height difference).

#### 2.3. Sample Preparation

Lingyang Ganmao tablets sample solution: Lingyang Ganmao tablets was accurately weighed 2.6823 g, added 40 mL water containing 80% methanol, extracted time of 24h at 21°C, filtered, washed and set the volume to 50 mL that was the Lingyang Ganmao tablets sample solution.

Arctiin standard solution: Arctiin was accurately weighed 0.0046 g, added 2 mL water.

### 3. Results and Discussion

#### 3.1. Selection electrophoresis conditions

The experiment was carried out at 16 kV voltage. UV detection wavelength was 277 nm.
Based on past experiment experience, 37.5 mmol/L borax solution containing 12.5% methanol was chosen as electrolyte solution.

3.2. Quantitative analysis

3.2.1. Standard curve. First, arctiin standard solution was prepared and its concentrations were 2.3, 1.15, 0.575, 0.2875, 0.1437, 0.0718, 0.0359 mg/mL. Each standard solution was run for three times under the above electrophoresis conditions and the results averaged. The chromatogram of arctiin 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 arctiin (peak area: \( y \mu V\cdot s \), density: \( x \) mg/mL) and the linear range was as follows: \( y = -2653.6 + 84674.5x \) \((r=0.998)\), 0.0359-2.3 mg/mL.

![Electrophorogram of arctiin standard solution](image)

**Fig.1** Electrophorogram of arctiin standard solution

1-arctiin

3.2.2. Precision test. A arctiin standard solution precisely drew and continuously injected for six times under electrophoretic separation conditions, the RSD of arctiin migration time and peak area were 2.25% and 4.91%, indicating good precision.

3.2.3. Determination of sample content. Under selected electrophoresis conditions, Lingyang Ganmao tablets sample solution was run. Separation chromatogram of the Lingyang Ganmao tablets sample solution was showed in Figure 2. Measured arctiin content in Lingyang Ganmao tablets was 12.81 mg/g \((\text{RSD}=3.5\%)\) \((n=6)\).
3.2.4. Recovery. After determination for five times, the recovery of arctiin in Lingyang Ganmao tablets sample was in the range of 81.3%-113.4% (n=5). The average recovery was 99.5%.

4. Conclusion

This paper investigated the determination of arctiin content in Lingyang Ganmao tablets by high performance capillary electrophoresis method. Measured arctiin content in Lingyang Ganmao tablets was 12.81mg/g (RSD=3.5%) (n=6).

Acknowledgments

This study were supported by the Natural Science Foundation of Shandong Province (No. ZR2010BL025), Open Project of State Key Laboratory of Supramolecular Structure and Materials (No. sklssm201323) (Jilin University), State Key Laboratory of Inorganic Synthesis and Preparative Chemistry (No. 2011-13) (Jilin University).

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