Determination of Forsythin in Ling yang Ganmao tablet by Capillary Electrophoresis

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Abstract. This paper set up the determination of forsythia content in ling yang Ganmao tablet by high performance capillary electrophoresis (HPCE) method. The borax solution was chosen as buffer solution, and its concentration was 10 mmol at a constant voltage of 20kV and injecting time of 10s. The content of forsythia in Ling yang Ganmao tablet was 4.176 mg/g (RSD = 4.7%) (n = 6). The recovery was in the range of 87.1% - 107.3% (n=4). This method is suitable for the detection of the content of forsythia in Ling yang Ganmao tablet.

1. Introduction
Ling yang Ganmao tablet consists of antelope horn, great burdock achene, fermented soybean, honeysuckle flower, fine leaf schizonepeta herb, weeping forsythia capsule, common lophatherum herb, platy codon root, liquoric root, peppermint Compound Preparation. It has the effect of clearing heat and detoxifying. It is used for treatment of influenza, colds and coughs, dizziness fever, sore throat, etc [1]. For observing the antiviral, antipyretic, ant cough and anti-inflammatory effect of Linyang Ganmao Tablet, the experimental study was used to observe the effect of the live rate of mouse infected epidemic cold virus the effect of body temperature of rabbit injected endotoxin heat model, the effect of rate of mouse stimulated by gasification ammonia, and the eff ected of the swell of rat’s Feet [2]. The results showed Linyang Ganmao tablet had the effect of antiviral, an ntipyretic, ant cough and anti-inflammatory effect of Linyang Ganmao Tablet, the experimental study was used to observe the effect of the live rate of mouse infected epidemic cold virus the effect of body temperature of rabbit injected endotoxin heat model, the effect of rate of mouse stimulated by gasification ammonia, and the eff ected of the swell of rat’s Feet [2]. The results showed Linyang Ganmao tablet had the effect of antiviral, an ntipyretic, ant cough and anti-inflammatory effect of Linyang Ganmao Tablet. Li et al [3] built up an HPLC method for determining arctiin in ling yang Ganmao Capsules. The ODS-A column (4.6 mm×150 mm, 5 μm) was used. The detection wavelength was 280 nm and a mixture of acetonitrile-water (25: 75) as mobile phase. The flow rate was 0.8 mL/min. Qiu et al [4] established an RP-HPLC method for the determination of chromogenic acid in ling yang Ganmao tablet. The diamonsil C18 column (4.6 mm×250 mm, 4.6 μm) was used, the methanol-water-glacial acetic acid-triethylaminc (18:85:1:0.3) as mobile phase. The detection wavelength was 324 nm. Wang et al [5] established an HPLC method for determination of arctiin and phillyrin in ling yang Ganmao oral liquid. The Agilent C18 column (4.6 mm×250 mm, 5 μm) was used, the mobile phase was composed of acetonitrile-water (25: 75) with flow rate of 1.0mL/min. the detection wavelength was 280nm, and the coulumn temperature was 30°C. The chromogenic acid and the glycyrrhizin acid contents in ling yang Ganmao table were determined by Hao et al [6] using RP-HPLC. The Zorbax SB-C18 column (5 μm, 250 mm×4.6 mm), methanol -0.2 mol/L NH4Ac-glacial acetic acid(67:33:1) as mobile phase, flow velocity at 1.0 ml/min, detection wave length at 250 nm and column temperature at 30°C. Gao et al [7] established a method to determine the arctiin in Linyang Ganmao Pills by SPE (solid-phase extraction).
-HPLC. The chromatographic column was Shim-pack CLC-ODS (6.0 mm ID × 15 cm). The mobile phase was methanol-water (1: 1.1), the flow rate was 1.0 mL/min. The detection wavelength at 280 nm. The column temperature was 30°C. In this paper, the forsythia content in ling yang Ganmao tablet 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.). Forsythin (Chinese Drugs and Biological Products); Lingyang Ganmao tablet (Shanxi hengshan pharmaceutical Co., Ltd.); 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 8 min. After three times running, capillary was cleaned again using the above method. Measurements were carded out at 20 kV voltage and experimental temperature at 20°C. UV detection wavelength was 277 nm. Injection time was 10 s (7.5 cm height difference).

2.3. Sample Preparation

Ling yang Ganmao tablet sample solution: ling yang Ganmao tablet powder was accurately weighed 1.8083 g, added 40 mL water with 30% ethanol, extracted time of 3 h at 60°C, filtered, washed and set the volume to 50 mL that was the ling yang Ganmao tablet sample solution.

Forsythin standard solution: Forsythin was accurately weighed 0.0018 g, added 4 mL water with 30% ethanol.

3. Results and Discussion

3.1. Selection electrophoresis conditions

Based on past experiment experience, we chose 10 mmol/L borax solution as a running buffer solution. According to the literature, forsythia maximum absorption wavelength was at 277 nm, so we chose the 277 nm detection wavelength.

3.2. Quantitative analysis

3.2.1. Standard curve. First, forsythia standard solution that the concentration were 0.45, 0.225, 0.112, 0.056, 0.028, 0.014 mg/mL was prepared. Each standard solution was run for three times under the above electrophoresis conditions and the results averaged. The chromatogram of forsythia 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 forsythia (peak area: y μV•s, density: x mg/mL) and the linear range was as follows: y = -927.3 + 36773.4x(r=0.987), 0.014 - 0.45 mg/mL.
3.2.2. **Precision test.** A forsythia standard solution precisely drew and continuously injected for six times under electrophoretic separation conditions, the RSD of forsythia peak area were 1.08%, indicating good precision.

3.2.3. **Determination of sample content.** Under selected electrophoresis conditions, Lingyang Ganmao tablet sample solution was run. Separation chromatogram of the Lingyang Ganmao tablet sample solution was showed in Figure 2. Measured forsythia content in Lingyang Ganmao tablet was 4.176 mg/g (RSD = 4.7%) (n = 6).

3.2.4. **Recovery.** After determination for six times, the recovery of forsythia in Lingyang Ganmao tablet sample was in the range of 87.1% - 107.3% (n=4).
4. Conclusion
This paper set up the determination of forsythia content in ling yang Ganmao tablet by high performance capillary electrophoresis method. The content of forsythia in ling yang Ganmao tablet was 4.176 mg/g (RSD = 4.7%) (n = 6).

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