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A Novel Design of Simulated Moving Bed (SMB) Chromatography for Separation of Ketoprofen Enantiomer

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Abstract A simulated moving bed (SMB) chromatography system is a powerful tool for preparative scale separation, which can be applied to the separation of chiral compound. We have designed our own lab-scale SMB chromatography using 5 HPLC pumps, 6 stainless steel columns and 4 multi-position valves, to separate a racemic mixture of ketoprofen in to its enantiomers. Our design has the characteristics of the low cost for assembly for the SMB chromatography and easy repair of the unit, which differs from the designs suggested by other investigators. It is possible for the flow path through each column to be independently changed by computer control, using 4 multi-position rotary valves and 5 HPLC solvent delivery pumps. In order to prove the operability of our SMB system, attempts were made to separate the (S)-ketoprofen enantiomer from a ketoprofen racemic mixture. The operating parameters of the SMB chromatography were calculated for ketoprofen separation from a batch chromatography experiment as well as by the triangle theory. With a feed concentration of 1 mg/mL, (S)-ketoprofen was obtained with a purity of 96% under the calculated operating conditions.

Keywords: SMB chromatography, rotary valve, chiral separation, ketoprofen enantiomer

INTRODUCTION

Simulated moving bed (SMB) chromatography technology is attracting interest as an alternative technique for the production of fine chemicals and pharmaceuticals [1]. The method is now well-known for binary separations and has been commercialized for a large number of separations [2]. The essential principle of SMB is based on the true moving bed chromatography system originated from the UOP patent, and many researchers have studied the countercurrent flow path of two components with steady state columns [3-5].

There are many ideas relating to the embodiment of continuous counter-current chromatography systems under high pressure conditions and realization of recycling flow in separation system [6,7]. The most popular modes are: closed loop SMB, the moving port system, recycling of an intermediate cut and second peak tail [8-11]. Of these, the method generally used is that of a 4-zone SMB chromatography system, which is composed of multiple columns, rotary valves and pumps. The Novasep Company, in France, has successfully designed a SMB chromatography system using multiple columns, with four valves placed between each column, which can be individually opened and closed to allow the flow path of the recycle flow to be discharged or flow in [5,12,13]. In this case, the SMB chromatography system requires four times as many solenoid valves as columns. However, high pressure solenoid valves are expensive, making the price of SMB chromatography system higher than that of other preparative chromatography systems.

On the other hand, multi-position rotary valves give a means by which the valve system of the Novasep can be replaced, which can then be controlled independently, with a lower cost than the aforementioned SMB system. The multi-position rotary valves enable the flow path between each column to be independently controlled by a combination of the position of the inlet and outlet ports. Therefore, a SMB chromatography system has been designed, with 4 multi-position rotary valves, and confirmed using ketoprofen enantiomer separation. Ketoprofen is an effective pharmaceutical drug agent, which has been widely usecd, as a racemic mixture, for the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylistis, but only the (S)-ketoprofen enantiomer has pain relieving activity [14]. However, it is difficult to separate the enantiomers by HPLC, as racemic ketoprofen has a lower resolution in chiral separation columns than other racemic mixture [15]. Therefore, there is a need to separate the (S)-ketoprofen enantiomer, which has been achieved using SMB chromatography despite the low resolution between the two components, and a method for calculating the flow rate required for the SMB operation established.

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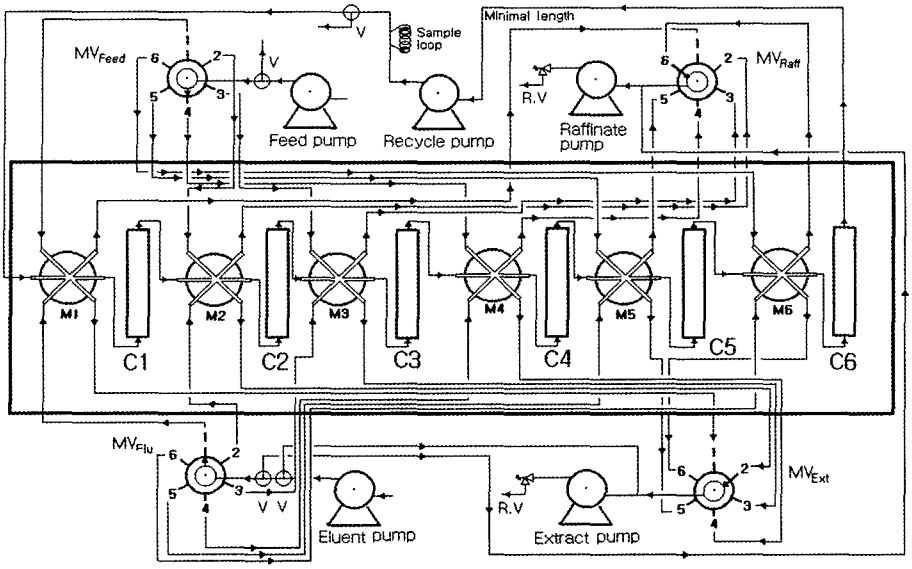

Fig. 1. Schematic diagram of the 6-column SMB chromatography; MF: rotary valve, C_i: HPLC column, M_j: manifold connecter, R.V: relief valve, i: column number; all columns are installed in constant heating chamber.

MATERIALS AND METHODS

SMB Chromatography System

The changes in the flow path of the recycle stream have to be independently performed, as this is essential to realize the simulated moving bed (SMB) effect. The valves for the SMB chromatography were selected with a view to installing an easy control capability. Four rotary valves (E-2-CSD12VW, Valco, USA) were installed to maintain the extract, raffinate, feed and eluent streams, as in Fig. 1. The 4 valves can direct the flow paths to 12 ports, of which 6 were utilized to reduce the number of columns. Twelve columns could be controlled in this SMB system, however, 6 columns (1/2/2/1) were sufficient to separate ketoprofen racemate. Recent SMB researches involving low number of columns are reported [16]. As shown in Fig. 1., HPLC pumps (M930, Younglin, Korea) were connected to the rotary valves, with the exception of the recycling pump and columns, which were equipped with 6-armed manifolds (1/16", Valco, USA). Four streams (the extract, raffinate, feed, and eluent ports) were located between the columns, so rotating the rotary valve made it possible to continuously change the flow path without disturbing the recycling flow. The recycle pump had a higher flow rate than the other pumps, as according to our calculation, the internal recycle flow had to be higher than that of the input and outlet flows.

Six columns (10 mm ID×100 mm length, Alltech, USA) were packed with chiral gel, combined with O,O-bis(4-tert-butylbenzoyl)-N,N'-diallyl-L-tartar diamide on silica particles (spherical type, 10 μm, 100 Å, Kromasil, Eka Chemical, Sweden), using the slurry packing method, and their performance individually verified by comparing the average retention times (raffinate of (S)-ketoprofen =13.7 min, extract of (R)-ketoprofen=15.9 min) of each ketoprofen enantiomer in each column to within 5%. After installing the six columns into our SMB system, a continuous separation of ketoprofen was initiated. In order to find the internal concentration profiles of the 6 columns during separation, an injection valve (Rheodyne 7125), with a 100 μL sample loop, was located inside the recycle flow line as a sampling valve, where a part of recycle flow could be collected without having to stop the pumps. As soon as the valves were rotated following each switch, the sample solution was collected in the sample loop, similarly to the principle of an injection valve in HPLC. In this case, the collected samples were representative of the internal concentration of the zone where the sampling valve was located.

A pressure drop was generated by the recycle flow through the columns allowing leakage flow to occur in the extract and raffinate pumps when these were not operating. Therefore, pressure relief valves were installed at the outlets of the extract and raffinate pumps. To gain an understanding of the pressure drop range, the pressure drop was measured throughout the 6 columns at various recycle flow rates.

Batch Chromatography Experiment and SMB Parameters Calculation

To obtain the appropriate mobile phase composition for separating (R)- and (S)-ketoprofen, batch experiments were performed with different compositions of n-hexane and *tert*-butyl methyl ether (t-BME). Once an optimized mobile phase composition had been set, the retention times of (R)- and (S)-ketoprofen were measured in another batch chromatography experiment. The column used in the batch experiment was the same as for the SMB, with a sample loading of 20 μL. The Henry constant could be calculated from Eqn. (1) to allow a plot

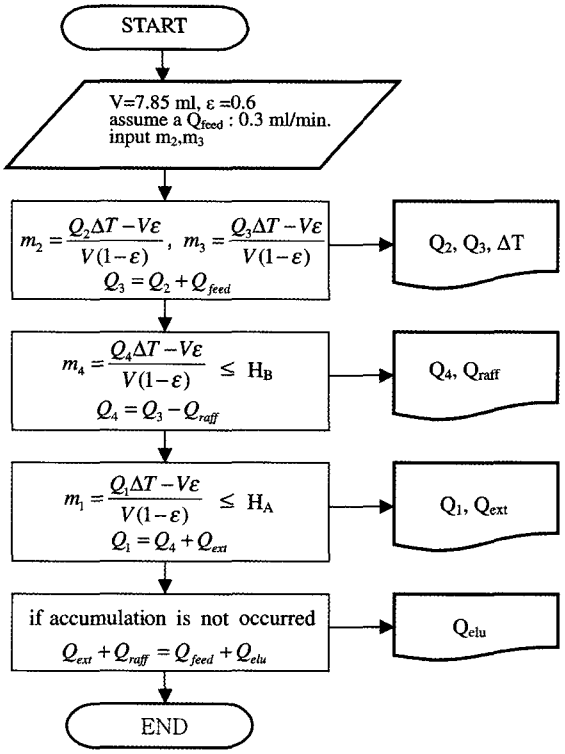

Fig. 2. Flow chart for the flow rate calculation from the m_2 *vs.* m_3 plane, using Eqns. (3-6); V=column volume, ε=void volume in column, ΔT = switching time.

of a triangle diagram for the case of a linear Langmuir isotherm range [17,18].

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

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

The parameter m_j, the so-called flow rate ratio, can be defined by Eqn.(2) as the ratio of the net fluid flow rate over the solid phase flow rate in each zone of the SMB [15].

$$m_j = \frac{Q_j \Delta t - V\varepsilon}{V(1-\varepsilon)} \quad (2)$$

where, j=number of zone,
Δt=switching time

Complete separation can be achieved with the following constraints (Eqns. (3)-(6)), defined as a region of the four dimensional spaces whose coordinates are the operating parameters m_1, m_2, m_3 and m_4.

$$H_A \leq m_1 \text{ (in zone I)} \quad (3)$$

$$H_B \leq m_2 \leq H_A \text{ (in zone II)} \quad (4)$$

$$H_B \leq m_3 \leq H_A \text{ (in zone III)} \quad (5)$$

$$m_4 \leq H_B \text{ (in zone IV)} \quad (6)$$

where, A=extract (more retained component on stationary phase)
B=raffinate (less retained component on stationary phase)

In order to find the operating conditions for the SMB, Eqns. (3)-(6) were used as guidelines for determining the flow rates of the four zones. First, the triangle diagram in the plane of m_2 *vs.* m_3 was plotted with the corresponding given constraints, with a positive feed flow rate implied at the condition of $m_3 > m_2$. Then, a point in the triangle diagram was assigned to decide the feed flow, as in Fig. 2. In this manner, the parameters for the SMB were calculated.

SMB Experiments

SMB experiments for various cases in the triangle diagram were performed under a constant temperature of 40°C. To establish a steady state condition, samples were withdrawn after the 2nd cycle, which means that all the rotary valves were fully rotated two times from the starting point. Extract and raffinate samples were with drawn from the outlet port of each pump through the sampling valve in the recycle line, as explained in the section on the SMB chromatography system. All samples were assayed by HPLC, with an analytical column (TBB, 4.6 mm ID× 250 mm L, Kromasil, Sweden), to obtain the internal concentration profiles of the 4 zones. To reduce the effect of the dead volume in the recycle line, the lag time generated by the dead volume of tubing and damper of the recycle line were taken into consideration. The switching times of the rotary valves were modified to take into account the lag time, with a column configuration of 1/2/2/1 for zones I/II/III/IV, respectively.

RESULTS AND DISCUSSION

Batch Chromatography Experiment and SMB Parameters Calculation

Experiments with different mobile phase compositions were used to determine the proper ratio of t-BME to hexane. As the ratio of hexane to t-BME was increased, the retention times of each enantiomer also increased. The resolution and selectivity trends are shown in Fig. 3. When the ratio was over 90%, the peak widths of each enantiomer broadened, so a ratio of 85% was chosen as the optimum. Pure t-BME was injected as a non-retained component by the stationary phase, with a t_0 of t-BME of 0.87 min. The void fraction was calculated as 0.53, using

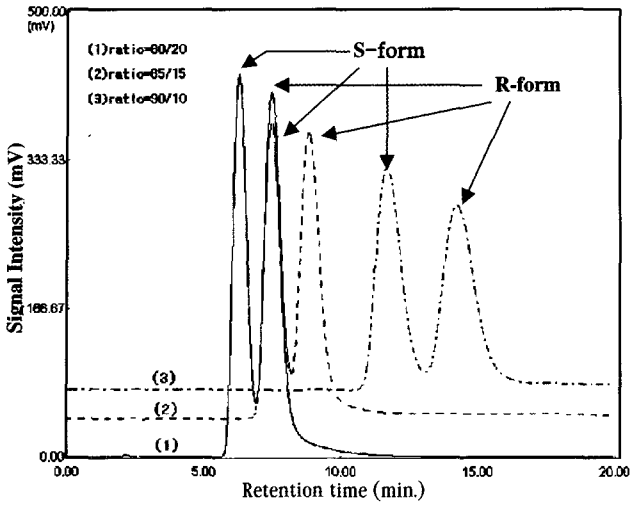

Fig. 3. Changes in the retention times for the 2-enantiomers with different hexane and t-BME compositions (sample loaded amount: 20 μL, wave length: 254 nm, flow rate: 4.7 mL/min).

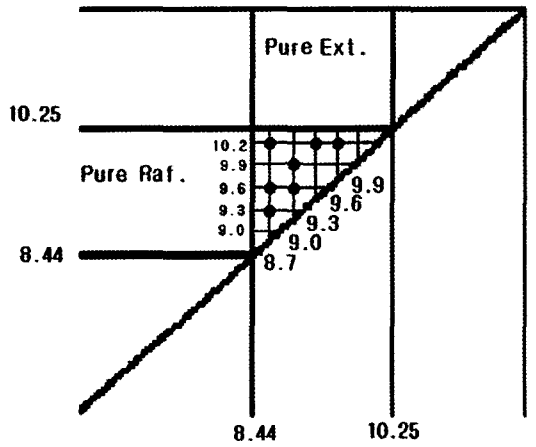

Fig. 4. Coordinates of selected points in the triangle diagram, as listed in Table 1.

Eq. (1). The (S)-ketoprofen was eluted earlier than (R)-ketoprofen, and their identification performed against standard (S)-ketoprofen. The Henry's constants of (S)- and (R)-ketoprofen, as calculated by Eq. (1), were 8.44 (H_S) and 10.25(H_R) when the feed concentration was 1 mg/mL.

Triangle diagram can be plotted from the H values, as shown in Fig. 4. Any point within the triangle will give conditions capable of separating each enantiomer; however, to perform SMB experiments for all cases within the diagram would be an enormous task. To limit the number of cases, only a few cases within the triangle were selected, with a fixed feed flow rate and concentration. Parameters calculated by Eq. (2) for some cases are listed in Table 1, which were selected from equally-spaced lattice points.

Table 1. Calculated parameters at the different positions of m_2 *vs.* m_3 in the triangle diagram using Eqns. (1) and (2)

	Case 1 (a)	Case 2 (b)	Case 3 (c)	Case 4 (d)	Case 5 (e)	Case 6 (f)	Case 7 (g)
m_2	9.0	9.0	9.6	8.7	8.7	9.3	8.7
m_3	9.9	9.6	10.2	9.3	9.6	10.2	10.2
Q_{feed} (mL/min.)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Q_{elu} (mL/min.)	0.60	0.91	0.91	0.91	0.61	0.61	0.36
Q_{raff} (mL/min.)	0.49	0.58	0.88	0.43	0.39	0.59	0.35
Q_{ext} (mL/min.)	0.42	0.63	0.33	0.78	0.52	0.32	0.31
Q_{rec} (mL/min.)	3.19	4.78	4.78	4.78	3.19	3.19	1.91
ΔT (min.)	11.1	7.38	7.38	7.38	11.1	11.1	18.45

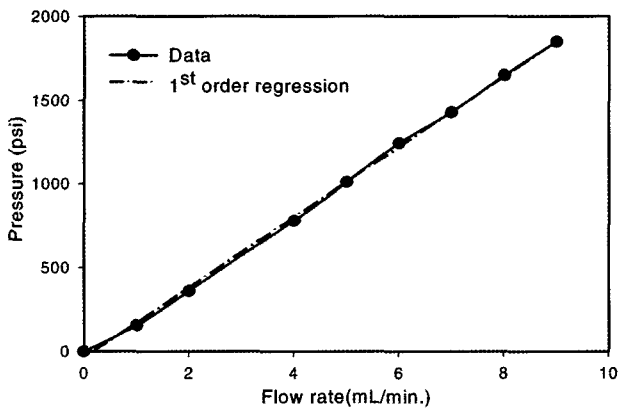

Fig. 5. Variation in the pressure drop with increments of the recycle flow rate.

SMB Experiment at Different Parameters

Fig. 5 shows the pressure drop with variation in the recycle flow rate, which was found to be proportional. The solid symbols in Fig. 5 indicate the experimental data and the dotted line corresponds to the 1st order regression line between the flow rate and the pressure drop. Pressure relief valves can be adjusted in relation to the regression data to prevent leakage from the raffinate and extract pumps. The pressure relief setting needed to be 1,200 psi with a recycle flow rate of 4.78 mL/min.

Fig. 6 shows the internal concentration profiles of the SMB chromatography for different cases within the triangular diagram. The horizontal axis indicates the num-

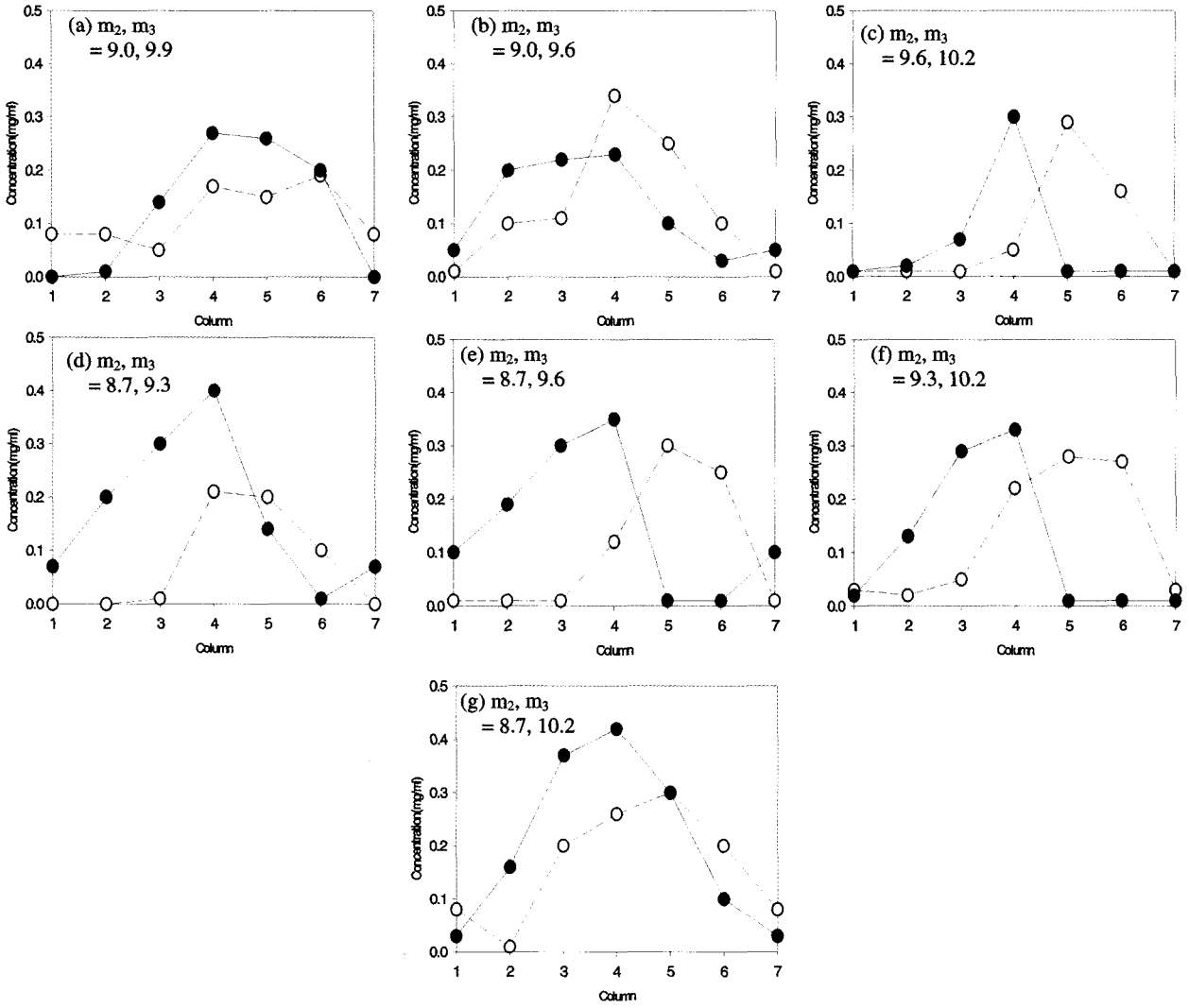

Fig. 6. Internal concentration profiles at different calculated parameters; eluent: 1, extract: 2, feed: 4, raffinate: 6; No. 7 is equivalent to No. 1; (a): case 1, (b): 2, (c): 3, (d): 4, (e): 5, (f): 6 (g): 7 in Table 1; ●: extract enantiomer ((R)-ketoprofen)○: raffinate enantiomer((S)-ketoprofen).

ber of columns and the vertical axis the concentration of each enantiomer obtained from the sampling valve located in the recycle line. Because all the columns were connected in a closed loop, the end of the 6th column was linked to the inlet of the 1st column. For brevity, the 7th means the 1st column. The eluent and feed streams were supplied to the 1st and 4th columns, respectively, with the extract and raffinate streams discharged simultaneously from the 1st and 5th columns, respectively. By switching valves, the internal concentration profile will shift to the right, while maintaining the shape of the profile during the SMB operation.

When the parameters of the SMB were set as in case 1(m_2, m_3=9.0, 9.9), the 2 components were unsatisfactorily separated, as shown in the internal concentration profile of Fig. 6(a). Since the two peaks were not separated at the outlet of the 5th column, the raffinate stream contained an almost equal mixture of (S)- and (R)-ketoprofen. The extract stream from the 1st column had more of the raffinate component ((S)-ketoprofen) than (R)-ketoprofen (purity: 11%), as shown in Table 2. Therefore, the flow rates of the extract and eluent were increased to 0.63 and 0.91 mL/min, respectively, to improve the purity of the extract. In case 2, the purity of the extract was enhanced to 83%, but the purity of the raffinate was still low (66%).

In the case of m_2=9.6 and m_3=10.2, the flow rate of the raffinate was increased to 0.88 mL/min and that of the extract decreased to 0.33 mL/min. As in Table 2, the purity of the raffinate was improved to 90%, since the peak widths of the two enantiomers narrowed, as shown in Fig. 6(c). The yield of the extract was as low as 20% because its concentration was low at the outlet of the 1st column, as shown in Fig. 6(c). In case 4 (m_2, m_3=8.7,

Table 2. Purification results of the SMB chromatography, with different values of m_2 and m_3 in the triangle diagram

		Case 1 (a)	Case 2 (b)	Case 3 (c)	Case 4 (d)	Case 5 (e)	Case 6 (f)	Case 7 (g)
m_2		9.0	9.0	9.6	8.7	8.7	9.3	8.7
m_3		9.9	9.6	10.2	9.3	9.6	10.2	10.2
Purity (%)	Raf	41	66	90	96	96	99	67
	Ext	11	83	88	99	90	75	91
Productivity (mg/min.)	Raf	0.09	0.14	0.15	0.10	0.11	0.10	0.10
	Ext	0.01	0.06	0.03	0.08	0.07	0.01	0.03
Yield (%)	Raf	59	93	99	67	73	67	67
	Ext	--	40	20	52	47	--	20

9.3), a point in the opposite position of the triangle diagram (Fig. 4) was selected, where the flow rates of the raffinate and extract were 0.43 and 0.78 mL/min. Fig. 6(d) shows that the concentration and purity of the extract increased to 0.2 mg/mL and 96%, respectively. As a result, the extract productivity was increased 2.5 times that of the previous case, as in Table 2, but the concentration of the raffinate decreased to 0.1 mg/mL compared with the 3rd case.

When the *m* points move toward vertices of the triangle (m_2, m_3=8.7, 9.6), the flow rates of the raffinate and extract decreased to 0.39 and 0.52 mL/min, respectively, compared to case 4. In this case, the internal concentration profile of the raffinate shifted to the right and the concentration increased to 0.2 mg/mL, but a little contamination due to the extract component occurred, as shown in Fig. 6(e). Because the desired product was the (S)-ketoprofen enantiomer (raffinate), this internal concentration profile was suitable for our experimental purpose. Furthermore, the flow rate of the raffinate was increased to 0.59 mL/min in order to recover a greater proportion of this component (Fig. 6(f)). In case 6, the raffinate stream gave a purity and recovery yield for the (S)-ketoprofen of 99 and 67% respectively, as in Table 2. To save the mobile phase, the flow rate of the eluent was decreased. In the 7th case (m_2, m_3=8.7, 10.2), the raffinate had the lower purity of 67%. Because the feed flow rate was fixed in all the experiments, a low eluent flow rate brought about a higher loading of feed. Also, the valve switching time was increased to 18.45 min, which led to inefficient operation of the SMB process.

CONCLUSION

A novel SMB chromatography system has been constructed with 5 HPLC pumps, 4 multi-position valves and 6 HPLC columns, and successfully operated for the separation of the (S)-ketoprofen enantiomer from a racemic ketoprofen mixture, based on the triangle theory. The suitable mobile phase condition was found to be 85 : 15 : 0.1(% v/v) hexane:*tert*-butyl methyl ether : acetic acid from batch experiments. To prevent leakage from the extract and raffinate pumps during sampling, two relief pressure valves were adjusted to 1200 psi, with a recycle flow rate of 4.78 mL/min. With raffinate and extract flow rates of 0.43 and 0.78 mL/min, the two enantiomers were reasonably separated, with yields of 67 and 52%, respectively. The lower left region in the triangle diagram was found to be an appropriate area for determining m values, resulting in a good separation of the chiral compounds.

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