Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 352–357
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Rapid separation and determination of structurally related anthraquinones in Rhubarb by pressurized capillary electrochromatography

Haixia Lü, Jiabin Wang, Xiaochun Wang, Xucong Lin, Xiaoping Wu, Zenghong Xie *

The Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University), Ministry of Education, and Department of Chemistry, Fuzhou University, Fuzhou, Fujian 350002, China

Received 18 April 2006; received in revised form 4 June 2006; accepted 12 June 2006
Available online 27 July 2006

Abstract

A pressurized capillary electrochromatography (pCEC) with monolithic column has been developed for the rapid separation and determination of five structurally related anthraquinones in Rhubarb. The possibility of rapid separation resulted from the unique pore structure with high permeability and favorable mass transfer characteristics of the monolithic stationary phase. The effect factors such as organic modifier, acidity and concentration of running buffer, separation voltage were investigated to acquire the optimum condition. In the 220 nm wavelengths, the five anthraquinones could be baseline-separated rapidly within 5 min with the separation voltage of −20 kV in 10 mmol/L phosphate buffer (pH 6.2) containing 65% acetonitrile. The calibration graphs of rhein, aloe-emodin, emodin chrysophanol and physcion were linear by plotting the peak area against the analytes concentration over the range of 0.2–65, 0.1–30, 0.1–55, 0.5–30 and 0.5–55 μg/mL, respectively. The detection limits of five anthraquinones were ranged from 0.06 to 0.2 μg/mL and the recoveries of Rhubarb samples were about 81.3–86.4% (R.S.D. ≤ 5.2%). This proposed method was successfully applied to determination of the five analytes in Rhubarb with satisfactory results.

Keywords: Anthraquinones; Monolithic column; Rhubarb; Pressurized capillary electrochromatography

1. Introduction

Rhubarb is one of the important ingredients in Chinese traditional prescriptions, in which the structurally related anthraquinone derivatives including emodin, aloe-emodin, rhein, physcion and chrysophanol, are the accepted important active components and have various pharmacological actions, such as purgation, antibacterial, curing mental and renal disorders, antitumor and antimutagenicity [1,2]. Recently continuing interest exists in studying the natural anthraquinones from many species of Rhubarb and commercial traditional Chinese drugs containing Rhubarb, which is also the foundation of evaluating its quality and the control of dosage during clinical studies. However, how to realize the rapid separation and determination of anthraquinone derivatives still represents a formidable challenge due to the very similar molecular structure. The structure of the five active anthraquinones is shown in Fig. 1.

The methods commonly used for the separation and determination of the anthraquinone compounds in Rhubarb are thin-layer chromatography (TLC) [3] and high performance liquid chromatography (HPLC) [4,5], But none of the methods are entirely adequate because of either poor resolution or consuming-time analysis. Although Liu et al. [6] developed a gradient elution HPLC method and successfully applied to separate and determine five anthraquinone derivatives in extract of Chinese herbal medicine Rhubarb, the separation of all analytes lasted 25 min. An isocratic elution HPLC method with a methanol −0.5% acetic acid (85:15 v/v) mobile phase was developed for the simultaneous separation the five anthraquinones in Rhubarb within 21 min [7]. In capillary zone electrophoresis [8], the best separation of the five anthraquinones could be obtained using 50 mmol/L borate buffer (pH 8.2) contain-

Abbreviations: ACN, acetonitrile; CEC, capillary electrochromatography; CZE, capillary zone electrophoresis; pCEC, pressurized capillary electrochromatography; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography; MEKC, micellar electrokinetic chromatography; SDS, sodium dodecyl sulfate; EOF, electroosmotic flow; IBMA, iso-butyl methacrylate; EDMA, ethylene dimethacrylate; MAA, methacrylic acid; MSMA, 3-trimethoxysilyl propyl methacrylate; AIBN, azobisisobutyronitrile

* Corresponding author. Tel.: +86 591 87893229.
E-mail address: zhxie@fzu.edu.cn (Z. Xie).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.06.023

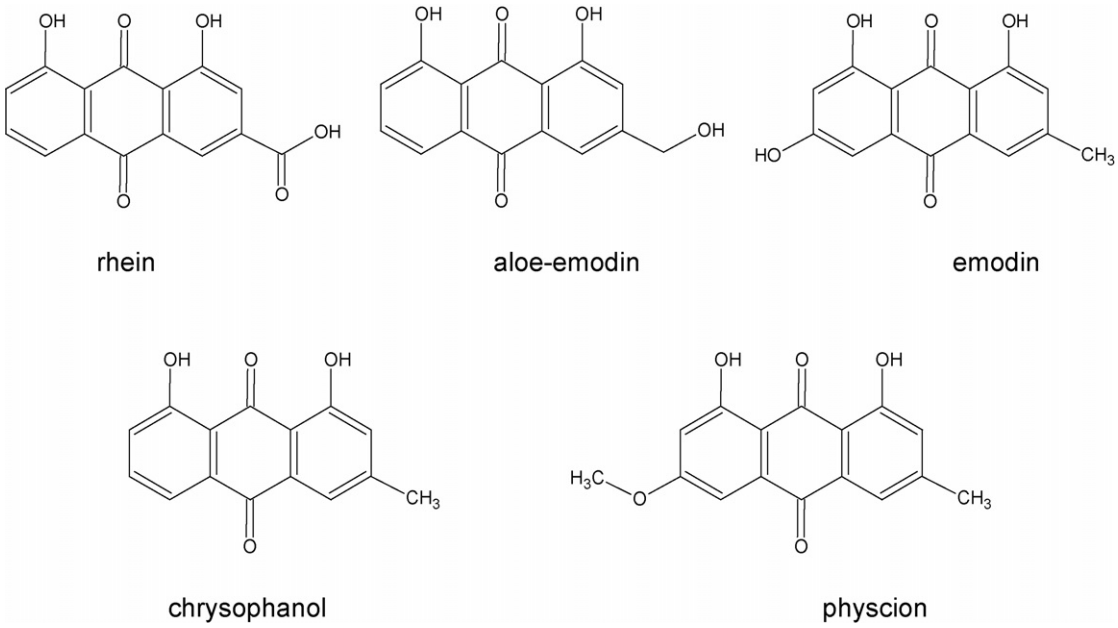

Fig. 1. Structures of anthraquinones analyzed.

ing 25% isopropyl alcohol and 25% acetontrile as modifier. The analysis was finished in 42 min with good recoveries. In addition, micellar electrokinetic chromatography (MEKC) [9–13] were also chosen as the alternative tool to finish the separation of anthraquinones in Rhubarb, Zong and Che [9] indicated that aloe-emodin, emodin, rhein, chrysophanol and physcion could be separated well within 13 min using 3-(cyclohexylamino)-1-propane sulfonic acid (0.025 mol/L) containing 0.025 mol/L sodium dodecyl sulfate (SDS) and acetonitrile (100:10, pH 10.96), but it was considered unsuitable for quantitative measurement for chrysophanol and physcion under MEKC system because of the unacceptably low linear correlation coefficients. Chai et al. [14] developed a MEKC method for the simultaneous separation of five anthraquinones within 10 min. However, it was focused on the analyte standards, thus the analysis of real samples has not been well documented.

Capillary electrochromatography (CEC), as a novel microcolumn separation technology, has been gained attractive attention in recent years. It is a new powerful separation technique, which combined the high efficiency of CZE and high selectivity of HPLC. Li et al. [15] and Ding et al. [16] established CEC method for separation of four anthraquinones from Rhubarb extract and commercial traditional Chinese drugs containing Rhubarb, in which the four analytes was baseline-separated within 12 min with ODS packed column. However, the intrinsic limitation of packed column such as bubble formation and "dry-out" problem encountered with "pure" CEC posted an obstacle to improve stability and reproducibility of experimental system. In order to minimize bubble formation, a micro-HPLC pump has been coupled to the CEC system and the result is a novel hybrid technique known as pressurized capillary electrochromatography (pCEC) [17–19], in which the mobile phase is propelled by an electroosmotic flow (EOF) of flat plug-like profile and a pressurized flow of parabolic profile like in HPLC, which can improve separation reliability and repeatability at the little expense of column efficiency, and increase the speed of separation and avoid bubble formation [20–22].

In this work, monolithic column with in situ polymerization of iso-butyl methacrylate (IBMA), ethylene dimethacrylate (EDMA), and methacrylic acid (MAA) was prepared, which offer unique pore structure with high permeability and favorable mass transfer characteristics for the stationary phase, and was suited for the rapid separation. Herein, we attempt to use the monoliths coupling pCEC for rapid separation of the structurally related anthraquinone derivatives in Rhubarb. The effects of analytical conditions were examined in detail. The mechanism of rapid separation and the possibility of pCEC with monolithic column for analysis of Rhubarb were simply discussed.

2. Materials and methods

2.1. Materials

Iso-butyl methacrylate (IBMA), ethylene dimethacrylate (EDMA), 3-trimethoxysilyl propyl methacrylate (MSMA), and methacrylic acid (MAA) were purchased from Acros (New Jersey, USA). Azobisisobutyronitrile (AIBN) was obtained from the Forth Chemical reagent Plant (Shanghai, China). 1-Propanol and 1, 4-butanediol were purchased from Tianjin Chemical Plant (Tianjin, China). Five standard anthraquinones, i.e., chrysophanol, rhein, aloe-emodin, emodin and physcion were all purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Commercial Rhubarb samples were bought from local herbal market in Fuzhou (Fujian, China). Acetonitrile (ACN) and methanol were of chromatographic grade. The other chemicals used were analytical grade and used without any further purification. The water

was purified with a Millipore Milli-Q purification system (Milford, MA, USA).

2.2. Instrumentation

pCEC was carried out on a Trisep™ 2100GV CEC system (Unimicro Technologies, Pleasanton, CA, USA) which comprised a solvent gradient delivery module, a high-voltage power supply (+30 and −30 kV), a variable wavelength UV–vis detector, a micro fluid manipulation module (including a six-port injector) and a data acquisition module [23]. A high-pressure syringe pump was used to provide supplementary flow to the CEC column. The mobile phase is driven by electroosmotic flow (EOF), as well as pressurized flow and enters into six-port injection valve. Samples injected are delivered to the injection valve and introduced in the internal 2 μL sample loop, and then be carried to the four-port split valve by the mobile-phase flow. After splitting in a four-port valve, the flow enters a capillary column under constant pressure controlled by a backpressure regulator. A negative voltage was applied to the outlet of column, and the inlet of column was connected to the split valve and grounded. In this experiment, the isocratic elution system was used and 220 nm was used as the measurement wavelength of the UV–vis detector. FS-1 Hi-speed homogenizer (Jintan Fuhua Instrument Corporation, Jiangsu, China) and BF2000 nitrogen evaporator (Beijing Bafang Century Science-Tech Corporation, Beijing, China) were also used.

2.3. Preparation of polymeric monolithic columns

In order to improve the stability of the monolithic columns, the inner wall of a capillary was treated with a bifunctional reagent, MSMA, according to the procedure which is similar to what has been previously reported [24]. After conditioning, the preparation of the monolithic column was carried according to the following procedure. The polymerization mixture containing 320 μL EDMA, 432 μL IBMA, 48 μL MAA, 2.4 mg AIBN (0.3 wt.% with respect to the monomers), and 1200 μL of porogenic solvent composed of 1-propanol and 1,4-butanediol (70/30 v/v) was sonicated for 15 min to obtain a homogeneous solution, and then was injected into the pretreated capillary to a total length of 20 cm by syringe injection. The capillary was sealed at both ends with silicon rubber stoppers and was submerged into a thermostated bath at 60 °C for 3–5 h. The resultant monolithic capillary column was washed with methanol for about 2 h using an HPLC pump to remove unreacted monomers and porogens. The detection window was created next to the end of the monolithic polymer by removing the coating using heater. The ashes of the organic monolith inside the capillary were flushed out by methanol for about 30 min with the HPLC pump.

2.4. Procedures

Standard stock solutions were prepared in methanol to give a final concentration of 1.0 mg/mL. A further dilution was performed by diluting stock solution with mobile phase to give a desired concentration before use. The mobile phase was prepared by mixing appropriate volume of ACN and phosphate buffer solution, and degassed in an ultrasonic bath for 15 min before use. All solutions were freshly prepared in water and passed through a 0.22 μm membrane filter before use. A supplementary pressure (100 psi) was applied to the column inlet during the separation. Before pCEC experiments, the column was conditioned on the instrument with the mobile phase for 1 h, applied voltage was first ramped from 0 to −20 kV and then operated at −20 kV. The column was equilibrated for about 30 min after the mobile phase was changed and the temperature of the column was kept at room temperature.

In our work, k^* can be expressed by the following equation [25]:

$$k^* = (t_r - t_0)/t_0 \quad (1)$$

where t_r and t_0 are the retention time of the solute and the void time, respectively. The void time was determined by thiourea in our experiment.

2.5. Sample preparation

A 6 mg sample of Rhubarb powder was extracted with 30 mL methanol in an ultrasonic bath at room temperature, then centrifuged at 1500 r/min for 5 min. The upper layer of the mixture was evaporated to dryness under N_2. The residue was dissolved by 3 mL mobile phase before analysis. Fixed amounts of pure chrysophanol, rhein, aloe-emodin, emodin and physcion were added to raw Rhubarb of known contents, respectively, and the mixtures were extracted according to the above-mentioned method. The extracts were used for recovery studies.

3. Results and discussion

3.1. Characteristics of the monolith

The SEM images of the cross section of the prepared monolith in the fused silica capillaries are shown in Fig. 2, in which the pore structure of the monolith and effective attachment of the ashes of the organic monolith to the capillary wall can be observed. Therefore, the low flow resistance and good permeability were possible due to the large through-pores or high porosity of the monolith. It may also have been a result of a high degree of connectivity of the through-pores, which has been shown to be an important factor affecting the permeability of a monolith in theoretical studies [26,27]. The monolithic bed with macropores linked to the pretreated capillary wall, which assured definite stability of the stationary phase.

3.2. Analytical conditions for the pCEC method

3.2.1. Effect of ACN concentration

The organic modifier in the mobile phase is an important parameter in CEC. In order to examine the effect of organic modifier on the separation of analytes by pCEC on the prepared monolith, different content of ACN, in the range of 60–74%

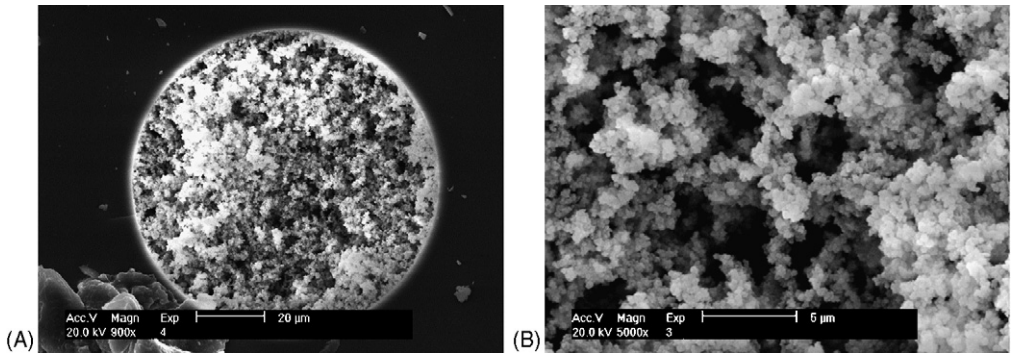

Fig. 2. Scanning-electron micrograph of the end of the poly (IBMA-co-EDMA-co-MAA) monolithic stationary phase in a fused-silica capillary column with 100 μm i.d. (A) 900×; (B) 5000×.

(v/v), were added to a 10 mmol/L phosphate buffer (pH 6.2). As can be seen from Fig. 3, the migration time and the resolution of these analytes decreased with the increasing content of ACN in the mobile phase, which maybe attributed to the change of EOF and chromatographic partitioning. The effect of ACN concentration in the mobile phase on the retention factors of five anthraquinones was also investigated. It was found that $\log k^*$ almost linearly decreased with the increasing concentration of ACN in the mobile phase ($r > 0.9829$). Because the hydrophobic groups in the monoliths are responsible for chromatographic interactions, it can be deduced that the separation of anthraquinones on the prepared monolithic column is mainly based on typical chromatographic retention mechanism.

3.2.2. Effect of concentration and pH of the buffer

The resolution of five anthraquinones is greatly affected not only by the percentage of organic modifier in the mobile phase but also by the pH of the buffer solution. The chemical structures of these analytes studied show that their apparent charged depends on their pK_a values tested to be approximately 8.5 for physcion, chrysophanol and aloe-emodin, 5.7 for emodin, and 4.7 for rhein in the aqueous solution, respectively [28]. The mobile phase containing 10 mmol/L phosphate at different pH values ranging from 4.9 to 7.0 was investigated to study the effect of pH value on the separation. As can be seen from the results, five anthraquinones had been baseline separation in the low-pH, with the increasing of pH, the resolution among the rhein; aloe-emodin; emodin; decreased. The reason is contributing to the negative charge of the rhein and the emodin with increasing of pH, which results in the increase of electrophoretic mobility, thereby, the capacity factor had been changed. Therefore, in our work, pH 6.2 was chosen as the optimal pH value since this pH value gave the good and relatively fast separation.

The effect of phosphate concentration on the separation of five anthraquinones was studied using 2–20 mmol/L of phosphate buffer at pH 6.2. The results indicated that with the increasing of buffer concentration, on the one hand, the migration time of five anthraquinone standard samples decreased. On the other hand, baseline separation between aloe-emodin and emodin could not be achieved. Considering the resolution and migration time, a 10 mmol/L ionic strength buffer was found to be suitable to separate in a proper analysis time.

3.2.3. Effect of applied voltage

The effect of the applied voltage on the resolution and analysis time of the five anthraquinones was determined in a mobile phase containing 65% (v/v) ACN, 10 mmol/L phosphate buffer (pH 6.2) at constant pressure. The applied voltage was varied from 0 to −25 kV. At −25 kV, peaks 1 and 2 is completely overlapped, while without applied voltage, the resolution between peaks 2 and 3 is poor, which is attributed to the migration mobility changes of rhein and emodin with the negative charge at pH 6.2. The carboxyl groups on the surface of the monolithic stationary phase contributed to the generation of cathodic electroosmotic flow (EOF) under pH 6.2 [29], which was contrary to the electrophoretic mobility of rhein and emodin. Because the degree of increase of migration mobility for emodin with the negative charge at pH 6.2 less than aloe-emodin without charge resulted in the resolution between peaks 2 and 3 becomes good, which is the same between rhein and aloe-emodin. In view of migration time and resolution, the best separation was obtained using −20 kV.

3.2.4. Optimized separation

After optimization of the mobile phase composition, pCEC separation of five anthraquinones has been performed in phosphate buffer (pH 6.2, 10 mmol/L ionic strength)/acetonitrile (35/65 v/v), applied voltage −20 kV, supplementary pressure 100 psi, flow rate 0.1 mL/min.

3.3. The linear range, detection limit and precision

The linear regression analysis of five anthraquinones was constructed by plotting the peak area of these analytes versus concentration in spiked Rhubarb sample at different concentrations under the optimum conditions. Each point on the calibration graph corresponded to the mean value obtained from independent peak area measurements. The corresponding regression equations, as well as other characteristic parameters for the determination of rhein; aloe-emodin; emodin; chrysophanol; physcion were listed in Table 1. The detection limits (LODs, S/N = 3) were also given.

Under optimized conditions, the reproducibility was evaluated with the intra-day R.S.D.s (≤0.36%, $n = 6$), inter-day R.S.D.s (≤1.60%, $n = 6$) and column-to-column R.S.D.s

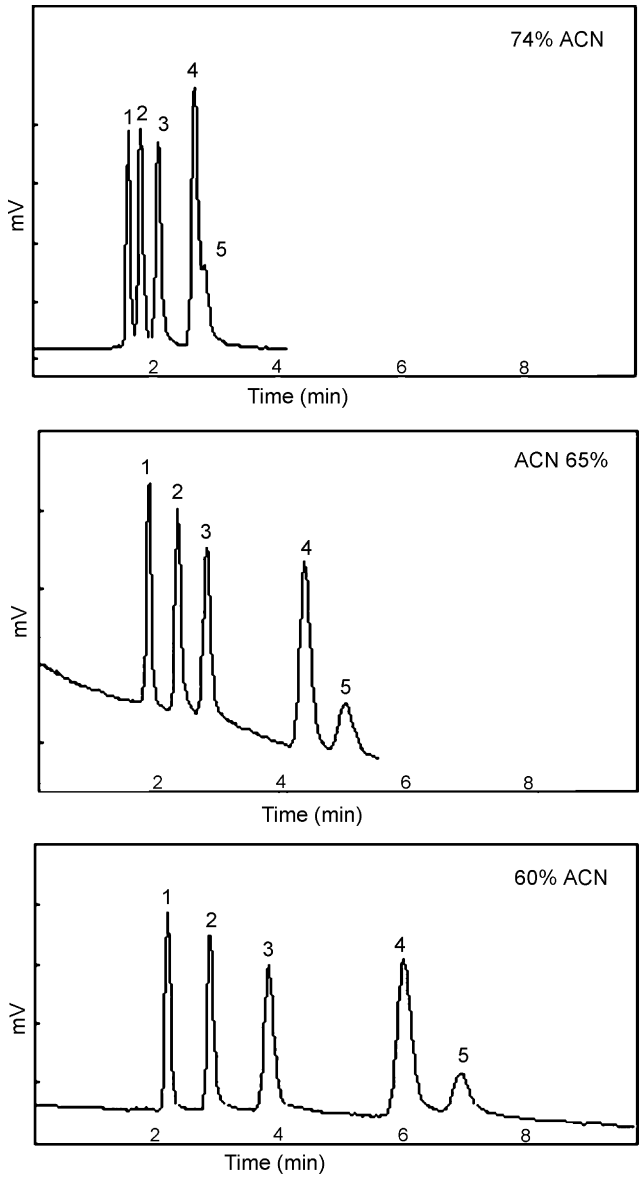

Fig. 3. Influence of the concentration of ACN on separation of antraquinone compounds. Experimental conditions: column, effective length 20.0 cm × 100 μm i.d. × 375 μm o.d.; mobile phase, 10 mmol/L phosphate buffer containing various volume fractions of acetonitrile, pH 6.2; applied voltage, −20 kV; detection wavelength, 220 nm; supplementary pressure, 100 psi; flow rate, 0.10 mL/min. Solutes: (1) rhein; (2) aloe-emodin; (3) emodin; (4) chrysophanol; (5) physcion.

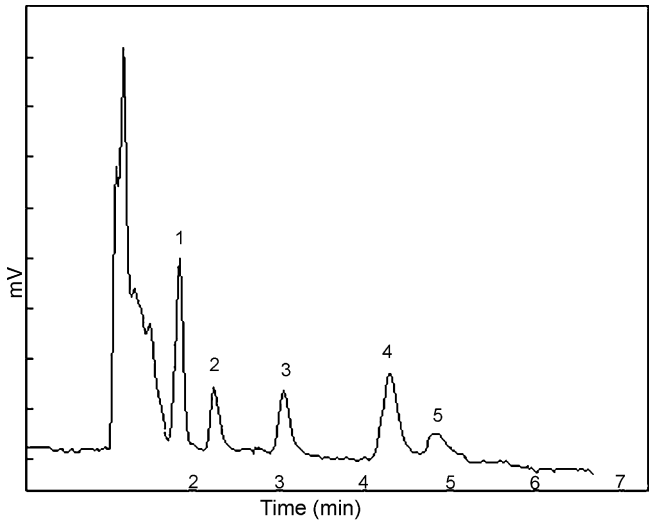

Fig. 4. Typical electrochromatogram obtain from Rhubarb extract under optimized conditions. Experimental conditions: mobile phase, 10 mmol/L phosphate buffer containing 65% acetonitrile, pH 6.2. Other conditions can be seen in Fig. 3.

(≤5.22%, $n = 5$) for the retention times of 1.0 μg/mL five anthraquinones standard solution, respectively. Intra-day repeatability, inter-day repeatability and column-to-column reproducibility in terms of peak areas were within 4.21, 6.05 and 8.22% (R.S.D.s), respectively.

3.4. Determination of anthraquinones in the Rhubarb sample

Under the optimized conditions, Rhubarb sample was analyzed by this proposed method. The electropherogram was given in Fig. 4 and no interference was observed at the migration time of the components in sample. By substituting the peak-area ratios of the individual peaks for y in the regression equations, the contents of the individual compounds in the test samples were obtained 1.41 μg/mg for rhein, 0.95 μg/mg for aloe-emodin, 1.49 μg/mg for emodin, 0.88 μg/mg for chrysophanol and 0.56 μg/mg for physcion and the R.S.D.s of three replicated injections were less than 5.35% for the five analytes in the extract of Rhubarb.

In order to examine the reliability of the method, the recoveries of five compounds were investigated. Rhubarb samples were spiked with 0.8 and 5.0 μg/mL of each standard solution of anthraquinones, then extracted and analyzed as described in Section 2.5. The recoveries were calculated to be 83.6–86.3%

Table 1
The linear range and detection limit

Analyte	Linear regression equation[a]	r	Linear range (μg/mL)	Detection limits[b] (μg/mL)
Rhein	$y = 126.19x + 2790.2$	0.9967	0.2–65	0.06
Aloe-emodin	$y = 202.44x + 897.78$	0.9970	0.1–30	0.06
Emodin	$y = 187.8x + 1448.1$	0.9914	0.1–55	0.06
Chrysophano	$y = 305.83x + 2656$	0.9954	0.5–30	0.1
Physcion	$y = 58.825x + 1148.7$	0.9946	0.5–55	0.2

[a] y: peak area (mV s); x: amount concentration (μg/mL).
[b] Calculated as three times of the signal-to-noise ratio.

Table 2
Results of recoveries ($n = 5$)

Analyte	Added (mg/L)	Found (mg/L) (mean ± S.D.)	Recovery (%)	R.S.D. (%)
Rhein	0.8	0.69 ± 0.01	86.3	2.7
	5.0	4.18 ± 0.04	83.6	3.2
Aloe-emodin	0.8	0.68 ± 0.01	85.0	4.6
	5.0	4.27 ± 0.04	85.4	2.2
Emodin	0.8	0.66 ± 0.02	82.5	3.0
	5.0	4.32 ± 0.06	86.4	1.7
Chrysophano	0.8	0.67 ± 0.02	83.8	3.4
	5.0	4.20 ± 0.04	84.0	2.1
Physcion	0.8	0.65 ± 0.02	81.3	5.2
	5.0	4.14 ± 0.03	82.8	2.8

for rhein, 85.0–85.4% for aloe-emodin, 82.5–86.4% for emodin, 83.8–84.0% for chrysophanol, 81.3–82.8% for physcion (see Table 2), which indicated the reliability of the method for the real sample analysis.

4. Conclusion

In this work, monolithic column with in situ polymerization of iso-butyl methacrylate (IBMA), ethylene dimethacrylate (EDMA), and methacrylic acid (MAA) was prepared, which has the unique pore structure with high permeability and favorable mass transfer characteristics of the monolithic stationary phase, and then suited for the rapid separation. Under the optimized conditions, five anthraquinones can be baseline-separated by isocratic elution pCEC within 5 min on the monolith. The proposed method was successfully applied to analysis of five active components in Rhubarb samples and it was performed without the use of a gradient, ion pairing reagents, or surfactants as the pseudostationary phase. However, it is possible to realize the rapid separation and determination of five structurally related anthraquinones in Rhubarb using conventional capillary LC mode by optimizing composition ratio of the prepared monolith. The further research will be developed in future work.

Acknowledgements

This work was supported by funding from Program for New Century Excellent Talents in University (NCET-04-0612), National Natural Science Foundation (20305004, 20575012), Specialized Research Fund for the Doctoral Program of Higher Education (20050386008) of China, and the Key Science and Technology project of Fujian province (2003Y005, 2005Y015).

References

[1] X.Y. Wu, Q.T. Wu, L.J. Liu, Acta Chin. Med. Pharmacol. 2 (1995) 54–55.
[2] Y.Zh. Tao, Y. Liu, Q.M. Guo, Y.X. Liu, J. Labour Med. 16 (1999) 28–29.
[3] L.Y. He, S.R. Luo, Acta Pharm. Sin. 15 (1980) 555–562.
[4] Dj. Djozan, Y. Assadi, Talanta 42 (1995) 861–865.
[5] W. Metzger, K. Reif, J. Chromatogr. A 740 (1996) 133–138.
[6] C.L. Liu, P.L. Zhu, M.C. Liu, J. Chromatogr. A 857 (1999) 167–174.
[7] M.Y. Ding, S.W. Ma, D.L. Liu, Anal. Sci. 19 (2003) 1163–1165.
[8] Y.X. Gong, S.P. Li, Y.T. Wang, P. Li, F.Q. Yang, Electrophoresis 26 (2005) 1778–1782.
[9] Y.Y. Zong, M.T. Yu, C.T. Che, J. Nat. Prod. 58 (1995) 577–582.
[10] S.W. Sun, P.C. Yeh, J. Pharm. Biomed. Anal. 36 (2005) 995–1001.
[11] X.Y. Shang, Z.B. Yuan, J. Pharm. Biomed. Anal. 31 (2003) 75–81.
[12] L.C. Chang, S.W. Sun, J. Pharm. Biomed. Anal. 40 (2006) 62–67.
[13] S.J. Sheu, H.R. Chen, Anal. Chim. Acta 309 (1995) 361–367.
[14] Y.F. Chai, S.G. Ji, Y.T. Wu, Biomed. Chromatogr. 12 (1998) 193–195.
[15] Y. Li, H.W. Liu, X.H. Ji, J.L. Li, Electrophoresis 21 (2000) 3109–3115.
[16] J. Ding, B. Ning, G. Fu, Y. Lu, S. Dang, Chromatographia 52 (2000) 285–288.
[17] T. Tsuda, Anal. Chem. 59 (1987) 521–523.
[18] C. Yan, D. Shaufelberger, F. Erni, J. Chromatogr. A 670 (1994) 15–23.
[19] J.T. Wu, P. Huang, M.X. Li, D.M. Lubman, Anal. Chem. 69 (1997) 2908–2913.
[20] P. Gfrörer, L.H. Tseng, E. Rapp, K. Albert, E. Bayer, Anal. Chem. 73 (2001) 3234–3239.
[21] D. Wistuba, K. Cabrera, V. Schurig, Electrophoresis 22 (2001) 2600–2605.
[22] Q.H. Ru, G.A. Luo, Y.R. Fu, J. Chromatogr. A 924 (2001) 331–336.
[23] Q.H. Ru, J. Yao, G.A. Luo, Y.X. Zhang, C. Yan, J. Chromatogr. A 894 (2000) 337–343.
[24] R.A. Wu, H.F. Zou, M.L. Ye, Anal. Chem. 73 (2001) 4918–4923.
[25] Z. Liu, K. Otsuka, S. Terabe, J. Chromatogr. A 959 (2002) 241–253.
[26] J.J. Meyers, A.I. Liapis, J. Chromatogr. A 852 (1999) 3–23.
[27] A.I. Liapis, J.J. Meyers, O.K. Crosser, J. Chromatogr. A 865 (1999) 13–25.
[28] D.X. Wang, G.L. Yang, X.R. Song, Electrophoresis 22 (2001) 464–469.
[29] W.H. Jin, H.J. Fu, X.D. Huang, H. Xiao, H.F. Zou, Electrophoresis 24 (2003) 3172–3180.