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Determination of Amygdalin in Cherry Seed by Capillary Electrophoresis

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Abstract. This paper investigated the determination of amygdalin content in Cherry Seed 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. Measured amygdalin content in Cherry Seed was 2.045 mg/g (RSD = 5.98%) (n = 6). The recovery of amygdalin in Cherry Seed sample was in the range of 97.7% - 120.5% (n=6). The average recovery was 109.7%. This method is suitable for the detection of the content of amygdalin in Cherry Seed.

1. Introduction

Chen [1] explored the application of polyethylene glycol ammonium sulfate aqueous two phase system in the separation and purification of total flavonoids from *Cerasus sachalinensis* Kom, and detected extraction conditions. Single factor exploration method was adopted to analyze the influence of four factors: Polyethylene glycol molecular weight, polyethylene glycol ammonium sulfate ratio, pH value, and temperature on the receiving rate of total flavonoids of the *C. sachalinensis* Kom. The optimum combination was filtered out and the best results to do the verification test were selected. In addition, the verification test was utilized to detect the purification effect of the polyethylene glycol ammonium sulfate aqueous two-phase extraction system. The volatile oils were extracted by Sun et al [2] from *Prunus tomentosa* Thunb's shell and kernel by steam distillation method, and their chemical compositions were then investigated and identified by GC/MS. The results indicate that 48 compounds were detected and 24 compounds of them were identified in shell, coming to 97.45% in gross; while 20 compounds of them were detected in the kernel volatile oil and 12 of them were identified, coming to 99.4% in gross. The scavenging rates of hydroxyl radicals and oxygen free radicals and reducing power were detected by Gong et al [3] using UV spectrophotometry. The methods of titration and UV spectrophotometry were applied in the experiments of hypoglycemic and lipid lowering. In the experiments, when the concentration of the ethanol extract increased to 1 g/mL, the scavenging rate of hydroxyl radical and oxygen free radical were 99.68% and 84.24% respectively, and the reduction ability was also increased. The inhibition rate of the activity of α -glucosidase and lipase activity was 96.73% and 67.9%, respectively. The scavenging rate of radicals and the inhibition rate of enzymes were increased with the ethanol extract concentration dependence. Cherry kernel ethanol extract has excellent antioxidant activity and favorable ability of hypoglycemic lipid-lowering. Sun [4] Studied on the extraction about the function elements of cherry seed-flavonoids and research into the best process conditions. With the yield of flavonoids for complete index, investigation ethanol concentration, the liquid-solid ratio, extraction temperature and time of extracting flavonoids were studied. The



optimized extraction conditions were tested by single factor experiment and orthogonal test. The results indicated that flavonoids best extraction conditions for: alcohol concentration 75 % (Volume fraction), the liquid-solid ratio 35:1 mL/g, extraction temperature of 65 °C and extraction time 2 h. Under these extraction conditions, the extractive amount of flavonoids can be as high as 0.803 %. The bioactive flavonoid compounds of cherry seed were extracted by ethanol and the optimal extraction conditions were explored by Zhang et al [5] using orthogonal test. The accelerated solvent extraction and the orthogonal experiment were chosen by Sun [6] to extract flavonoids from the cherry pits to optimize extraction condition. The total antioxidant capacity of flavonoids and DPPH· radical scavenging capacity were measured by the method of in vitro antioxidant activity. Cherry can be processed to juice, wine, vinegar and preserve, but the cherry stone is often discarded as a waste. The major components in the stone of Dazhihong cherry and Laoshan red cherry were measured by Zhen et al [7] and the antioxidant capabilities of the aqueous extract, petroleum extract, ethyl acetate extract, and n-butanol extract of Laoshan red cherry stone were detected. Yang et al [8] established a HPLC method for the determining the amygdalin content in Zhike Dingchuan Pills. The Agela Venusil MP C18 column(250 mm×4.6 mm, 5 μm) was adopted with the mobile phase of methanol-water (20:80) at flow rate of 1.0 mL/min, the detection wavelength was 210 nm. Liu et al [9] established an HPLC method for the determining the amygdalin content in Zhike Huatan Granules. Amygdalin was analyzed by HPLC with ZORBAX SB-C18 column (250 mm× 4.6 mm, 5 μm). The mobile phase consisted of methanol-1% phosphoric acid water (30:70) at flow rate of 1 mL/min. The detection wavelength was 210 nm and the column temperature was room temperature. Sun [10] established a HPLC method for determining amygdalin, paeoniflorin, ferulic acid, kaempferol and imperatorin in Huaxuexiaozhong pills. A stable HPLC method was explored and the chromatography was analyzed on a Waters Symmetry C18 column, with a mobile phase composed of methanol-0.1%(W) phosphoric acid in a gradient mode at a flow rate of 1 mL/min. The detection wavelength was set at 210 nm for amygdalin and kaempferol, 230nm for paeoniflorin, 300nm for ferulic acid, 370nm for imperatorin, respectively. High purity amygdalin was obtained by Chen et al [11] from walnut kernel by the process of enzyme destruction, degreasing and crystallization. The effects of different volume and concentration of ethanol, temperature and extraction time were studied. The optimized conditions were obtained as follows: 120 mL of ethanol with concentration of 95% was added, the extraction time 30 min under 60°C, the process repeat four times. A HPLC method was developed by Lin et al [12] for the determining eight active components including hydroxysafflor yellow A, safflor yellow A, asperosaponin VI, amygdalin, paeoniflorin, brazilin, (±)protosappanin B and tetrahydropalmatine in Huoxue Lishang Wan. An Agilent SB C18 column was adopted, with the mobile phase consisting of methanol:acetonitrile (2:1):0.5% phosphoric acid for gradient elution. Gou et al [13] established HPLC method for the determining amygdalin content in Huoxuetongmai Capsules. The chromatographic column was C18 (250 mm× 4.6 mm, 5 μm). The mobile phase was methanol-0.1% phosphoric acid(25:75) at flow rate of 1 mL/min. The detection wave length was 210 nm. In this paper, the amygdalin content in Cherry Seed 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); Cherry Seed (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·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 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

Cherry Seed sample solution: Cherry Seed powder was accurately weighed 1.1934 g, added 40 mL water, extracted time of 24h at 30°C , filtered, washed and set the volume to 50 mL that was the Cherry Seed 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: $y \text{ } \mu\text{V}\cdot\text{s}$, density: $x \text{ mg/mL}$) and the linear range was as follows: $y = -181 + 149457x$ ($r = 0.998$), 0.041-2.6 mg/mL.

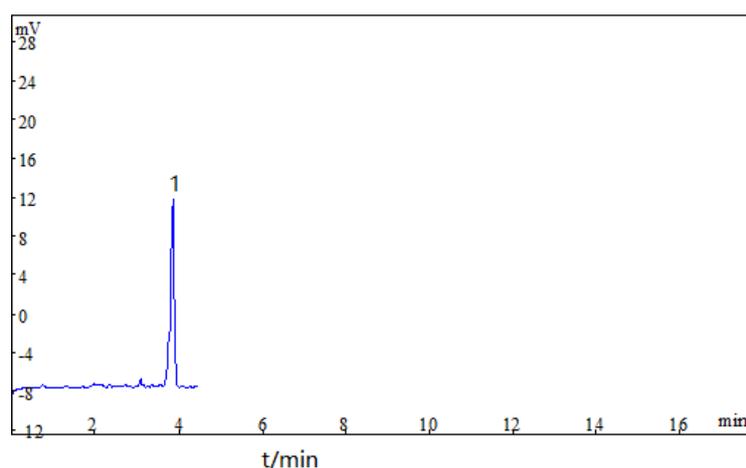


Fig.1 Electrophorogram of amygdalin standard solution
1-amygdalin

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, Cherry Seed sample solution was run. Separation chromatogram of the Cherry Seed sample solution was showed in Figure 2. Measured amygdalin content in Cherry Seed was 2.045 mg/g (RSD = 5.98%) (n = 6).

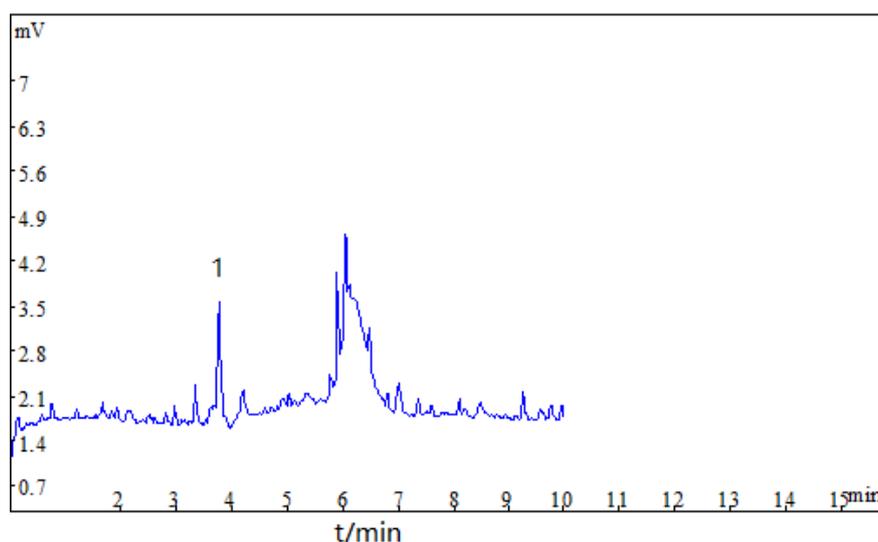


Fig.2 Electropherogram of Cherry Seed sample solution
1-amygdalin

3.2.4 Recovery

After determination for six times, the recovery of amygdalin in Cherry Seed sample was in the range of 97.7% - 120.5% (n=6). The average recovery was 109.7%.

4. Conclusion

This paper investigated the determination of amygdalin content in Cherry Seed by high performance capillary electrophoresis method. Measured amygdalin content in Cherry Seed was 2.045 mg/g (RSD = 5.98%) (n = 6).

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