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Heart-cut two-dimensional separation method via hyphenation of micellar electrokinetic capillary chromatography and capillary zone electrophoresis using analyte focusing by micelle collapse

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ABSTRACT

A novel two-dimensional (2D) separation method, which hyphenated micellar electrokinetic capillary chromatography (MEKC) and capillary zone electrophoresis (CZE), was developed for analysis of flavonoids in Leonurus cardiaca. The Leonurus cardiaca sample was separated and purified in first dimension by MEKC. Then only a selected portion of the first dimension separation was transferred into the second dimension by pressure. Finally, the zone of flavonoids was separated by CZE. As the key to successful hyphenation of MEKC and CZE, an analyte focusing by micelle collapse (AFMC) concentration method was employed between the two dimensions to release analytes from the micelle interior to a liquid zone and to overcome the sample zone diffusion caused by mobilization pressure. The whole heart-cut 2D separation process can be performed in a conventional CE analyzer. The relative standard deviation of peak height, peak area and migration time were in the range of 2.3–4.2%, 1.5–3.8% and 3.6–5.5%, respectively, and detection limits (S/N = 3) were 15–55 ng/mL. The new methodology was applied with success for the flavonoids separation of Leonurus cardiaca.

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1. Introduction

For complex samples, two-dimensional (2D) capillary electrophoresis (CE) has aroused great interest in recent years due to the high separation power and peak capacity. Dovichi's group combined submicellar CE at pH 7.5 with electrophoresis at pH 11.1 [1], capillary sieving electrophoresis (CSE) with MEKC [2–5] using a cross interface with aligned capillaries for proteomic analysis by 2D CE. Sheng and Pawliszyn developed a 2D separation system based on coupling MEKC to capillary isoelectric focusing (cIEF) by a 10-port valve with two conditioning loops for protein digests analysis [6]. Zhang's group and Mohan et al. coupled cIEF to capillary gel electrophoresis (CGE) [7], CSE [8] and CZE [9–12] for proteome analysis with a microdialysis interface [7–11] and an etched fused-silica porous junction [12]. Zhang and El Rassi used a nanoinjector valve for connecting of cIEF and capillary electrochromatography (CEC) for 2D separation of proteomics [13]. Sahlin reported a CZE–MEKC system for the separation of peptides using tangentially connected capillaries [14]. In summary, the similarity in the above-mentioned 2D CE separation systems lies in the construction of a reliable interface to switch the effluents from the first-dimension column to the second one.

Cottet's group demonstrated the interest of using a single capillary for performing heart-cutting 2D CE [15–17]. The methodology has been applied for the charge- and size-based characterization of synthetic polymer mixtures by CZE–CGE [15,16] and then used for on-line purification and separation of 12 derivatized amino acids using 2D CZE–MEKC separation [17]. Recently, they investigated heart-cutting 2D-CE of native amino acids incorporating on-line preconcentration step using transient moving chemical reaction boundary (tMCRB) and the use of multiple detection points [18]. However, the use of hydrodynamic flow between the two dimensions reduced peak efficiency due to peak broadening by Taylor dispersion [17]. To limit the loss in peak efficiency related to the hydrodynamic mobilization, on-line concentration step including field amplified sample stacking (FASS) [17] and tMCRB [18] and a 10 μm id capillary with high sensitive detector were used [19].

In previous works, we also demonstrated the interest of incorporating a sample concentration step between the two dimensions to avoid sample zone diffusion at the interface [20–22]. Weak basic drug mixtures were subjected to 2D CZE–MEKC separation with a microhole interface and pH junction and sweeping to connect the two separation modes [20]. Then we incorporated sample

Abbreviations: 2D, two-dimensional; MEKC, micellar electrokinetic capillary chromatography; CZE, capillary zone electrophoresis; AFMC, analyte focusing by micelle collapse.

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preconcentration and 2D CZE–MEKC separation for simultaneous enhancement of resolution and sensitivity [21]. Electroaccumulation focusing method was utilized at the junction between the two capillaries to avoid the primarily preconcentrated and separated analyte bands diffusion at the interface. These methodologies demonstrated the availability of on-line concentration method to overcome the problems lie in 2D CE such as dead volume, peak broadening and low sensitivity. Recently, heart-cutting 2D CZE–MEKC in a single capillary has been applied for blood samples with electrochemical detection [22]. On-line dual concentration method, FASS and sweeping, was employed between the two dimensions to accumulate the purified components that effectively counteracted the sample zone diffusion during the mobilization pressure step.

In this paper, we developed a new heart-cut 2D MEKC–CZE separation scheme for analysis of flavonoids in Leonurus cardiaca. MEKC was used as the first dimension, from which the selected effluent fractions were further analyzed by CZE acting as the second dimension. To release analytes from the interior of SDS micelle and to counteract the primarily separated analyte bands diffusion during the hydrodynamic mobilization process, an AFMC [23,24] step was utilized between the two dimensions. Leonurus cardiaca sample was selected as model sample to evaluate the effectiveness and reliability of the proposed method. To the best of our knowledge, this work represented the first demonstration of heart-cutting 2D CE separation via hyphenation of MEKC and CZE with AFMC between the two dimensions.

2. Experimental

2.1. Reagents and solutions

The flavonoids including kaempferol, hesperetin, apigenin, rutin, hyperoside and quercetin were obtained from Zelang Medicine Ltd. (Nanjing, China). The Leonurus cardiaca was purchased from the Central Hospital of Qingdao City (Qingdao, China). Sodium tetraborate, sodium acetate, sodium phosphate, etc. were obtained from Shanghai Chemical Plant (Shanghai, China). Sodium dodecyl sulfate (SDS) was from Sigma. Standard stock solutions of six flavonoids at a concentration of 1.0 g/L were prepared in ethanol and diluted to the desired concentration with running buffer just prior to use. The phosphate buffer solution was prepared by adjusting the acidity of sodium dihydrogen phosphate (concentrations ranging from 5 to 150 mmol/L) solution to a desired pH with concentrated sodium hydroxide, and the pH value of the buffer solution was measured by a PHS-3C pH meter (Shanghai Dapu Instrument Co., Shanghai, China). All aqueous solutions were prepared with double-distilled water and filtered through 0.22 μm cellulose acetate membrane filters (Shanghai Yadong Resin Co., Shanghai, China) before use.

2.2. Instrumentation

The 2D CE was performed using a set of model MPI-A electrophoresis system (Xi'an Remax Electronics Inc., Xi'an, China), equipped with a high-voltage power supplier (0–30 kV) for electronic sampling and separation and an electrochemical (EC) potentiostat (0–2.5 V) for EC detection. A 60 cm fused-silica capillary (50 μm i.d. and 375 μm o.d.) was obtained from Ruiyang Chromatographic Co. Ltd. (Yongnian, China). A laboratory-built on-column EC detection system, including a field decoupler joint and a three-electrode system was employed in the experiment. The details of this system have been described in previous work [22]. In brief, 5 mm of capillary wall started 1.5 cm from the capillary outlet was etched by laser to construct the field decoupler joint. The field decoupler joint and a Pt electrode served as the ground electrode of electrophoresis were inserted into the ground cell filled with buffer solution. A three-electrode system (a 20 μm diameter Pt wire working electrode, an Ag/AgCl reference electrode, and a Pt wire auxiliary electrode) was used in combination with an EC detector. The Pt working electrode was inserted into the capillary outlet at a depth of 0.5 cm and adjusted to align with the decoupler and separation capillary.

2.3. Leonurus cardiaca sample solution preparation

Leonurus cardiaca samples were dried in a drying oven at 50 °C for 4 h, and then crushed into powder. A 0.50 g Leonurus cardiaca sample was weighed in a 100 mL glass conical flask. After adding 40 mL ethanol, the flask was capped and the sample was extracted with ultrasonic cleaner for 30 min. The extract was filtered with a 0.22 μm acetic cellulose membrane, and then concentrated to about 2.0 mL at 50–60 °C. The treated solution was stored in a refrigerator and 5-fold diluted with sodium phosphate buffer just prior to analysis.

2.4. Procedure of monodimensional CE separation and EC detection

The capillary was flushed daily in the order of H_2O (1 min), 1.0 mol/L NaOH (15 min), H_2O (1 min) and conditioned with running buffer for 10 min by pressure. Between two runs, the capillary was conditioned with running buffer for 10 min. After the EC signal reached a constant value, the mixed solution of the standard flavonoids (or the Leonurus cardiaca sample) was injected with an injection voltage of 15 kV and an injection time of 8 s. Then a separation voltage of 15 kV was applied across the capillary. The detection potential of 0.85 V was applied at the working electrode and the EC responses were recorded.

2.5. Procedure of heart-cut 2D MEKC–CZE separation and EC detection of Leonurus cardiaca

After flushed with H_2O and 1.0 mol/L NaOH solution, the capillary was conditioned with MEKC buffer for 10 min. Leonurus cardiaca sample was electrokinetically injected at 15 kV for 8 s. By replacing the sample solution with MEKC running buffer, the sample was separated by MEKC at 15 kV in the first dimension. Once the EC responses signal of the selected fraction was appeared, the MEKC separation voltage was stopped immediately. The detection cell was changed with CZE buffer solution and a 2.0 psi nitrogen pressure was applied to the detection cell. After 295 s, the nitrogen pressure was stopped and the capillary inlet reservoir was changed with CZE buffer solution. A 15 kV voltage was applied for AFMC concentration, and analyte molecules bound hydrophobically to the interior of the micelle were released to the micellar dilution zone. Finally, the neutral analytes were negatively charged in the CZE buffer and separated by CZE mode.

3. Results and discussion

3.1. Analysis of standard flavonoids and Leonurus cardiaca sample with CZE separation

Leonurus cardiaca, a commonly used medicinal herb, has the effects of invigorating blood circulation and regulating the menstrual function, dissolving blood stasis and promoting tissue regeneration, enhancing urine excretion and reducing swelling, and therefore is used to treat such diseases as menoxenia, dysmenorrheal, dystocia, postpartum blood stasis, to name only a few. Flavonoids including kaempferol, hesperetin, apigenin, rutin,

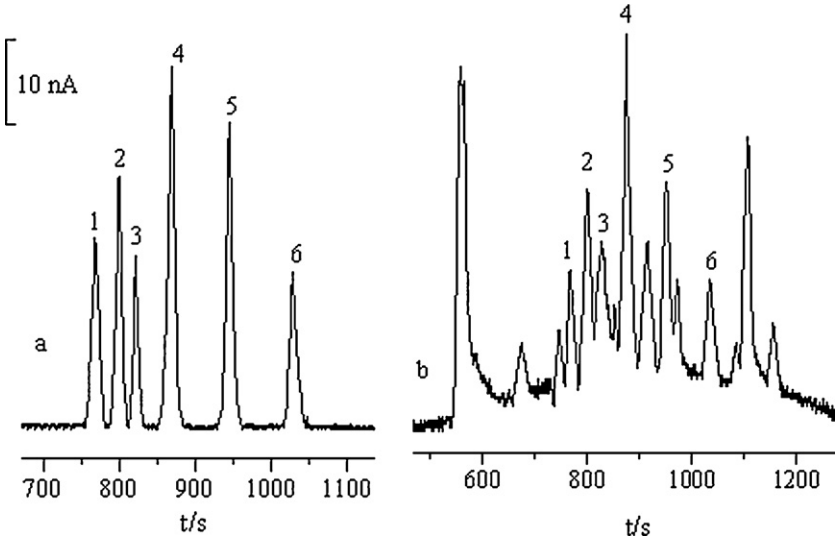

Fig. 1. Electropherograms of standard flavonoids mixtures (a) and Leonurus cardiaca sample (b) by CZE separation. Experimental conditions: injection, 15 kV for 8 s. Capillary, 50 μm i.d. × 375 μm o.d., 60 cm length; six standard flavonoids concentration of (a) were all 2 mg/L. Buffer, 40 mmol/L sodium phosphate (pH 8.5); detection potential, 0.85 V. Peak identification: 1, kaempferol; 2, hesperetin; 3, apigenin; 4, rutin; 5, hyperoside; 6, quercetin.

hyperoside and quercetin and so on are one of the largest groups of naturally occurring phenols in Leonurus cardiaca and have antiinflammatory, antitumor, antivirus, antibacteria, and antioxidation functions. Firstly, the mixture of standard flavonoids was separated by CZE. In order to optimize the separation conditions, buffer solutions at different concentrations and pH values, including sodium acetate, sodium phosphate, sodium borate, sodium citrate and NH_3/NH_4Cl, etc. were studied. Among them, 40 mmol/L sodium phosphate buffer solution was proved to be best in terms of resolution and separation efficiency and was used in subsequent studies. The pH of 40 mmol/L sodium phosphate buffer solution was optimized. The experimental results showed that, at pH 8.5, these compounds could be separated with the best resolution. Under the optimal conditions, the electropherogram of the standard flavonoids mixture was shown in Fig. 1a. Each compound was identified by adding a pure standard to the mixed solution so that the peak height and area of the corresponding compound was increased significantly.

With the optimal conditions above used, the Leonurus cardiaca sample solution was introduced into capillary and separated by CZE, the typical electropherogram is shown in Fig. 1b. The flavonoids in Leonurus cardiaca sample were accurately identified by comparing their migration times with those of standards and by adding a pure standard to the sample. From the electropherogram we could see that a number of interfering ingredients in Fig. 1b comigrated with the flavonoids. These undesirable ingredients were related to some alkaloids, diterpenoids, secoiridoid glycosides and organic acids that existed in Leonurus cardiaca in a large quantity. Although these compounds were not all identified, some examples of Leonurine hydrochloride and ferulic acid were identified by adding a pure standard to the sample. Except for some alkaloids such as Leonurine hydrochloride, most of interfering ingredients were neutral or anionic in the running buffer and co-migrated with the flavonoids.

3.2. Analysis of Leonurus cardiaca sample with MEKC separation

Then the Leonurus cardiaca sample was separated by MEKC with SDS micellar in running buffer. The effect of different types and concentrations of running buffer including sodium phosphate, sodium borate, sodium acetate, sodium citrate and sodium tartrate, etc. were examined for separation of Leonurus cardiaca sample. The results showed that sodium phosphate buffer solution was more suitable because the peak-to-peak resolution (R) of flavonoids and interfering ingredients excel than those of other buffer solutions. The relationships of resolution to the concentration of sodium phosphate (varied from 5 to 150 mmol/L) and the buffer pH (ranging from 3.0 to 10.0) were tested. From the results obtained, when the concentration of phosphate was 90 mmol/L and the pH was 7.0, the best resolution of these compounds could be obtained. SDS micelle (3–40 mmol/L) was added to 90 mmol/L phosphate (pH 7.0) buffer solution to carry out MEKC. As higher concentrations of SDS were added to buffer solution, improvement in the resolution was observed. When the concentration of SDS was 5 mmol/L, the flavonoids and interfering ingredients were entirely separated. Further increase of the SDS concentration, the migration time and current were observed to obviously increase in spite of improvement in the resolution. So 5 mmol/L SDS in 90 mmol/L phosphate buffer was used in subsequent studies. The typical electropherogram was shown in Fig. 2. Although not all the flavonoids were separated on baseline, the flavonoids were isolated well from that of the interfering ingredients.

As can be seen from Figs. 1b and 2, when Leonurus cardiaca sample was analyzed in one CZE run, the flavonoids were separated well while some peaks of interfering ingredients were overlapped

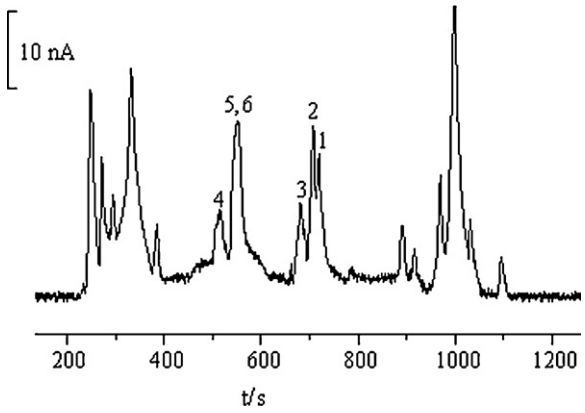

Fig. 2. Electropherogram of Leonurus cardiaca sample by MEKC separation. Running buffer, 5 mmol/L SDS in 90 mmol/L sodium phosphate buffer (pH 7.0). Peak identification and other conditions were the same as in Fig. 1.

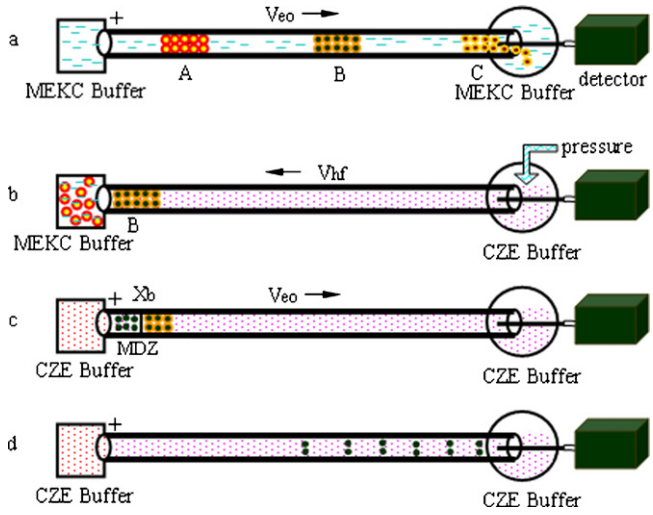

Fig. 3. Evolution of heart-cut 2D MEKC–AFMC/CZE for purification and separation of flavonoids in Leonurus cardiaca sample. (a) MEKC separation of Leonurus cardiaca sample in the first dimension and elimination of interfering fraction C from the detection end of the capillary; (b) elimination of interfering fraction A and return of the objective fraction B to the inlet end of the capillary; (c) AFMC concentration and releasing of analytes from the interior of SDS micelle to the MDZ; (d) CZE separation of the flavonoids in the second dimension. Veo is electroosmotic flow. Vhf is hydrodynamic flow. Xb is the boundary which separates the sample zone and the left CZE buffer zone. MDZ is the zone closest to Xb in left CZE buffer solution. MEKC buffer is 5 mmol/L SDS in 90 mmol/L sodium phosphate buffer (pH 7.0); CZE buffer is 40 mmol/L sodium phosphate buffer (pH 8.5).

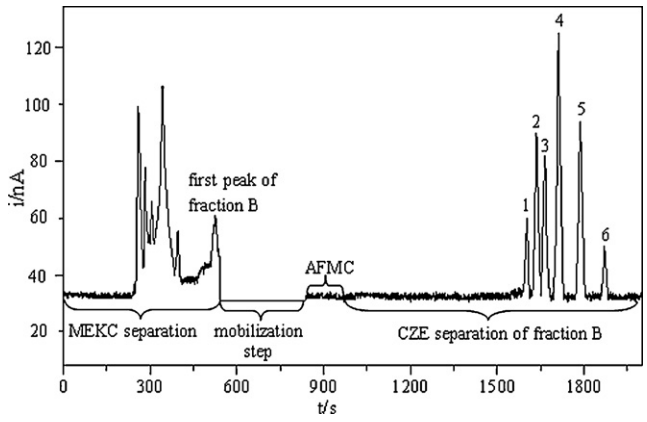

Fig. 4. Entire process of heart-cut 2D MEKC–AFMC/CZE separation of flavonoids in Leonurus cardiaca sample. The process includes MEKC separation of Leonurus cardiaca acting as the first dimension until the first peak of fraction B detected (15 kV voltage), the mobilization step (2.0 psi nitrogen pressure at detection end), AFMC and CZE separation of flavonoids acting as the second dimension (15 kV voltage). Peak identification and other conditions were the same as in Figs. 1 and 3.

with those of flavonoids; however, when the sample was analyzed in one MEKC run, the flavonoids were isolated well from that of the interfering ingredients in spite of not all the flavonoids separated completely. Therefore, the design of heart-cut 2D MEKC–CZE was motivated by the desire to analyze Leonurus cardiaca sample by evacuating the interfering ingredients in a first dimension with MEKC separation and, next, to separate the flavonoids in a second dimension with CZE mode.

3.3. Principle and methodology of the heart-cut 2D MEKC–AFMC/CZE

The main idea of heart-cut 2D MEKC–AFMC/CZE for purification and separation of flavonoids in Leonurus cardiaca sample was illustrated in Fig. 3. The capillary was initially filled with MKEC buffer solution. Leonurus cardiaca sample was electrokinetically pumped into capillary at 15 kV for 8 s. By replacing the sample solution with MEKC buffer solution, the Leonurus cardiaca sample was separated by MEKC in the first dimension at 15 kV (Fig. 3a). During this separation stage, the sample was separated into three main fractions. The fraction A corresponded to the undesirable hydrophobic species such as secoiridoid glycosides, diterpenoids and the cationic alkaloids. Fraction B was related to the objective flavonoids, and fraction C was some hydrophilic neutral species that comigrated with EOF. At the end of this step, the undesirable fraction C was evacuated out of capillary by the detection end. Once the fraction B reached outlet end, the EC responses signal was appeared and the separation voltage was stopped immediately. Then the detection cell was changed with CZE buffer solution and a 2.0 psi nitrogen pressure was applied. The undesirable fraction A was evacuated out of capillary by the inlet end. At the same time, the CZE buffer solution entered the capillary by hydrodynamic flow directed from the outlet to the inlet end of the capillary (Fig. 3b). According to our previous work, the desirable fraction B was pushed to the inlet end at 295 s without sample loss [22]. So the nitrogen pressure was stopped for 295 s and the capillary inlet reservoir was changed with CZE buffer solution. After 15 kV voltage was applied, the high electrophoretic mobility anionic phosphate and SDS micelle moved in the direction of the anode relative to the Xb boundary (Fig. 3c). The zone closest to Xb in the left CZE buffer solution was called micellar dilution zone (MDZ) [23,24]. At the MDZ, the phosphate anions and SDS micelles from sample zone progressively replaced the original phosphate anions of the left CZE buffer solution. Initially, the MDZ was completely filled by high electrophoretic mobility phosphate anions from sample zone. Then SDS micelles moved into this zone and its concentration increased from an initial value of zero. The approximated critical micelle concentration (cmc) of SDS in the systems we used was 3 mmol/L measured by conductivity method. Therefore, the SDS micelle in MDZ collapsed due to the concentration less than its cmc. Subsequently, analyte molecules bound hydrophobically to the interior of the micelle were released to the MDZ. With more and more micelles from sample zone reached the MDZ and collapsed, neutral analytes of fraction B were all transported and accumulated to the boundary. After the AFMC concentration step, the neutral analytes were negatively charged in the CZE buffer and separated by CZE mode (Fig. 3d). The entire process of heart-cut 2D MEKC–AFMC/CZE separation of flavonoids in Leonurus cardiaca was shown in Fig. 4. As expected, the Leonurus cardiaca sample was well purified by the first dimension MEKC and the interfering ingredients were not detected. By comparing Figs. 4 and 1a we can see, the resolution of flavonoids in Leonurus cardiaca sample obtained by heart-cut 2D MEKC–AFMC/CZE is similar to that obtained by a single CZE separation of standard flavonoids. This is mainly due to the Taylor dispersion during the hydrodynamic mobilization step and AFMC concentration between the two dimensions. In step 2 (Fig. 3b), the partial separated analytes in fraction B (see Fig. 2) recombined again due to Taylor dispersion. Furthermore, neutral analytes bound to the interior of the micelle were released to the MDZ, rejoined and accumulated to the boundary in step 3 (Fig. 3c). After the AFMC concentration, the neutral analytes were negatively charged and separated by CZE. Therefore, a satisfactory resolution on the 2D-CE electropherogram in Fig. 4 was obtained although the first dimension performed in MEKC leads to a reversed order of migration of the six flavonoids.

3.4. Effect of the AFMC between the two dimensions

For the first time, MEKC acting as the first dimension was integrated with CZE as the second dimension for 2D separation. To release analytes from the interior of SDS micelle into a

Table 1
Analytical characteristic parameters and analysis of flavonoids in Herba Leonuri samples.

Analytes	Regression equation ($nA \sim$ mg/L)	Correlation coefficient (r)	Linear range (mg/L)	LOD (mg/L)	Concentration (μg/g)	Recovery (%)	RSD (%, $n = 10$)
Kaempferol	$I = 10.6C + 0.34$	0.9948	0.10–230	0.047	50.4	93.8	5.7
Hesperetin	$I = 17.5C + 0.83$	0.9975	0.08–210	0.028	62.4	96.4	4.2
Apigenin	$I = 12.1C + 0.25$	0.9991	0.10–230	0.033	77.2	104.5	6.1
Rutin	$I = 23.7C + 0.46$	0.9956	0.04–160	0.016	93.6	102.6	2.9
Hyperoside	$I = 21.4C + 0.72$	0.9982	0.06–190	0.019	56.0	105.7	3.8
Quercetin	$I = 9.8C + 0.17$	0.9979	0.16–270	0.054	22.6	97.3	4.6

liquid phase, AFMC step was utilized between the two dimensions. AFMC is based on the transport, release, and accumulation of molecules from the hydrophobic core of an anionic micelle into a hydrophilic phase [23,24]. In electrophoresis with strong electrolytes, the Kohlrausch regulating function of a zone (x) is given by the following equation [25]. The total flux of ions in and out of a zone (x) is conserved by a value equal to the KRF.

$$\mathrm{KRF}(x) = \sum_{i=1}^{N} \frac{|z_i|\, c_i(x, t)}{\mu_i}$$

where z_i is the charge of ion i, $c_i(x, t)$ is the concentration of i at zone x at time t, and μ_i is the electrophoretic mobility of i. The sample molecules are transported inside micelles under an applied electric field in a micellar electrolyte solution containing an anionic surfactant (SDS) and an additional anion having high electrophoretic mobility (phosphate). With an increase in the concentration of anions in the sample zone, the surfactant micelles are continuously diluted to below its cmc and collapsed into a liquid phase zone, thereby releasing and accumulating the transported molecules. The effect of AFMC step on the second dimension separation can be contemplated in Fig. 5. The migration time of the first dimension separation increased with increasing of SDS concentration in MEKC buffer solution. For electropherogram (a) in Fig. 5, with 30 mmol/L SDS micelles in the sample zone, the resulting concentration of SDS in the MDZ is predicted to be above the cmc. The flavonoids were not released and further separated by CZE but retained and transported by the SDS micelles. Correspondingly, with 5 mmol/L SDS micelles in the sample zone, the concentration of SDS in the MDZ was below the cmc, so that the micelles collapsed and the flavonoids were released from the interior of SDS micelles to the MDZ and further separated by CZE as shown in the electropherogram (b) of Fig. 5. Comparing of electropherogram (a) and (b) of Fig. 5, the AFMC step between the two dimensions has the functions of coupling MEKC with CZE for 2D separation by release of analytes from micelle to a liquid phase zone, effective contraction of the peak broadening related to the hydrodynamic flow in Fig. 3b, and further compression of the analyte zone to improve the separation efficiency and sensitivity.

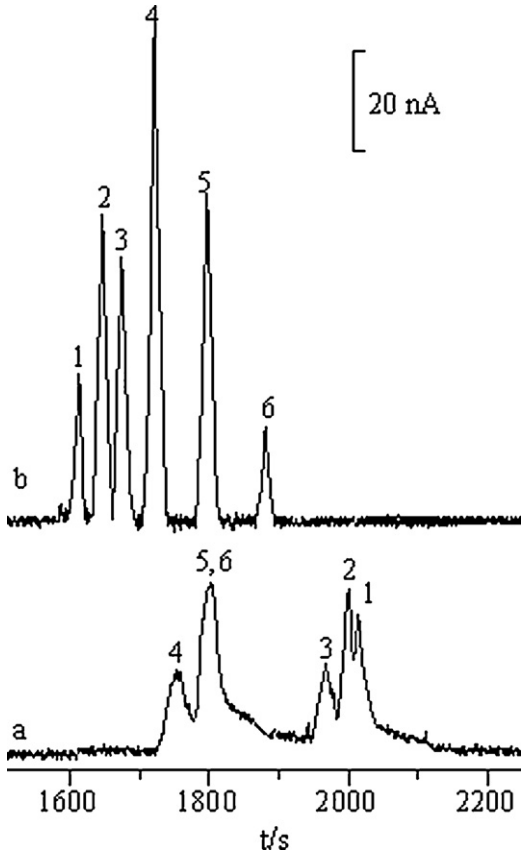

Fig. 5. Effect of AFMC between the two dimensions on the second dimension separation by heart-cutting 2D CE analysis of Leonurus cardiaca sample. (a) Is the electropherogram of flavonoids in Leonurus cardiaca sample without AFMC in heart-cutting 2D CE. Buffers: MEKC separation, 30 mmol/L SDS in 90 mmol/L sodium phosphate, pH 7.0; CZE separation, 40 mmol/L sodium phosphate, pH 8.5. (b) Is the electropherogram of flavonoids in Leonurus cardiaca sample with AFMC step between the two dimensions. Buffers: MEKC separation, 5 mmol/L SDS in 90 mmol/L sodium phosphate, pH 7.0; CZE separation, 40 mmol/L sodium phosphate, pH 8.5. Peak identification and other conditions were as in Fig. 4.

3.5. Analytical characteristics and quantification of flavonoids in Leonurus cardiaca sample

Under the optimum conditions, the equation of linear regression, concentration linear range and limit of detection (LOD) (S/N = 3) were investigated and listed in Table 1. The calibration curves were acquired by plotting the peak currents against individual analyte concentrations. In regression equations, I was peak current and C was analyte concentration (mg/L). The calibration curves for all analytes using the proposed system at each concentration level were linear with a correlation coefficient, r, greater than 0.99. The LODs of six flavonoids are in the range of 15–55 ng/mL. To determine the precision of the proposed method, 10 Leonurus cardiaca samples were determined and the results indicated that the migration times and peak shapes of analytes were reproducible. The relative standard deviation (RSD) values of peak height, peak area and migration time were 2.3–4.2%, 1.5–3.8% and 3.6–5.5%, respectively. Intraday repeatability of migration time was 3% for a series of 10 repetitions. The quantitative determination is based on the calibration curves, as shown in Table 1. The mixed calibration solutions and the samples were detected in one day. Accurate amounts of the six flavonoids were added to the extract of the Leonurus cardiaca sample, and the recovery values were obtained using their peak currents from the calibration curves under the same conditions. The average recoveries range from 93 to 106%.

4. Conclusions

In the present work, a novel heart-cut 2D separation system has been developed for determination of flavonoids in Leonurus

cardiaca. MEKC was used as the first dimension and CZE as the second dimension. An AFMC step was utilized between the two dimensions that not only released of analytes bound to micelle into a liquid phase zone but also accumulated analytes and effective counteracting analytes diffusion during the mobilization pressure step. This method has been successfully applied to the determination of flavonoids in Leonurus cardiaca. It is proposed that this technique could be developed into a useful and powerful way for the analysis of objective species in complex samples. Nevertheless, the proposed system has several limitations. First, the method can only be used for heart-cut 2D analysis. Second, the modest reproducibility in migration time makes identification of components challenging, unless spiking or internal standard are used. Finally, the method is only useful if a first-dimension separation is available that separates classes of molecules into discrete bands and if a higher resolution separation is available for the second dimension.

Acknowledgements

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