Determination of Amygdalin in Lianhua Qingwen Granules by Capillary Electrophoresis

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Abstract: This paper investigated the determination of amygdalin content in Lianhua Qingwen Granules by high performance capillary electrophoresis (HPCE) method. The borax solution of 20 mmol concentration containing 15% methanol was chosen as buffer solution. The experiment was performed at a constant voltage of 18kV and UV detection wavelength of 210 nm. The content of amygdalin in Lianhua Qingwen Granules was 9.906 mg/g (RSD = 3.8%) (n = 7). The recovery was in the range of 79.1% - 122.3% (n=6). This method is suitable for the detection of the content of amygdalin in Lianhua Qingwen Granules.

1. Introduction

Lianhua Qingwen Granules consists of weeping forsythiae capsule, honeysuckle flower, bitter apricot seed, liquoric root 13 traditional Chinese medicine etc. It has the effect of heat-clearing, detoxifying, diffusing the lung and disperse heat. It is used for treatment of pulmonary infection, upper respiratory tract infection, common cold, etc. [1, 2]. Li et al [3] established an HPLC method for the determining chlorgenic acid in Lianhua qingwen granules and capsules. The Purospher Star RP-18 endcapped (55 mm×4 mm, 3 μm) chromatographic column was adopted. The mobile phase was composed of methanol-5% glacial acetic acid (15:85) with flow rate of 1.0 ml/min. The detection wavelength was at 327 nm. The column temperature was 30 ℃. Guo et al [4] established a RP-HPLC method for the content determining 4 active compounds in Lianhua Qingwen granules, including phillyrin, forsythoside A, chlorogenic acid, crytochlorogenic acid and fingerprint analysis. The separation was carried out on Swell chromplus TM-C_{18} column with a temperature at 30℃, mobile phase composed of methanol–acetonitrile-0.3% phosphoric acid (gradient elution) with flow rate of 1.0 mL/min, and detection wavelength at 278 nm. For solving the agglomeration problem in the former process, dry granulation technology was applied by Wang et al [5] to prepare the granules of Lianhua Qingwen Capsules. Complexity and granule yield coefficient were set as inspection indexes. The optimum subsidiary material and its amount were optimized. The parameters of dry granulation technology were investigated by orthogonal test. Then, granule yield, angle of repose, and bulk density were compared with those of wet granulation technology. Starch was set as subsidiary material. The optimum technology is roll pressure of 12 MPa, rotation speed of 5 r/min, and feed speed of 10 r/min. The yield of granules prepared by dry granulation technology was significantly higher than that of wet granulation technology. For providing a better reference for the quality control of Lianhua Qingwen Granules, the HPLC method was established by Cai et al [6] for the content determining Forsythoside A in Lianhua Qingwen Granules. The chromatographic condition was used Agilent C18 as chromatographic column with acetonitrile-0.4% acetic acid (15:85) as the solvent system. The flow rate was 1.0 mL/min. The column temperature was at room temperature. The detection wavelength was 330 nm. Xu et al [7] established an LC-MS/MS method for simultaneous content determining the ten components (Ginsenoside Rc, Ginsenoside Rd, Ginsenoside Rg1, Quercetin, Quercitrin, Isoquercitrin, Latetrile, Norisoboldine, Linderane, Arecoline). The HPLC analysis was investigated on an a Waters BEH C18 column (2.1mm×100mm, 1.7μm) with gradient elution using 0.5‰ formic

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acid-water and acetonitrile as the solvent system and flow rate of 0.2mL/min, the column temperature was room temperature. Xue et al [8] established the method for simultaneous determining 9 components in Huichun yuzi granules. LC-MS/MS method was applied. The determination was carried out on Shim-pack XR-ODS C18 column with column temperature of 30℃. The sample size was 5 μL, and ion source as electrospray ion source, MRM mode was used. The acetonitrile-water was utilized as mobile phase for ferulic acid, rutin, paeonol, icariin and schisandrin (gradient elution), positive ion mode monitoring was conducted. The methanol-0.1% fomic acid water was utilized as mobile phase for naringin, verbascoside, amygdalin and protocatechuic acid (gradient elution), negative ion mode monitoring was conducted. Li et al [9] developed an LC-MS/MS method for the simultaneous determining amygdalin and paeoniflorin in urine samples, and to test their urinary excretion characteristics in healthy volunteers after intravenous infusion administration of Huoxue-Tongluo lyophilized powder for injection (HTLPI). The urine samples were extracted by methanol, and then analyzed on a Hedera ODS-2 column with a mobile phase of acetonitrile and 5 mmol/L ammonium acetate buffer solution containing 0.05% formic acid (20:80). Electrospray ionization source was adopted and operated in the positive ion mode using MRM. Cai et al [10] established a method for determining amygdalin in Juhong Huatan Wan. A RP-HPLC method was developed. The chromatographic column was Diamonsil C18. The mobile phase consisted of methanol-0.1 % phosphoric acid (20:80, *v/v*) with flow rate of 1.0 mL/min. The detection wavelength was 210 nm. The column temperature was 30℃. Li et al [11] established a RP-HPLC method for the content determining 9 kinds of water-soluble ingredients (amygdalin, hydroxysafflor yellow A, catalpol, rehmaionoside A, ferulic acid, paeoniflorin, protocatechuic acid, chlorogenic acid and gallic acid) from Tao Hong Siwu Decoction using RP-HPLC. The Hypersil GOLD AQ chromatographic column (5 μm, 4.6 mm×250 mm) was adopted. The 0.05% phosphoric acid solution and acetonitrile were utilized as the mobile phase to separate the 9 kinds of ingredients by gradient elution. The flow rate was 1 mL/min. The column temperature was set at 30 ℃, and the detection wavelength was set at 215 nm. Yi [12] established an RP-HPLC method for determining amygdalin in Xiaoer Qingfei Huatan Liquid. A HPLC method was developed. The chromatographic column was Diamonsil-C18. The mobile phase consisted of methanol-0.1% phosphoric acid (10:90, v/v) with flow rate of 1.0mL/min.Tthe detection wavelength was 215nm. Wang et al [13] established the method for simultaneous determining paeoniflorin, amygdalin, ferulic acid and ligustrazine in Modified buying huanwu decoction. The RP-HPLC method was used. The determination was carried out on YMC C18 column with the mobile phase consisting of acetonitrile-0.1％ phosphoric acid (gradient elution) at the flow rate of 1.0 mL/min. The detection wavelengths were set at 320 nm (ferulic acid), 230 nm (paeoniflorin), 207 nm (amygdalin), 280 nm (ligustrazine). The column temperature was 30℃. In this paper, the amygdalin content in Lianhua Qingwen 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.).

Amygdalin (Chinese Drugs and Biological Products); Lianhua Qingwen Granules (Beijing Yiling Pharmaceutical Co., Ltd., Batch number: 17090227); 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 $mol \cdot L^{-1}$ hydrochloric acid solution, double-distilled water, 1 $mol \cdot L^{-1}$ 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 18 kV voltage and experimental temperature at 30°C. UV detection wavelength was 210 nm. Injection time was 10s (7.5 cm height difference).

2.3 Sample Preparation

Lianhua Qingwen Granules sample solution: Lianhua Qingwen Granules was accurately weighed 3.7749 g, added 40 mL water, extracted time of 24h at 30°C, filtered, washed and set the volume to 50 mL that was the Lianhua Qingwen Granules sample solution.

Amygdalin standard solution: Amygdalin was accurately weighed 0.0026 g and 1 mL water was added.

3. Results and Discussion

3.1 Selection electrophoresis conditions

Considering the factors of voltage and current, 20mmol/L borax solution was chosen first in the CE experiment. Under the condition, the running is stable basically and the current size is appropriate, However, amygdalin in Lianhua Qingwen granules overlapped with the other peak and did not separate completely. Thus organic substance (methanol) was considered to put in the buffer for changing the size of electroosmotic flow and the rate of substance migration. The buffer solution containing 5%, 10%, 15%, 20% and 25% methanol was prepared, and the sample solution of Lianhua Qingwen granule was run respectively. At the beginning, it is found that the sample separation effect is better as the increase of methanol content. However, the separation effect of Lianhua Qingwen granule sample is worse as methanol content too high. So the buffer containing 15% methanol was chosen, and amygdalin was basically separated with the other substances in Lianhua Qingwen granules. Finally, 20mmol/L borax solution containing 15% methanol was chosen as electrolyte solution.

3.2 Quantitative analysis

3.2.1 Standard curve

First, amygdalin standard solution was prepared and its concentrations were 2.6, 1.3, 0.65, 0.325, 0.162, 0.085, 0.041 mg/mL. Each standard solution was run for three times under the above electrophoresis conditions and the results averaged. The chromatogram of amygdalin 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 amygdalin (peak area: y μV•s, density: x mg/mL) and the linear range was as follows: y= -181+149457x(r=0.998), 0.041-2.6 mg/mL.

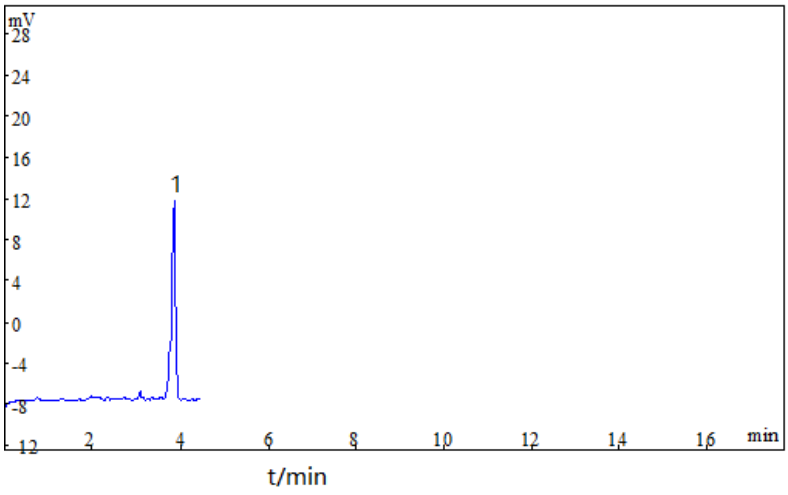

Fig.1 Electrophorogram of amygdalin standard solution 1-amygdalin

3.2.2 Precision test

A amygdalin standard solution precisely drew and continuously injected for sixt times under electrophoretic separation conditions, the RSD of amygdalin migration time and peak area were 0.28% and 3.1%, indicating good precision.

3.2.3 Determination of sample content

Under selected electrophoresis conditions, Lianhua Qingwen Granules sample solution was run. Separation chromatogram of the Lianhua Qingwen Granules sample solution was showed in Figure 2. Measured amygdalin content in Lianhua Qingwen Granules was 9.906 mg/g (RSD = 3.8%) (n = 7).

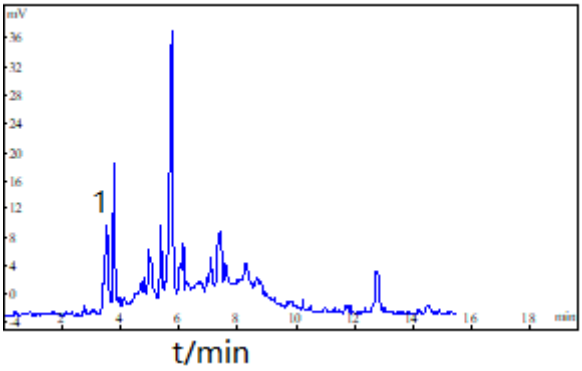

Fig.2 Electrophorogram of Lianhua Qingwen Granules sample solution 1-amygdalin

3.2.4 Recovery

After determination for six times, the recovery of amygdalin in Lianhua Qingwen Granules sample was in the range of 79.1% - 122.3% (n=6).

4. Conclusion

This paper investigated the determination of amygdalin content in Lianhua Qingwen Granules by high performance capillary electrophoresis method. Measured amygdalin content in Lianhua Qingwen Granules was 9.906 mg/g (RSD = 3.8%) (n = 7).

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