Determination of paeonol in huangliansu tablets by Capillary Electrophoresis

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Keywords: capillary electrophoresis, paeonol, huangliansu tablets

Abstract: This design uses High Performance Capillary Electrophoresis (HPCE) to determine the Paeonol's content in huangliansu tablets. Electrophoresis separation controlled conditions for uncoated fused silica capillary column (75μm×52 /60cm) injection height 7.5cm, and using a concentration of 40 mmol/L borax solution as buffer. Using separate voltage 20KV to detect wavelength 270nm, temperature is 17℃, and the injection time control 10s. The Paeonol is linear relationship(r=0.998) within concentration of 3~400mg/L, Measured paeonol content in huangliansu tablets was 13.2 mg/g (RSD = 14.4%) (n = 6). The recovery of paeonol in huangliansu tablets sample was in the range of 73.1% - 118.1% (n=4).

Introduction

Huangliansu tablet consists of berberine hydrochloride,combination, evodia rutaecarpa,Radix paeoniae alba and so on, with sissipating heat, flowing gas, antibacterial an-tiflammatory, relieving pain and diarrhea role. Simultaneous determination method of the content of six components in Fufang Huangliansu tablets using HPLC wavelength switching technology was established by LIN et al [1], The quantitative analysis of paeoniflorin, evodine, evodiamine, rutaecarpine, costunolide and dehydrocostuslacton was executed on a column of Kromasil C_{18} (250mm×4.6mm, 5μm) by simultaneous HPLC wavelength switching method, using a mobile phase of acetonitrile(A)-0.033mol·L^{-1} potassium phosphate monobasic solution. WU et al [2] established an HPLC method for determination of the eostunolide and dehvdrocostuslactone in Fufang Huang1iansu Tablets. The HPLC system consisted of century SIL C_{18} (250mm× 4.6mm, 5μm) and acetonitrile-0.1% H_3PO_4 (53:47) as the mobile phase and the detection wavelength was 225 nm. LIU et al [3] established a method for simultaneous detection peoniflorin, costunolide and dehydrocostus lactone in compound Fufang Huang1iansu tablets. Three components (peoniflorin, costunolide, and dehydrocostus lactone) were separated by C_{l8} chromatographic column with a gradient mobile phase consisting of aeetonitrile and 0.2% phosphoric acid solution. ZHANG et al [4] compared HPLC and UPLC methods in the determination of evodiamin and rutacarpine in compound Huangliansu tablet. The HPLC condition: Atlantis C_{18} column(4.6 mm×250 mm, 5μm), 10 mmol·L^{-1} ammonium acetate (pH=3.5) as mobile phase A, acetonitrile as mobile phase B. WANG et al [5] developed a method for the dissolution determination of berberine hydrochloride in compound Huangliansu tablets. The determination was conducted on Lichrospher ODS column with mobile phase consisted of 0.033 mo1·L^{-1} acetonitrile-monobasic potassium phosphate solution (40:60) at detection wavelength of 265 nm. To study the antioxidant activity of Huangliansu, the MDA determination, agarose gel electrophoresis and SDS-PAGE are utilized to detect the protections against the lipid peroxidation, DNA and protein oxidation degradation caused by free radicals [6]. The DMPD* method is used for the detection of the scavenging action of DMPD*. The antimicrobial effects of Huangliansu were studied by Wang et al [7] using disc diffusion method. The results indicated that Huangliansu has antibacterial spectrum, inhibitory effect of Huangliansu on bacteria gram negative Escherichia coli and gram positive bebacillus subtilis, Staphylococcus aureus, inhibitory effect on disease fungus Aspergillus niger. Zhang et al [8] explored the effects of

Huangliansu on the weight, blood glucose, lipid metabolism and serum insulin in KKAY mice and to study its possible glucoce-lowing and lipid-regulating mechanism. In this paper, the paeonol content in huangliansu tablets was determined by 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); huangliansu tablets (Jilin yinhe pharmaceutical Co., Ltd.); 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 $mol \cdot L^{-1}$ hydrochloric acid solution, double-distilled water, 1 $mol \cdot L^{-1}$ 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 17 °C experimental temperature. UV detection wavelength was 270 nm. Injection time was 10s (7.5 cm height difference).

Sample Preparation. Huangliansu tablets sample solution: huangliansu tablets powder was accurately weighed 0.3413 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 huangliansu 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 μV•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.

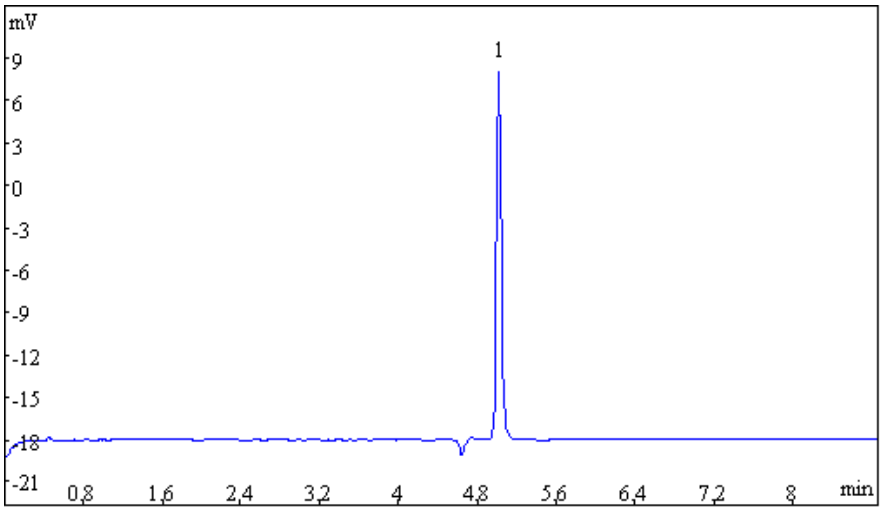

Fig.1 Electrophorogram of paeonol standard solution 1-paeonol

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, huangliansu tablets sample solution was run. Separation chromatogram of the huangliansu tablets sample solution was showed in Figure 2. Measured paeonol content in huangliansu tablets was 13.2 mg/g (RSD = 14.4%) (n = 6).

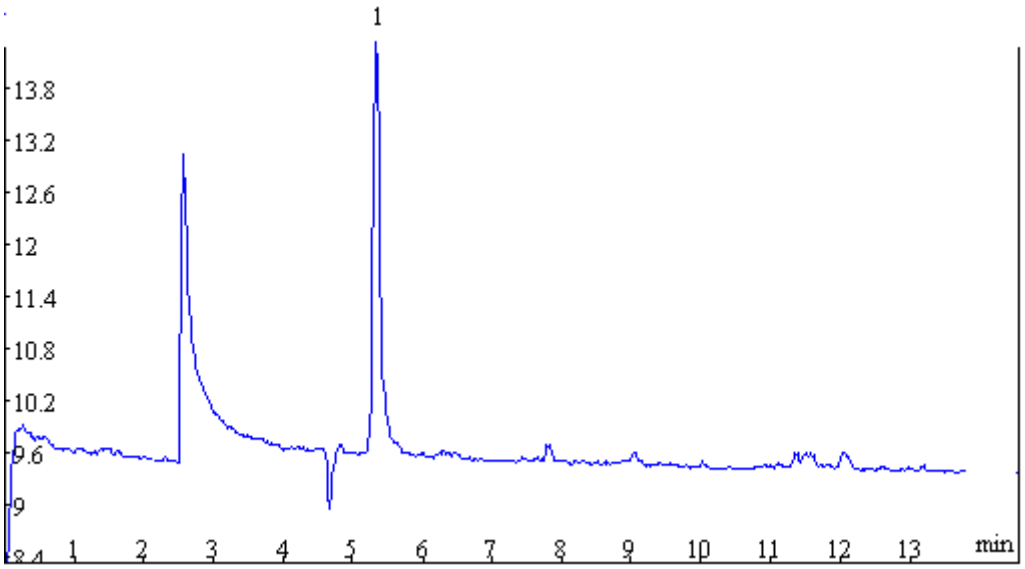

Fig.2 Electrophorogram of huangliansu tablets sample solution 1-paeonol

Recovery

After determination for four times, the recovery of paeonol in huangliansu tablets sample was in the range of 73.1% - 118.1% (n=4).

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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