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Review

Separation procedures for the pharmacologically active components of rhubarb

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Abstract

Rhubarb, as an important Chinese medicine, has many functions owing to containing anthraquinone derivatives. The analysis of anthraquinone derivatives in Chinese rhubarb is reviewed. The analytical techniques include high performance liquid chromatography, capillary electrophoresis, thin-layer chromatography and so on. The main operation parameters in every technique were given. The structures of anthraquinone derivatives and the classification of Chinese rhubarb were summarized too.

Keywords: Review; Rhubarb; Anthraquinones; Perivatization

Contents

1. Introduction

Rhubarb, one of the ancient and best-known Chinese herbal medicines, has been used for thousands of years in china. It has the effect of purgation, purging heat, loosening the bowels, curing gastric and renal disorders, removing bacterial dysentery, removing heat from the blood, clearing toxins away, promoting blood circulation and removing blood stasis. Rhubarb has the action of antitumor and antimutagenicity too [1]. Rhubarb existed as an important component in a lot of Chinese patent medicine or Chinese compound medicine. Besides its pharmacological values, rhubarb can be made as nourishing food too. Most *Rheum* species are produced in China, and are exported under various commercial names according to their appearance, quality or place of

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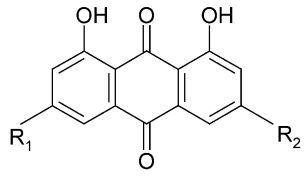

Fig. 1. Structures of important anthraquinone derivatives. Aloe-emodin R_1 = H, R_2 = CH_3OH; rhein R_1 = H, R_2 = COOH; emodin R_1 = OH, R_2 = CH_3; physcion R_1 = CH_3O, R_2 = CH_3; chrysophanol R_1 = H, R_2 = CH_3.

production. In addition to the species listed in the Chinese Pharmacopoeia (*Rheum palmatum* L., *Rheum tanguticum* Maxim. et Baill, and *Rheum officinale* Baill), more than 50 other species of rhubarb grow in China [2] and several of them have been found in use as Chinese medicine. Usually, these species of rhubarb are divided into seven groups: Sect. Acuminata, Sect. Deserticola, Sect. Rhapontica, Sect. Palmata, Sect. Spiciforma, Sect. Globulosa and Sect. Nobilia.

A variety of constituents have been isolated from rhubarb. They are classed as anthraquinones, dianthrones, stilbenes, anthocynins, falvonoids, anthraglycosides, polyphenols, essential oil, organic acids, chromenes, chromanone, chromone glycosides and vitamins [3], in which anthraquinone derivatives including emodin, aloe-emodin, rhein, physcion, chrysophanol and their glucosides are the accepted important active components. Fig. 1 shows the structures of important anthraquinone derivatives.

The methods commonly used for the separation of anthraquinone compounds in rhubarb are high performance liquid chromatography (HPLC), capillary electrophoresis (CE), and thin-layer chromatography (TLC). This review includes these characteristic methods and the literatures included came mainly from Sciencedirect [4] and Vip [5] database. The review would not try to include all the related papers because there are so many papers present, i.e., there are more than 400 articles related with this topic during 1993–2003.

2. Analytical methods in anthraquinone derivatives

2.1. Capillary electromigration techniques

2.1.1. Capillary zone electrophoresis (CZE)

All the different modes of electromigration separations were applied to the analysis of anthraquinone derivatives. In capillary zone electrophoresis [6], 12 anthraquinones in *Rhei rhizoma* were separated in 0.05 mol/L NaOH–acetonitrile (9:1). The analysis was finished in 50 min with good correlation coefficients in the linear range (Fig. 2).

2.1.2. Micellar electrokinetic chromatography (MEKC)

Some authors did not find capillary zone electrophoresis is a good tool to analyze anthraquinones and Micellar electrokinetic chromatography was chosen as the last tool to finish the separation of anthraquinones in rhubarb [7]. In Fact, Micellar electrokinetic capillary chromatography is employed most widely to separate anthraquinones.

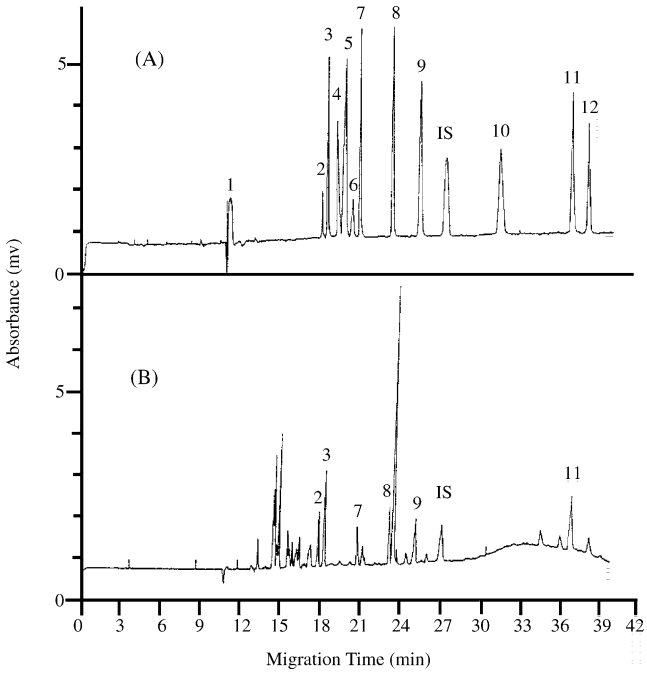

Fig. 2. CE electrophergrams of (A) 12 authentic compounds and (B) *Rhei rhizoma* extract. Adapted and reprinted by permission of authors of Ref. [6]. Copyright (2000) WILEY-VCH Verlag GmbH Publishing. Conditions are shown in Table 1.

Micellar electrokinetic chromatography of aloe-emodin, emodin, and rhein in rubarb samples were reported by Zong and Che [7]. 3-(Cyclohexylamino)-1-propane sulfonic acid (0.025 M) containing 0.025 M sodium dodecyl sulfate (SDS) and acetonitrile (100:10, pH 10.96) was used (1,8-dihydroxy-anthraquinone served as internal standard). The authors found aloe-emodin, emodin, rhein, chrysophanol and physcion could be separated well within 13 min but the linear correlation coefficients be unacceptably low and therefore considered unsuitable for quantitative measurement for chrysophanol and physcion under the optimal conditions. It was thought such poor responses might arise from the fact that these compounds were incompletely dissolved in the buffer solution. pH was critical in the experiment and even pH 11 led to bad separation. The analyzed five samples included *Rheum palmatum*, *Rheum tanguticum*, and *Rheum emodi*. Similarly, the same results for chrysophanol and physcion were gotten with another system of Micellar electrokinetic chromatography [8–9]. The buffer used was 50 mmol/L H_3BO_3–NaOH (pH 11) containing 25 mmol/L sodium deoxycholate (SDC) and the internal standard was paracetamol.

Sheu and Chen [10] developed a MEKC method for the simultaneous assay of three anthraquinones and two anthrones in seven Chinese herbal preparations (Table 1). The separation was finished within 30 min. The effects of surfactant concentration and organic modifier (acetonitrile) concentration

Table 1
Analytes and Conditions from Sheu et al.

Methods	Conditions	Analytes	Samples	References
CZE	0.05 mol/L NaOH and acetonitrile (9:1)	Anthraquinone, emodin, chrysphanol, quinalizarin, alo-emodin, rhein, alizarin, anthraflavic acid, anthraquinone-2-carboxylic acid, purpurin, sennoside A and B	*Rhei rhizoma*	[6]
HPLC	Gradient, A: phosphate buffer, pH 2.91; B: methanol	Same as above	Same as above	[6]
MEKC	A buffer solution (10 mM sodium dodecyl sulfate, 12.5 mM sodium dihydrogenphosphate and 15 mM sodium borate) and acetonitrile (3:1)	Rhein, emodin, aloe-emodin, sennoside A and B	Two kinds of rhubarb, pa-cheng-san, yin-chen-hao-tang, tao-ho-cheng-chi-tang ta-huang-kan-tsao-tang, fu-yuan-huo-hsieh-tang	[10]
HPLC	Gradient, A: solution of buffer–CH_3OH–CH_3CN (8:1:1, v/v), pH 3.5; B: solution of buffer–CH_3OH–CH_3CN (1:2:2, v/v), pH 4.2; the buffer was consisting of 3.0 mM TBA and 7.3 mM KH_2PO_4	Aloe-emodin, emodin, sennoside B and A, oroxylin A 7-*O*-glucuronide, baicalein, wogonin 7-*O*-glucuronide, Baicalin, W-wogonin, ferulic acid, caffeic acid, glycyrrhizin	I-tzu-tang	[11]
MEKC	18 mM SDS, 2 mM SC, 12.5 mM $Na_2B_4O_7$ and 10 mM NaH_2PO_4	Same as above	Same as above	[11]
HPLC	Gradient, A: buffer–CH_3CN (9:1), the buffer consisting of 0.02M sodium acetate and 0.4197 M acetic acid; B: CH_3OH–CH_3CN–1% acetic acid (9:9:2)	Gallic acid, sennoside B and A naringin, hesperidin, honokiol, magnolol, emodin	Hsiao-cheng-chi-tang	[12]

TBA: tetrabutylammonium bromide.

of the carrier on the migration and separation of the solutes were also studied. All of the relative works from same authors were summarized in Table 1.

A series of reports on the determination of active components in various samples containing rhubarb was given by Shang and Yuan [1,13–18]. The experimental conditions are given in Table 2.

Kuo and Sun [19] analyzed nine anthraquinones and bianthrones, including aloe-emodin, emodin, chrysophanol, physcion, rhein, sennoside A, sennoside B, sennidin A and sennidin B in *R. officinale* Baill. In the work, a chemometric approach was employed to systematically optimize the relevant operating parameters. Factors having significant effect were further optimized through the central composite face-centered (CCF) design. At last, the samples pretreated by an Oasis HLB SPE cartridge and with the optimized conditions: 15 mM sodium tetraborate/15 mM sodium dihydrogenphosphate buffer, 30 mM sodium deoxycholate, pH 8.6,

Table 2
Conditions from Shang and Yuan

Conditions	Components	Samples	References
20 mmol/L SC and 20 mmol/L STC with 15 mmol/L β-CD in the background electrolyte (BEG, which was 20 mmol/L borax)	Emodin, aloe-emodin, rhein, physcion, chrysophanol, rhaponticin	Raw rhubarb; Mongolian rhubarb and *R. tanguticum*	[1]
50 mmol/L NaOH + H_3BO_3 containing 20 mmol/L SDS, pH 10	Emodin, rhein, physcion, chrysophanol	Raw rhubarb	[13]
50 mmol/L NaOH–NaH_2PO_4 and 25 mmol/L AC with 20 mmol/L β-CD, pH 9.5	Emodin, aloe-emodin, rhein, physcion, chrysophanol	Raw rhubarb, Mongolian rhubarb, *R. tanguticum*	[14]
20 mmol/L NaOH +H_3BO_3 containing 20 mmol/L SDS and 20 mmol/L STC with 15 mmol/L β-CD, pH 11	Emodin, aloe-emodin, rhein, physcion, chrysophanol, rhaponticin	Kan-su *Rhei rhizoma* Cruzoma, *R. palmatum* L., Da-huang-Zhe-Chong capsules	[15]
20 mmol/L SDS and 20 mmol/L SC with 10 mmol/L β-CD in phosphate buffer, pH 10.4	Emodin, aloe-emodin, rhein, physcion, chrysophanol	Hua-pe-da-huang and R. *palmatum* L.	[16]
15 mmol/L NaH_2PO_4 + 20 mmol/L borax and 15% ethanol (v/v) with SDS or SDS-free	Emodin, aloe-emodin, rhein, physcion, chrysophanol	*R. palmatum* L., Hua-pe-da-huang, Niu Huang Jie Du Pian and Jin Yi Niao Shi granules	[17]
20 mmol/L SDS and 10 mmol/L β-CD in phosphate buffer (pH 10-10.4), 10–15% MeOH	Emodin, aloe-emodin, rhein, physcion, chrysophanol	Raw rhubarb; Mongolian rhubarb	[18]

SC, sodium cholate; STC, sodium taurocholate; CD, β-cyclodextrin; SDS, sodium dodecyl sulfate.

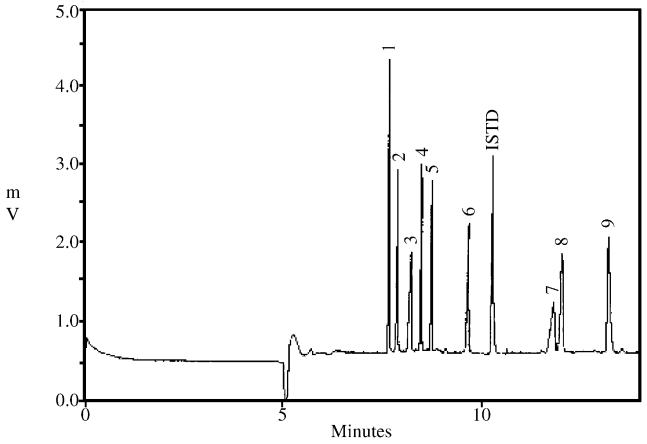

Fig. 3. Electropherogram of the anthraquinone and bianthrone analytes obtained with optimum separation conditions. Fused-silica capillary 72 cm (60 cm detection length) × 50 μm i.d.; 15 mM $Na_2B_4O_7$/15 mM NaH_2PO_4, 30 mM SDC, pH 8.6 and 17 vol.% ACN; 28 kV; 30 °C; 270 nm; injection, 50 mbar, 4.8 s; sample dissolved in 70 vol.% ACN. Adapted and reprinted by permission of Ref. [19].

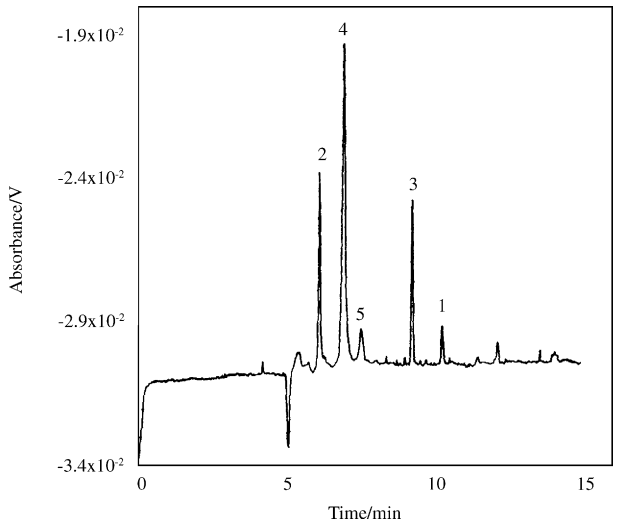

Fig. 4. Eletropherogram of *Rheum* plant extract. 1 V = 1 absorbance unit. Microemulsion content in buffer: 0.7% SDS, 0.64% ethyl acetate, 0.16% butan-1-ol. Buffer composition, 5 mM phosphate (pH 7.20); applied voltage, 15 KV, 25 ± 1 °C. (1) rein; (2) aloe-emodin; (3) emodin; (4) chrysophanol; and (5) physcion. Adapted and reprinted by permission of the authors of Ref. [22] and The Royal Society of Chemistry.

17 vol.% acetonitrile. Nine tested analytes were baseline-separated within 14 min (Fig. 3).

2.1.3. Capillary electrochromatography (CEC) and microemulsion electrokinetic chromatography (MEEKC)

A capillary electrochromatography (CEC) method with diode-array detection for the separation of four anthraquinones from rhubarb extract and commercial traditional Chinese drugs containing rhubarb established by Li et al. [20]. Aloe-emodin, emodin, chrysophanol, and physcion were separated with baseline in 12 min using a background electrolyte consisting of 5 mM acetic acid (pH 4.5) with 80% acetonitrile.

The separation of four important anthraquinones with a packed CEC in 12 min was reported by Ding et al. [21]. YWG-ODS (5 um) was selected as stationary phase and the mobile phase comprised 2.0 mM phosphate, 4.0 mM 2-(*N*-morpholine) ethanesulphonic acid and 80% (v/v) acetonitrile, pH 5.5. Li et al. [22] established a MEEKC method to analyze hydroxyanthraquinones in *Rheum* natural products. The buffer was 5 mM phosphate (pH 7.20) containing 0.7% SDS, 0.64% ethyl acetate, 0.16% butan-1-ol with 30% acetonitrile. Acetonitrile concentration was studied in detail owing to its contribution to the separation (Fig. 4).

2.2. High performance liquid chromatographic method

Liu et al. [23] set up a HPLC method to separate and determine five hydroxyanthraquinone derivatives in extract of Chinese herbal medicine rhubarb under the help of software DryLab. In the case of gradient elution, the data from two scouting runs with a sample are input to simulate retention behavior of each component in the sample as a function of gradient conditions. Based on the prediction from DryLab simulation and under the optimized conditions (solvent A, 36 mM triethylamine phosphate (TEAP), pH 2.5; solvent B, Acetonitrile; gradient, 36%/36%/80%/80% B at 0 min/5.5 min/20.5 min/25.5 min; flow-rate, 1.00 mL/min; temperature, ambient), the method was successfully applied to monitor the quality of rhubarb from different sources and the separation lasted 25 min (Fig. 5). Li et al. [24] established a HPLC/MS/MS method for the characterization of anthraquinone derivatives in rhubarb.

Anthraquinones exsit not only in rhubarb but other Chinese herbs, such as *Polygonum cuspidatum* sieb et zucc [25], *Cassia tora* L. [26], *Polygonum multiflorum* [27] and *Artemisia capillaries* [28] so on. Metzger and Reif [29] described the determination of 17 different 1,8-dihydroxyanthranoids (anthraquinones and their bianthrys) in fruits and leaves of *Psenna angustifolia* and *Senna acutifolia*. The anthranoids are extracted using a mixture of acetonitrile and a solution of sodium hydrogencarbonate. The different compounds are separated and detected by HPLC with gradient elution using an Rp-8 column and a photodiode array detector. The method was optimized by means of the DryLab too. The conditions were shown in Table 3.

Djozan and Assadi [30] used a 5 μm Spherisorb-CN column (250 × 4.6 mm i.d.) for the determination of the monomeric anthraquinones and their glycosides (rhein, emodin and chrysophanol) present in rhubarb roots, senna leaves and dock flower. The mobile phase was chloroform-concentrated (96%) acetic acid (95:5, v/v). The absorption of the column eluate was monitored at 254 nm and the separation is less than 8 min.

Determination of five hydroxyanthraquinones in rhubarb and experimental animal bodies with HPLC method were reported [31]. A Zorbax SB-C18 column (250 mm × 4.6 mm

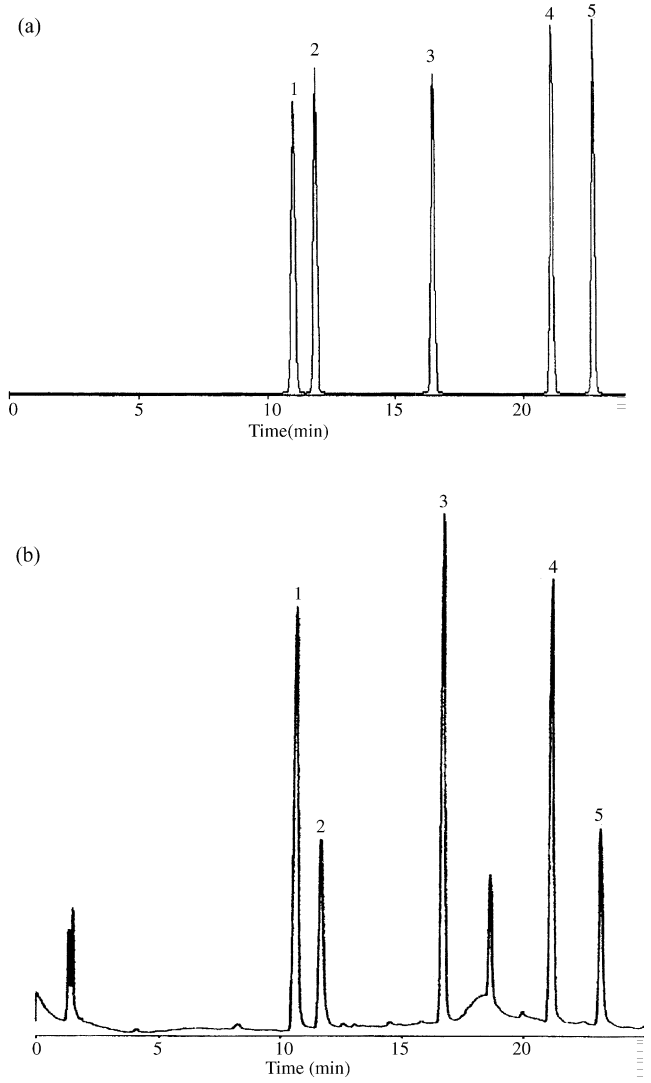

Fig. 5. (a) Predicted and (b) actual separations. Compounds: (1) aloe–emodin; (2) rhein; (3) emodin; (4) chrysophanol; and (5) physcion. Chromatographic conditions: solvent A: 36 mM triethylamine phosphate (TEAP), pH 2.5; solvent B: acetonitrile; gradient: 36%/36%/80%/80% B at 0 min/5.5 min/20.5 min/25.5 min; flow-rate: 1.00 mL/min; temperature: ambient. Adapted and reprinted with permission from Ref. [23].

i.d., 5 μm) and a methanol–0.5% acetic acid (85:15, v/v) mobile phase were used for the separation. The detection wavelength of a diode array detector (DAD) was set at 254 nm. The detection limits (S/N = 3) ranged from 0.35 to 3.13 ng, and the recoveries ranged from 83 to 103% for different hydroxyanthraquinones.

A lot of papers in Chinese [32–34] were related with the determination of one or more hydroxyanthraquinone derivatives in Chinese patent medicine consisting of rhubarb, such as shen-kang-jiao-nang, zhi-gan-jing-jiao-nang, da-bai-du-jiao-nang. In these study, the HPLC conditions consisting a mobile phase MeOH (or acetonitrile)–H_2O (adjusted to a certain pH with H_3PO_4, CH_3COOH, $HClO_4$) and a C_8 or C_{18} column is in common. The ratio of MeOH (or acetonitrile) to H_2O was changed owing to the different samples.

2.3. Thin-layer chromatography

Thin-layer chromatography is a technique widely used to determine hydroxyanthraquinones in rhubarb. During the period, 143 papers related with the topic. Danielsen and Francis [35] got a satisfied solvent system to separate five important anthraquinone aglycones simultaneously from rhubarb on silica thin layers. The solvent consisting of hexane: acetone: *tert*-butanol (85:10:5) gave excellent results on both analytical and preparative scales. Other solvent systems present were only suitable to the isolation and preparation of less than five anthraquinone derivatives. Some solvent systems listed in Table 4.

In addition, Wu and Yang [42] used a TLC–fluorescence quenching method to determine the content of Physcion and Emodin in three kinds of Chinese patent medicine (san-huang-pian, shang-qing-wan, niu-huang-jie-du-wan). The optimal fluorescence quenching system was 7×10^{-4} mol/L luminal, 1.0×10^{-2} mol/L H_2O_2, 2.5×10^{-9} mol/L Cr^{3+}, 1.0×10^{-3} mol/L EDTA, 0.1 mol/L $NaHCO_3$–Na_2CO_3 buffer, pH 10.5. The results were agreed with those from other method. Huang and Kong [43] established a two dimension TLC method to analyze five kinds of hydroxyanthraquinones in 18 kinds of rhubarb. The first developing solvent was ethyl acetate–methanol–H_2O (100:16.5:13.5) and the second developing solvent was petroleum ether–hexane–ethyl formate–formate acid (1:3:1.5:0.2). It was found that the content of hydroxyanthraquinones were in a great diversity and the method was simple and reliable. Wang et al. [44] determined the formation constants for the inclusion complexes between emodin, aloe-emodin and cyclodextrins (CD) by thin-layer chromatography. The interaction between emodin, aloe-emodin, and six cyclodextrins has been studied by reversed-phase thin-layer chromatography. The $NH_3 \cdot H_2O$–NH_4Cl buffer containing various CDs (pH 9.7, 20 °C) and polyamide plate were selected as mobile phase and stationary phase, respectively. CDs except for α-CD and carboxymethyl-β-CD can form inclusion complex with these two compounds. The comparison of inclusion capacity of different CDs indicates that for the ionic cyclodextrins, the charge interaction plays an important role in the inclusion procedure. The thermodynamic parameters of interaction imply that the inclusion process shows the enthalpy–entropy compensation effect at compensation temperature of 296.5 K.

2.4. High-speed counter-current chromatography

High-speed counter-current chromatography (HSCCC), being a support-free liquid–liquid partition chromatography, eliminates irreversible adsorption of sample onto the solid support, and is considered as a suitable alternative for the separation of phenolic compounds such as flavonoids and hydroxyanthraquinones. A HSCCC method for isolation and purification of four hydroxyanthraquinones from *Rheum officinale* Baill using pH-modulated stepwise elution was reported [45]. The separation column was 50 m × 0.85 mm

Table 3
Conditions and results obtained in Ref. [29]

Eluent	Time program	Analytes	Recoveries (%)	Range (mg/100mL)
A: 0.02 M KH_2PO_4 in H_2O, pH 3	0–10 min, 86% A	Quantitative standard		
	15–25 min, 80% A	Aloe-emodin	108 ± 1	0.010–9.88
	26 min, 79% A	Rhein	102 ± 1	0.010–13.3
	55 min, 20% A	Emodin	100 ± 0.4	0.010–10.2
B: acetonitrile	57–70 min, 86% A	Physcion	82 ± 4	0.003–4.80
		Chrysophanole	95 ± 1	0.003–4.20
		Sennosid B	99 ± 5	0.006–18.0
		Qualitative standard Sennosid A, A_1, B, C, D, D_1, Sennidin A/B Aloe-emodin-8-glucoside Rhein-8-glucoside Sennidin-A-glucoside Sennidin-B-glucoside		

Table 4
Some solvent systems of TLC for separation of anthraquinone derivatives

Solid phase	Solvent	Analytes	Samples	References
Silica	Benzene–ethyl acetate-methanol (5:3.5:1.5)	Rhapontin	*R. hotaoense*	[36]
Silica	Petroleum–ether–hexane–ethyl acetate–acetic acid–methanol (150:300:150:10:10)	Rhein, aloe-emodin, emodin	Plasma	[37]
Silica	Benzene–ethyl formate–formate acid–methanol–water (3:1:0.05:0.1:0.5)	Emodin	Qing-re-ming-mu-jiao-nang	[38]
Silica	Petroleum ether–ethyl acetate–acetic acid (87:6.4:6)	Emodin	Rhubarb	[39]
Silica	Petroleum ether–ethyl formate–formate acid (15:5:1)	Emodin	Jian-fei-jiang-zhi-jiao-nang	[40]
Polyamide film	Six kinds of SDS/*n*-C_4H_9OH/*n*-C_7H_{16}/H_2O	Emodin, chrysophanol	Fangfeng tongshen Pill	[41]

i.d. and the preparative column 110 m × 1.6 mm i.d., respectively. The stationary phase was diethyl ether and H_2O as the mobile phase (Fig. 6). Wang et al. [46] isolated and purified aloin and aloe-emodin with a solvent system consisting chloroform–methanol–H_2O (9:10.5:8, v/v/v), the upper phase was as stationary phase and the lower phase as mobile phase. The purities of aloin and aloe-emodin were analyzed by HPLC, MS, IR and UV.

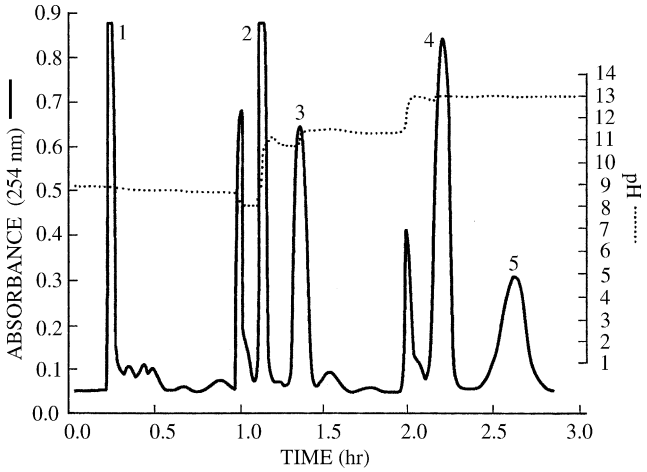

Fig. 6. Chromatogram of the crude sample of hydroxyanthraquinones from *R. officinale* Baill by preparative HSCCC with a diethyl ether–aqueous base system. Stationary phase: upper organic phase; mobile phase: 120 mL of 4.0% $NaHCO_3$, 240 mL of 0.7% Na_2CO_3 and 480 mL of 0.2% NaOH; flow-rate: 2.0 mL/min; revolution speed: 800 rpm; sample size: 300 mg dissolved in 200 mL stationary phase, retention of stationary phase: 50%. Peaks: (1) rhein + unknown; (2) emodin; (3) aloe-emodin; (4) chrysophanol; and (5) physcion. Adapted and reprinted with permission from Ref. [45].

3. Preparation of anthraquinones

Most authors got the total hydroxyanthraquinones by simple liquid extraction. Typically, a mixture of 20% H_2SO_4 and $CHCl_3$ (1:100, v/v) was used. The obtained $CHCl_3$ solution was washed and dried to get total hydroxyanthraquinones [1]. Xia et al. [47] utilized H_2O as solvent to extract crude hydroxyanthraquinones from rhubarb samples, then used polyamide as sorbent absorbing hydroxyanthraquinones from aqueous extraction. Pure hydroxyanthraquinones mixtures eluted from polyamide by absolute ethanol. Hydroxyanthraquinones were isolated further after the extraction with 20% H_2SO_4–$CHCl_3$ solution [48]. Firstly, 5% $KHCO_3$ was added to the $CHCl_3$ solution and rhein was deposited from aqueous phase. Secondly, 5% Na_2CO_3 was added to the above $CHCl_3$ solution and aloe-emodin was gotten from aqueous phase. Thirdly, 5% NaOH added to $CHCl_3$ to get the mixture of phycion and chrysophanol. The mixture was separated from a silica column. Supercritical fluid extraction (SFE) was used to prepare hydroxyanthraquinones too [49]. The mixture of aloe-emodin, emodin and rhein obtained under the conditions: 0.05 mL HCl, 0.4 mL methanol, 41.4 MPa, 50 °C, 5 min with SFE.

4. Conclusion

There existed various of techniques to determine hydroxyanthraquinoids and still will have some new methods to finish the same work owing to rhubarb present in a lot of Chinese medicine compounds. Although there are so many articles related with determination of hydroxyanthraquinones but quite a bit authors only gave different modification of the same method. It seems capillary electrophoresis is the best among these satisfied methods to determine interested substances in various samples.

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