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Two-dimensional counter-current chromatography: 1st Traditional counter-current chromatography, 2nd acid–base elution counter-current chromatography

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ABSTRACT

An on-line column-switching counter-current chromatography (CCC) with solid-phase trapping interphase is presented in this paper. The large volume injection is avoided using solid-phase trapping interphase. Thereby, totally different chemical composition biphasic solvent system can be utilized to enhance system orthogonality. In the present work, a 140 mL-capacity CCC instrument was used in the first dimension, and a parallel three-coil CCC centrifuge (40 mL each coil) was used in the second dimensional separation allowing three injections at the same time. With biphasic solvent system composed of n-hexane: ethyl acetate: methanol: water (1:1:1:1, v/v), five well-separated fractions were obtained in the first dimension. Two fractions were online concentrated and further separated in the second dimension with solvent system composed of methyl tert-butyl ether: acetonitrile: water (2:2:3, v/v), where trifluoroacetic acid (10 mM) was added to the upper organic phase as a retainer and triethylamine (10 mM) to the aqueous mobile phase as an eluter. Four hydroxyanthraquinones were successfully separated and purified from Chinese medicinal plant *Rheum officinale* in only one step.

1. Introduction

Counter-current chromatography (CCC) was a continuous liquid–liquid partition method which was developed by Ito in the late 1960s [1]. Both the stationary phase and mobile phase used in CCC are liquids, which relies on the partition of a sample between biphasic solvent systems to achieve separation. Therefore, CCC benefits from great advantages when compared with the traditional liquid–solid separation methods: it eliminates the complications resulting from the solid support matrix, such as irreversible adsorptive sample loss and deactivation, tailing of solute peaks, and contamination. In addition, CCC has the unique features of high recovery, high efficiency and ease to scale up [2–4]. Regarding the recent numerous literature about CCC development and applications, CCC has been widely used in the separation and purification of various natural products [5–10]. However, since the crude natural product extract is a complex mixture which contains extremely large number of molecules with a wide range of polarity and different molecular weight, it is a significant challenge for conventional CCC technique due to the narrow hydrophobicity window of any single biphasic solvent system in the isocratic elution mode [11,12].

Multiple-dimensional CCC (MDCCC) method is one of the promising approaches to deal with the crude natural plant extracts. MDCCC was first developed by Ito and co-workers [13–15]. Using two same multilayer coil planet centrifuges and a commercial six-port valve, the interested effluent in the first column was directly introduced to the second column. It is proved to be an effective approach to improve the peak resolution when two peaks are overlapped in 1 D CCC separation. As a result, it is predictable that MDCCC method has great potential in separation of complex natural plant extracts.

In present study, a MDCCC system with solid-phase trapping interface has been developed, including a semi-preparative CCC instrument (V_c = 140 mL) in the first dimension and a parallel three-coil CCC instrument (40 mL each coil) in the second dimension (Fig. 1). Using solid-phase trapping column and switching valves, the interested fractions in the first column were concentrated and on-line introduced to the second column. It is promising to use totally different biphasic solvent system in present MDCCC system. Up to three independent CCC separations can be performed at the same time in the second dimension, which greatly increases separation efficiency. The crude extract of *Rheum officinale* was used as testing materials to evaluate the present method. *R. officinale* is a traditional Chinese medicine, and is widely used for the treatment of gastrointestinal hemorrhage, abdominal pain, gum pain, sore throat, amenorrhea and so on [16–18]. The phytochemical and pharmacological studies have demonstrated that

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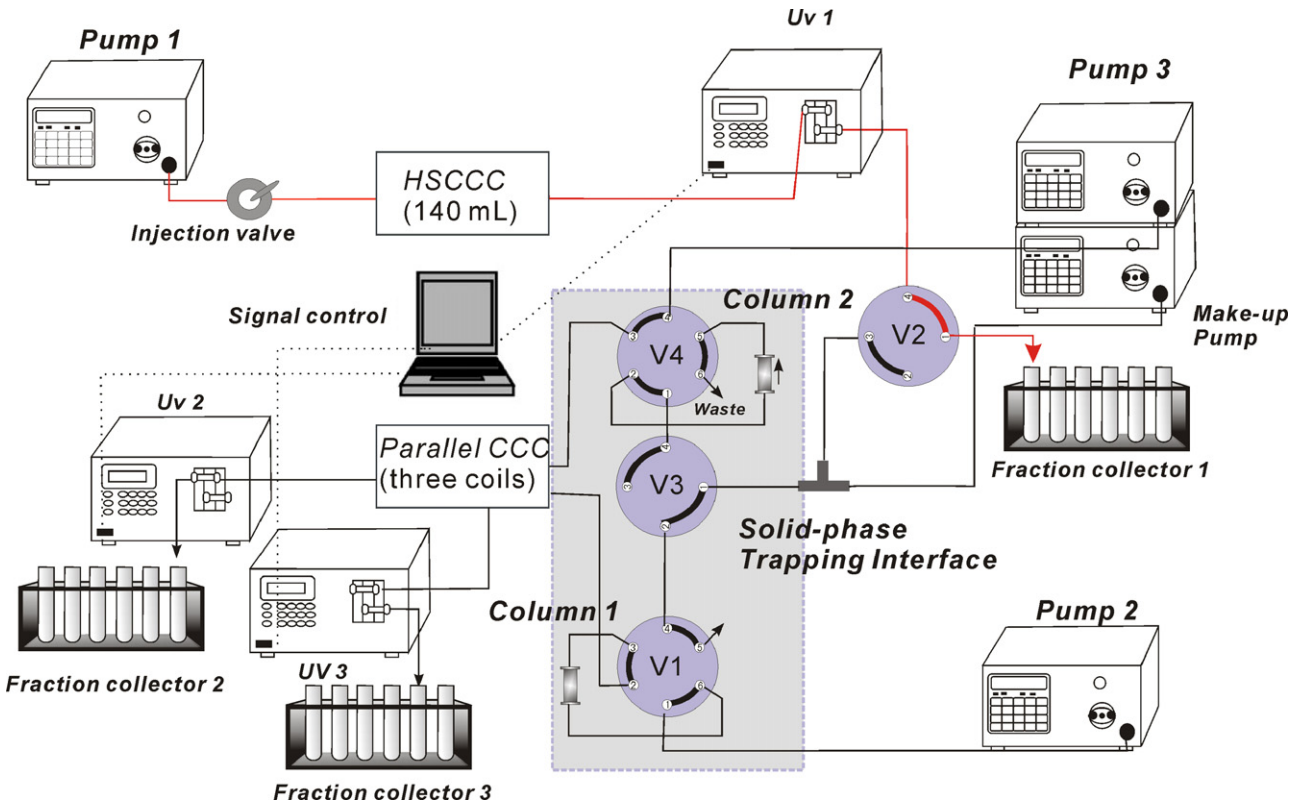


Fig. 1. Scheme of MDCCC system set-up.

hydroxyanthraquinones and their derivatives are the major bioactive constituents in *R. officinale* which have antitumor, antioxidant and anti-inflammatory activities [19]. In this work, elution-extrusion CCC (EECCC) was selected in the first dimension to fast fractionate the crude extract. The interested fractions containing overlapped constituents in the first dimension were further separated by acid–base elution. The crude extract of *R. officinale* was monitored by LC/MS/MS analysis, providing the primary structural information.

2. Experimental

2.1. 2D-CCC instrumentation

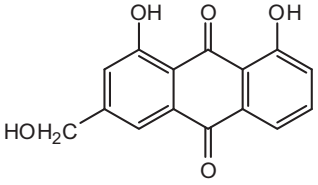
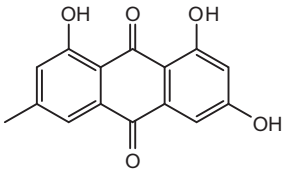
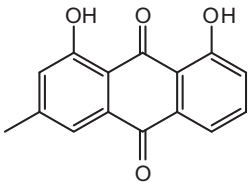
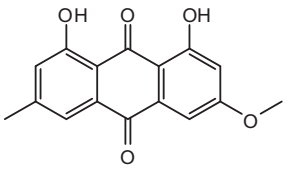
A 140 mL Ito scheme-IV CCC column (Zhejiang University machine shop) with one 140 mL coil and a counter-weight both held in a 12 cm rotor was used in the first dimension. The coil was made winding 26.4 m of 2.6 mm I.D. (3.2 mm O.D.) PTFE tubing in multiple layers. The β-value of the multilayer coil varies from 0.33 at the internal terminal to 0.60 at the external terminal. The rotation speed can be regulated with a speed controller in the range of 0–1000 rpm able to produce 100 G gravitational field at most. Sample injection was accomplished through an injection valve with a 5-mL sample loop. The second dimension was performed on an analytical-scale integrated parallel CCC separation system manufactured by the Zhejiang University machine shop (Hangzhou, China). The apparatus holds three independent identical multi-layer coils in the symmetrical position around a rotary frame at a distance of $R = 6$ cm from the central axis of the centrifuge to keep balance without any rotary seal. Each coil is a 40 mL CCC column made by winding 22.6 m of 1.5 mm i.d. PTFE tube. The β-value of the multilayer coil varies from 0.34 at the internal terminal to 0.75 at the external terminal ($\beta = r/R$ where r is coil radius, and R, the revolution radius or the distance between the holder axis and central axis of the centrifuge). The three multilayer coils are connected as three independent CCC columns with parallel connecting flow tubes made of 0.75 mm i.d. PTFE tubing. The revolution speed of the apparatus can be regulated with a speed controller in the 0–1000 rpm range able to produce a maximum gravitational field of about 60 G. Three model 2W-2B constant-flow pumps (Beijing Xingda Equipment, Beijing, China), three HD-9704 UV spectrophotometers (Jingke Equipment, Shanghai, China), and three BSZ-100 fraction collectors were equipped to the integrated parallel CCC system. The N2000 data analysis systems (Institute of Automation Engineering, Zhejiang University, Hangzhou, China) were used to record and process the CCC chromatograms.

The coupling of the two columns was accomplished by solid-phase trapping column and switching valves. According to precious study [20], Oasis HLB (poly(*N*-vinylpyrrolidonedivinylbenzene) copolymer, Waters, Shanghai, China) was selected as sorbent. The sorbent was laboratory-packed in a 25 mm × 10 mm i.d. (~600 mg sorbent mass) column holder (Michrom Bioresources, Auburn, CA). All connections were through 0.50 mm i.d. PTFE tubing until the outlet of UV detector. Moreover, a model 2W-2B constant-flow pump (Xingda Equipment, Beijing, China) was used for the addition of water as makeup fluid through a tee union (VICI, Schenkon, Switzerland). N2 was used to dry the trapping sorbent.

All the HPLC analysis was performed by an Agilent 1100 HPLC system equipped with a G1311A Quatpump, a G1322 Degasser, a G1314A UV detector, a Rheodyne 7725i manual injection valve with a 20 μL loop and an Agilent Chemstation for data treatment.

LC/ESI/MS peak identification was performed using the above described Agilent HPLC system coupled with a Bruker Esquire 3000 plus ion trap mass spectrometer (Bruker–Franzen Analytik, Bremen, Germany) equipped with an electrospray ionization (ESI). Instrument control and data acquisition were performed using Esquire 5.0 software.

Table 1
LC/MS/MS information of four hydroxyanthraquinones.

Compound no.	Structures	HPLC retention (min)	$[M-H]^-$	MS^2
1	 Aloe-emodin	27.8	269	240 (100)
2	 Emodin	30.6	269	225 (100), 241 (52)
3	 Chrysophanol	32.6	253	225 (100)
4	 Physcion	33.6	283	240 (100), 268 (27)

2.2. Reagents and materials

All organic solvents used for CCC were of analytical grade and purchased from Huadong Chemicals, Hangzhou, China. Reverse osmosis Milli-Q water (18 MΩ) (Millipore, Bedford, MA, USA) was used for all solutions and dilutions. Acetonitrile used for HPLC analysis was of chromatographic grade and purchased from Merck, Darmstadt, Germany. The dried stems of *R. officinale* were purchased from a local drug store and identified by Professor Chengxin Fu (Zhejiang University, Hangzhou, China). The standard phenolic compounds (see Table 1) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Health, Beijing, China. The 0.5 mg/mL stock solutions were prepared by dissolving 5.0 mg of each standard compound in 10.0 mL of methanol, and then stored in a refrigerator. The working solutions were prepared by suitable dilution of the stock solutions with methanol.

2.3. Preparation of crude extract

The stems of *R. officinale* (3 kg) were bought from Hangzhou local open market and dried to constant mass at 55 °C in a vacuum oven and then pulverized. One kilogram of powder was extracted by 5 L 95% ethanol for 2 h under reflux. The procedure was repeated for three times. The combined 15 L were concentrated to dryness under reduced pressure at 45 °C producing about 100 g crude extract and the extract was stored at 4 °C for further analysis and CCC separation.

2.4. CCC procedures

The present MDCCC system was performed by different combination of switching valve positions (V_1, V_2, V_3 and V_4) as follows: First, the switching valves were initially set as shown in Fig. 1, and the four CCC columns were filled with suitable upper stationary phases, respectively. Then the HSCCC instrument was rotated at 600 rpm and the corresponding lower phase was pumped at 2.0 mL/min in the head-to-tail direction. When the hydrodynamic equilibrium was established, suitable amount sample solution was injected through the injection valve, and the effluents from HSCCC column was monitored on-line at 254 nm and automatically collected by a fraction collector. When the target peak appeared, V_1, V_2 were switched to introduce the target effluents into the trapping column. At the same time, water was added to the flow tube with the makeup pump at defined flow rate, to provide proper retention of the peaks. In a second step, V_2 was switched back and the trapping column was dried with nitrogen gas to remove all residual solvents. Then the trapped analytes were subsequently back-flushed to parallel CCC for further separation by switching V_1 to the wash position, at the same time parallel CCC was rotated at 650 rpm and the corresponding mobile phase was pumped at selected flowrate in the head-to-tail direction. The effluents were continuously monitored at 254 nm and automatically collected in a fraction collector. When another target peak appeared, the effluents were introduced to another trapping column by simply changing the position of V_3. Then the similar operation procedure was performed to accomplish the injection.

2.5. LC/MS analysis

The crude extract was analyzed by HPLC/MS/MS. HPLC condition are as follows: Yilite-C18 column (250 mm × 4.6 mm i.d., 5 μm); gradient elution was performed using A (acetonitrile) and B (0.5% acetate acid in water) as mobile phase: 0 min 18% A; 20 min 34% A; 28 min 80% A. Flow rate was 0.8 mL/min and was monitored at 254 nm. A split valve was used to reduce the flow rate from outlet

Table 2
The *K* (partition coefficient) values of the target compounds in several solvent systems.

Solvent systems (v/v)	*K* values			
	Aloe-emodin	Emodin	Chrysophanol	Physcion
n-Hexane–ethyl acetate–methanol–water (2:5:2:5)	13.6	24.8	31.9	47.9
n-Hexane–ethyl acetate–methanol–water (1:1:1:1)	2.2	4.22	4.43	5.06
n-Hexane–ethyl acetate–methanol–water (5:2:5:2)	0.16	0.59	3.73	3.74
Methyl tert-butyl ether–acetonitrile–water (2:2:3)[a]	0.009	0.32	2.9	5.34
Methyl tert-butyl ether–acetonitrile–water (2:2:3)[b]	0.08	2.01	24.6	39.4
Methyl tert-butyl ether–acetonitrile–water (2:2:3)[c]	37.8	54.65	48.21	43.71

[a] 10 mM NaOH was added.
[b] 10 mM TEA was added.
[c] 10 mM TFA was added.

of the HPLC to the inlet of ESI, and the flow rate was reduced to one third. The MS system was operated in negative ion mode. The ion source temperature was 250 °C, and needle voltage was always set at 4.0 kV. Nitrogen was used as the drying and nebulizer gases at a flow rate of 10 L min^{-1} and a back-pressure of 30 psi.

3. Result and discussion

3.1. MDCCC system

The major difficulty in application of MDCCC was the large volume injection into the second dimension separation. In Yang's system [13], large volume introduction is always inevitable (almost 1/3 column volume), which directly results in limitation of improvement of peak resolution owing to the same biphasic liquid systems used. In our previous work, a MDCCC system using a small capacity coil (V_c = 140 mL) in the first and a preparative upright CCC (V_c = 1500 mL) as the second column [21] has been developed. This approach has been demonstrated to be a practical solution for large-volume introduction. However, longer separation (10 h) duration is always needed. Additionally, the two biphasic liquid systems used are chemically identical, only different in compositions. Recently, an integrated MDCCC system has been developed [20]. In conjunction with a solid-phase trapping interface, it results in a considerable orthogonality improvement. Two same columns were integrated in one centrifuge to simplify instrumentation. However, the small column (40 mL) in the first dimension results in the limitation of sample injection amount. In order to enlarge the preparative capacity, we make an improvement of the previous design, where a semi-preparative CCC instrument was used as the first column (V_c = 140 mL) to increase the sample loading. Furthermore, using additional four-port valves, the effluents from the first dimension can be easily introduced to different solid-phase trapping columns and following columns. The integrated parallel CCC with three columns (40 mL each column) used in the second dimension allows up to three independent injections. As a result, more interested effluents can be separated in the second dimension separation which greatly improved the separation efficiency.

3.2. LC/MC analysis

LC/MS/MS has been widely used in natural products analysis. Mass spectrometry can provide abundant information for structural elucidation of the compounds especially when tandem mass spectrometry is applied. In this study, the crude extract of *R. officinale* was first analyzed by LC/MS/MS analysis. As the major components are phenolic compounds, negative ion mode was selected. The HPLC chromatogram and total ion chromatogram are shown in Fig. 2, the mass information of the major com-

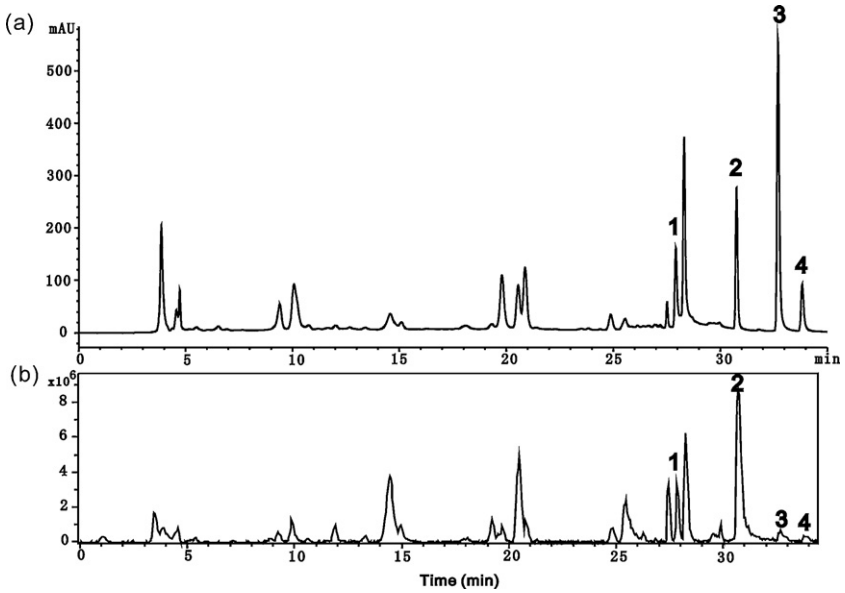


Fig. 2. The HPLC chromatogram and TIC chromatogram of *R. officinale*. HPLC condition are as follows: Yilite-C18 column (250 mm × 4.6 mm i.d., 5 μm); gradient elution was performed using A (acetonitrile) and B (0.5% acetate acid in water) as mobile phase: 0 min 18% A; 20 min 34% A; 28 min 80% A. Flow rate was 0.8 mL/min and was monitored at 254 nm. The MS system was operated in negative ion mode. The ion source temperature was 250 °C, and needle voltage was always set at 4.0 kV. Nitrogen was used as the drying and nebulizer gases at a flow rate of 10 L min^{-1} and a back-pressure of 30 psi.

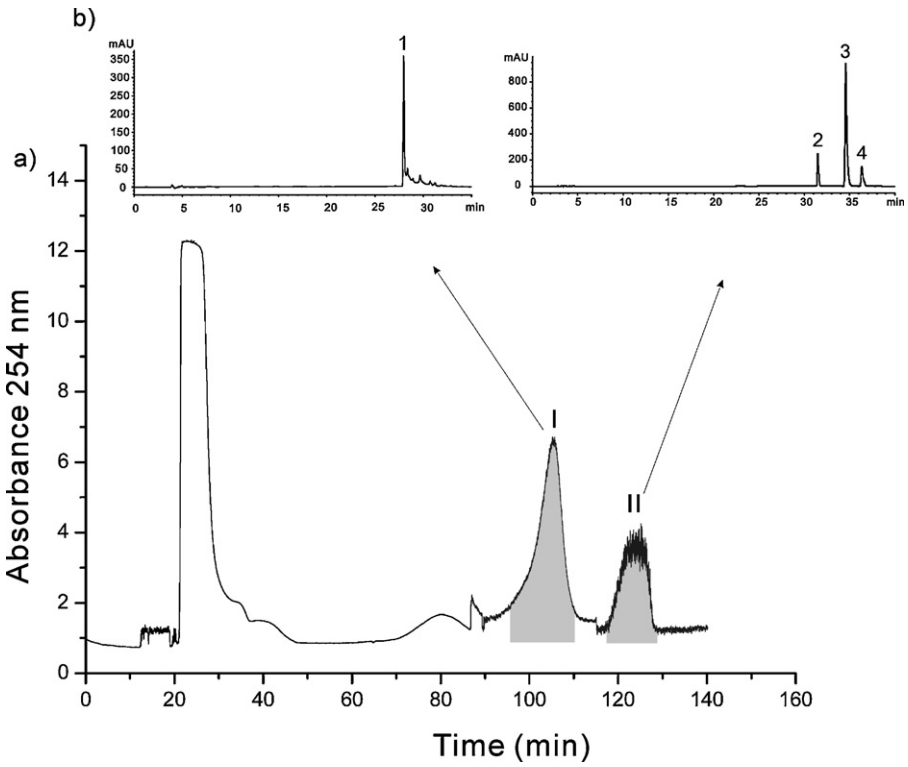


Fig. 3. (a) 1st-D HSCCC separation of *R. officinale*. Column capacity: 140 mL; solvent system: n-hexane–ethyl acetate–methanol–water (1:1:1:1, v/v); Flow rate: 2.0 mL/min of lower phase up to 140 mL immediately followed by 2.0 mL/min of upper phase; resolution speed: 600 rpm; injected sample: 100 mg of crude sample in 2 mL upper phase and 2 mL lower phase; detection wavelength: 254 nm. (b) HPLC chromatograms of shaded peaks.

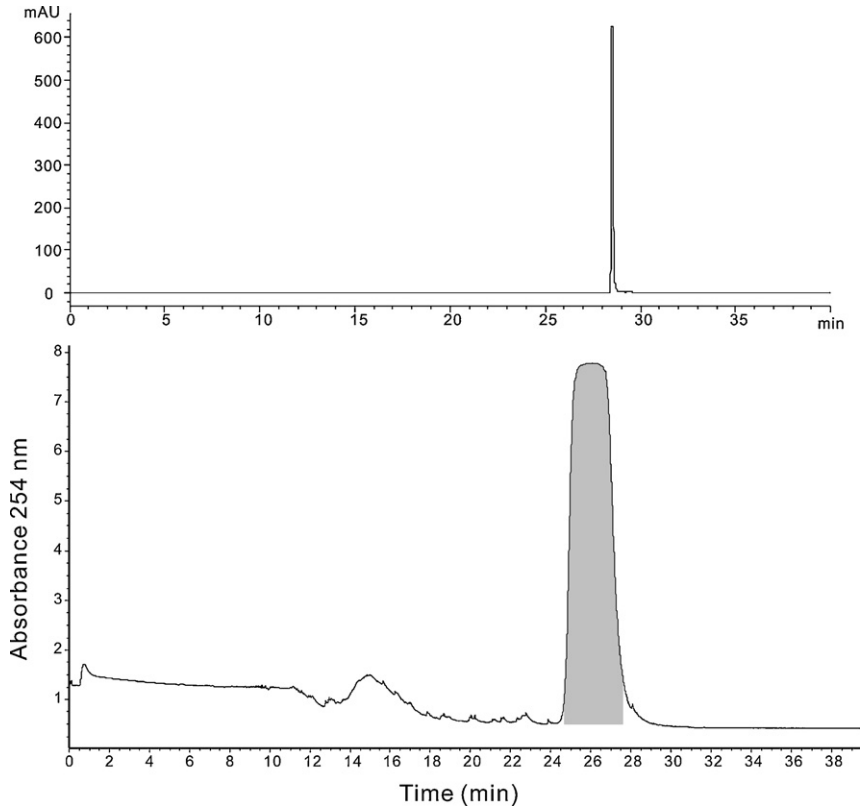


Fig. 4. 2nd-D HSCCC separation of fraction I in 1st dimension. Column capacity: 40 mL; solvent system: methyl tert-butyl ether–acetonitrile–water (2:2:3, v/v), 10 mM TFA was added to the upper phase and 10 mM TEA was added to the lower phase; Flow rate: 2.0 mL/min; resolution speed: 650 rpm; detection wavelength: 254 nm.

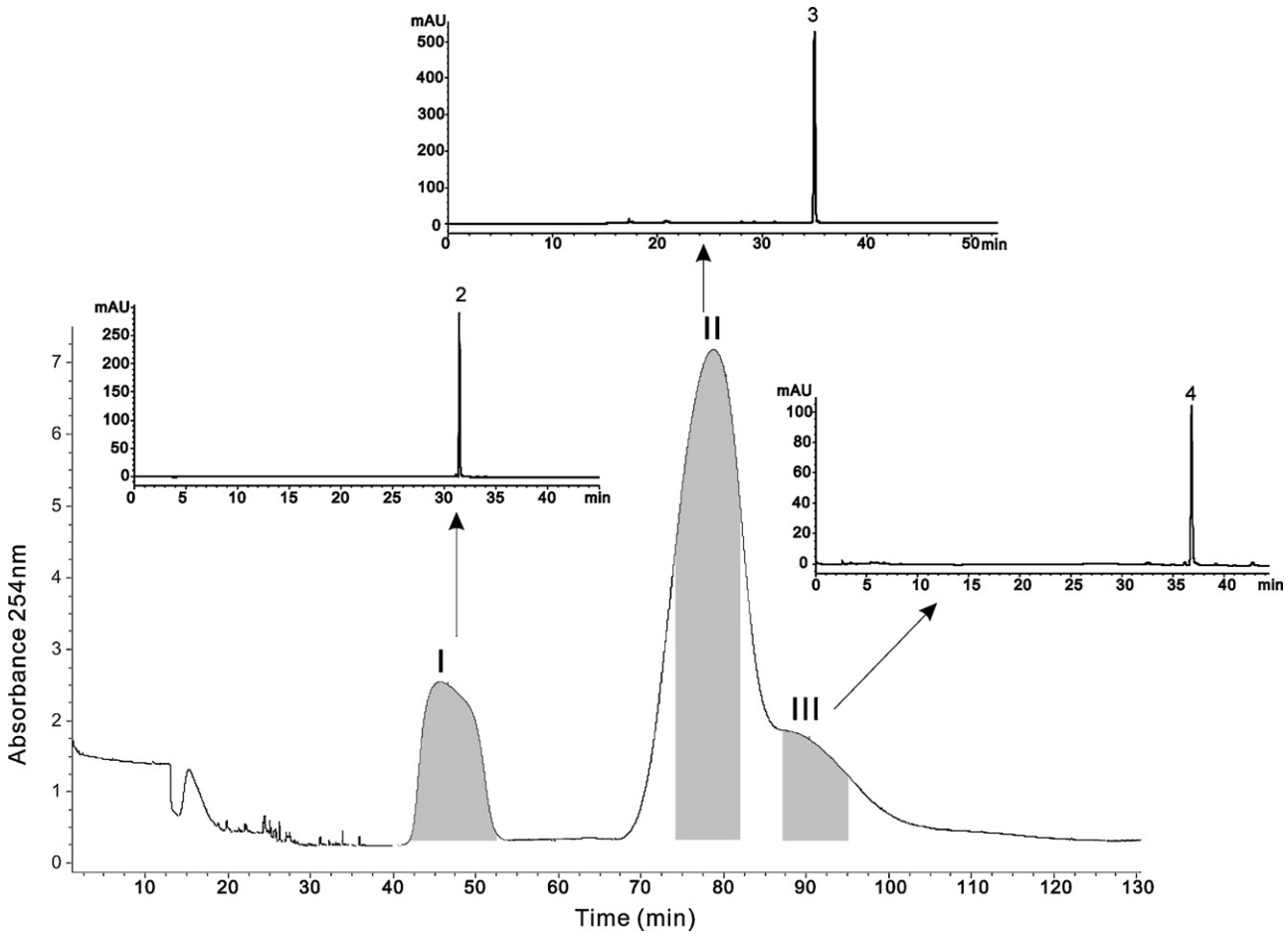


Fig. 5. 2nd-D HSCCC separation of fraction II in 1st dimension. Column capacity: 40 mL; solvent system: methyl tert-butyl ether–acetonitrile–water (2:2:3, v/v), 10 mM TFA was added to the upper phase and 10 mM TEA was added to the lower phase; Flow rate: 1.5 mL/min; resolution speed: 650 rpm; detection wavelength: 254 nm.

pounds are listed in Table 1. The HPLC chromatogram indicated that the crude extract possesses various components with a wide range of hydrophobicities. The fragmentation information of mass spectrometry listed in Table 1 gives essential structural information. The $[M-H]^-$ ion of compound **1** only produced one fragment at *m*/*z* 240 ($[M-H-CHO]^-$). The $[M-H]^-$ ion of compound **2** produced one fragment ion at *m*/*z* 240 ($[M-H-CO]^-$), and followed by loss of 16 Da to form an ion at *m*/*z* 225. In the MS/MS spectrum of compound **3**, only one product ion was observed at 225 ($[M-H-CO]^-$). The $[M-H]^-$ ion of compound **4** was initiated by elimination of CH_3 radical to produce *m*/*z* 268, and followed by loss of CO to give *m*/*z* 240. According to previous ESI-MS/MS study in *R. officinale* b [22], compounds **1**–**4** were speculated as aloe-emodin, emodin, physcion and chrysophanol, respectively. Comparing with the retention time and fragmentation information, the structures were further identified by reference compounds.

3.3. Selection of two-phase solvent systems in 1st and 2nd DHSCCC

Selection of suitable solvent systems plays a crucial role in one successful CCC separation. There are mainly two ways to screen the appropriate biphasic solvent systems. One approach is to measure the partition coefficient values of each compound by HPLC. The other approach is run a trial separation by a small volume CCC column (10–40 mL). In the present study, a parallel three-coil CCC (40 mL for each coil) was used for selection of the biphasic solvent systems. The biphasic solvent screen strategy has been already discussed in previous research [23]. The HEMWat solvent system (1/1/1/1, v/v), (2/5/2/5, v/v) and (5/2/5/2, v/v) was first evaluated. With relative hydrophobic solvent system 5/2/5/2, a great portion had already been eluted in the classic elution; only a small peak was eluted in extrusion stage. With relative hydrophilic solvent system 2/5/2/5, a great amount of components were eluted in one peak with low resolution in the extrusion stage. When using the solvent system 1/1/1/1, the crude extract can be well separated into five fractions. Therefore, we chose HEMWat solvent system (1/1/1/1, v/v) as the solvent in the 1st dimension separation.

The solvent system MtBE/ACN/Water (2/2/3, v/v) has been widely used in separation of ionic compounds. We prefer to choose this solvent system as the basic solvent system in 2nd DHSCCC separation. 10 mM TFA was added to the upper phase and different elution base was optimized. Commonly used base NaOH, and TEA were evaluated by trial separation. Compound **1** can be well separated by using 10 mM NaOH as an eluter, a sharp peak was formed. However, the compound **2**, **3**, and **4** could not be separated, and were concentrated in one peak by using 10 mM NaOH in the lower phase. The results showed that, compound 1, 2, 3, 4 can be separated when using 10 mM TEA in the lower phase. Therefore, we chose MtBE/ACN/Water (2/2/3, v/v) as the solvent system in the second dimension, where 10 mM TEA was added in the lower phase and 10 mM TFA was added in the upper phase. The partition coefficients of the four target compounds were analyzed by HPLC method and listed in Table 2.

3.4. 2D-CCC separation of ethanol extract of R. officinale

The HEMWat (1/1/1/1, v/v) solvent system was used in the first dimension, and 100 mg crude sample was dissolved in 4 mL solution (2 mL upper phase and 2 mL lower phase) and then separated by a semi-preparative CCC instrument using EECCC method. The switch volume was equal to one column (140 mL). Five fractions were obtained in the first dimension. According to HPLC analysis (Fig. 3), the hydroxyanthraquinones are mainly present in fraction IV and V. Since the solid-phase trapping column used in present study has a limited sample loading, the two fractions cannot be trapped in one column. These two fractions were separately online concentrated by the solid-phase trapping interface and further separated in the second dimension. As the mobile phase used in the extrusion stage was the upper phase of HEMWat (1/1/1/1, v/v) system containing high contents of organic solvents. According to previous study [20], the loading flow rate was then set at 22 mL/min (mobile phase flow rate 2 mL/min) to ensure the retention of the target compounds in the solid trapping interface. The acid–base elution was then applied in the second dimension separation. The biphasic solvent system MtBE:ACN:Water (2:2:3, v/v) was selected as the solvent system. 10 mM TFA was added to the upper phase as a retainer and 10 mM TEA was added to the aqueous phase as an eluter. The 2nd-D CCC chromatograms and HPLC chromatograms of purified peaks were shown in Figs. 4 and 5. The compound **1** in corresponding fraction was further purified with a higher purity (see Fig. 4). The three compounds overlapped were further separated to three peaks in the second dimension (see Fig. 5). During ~4 h separation, four hydroxyanthraquinones, 12.3 mg aloe-emodin, 14.5 mg emodin, 19.7 mg chrysophanol and 7.6 mg physcion were separated at purities over 95% in one step. The structures were unambiguously characterized by comparing their HPLC retention times and mass spectra with reference compounds.

Compared with regular one dimension approach, the present MDCCC system allows the usage of different biphasic solvent systems with different chemical compositions which improves the column selectivity, capacity and efficiency. In addition, different separation mode used in the two dimensions can enhance the separation orthogonality, such as traditional CCC and acid–base elution CCC. EECCC provides an extended sweet spot of separation and offers high-resolution separation of a wider polarity range of analytes. All the solutes retained in the stationary phase can be eluted in the extrusion stage. Therefore, EECCC was first used to fast fractionate the crude extract. Acid–base elution CCC has been widely used to analyze the ionizable compounds. In addition, the sample injection amount can be greatly increased compared with traditional CCC. The separation mechanism of this mode is also different from the traditional CCC. Thus, we choose this mode in the second dimension. The integrated parallel three-coil centrifuge in the second dimension can further improve the separation efficiency as up to three independent separations can be performed at most. Two of the three columns were used in this study as only two interested fractions needed to be separated. The four target compounds involved in two fractions were successfully separated in the second dimension with present method. We believe this method is promising to deal with the complex natural products.

4. Conclusions

An MDCCC system was constructed using a semi-preparative instrument in the first dimension and an integrated parallel three-coil centrifuge in the second dimension. Different biphasic solvent system and elution mode can be used in present system. The ethanol extract of *R. officinal* was used as test material to evaluate the present system. Four hydroxyanthraquinones were successfully separated with high purities which indicated that the present method was efficient in separation of natural products. This method gives a new option in separation of complex natural products: The crude extract can be first fractionated by extrusion method to get several fractions. Then, the interested fractions can be further separated in the second dimension with another solvent system.

Acknowledgements

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References

[1] Y. Ito, R.L. Bowman, Science 167 (1970) 281.
[2] I.A. Sutherland, J. Chromatogr. A 1151 (2007) 6.
[3] P. Wood, S. Ignatova, L. Janaway, D. Keay, D. Hawes, I. Garrard, I.A. Sutherland, J. Chromatogr. A 1151 (2007) 25.
[4] I.A. Sutherland, G. Audob, E. Bourton, F. Couillard, D. Fisher, I. Garrard, P. Hewitson, O. Intes, J. Chromatogr. A 1190 (2008) 57.
[5] A. Berthod (Ed.), Countercurrent Chromatography, the Support Free Liquid Stationary Phase (Comprehensive Analytical Chemistry, Vol. 38), Elsevier, Amsterdam, 2002.
[6] Y. Ito, W.D. Conway (Eds.), High-Speed Countercurrent Chromatography (Chemical Aanlysis, Vol. 132), Wiley-Interscience, New York, 1996.
[7] R.J. Case, Y. Wang, S.G. Franzblau, D.D. Soejarto, L. Matainaho, P. Piskaut, G.F. Pauli, J. Chromatogr. A 1151 (2007) 169.
[8] Y.W. Ha, S.S. Lim, I.J. Ha, Y.C. Na, J.J. Seo, H. Shin, S.H. Son, Y.S. Kim, J. Chromatogr. A 1151 (2007) 37.
[9] X. Chu, A. Sun, R. Liu, J. Chromatogr. A 1097 (2005) 33.
[10] R. Liu, A. Li, A. Sun, J. Chromatogr. A 1052 (2004) 217.
[11] W.D. Conway, J. Liq. Chromatogr. Relat. Technol. 24 (2001) 1555.
[12] J.B. Friesen, G.F. Pauli, J. Liq. Chromatogr. Relat. Technol. 28 (2005) 2777.
[13] F. Yang, J. Quan, T.Y. Zhang, Y. Ito, J. Chromatogr. A 803 (1998) 298.
[14] G. Tian, T.Y. Zhang, Y. Zhang, Y. Ito, J. Chromatogr. A 945 (2002) 281.
[15] J. Wei, T.Y. Zhang, Y. Ito, J. Liq. Chromatogr. Relat. Technol. 28 (2005) 1903.
[16] Pharmacopoeia of China, The State Pharmacopoeia Commission of PR China, Chemical Industry Press, Beijing, 2005.
[17] W. Tang, G. Eisenbrand, Chinese Drugs of Plant Origin, Springer, Berlin, Heidelberg, 1992, p. 855.
[18] J.A. Duke, Handbook of Medicinal Herbs, 2nd edn., CRC, Boca Raton, FL, 2002, p. 621.
[19] P. Xiao, L. He, L. Wang, J. Ethnopharmacol. 10 (1984) 275.
[20] Y. Lu, R. Hu, Y. Pan, Anal. Chem. 82 (2010) 3081.
[21] Y. Lu, C. Sun, R. Liu, Y. Pan, J. Chromatogr. A 1146 (2007) 125.
[22] M. Ye, J. Han, H. Chen, J. Zhen, D. Guo, J. Am. Soc. Mass Spectrom. 18 (2007) 82.
[23] R. Hu, Y. Lu, X. Dai, Y. Pan, J. Sep. Sci. 33 (2010) 1595.