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Short communication

Separation and determination of alpinetin and cardamonin by reverse micelle electrokinetic capillary chromatography

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Abstract

A novel electokinetic capillary chromatography method, reverse sodium dodecyl sulfate (SDS) micelles as pseudo-stationary phase, was developed for separation and detection of alpinetin and cardamonin. In this work, reverse micelles (RMs) have been firstly introduced into background electrolyte for electrophoresis separation. The optimum reverse SDS micelle system was formed with *n*-butyl chloride as continuous phase, SDS (20.9%, w/v) as the surfactant, W_0 (13.0, water–surfactant molar ratio), 18.0% (v/v) 1-butanol as the co-surfactant, 8.0% (v/v) acetonitrile (ACN), 1.5% (v/v) heptane, and a 60 mol L^{-1} tris-(hydroxymethyl)aminomethane (Tris) buffer, as dispersed phase. Linear relationships (correlation coefficients: 0.9961 for cardamonin and 0.9991 for alpinetin) between the peak areas and concentration of the two compounds were obtained (5.0–350.0 μg mL^{-1} for cardamonin and 1.25–350.0 μg mL^{-1} for alpinetin). The detection limits (S/N = 3) for cardamonin and alpinetin were 0.19 and 0.14 μg mL^{-1}, respectively. The method was successfully applied for the quantification of alpinetin and cardamonin in Alpinia katsumadai Hayata and kuaiwei tablet with satisfactory recoveries in the range of 95.9–100.2%.

Keywords: Reverse SDS micelle; Alpinetin; Cardamonin; Pseudo-stationary phase; Reverse micelle electokinetic capillary electrophoresis

1. Introduction

Cardamonin (2′,4′-dihydroxy-6′-methoxychalcone) and alpinetin (7-hydroxy-5-methoxyflavanone) (Fig. 1), the major effective components from the fruits of Alpinia katsumadai Hayata, belong to chalcone and flavonoid, respectively [1]. Cardamonin has numerous biological roles, including anti-tumour promoting property, insecticidal effect, anti-mutagenic activity and inhibition of arachidonic acid, collagen, adenosine diphosphate and ristocetin-induced platelet aggregation [2–5]. Recently, cardamonin has been shown to exhibit an appreciable anti-HIV-1 protease activity with an IC_{50} value of 31 μg mL^{-1} [6], and the interaction between cardamonin and protein has been studied [7]. Alpinetin has antibacterial, anti-inflammatory and other important therapeutic activities of significant potency. Although their biomedical effects have been extensively studied, the determination methods for cardamonin and alpinetin are scanty. As far as our knowledge is concerned, only two determination methods, chemiluminescent flow-injection method for cardamonin and high performance liquid chromatography (HPLC) method for cardamonin and alpinetin, were reported [8,9]. The present paper firstly developed a reverse micelle electrokinetic capillary chromatography method in which reverse SDS micelle solutions were used as background electrolytes to separate and determine cardamonin and alpinetin.

Surfactant molecules when dissolved in nonpolar solvents, self-assemble to form reverse micelles. W_0 ($W_0 = [H_2O]/[S]$, where $[H_2O]$ and [S] are the molar concentration of water and surfactant, respectively [10,11]), the water–surfactant molar ratio, was usually used to characterize the reverse micelles. These surfactant aggregates containing a small amount of water ($W_0 < 15$) are called reverse micelles whereas water-in-oil (w/o) microemulsions correspond to droplets containing a large amount water ($W_0 > 15$) [10,11]. In these systems, the polar groups present in the surfactant molecules constitute the inner core of the micelles and the hydrocarbon chains form the outer layer [12,13]. According to the concept of reverse micelle [10–15], the reverse micelle systems are similar to the w/o emulsion systems, and the major difference is W_0. The size

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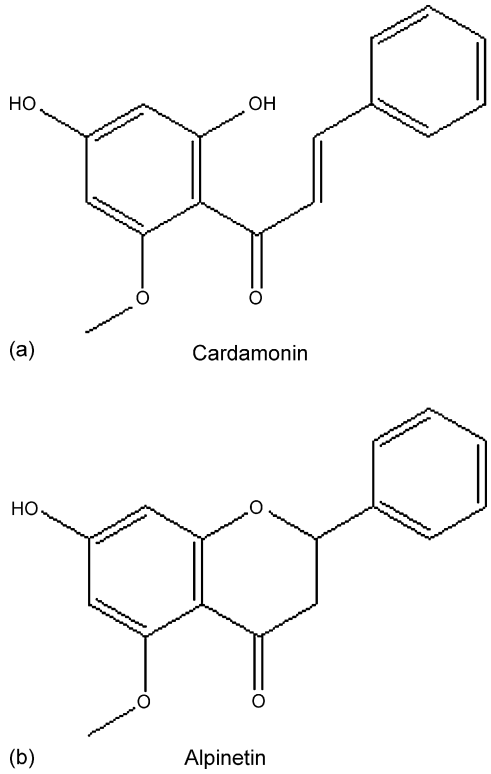

Fig. 1. The structures of cardamonin and alpinetin.

of reverse micelles increased and the micellar concentration decreased while W_0 increased [10]. Reverse micelle solutions were used as background electrolytes for reverse micelle electrokinetic capillary chromatography (RMEKC), which is different from reversed migration micellar electrokinetic chromatography (RM-MEKC) [16]. The reversed migration micelles proposed in RM-MEKC, the hydrocarbon chains present in the surfactant molecules constituting the inner core of the micelles and the polar groups with negative changes forming the outer layer, are the normal micelles migrating in the opposite migration direction at the low pH [17]. Reverse micelle systems appear as homogeneous, transparent solutions, and thermodynamic stability that can solvate a wide range of hydrophilic and hydrophilic compounds [18,19]. The solutes can be partitioning between the continuous phase and the reverse micellar phase, and the partition equilibrium in reverse micellar solutions is considered to achieve rapidly, because collision frequency among reverse micelles is 10^9 to 10^{11} s^{-1} [15].

The aim of this study was to develop a reverse micelle electokinetic capillary chromatography (RMEKC) method to investigate the effects of separation conditions on cardamonin and alpinetin, and develop an efficient and feasible RMEKC method to analysis the two compounds in real samples.

2. Experimental

2.1. Apparatus and procedures

All experiments were performed using a P/ACETM MDQ system (Beckman Coulter Instrument, Fullerton, CA, USA) with PDA detector. The system was controlled by 32 Karat^{TM} software (Version 7.0). The separation was carried out on a 60.2 cm (50.2 cm from inlet to the detector) × 50 μm i.d. fused-silica capillary (Yongnian Photoconductive Fiber Factory, Hebei, China).

Prior to its first use, the capillary was washed successively with methanol for 5 min, 1.0 mol L^{-1} HCl for 5 min, water for 5 min, 0.50 mol L^{-1} NaOH for 15 min, water for 5 min, and the background electrolyte for 5 min. Between two runs, a rinse-cycle was used with 1.0 mol L^{-1} HCl for 2 min, distilled water for 2 min, 0.5 mol L^{-1} NaOH for 3 min, distilled water for 2 min, and running buffer for 2 min. Samples were injected by applying a pressure of 0.5 psi for 3 s. The applied voltage was −30 kV (anode at the detection end) for separation. The capillary was maintained at 25 °C, while 310 nm was selected as the detection wavelength.

2.2. Materials

SDS of chemical purity was purchased from Huyi reagent factory in Shanghai. Alpinia Katsumadai Hayata and kuaiwei tablet were purchased from local drug stores. Cardamonin and alpinetin were of analytical grade and purchased from the National Institute for Control of Pharmaceutical and Bioproducts, China. All other chemicals were of analytical grade. The stock solutions of cardamonin and alpinetin were prepared in reverse micelle buffer at 1.0 mg mL^{-1} and filtered through a 0.45 μm filter before use. All stock solutions were stored at 4 °C.

2.3. Preparation of the electrolytes

The order of addition was important in the formation of the reverse micelle solutions. The optimum buffer was prepared as following: SDS (20.9 g) was mixed with organic solvent (*n*-butyl chloride (37 mL), heptane (1.5 mL), 1-butanol (18.0 mL) and sonicated for 5 min, and then the water (7.0 mL) and Tris solution (60 mmol L^{-1}, 10 mL) were added and sonicated for 15 min. Finally, ACN (8.0 mL) was added into reverse micelle solutions. Thus, optically transparent reverse micelle solutions were formed.

2.4. Sample extraction

All the samples were powdered, and then 1.00 g Alpinia katsumadai Hayata and 5.0 g kuaiwei tablets with 25.0 mL ethanol were extracted in ultrasonic bath for 60 min, respectively. After extract solution of kuaiwei tablets removing ethanol, the dried extracts were treated with 2.0 mL ethanol. All extract solutions were filtered through a 0.45 μm filter prior to use.

3. Results and discussion

3.1. Optimization of reverse micelle solutions

Several physical characteristics of reverse micellar solution (drop size, critical micelle concentration, and viscosity) were

investigated. The critical micelle concentration can be determined through the variation of dye color by the spectrum method [20]. The critical micelle concentration was approximate 37 mmol L^{-1} with eriochrome black T as the maker. The drop size was determined by the constituents of the system. The shape and drop size will vary when the surfactant concentration is above 10-fold critical micelle concentration [20]. Different drop sizes of reverse micelles (1–200 nm) have been reported in literatures [10,21]. In this work, the drop size was in the range of 95.03–97.72 nm, and the viscosity was 5.7165 mPa s for the optimum buffer. The stability of the reverse micelle solution was investigated through the migration times of the studied compounds with the same reverse micelle solution (inter-day, for a 14-day period). The RSD values of the migration times were <3.2%. In addition, the reverse micellar solutions were stable which were diluted by *n*-butyl chloride and ACN (in the investigated range of 4.0–12.0%).

3.1.1. Effects of heptane and Tris concentration on the stability of reverse micelle solutions

Heptane and Tris were introduced into the reverse micelle system to improve the stability and generate stable current in RMEKC. A series of different concentrations for heptane (0.5–1.75%, v/v) and Tris (30–70 mmol L^{-1}) were investigated under the conditions: *n*-butyl chloride as continuous phase, SDS (21.6%, w/v), W_0 (12.0), 1-butanol (20.0%, v/v). The results implied that the current remained stable at 1.5% (v/v) heptane and 60 mmol L^{-1} Tris. Hence, 1.5% (v/v) heptane and 60 mmol L^{-1} Tris were selected for subsequent experiments.

3.1.2. Effect of n-butyl chloride and 1-butanol as continuous phase

The effect of *n*-butyl chloride and 1-butanol were investigated, respectively. Sixty-three milliliters of 1-butanol and 43 mL *n*-butyl chloride were used as continuous phase in the running buffer (total volume, 100 mL), respectively. The results were shown in Fig. 2. It could be seen that the migration times for cardamonin and alpinetin obviously prolonged with 1-butanol used as continuous phase (Fig. 2b) than those with *n*-butyl chloride used as continuous phase (Fig. 2a). A reason was that the viscosity of 1-butanol (2.57 mPa s) is 6-fold over that of acetonitrile (0.42 mPa s) resulting in the lower migration velocity of the analytes. The other reason was the interaction between hydroxyl groups of the compounds and the hydroxyl groups of 1-butanol became stronger because of forming hydrogen bond, then the analyte molecules were difficult to dissociate. Based on the discussion above, *n*-butyl chloride was used as the continuous phase for further study.

3.2. Method optimization

The effect of SDS concentration from 18.7 to 21.6% (w/v) in the running buffers on the separation was studied. The results indicated that the migration time increased with the increasing of SDS concentration. The possible reason may be that the concentration of reveres micelle increased with the increasing of

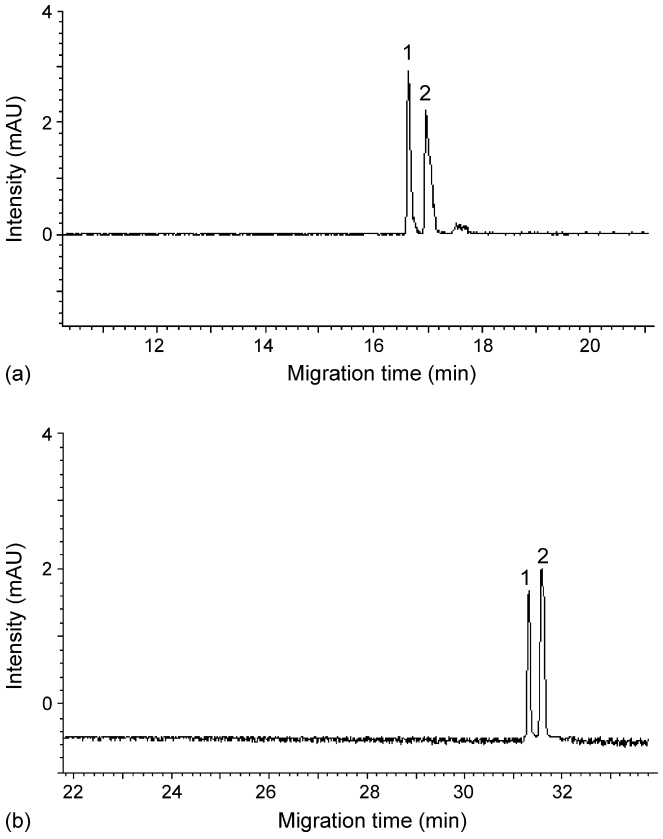

Fig. 2. (a) Separation condition: reverse micelle solutions composed of SDS (21.6%, m/v), W_0 (12.0), 1-butanol (20.0%, v/v), Tris (60 mmol L^{-1}), heptane (1.5%, v/v), and *n*-butyl chloride as continuous phase with detection wavelength at 310 nm. −30 kV voltage was applied to a fused silica capillary tube with 50 μm i.d. and 60.2 cm of capillary column length, and the current was 16.54 μA (50 cm for inlet end to the detective window). Capillary temperature was maintained at 25 °C. (1) cardamonin and (2) alpinetin. (b) Separation condition: 1-butanol was used as continuous phase. All the other separation conditions were the same as in (a). The current was 15.37 μA.

SDS concentration, which would result in change of migration times and resolutions of the analytes. SDS (20.9%) was selected for the subsequent experiments in considering analytical times and separation resolutions.

W_0 was considered an important parameter to control the selectivity. The effect of W_0 was investigated in the range of 12.0–14.0. The results indicated that the migration times of two compounds decreased with the increasing of W_0 in running buffer. The peak width of the two compounds increased from 0.19 to 0.35 min for cardamonin and from 0.28 to 0.53 min for alpinetin with the increasing of W_0. The resolution reached the highest (2.5916) while W_0 was 13.0. Therefore, W_0 13.0 was selected.

The effects of other parameters such as 1-butanol concentration, ACN concentration and the acidity of running buffer on the separation were investigated. With respect to resolutions and analysis time, the optimum separation conditions were selected as follows: SDS (20.9%, m/v), W_0 (13.0), 1-butanol (18%, v/v) and ACN (8.0%, v/v), heptane (1.5%, v/v) and Tris (60 mmol L^{-1}). Under the optimum conditions, the two analytes were well separated within 15 min with symmetrical peaks. The

Table 1
The determined results and average recoveries of the found analytes in samples ($n = 3$)

Sample	Compound	Content (mg g^{-1})	Added amount (μg mL^{-1})	Recovery (%)
Alpinia katsumadai	Cardamonin	0.18	5, 7.5, 10	(97.1, 95.5, 97.6)[a] 96.7
Hayata	Alpinetin	0.97	20, 40, 60	(98.5, 98.3, 103.8) 100.2
Kuaiwei tablet	Alpinetin	0.0046	10, 15, 20	(94.6, 95.7, 97.4) 95.9

[a] The data in parentheses refer to the correspondent recovery for each of the three added amount.

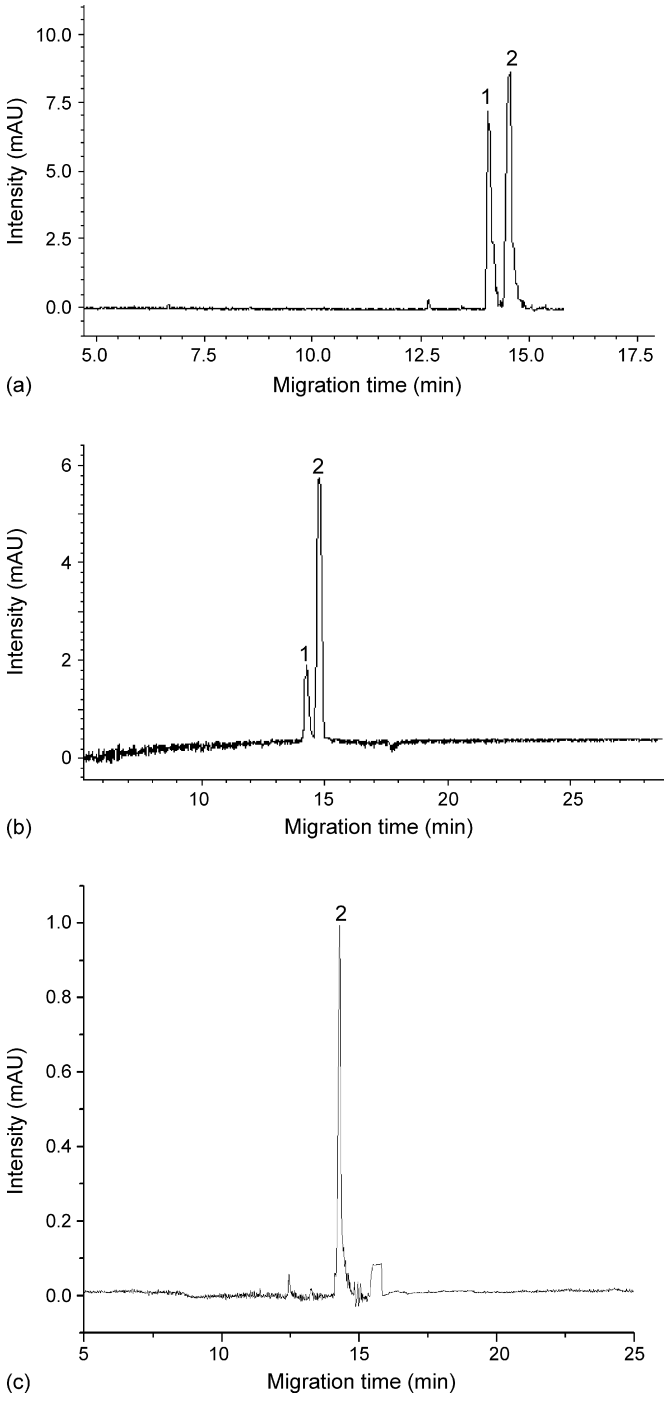

Fig. 3. Electropherograms of the standard sample mixture and real samples: (a) standard sample; (b) kuaiwei tablet; (c) Alpinia katsumadai Hayata. Separation condition: reverse micelle solutions composed of SDS (20.9%, m/v), W_0 (13.0), 1-butanol (18.0%, v/v), and ACN (8.0%, v/v). Other separating conditions were the same as in Fig. 2a.

typical electropherogram for the standard mixture was shown in Fig. 3a.

3.3. Method validation

For evaluation of the quantitative applicability of the method, the standard solutions in the tested concentration ranges were analyzed under the optimum separation conditions. The calibration curves were found to have excellent linearity over the concentration range of 5.0–350 μg mL^{-1} with correlation coefficient (R) 0.9961 for cardamonin and 1.25–350 μg mL^{-1} with correlation coefficient (R) 0.9991 for alpinetin. The detection limits (S/N = 3) for cardamonin and alpinetin were 0.19 and 0.14 μg mL^{-1}, respectively. The reproducibility of the method was determined with a standard mixture solution at the level of 100 μg/mL for each analyte, respectively. The relative standard deviations (R.S.D., $n = 5$) of the migration times and peak areas were 1.1–1.5, 2.2–2.7% (intra-day), and 2.4–3.8, 2.9–4.2% (inter-day, for a 3-day period), respectively.

Cardamonin and alpinetin were separated well in less than 15 min with theoretical plates 2.0×10^5 for alpinetin and 5.8×10^5 for cardamonin. The studied compounds were separated in 7 min with theoretical plates 4500 for alpinetin and 7800 for cardamonin by HPLC method in literature [9]. Compared to the results, the separation efficiency was higher than the HPLC method, though the analysis time was longer.

3.4. Application

The analysts extracted from Alpinia katsumadai Hayata and kuaiwei tablets, respectively, were analyzed by the present method under the optimum conditions. The peaks were identified by comparing the migration times and spiking the standards to the real sample solutions. The typical electropherograms of Alpinia katsumadai Hayata and kuaiwei tablets were shown in Fig. 3b and c. The contents of the two compounds in the real sample and the recoveries are shown in Table 1.

4. Conclusions

It has been proved that reverse micelles can be used as pseudo-stationary phases in RMEKC. Reverse micelle solutions generated a low separation current, which made the application of high voltage was possible. There are two phases in the reverse micelle buffer, which are the reverse micelle phase containing water and organic continuous phase, so hydrophobic and hydrophilic substances can dissolve easily in the reverse micelle solution. The RMEKC method developed is efficient, repro-

ducible and applicable, and it has been successfully applied to analyze cardamonin and alpinetin in Alpinia katsumadai Hayata and kuaiwei tablet. This RMEKC method is a promising alternative for analysis and processing for quality control of other preparations containing cardamonin and alpinetin.

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