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Selective isolation of components from natural volatile oil by countercurrent chromatography with cyclodextrins as selective reagent

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ABSTRACT

Selective separation of chemical components from seven kinds of volatile oil by countercurrent chromatography with three types of cyclodextrins as selective reagent was investigated in this work. Preparative separation of chemical components from volatile oil is generally quite challenging due to their extremely complexity of the composition. A biphasic solvent system *n*-hexane-0.10 mol L^{-1} cyclodextrin (1:1, v/v) was selected for separation of components from volatile oil and three types of cyclodextrins were investigated, including β-cyclodexrin, methyl-β-cyclodexrin and hydroxypropyl-β-cyclodexrin. All kinds of volatile oils are from seven kinds of traditional Chinese herb. Results showed that some chemical components could be well separated with high purity from each kind of volatile oil using different type of cyclodextrin as selective reagent. For example, germacrone and curcumenone could be selectively separated from volatile oil of *Curcumae Rhizoma* with methyl-β-cyclodexrin and hydroxypropyl-β-cyclodexrin as selector respectively, and other five components were selectively separated from volatile oil of *Chuanxiong Rhizoma*, *Myristicae Semen*, *Aucklandiae Radix* and *Angelicae Sinensis Radix* by countercurrent chromatography with different cyclodexrin as selective reagent. Separation mechanism for separation of components from volatile oil by countercurrent chromatography with cyclodextrin as selective reagent was proposed. Peak resolution of the present separation method could be greatly influenced by the chemical compositions of volatile oil.

1. Introduction

Chemical compositions of volatile oils extracted from traditional Chinese herbs are generally very complex and they are difficult to be separated with high purity. Separation of components from volatile oil of natural products is generally conducted by repeated column chromatographic technologies, which is very tedious, time and solvent-consuming. Modern countercurrent chromatography technologies, mainly including high speed countercurrent chromatography and high performance centrifugal partition chromatography, have been widely used in separation and purification of chemical constituents from natural products [1]. Compared with conventional chromatographic methods, countercurrent chromatography is an ideal method for preparative separation of chemical components from natural products due to its distinctive advantages, i.e., preparative capacity and no solid support for the stationary phase. However, only a small number of literatures reporting on separation of volatile oil by countercurrent chromatography could be found and peak resolutions were generally very low because of their extremely complexity [2–9].

In the recent years, molecular recognition inclusion crystalline method has been explored to isolate specific chemical constituent from volatile oil using a host chemical structure [10–12]. For instance, selective isolation of anethole from volatile oil of *Foeniculum Vulgare Mill* could be completed using an inclusion crystalline method with 1,1,6,6-tetraphenylhexa-2,4-diyne-1,6-diol as a host molecule and anethole was believed to be a guest molecule, in which the host molecule could selectively form an inclusion complex with anethole [12]. Meanwhile, molecular imprinting technique is a simple and efficient method for separation of chemical components, in which a molecularly imprinted polymer was prepared with tailor-made recognition sites for certain target molecules. The polymers have proven to be versatile synthetic receptors due to their high specific recognition ability, making it

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very promising candidates for many applications, including chromatographic stationary phase, solid phase extraction and drug development [13,14]. Inspired by the above two methods, selective isolation of components from volatile oil by countercurrent chromatography with a host chemical structure as additive was investigated in our lab. Although the separation method in our manuscript was different with the above two methods, all of them shared the same phenomenon of molecule recognition. Cyclodextrins used in our present research could be thought as a kind of natural molecularly imprinted polymer.

Cyclodextrins can form inclusion complexes with hydrophobic compounds in the aqueous phase because they are hydrophobic inside and hydrophilic outside. Solubility of volatile oils of natural products could be generally improved using cyclodextrins as the host chemical structure added in an aqueous phase, and bioavailability of volatile oil of traditional Chinese medicine could be greatly improved using the inclusion method. Furthermore, major difference of inclusion formation constant between cyclodextrin and chemical component could be found due to different specific stereochemical structures. For instance, substituted β-cyclodextrins have been widely used as chiral selectors for enantioseparations, which indicates specific type of cyclodextrin shows highest inclusion interaction with a specific stereochemical molecule. So this method might also be applied to selective separation of components from volatile oil. In the present paper, different type of cyclodextrin was used as selective reagent added in the biphasic solvent systems to selectively isolate chemical components from volatile oil by countercurrent chromatography. Seven kinds of volatile oils of traditional Chinese herb were investigated, including volatile oil of *Curcumae Rhizoma*, *Chuanxiong Rhizoma*, *Myristicae Semen*, *Aucklandiae Radix*, *Angelicae Sinensis Radix*, *Artemisiae Annuae Herba* and *Foeniculi Fructus*. Selection of the above seven types of volatile oils was based on the reported literatures [15–22], in which they had been investigated for the interactions between the chemical components of volatile oil and cyclodextrins. Meanwhile, in our preliminary experiments, liquid–liquid partition experiment had been conducted to estimate the partition performance of chemical constituent in the volatile oil of *Curcumae Rhizoma* in a biphasic solvent system *n*-hexane-0.10 mol L^{-1} cyclodextrin aqueous solutions. It was found that the methyl-β-cyclodextrin have a high recognition for the component germacrone. Therefore, the other six kinds of volatile oils which have binding forces to the cyclodextrin were also selected in the present work.

2. Experimental

2.1. Apparatus

The high performance liquid chromatography (HPLC) used was a Waters Breeze system (Waters Corporation, Milford, USA) comprised of a Waters 2487 Dual λ Absorbance Detector, a Waters 717 plus Autosampler, a Waters 1525 controller, a Waters 1525 Binary HPLC pump and a Breeze workstation.

Gas chromatography-mass spectrometry (GC–MS) was performed with an Agilent 6890 N gas chromatography instrument coupled to an Agilent 5973 mass spectrometer and an Agilent ChemStation software (Agilent Technologies, Palo Alto, CA, USA). 7683 series injector and 6890 N Network GC system were used. Volatile oils were separated on a 30 m × 0.25 mm i.d. capillary column coated with 0.25 μm film 5% phenyl methyl siloxane.

A model of TBE–200 V type-J high speed countercurrent chromatographic apparatus was used for selective separation (Shanghai Tauto Biotechnique, Shanghai, China). Detailed parameters of this apparatus had been described in our previous work [23]. The apparatus was installed within a vessel that maintains column temperature by a Model SDC-6 constant-temperature controller (Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China). The solvents were pumped into the column with a model s-1007 constant-flow pump (Beijing Shengyitong Technique, Beijing, China). Continuous monitoring of the effluent was achieved with a model UVD-200 detector (Shanghai Jinda Biotechnology Co., Ltd., Shanghai, China), and SEPU3010 workstation (Hangzhou Puhui Technology, Hangzhou, China) was employed to record the chromatogram.

2.2. Reagents

β-Cyclodextrin (β-CD), methyl-β-cyclodextrin (Me-β-CD) and hydroxylpropyl-β-cyclodextrin (HP-β-CD) were purchased from Zibo Qianhui Fine Chemical & Co. Inc. (Shandong, China). The average substitution degree of ME-β-CD and HP-β-CD were around 6.5. Seven kinds of volatile oils were purchased from Jishui medicinal spices oil extraction plant (Jiangxi, China). All organic solvents used for selective separation were of analytical grade. Acetonitrile and methanol used for HPLC analysis were of chromatographic grade. Water used for HPLC study was redistilled.

2.3. Liquid–Liquid partition experiments

Liquid-liquid partition experiment was used to determine the partition coefficient of components in volatile oil. HPLC analysis would be more convenient than GC–MS because one phase of the biphasic solvent system was an aqueous phase. The separation column of GC–MS could be damaged by aqueous samples. The liquid–liquid partition experiment was conducted as follows: in a 10 mL tube with a glass stopper, 2 mL of *n*-hexane was added and 2 drops of volatile oil was dissolved. HPLC analysis of this solution gave the first chromatogram for the composition of volatile oil. Then 2 mL of water containing 0.10 mol L^{-1} cyclodextrin was added into the same tube and shaken it violently. After equilibration the aqueous phase was analyzed by HPLC and the second chromatogram was obtained. Compared with the first chromatogram, the second chromatogram could indicate how much and how many components had been partitioned into the aqueous phase. HPLC results gave us a preliminary estimation of the partition performance of chemical components of volatile oil in the biphasic solvent system.

2.4. Separation procedure

For the present study, a biphasic solvent system composed of *n*-hexane-0.10 mol L^{-1} cyclodextrin aqueous phase was selected. The solvent mixture was thoroughly equilibrated in a separation funnel at room temperature and the two phases were separated shortly before use. The multilayer-coiled column was first entirely filled with the lower aqueous phase as stationary phase. The upper organic mobile phase was then pumped into the tail end of the column inlet at a flow rate of 2.0 mL min^{-1}, while the apparatus was run at a revolution speed of 800 rpm. After hydrodynamic equilibrium was reached, as indicated by a clear mobile phase eluting at the head outlet, the sample solution (about 500 mg dissolved in 8 mL mixture solution of lower phase and upper phase (1:1, v/v) of the solvent system) was injected through the sample port. The effluent from the head end of the column was continuously monitored with a UV detector at 254 nm. Each peak fraction was manually collected according to the elution profile and determined by GC–MS. As for the fractions with no UV absorbance, a silica gel thin layer chromatography was used for analysis with concentrated sulfuric acid spray as indicator. After the separation was completed, retention of the stationary phase was measured by collecting the