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Modeling and optimization of two phase system for recycling high-speed counter-current chromatographic separation of ketoconazole enantiomers

Panliang Zhang [a], Na Xie [b], Kewen Tang [a,*], Xiaoming Chen [b], Weifeng Xu [a,*]

[a] *Department of Chemistry and Chemical Engineering, Hunan Institute of Science and Technology, Yueyang, China*
[b] *College of Chemistry, Xiangtan University, Xiangtan, China*

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ABSTRACT

An efficient method using recycling high speed counter-current chromatography (HSCCC) with hydroxy propyl-β-cyclodextrin (HP-β-CD) as chiral selector (CS) is developed for the separation of ketoconazole enantiomers. The separation process was performed with a two-phase solvent system composed of *n*-hexane/acetic acid isobutyl ester/0.1 mol L^{-1} phosphate buffer solution with pH = 8.5 containing 0.1 mol L^{-1} HP-β-CD (2:3:5, v/v/v). Important factors that influence the separation efficiency, including organic solvent system, temperature, the pH value of the aqueous phase as well as type and concentration of chiral selector were investigated experimentally. Influence of the pH value and concentration of chiral selector was modeled and the conditions were further optimized by the model. Pure enantiomers with a purity >99% were obtained by separating 20 mg racemate via HSCCC. The recovery rates ranged from 85% to 89%.

1. Introduction

Nowadays, there is a great demand for enantiopure drugs. As it is well known that although enantiomers share the same chemical properties, they may manifest quite different biological activity and bring about unwanted side effects [1]. To obtain enantiopure compounds, some impressive separation methods such as liquid membrane [2], chromatographic techniques [3,4], crystallization techniques [5], chiral extractions [6], have been proposed. But these above methods have some limitations. The stability and lifetime of liquid membrane is not easy to control. The high cost and limited large-scale application is the main weakness in conventional chromatographic techniques. Long time consumption and low versatility is the main disadvantages of crystallization techniques. Chiral extraction is a promising method while the single-sage extraction can hardly give satisfactory separation efficiency and the multistage extraction is usually required. High-speed counter-current chromatography (HSCCC) is a liquid–liquid partitioning chromatography and unlike the conventional chromatographic techniques, it has no solid supporter which permits repetitive use of the same column for a variety of chiral separation [7–10]. As is well known, it is difficult to completely isolate enantiomers in single HSCCC operation mode when the separation factor for enantiomers is relatively low because the number of plate in HSCCC is limited. Recently, work concerning recycling mode of HSCCC has been reported [8,10]. Application of recycling elution mode can considerably improve the number of plate, which makes enantiomers with quite low separation factor be resolved entirely. Therefore, recycling elution mode of HSCCC has good prospect in chiral separation. Another main point in HSCCC separation of enantiomers key problem is to find a suitable chiral selector which is highly selective in the two-phase solvent system and possesses other essential properties [11–13]. So far, several chiral selectors have been successfully applied in the chiral separation by CCC [14–17], among which β-cyclodextrin derivatives [18–20] attract much attention because they are high-performance, economical and environmentally friendly. In our previous work, enantioselective extraction with a variety of β-cyclodextrins as CS was investigated [21], which is the basis of two-phase solvent system selection and optimization.

Ketoconazole, [cis-l-acetyl-4[4-[2-(2,4-dichlorophenyl)-2-(1H-imidazole-l-ylmethyl)-1,3-dioxolan-4-yl]methoxyphenyl] piper-

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Abbreviations: HSCCC, high-speed counter-current chromatography; CS, chiral selector; HP-β-CD, hydroxypropyl-β-cyclodextrin; He-β-CD, hydroxyethyl-β-cyclo dextrin; Me-β-CD, methyl-β-cyclodextrin; SBE-β-CD, sulfobutyl ether-β-cyclodextrin.

* Corresponding authors.

E-mail addresses: tangkewen@sina.com (K. Tang), lotus8870@126.com (W. Xu).

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azine, is an oral antifungal agent that has a broad spectrum activity (Fig. 1). Although it has two chiral centers, ketoconazole only exhibits a pairs of enantiomers, (+)-2R,4S-ketoconazole and (−)-2S,4R-ketoconazole [22,23]. This is because hydrogen atom and 2,4-dichlorophenyl in the two chiral centers must share one side as a result of space steric effect. It has been found that R-ketoconazole ((−)-2S,4R-ketoconazole) shows some times of pharmacological activity than that of S-ketoconazole ((+)-2R,4S-ketoconazole) [24,25]. Currently, ketoconazole is prescribed in its racemate form, which links to liver damage and other complications. Therefore, the chiral separation of ketoconazole enantiomers is not only essential but also of great significance.

2. Experimental

2.1. Apparatus

First of all, the TBE-300B HSCCC (Tauto Biotechnique, Shanghai, China) with three multilayer coil separation column connected in series was used. The TBE-300B preparative column consists of 1.6 mm ID PTFE tubing with a total capacity of 270 mL. The β value of the preparative columns ranged from 0.46 to 0.73, ($\beta = r/R$, $R = 6.5$ cm for preparative columns, where r is the distance from the coil to the holder shaft, and R, the revolution radius or the distance between the holder shaft and central axis of the centrifuge). In addition, the instruments listed below were also applied, including YKKY circulating water bath, a variable wavelength UV/Vis detector, HPLC (Waters1525, USA), TBP5002 type pressure cross-flow pump, PHS-29A pH meter and TE214S electronic balance.

2.2. Reagents

Hydroxypropyl-β-cyclodextrin (HP-β-CD), Sulfobutylether-β-cyclodextrin (SBE-β-CD), hydroxyethyl-β-clodextrin (He-β-CD) and methyl-β-cyclodextrin (Me-β-CD) were purchased from Shandong Binzhou Zhiyuan Bio-Technology Co., Ltd., Shandong, China. Ketoconazole racemate was obtained from Nanjing Enming Pharmaceutical Technology Co., Ltd., Nanjing, China. Solvents used such as *n*-hexane, dichloromethane, *n*-octanol, ethyl acetate, acetonitrile, were of analytical grade and from different suppliers.

2.3. Liquid–liquid extraction experiments

Determining the distribution ratio of ketoconazole is essential for selection of the two-phase solvent system previous to HSCCC study. Distribution ratios for ketoconazole enantiomers were calculated by the concentration of ketoconazole enantiomer in the organic phase divided by the concentration of ketoconazole enantiomer in aqueous phase. The quantitative distributions of enantiomers in the biphasic solvent system were determined by means of liquid–liquid extraction experiments under 5 °C. The organic phase was prepared by dissolving racemic ketoconazole and the aqueous solution was prepared by dissolving 0.1 mol L^{-1} phosphate buffer solution containing (SBE-, HE-, Me-, or HP-) β-cyclodextrin. The whole extraction process was performed in a 10 mL plastic centrifuge tube. Equal volumes (3 mL) of aqueous phase (containing hydrophilic CSs) and organic phase were placed into the tube and were shaken vigorously for at least 10 h before being kept in a water bath at a constant temperature for 5 h to reach extraction equilibrium. The distributions of enantiomers were analyzed by HPLC. The solvent systems that gave the suitable distribution ratio for (+)-enantiomer and (−)-enantiomer were selected to investigate the influence factors including equilibrium temperature, concentration of HP-β-CD and pH of buffer solution for the development of CCC separation.

2.4. Preparation of two-phase solvent system and sample solution

Solvent system consisting of *n*-hexane-acetic acid isobutyl ester-0.1 mol L^{-1} phosphate buffer solution with pH = 8.5 containing 0.1 mol L^{-1} HP-β-CD (2:3:5, v/v/v) was used for separating ketoconazole racemate by HSCCC. Before being put into use, the solvent mixture was vigorously shaken and thoroughly equilibrated in a separatory funnel then the two phases were degassed by ultrasound for 30 min separately. The sample solution was prepared by dissolving 20 mg ketoconazole racemate in 20 mL of organic phase (upper phase) for preparative separation.

2.5. HSCCC separation procedure

Conventional HSCCC: preparative separation was initiated by filling the column with the upper phase. The mobile phase was pumped into the column while the column was rotated at 850 rpm in a "head to tail" mode in "forward" direction. The sample solution was injected after reaching hydrodynamic equilibrium, as indicated by a clear mobile phase eluting at the tail outlet. Temperature of the whole HSCCC separation procedure was set at 5 °C. The absorbance of the effluent was continuously monitored at 220 nm.

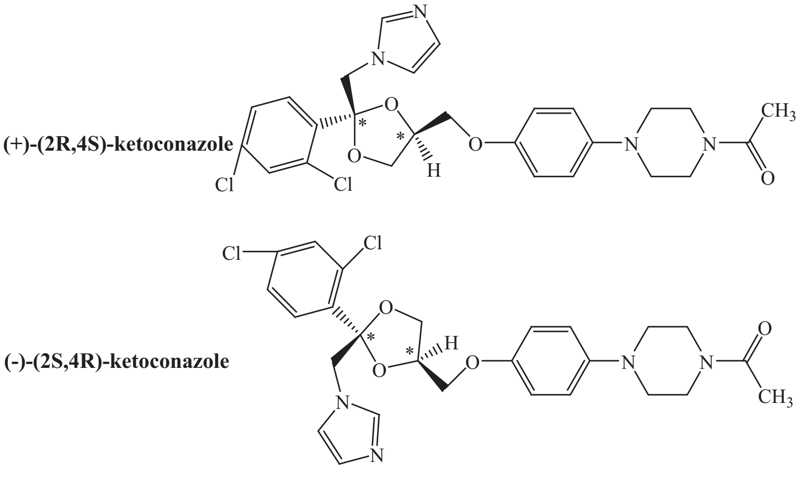

Fig. 1. Chemical structure of ketoconazole.