*Korean J. Chem. Eng.*, **25**(2), 285-290 (2008)

# The enrichment of loxoprofen enantiomer by 6-columns simulated moving bed chromatography

**Tae Ho Yoon, Eun Lee, Jong Min Kim*, Woo-Sik Kim** and In-Ho Kim†**

Department of Chemical Engineering, Chungnam National University, Daejeon 305-764, Korea
*Department of Chemical Engineering, Dong-A University, Busan 604-714, Korea
**Department of Chemical Engineering, Kyunghee University, Yongin 446-701, Korea
(*Received 29 May 2007 • accepted 26 June 2007*)

**Abstract**−Simulated moving bed (SMB) chromatography is very useful for the separation of binary systems such as chiral compounds. Because loxoprofen racemate has four enantiomers owing to its two chiral centers, to gain pure enantiomer it is impossible with only one step. To apply enrichment of loxoprofen using SMB, extract and raffinate should be separated as a binary mixture. In order to enrich loxoprofen racemate as binary mixture among four mixtures and to characterize its enantiomer, we performed experiments with two types of columns. When TBB® column was used as CSP, the mixture of (1′R,2S) and (1′S,2S) forms was eluted as a raffinate and that of (1′R,2R) and (1′S,2R) forms was discharged as a extract under linear adsorption isotherm range. When the feed flow rates were 0.1 and 0.3 mL/min, purities of raffinate and extract were 98 and 95%, respectively. In this case, productivity of raffinate was 7.81 g/h·g-CSP and that of extract was 6.95 g/h·g-CSP.

Key words: Loxoprofen, SMB, Chiral Enrichment, TBB® Column, (1R,2S) & (1S,2S)

## INTRODUCTION

Loxoprofen, 2-[4-(2-oxocyclopentylmethyl)phenyl]-propionic acid is a non-steroid pain-killer drug of increasing importance owing to low side effects and 10-20 times higher efficacy compared with ketoprofen in the human body [1]. The mechanism of this drug of pain alleviation is inhibition of prostaglandin synthesis by trans-alcohol formation that is reduced intermediate metabolite of 1R-2S loxoprofen in the human body as shown in Fig. 1 [2-4]. Since this form has short half life cycle, loxoprofen should be taken frequently as administration of the drug [5]. In this case, there have been some possibilities related on bad effects on the stomach; therefore many DDS (drug delivery system) researches through human skin have been investigated [6]. Because conventional SMB chromatography can be applied on binary mixtures as a feed stream especially, we can separate two kinds of products containing a binary mixture from tetra mixture racemate [7-9]. If loxoprofen enantiomer, which has pharmaceutical activity, can be enriched into a binary mixture, there are the following advantages: a large amount of 1R-2S loxoprofen enantiomer can be reserved in the same capacity of patch raw material, and total uptake amount into humans can be decreased to maintain the same medical treatment compared with the raw material. To separate these chiral compounds, many kinds of the chiral stationary phases (CSP) were developed by researchers and companies, especially, Daicel chiral column has been based on the polysaccharide chiral stationary phase, which is recognized as a well known chiral column. Several kinds of Daicel CSP are documented. Kromasil CSP, less known CSP, is manufactured with TBB modified silica. Silica-based CSP has the advantage of easy and cheap packing into empty HPLC columns by a slurry packer.

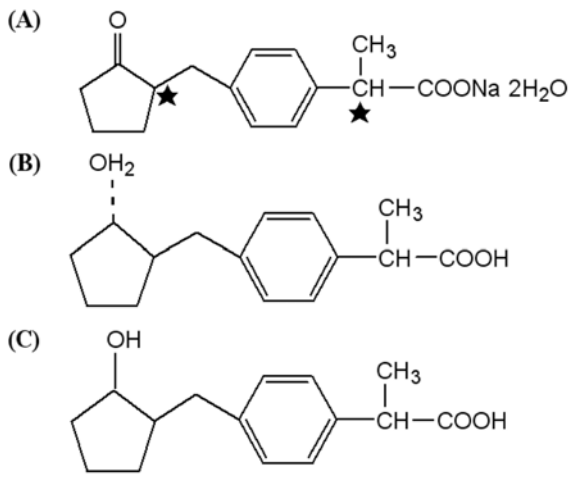


**Fig. 1. Molecular structure of loxoprofen (A), trans- (B) and cis-alcohol metabolite (C); *s are chiral centers.**

In this paper, we dealt with the application of loxoprofen racemate on an in-house SMB chromatography system to separate into a binary mixture among a tetra mixture system. To get operating parameters for SMB, Henry's constants were calculated through batch experiments with a single column, and the value of $m_2$ and $m_3$ was chosen from a Triangle diagram suggested by Morbidelli and Mazzotti [10,11]. In addition, the switching time interval and the flow rates of four zones differ from those for the other ketoprofen's SMBs in terms of the operating condition location at the triangle diagram [12].

## MATERIAL AND METHODS

### 1. Chemicals and Equipments

†To whom correspondence should be addressed.
E-mail: inkim@cnu.ac.kr

Loxoprofen used in experiments was a powdered form of sodium loxoprofen racemate and provided from Sun Fine Chem. Ltd. (Osong, Chungbuk, Korea). A sample was dissolved in mobile phase at given composition and filtered with syringe membrane filter (0.22 μm GVPP, Millipore, USA). Validation of each enantiomer of loxoprofen was based on the method of reference at the same conditions [13]. Solvents used in experiments mainly were ethanol (99.9%, Aldrich, USA), 2-propanol (99.8%, Merck, USA) and hexane (95%, J. T. Baker, USA), and trifluoracetic acid (99%, Acros Organic, USA) and acetic acid (1st grade, Dongyang Chem, Korea) were added for pH adjustment. Two kinds of stationary phase were employed in experiments: the one is ChiralCel OJ column (dp: 5 μm, I.D.: 4.6 mm and L.: 250 mm, Daicel, Japan), which is bound with a derivative of cellulose for the identification of four enantiomers; and the other is a Kromasil CHI-II column, which is bound with O,O′-bis(4-tert-butylbenzoyl)-N,N′-diallyl-L-tartar diamid on silica surface (dp: 10 μm, Eka Nobel, Sweden) for purity analysis and operation of SMB chromatography. An analytical CHI-II column (I.D.: 4.6 mm and L.: 250 mm) was used to check the purities of products, and preparative CHI-II columns packed with CHI-II gel by slurry packing device (Model 1666 Slurry packer, Alltech, USA) into an empty stainless steel column (I.D.: 10 mm and L.: 100 mm, Alltech, USA) were installed in the SMB chromatography system.

Loxoprofen racemate has very low solubility in hexane and 2-proponol; therefore, loxoprofen racemate was dissolved in pure ethanol to a concentration of 10 mg/ml as a stock solution. All samples were diluted to 0.1 mg/ml with mobile phase for injection through a Rheodyne injector. Flow rates were 1 ml/min for analysis in the Kromasil and Chiral OJ column with loading amount 20 μl and 3 ml/min for a packed column for SMB chromatography with loading amount 100 μl. The wavelength of the UV detector was set as 225 nm.

A solvent delivery pump (M-930, Younglin, Korea or 6000A, Waters, USA), UV detector (M720, Younglin, Korea) and data acquisition system (Clarity, DataApex, Czech) were used for HPLC experiments.

**2. Batch Experiments for Pulse Input Methods (PIM)**

To identify four enantiomers in loxoprofen racemate, a sample was dissolved in 95/5/0.1 (%, v/v) of hexane/2-propanol/trifluoracetic acid mixture, and injected into the ChiralCel OJ column with 20 μl of loading volume. To apply SMB chromatography separation into binary mixtures of loxoprofen enantiomers, a mixture of hexane/ethanol/acetic acid was used in the Kromasil CHI-II as a mobile phase, with 0.01-10.0 mg/ml of sample concentrations with 100 μl of injection volume, and the ratios of hexane and ethanol were also varied to show selectivity of samples.

**3. SMB Chromatography Experiments**

The in-house SMB chromatography used in the experiments had six preparative HPLC columns and four 12-way multi-position rotary valves controlled independently by computer with time duration sequences. Column configuration was 1/2/2/1, because the lengths of zone 2 and 3 were required longer than that of the other zone to separate efficiently, and detailed schemes are listed in a previous report [13]. During the duration of SMB chromatography experiments, a pressure drop was generated by the recycle flow through the columns allowing leakage flow to occur in the extract and the raffinate pumps even though both pumps were not switched. Therefore, some pressure barrier had to be made at the outlet ports of the extract and the raffinate pumps, so pressure relief valves were installed in them. To gain a pressure drop occurring by increasing of flow rate of recycle flow through six columns, the pressure drop was measured throughout the six columns at various recycle flow rates.

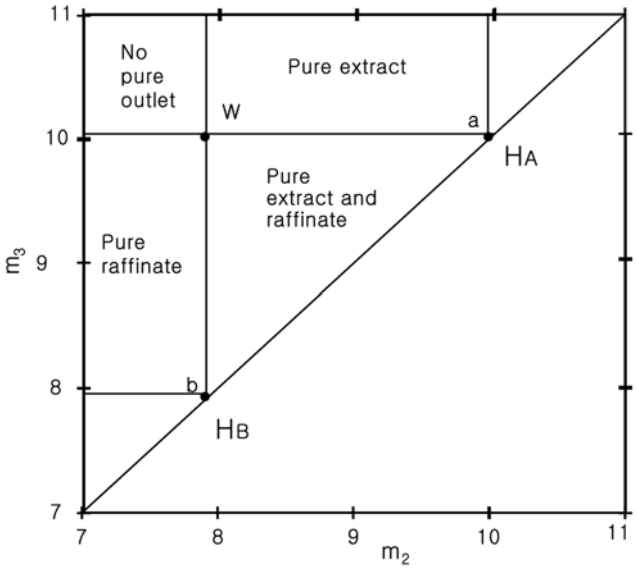


**Fig. 2. $m_2$-$m_3$ diagram of linear isotherm; $H_B$=7.90, $H_A$=10.07.**

For the triangle diagram, Henry's constants are calculated by the following Eq. (1). And void volume of column was estimated by injection of non-retained component into packed columns at given flow rate. Two H values generate horizontal and vertical lines, and a diagonal line completes the so-called triangle diagram (Fig. 2). With the aid of the triangle, we selected two points from which two operating parameters of SMB could be calculated [14].

$$H_i(\text{Henry constant}) = \frac{t_{ri} - t_0}{t_0} \cdot \left(\frac{\varepsilon}{1-\varepsilon}\right) \quad (1)$$

$$t_0 = \frac{\varepsilon \cdot V}{Q}$$

where, $t_r$=retention time, $t_0$=zero retention time, i=component, $\varepsilon$=void fraction, V=column volume, Q=flow rate.

## RESULTS AND DISCUSSION

**1. Effect of Eluent and Stationary Phase in Batch Experiments**

Fig. 3 shows chromatograms obtained by the Kromasil and Daicel columns. Two peaks were observed in the Kromasil column, and each peak was reanalyzed through collection and re-injection of them into Daicel column, where four peaks were identified. Raffinate fraction from Kromail contains 1′R-2S and 1′S-2S enantiomers, and extract fraction corresponds to 1′R-2R and 1′S-2R enantiomers. The elution sequence of the Kromasil column was reverted in the case of the Daicel column. Kromasil TBB chiral phase showed good selectivity as the popular polysaccharide-based chiral phase [15]. Loxoprofen has a better separation factor ($\alpha$=1.29) than that of ketoprofen ($\alpha$=1.20) [12].

To study the effect of hexane in eluent hexane ratios in eluent

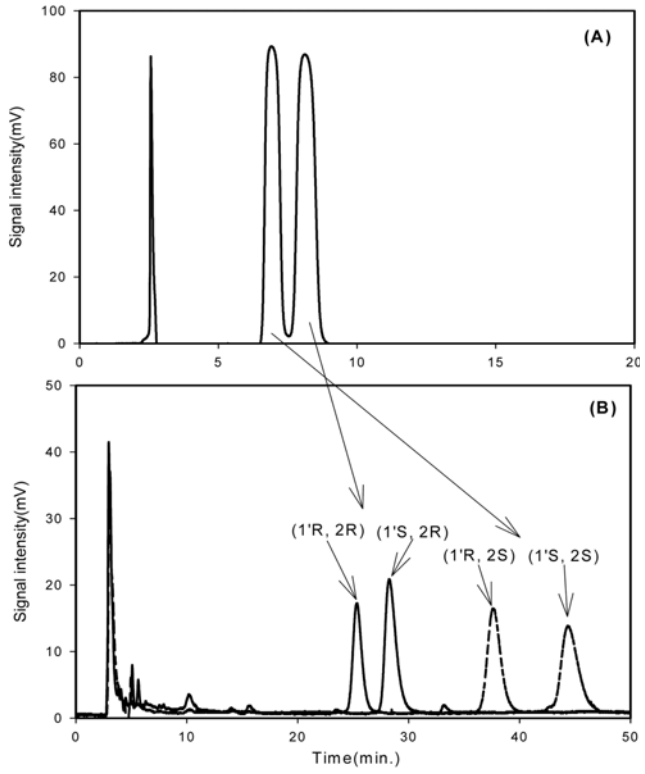


**Fig. 3. Identification of loxoprofen enantiomers ((A) Kromasil CHI-II analytical column, hexane : ethanol : acetic acid=95 : 5 : 0.1; (B) ChiralCel OJ column, hexane : 2-propanol : trifluoracetic acid=95 : 5 : 0.1, Sample loading volume=20 μl, flow rate=1.0 ml/min).**

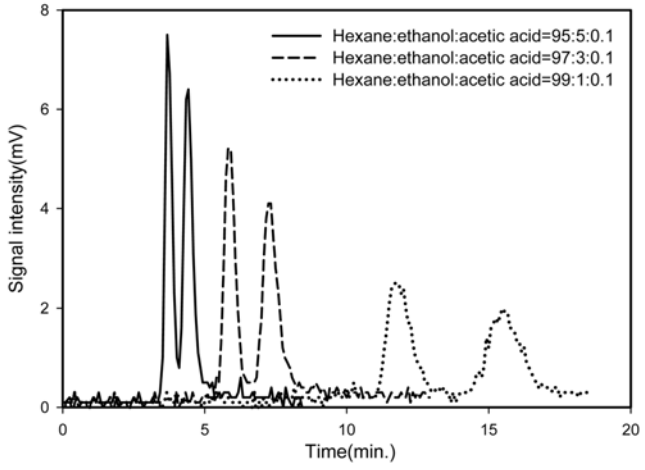


**Fig. 4. Effect of ethanol compositions in mobile phase (sample loading amount=20 μl, mobile phase flow rate=3.0 ml/min).**

were increased from 95% (v/v) to 99% (v/v) by packed column (10 mm ID×100 mm L). Fig. 4 shows that the $t_R$ of each peak increases when hexane ratios in eluent are increased. Small amount of ethanol in eluent makes H values change by effect of the interaction between CSP and each enantiomer. In the case of 99/1/0.1 (%v/v n-hexane/Et-OH/acetic acid) of the eluent, the composition has better separation factor ($\alpha$=1.38) and resolution (R=3.87) than that of

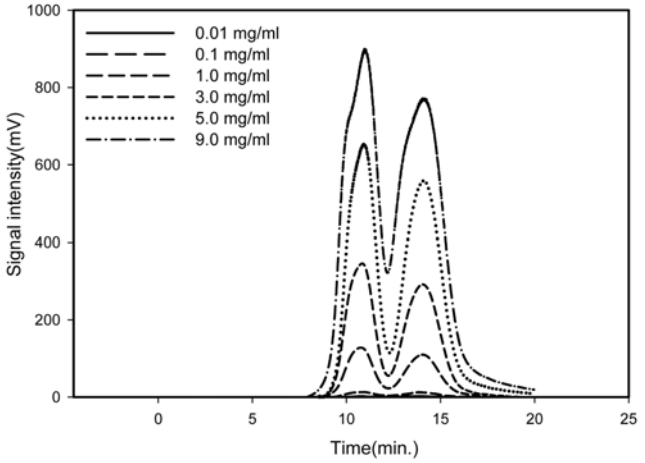


**Fig. 5. Chromatograms of loxoprofen racemate at different sample concentrations from SMB columns packed with CHI-II CSP, (sample loading amount=100 μl, mobile phase flow rate=3.0 ml/min, mobile phase=n-hexane/Et-OH/acetic acid =99/1/0.1).**

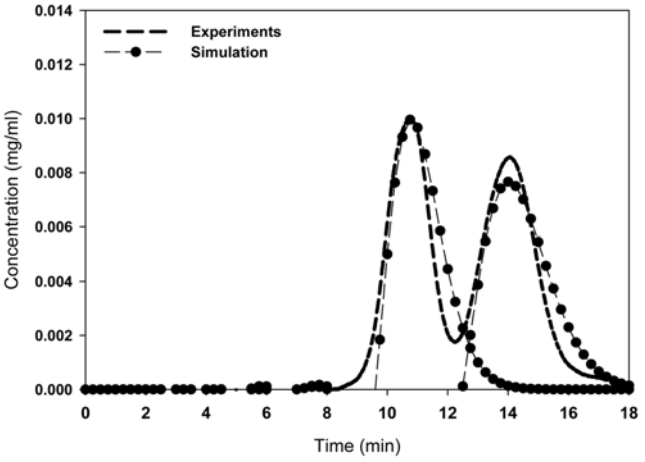


**Fig. 6. Comparison of experimental and simulated results by ASPEN chromatography (sample concentration=0.1 mg/ml, sample loading amount=100 μl, mobile phase flow rate =3.0 ml/min, mobile phase=n-hexane/Et-OH/acetic acid= 99/1/0.1).**

composition of 95/5/0.1 ($\alpha$=1.29 and R=2.51). As a result, the eluent was determined at the composition of n-hexane/Et-OH/acetic acid (99/1/0.1% (v/v)) for the SMB process.

## 2. Pulse Input Method

Henry constants were obtained from the packed column with an eluent composition (n-hexane/Et-OH/acetic acid=99/1/0.1). Since the concentration of loxoprofen in the SMB feed was quite low, a linear adsorption isotherm could be adapted. Fig. 5 shows that retention times showed no deviation at 11.11 and 13.78 min even though sample concentrations were increased from 0.01 mg/ml to 10.0 mg/ml. Henry's constant was calculated as follows:

$$q_A=10.07\ C_A \text{ and } q_B=7.90\ C_B \quad (2)$$

Where $q_i$ is the concentration of components in the solid phase, and $C_j$ is the concentration of components in the liquid phase; sub-

scripts B and A denote raffinate stream (1′R-2S and 1′S-2S) and extract stream (1′R-2R and 1′S-2R), respectively. Fig. 6 shows good agreement of chromatograms between experiments and simulation results by ASPEN Chromatography simulator (ASPEN 2006, Aspen Tech. USA) with calculated Henry constants at the given conditions. 1′R-2S loxoprofen enantiomer enriched portion is the target to be obtained and recovered in the raffinate stream of SMB chromatography.

### 3. SMB Experiments and Simulations

Fig. 7 shows the pressure drop at different recycle flow rates, and the pressure drop is proportional to the recycle flow rate. The solid symbols in Fig. 7 indicate the experimental data and the dotted line corresponds to the 1st order regression result between the flow rate and the pressure drop. Pressure relief valves should be adjusted according to the calculated pressure drop at given flow rate to prevent eluent leakage which occurs by internal pressure from raffinate and extract pumps. The pressure relief setting reads to be 1,200 psi when the recycle flow rate during SMB chromatography operation was 4.78 ml/min.

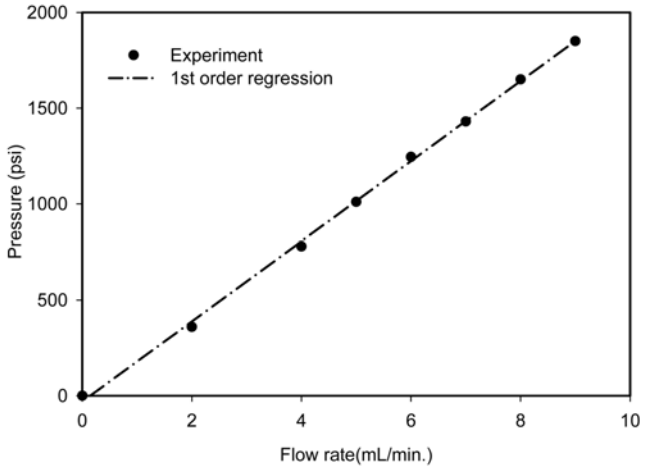


**Fig. 7. Pressure increment related with flow rate in SMB chromatography; eluent composition=hexane : ethanol : acetic acid =99 : 1 : 0.1 (%, v/v).**

**Table 1. Operation parameters of SMB chromatography at different positions of $m_2$ and $m_3$ and feed flow rates**

| Exp. no. | 1 | 2 | 3 |
|---|---|---|---|
| $m_2$ | 8.46 | 7.90 | 7.90 |
| $m_3$ | 9.46 | 10.07 | 10.07 |
| $Q_{feed}$ (ml/min) | 0.30 | 0.30 | 0.50 |
| $Q_{eluent}$ (ml/min) | 0.65 | 0.30 | 0.50 |
| $Q_{extract}$ (ml/min) | 0.48 | 0.30 | 0.50 |
| $Q_{raffinate}$ (ml/min) | 0.47 | 0.30 | 0.50 |
| $Q_I$ (ml/min) | 3.36 | 1.55 | 2.58 |
| $Q_{II}$ (ml/min) | 2.88 | 1.25 | 2.08 |
| $Q_{III}$ (ml/min) | 3.18 | 1.55 | 2.58 |
| $Q_{IV(Recycle)}$ (ml/min) | 2.71 | 1.25 | 2.08 |
| Δt (min) | 12.3 | 26.69 | 16.01 |
| Time delay (sec)* | 27 | 58 | 35 |

*Switching time delay was added on from column 6 to column 1. Operation parameters of SMB chromatography at different positions of $m_2$ and $m_3$ and feed flow rates.

A feed solution was prepared from the 10 mg/ml of loxoprofen in ethanol stock solution by adding 1% (v/v) into hexane/acetic acid (100/0.1 (%v/v)), because of low solubility of loxoprofen in n-hexane. Therefore, final concentration of loxoprofen racemate in feed was 0.1 mg/ml; the concentration of each extract and raffinate component was 0.05 mg/ml.

SMB conditions were set as an arbitrary point ($m_2$, $m_3$=8.46, 9.46) within $m_2$-$m_3$ diagram and the W ($m_2$, $m_3$=7.90, 10.07) in Fig. 2 with a feed flow rate of 0.3 ml/min and 0.5 ml/min respectively. Table 1 shows operating parameters of the SMB process at these points.

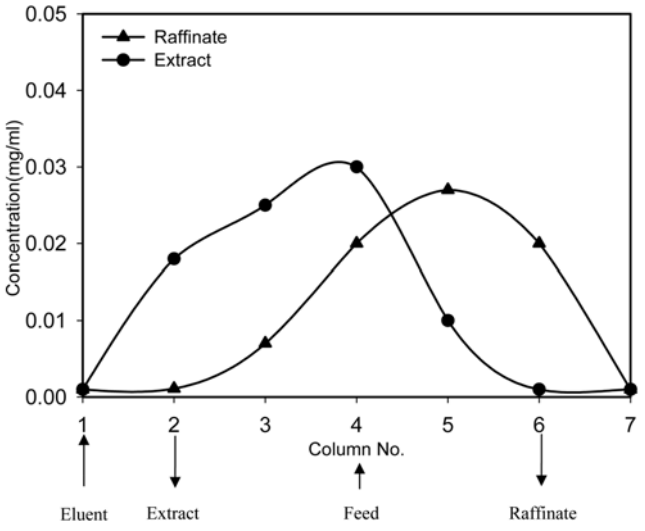


**Fig. 8. Internal concentration profiles of loxoprofen at $m_2$=8.46 and $m_3$=9.46 (feed amount=0.1 mg/ml, $Q_{feed}$=0.3 ml/min, $Q_{eluent}$ =0.65 ml/min, $Q_{extract}$=0.48 ml/min, $Q_{raffinate}$=0.47 ml/min, $Q_{recycle}$ =2.71 ml/min, Δt=12.3 min, eluent composition=n-hexane/ Et-OH/acetic acid=99/1/0.1).**

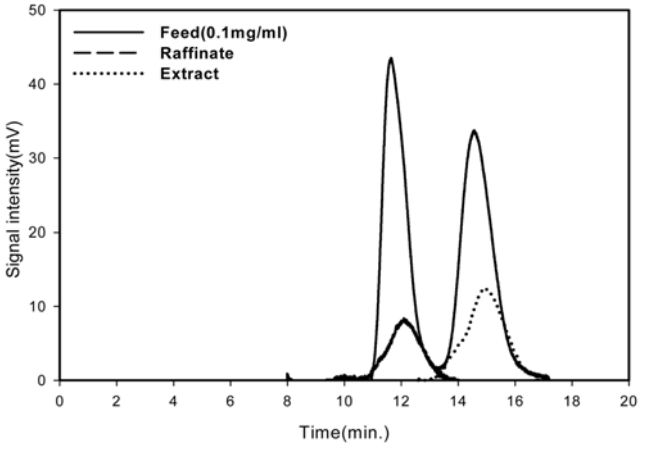


**Fig. 9. Chromatograms of feed, raffinate and extract in the case of $m_2$=8.46, $m_3$=9.46 (feed amount=0.1 mg/ml, $Q_{feed}$=0.3 ml/ min, $Q_{eluent}$=0.65 ml/min, $Q_{extract}$=0.48 ml/min, $Q_{raffinate}$=0.47 ml/min, $Q_{recycle}$=2.71 ml/min, Δt=12.3 min, eluent composition=n-hexane/Et-OH/acetic acid=99/1/0.1).**

The SMB experiment was performed at 8.46 and 9.46, which is an expected condition of complete separation. Fig. 8 shows that the internal concentration profile of loxoprofen racemate at the end of a switching period in SMB and the extract stream (column No. 2) and the raffinate stream (column No. 6) are well separated. Samples of raffinate and extract ports were analyzed under the analytic condition using the Kromasil CHI-II column. Purities of raffinate and extract were above 98%, and average concentrations of raffinate and extract 0.01 mg/ml and 0.015 mg/ml as shown in Fig. 9. Eluent was consumed at 40 ml per hour, and productivities of raffinate and extract were 2.7 μg/h·g-CSP and 3.1 μg/h·g-CSP.

At the point W ($m_2$, $m_3$=7.90, 10.07) of minimum eluent consumption condition (No. 2 in Table 1), the extract and raffinate stream were successfully separated as shown in the internal concentration profile of Fig. 10. The eluent rate decreased from 0.65 ml/min to 0.3 ml/min (Table 1), and the purity of raffinate and extract was 98% and 95%, respectively, shown in Fig. 11. Comparing Fig. 8 and Fig. 10, we find that the raffinate profile moves toward the extract port. As a result, the extract was contaminated by the raffinate stream and extract purity was lowered. Productivities increase from 2.7 μg/h·g-CSP to 7.81 μg/h·g-CSP for raffinate and from 3.1 μg/h·g-CSP to 6.95 μg/h·g-CSP for extract.

Another SMB experiment was performed at $Q_{feed}$=0.5 ml/min in order to increase the feeding rate (No. 3 in Table 1). Fig. 12 shows the internal concentration profile in SMB; the overlapped area of

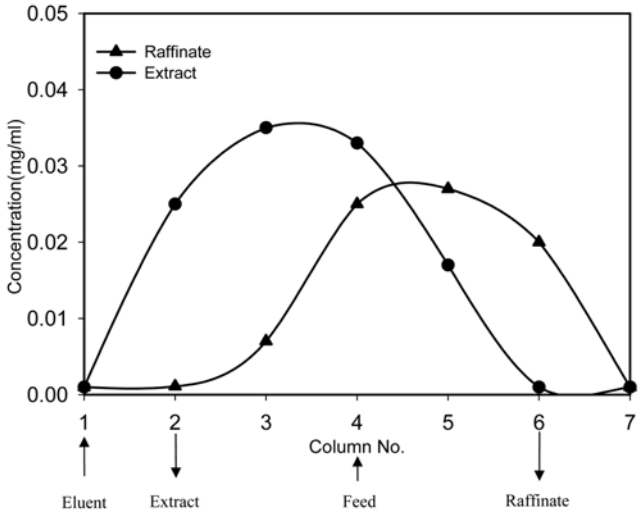


**Fig. 10. Internal concentration profiles of loxoprofen at $m_2$=7.90, $m_3$=10.07 (feed amount=0.1 mg/ml, $Q_{feed}$=0.3 ml/min, $Q_{eluent}$ =0.3 ml/min, $Q_{extract}$=0.3 ml/min, $Q_{raffinate}$=0.3 ml/min, $Q_{recycle}$ =1.25 ml/min, Δt=26.69 min, eluent composition=n-hexane/Et-OH/acetic acid=99/1/0.1).**

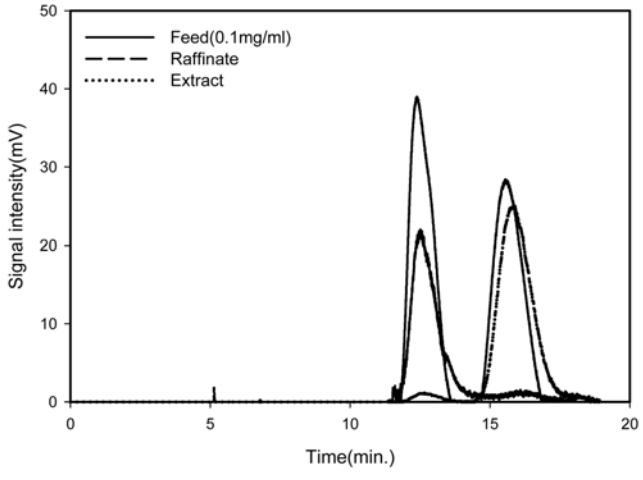


**Fig. 11. Chromatograms of feed, raffinate and extract in the case of $m_2$=7.90, $m_3$=10.07 (feed amount=0.1 mg/ml, $Q_{feed}$=0.3 ml/min, $Q_{eluent}$=0.3 ml/min, $Q_{extract}$=0.3 ml/min, $Q_{raffinate}$=0.3 ml/min, $Q_{recycle}$=1.25 ml/min, Δt=26.69 min, eluent composition=n-hexane/Et-OH/acetic acid=99/1/0.1).**

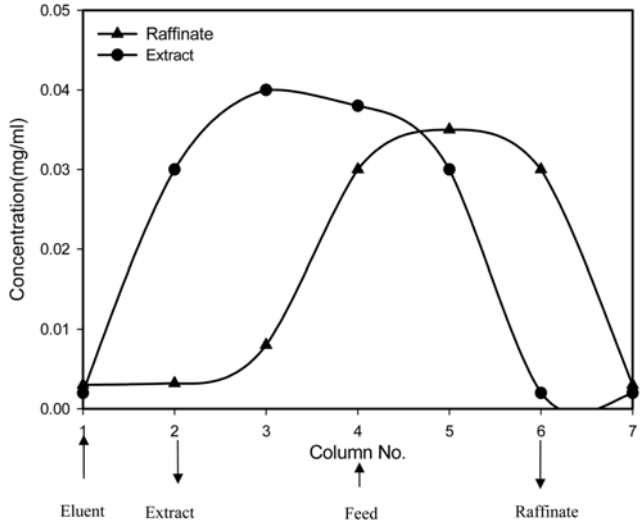


**Fig. 12. Internal concentration profiles of loxoprofen at $m_2$=7.90, $m_3$=10.07 (feed amount=0.1 mg/ml, $Q_{feed}$=0.5 ml/min, $Q_{eluent}$ =0.5 ml/min, $Q_{extract}$=0.5 ml/min, $Q_{raffinate}$=0.5 ml/min, $Q_{recycle}$ =2.08 ml/min, Δt=16.01 min, eluent composition=n-hexane/Et-OH/acetic acid=99/1/0.1).**

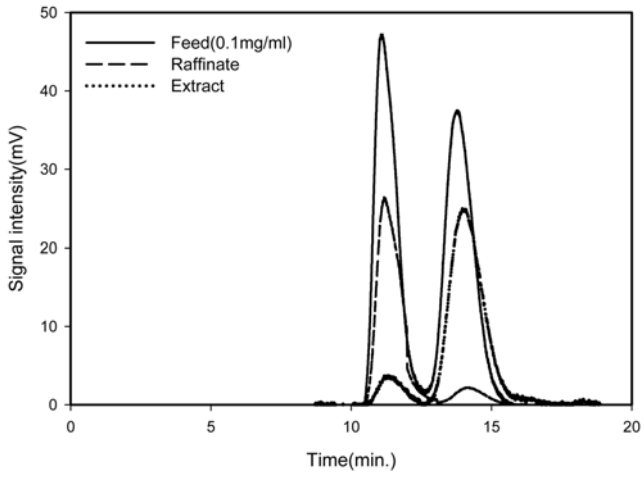


**Fig. 13. Chromatograms of feed, raffinate and extract in the case of $m_2$=7.90, $m_3$=10.07 (feed amount=0.1 mg/ml, $Q_{feed}$=0.5 ml/min, $Q_{eluent}$=0.5 ml/min, $Q_{extract}$=0.5 ml/min, $Q_{raffinate}$=0.5 ml/min, $Q_{recycle}$=2.08 ml/min, Δt=16.01 min, eluent composition=n-hexane/Et-OH/acetic acid=99/1/0.1).**

raffinate and extract increases more than that of Fig. 10. As feed flow rate increases, $Q_3$ is also increasing with less separation time in zone. This results in the largest productivity, 13 μg/h·g-CSP for raffinate and 12 μg/h·g-CSP for extract; however, purities of raffinate and extract are decreased to 95% and 90% as shown in Fig. 13.

## CONCLUSIONS

Loxoprofen racemate was experimentally separated into four enantiomers by a Daicel column and into two enantiomers mixture by a Kromasil TBB column. Loxoprofen enantiomer, 1′R-2S, can be enriched double times to raffinate component, and 1′R-2R and 1′S-2R enantiomers gathered as an extract component with TBB CSP in low concentration (0.1 mg/ml) by SMB chromatography. SMB experiments were performed with three operating conditions in the $m_2$-$m_3$ diagram. When the feed flow rate was 0.3 ml/min, the purity of each racemate was above 95% at the vertex point in the triangle region. To increase productivity, the feed flow rate was increased to 0.5 ml/min. In this case, raffinate can be separated with 95% purity and 13 μg/h·g-CSP productivity.

## ACKNOWLEDGMEMTS

This study was supported by KOSEF grant (R01-2005-000-10245-0) as well as Advanced Bioseparation Engineering Research Center of Inha University.

## REFERENCES

1. A. Terada, S. Naruto, K. Wachi, S. Tanaka, Y. Iizuka and E. Misaka, *J. Med. Chem*., **27**, 212 (1984).
2. K. Matsuda, Y. Tanaka, S. Ushiyama, K. Ohnishi and M. Yamazaki, *Biochem. Pharmacol.*, **33**, 2473 (1984).
3. H. Nagashima, Y. Tanaka and R. Hayashi, *J. Chromatogr.*, **345**, 373 (1985).
4. M. Sugimoto, T. Kojima, M. Asami, Y. Iizuka and K. Matsuda, *Biochem. Pharmacol*., **42**, 2363 (1991).
5. K. S. Choo, I. W. Kim, J. K. Jung, Y. G. Suh, S. J. Chung, M. H. Lee and C. K. Shim, *J. Pharm. Biomed. Anal*., **25**, 639 (2001).
6. T. S. Kim and I. G. Chun, *J. Applied Pharmacology*, **9**, 298 (2001).
7. L. Miller, C. Grill, T. Yan, O. Dapremon, E. Huthmann and M. Juza, *J. Chromatogr. A*, **1006**, 267 (2003).
8. M. Juza, M. Mazzotti and M. Morbidelli, *Tibtech.*, **18**, 108 (2000).
9. E. Francotte, P. Richert, M. Mazzotti and M. Morbidelli, *J. Chromatogr. A*, **796**, 239 (1998).
10. C. Migliorini, M. Mazzotti and M. Morbidelli, *J. Chromatogr. A*, **827**, 161 (1998).
11. M. Pedeferri, G. Zenoni, M. Mazzotti and M. Morbidelli, *Chem. Engng. Sci*., **54**, 3735 (1999).
12. T. H. Yoon, B. H. Chung and I. H. Kim, *Biotechnol. & Bioproc. Eng*., **9**, 285 (2004).
13. H. Kanazawa, A. Tsubayashi, Y. Nagata, Y. Matsushima, C. Mori, J. Kizu and M. Higaki, *J. Chromatogr. A*, **948**, 303 (2002).
14. L. S. Pais and A. E. Rodriues, *J. Chromatogr. A*, **1006**, 33 (2003).
15. B.-A. Persson and S. Andersson, *J. Chromatogr. A*, **906**, 195 (2001).