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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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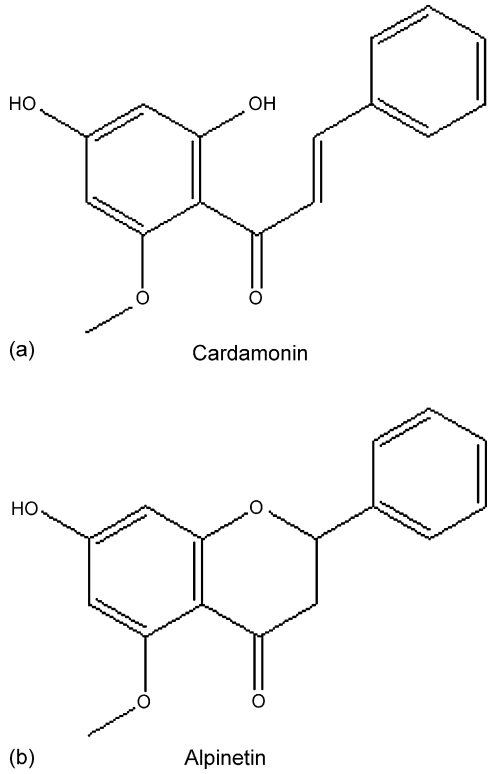

Fig. 1. The structures of cardamonin and alpinetin.

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