RESEARCH ARTICLE | JANUARY 10 2019

Determination of amygdalin in Keke Pian by capillary electrophoresis FREE

Xiaojing Zhou; Yan Pang; Haixing Liu

AIP Conf. Proc. 2058, 020038 (2019)
https://doi.org/10.1063/1.5085551

Determination of Amygdalin in Keke Pian by Capillary Electrophoresis

Xiaojing Zhou[1)], Yan Pang[1)] and Haixing Liu[1, a)]

[1]*Chemistry & Chemical and Environmental Engineering College, Weifang University, Weifang 261061, P.R. China.*

[a)]Corresponding author e-mail: haixingliu@tom.com

Abstract. This paper investigated the determination of amygdalin content in Keke Pian 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 Keke Pian was 11.351 mg/g (RSD = 4.1%) (n = 6). The recovery was in the range of 88.0% - 115.9% (n=6). This method is suitable for the detection of the content of amygdalin in Keke Pian.

Key words: Capillary Electrophoresis, Amygdalin, Keke Pian.

INTRODUCTION

Keke Pian consist of ephedra, poppy shell, bitter almond, licorice root, radish seed, platycodon grandiflorum and gypsum. It is used for treatment of cough, shortness of breath. Zhu et al [1] Established a method for determination of amygdalin in loquat cough syrup by HPLC. The HPLC with Ultimate XB-C18 (250mm×4.6 mm, 5 μm) column was employed. The mobile phase was composed of methanol-water (20:80) with flow rate of 1 mL/min. The detection wavelength was set at 210nm. Zhu [2] established an HPLC method for the simultaneous determining five components (chlorogenic acid, amygdalin, puerarin, aicalinand, phillyrin) in Chaiyin Oral Liquid. Using Zorbax SB-C18 column (4.6 mm×250 mm, 5 μm), acetonitrile-0.1% phosphoric acid solution as solvent system. The flow rate was 1 mL/min, the detection wavelength was at 210 nm. Liu [3] established an HPLC method for the simultaneous determining 5 main components (amygdalin, honokiol, magnolol, hesperidin and nobiletin) in dingchuanzhike syrups. A stable HPLC method was tested and the chromatography was analyzed on an Hypersil C18 column, with a mobile phase of acetonitrile and 0.2% glacial acetic acid solution in a gradient elution and flow rate of 0.8 mL /min. The column temperature was set at 30 ºC. Amygdalin was analyzed at 210 nm, honokiol and magnolol were measured at 254 nm, hesperidin and nobiletin were analyzed at 283 nm. Fang [4] established an HPLC method for Simultaneous content determining dan phenol, paeoniflorin and peach kernel in guizhifuling capsule. The content of dan phenol, paeoniflorin and peach kernel in guizhifuling capsule was obtained by HPLC method. The chromatographic condition was C18 column with mobile phase of methanol-water (55:45) elution and flow rate of 1.0ml/min. The detection wavelength was 274nm and the column temperature of 30ºC. Liu et al [5] established the quantitative analysis method for determining the 8 components (amygdalin, baicalin, wogonoside, saikosaponin A, saikosaponin D, baicalein, wogonin and oroxylin A) in extract of modified Xiaochaihu Decoction simultaneously by HPLC method. The kromasil C18 (250mm×4.6 mm, 5 μm) column was adopted. The mobile phase consisted of acetonitrile-0.005% phosphoric acid with flow rate of 1 mL/min. The detection wavelength was at 210nm. Xu et al [6] established an HPLC method for the determining ephedrine hydrochloride and amygdalin in Kangzhi syrup. The separation was tested on a Waters ODS2 (250 mm× 4.6 mm, 5 μm) column. The mobile phase was acetonitrile-0.2% phosphoric acid solution (containing 0.1% triethylamine) with gradient elution with flow rate of 1.0 ml/min, the detection wavelength was at 205 nm, and the column temperature was 30 ºC. Jiang et al [7] established the method for simultaneous determining ephedrine, pseudoephedrine, amygdalin, liquiritin and glycyrrhizic acid in Maxing Shigan Decoction by HPLC. The

International Conference on Frontiers of Biological Sciences and Engineering (FBSE 2018)
AIP Conf. Proc. 2058, 020038-1–020038-5; https://doi.org/10.1063/1.5085551
Published by AIP Publishing. 978-0-7354-1786-1/$30.00

Agilent ZORBAX SB-Aq C18 column maintained at 30ºC with a gradient mobile phase system composed of ACN-0.1% phosphoric acid solution was utilized for sample analysis. The wavelength of the UV detector was set at 207nm for ephedrine, pseudoephedrine and amygdalin determination, whilst 237 nm for liquiritin and glycyrrhizic acid determination with a flow rate of 1mL/min. Zhang et al [8] established an HPLC method for the simultaneous determining the contents of ephedrine hydrochloride, nitrilosides, naringin, lobetyolin, schisandrin, senegenin and tussilagone in Qiguanyan Granules. The analysis was carried out on Agilent ZORBAX SB-C18 column (4.6 mm×150 mm, 5 μm), mobile phase consisted of acetonitrile-0.1% phosphoric acid solution with gradient elution, flow rate was 1 mL /min, and detection wavelength was set at 210 nm. Xue et al [9] determined the three constituents (ephedrine hydrochlorid, pseudoephedrine hydrochloride, amygdalim, baicalin) in Qingfei Huatan oral solution. Three constituents were discussed by ZORBAX SB-C18, with acetonitrile-0.1% phosphoric acid as solvent system in gradient elution, 0.8mL/min as flow-rate, 30 ºC as temperature of column and the detection wavelength were at 210nm. Zhou [10] established an HPLC method for the simultaneous content determining amygdalin, narirutin, naringin, rhoifolin, praeruptorin A, praeruptorin B and praeruptorin E in Sanfeng Ningsou Syrup (Armeniacae Semen Amarum, Citri grandis Exocarpium, Peucedani Radix, etc.). The analysis of methanol extract of this drug was tested on a 25 ºC Agilent SB-C18 column (4.6 mm× 250 mm, 5 μm), with the mobile phase consisting of acetonitrile-methanol (1:1)-0.1% phosphoric acid flowing at 0.9 mL /min in a gradient elution manner, and the detection wavelengths were set at 207, 283 and 321 nm. Zhu et al [11] established an HPLC method for determination of the contents of ephedrine hydrochloride, (R,S)-goitrin, laetrile, chlorogenic acid, licorice glycosides and glycyrrhizic acid in Xiao'er Kechuanling granule, The chromatography conditions were as follows: an Agilent Eclipse XDB -C18 (250 mm× 4.6 mm, 5 μm) column was utilized, and acetonitrile-0.1% phosphoric acid was used as the mobile phase with flow rate of 1.0 ml/min by gradient elution. The wavelength was 207 nm for ephedrine hydrochloride and laetrile, 237 nm for glycyrrhizic acid, licorice glycosides and chlorogenic acid and 245 nm for (R,S)-goitrin. Tan et al [12] established the quantitative method for the determination of six main constituents (amygdalin, harpagide, angoroside C, harpagoside, 24-acetate alisol A, 23-acetate alisol B) simultaneously in Jinsangsanjie capsules by HPLC gradient elution. The separation was tested on Venusil MP C18 chromatographic column by HPLC gradient elution. The mobile phase was acetonitrile and 0.03% phosphoric acid solution with flow rate of 0.8 mL/min. The column temperature was set at 30 ºC. The detection wavelength was set at 210 nm (amygdalin, harpagide), 280 nm (angoroside C, harpagoside) and 208 nm (24-acetate alisol A, 23-acetate alisol B). Chen et al [13] established an HPLC method to determine pinoresinol diglucoside, amygdalin, and cinnamylaldehyde in Bushen Huoxue granule. Agilent Zorbax Extend-C18(4.6 mm×150 mm, 5 μm) was used, the mobile phase was acetonitrile-water with gradient elution at a flow rate of 1.0 mL/min, and the detection wavelength was 210 nm(0-5 min) for amygdalin, 227 nm(5-10 min) for pinoresinol diglucoside, 290 nm(10-14 min) for cinnamylaldehyde. In this paper, the amygdalin content in Keke Pian 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); Keke Pian (Jiangxi Minze Pharmaceutical Co., Ltd., Batch number: 170413); 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

Keke Pian sample solution: Keke Pian was accurately weighed 2.0179 g, added 40 mL water, extracted time of 24h at 30°C, filtered, washed and set the volume to 50 mL that was the Keke Pian 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.

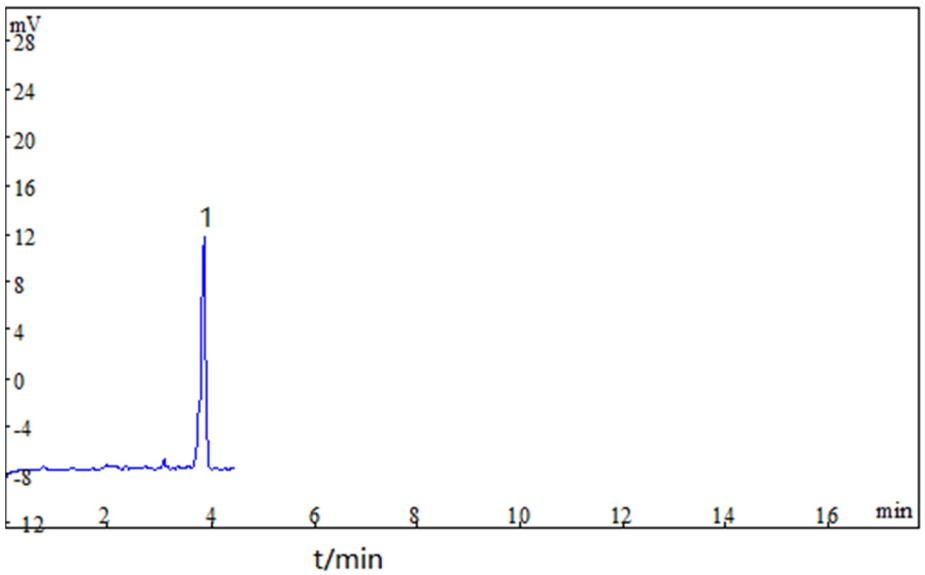

FIG 1. Electrophorogram of amygdalin standard solution 1-amygdalin

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, Keke Pian sample solution was run. Separation chromatogram of the Keke Pian sample solution was showed in Figure 2. Measured amygdalin content in Keke Pian was 11.351 mg/g (RSD = 4.1%) (n = 6).

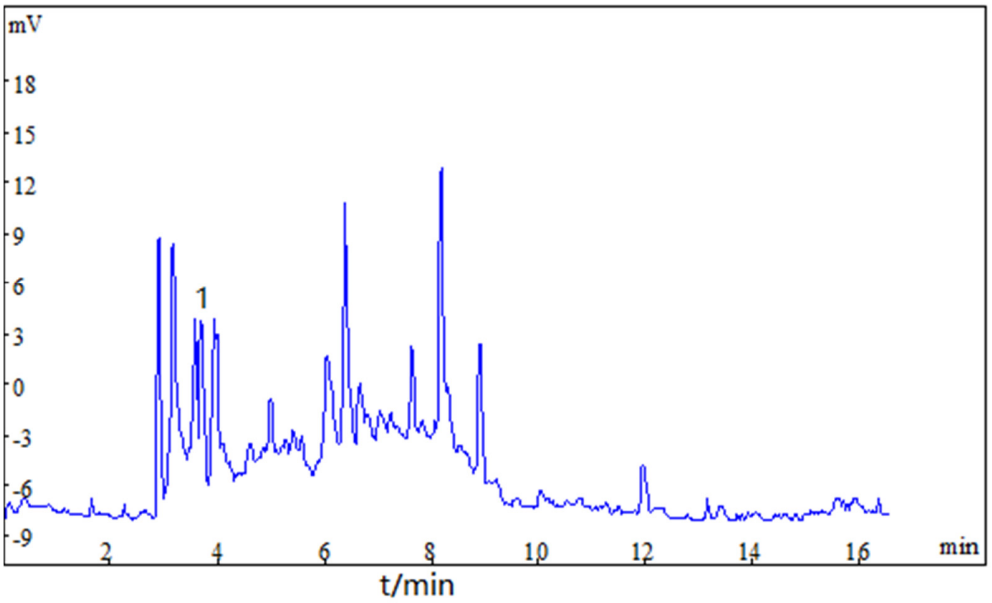

FIG 2. Electrophorogram of Keke Pian sample solution 1-amygdalin

Recovery

After determination for six times, the recovery of amygdalin in Keke Pian sample was in the range of 88.0% - 115.9% (n=6). The average recovery was 100.1%.

CONCLUSION

This paper investigated the determination of amygdalin content in Keke Pian by high performance capillary electrophoresis method. Measured amygdalin content in Keke Pian was 11.351 mg/g (RSD = 4.1%) (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).

REFERENCES

1. Zhu Yueyue, Fu Jing, Nie Shiming, Content Determination of Amygdalin in Loquat Cough Syrup by HPLC, Journal of Mathematical Medicine, 28(11), 1672-1674(2015).
2. Zhu Ri-ran, Simultaneous determination of five components in Chaiyin Oral Liquid by HPLC, Journal of Pharmaceutical Research, 34(9), 509-511(2015).
3. Liu Xiao-feng, Simultaneous determination of amygdalin, honokiol, magnolol, hesperidin and nobiletin in dingchuanzhike syrups by HPLC, Practical Pharmacy And Clinical Remedies, 19(2), 219-222(2016).

4. Fang Liqin, Simultaneous content determination of dan phenol, aeoniflorin and peach kernel in Guizhifuling capsule by HPLC method, Journal of North Pharmacy, 12(5), 5-6(2015).
5. Liu Junhong, Zhang Shukun, Li Dihua, Lu Yuanshan, Simultaneous content determination of 8 components in modified Xiaochaihu Decoction simultaneously by using HPLC, Chinese Journal of traditional Chinese Medicine, 31(3), 979-981(2016).
6. Xu Guijun, Qi Wei, Wang Xuesong, Zhao Min, Li Zhijun, Wang Youpeng, Simultaneous Determination of Ephedrine Hydrochloride and Amygdalin in Kangzhi Syrup by HPLC, China Pharmacist, 21(3), 499-501(2018).
7. Jiang Li, Li Hui-lan, Zhang Qi-yun, Rao Yi, Wang Yue-sheng, Xu Guo-liang, Simultaneous determination of 5 components in Maxing Shigan Decoction with HPLC method, Chinese Journal of traditional Chinese Medicine, 30(2), 537-540(2015).
8. Zhang Jian-chun, Gou Ya-jun, Deng Yong-qiang, Simultaneous determination of seven effective constituents in Qiguanyan Granules by HPLC, Chinese Traditional Patent Medicine, 38(4), 806-809(2016).
9. Xue Ping, Li Li, Determination of Four Ingredients in Qingfei Huatan Oral Solution by HPLC, Strait Pharmaceutical Journal, 29(8), 50-51(2017).
10. Zhou Min, Simultaneous determination of seven constituents in Sanfeng Ningsou Syrup by HPLC, Chinese Traditional Patent Medicine, 39(11), 2301-2304(2017).
11. Zhu Zebing, Zhang Dewei, Mou Lamei, Guo Ya, Simultaneous Determination of Six Components in Xiao'er Kechuanling Granule by HPLC, China Pharmacist, 21(2), 354-357(2018).
12. Tan Xiong-si, Pang Wu-yao, Simultaneous determination of six main constituents in Jinsangsanjie capsules by HPLC gradient elution, Chinese Journal of Drug Application and Monitoring, 14(5), 263-266(2017).
13. Chen Weifei, Li Yuanzhen, Zhu Xi, Wang Ling, Yin Hua, Simultaneous Determination of Pinoresinol Diglucoside, Amygdalin and Cinnamylaldehyde in Bushen Huoxue Granule, Chin J Mod Appl Pharm, 32(4), 463-466(2015).