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Online capillary solid-phase microextraction coupled liquid chromatography-mass spectrometry for analysis of chiral secondary alcohol products in yeast catalyzed stereoselective reduction cell culture

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ABSTRACT

An online solid-phase microextraction coupled liquid chromatography-electrospray ionization-ion trap mass spectrometry was developed for the analysis of trace *R*- and *S*-4-phenyl-2-butanol (*R*- and *S*-pbol) in salt rich cell culture of *Saccharomyces cerevisiae* catalyzed stereoselective reduction of 4-pheny-2-butanone (pbone). A Supel-Q PLOT capillary column was used for the extraction and deionized distilled water was used as the extraction mobile phase. The extraction flow rate and extraction time were at 0.1 mL min^{-1} and 0.95 min, respectively. The three target analytes, pbone, *R*-pbol, and *S*-4-pbol, were desorbed and eluted by the mobile phase of water/methanol/isopropanol (55/25/20, v/v/v) with a flow rate of 0.5 mL min^{-1} and analyzed by a chiral column. The mass spectrometric detection of the three target analytes was in positive ion mode with the signal $[M+Na]^+$. The matrix-matched external standard calibration curves with linear concentration range between 0 and 50 μg mL^{-1} were used for quantitative analysis. The linear regression correlation coefficients (r^2) of the standard calibration curves were between 0.9950 and 0.9961. The yeast mediated reduction was performed with a recation culture of yeast incubation culture/glycerol (70/30, v/v) for 4 days. This biotransformation possessed 82.3% yield and 92.9% *S*-enantomeric excess. The limit of detection (LOD)/limit of quantification (LOQ) for pbone, *R*-pbol, and *S*-pbol was 0.02/0.067, 0.01/0.033, and 0.01/0.033 μg mL^{-1}, respectively. The intra-day and inter-day precisions from repeated measurements were 10.8–21.1% and 11.6–18.7%, respectively. The analysis accuracy from spike recovery was 84–91%.

1. Introduction

The asymmetric synthesis has been important strategy in pharmaceutical industry for chiral drugs development [1,2]. The utilization of microbes in asymmetric synthesis is interested by chemists for reasons: (1) the reaction is environmentally friendly; (2) the reaction conditions are mild; and (3) the reaction is substrate specificity and product selectivity. Among the various microbes, the baker yeast *Saccharomyces cerevisiae* has been widely used for various prochiral ketones reduction in the beginning of 20th century [2,3]. For instances, the catalysis of ketones was performed by adding yeast powder to pure glycerol medium with a high yield and a high enantiomeric excess (e.e.) value [4–6], the yeast mediated bio-reduction was proceeded by using hexane/water biphasic cell culture to improve the enantioselectivity and yield [7].

For chiral drug synthesis, FDA requires that the enantiomer with unwanted side effects to human body even though it is in very small amount must be eliminated in order to be commercialized without causing harmful effects to people. For instances, the *S*-form of sedative thalidomide causes infants with phocomelia [8] and the *S*,*S*-form of antimycobacterial drug ethambutol prescribed to treat tuberculosis can cause problems with vision [9] so that they must be separated from their counter enantiomers. From this point of view, the study of the enantioselective yeast mediated production of chiral compounds in complicated medium, efficient methods including sample pretreatment for chiral products separation and sensitive detection of trace chiral products are necessary and important.

It is usually a difficult task for analytical chemists to separate chiral compounds [10]. However, the development of a variety of

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chiral selectors not only made a breakthrough in chiral compound separation but also let the chiral compound analysis a routine work. In recent years, the separation and analysis of chiral compounds using liquid chromatography (LC) [11–13], gas chromatography (GC) [14–16], and capillary electrophoresis (CE) [11,17] or micellar electrokinetic chromatography (MEKC) [18] have been successively reported to explore many new chiral compounds. In these studies, LC was the first to apply for the chiral compound analysis that leads a rapid and more extensive development of chiral stationary phases (CSPs) than other chromatographies [10,19].

The solid-phase microextraction (SPME) developed by Arthur and Pawliszyn [20] based on the solid-phase extraction (SPE) has been run by off-line process with two major extraction modes in early days [21–24]. An automatically sampling SPME was then developed and online combined with high-performance liquid chromatography (HPLC) using a GC capillary column as the extraction device that a maximal analytes can be absorbed by repeated draw/ejection operation and directly desorbed by the desorption mobile phase [25]. Nowadays, the SPME technique has been extensively applied to various samples such as environmental waters [25–31], wine samples [22,23], nuts and grains [32], tea products and crude drugs [33], human urine [34–38], human saliva [38,39], liquid medicines and intravenous injection solutions [40], and human hair [35]. Recently, in order to increase the detection sensitivity for ultra-trace sample analysis, the SPME coupled liquid chromatography has switched its detector from spectrometers such as ultraviolet (UV) detector [30,37,40–42] and fluorescence (FL) detector [33] to high sensitivity mass spectrometer (MS) [26,29,32,34–36,38,39,43–45] and tandem mass spectrometer (MS/MS) [25,27,28,31,46,47].

This paper focuses on the development of online SPME coupled liquid chromatography-electrospray ionization-ion trap mass spectrometric method for performing efficient chiral separation and analysis [7,48–52] of trace secondary alcohol products *R*- and *S*-4-phenyl-2-butanol in a salt rich cell culture which was associated with the baker yeast *S. cerevisiae* catalyzed stereoselective biotransformation of the model compound 4-phenyl-2-butanone. In this method manual one-time fixed amount sample injection and online dual column switching technique were used to reach the goals of salt interference reduction, accurate, precise, and sensitive analysis.

2. Experimental

2.1. Materials and chemicals

S. cerevisiae CCRC 21443 was purchased from Culture Collection Research Center of Food Industry Research and Development Institute (Hsinchu, Taiwan, ROC). Reagent grade KH_2PO_4, K_2HPO_4, $(NH_4)_2SO_4$, $MgSO_4{\cdot}7H_2O$, $CaCl_2{\cdot}2H_2O$, $FeSO_4{\cdot}7H_2O$, $ZnSO_4{\cdot}7H_2O$, D-glucose (D-$C_6H_{12}O_6$), and methanol (CH_3OH, MeOH) of LC grade were all bought from Merck (Darmstadt, Germany). Reagent grade NaOH, H_2SO_4, and glycerol ($C_3H_5(OH)_3$) were obtained from Showa (Tokyo, Japan). Isopropanol ($(CH_3)_2CHOH$, IPA) was supplied by Tedia (Fairfield, Ohio, USA). 4-Phenyl-2-butanone ($C_{10}H_{12}O$, 98%, pbone), *R*-4-phenyl-2-butanol ($C_{10}H_{14}O$, >99%, *R*-pbol), *S*-4-phenyl-2-butanol ($C_{10}H_{14}O$, >99%, *S*-pbol) were all purchased from Alfa Aesar (Karlsruhe, Germany). Deionized distilled water (18.2 MΩ) used for cell incubation, stereoselective reduction, standard calibration curve preparation, and mobile phase of HPLC and liquid chromatography-mass spectrometer (LC-MS) was purified from double distilled water through EASY pure® II RF/UV ultrapure water system (Barnstead International, Dubuque, IA, USA).

2.2. Instrumentation of online SPME-LC-MS and operating conditions

The liquid chromatographic system including three solvent delivery pumps: Jasco PU-2085 dual piston solvent delivery pump (Tokyo, Japan), Jasco PU-2080 dual piston solvent delivery pump (Tokyo, Japan), and Waters 515 dual piston solvent delivery pump (Milford, MA, USA); a sample injection valve (Rheodyne 7725i, Cotati, Ca, USA) with a 20 μL sampling loop, a column oven (Colbox, Kaoshiong, Taiwan, ROC), an chiral analytical column (Phenomenex Lux 3u cellulose-1 chiral OD, 150 mm × 4.6 mm i.d., 10 μm), a guard column (5 mm × 4.6 mm i.d., 10 μm) (Phenomenex, Torrance, CA, USA), and a variable wavelength ultraviolet detector (Jasco, 2075-plus, Tokyo, Japan) set at 221 nm. The mass spectrometer was an Esquire 2000 ion trap mass spectrometer (Bruker Daltonics, Billerica, MA, USA) equipped with an electrospray ionization (ESI) interface characterized as a soft ionization technique. The detection of pbone by the ion trap mass spectrometer was in positive ion mode and the operation conditions were the applied voltage at the entrance of the capillary column: 4000 V, the nebulizer gas (N_2) pressure: 10 psi, the drying gas (N_2) flow rate: 6 L min^{-1}, the drying gas temperature: 300 °C, the mass scan range of the ion trap: *m/z* 50–300, and the volume split ratio: 1:9 (eluate/waste, v/v). The detection of *R*- and *S*-pbol by the ion trap mass spectrometer was also in positive ion mode and the operation conditions were the applied voltage at the entrance of the capillary column: 4000 V, the nebulizer gas (N_2) pressure: 8 psi, the drying gas (N_2) flow rate: 3 L min^{-1}, the drying gas temperature: 300 °C, the mass scan range of the ion trap: *m/z* 50–300, and the volume split ratio: 1:9 (eluate/waste, v/v). Other parameters were adjusted automatically to the optimal value using the software smart mode.

2.3. Solid-phase microextraction system and operating procedure

The SPME system shown in Fig. 1 consisted of a Supel-Q PLOT capillary column (divinylbenzene, 60 cm × 0.32 mm i.d., 12 μm film thickness; Supelco, Bellefonte, PA, USA) and two six-port column switching valves (Rheodyne 7000, Cotati, CA, USA) to function the adsorption, desorption, separation, salt elimination, and analysis of analytes. The connection of microextraction Supel-Q PLOT capillary column to the switching valve was facilitated by the use of a 2.5 cm polyetheretherketone (PEEK) tubing sleeve (0.025″ o.d. and 0.018″ i.d.), PEEK MicroFingertight I fittings (0.002–0.003″ i.d.), and PEEK Microtight adapter (suitable for 1/16–1/32″ fitting) at both ends of the capillary column. The adaptor at both ends of the capillary column was then connected to the 1/8″ stainless tubing of the switching valve through one-piece PEEK Fingertight fitting to complete the connections.

The extraction and desorption of analytes of the SPME system were operated at room temperature without controlling the capillary column temperature. In Fig. 1A, the sample was loaded to the 20 μL sampling loop with a microsyringe. The loaded sample (20 μL) was then injected by turning the sample injection valve to the injection position as shown in Fig. 1B and the loaded sample was subsequently eluted by the extraction mobile phase (deionized distilled water) at a flow rate of 0.1 mL min^{-1} to the extraction capillary column. At this moment, the two switching valves were kept at the extraction configuration as shown in both Fig. 1A and B. After extraction for 0.95 min, the switching valve 1 was switched to the desorption configuration as shown in Fig. 1C to desorb, separate, and detect analytes with desorption (viz. separation) mobile phase (H_2O/MeOH/IPA, 55/25/20, v/v/v) for 38 min. The desorbed analytes were directly delivered to the analytical column for separation using desorption mobile phase where the column temperature was kept at 30 °C. During the separation, the first 18 min salt containing eluate was delivered to the waste collector to reduce salt