Determination of Arctiin in Yinqiaojiedu Granules by Capillary Electrophoresis

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Abstract. This paper investigated the determination of arctiin content in Yinqiaojiedu granules 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 Yinqiaojiedu granules was 1.99 mg/g (RSD=2.44%) (n=7). The recovery was in the range of 87.1%-121.6% (n=5). This method is suitable for the detection of the content of arctiin in Yinqiaojiedu granules.

1. Introduction
Yinqiaojiedu granules consists of, honeysuckle flower, weeping forsythiae capsule, peppermint, fineleaf schizonepeta herb, fermented soybean, great burdock achene] (fried), platycodon root, common lophatherum herb and liquoric root 9 traditional Chinese medicine etc. It has the functions of cooling and relieving exterior, clearing away heat and detoxification. It is used for the treatment of for wind-heat cold, fever, headache, cough, dry mouth and sore throat. The fusion high performance liquid chromatographic fingerprint combined with quantification and principal component analysis (PCA) was successfully explored by Ma et al [1], which was used to assess the quality consistency of 25 batches Yinqiaojiedu tablets. The resolution index I was utilized as an objective function to evaluate chromatographic conditions. The chromatographic fingerprints were built by reversed-phase high performance liquid chromatography. The wavelengths were set at 230, 279 and 327 nm, separately. Then, the three wavelength fusion fingerprint was explored by the technique of multi-wavelength fusion fingerprint. The linearity, precision, repeatability, stability and accuracy of the method were consistent with the fingerprint analysis criteria. Subsequently, the systematic quantified fingerprint method was adopted for integrative quality discrimination of Yinqiaojiedu tablets from both qualitative and quantitative perspectives. Feng et al [2] established the fingerprint identification method by HPLC and provided basis of quality integral control and evaluation for Yinqiaojiedu Tablet. 10 batches of samples from 4 factories were measured using HPLC. The analysis were performed on Hypersil ODS C18 column (200 mm × 4.6 mm, 2.5 μm) with acetonitrile-0.1% phosphoric acid solvent system gradient elution at 30°C. The detecting wavelength was 230 nm. The samples quality was estimated with similarity evaluation system for chromatographic fingerprint of TCM 2004 A. Wang et al [3] developed a unified method for the determination of seven phenolic acids (neochlorogenic acid, chlorogenic acid,
4-caffeoylquinic acid, caffeic acid, isochlorogenic acid B, isochlorogenic acid A and isochlorogenic acid C) contained in honeysuckle flower that was the monarch drug of all the eight Yinqiaojiedu serial preparations by quantitative analysis of multi-components using single-marker (QAMS). Firstly, chlorogenic acid was applied as a reference to obtain the average relative correction factors (RCFs) of the other phenolic acids in ratios to the reference. The columns and instruments from different companies were adopted to validate the durability of the achieved RCFs in different levels of standard solutions; and honeysuckle flower extract was applied as the reference substance to fix the positions of chromatographic peaks. Secondly, the contents of seven phenolic acids in eight different Yinqiaojiedu serial preparations samples were analyzed by the RCFs durability. Finally, the quantitative results were compared between QAMS and the external standard (ES) method. Huo et al [4] developed a method for quality control of Menthae Haplocalycis Herba and Schizonepetae Herba in all preparations of Yinqiaojiedu serial products. The analysis was carried out on HP-5 column (30 m × 0.32 mm, 0.25 μm) by temperature programmed from 50°C to 240°C using menthone, menthol, and pulegone as markers. The methodology validation was made and determination was performed for 38 batches sample of five different preparations of Yinqiaojiedu products. He et al [5] established a rapid GC method for determining organic chloride pesticide residue in Yinqiaojiedu tablets. With different solvents, different extraction methods, extraction and purification of samples was pre-processed, the recovery of peak area was compared to achieve the best pre-processing method. Zhang et al [6] established a HPLC method for determining forsythia and arctiin in Yinqiaojiedu tablet. The Kromasil C18 column (4.6 mm × 150 mm, 5 μm) was adopted with a mixture of acetonitrile-0.1% iceacetic acid (25: 75) as mobile phase and the flow rate of 1.0 mL/min and the detection wavelength of 280 nm. Sun et al [7] developed a digitized HPLC fingerprint (HPLC-FP), logarithm fingerprint (ln-FP), reversed fingerprint(R-FP) and square fingerprint(S-FP) to determine the quality of Yinqiaojiedu pills. The sample fingerprints were characteristically digitized and evaluated by Digitized Evaluation System of Traditional Chinese Medicine Fingerprint 4.0 to get 46 parameters to excavate the potential information, in which systematically qualified fingerprint method (SQFM) was used to estimate the quality levels of the sample by HPLC-FP, ln-FP, R-FP and S-FP. Qiu et al [8] established an HPLC method for determining paracetamol, chlorogenic acid, forsythia and arctiin in Jingzhi Yinqiaojiedu capsules. The Capcell Pak C18 (150 mm×4.6 mm, 5 μm) column was used. The mobile phase was composed of acetonitrile-water (contained 0.25% glacial acetic acid) with flow rate of 1 mL/min at gradient elution. The detection wavelengths were 300 nm and 228 nm. The column temperature was 30°C. Wan et al [9] compared the dissolution rates of Yinqiaojiedu tablets, capsules and pills from eight manufactures in order to provide reference for clinical medication. The absorbance of chlorogenic acid was detected using ultraviolet spectrophotometry. The dissolution was measured with rotating basket method. The parameters on stripping curves were fitted by Weibull distribution model. In this paper, the arctiin content in Yinqiaojiedu granules 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); Yinqiaojiedu granules (Guangxi Zhengtang Pharmaceutical limited company, Batch number: 20171206); 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
Yinqiaojiedu granules sample solution: Yinqiaojiedu granules was accurately weighed 2.8776 g, added 40 mL water containing 80% methanol, extracted time of 48h at 21°C, filtered, washed and set the volume to 50 mL that was the Yinqiaojiedu granules 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.

![Figure 1. Electrophorogram of arctiin standard solution](image)

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, Yinqiaojiedu granules sample solution was run. Separation chromatogram of the Yinqiaojiedu granules sample
solution was showed in Figure 2. Measured arctiin content in Yinqiaojiedu granules was 1.99mg/g (RSD=2.44%) (n=7).

![Electrophorogram of Yinqiaojiedu granules sample solution 1-arctiin](image)

**Figure 2.** Electrophorogram of Yinqiaojiedu granules sample solution 1-arctiin

3.2.4. **Recovery.** After determination for five times, the recovery of arctiin in Yinqiaojiedu granules sample was in the range of 87.1%-121.6% (n=5). The average recovery was 97.6%.

4. **Conclusion**

This paper investigated the determination of arctiin content in Yinqiaojiedu granules by high performance capillary electrophoresis method. Measured arctiin content in Yinqiaojiedu granules was 1.99mg/g (RSD=2.44%) (n=7).

**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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