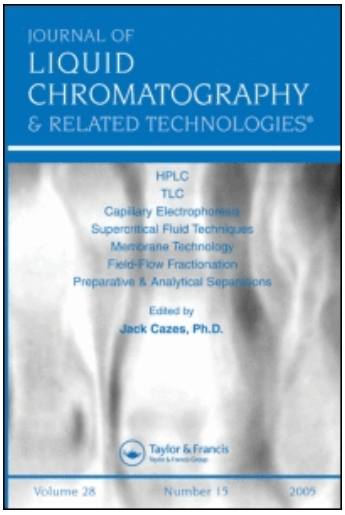
This article was downloaded by:
On: *23 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Column Switching Technique, Assisted On-Line Matrix Elimination, and Chiral Analysis of *R*- and *S*-1-Phenyl-1-butanol in Biphasic *Saccharomyces cerevisiae* Mediated Culture

Shiang-Rong Tsai[a]; Cheanyeh Cheng[a]
[a] Department of Chemistry, Chung Yuan Christian University, Chungli, Taiwan, R.O.C.

Online publication date: 27 October 2003

To cite this Article Tsai, Shiang-Rong and Cheng, Cheanyeh(2003) 'Column Switching Technique, Assisted On-Line Matrix Elimination, and Chiral Analysis of *R*- and *S*-1-Phenyl-1-butanol in Biphasic *Saccharomyces cerevisiae* Mediated Culture', Journal of Liquid Chromatography & Related Technologies, 26: 19, 3249 — 3264
To link to this Article: DOI: 10.1081/JLC-120025521
URL: http://dx.doi.org/10.1081/JLC-120025521

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 19, pp. 3249–3264, 2003

Column Switching Technique, Assisted On-Line Matrix Elimination, and Chiral Analysis of *R*- and *S*-1-Phenyl-1-butanol in Biphasic *Saccharomyces cerevisiae* Mediated Culture

Shiang-Rong Tsai and Cheanyeh Cheng*

Department of Chemistry, Chung Yuan Christian University, Chungli, Taiwan, R.O.C.

Downloaded At: 19:44 23 January 2011

ABSTRACT

The matrix interference subjected by *R*- and *S*-1-phenyl-1-butanol in the biphasic *Saccharomyces cerevisiae* CCRC 21443 mediated culture cannot be eliminated by chiral high-performance liquid chromatography (HPLC) with a single column of either Chiralcel OB or Chiralpak AD. The use of a single column also results in a long analysis time. The application of the column switching technique can hyphenate the Chiralcel OB column and the Chiralpak AD column together, to resolve not only the matrix interference, but a reduction of the analysis time for these chiral products in a complex medium. This coupled column system has an accuracy greater than 98.6% and a high precision of less than 1.6% relative

*Correspondence: Cheanyeh Cheng, Department of Chemistry, Chung Yuan Christian University, Chungli, Taiwan 320, R.O.C.

DOI: 10.1081/JLC-120025521
1082-6076 (Print); 1520-572X (Online)
Copyright © 2003 by Marcel Dekker, Inc.
www.dekker.com

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

standard deviation (RSD). The developed technique for on-line sample clean-up and analysis of phenyl-*n*-propyl ketone, *R*- and *S*-1-phenyl-1-butanol in the culture is successful.

Key Words: Column switching; Matrix elimination; Chiral; *Saccharomyces cerevisiae*.

INTRODUCTION

High-performance liquid chromatography (HPLC), gas chromatography (GC), and capillary electrochromatography (CEC) have been found useful for the separation of chiral molecules. In the interest of a direct separation for chiral molecules, the various designs of stationary phase for columns have made HPLC the most practical method of all. Today, the commercially available derivatized polysaccharide stationary phase columns, Chiralcel OB and Chiralpak AD, developed for HPLC, have led to many applications in the agrochemical, food, and drink industry and, especially, in the pharmaceutical industry.[1–4] However, the sample pretreatment for eliminating matrix interference or for enriching the concentration of analytes cannot be performed together with the separation and, sometimes, tedious procedures of the sample pretreatment should be followed in these applications.

The column switching technique used in coupled column liquid chromatography (LC) system is useful and powerful for the clean-up and separation of multicomponent mixture. The advantages of the column switching technique for LC system described in previous papers[5] are less sample handling, greater accuracy and precision, obvious time reduction, on-line sample analysis, possibility of full automation, and flexibility in combining different chromatographic systems. This technique used in either a GC system or a CEC system shows difficulty by their intrinsic design of instrumentation. Therefore, many chemists use the column switching technique with the LC system to analyze drugs and their metabolites in urine and blood plasma,[6,7] trace components in the environment,[8,9] trimethoprim in milk,[10] aromatic compounds in gasoline,[11] and amino acids in enzymatic biotransformation.[12] Most importantly, the joining of the column switching HPLC system and a tandem mass spectrometry (MS/MS) has been used to analyze the analytes of interest qualitatively and quantitatively.[13,14]

The target chiral compounds *R*- and *S*-1-phenyl-1-butanol are the metabolites of *Saccharomyces cerevisiae* produced from the reduction of prochiral phenyl-*n*-propyl ketone. This bioconversion was performed under a biphasic culture that is due to the water insoluble property of the reaction substrate phenyl-*n*-propyl ketone. However, the complication of the yeast mediated reduction culture makes the qualitative separation of the chiral products *R*- and

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

S-1-phenyl-1-butanol difficult with either the Chiralcel OB or Chiralpak AD column. The difficulty usually comes from the matrix interference produced by the unreacted reactant phenyl-*n*-propyl ketone and other unknown peaks in the culture. Similarly, this kind of interference is also observed in the bioreduction of phenyl-*n*-propyl ketone with *Candida utilis* and *Pseudomonas dacunhae* in our laboratory. Therefore, the successful application of this kind of biotransformation depends strongly upon an efficient analytical technique to separate and analyze the corresponding chiral products in the complex medium. Thus, a coupled dual-analytical column system was developed to analyze the yeast mediated biphasic bioreduction with the assistance of a column switching technique.

EXPERIMENTAL

Materials and Chemicals

D-(+)-glucose, KH_2PO_4, K_2HPO_4, $(NH_4)_2SO_4$, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, and $ZnSO_4 \cdot 7H_2O$ of reagent grade, *n*-hexane of LC grade, extract of yeast powder, malt extract for microbiology, and peptone from digested casin trypsin were all bought from Merck (Darmstadt, Germany). Reagent grade *R*-(+)-1-phenyl-1-butanol and *S*-(−)-1-phenyl-1-butanol were purchased from Aldrich Chemical (Milwaukee, WI). Phenyl *n*-propyl ketone was obtained from Tokyo Chemical Industry Co. (Tokyo, Japan). Yeast extract and agar for culture media were supplied by DIFCO (Detroit, MI). Isopropyl alcohol of LC grade was from Tedia (Fairfield, OH). High purity (98%) β-NADH was bought from Sigma (St. Louis, MO). Reagent grade ethanol was from Fisher Scientific (Fair Lawn, NJ). $FeSO_4$ of reagent grade was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Anhydrous diethyl ether was supplied by J. T. Baker (Phillipsburg, NJ). Freeze dried *S. cerevisiae* CCRC 21443 was bought from the Culture Collection Research Center of Food Industry Research and Development Institute (Hsinchu, Taiwan). Water used for the culture media was purified from tap water by deionization and distillation once.

HPLC Instrumentation

The HPLC system consisted of two PU-1580 dual-piston intelligent pumps (JASCO, Tokyo, Japan), a Rheodyne model 7125 injection valve (Cotati, CA) fitted with a 20-μL sampling loop, a Rheodyne model 7000 six-position switching valve (Cotati, CA), a CTO 6A column oven (Shimadzu, Kyoto, Japan), a SPD 10A variable wavelength UV detector (Shimadzu,

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Kyoto, Japan), and a chromatography data processing software developed by Scientific Information System Co. (Taipei, R.O.C.). The analytical Chiralcel OB and Chiralpak AD columns (Daicel Chemical Industries, Ltd., Tokyo, Japan), packed with cellulose tribenzoate and amylose tri-(3,5-dimethylphenyl carbamate) coated on silica gel, respectively, were both 25×0.46 cm i.d.

Cell Growth

Freeze dried *S. cerevisiae* was dissolved in a 0.3–0.5 mL of a sterilized medium containing 3.0 g yeast extract, 3.0 g malt extract, 5.0 g peptone, 10.0 g *D*-(+)-glucose, and 1.0 L deionized and distilled water. Then the cell suspension was injected onto agar slants prepared by adding 2% (w/v) agar into the above incubation medium. The incubation of cells on the agar slant was at 30°C. Yeast grown on agar slants was injected into 100 mL of the following synthetic growth medium for mass growing: 1.5 g KH_2PO_4, 2.9 g K_2HPO_4, 1.3 g $(NH_4)_2SO_4$, 1.8 g $MgSO_4 \cdot 7H_2O$, 0.0175 g $CaCl_2 \cdot 2H_2O$, 0.1 mg $ZnSO_4 \cdot 7H_2O$, 0.1 mL $FeSO_4$ (1.25%, w/v), 6.0 g *D*-(+)-glucose, and 1.0 L deionized and distilled water. The inoculated synthetic growth medium was incubated at 30°C in an orbital shaking incubator for 7 days at 150 rpm. This 100 mL yeast grown cell culture was subsequently used for the reduction reaction.

Bioreduction of Phenyl-*n*-propyl Ketone

Shaking flask type reduction of phenyl-*n*-propyl ketone with yeast was performed in a biphasic medium of *n*-hexane and water with a 10/90 volume ratio. An 11 mL *n*-hexane was added directly into the 100 mL yeast grown cell culture. Immediately, a 0.5 mL aliquot of the solution mixture that was made up with 0.0565 g phenyl-*n*-propyl ketone, 0.0165 g β-NADH, and 5.0 mL absolute ethanol was added into this biphasic *n*-hexane-aqueous culture. The cell culture was put into an orbital shaking incubator and shaken at a constant temperature of 30°C and a rate of 150 rpm for a period of 7 days.

Except for the shaking flask type reduction, a scale-up experiment was performed for the reaction in a bench type 2L fermentor. The 100 mL inoculated synthetic growth medium was incubated at 30°C in the orbital shaking incubator for 2 days at a 150 rpm shaking rate. This yeast grown medium was subsequently transferred into the 2-L capacity stirred tank fermentor (Rikakai, Model M-100, Tokyo, Japan) containing the same 0.9 L synthetic medium for further growth and reaction. The culture was agitated at a stirring rate of 150 rpm and air was bubbled through the system at a flow rate of 0.5 L/min (Rikakai Model FM-110, Tokyo, Japan). The fermentor tem-

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

perature was controlled at 30°C and the pH of the culture was controlled at 5.0 with a pH controller (Rikakial, Model MOB-2, Tokyo, Japan). The cell mass reached a maximum within about 2 days. Then, 100 mL *n*-hexane was added into the cell culture to make the volume percentage of *n*-hexane approximately 10%. Instantaneously, 5.0 mL solution mixture that was made up with 0.0565 g phenyl-*n*-propyl ketone, 0.0165 g β-NADH, and 5.0 mL absolute ethanol was directly added into the above biphasic culture. The batch type reaction was performed for a period of 7 days and without air bubbling through the system during the reaction period.

Sample Pretreatment

After the reaction was stopped, the *n*-hexane layer of the biphasic culture was drawn and dehydrated with anhydrous $MgSO_4$. The aqueous layer of the biphasic culture was extracted with anhydrous ethyl ether. The ethyl ether extract was dehydrated with anhydrous $MgSO_4$ and combined with the *n*-hexane portion. The overall organic portion of the culture was concentrated with a rotary vacuum evaporator (Eyela N-1N, Tokyo, Japan) to about 1 mL. Then, more *n*-hexane was added to make a total volume of 10 mL. Subsequently, the final 10 mL organic portion can be analyzed by a chiral HPLC system.

HPLC Analysis with Column Switching Technique

The qualitative identification of phenyl-*n*-propyl ketone, *R*- and *S*-1-phenyl-1-butanol in the cell culture was proceeded by a standard addition method with corresponding standards and a Chiralcel OB column. The UV detection was at a wavelength of 221 nm, at which the analytes have the largest UV absorbance. The commercially suggested mobile phase has a composition of *n*-hexane/isopropanol = 9/1 (v/v) and runs at an elution rate of 0.5 mL/min. However, the elution conditions could not provide a successful elimination of matrix interference for the sample. Therefore, the composition of the mobile phase was varied to try to eliminate the matrix interference and to avoid the overlap of analyte peaks. Moreover, we found that the addition of a small amount of glacial acetic acid to the binary mobile phase can help greatly in eliminating the matrix effect. During the analysis, the column was maintained at ambient temperature. Then, the cell culture sample was also analyzed by a Chiralpak AD column with a binary composition of *n*-hexane and isopropanol. However, the sample was so complicated that the matrix interference affected analyzes greatly and cannot be eliminated by changing the composition of mobile phase.

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

The use of the column switching technique to assist the hyphenation of the Chiralcel OB column and the Chiralpak AD column was the solution to resolve these problems. The coordination of the two chiral analytical columns cannot only separate the analytes well, but also reduces the analysis time. The dual-analytical column coupled HPLC system, with a switching valve, is shown in Fig. 1, which contains two pumps, an injection valve, a Chiralpak AD column, a Chiralcel OB column, a UV detector, and one column switching valve. The column switching valve is used to connect the two solvent delivery pumps and the two columns for selecting the portion of sample of interest. The Chiralpak AD column plays a role as the pre-separation column for crude separation of the sample. The role of the Chiralcel OB column is to serve as the analytical column for final analysis. With this assembly, the selection of the specific part of the sample containing the phenyl-*n*-propyl ketone and *R*- and *S*-1-phenyl-1-butanol through the Chiralpak AD column can be accomplished by rotating the switching valve. The selected portion of the sample can then be directed to the Chiralcel OB column.

Thus, the composition of the two mobile phases going through the two columns, respectively, has been optimized to obtain a best separation. Standard solutions containing 50 ppm each of the phenyl-*n*-propyl ketone, *R*-1-phenyl-1-butanol, and *S*-1-phenyl-1-butanol were prepared to test the precision and accuracy of the column switching assisted dual-analytical column coupling

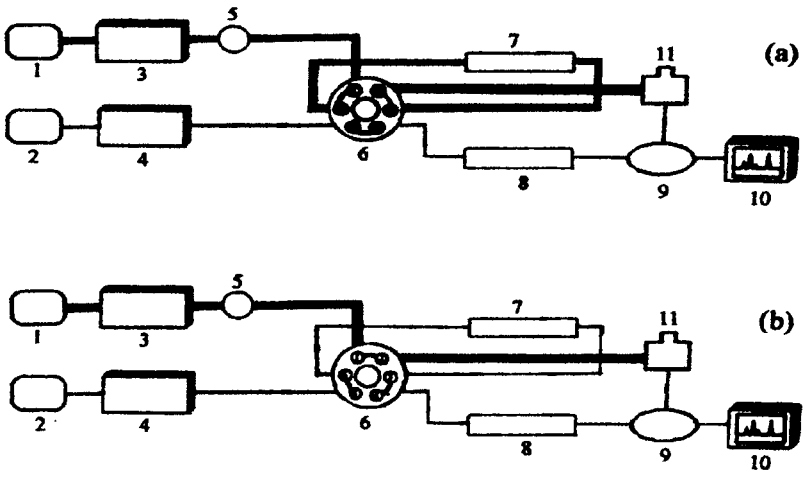


Figure 1. Schematic diagram of the column switching HPLC system. 1, container of mobile phase for Chiralpak AD column; 2, container of mobile phase for Chiralcel OB column; 3, pump for Chiralpak AD column; 4, pump for Chiralcel OB column; 5, injection valve; 6, switching valve; 7, Chiralpak AD column; 8, Chiralcel OB column; 9, UV detector; 10, computer (data station); 11, waste container.

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

system by the standard addition method. The reaction cultures were also analyzed qualitatively and quantitatively by this column coupling system with the optimized conditions and the standard addition procedure. Five replicate measurements were made for each sample during the assay.

RESULTS AND DISCUSSION

Chiral Separation of *R*- and *S*-1-Phenyl-1-butanol by Chiralcel OB

The commercially suggested mobile phase, Chiralcel OB column, for the separation of secondary alcohol was, in general, a composition of *n*-hexane/isopropanol [90/10 (v/v)]. The chromatogram in Fig. 2 was obtained for the reaction medium in a shaking flask type reduction, with a mobile phase *n*-hexane/isopropanol = 90/10 (v/v) that shows a partial overlap of the peaks

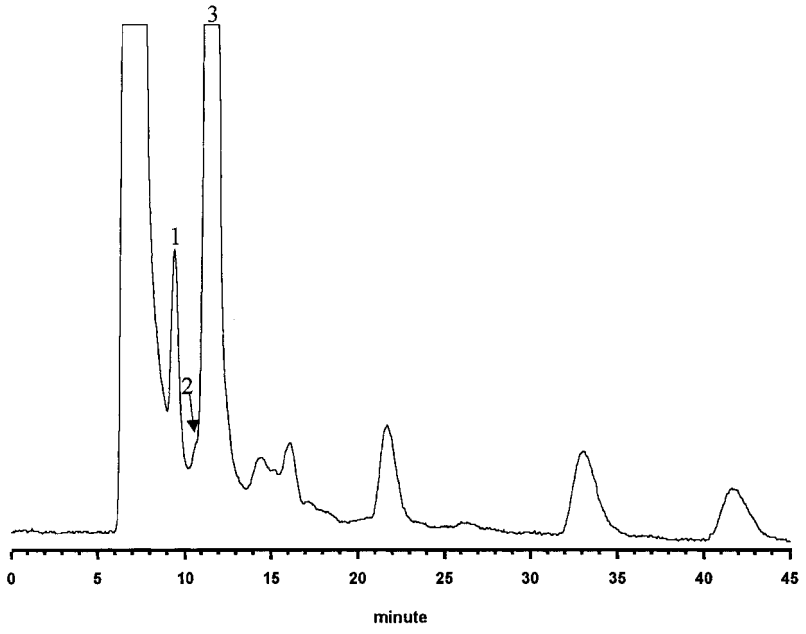


Figure 2. Chromatogram of the biphasic *S. cerevisiae* mediated shaking flask type reduction culture. Mobile phase: *n*-hexane/isopropanol = 90/10 (v/v); column: Chiralcel OB; column temp.: ambient; elution rate: 0.5 mL/min; UV detection: 221 nm. Peak 1, *S*-1-phenyl-1-butanol; Peak 2, *R*-1-phenyl-1-butanol; Peak 3, phenyl *n*-propyl ketone.

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

between phenyl-*n*-propyl ketone and *R*-1-phenyl-1-butanol and also a partial overlap of the peaks between *S*-1-phenyl-1-butanol and the solvent front. This is due to the large proportion of the polar isopropanol in the mobile phase that elutes the peaks of *R*- and *S*-1-phenyl-1-butanol quickly out of the column. The interaction of isopropanol with both *R*- and *S*-1-phenyl-1-butanol is through hydrogen bonding; thus, it is stronger than its interaction with phenyl-*n*-propyl ketone. Therefore, reducing the volume percentage of isopropanol in the binary mobile phase will shift the *R*- and *S*-1-phenyl-1-butanol to much longer retention times than the phenyl-*n*-propyl ketone and could provide a method to improve the separation among the three analytes. The chromatogram of Fig. 2 elucidates that the ideal appearance for the peaks of *R*- and *S*-1-phenyl-1-butanol should be located around a retention time of 25–30 min. When the composition of the mobile phase is finally changed to the volume percentage of *n*-hexane/isopropanol = 99.3/0.7 (v/v), an ideal separation can be obtained for *R*- and *S*-1-phenyl-1-butanol without matrix interference. However, the addition of 0.1% glacial acetic acid equal in volume to the binary mobile phase of *n*-hexane/isopropanol = 99.3/0.7 (v/v), to make a ternary mobile phase of *n*-hexane/isopropanol/acetic acid = 99.2/0.7/0.1 (v/v/v), can reduce the elution time and decrease peak tailing.

As the ternary mobile phase was used to analyze the culture from a 2-L bench scale *S. cerevisiae* mediated bioreduction, the chromatogram in Fig. 3 shows a partial overlap among *R*- and *S*-1-phenyl-1-butanol and an unknown peak. However, another unknown peak appeared at a retention time of about 180 min in the chromatogram. Because the Chiralcel OB column is a normal-phase column, we assumed the unknown peak should be a very polar substance. Nevertheless, the very long retention time of the unknown peak makes the analysis of the culture unfavorable. The reduction of the retention time for the unknown peak can be made by increasing the polarity of the mobile phase, that is by an increase of isopropanol. We, thus, tried changing the volume ratio of mobile phase for *n*-hexane to isopropanol as 99/1; however, the unknown peak was shifted a retention time of around 190 min. Continuously increasing the volume ratio of isopropanol in the mobile phase to 5% can decrease the retention time of the unknown peak to about 140 min; however, this will sacrifice the separation for phenyl-*n*-propyl ketone, *R*- and *S*-1-phenyl-1-butanol. Therefore, the use of a single Chiralcel OB column is not very satisfactory for the analysis of the reduction culture.

Chiral Separation of *R*- and *S*-1-Phenyl-1-butanol by Chiralpak AD

The Chiralpak AD column has been found to be useful for the analysis of yeast mediated reduction of prochiral ketone in our laboratory. In the analysis

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

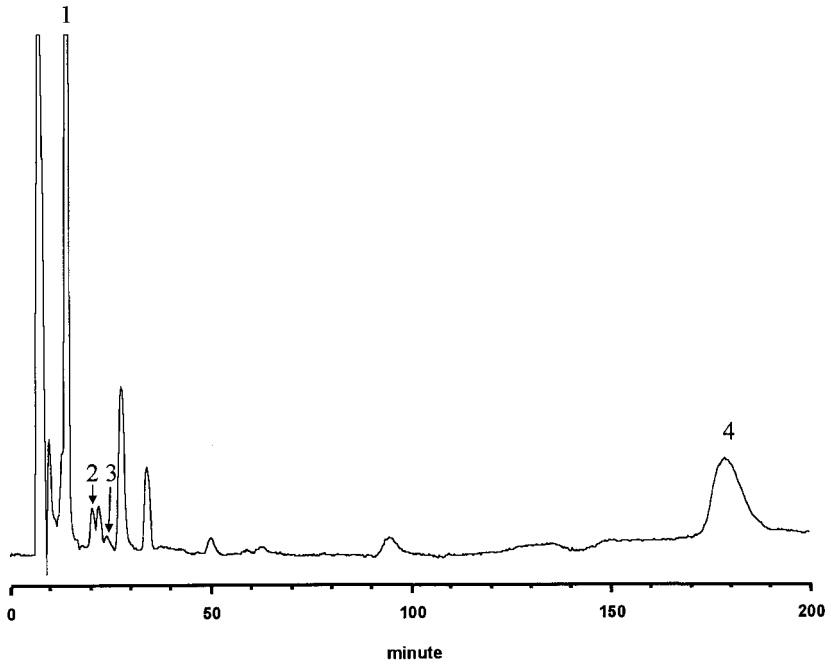


Figure 3. Chromatogram of the biphasic *S. cerevisiae* mediated 2-L bench scale reduction culture. Mobile phase: *n*-hexane/isopropanol/acetic acid = 99.2/0.7/0.1 (v/v/v); column: Chiralcel OB; column temp.: ambient; elution rate: 0.5 mL/min; UV detection: 221 nm; Peak 1, phenyl *n*-propyl ketone; Peak 2, *S*-1-phenyl-1-butanol; Peak 3, *R*-1-phenyl-1-butanol; Peak 4, unknown.

of the biphasic *S. cerevisiae* mediated 2-L bench scale reduction culture by Chiralpak AD, we first tried the mobile phase with composition *n*-hexane/isopropanol/acetic acid = 99.2/0.7/0.1 (v/v/v). We found partial peak overlapping for phenyl-*n*-propyl ketone, *R*- and *S*-1-phenyl-1-butanol, and there is also an unknown peak at the retention time of about 140 min, as indicated by chromatogram of Fig. 4(a). When the composition of mobile phase is changed to *n*-hexane/isopropanol = 99/1 (v/v), an unknown peak appears at a retention time of 200 min, as shown by chromatogram of Fig. 4(b). Increasing the percentage of isopropanol to 3% and 6% in volume of the binary mobile phase, can largely reduce the analysis time, however, the separation for *R*- and *S*-1-phenyl-1-butanol cannot be made, as shown by the chromatogram of Fig. 5.

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

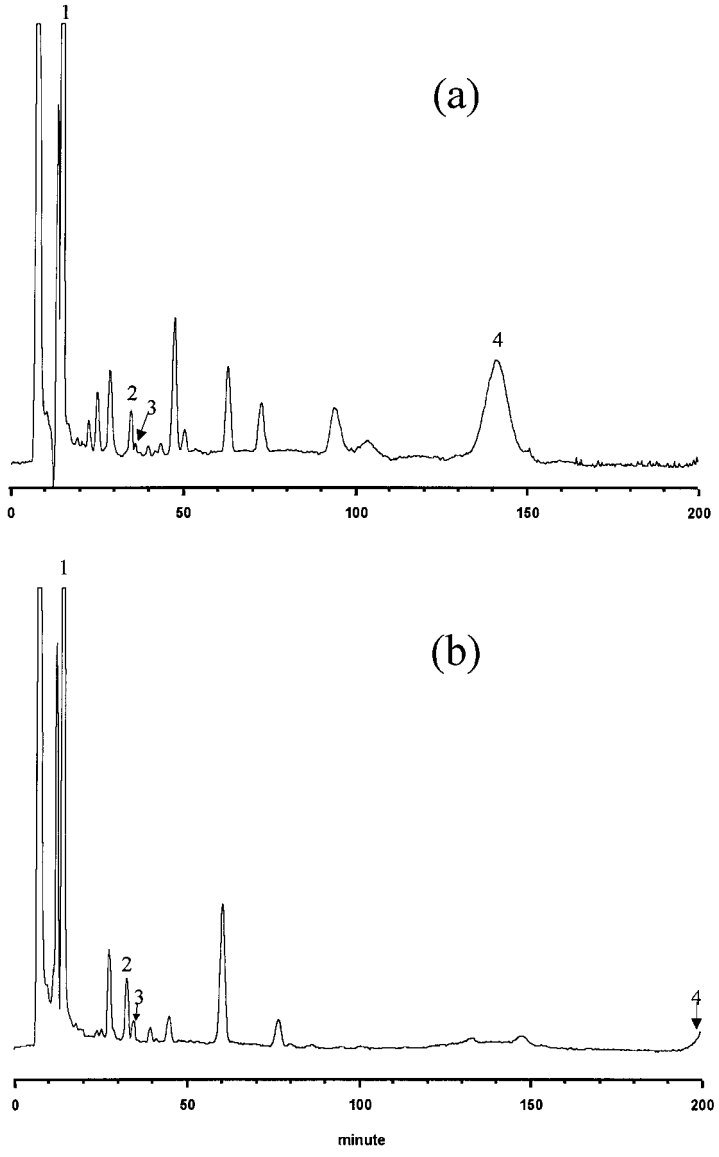


Figure 4. Chromatograms of the biphasic *S. cerevisiae* mediated 2-L bench scale reduction culture. Mobile phase: (a) *n*-hexane/isopropanol/acetic acid = 99.2/0.7/0.1 (v/v/v); (b) *n*-hexane/isopropanol = 99/1 (v/v); column: Chiralpak AD; column temp.: ambient; elution rate: 0.5 mL/min; UV detection: 221 nm; Peak 1, phenyl *n*-propyl ketone; Peak 2, *S*-1-phenyl-1-butanol; Peak 3, *R*-1-phenyl-1-butanol; Peak 4, unknown.

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

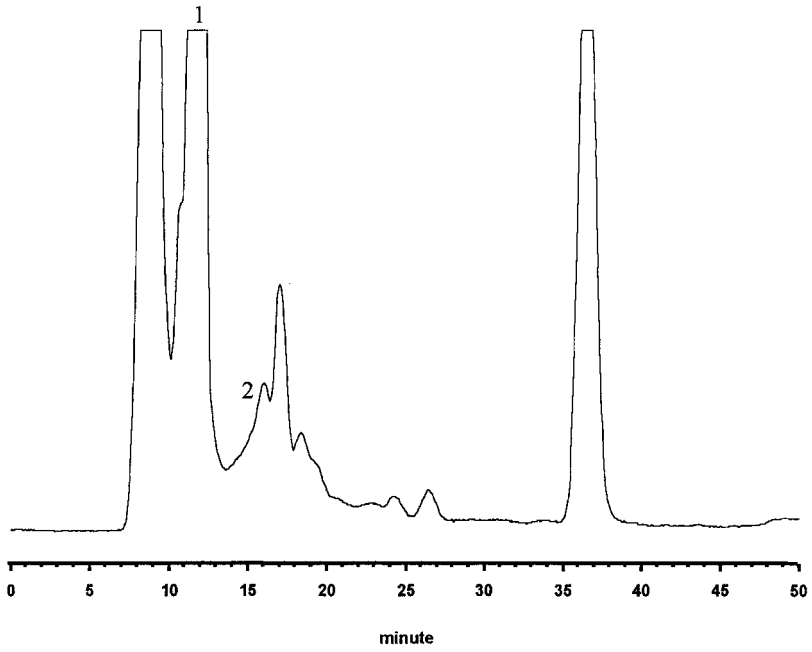


Figure 5. Chromatogram of the biphasic *S. cerevisiae* mediated 2-L bench scale reduction culture. Mobile phase: *n*-hexane/isopropanol = 94/6 (v/v); column: Chiralpak AD; column temp.: ambient; elution rate: 0.5 mL/min; UV detection: 221 nm; Peak 1, phenyl *n*-propyl ketone; Peak 2, *R*- and *S*-1-phenyl-1-butanol.

Separation by Column Switching Assisted Column Coupling System

The separation of *R*- and *S*-1-phenyl-1-butanol and phenyl-*n*-propyl ketone in the biphasic *S. cerevisiae* mediated reduction culture cannot be performed satisfactorily by using a single column HPLC system. However, a coupled column system, plus the column switching technique, could solve the problem. In the coupled column system, the Chiralpak AD column serves as the pretreatment column for a rough separation of the culture, and the Chiralcel OB column is the analytical column. In order to obtain the best separation, the composition of mobile phases for the two coupled columns were varied and investigated. At present, the optimum combination of the two mobile phases for Chiralpak AD column and Chiralcel OB column is *n*-hexane/isopropanol = 94/6 (v/v) and 95/5 (v/v), respectively. According to Fig. 5, the piece of chromatogram containing phenyl-*n*-propyl ketone,

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

R- and *S*-1-phenyl-1-butanol is around the 9 ~ 17 min time interval. This part of the chromatogram can be selected by the switching valve and sent to the Chiralcel OB column for final analysis. The part of the chromatogram around the 0 ~ 9 min time interval, is, thus, eluted to the waste container, as shown by the diagram (a) of Fig. 1. As the column switching valve is switched back at the time of 17 min, the flow rate of the mobile phase for the Chiralpak AD column is increased to 1.0 mL/min to quickly elute the rest of the components in order to avoid any contamination by the late eluting peaks from the next run. The part of chromatogram around 9 ~ 17 min in Fig. 5 was then switched to and separated by the Chiralcel OB column. The final chromatogram obtained by the column switching technique is shown by Fig. 6. The peak around 16.5 min is due to the merging of the two mobile phases to make a higher volume ratio of isopropanol in the mobile phase through the Chiralcel OB column, rather than its normal composition and, thus, results in an increase

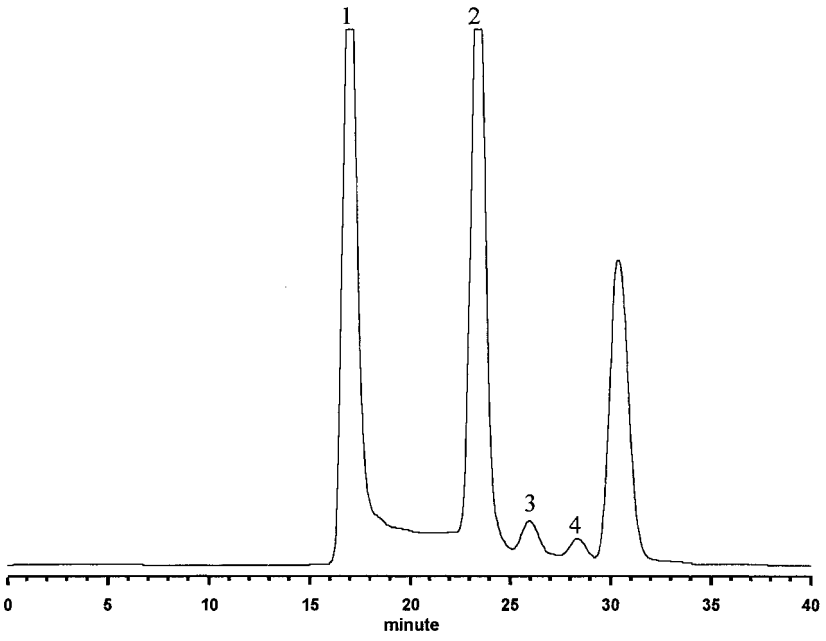


Figure 6. Chromatogram of the biphasic *S. cerevisiae* mediated 2-L bench scale reduction culture from the coupled column system. Mobile phase: *n*-hexane/isopropanol = 94/6 (v/v) (Chiralpak AD column); *n*-hexane/isopropanol = 95/5 (v/v) (Chiralcel OB column); column temp.: ambient; elution rate: 0.5 mL/min; UV detection: 221 nm; Peak 1, the solvent peak; Peak 2, phenyl *n*-propyl ketone; Peak 3, *S*-1-phenyl-1-butanol; Peak 4, *R*-1-phenyl-1-butanol.

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

of the UV absorbance. By eliminating most of the matrixes from the original chromatogram in Fig. 6 with the column switching technique, the separation is efficient and successful for phenyl-*n*-propyl ketone, *R*- and *S*-1-phenyl-1-butanol in the culture, and the analysis time is obviously reduced to 33 min.

PRECISION, ACCURACY, AND QUANTITATION OF THE ANALYSIS BY THE COLUMN SWITCHING TECHNIQUE

Due to the excellent separation for phenyl-*n*-propyl ketone, *R*- and *S*-1-phenyl-1-butanol in the reaction culture by the column switching technique, we can test the precision and accuracy of this technique for the three substances, simultaneously, by the standard addition method. The results from the analysis of a standard solution containing 50 ppm of each analyte are shown in Table 1. The precision corresponding to the relative standard deviation (RSD) in Table 1 for phenyl-*n*-propyl ketone, *R*- and *S*-1-phenyl-1-butanol are 1.6%, 1.0%, and 0.2%, respectively. These low values of RSD indicate accurate precision can be obtained with the use of the technique. The recovery rate shown in Table 1 for phenyl-*n*-propyl ketone, *R*- and *S*-1-phenyl-1-butanol are 98.6%, 99.8%, and 98.8%, respectively. The recovery rates are all close to 100%, indicating that accurate results can also be made by this technique. The 98.6% recovery for phenyl-*n*-propyl ketone is probably influenced by the baseline shifting from the beginning solvent front peak. The amounts of the three analytes in the reaction culture, determined by the standard addition method with the column switching technique, are shown in Table 2 as about 1.09, 0.15, and 2.06 mg, respectively. Their RSD values are

Table 1. The precision and accuracy of the coupled column system.

	Component		
	Phenyl-*n*-propyl ketone	*R*-1-phenyl-1-butanol	*S*-1-phenyl-1-butanol
Original concentration (ppm)	50	50	50
Concentration determined (ppm)	49.3 ± 0.8	49.9 ± 0.5	49.4 ± 0.1
No. of measurement (*n*)	5	5	5
Recovery rate (%)	98.6	99.8	99.8
RSD (%)	1.6	1.0	0.2

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 2. The analytical result in the culture from a 2-L bench scale *S. cerevisiae* mediated bioreduction by the coupled column system.

Component	Total amount (mg)	RSD (%)
Phenyl *n*-propyl ketone	1.091 ± 0.015	1.4
R-1-phenyl-1-butanol	0.154 ± 0.002	1.3
S-1-phenyl-1-butanol	2.062 ± 0.019	0.9

all below 1.5% and are consistent with the results of a standard solution. Quantitative assay of the enantiomeric excess value (e.e., %) in excess of the *S*-enantiomer, is 86.1% and the yield for the total production of alcohol is about 19.5%. Both the e.e. value and the yield are much better than previous results that were obtained with a synthetic medium without zinc(II) ion in it. The Zn^{2+} ion plays a role in the cofactor for alcohol dehydrogenase in this kind of yeast mediated reduction,[15] and can help the substrate binding. The low production yield shows that the bioreduction of phenyl-*n*-propyl ketone with *S. cerevisiae* is probably not the major reaction.

CONCLUSION

The ternary mobile phase of *n*-hexane/isopropanol/acetic acid (99.2/0.7/0.1, v/v/v) has been used in the Chiralcel OB column to separate the chiral products, *R*- and *S*-1-phenyl-1-butanol, in a small scale shaking flask type reaction. However, the ternary mobile phase used for the analysis of the analytes in the biphasic culture from a 2-L bench scale *S. cerevisiae* mediated bioreduction is not satisfactory. In fact, the very long elution peak, around 180 min, makes the use of single Chiralcel OB column for the separation of *R*- and *S*-1-phenyl-1-butanol in the cell culture impractical by simply adjusting the composition of mobile phase. The same situation applies to the analysis of the cell culture with a single Chiralpak AD column. However, the use of the column switching technique to hyphen the Chiralpak AD column and the Chiralcel OB column can solve these problems. The optimum composition of the two mobile phases in the coupled column system is *n*-hexane/isopropanol = 94/6 (v/v) and 95/5 (v/v) for the Chiralpak AD column and Chiralcel OB column, respectively. The precision and accuracy of the coupled column system tested by standard solutions are both very good. Therefore, the column switching technique assisted column coupling system is successful for the on-line sample clean-up and the analysis of chiral products in the complex biphasic culture. A future extension of this chiral column switching HPLC

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

could be used to hyphen a MS spectrometer for the identification and determination of chiral compounds in a complex medium.

ACKNOWLEDGMENT

The authors gratefully thank the National Science Council, under grant No. NSC 89-2113-M-033-016, for their financial support.

REFERENCES

1. Cherrak, D.E.; Khattabi, S.; Guiochon, G. Adsorption behavior and prediction of the enantiomers of 3-chloro-1-phenyl-1-propanol influence of the mass transfer kinetics. J. Chromatogr. A **2000**, *877*, 109–122.
2. Cheng, C.; Ma, J.H. Enantioselective synthesis of S-(−)-1-phenylethanol in *candida utilis* semi-fed-batch cultures. Proc. Biochem. **1996**, *31*, 119–124.
3. Kartozia, I.; Kanyonyo, M.; Happaerts, T.; Lambert, D.M.; Scriba, G.K.E.; Chankvetadze, B. Comparative HPLC enantioseparation of new chiral hydantoin derivatives on three different polysaccharide type chiral stationary phases. J. Pharm. Biomed. Anal. **2002**, *27*, 457–465.
4. Ning, J.G. Direct chiral separation with Chiralpak AD converted to the reversed phase mode. J. Chromatogr. A **1998**, *805*, 309–314.
5. Campíns-Falcó, P.; Herráez-Hernández, R.; Sevillano-Cabeza, A. Column-switching techniques for high-performance liquid chromatography of drugs in biological samples. J. Chromatogr. **1993**, *619*, 177–190.
6. Fujitomo, H.; Nagaoka, T.; Nishino, I.; Umeda, T. Determination of a new oral cephalosporin, s-1090, in human plasma and urine by direct injection high-performance liquid chromatography with ultraviolet detection and column switching. J. Chromatogr. B **1999**, *728*, 125–131.
7. Lamprecht, G.; Kraushofer, T.; Stoschitzky, K.; Lindner, W. Enantio-selective analysis of (R)- and (S)-atenolol in urine samples by a high-performance liquid chromatography column-switching setup. J. Chromatogr. B **2000**, *740*, 219–226.
8. Murahashi, T.; Hayakawa, K. A sensitive method for the determination of 6-nitrochrysene, 2-nitro-fluoranthene and 1-, 2- and 4-nitropyrenes in airborne particulates using high-performance liquid chromatography with chemiluminescence detection. Anal. Chim. Acta. **1997**, *343*, 251–257.

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

9. Huang, Y.; Mou, S.F.; Riviello, J.M. Determination of ammonium in seawater by column-switching ion chromatography. J. Chromatogr. A **2000**, *868*, 209–216.
10. Blahová, E.; Bovanová, L.; Brandšteterová, E. Direct HPLC analysis of trimethoprim in milk. J. Liq. Chrom. & Rel. Technol. **2001**, *24* (19), 3027–3035.
11. Wang, H.; Xu, H.; Guan, Y. Column switching-back flushing technique for the analysis of aromatic compounds in gasoline. J. Chromatogr. A **2002**, *972*, 253–258.
12. Cheng, C.; Wu, S.C. Analysis of the enzymatic racemization of *D*-aspartic acid to *L*-aspartic acid by the on-line coupling of a solid-phase extraction column and a ligand-exchange high-performance liquid chromatography column. J. Chromatogr. A **2000**, *896*, 299–310.
13. Oertel, R.; Richter, K.; Fauler, J.; Kirch, W. Increasing sample throughput in pharmacological studies by using dual-column liquid chromatography with tandem mass spectrometry. J. Chromatogr. A **2002**, *948*, 187–192.
14. Zeng, H.; Wu, J.T.; Unger, S.E. The investigation and the use of high flow column-switching LC/MS/MS as a high-throughput approach for direct plasma sample analysis of single and multiple components in pharmacokinetic studies. J. Pharm. Biomed. Anal. **2002**, *27*, 967–982.
15. Mathews, C.K.; van Holde, K.E.; Ahern, K.G. Enzymes: biological catalysts. In *Biochemistry*, 3rd Ed.; Addison Wesley Longman, Inc.: San Francisco, CA, 2000; 391 pp.

Received April 20, 2003
Accepted June 26, 2003
Manuscript 6153

Downloaded At: 19:44 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016