

Determination of paeonol in yanyan tablets by Capillary Electrophoresis

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Abstract: This design uses High Performance Capillary Electrophoresis (HPCE) to determine the Paeonol's content in yanyan tablets. Electrophoresis separation controlled conditions for uncoated fused silica capillary column (75 $\mu\text{m}\times 52/60\text{cm}$) injection height 7.5cm, and using a concentration of 40mmol/L borax solution as buffer. Using separate voltage 20KV and detect wavelength 270nm, temperature is 14°C, and the injection time control 10s. The Paeonol is linear relationship($r=0.998$) within concentration of 3~400mg/L, Measured paeonol content in yanyan tablets was 6.14 mg/g (RSD = 11.6%) (n = 5). Measuring recovery of paeonol was in the range of 77.6%-112.8% (n=5). The method is simple, accurate and efficient with good reproducibility.

Introduction

Yanyan tablets were a clinical medicine commonly used for the curing of angina, pharynx dryness and irritable cough induced by chronic pharyngitis for many years. It consisted of 12 wellknown Chinese herbal medicines, such as Radix Scrophularia, Cortex Moutan, Radix Rehmanniae, Semen Oroxyli, etc. Radix Scrophularia was the monarch drug, which played a principle role in pharmacology effects. Harpagoside and cinnamic acid were known as the primary bioactive components of Radix Scrophularia in Yanyan tablets [1,2]. Zhao et al [3] established an RP-HPLC method for determination of the contents of paeoniflorin, paeonol and baicalin in yanyan tablets. Using Zorbax SB-C₁₈ column (250mmX 4.6mm, 5 μm) with mobile phase consisted of methanol(A)-1.5% acetic acid(B) (gradient elution). The detection wavelength was set at 244 nm. ZHANG et al [4] established an HPLC method for simultaneous determination of the contents of seven kinds of flavonoids in Jinhuang yanyan tablets. The separation was carried on Phenomenex Synergi C₁₈ column (250mmX 4.6mm, 5 μm) with mobile phase consisted of methanol(A)-0.2% phosphoric acid(B). The detection wavelength was set at 349 nm. LI et al [5] set up a control method of Yanyan tablets quality. A HPLC method was used to quantitative determination of Harpagoside. Using Agilent TC-CIH (250mmX4.6mm, 5 μm). The mobilic phase was acetonitrile-1% acetic acid solution in linear gradient elution. Guo et al [6] investigated the effects on the extraction rate of paeonol by different extraction methods and to set up a method for the determination of paeonol in yanyan Tablets. FU Yong-hui et al [7] developed an HPLC method for determination the content of harpagoside, cinnamic acid, paeonol, rutoside and baicalin in Yanyan ablets. A Kromacil C₁₈ column (200mmX 4.6mm, 5 μm) was utilized, the mobile phase was methanol-water (contained 0.05% phosphoric acid) for gradient elution with flow rate of 1.0 mL $\cdot\text{min}^{-1}$. The detection was set at 278 nm. Qin Xiang-qin et al [8] established a method of Paeoniflorin for yanyan tablets by high performance liquid chromatography. The method used a C₁₈ column, acetonitrile-0.1% phosphoric acid (15:85) as the mobile phase. The detection wavelength was set at 230 nm. In this paper, the paeonol content in yanyan tablets was determined by High

Performance Capillary Electrophoresis.

Experimental section

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, 60 cm overall length, 52 cm effective length) from Hebei Yongnian Ruifeng Chromatographic Devices Co., Ltd.).

paeonol (Chinese Drugs and Biological Products); yanyan tablets (Central Hospital of Weifang, Shandong Province); Other reagents used in the experiments were all analytical grade; Double-distilled water was used.

Experimental Methods

Before the start of the experiment, capillary was successively washed with 1 $\text{mol}\cdot\text{L}^{-1}$ hydrochloric acid solution, double-distilled water, 1 $\text{mol}\cdot\text{L}^{-1}$ 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 14 $^{\circ}\text{C}$ experimental temperature. UV detection wavelength was 270 nm. Injection time was 10s (7.5 cm height difference).

Sample Preparation

yanyan tablets sample solution: yanyan tablets powder was accurately weighed 0.4114 g, added 30 mL water with 25% ethanol, cold soak time of 24 h, filtered, washed and set the volume to 50 mL that was the yanyan tablets sample solution.

paeonol standard solution: paeonol was accurately weighed 0.0012 g, added 3 mL water with 25% ethanol.

Results and Discussion

Selection electrophoresis conditions

Based on past experiment experience, we chose 40 mmol/L borax solution as a running buffer solution.

According to the literature, Paeonol maximum absorption wavelength was at 274 nm, so we chose the 270 nm detection wavelength.

Quantitative analysis

Standard curve

First, paeonol standard solution that the concentration were 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125 mg/mL was prepared. Each standard solution was run for three times under the above electrophoresis conditions and the results averaged. The chromatogram of paeonol 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 paeonol (peak area: y $\mu\text{V}\cdot\text{s}$, density: x mg/mL) and the linear range was as follows: $y = -3147.5 + 420341x$ ($r = 0.998$), 0.003-0.4 mg/mL.

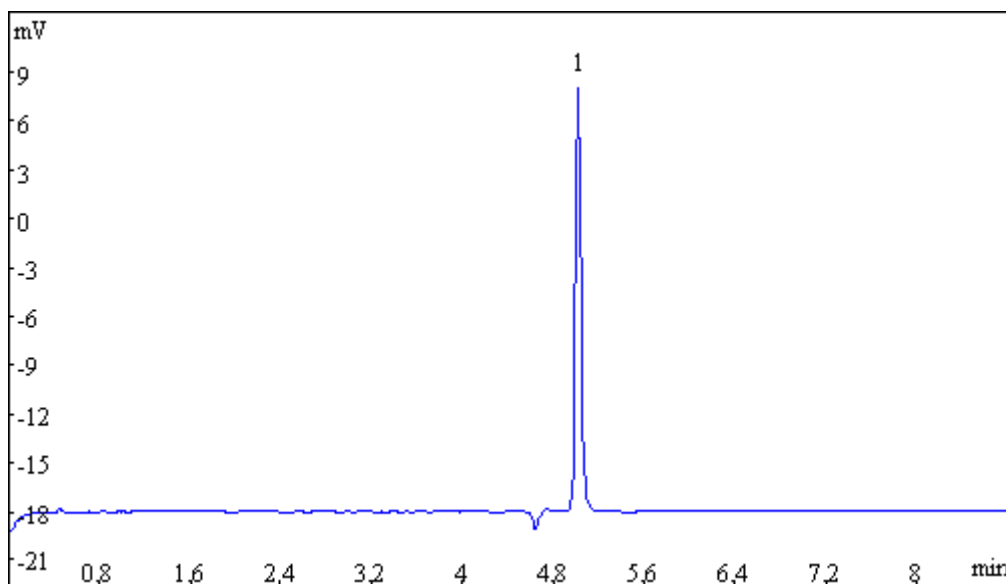


Fig.1 Electropherogram of paeonol standard solution 1-paeonol

Precision test

paeonol standard solution precisely drew and continuously injected for six times under electrophoretic separation conditions, the RSD of paeonol peak area was 3.3%, indicating good precision.

Determination of sample content

Under selected electrophoresis conditions, yanyan tablets sample solution was run. Separation chromatogram of the yanyan tablets sample solution was showed in Figure 2. Measured paeonol content in yanyan tablets was 6.14 mg/g (RSD = 11.6%) (n = 5).

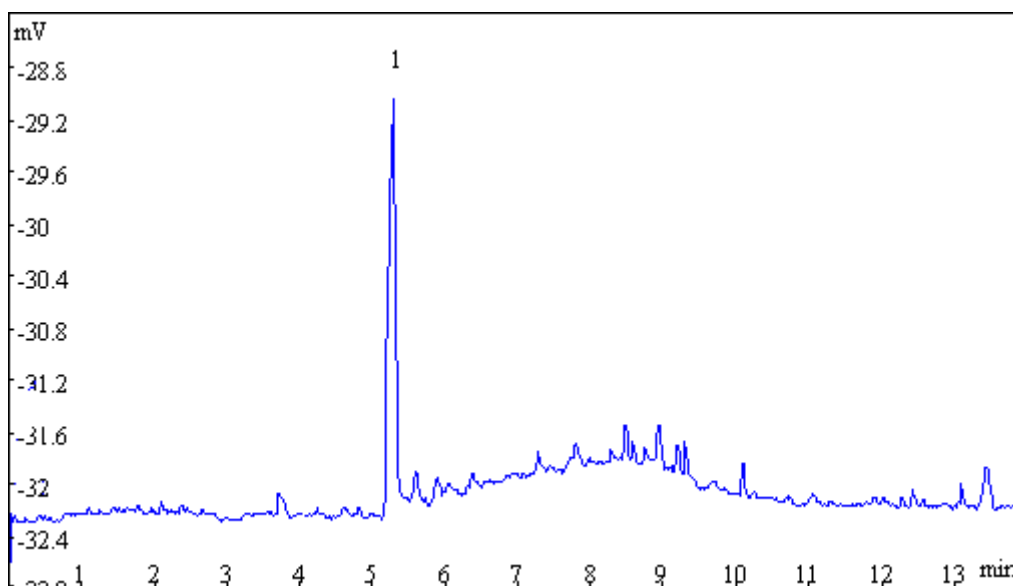


Fig.2 Electropherogram of yanyan tablets sample solution 1-paeonol

Recovery

After determination for five times, the recovery of paeonol in yanyan tablets sample was in the range of 77.6%-112.8% (n=5).

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