Determination of Amygdalin in Feilike Capsule by Capillary Electrophoresis

Yimin Feng[a], Haixing Liu[b], and Lintong Wang[c, *]

Chemistry & Chemical and Environmental Engineering College, Weifang University, Weifang 261061, P.R. China

[a]2_haixingliu@tom.com, [b]haixingliu@tom.com, [c]wanglt6408@sina.com

Keywords: capillary electrophoresis, amygdalin, Feilike Capsule

Abstract: This paper investigated the determination of amygdalin content in Feilike Capsule 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 Feilike Capsule was 10.699 mg/g (RSD = 5.61%) (n = 6). The recovery was in the range of 79.1% - 122.8% (n=7). This method is suitable for the detection of the content of amygdalin in Feilike Capsule.

1. Introduction

Feilike Capsule consist of radix scutellariae, radix acutus, hedyotis diffusa, etc.. It has the function of clearing away heat and detoxicating, relieving cough and eliminating phlegm. It is used treatment of cough with asthma, difficulty in breathing. Zhou et al [1] developed an HPLC-DAD method for determining amygdalin, hydroxysafflor yellow A, paeoniflorin, ferulic acid, salvianolic acid B, and tanshinoneIIA in Naoxueshuan Tablets. The HPLC-DAD chromatography was adopted. The analysis was studied on an Agilent Poroshell 120 SB-C18 column (100 mm×4.6 mm, 2.7 μm). The mobile phase was composed of methanol - 0.2% phosphoric acid with flow rate of 0.7 mL/min for gradient elution. Detection with variable wavelength were adopted, and set at 210 nm for amygdalin, 403 nm for hydroxysafflor yellow A, 230 nm for paeoniflorin, 321 nm for ferulic acid, 278 nm for salvianolic acid, and 270 nm for tanshinoneIIA. The column temperature was 30℃ with injection volume of 3-5 μL. A high performance liquid chromatographic method with evaporative light scattering detector (ELSD) was obtained for determination of Amygdalin in Bitter Almond by Li et al [2]. Amygdalin was successfully analyzed by a XDB-C18 column (4.6×150mm, 5um) at 40℃. The mobile phase was composition of a binary solvent mixture of methanol-water (40:60, v/v) with flow rate of 1.0mL/min. The ELSD drift tube temperature was 60^{o}C, carrier air pressure was 0.2MPa. Peng et al [3] developed an HPLC-ELSD method for the determining amygdalin, aucubin, harpagide, peimisine, peimine and peiminine in Keling capsules simultaneously. An Ultimate XB C18 (250 mm×4.6 mm, 5 μm) chromatographic column was used with an ELSD (the drift tube temperature was 105℃, the flow rate of nitrogen was 2.0 L/min). The mobile phase was acetonitrile-methanol (1:1) and 0.4% acetic acid solution with gradient elution with flow rate of 0.7 ml/min, and the column temperature was set at 35℃. Jia et al [4] established a method for determining rutin, chlorogenic acid, forsythin, platycodin D and amygdalin in Sangju Ganmao Tablets by HPLC-MS /MS. The determination was investigated with Waters Atlantis C18 column (2.1 mm× 150 mm, 5 μm). The mobile phase was composed of acetonitrile -0.1% formic acid solution by gradient elution with flow rate of 0.2 mL/min and column temperature of 35℃. The cation scanning was carried out by tandem mass spectrometry under the the electrospray ionization (ESI) source combined with multiple reaction monitoring (MRM) mode. Hu [5] developed a HPLC method for determination of five constituents (ephedrine hydrochlorid, pseudoephedrine hydrochloride, amygdalim, baicalin and praeruptorin A) in Xiaoer Qingfei Huatan oral solution. Five constituents were studied by ZORBAX SB-C18 (4.6 mm× 250 mm, 5 μm) with acetonitrile-water (containing 0.1% phosphonic acid) as solvent system in gradient elution, 0.8

  DOI: 10.25236/iwass.2018.052

mL/min as flow-rate and 35℃ as temperature of column. The detection wavelengths were at 210 nm for ephedrine hydrochloride and pseudoephedrine hydrochloride, 225 nm for amygdalim, 277 nm for baicalin and 321 nm for praeruptorin A respectively. Jin [6] established a HPLC method for the determining amygdaloside in Baibu Zhike Syrup. The HPLC method was utilized at Grace Alltima C18 column (4.6mm×150mm, 5 μm) with mobile phase of methanol-water (14:86). The detective wavelength was 215nm. Zhang et al [7] developed an HPLC for quantitative analysis of amygdalin in Ganshi granules. Mobile phase was consisted of water, methanol, and acetonitrile at a rate of 70:15:10. The detection wavelength was set at 210 nm. Wang et al [8] established a method for determining Amygdalin and Baicalin in Gongliuqing Tablets by HPLC. The analysis method was carried out using AgiLent 1100 HPLC system, the analysis was performed with an AgiLent HC-C18 column (4.6mm×250mm, 5μm), the flow rate was 1 mL/min and the column temperature maintained at 30℃. Amygdalin: the mobile phase of ethanol-water (20:80), the detection wavelength was set at 210nm. Baicalin: the mobile phase of methanol-0.2% phosphoric acid (47:53), the detection wavelength was set at 280nm. Liu et al [9] established the determining method for amygdalin and paeoniflorin in the active fraction of Guizhi Fuling pills by HPLC. The Diamonsil C18 (250 mm×4.6 mm, 5 μm) was utilized with methanol-water (35:65) as the solvent system. The UV detection wavelength was set at 215 nm, the flow rate was 1.0 mL/min, and the column temperature was 30℃. Bi et al [10] established a method for the content determining of amygdalin in Lianhua qingwen capsule. The HPLC method was carried out on the column of Phenomenex Kinetex XB-C18 with mobile phase of acetonitrile-0.2％ phosphoric acid solution(6:94, *V*/*V*)at a flow rate of 1.0 ml/min, the detection wavelength was 207 nm, column temperature was 30 ℃. A HPLC method for determining amygdalin in Maxing cougy syrup was explored by Luo et al [11]. The Kromasil C18 column (250mm×4.6mm, 5μm) was applied. The mobile phase was composed of acetonitrile-0.1% phosphoric acid solution (8:92) with the flow rate of 1.0mL/min. The detecting wavelength was at 207nm. Li et al [12] determined amygdalin contents in heat-clearing dryness-moistening oral liquid by HPLC. The column utilized DiKma C18-EP (4.6mm×250mm, 5μm), mobile phase consisted of methanol-0.1％ phosphoric acid solution (7:93) with flow rate of 1.0mL/min, detection wavelength was 207nm and the column temperature was 25℃. Wang [13] evaluated the feasibility of determining amygdalin in Infantile Feirekechuan oral liquid by HPLC. Thermo-C18 column (250× 4.6 mm, 5μm) was used with methanol-water (13:87) as solvent system and flow rate of 1.0 ml /min and detection wavelength of 196 nm. In this paper, the amygdalin content in Feilike Capsule 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); Feilike Capsule (Guizhou Jianxing Pharmaceutical Co., Ltd., Batch number: 20171130); 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

Feilike Capsule sample solution: Feilike Capsule was accurately weighed 0.9149 g, added 40 mL water, extracted time of 24h at 30°C, filtered, washed and set the volume to 50 mL that was the Feilike Capsule 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.

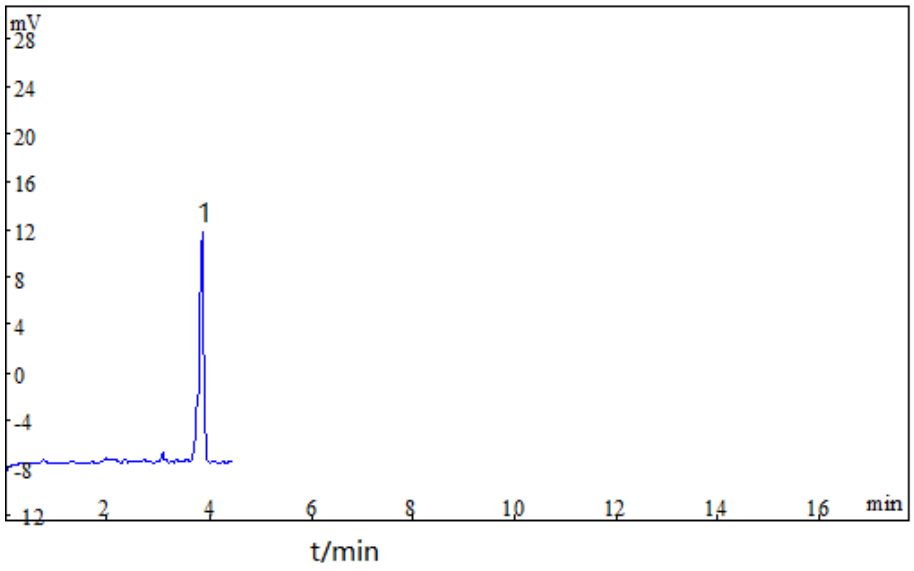

Fig.1 Electrophorogram of amygdalin standard solution 1-amygdalin

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, Feilike Capsule sample solution was run. Separation chromatogram of the Feilike Capsule sample solution was showed in Figure 2. Measured amygdalin content in Feilike Capsule was 10.699 mg/g (RSD = 5.61%) (n = 6).

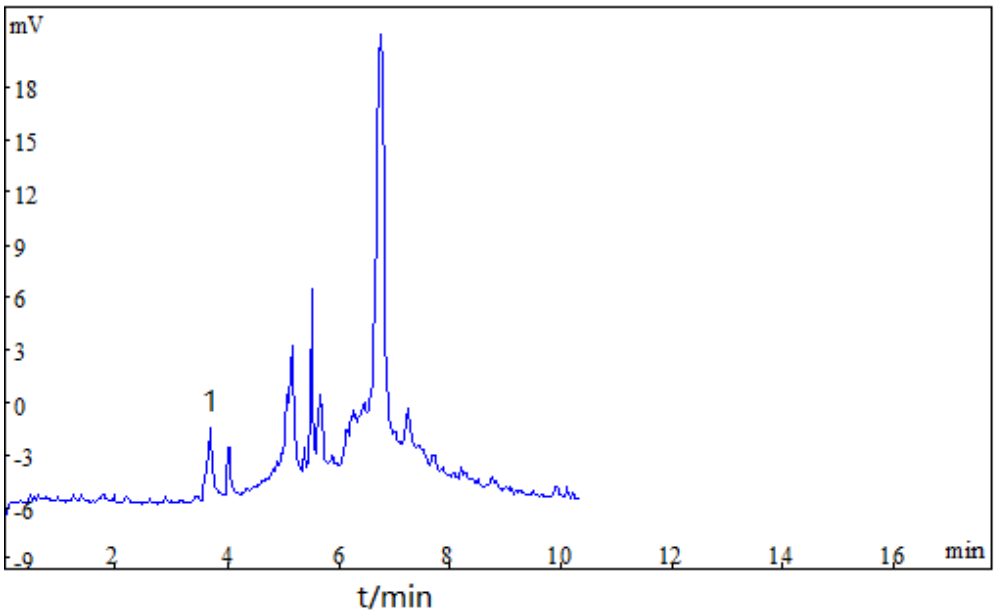

Fig.2 Electrophorogram of Feilike Capsule sample solution 1-amygdalin

3.2.4 Recovery

After determination for seven times, the recovery of amygdalin in Feilike Capsule sample was in the range of 79.1% - 122.8% (n=7).

4. Conclusion

This paper investigated the determination of amygdalin content in Feilike Capsule by high performance capillary electrophoresis method. Measured amygdalin content in Feilike Capsule was 10.699 mg/g (RSD = 5.61%) (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).

Corresponding author e-mail: wanglt6408@sina.com

References

[1] Zhou Jun, Zhang Lei, Wang Jie, (2015). Determination of amygdalin, hydroxysafflor yellow A, paeoniflorin, ferulic acid, salvianolic acid B, and tanshinoneIIA in Naoxueshuan Tablets by HPLC-DAD, Drugs & Clinic, 30(3), 262-266

[2] Li Hailong, Wang Jing, Zhang Yingchun, Jin Hongdao, Liu Shuming, Hao Haidong, (2007). Determination of Amygdalin in Semen Bitter Almond by High Performance Liquid Chromatographic Method with Evaporative Light Scattering Detector, FOOD AND FERMENTAT ION INDUSTRIES, 33(1), 109-112

[3] Peng Wei, Wang Lisi, (2017). Simultaneous Determination of Aygdalin, Aucubin, Harpagide, Peimisine, Peimine and Peiminine in Keling Capsules by HPLC-ELSD, China Pharmacist, 20(8), 76-88

[4] Jia Shoushi, Wang Mengmeng, Wang Chaozhong, (2017). Determinations of Five Components in Sangju Ganmao Tablets by HPLC-MS/MS, Drug Standards of China, 18(6), 455-460

[5] Hu Ying-jie, (2015). Quantitative Determination of Five Ingredients in Xiaoer Qingfei Huatan Oral Solution by HPLC, Chinese Journal of Experimental Traditional Medical Formulae, 21(13), 62-65

[6] Jin Tao, (2015). Determination of Amygdaloside in Baibu Zhike Syrup by HPLC, Strait Pharmaceutical Journal, 27(12), 55-57

[7] Zhang Ying, Zhang Wenjun, (2015). Determination of amygdalin content in Ganshi granules by HPLC, Tianjin Pharmacy, 27(4), 5-6

[8] Wang Ding-feng, Hu Bing, (2014). Detetmination of Amygdalin and Baicalin in Gongliuqing Tablets by HPLC, Strait Pharmaceutical Journal, 26(5), 40-43

[9] Liu Guohong, Zhao Lu, Wu Chunwei, Wang Shumei, Liang Shengwang, (2015). Determination of amygdali and paeoniflorin of the active fraction in Guizhi Fuling pills by HPLC, Journal of Guangdong Pharmaceutical University, 31(1), 50-53

[10] Bi Dan, Chen Yupeng, Wang Shujing, Wang Guijin, Zhao Qian, Wei Feng, (2016). Content Determination of Amygdalin in the Lianhua Qingwen Capsule by HPLC, China Pharmacy, 27(21), 2978-2979

[11] Luo Pei-he, Zhu Qi-qiong, (2014). Determination of Amygdalin in Maxing Cough Syrup by HPLC, Technology & Development of Chemical Industry, 43(2),42-44

[12] Li Yueting, Chen Lingyun, Wei Danxia, Yu Xiaoling, (2015). Determination of amygdalin contents in heat-clearing drynessmoistening oral liquid by HPLC, CHINA MEDICINE AND PHARMACY, 5(23), 69-71

[13] Wang Qian, (2015). Determination of Laetrile in Infantile Feirekechuan oral liquid by HPLC, Chin J of Clinical Rational Drug Use, 8(8), 37-39