Determination of Amygdalin in Apple Core by Capillary Electrophoresis

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Abstract. This paper investigated the determination of amygdalin content in Apple Core 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 Apple Core was 3.432 mg/g (RSD = 4.5%) (n = 6). The recovery was in the range of 88.1% - 115.7% (n=5). This method is suitable for the detection of the content of amygdalin in Apple Core.

1. Introduction

Lu et al [1] established an RP-HPLC method for the simultaneous determining four constituents in Qinfei Huatan Pills (Ephedrae Herba, Armeniacae Semen Amarum, Citri Reticulatae Pericarpium, etc.). The analysis of Qinfei Huatan Pills methanolic extract was carried out on a 25℃ thermostatic Agilent TC-C18(2) column (250 mm×4.6 mm, 5 μm), with the mobile phase consisted of acetonitrile-0.2% phosphoric acid at a flow of 0.8 mL/min in a gradient elution manner. And the detection wavelengths were set at 207 nm for ephedrine and laetrile, and 278 nm for hesperidin and baicalin. A method was studied by Gao et al [2] for the determining amygdalin and its metabolite prunasin in rat plasma after intragastric administration of Maxing shigan decoction. The analytes were identified by ultra-high performance liquid chromatography-tandem quadrupole time of flight mass spectrometry and quantitatively tested by ultra-high performance liquid chromatography-tandem triple quadrupole mass spectrometry. After purified by liquid-liquid extraction, the qualitative analysis of amygdalin and prunasin in the plasma sample was carried out on a shim-pack XR-ODS HPLC column (75 mm×2.0 mm, 1.6 μm), using acetonitrile-0.1%(v/v) formic acid aqueous solution. The detection was carried out on a Triple TOF 5600 quadrupole time of flight mass spectrometer. A UPLC method was developed by Chen [3] for the determining amygdalin, liquiritin and glycyrrhizic acid of Chuanbei Qingfei syrup. An Agilent Eclipse Plus C18 column was adopted, with the mobile phase of acetonitrile: 0.1% phosphoric acid by gradient elution and double-wavelength switching detection. The detection wavelength was set at 210 nm during 0-7 min for the determination of amygdalin and liquiritin, and then changed to 250 nm for the determination of glycyrrhizic acid during 8-14 min. Huang [4] established a multiple UV wavelengths HPLC method for simultaneously determining the contents of 3,5-O-dicaffeoylquinic acid, chlorogenic acid, forsythin, forsythoside A, arctiin, and amygdalin in SangJu Yinqiao powder. The analysis was performed on the ODS-SP C18 column (4.6× 250mm, 5μm), with 0.1% (v/v) phosphoric acid-acetonitrile in a gradient elution mode as the solvent system at a flow rate 1.0mL/min. The detection wavelengths were set at 210nm for amygdalin, 277nm

for arctiin and forsythin, and 327nm for 3,5-O-dicaffeoylquinic acid, chlorogenic acid, forsythoside A. The temperature was maintained at 35℃. Liu et al [5] applied the RP-HPLC method for the content determining amygdalin and peoniflorin in maren pills. The chromatographic column used Aglient TC-C18 (4.6× 250mm, 5μm), with 0.1% (v/v) formic acid-acetonitrile (89:11) in a gradient elution mode as the mobile phase at a flow rate 1. 0mL/min. The detection wavelength was set at 221nm. A RP-HPLC method was established by Kou et al [6] for the determining amygdalin in amygdalus pedunculatus pall products. The sample of amygdalin in amygdalus pedunculatus pall was extracted in methanol. The separation was tested with a mobile phase of methanol and water and acetonitrile (2:85:13) and a detection wavelength of 220nm. Feng [7] established a method for determining amygdalin, chlorgenic acid and forsythin in Fengreganmao granules by HPLC with different UV wavelengths. The analysis was carried out on an SHISEIDO capcell pak-C18 column (4.6 mm×250 mm, 5 μm). The mobile phase composed of methanol-0.1% phosphoric acid solution with gradient elutionat at flow rate of 1 mL/min. The column temperature was 35℃. The detection wavelength was set as 207 nm (amygdalin), 327 nm (chlorgenic acid) and 279 nm (forsythin). A HPLC method with gradient elution and three wavelength detection was established by Liang et al [8] for the simultaneous determining amygdalin, liquiritin and naringin in Juhong Huatan Wan. The separation was tested on a Diamonsil-C18 column with methanol and 0.1% phosphoric acid as the solvent system of gradient elution with flow rate of 1.0 mL/min. The detection wavelengths were set at 210, 276, 283 nm. Hu [9] determined the content of ephedrine hydrochloride, pseudoephedrine hydrochloride and amygdalin in Xiao'er Feire Kechuan Oral Solution by HPLC. A RP-HPLC method was developed, with emethanol-acetonitrile-water (v:v:v=9:9:82, adding 1% triethylamine) used as the mobile phase, and 210 nm was the detecting wave length. Tian et al [10] established a method for determining Qiangli wuhu heji index components of amygdalin, ephedrine hydrochloride by HPLC. The column used WondaSil C18 (4.6mm× 250mm, 5 m). The mobile phase consisted of methanol, acetonitrile, 0.1% phosphoric acid (16:4:80) (at 207 nm wavelength determination of amygdalin), 0.1% phosphoric acid and acetonitrile (95:5)(at 207 nm wavelength determination of ephedrine hydrochloride). Wu et al [11] established the determining amygdalin content in pinellia almond soup using HPLC. The chromatographic column adopted Thermo C18 (4.6× 250mm, 5μm), with methanol-acetonitrile-water (10:10:80) as the mobile phase at a flow rate 1.0mL/min. The detection wavelength was set at 210nm. Zhang et al [12] established a method for determination of amygdalin in honeyed folium eriobotryae using HPLC. The Take Welch Ultimate XB-C18 (4.6× 250mm, 5μm) as solid phrase, methanol-water (20:80) as mobile phrase to determine the content at UV wavelength of 210nm. The amygdalin in qinfei huatanwan separated on C18 column was developed by Zhu et al [13] using HPLC with mobile phase of methanol-water (14:86) and detection wavelength of 214nm. In this paper, the amygdalin content in Apple Core 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); Apple Core (purchase in weifang market); 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

Apple Core sample solution: Apple Core powder was accurately weighed 0.3918 g and 9 mL water was added, extracted time of 24h at 30°C, filtered, washed and set the volume to 10 mL that was the Apple Core 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

The experiment was carried out at 18 kV voltage. UV detection wavelength was 210 nm.

Based on past experiment experience, 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.

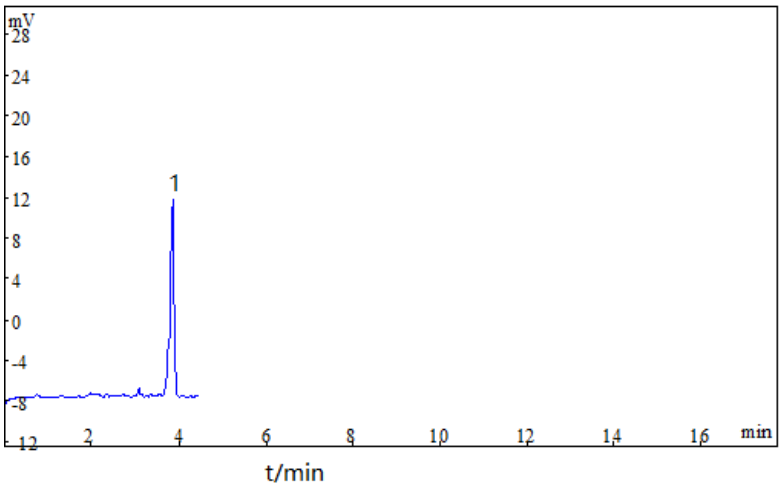

Fig.1 Electrophorogram of amygdalin standard solution
1-amygdalin

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, Apple Core sample solution was run. Separation

chromatogram of the Apple Core sample solution was showed in Figure 2. Measured amygdalin content in Apple Core was 3.432 mg/g (RSD = 4.5%) (n = 6).

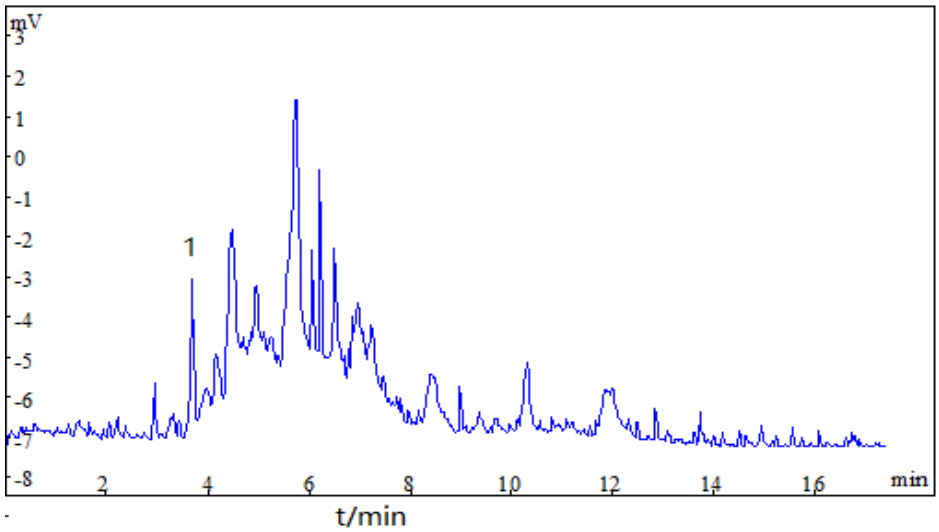

Fig.2 Electrophorogram of Apple Core sample solution
1-amygdalin

3.2.4 Recovery

After determination for five times, the recovery of amygdalin in Apple Core sample was in the range of 88.1% - 115.7% (n=5). The average recovery was 103.9%.

4. Conclusion

This paper investigated the determination of amygdalin content in Apple Core by high performance capillary electrophoresis method. Measured amygdalin content in Apple Core was 3.432 mg/g (RSD = 4.5%) (n = 6).

Acknowledgments

This study was 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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