RESEARCH ARTICLE | JANUARY 10 2019

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AIP Conf. Proc. 2058, 020039 (2019)
https://doi.org/10.1063/1.5085552

Determination of Amygdalin in Cassia Obtusifolia L. by Capillary Electrophoresis

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Abstract. This paper investigated the determination of amygdalin content in Cassia obtusifolia L. 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 Cassia obtusifolia L. was 5.586 mg/g (RSD = 8.56%) (n = 6). The recovery was in the range of 88.2%-111.0% (n=6). This method is suitable for the detection of the content of amygdalin in Cassia obtusifolia L...

Key words: Capillary Electrophoresis, Amygdalin, Cassia Obtusifolia L.

INTRODUCTION

Cassia obtusifolia L. is dry mature seeds of Cassia obtusifolia L. or Cassia tora L,. It is sweet, bitter, salty and slightly cold. It has the functions of clearing liver and improving eyesight and moistening the intestines and relaxing the bowels. Its main active ingredients include anthraquinones and naphthopyrones. It has hypolipidemic, blood pressure regulation, liver preservation, antimicrobial activiyies and antioxidantion pharmacological action. Folin-Ciocalteu method, ultraviolet spectrophotometry and DPPH radical-scavenging capacity analytical method were employed by Pan et al [1] to measure the total phenolic and flavonoid contents and the DPPH scavenging capacity of 8 Chinese edible herbal materials (Semen ziziphi Spinosae, Gardenia jasminoides Ellis., Glycyrrhiza uralensis Fisch., Momordica grosvenori Swingle, Cassia obtusifolia L., Raphanus sativus L., Chrysanthemum morifolium Ramat., Prunus japonica Thunb.). Wang et al [2] established a simultaneous determining six free anthraquinones in Semen Cassiae by HPLC, including chrysophanol, emodin, rhein, physcion, obtusifolin and aurantio-obtusin. The analysis was carried out on a DiamonsilTM C18 column (5μm, 250 mm×4.6 mm) by gradient elution with the mobile phase comprising of 0.1% orthophosphoric acid in water and acetonitrile. The flow rate was 1.0 mL/min. The detection wavelength was set at 285 nm and at room temperature. Luo et al [3] established the method of HPLC for the determining the content of three naphthopyrone constituents including cassiaside B2, rubrofusarin gentiobioside and cassiaside C in Semen cassiae. The analysis was investigated on a Sunfire C18 column (250mm×4.6mm, 5μm) at 35℃. The mobile phase was composed of methanol and acetonitrile (2:1) and water as by gradient elution with flow rate of 1.0mL/min. The detection wavelength was 278nm. Zhang et al [4] established methods of high performance liquid chromatography to measure content of rubrofusarin gentiobioside, assiaside C and Aurantio- obtusifolin- 6- O- β- D- glucopyranoside of cassia seed. Performed Zorbax Eclipse XDB-C18 column was used at the temperature of 30℃. The mobile phase was composed of acetonitrile and tetrahydrofuran-1% acetic acid(30:70) as by isocratic elution with flow rate of 1.0 mL/min and detection wavelength of 278 nm. Analysis of polysaccharides and their hydrolyzed products in Cassia obtusifolia L. was studied by Wan et al [5] using UV spectrometry and high performance liquid chromatography, respectively. The composition characteristics of polysaccharides were investigated, including 12 batches of Cassia obtusifolia L. Zhan et al [6] established for simultaneously determination of aurantio-obtusifolin, emodin, chrysophanol and physcione in Semen Cassiae by UPLC. The optimal conditions of separation and detection

International Conference on Frontiers of Biological Sciences and Engineering (FBSE 2018)
AIP Conf. Proc. 2058, 020039-1–020039-5; https://doi.org/10.1063/1.5085552
Published by AIP Publishing. 978-0-7354-1786-1/$30.00

were obtained on an ACQUITY UPLC BEH-C18 column (2.1 mm×50 mm×1.7 μm). The mobile phase was composed of acetonitrile and 0.1% phosphoric acid with gradient elution with flow rate of 0.6 mL/min. The detection wavelength was 284 nm. Luo et al [7] developed a facile method for identification of components in Cassiae Semen by HPLC-MS. The method was carried out on HPLC-IT-TOF MS with electrospray ionization in both positive and negative ion modes. The mobile phase was composed of methanol and acetonitrile in proportion (2:1)-water by gradient elution with flow rate of 1.0 mL/min. The column temperature was at 35℃. A total of 17 components were identified, including 7 naphthopyrones, 7 anthraquinones and 3 naphthols. The total flavonoid of cassia seed tea were extracted by Ding et al [8] with water or different ethanol water solution using ultrasonic or reflux. The total flavonoid contents were determined with absorption spectrophotometer in 494 nm by using NaNO2-AlCl3 method. The anti-free radical activity of the extracts was detected by using DPPH method. The influence of different Cassia obtusifolia L. extracts on contents of eight kinds of the natural taste substances (glucose, fructose, mannitol, cassiaside, cassiaside Ⅴ, cassiaside IV, obtusifolin and Cassia obtusifolia L. benzoic acid esters) in Cassia obtusifolia L. extracts was compared by Zhao et al [9] using HPLC. The experiment was based on Waters symmetry C18 column (250 mm×4.6 mm, 5 μm) with mobile phase A of 80% acetonitrile aqueous solution (containing 0.01% trifluoroacetic acid and 2.0 mmol/L ammonium acetate aqueous solution) and mobile phase B of 0.1% trifluoroacetic acid and 2.0 mmol/L ammonium acetate, and gradient eluted with the flow rate of 0.8 mL/min, DAD detection wavelength of 254 nm and column temperature of 45℃. Chen [10] established the method for simultaneous determining amygdalin, licorice glycosides and glycyrrhizic acid in Pediatric oral Qingrezhike by HPLC. The Hypersil C18 column maintained at 35℃ with a gradient mobile phase composed of ACN-0.1% phosphoric acid solution was utilized for sample analysis. The wavelength of the UV detector was set at 207nm for amygdalin determination, whilst 237nm for liquiritin and glycyrrhizic acid determination. Gou et al [11] established an HPLC method for the determining amygdalin in asthma granules. The procedure of HPLC was analyzed on the chromatographic column of Agilent C18 (250 mm×4.6 mm, 5 μm), the mobile phase consisting of methanol-1% phosphoric acid water (21:79). The flow rate was l mL/min and detection wave length was 210 nm. Han et al [12] established the HPLC method for quantitative analysis of amygdalin in Xinjiang Amygdalus persica L., Amygdalin was extracted by ethyl alcohol reflux method. The content of amygdalin was investigated by use of the HPLC method. In the test, stationary phase was Zirchrom C18 column (250mm×4.6 mm, 10 μm), the mobile phase was composed of combination of methanol, water, glacial acetic acid in the volume ration of 20:80:05, the flow velocity was 1.0 mL/min, the wavelength of UV detector was 225 nm and the column temperature was 35℃. Shi et al [13] explored the application of RP-HPLC in determination of the contents of amygdalin and rosemary acid in XingSuSan extracts. The gradient elution with HPLC reversed chromatographic column C18 (250mm×4.6 mm, 5 μm) was adopted with the dual wavelengths respectively (222nm, 330nm), the flow rate 1.0 mL/min. Qin et al [14] established an HPLC method for the determining amygdalin in Xuehua Zhike. The procedure of HPLC was carried out on the chromatographic column of Zorbax SB-C18 (250 mm× 4.6 mm, 5 μm), the mobile phase was acetonitrile-1% phosphoric acid water (14:86). The detection wave length was 207 nm. In this paper, the amygdalin content in Cassia obtusifolia L. was determined by High Performance 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, 52 cm overall length, 44 cm effective length) from Hebei Yongnian Ruifeng Chromatographic Devices Co., Ltd.).

Amygdalin (Chinese Drugs and Biological Products); Cassia obtusifolia L. (purchase in weifang pharmacy); 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·L-1 hydrochloric acid solution, double-distilled water, 1 mol·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).

Sample Preparation

Cassia obtusifolia L. sample solution: Cassia obtusifolia L. power was accurately weighed 3.9779 g, added 40 mL water, extracted time of 24h at 30°C, filtered, washed and set the volume to 50 mL that was the Cassia obtusifolia L. sample solution.

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

RESULTS AND DISCUSSION

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.

Quantitative Analysis

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.

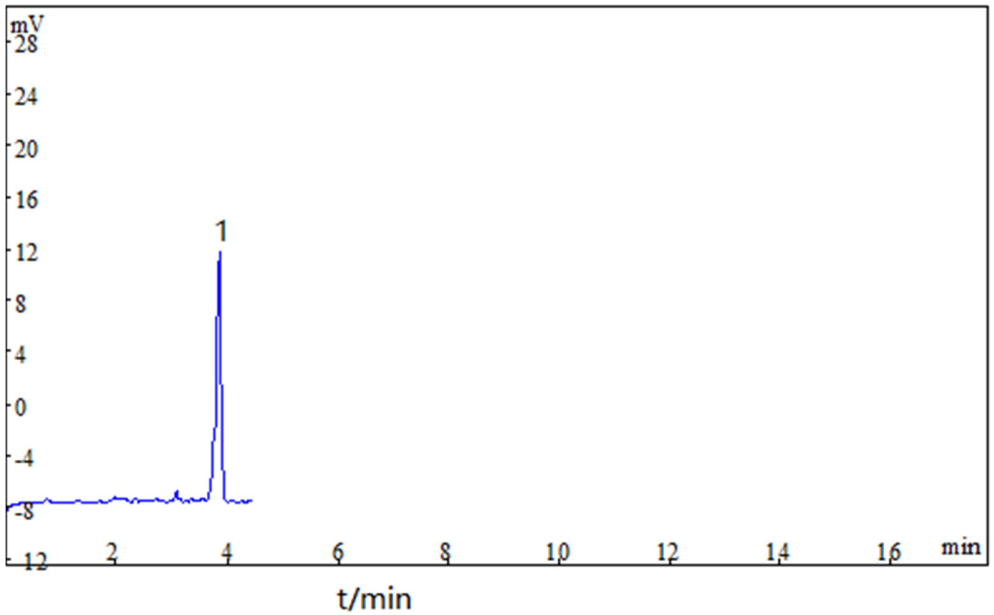

FIG 1. Electrophorogram of amygdalin standard solution 1-amygdalin

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.

Determination of Sample Content

Under selected electrophoresis conditions, Cassia obtusifolia L. sample solution was run. Separation chromatogram of the Cassia obtusifolia L. sample solution was showed in Figure 2. Measured amygdalin content in Cassia obtusifolia L. was 5.586 mg/g (RSD = 8.56%) (n = 6).

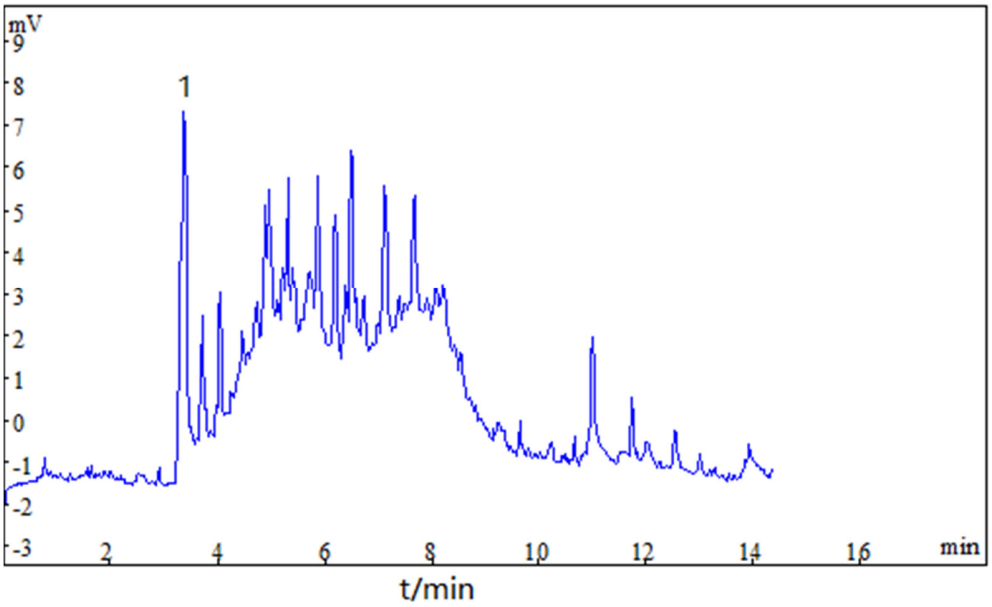

FIG 2. Electrophorogram of Cassia obtusifolia L. sample solution 1-amygdalin

Recovery

After determination for six times, the recovery of amygdalin in Cassia obtusifolia L. sample was in the range of 88.2% - 111% (n=6). The average recovery was 100.8%.

CONCLUSION

This paper investigated the determination of amygdalin content in Cassia obtusifolia L. by high performance capillary electrophoresis method. Measured amygdalin content in Cassia obtusifolia L. was 5.586 mg/g (RSD = 8.56%) (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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