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Two-stage fractionation of polar alkaloids from *Rhizoma coptidis* by countercurrent chromatography considering the strategy of reactive extraction

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ABSTRACT

Separation of polar alkaloids by countercurrent chromatography (CCC) is challengeable due to their close partition behaviors in solvent system. In this paper, a two-stage method for isolation of epiberberine, jatrorrhizine, palmatine, coptisine, and berberine from *Rhizoma coptidis* was presented. The first stage separation performed on CCC was based on the principle of reactive extraction. Trifluoroacetic acid was acted as a modulator to selectively react with alkaloids, which changed their partition coefficients in solvent system. Purified epiberberine and other partially separated targets were eluted by ammonium adjusted mobile phase. In the second stage, four alkaloids were purified in pH-zone-refining CCC mode. All the targets collected were over 97% pure determined by HPLC. The method developed demonstrates performing of reactive extraction on standard CCC as an option for separation of polar alkaloids from medicinal plants.

1. Introduction

Natural products remain one of the key sources of leading compounds since their active role in development of potential drugs [1]. Considerable attention from research and industry has been paid to water-soluble components in medicinal plants and the combinations because of their function of prevention and cure of human diseases. Based on this fact, water is the most frequently used solvent to extract hydrophilic components from medicinal plants, but meanwhile more ingredients can be extracted than what are needed. Therefore, how to concentrate and separate the interests from other impurities in aqueous solution is one of the most challengeable issues.

Countercurrent chromatography (CCC), conceptualized as a series of dynamic chained separatory funnels, has been already accepted as an alternative technique for preparative separation of components from natural and synthetic sources [2–4]. The rule of separation performing on CCC is based on liquid–liquid partition which is commonly named as physical extraction. Prior to a successful separation on CCC, one should conduct several or even more experiments to select a proper biphasic solvent system (SS). When a candidate SS is chosen, target compounds are differentially partitioned in two phases in test tube. Then, the targets will be discriminately fractionated according to their partitioning coefficients (K_D) on CCC [5,6]. In some particular cases, when two or more compounds having very close K_{Ds} are mixed in a sample, the process of looking for a qualified SS will be even challengeable. To meet the difficulty, extra selection of SS will be done among the limited solvent combinations.

Reactive extraction offers another way out for extraction of compounds having close K_{Ds} which cannot be separated by physical extraction. In order to enhance the selectivity of SS to the targets, a modifier is added to the SS which can selectively react with target and increase its partition in the desired phase. The modified target is then repartitioned into phases of the SS. In this way, compounds can be discriminated and separated. After separation, the modified targets are reextracted either by shift of temperature or acidity/basicity, or ionic strength to remove the modifier [7]. In recent years, based on the principles of complexation, ion-pair association, and ion exchange effect various reaction extraction methods have been developed [8–11]. Scientists in CCC field have

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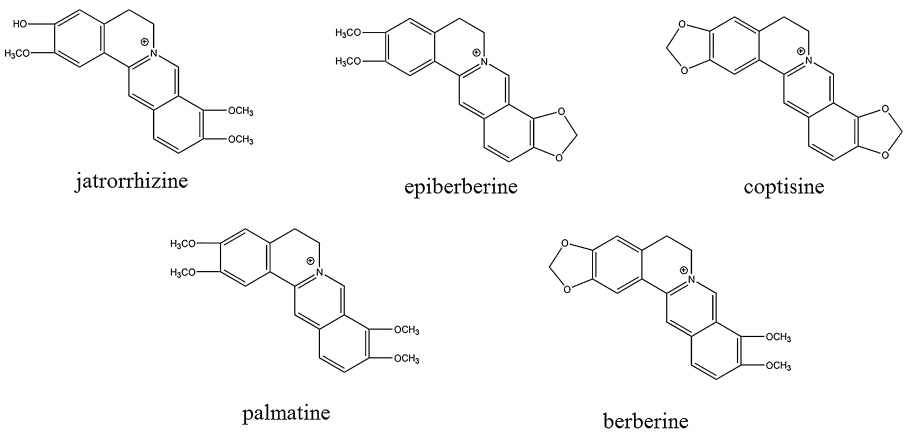

Fig. 1. Chemical structures of alkaloids in *R. coptidis*.

already put efforts in investigation of reactive extraction to tackle the problem of separation of compounds having close K_{Ds}. Winterhalter et al. have developed ion-pair HSCCC method to obtain pure betacyanins which offered another option for extraction of polar compounds by CCC [12–14].

Protoberberine alkaloids are the major bioactive compounds in *Rhizoma coptidis*. They have been proved to possess antimicrobial activity [15] and show effects in diabetes treatment [16]. Results have shown that DNA-binding alkaloids are attractive as promising drugs [17,18]. In the present research, five polar alkaloids (structures shown in Fig. 1) extracted from *R. coptidis* were selected to illustrate the mechanism of reactive extraction on CCC. These alkaloids are quaternary iminium protoberberine alkaloids. The log *P* values of epiberberine, jatrorrhizine, palmatine, coptisine, and berberine calculated by ChemSketch were −1.89, −0.99, −0.87, −1.12, and −0.99, respectively. Attempts have already been made to gain the above pure alkaloids by CCC [19–23]. Separation of them is even challengeable because of their strong polarities but little difference of K_{Ds} in biphasic SS. Here, we present a two-stage CCC fractionation method to totally separate five pure alkaloids from *R. coptidis* based on reactive extraction. The main aim of this work is to develop a new CCC method considering the strategy of reactive extraction and to illustrate the possible mechanism. The strategy of reactive extraction on CCC could provide an alternative way-out for selective purification of polar ionic components using an already fully developed solvent system on a classical CCC apparatus.

2. Experimental

2.1. Apparatus

Separations were performed on a TBE-300B high speed countercurrent chromatography (HSCCC) equipped with three polytetrafluoroethylene coils (tubing I.D. = 1.6 mm, column capacity = 300 mL) and a full wavelength of UV-500 ultraviolet detector (Tauto Biotech, Shanghai, China). A TBP-5002 constant-flow pump (Tauto Biotech, Shanghai, China) was used to deliver solvents. A DC-0506 chiller (Sunny Henping, Shanghai, China) was supplied to adjust the temperature inside the HSCCC. The HSCCC was operated at a rotational speed of 850 rpm. Samples were analyzed by an Agilent 1200 HPLC system equipped with a G1315 diode array detector and a G1329A autosampler (Agilent, U.S.).

2.2. Reagents and materials

R. coptidis was purchased from Shanghai Hongqiao Pharmaceutical Co., Ltd. (Shanghai, China). Standards of jatrorrhizine and palmatine were both from Chinese National Institutes for Food and Drug Control (Beijing, China). Standard of coptisine was booked from Shanghai Jingke Chemical Co., Ltd. (Shanghai, China). Standard of berberine was provided by Shanghai Winherb Medical Technology Co., Ltd. (Shanghai, China). Standard of epiberberine was purchased from Shanghai Haling biotechnology Co., Ltd. (Shanghai, China). Chemicals for CCC were of analytical grade and supplied

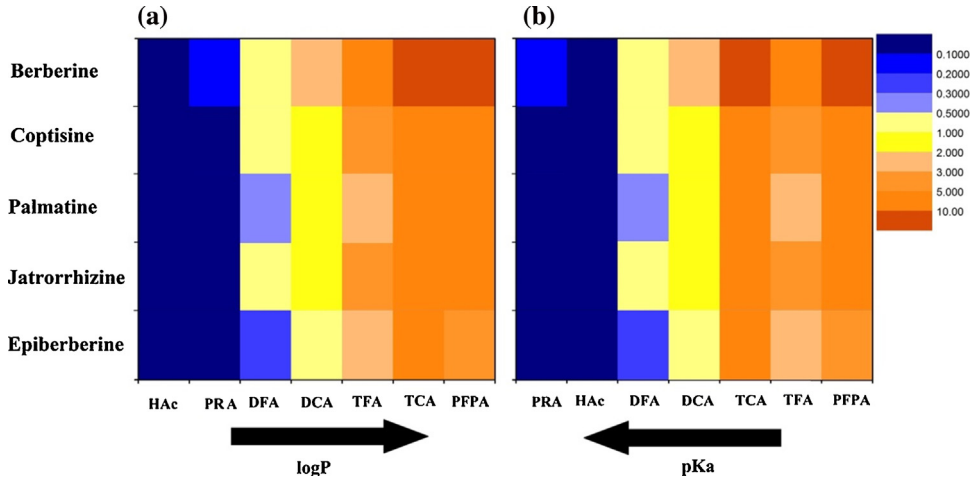

Fig. 2. The relationships between *K* values of alkaloids and the properties of acids. (a) log *P*∼*K* (b) pKa∼*K*.