Determination of Arctiin in Fengre Ganmao Granules by Capillary Electrophoresis

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Abstract. This paper investigated the determination of arctiin content in Fengre Ganmao 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 Fengre Ganmao granules was 10.307 mg/g (RSD=5.8%) (n=6). The recovery was in the range of 90.5%-115.1% (n=5). This method is suitable for the detection of the content of arctiin in Fengre Ganmao granules.

1. Introduction
Fengre Ganmao granules consists of indigowoad root, weeping forsythiae capsule, peppermint, great burdock achene, chrysanthemum, bitter apricot seed, mulberry twig, reed rhizome, mulberry leaf, medicated leaven and the ear of fineleaf schizonepeta herb 11 traditional Chinese medicine etc. It has the effect of clearing away heat and detoxification, promoting lung and alleviating pharynx. It used for the treatment of wind-heat, cold, fever, nasal congestion, headache, cough, multi-phlegm, etc. Feng Jiangjiang [1] established a method for determining amygdalin, chlorgenic acid and forsythin in Fengre Ganmao granules using HPLC at different UV wavelengths. The analysis was carried out an SHISEIDO capcell pak-C18 column (4.6 mm×250 mm, 5 μm). The mobile phase consisted of methanol-0.1% phosphoric acid solution with gradient elution and flow rate of 1 mL/min. The column temperature was set at 35℃. The detection wavelength was set at 207 nm (amygdalin), 327 nm (chlorogenic acid) and 279 nm (forsythin). The HPLC method was accomplished by Zou et al [2] for determining arctiin in Fengre Ganmao granules. The CLC-DOS (M) C18 column (250 mm×4.6 mm, 5 μm) was adopted at 30 ℃. The solvent system was CH3OH-Water (1:1). The flow rate was 1.0 mL /min and the detection wavelength was set at 280 nm. The content for arctiin in Fengre Ganmao granules was detected by shi et al [3] using HPLC. The hanbon science C18 column (250 mm×4.6 mm, 5 μm) was used. The mobile phase consisted of a mixture of acetonitrile-water (25:75) with the flow rate of 1.0mL/min and the detection wavelength of 275 nm. Ionic liquid-based ultrahigh pressure-assisted extraction (IL-UPE) combined with HPLC was adopted by Wang et al [4] at extraction of arctiin and arctigenin in Fructus Arctii. The influences of experimental factors including ionic liquids with different cations and anions, ionic liquid concentration, extraction pressure, extraction time and...
ratio of solid to liquid, were tested. The optimal condition was obtained with extraction solvent of 0.80 mol/L 1-lauryl-3-methylimidazolium bromide ionic liquid solution and extraction pressure of 200 MPa and extraction time of 2 min and solid/liquid ratio of 1:20(g/mL). The yields of arctiin and arctigenin were 37.15 mg/g and 8.04 mg/g using the optimum condition, respectively. Xue et al [5] investigated the effect of arctigenin on the invasion and migration of human prostate cancer PC3 cells. PC3 cells were cultured in vitro, the inhibition rate of arctigenin (ARG) on the proliferation of PC3 cells was investigated by MTT assay, the cell invasion was determined by Transwell assay, and the cell migration was measured by scratch test. The expression of matrix metallo proteinases (MMP-9), MMP-2 and CXCR4 genes, which were used as biomarkers for reflecting cell invasion and migration, were measured by RT-PCR and their protein expression were measured by Western blot. Liu et al [6] established a method for determining the content of seven bioactive components in Forsythia suspense by UPLC. A quantitative analysis of multi-component by a single-marker (QMAS) analytical method was obtained. The linear range, recovery rate, precision, stability and repeatability were investigated. Tan Xiongsi [7] established a HPLC method for determining the content of arctiin, arctigenin, forsythoside A, narirutin, naringin, rhoifolin, rim-O-glucosylcimifugin and 5-O-methylvisammioside simultaneous in Wuli Huichun Pills. The analysis of 70% methanol extract of this drug was carried out on thermostatic Agilent TC-C18 column (4.6 mm × 250 mm, 5 μm) at 25 ℃. The mobile phase was methanol-0.4% glacial acetic acid with flow rate of 0.9 mL/min at gradient elution. In this paper, the arctiin content in Fengre Ganmao 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); Fengre Ganmao granules (Yunnan Baiyao Pharmaceutical limited company, Batch number: ZF A1737); 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℃. UV detection wavelength was 277 nm. Injection time was 10s (7.5 cm height difference).

2.3. Sample Preparation

Fengre Ganmao granules sample solution: Fengre Ganmao granules was accurately weighed 3.1165 g, added 40 mL water containing 80% methanol, extracted time of 24h at 21℃, filtered, washed and set the volume to 50 mL that was the Fengre Ganmao 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 μV•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.

![Fig.1 Electrophorogram of arctiin standard solution](image1)

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, Fengre Ganmao granules sample solution was run. Separation chromatogram of the Fengre Ganmao granules sample solution was showed in Figure 2. Measured arctiin content in Fengre Ganmao granules was 10.307mg/g (RSD=5.8%) (n=6).

![Fig.2 Electrophorogram of Fengre Ganmao granules sample solution](image2)
3.2.4. Recovery. After determination for five times, the recovery of arctiin in Fengre Ganmao granules sample was in the range of 90.5%-115.1% (n=5). The average recovery was 101.9%.

4. Conclusion
This paper investigated the determination of arctiin content in Fengre Ganmao granules by high performance capillary electrophoresis method. Measured arctiin content in Fengre Ganmao granules was 10.307mg/g (RSD=5.8%) (n=6).

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