Determination of Amygdalin in Nectarine Nucleolus by Capillary Electrophoresis

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Abstract. This paper investigated the determination of amygdalin content in Nectarine Nucleolus 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 Nectarine Nucleolus was 108.01 mg/g (RSD = 5.9%) (n = 6). The average recovery was 95.7%. This method is suitable for the detection of the content of amygdalin in Nectarine Nucleolus.

1. Introduction

Zhang et al [1] established HPLC coupled with wavelength switching and gradient elution method (HPLC-DVD) for simultaneous determining ten main components (calycosin-7-glucoside, ruscogenin, amygdalin, ginsenoside Rb1, ginsenoside Re, ferulic acid, crocin I, salvianolic acid B, acetyl-11-keto-β-boswellic acid, and tanshinone IIA) in Shuangshenlong Capsule (SSLC). The chromatographic separation was obtained on Hypersil ODS C18 (150 mm× 4.0 mm, 3 μm) column with methanol water (8:2)-0.1% phosphoric acid solution as mobile phases for gradient elution, at the flow rate of 0.6 mL/min. The detection wavelength was set at 260 nm for α-calycosin-7-glucoside, 280 nm for ruscogenin, 210 nm for amygdalin, 203 nm for ginsenoside Rb1 and ginsenoside Re, 320 nm for ferulic acid, 440 nm for crocin I, 286 nm for salvianolic acid B, 250 nm for acetyl-11-keto-β-boswellic acid, and 270 nm for tanshinone IIA. He et al [2] established a method for the determining dissolution of 8 active ingredients in Guizhi Fuling capsules. The UPLC was adopted. The mobile phase of gallic acid, paeoniflorin, benzoic acid, benzoylpaeoniflorin, paeonol, cinnamic acid and cinnamaldehyde was 0.02% trifluoroacetic acid aqueous solution-acetonitrile for gradient elution, their detection wavelengthes were 230 nm and 275 nm. The mobile phase of amygdalin was water-methanol (80:20) and its detection wavelength was 218 nm. Lin et al [3] established an HPLC fingerprint of Xiaochuan Granula, and to make a quantitative analysis of seven components by fused-core column. The Kromasil C18 (150 mm× 4.6 mm, 3.5 μm) was adopted with the mobile phase of methanol-acetonitrile-water and flow rate of 1.0 mL/min. The detection wavelength was set at 210 nm for amygdalin and magnolin, 240 nm for morroniside, loganin, prim-*O*-glucosylcimifugin, 4′-*O*-β-glucopyranosyl-5-*O*-methylvisamminol, schizandrin, and fingerprint. The column temperature was maintained at 40 ℃. Li et al [4] formulated a quality standard for Kujukechun Mistura. TLC method was developed for ephedra herba, citri exocarpium rubrum, and HPLC method was performed for determining laetrile in kujukechuan Mistura. Hypersil ODS (150 mm× 4.6 mm, 5 μm) column was adopted with acetonitrile-0.1% phosphoric acid (6:94) as the solvent system at flow rate of 1.0

mL/min. The detection wavelength was 207 nm. Wang et al [5] explored the HPLC fingerprint chromatograms analytical methods of different processed products of Armeniacae Semen Amarum with the personality characteristics. Methanol(50%) was applied for ultrasonic extraction of three processed products of Armeniacae Semen Amarum. HPLC was used with mobile phase of acetonitrile-0.1% phosphoric acid water solution for gradient elution, the column temperature at 25℃, the detection wavelength at 225 nm, the flow rate of 1.0 mL/min. Liu et al [6] screened amygdalin gel matrix to detect the preparation process. Using transdermal diffusion test instrument in vitro, the content of amygdalin in the received solution was obtained by HPLC. Steady-state penetration rate was served as investigation indexes by orthogonal test. Zhou et al [7] optimized the preparation process and studied the quality standard of Prunus armeniaca dispensing granules. Nine experiments were performed by L9 (3^4) orthogonal design. Prunus armeniaca dispensing granules was identified by TLC and the content of amygdalin was obtained by HPLC. The best extraction technology of amygdalin in armeniacae semen amarum was investigated by Fan et al [8] and different processing methods content of amygdalin in armeniacae semen amarum were compared. The HPLC method was applied for determining the content of amygdalin in armeniacae semen amarum, the effects of different solvents, extraction time and extraction times on the extraction process were tested, and the best processing technology was obtained. The content of amygdalin of Prunus humilis Bunge was determined by Wu et al [9] using HPLC and anti-oxidative activity of amygdalin was tested by DPPH method. Wang et al [10] established a method for determination of Wuhu mixture index components including amygdalin and ephedrine hydrochloride. The HPLC was adopted: column chromatography WondaSil C18 (4.6 mm× 250 mm, 5 μm), methanol acetonitrile-0.1% phosphoric acid 16:4:80, at 207 nm wavelength to detect amygdalin and 0.1% phosphoric acid and acetonitrile (95:5), at 207 nm wavelength to detect ephedrine hydrochloride. Jiang et al [11] studied the effects of gypsum on the contents of ephedrine, pseudoephedrine, amygdalin, liquiritin and glycyrrhizic acid in Maxingshigan decoction. The HPLC method was applied to detect the five components in both decoctions with and without gypsum. The Agilent ZORBAX SB-Aq C18 column (4.6 mm× 250 mm, 5 μm) maintained at 30℃ with a gradient mobile phase system consisted of ACN-0.1% phosphoric acid solution was applied for sample analysis. The wavelength was set at 207 nm for ephedrine, pseudoephedrine and amygdalin, while 237 nm for liquiritin and glycyrrhizic acid with a flow rate of 1.0 mL/min. Wang et al [12] optimized extraction technology of effective ingredients from Majie Pingchuan cataplasma. With extraction rate and transmittance of amygdalin and pseudoephedrine hydrochloride as comprehensive evaluation index, extraction technology was optimized by orthogonal test, transdermal test of extract was applied by Franz diffusion cell, the contents of amygdalin and pseudoephedrine hydrochloride were detected by HPLC. Fruit characteristics and nutritional components were comprehensively measured by Zhao et al [13] for three Xinjiang paddan almond samples (Shuangguo, Shuangren, and Zhipi). Atomic absorption spectrophotometry was adopted to detect the content of trace elements and major elements. A method described by the Chinese Pharmacopeia was applied to determine amygdalin content. The nutritional value of proteins was evaluated as the score of the ratio coefficient of amino acid. The gas chromatography mass spectrometry was adopted to detect the components and content of fatty acids. A HPLC method for determining amygdalin in Xingbei Zhike granules was developed by Jing et al [14]. A Kromasil C18 column was adopted with the mobile phase of methanol-water (24:76) at the detection wavelength of 218nm. Liu et al [15] established a method for determining the concentration of amygdalin in almond skin. The optimization of detoxification also was finished. With Ultraviolet spectrometry, the maximum absorption wavelength detected at 219 nm, and the calibration curve was linear in a certain range, and with the standard of amygdalin as reference, amygdalin contents in samples were determined. Based on this method, the single factors and orthogonal optimization were adopted for the detoxification of soaking. The result was as follows: 0.1% citric acid soaking reagent, 8 hours for smoking, 70℃ soaking temperature, the rate of solid to liquid 1:15 (g/mL). Wang et al [16] established a qualitative and quantitative method for kidney capsules. Salvia miltiorrhiza radix and Curcuma longa rhizome of kidney capsules were identificated by TLC. The HPLC was applied to detect the content of loganin, laetrile and syringin. In this paper,

the amygdalin content in Nectarine Nucleolus 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); Nectarine Nucleolus (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

Nectarine Nucleolus sample solution: Nectarine Nucleolus powder was accurately weighed 0.0681 g, added 9 mL water, extracted time of 48h at 30°C, filtered, washed and set the volume to 10 mL that was the Nectarine Nucleolus 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.

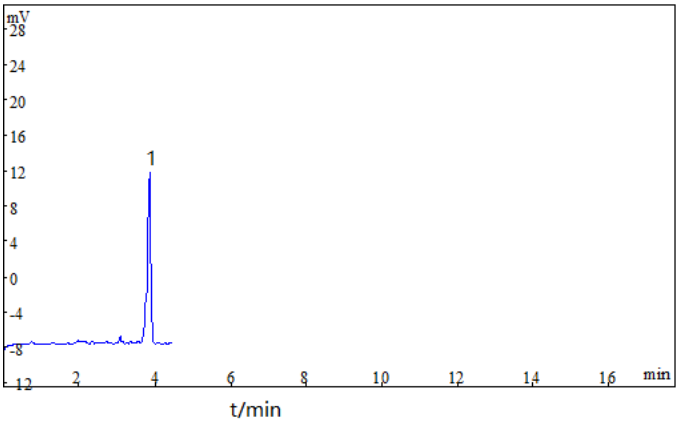

Fig.1 Electrophorogram of amygdalin standard solution
1-amygdalin

3.2.2 Precision test

A amygdalin standard solution precisely drew and continuously injected for six 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, Nectarine Nucleolus sample solution was run. Separation chromatogram of the Nectarine Nucleolus sample solution was showed in Figure 2. Measured amygdalin content in Nectarine Nucleolus was 108.01 mg/g (RSD = 5.9%) (n = 6).

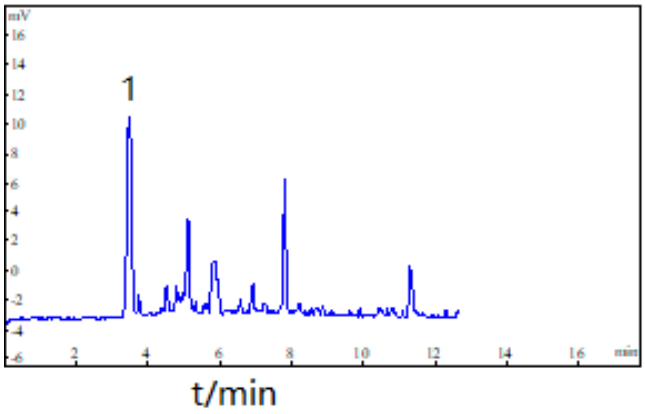

Fig.2 Electrophorogram of Nectarine Nucleolus sample solution
1-amygdalin

3.2.4 Recovery

After determination for six times, the recovery of amygdalin in Nectarine Nucleolus sample was in the range of 76.8% - 125.0% (n=6). The average recovery was 95.7%.

4. Conclusion

This paper investigated the determination of amygdalin content in Nectarine Nucleolus by high performance capillary electrophoresis method. Measured amygdalin content in Nectarine Nucleolus was 108.01 mg/g (RSD = 5.9%) (n = 6).

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