Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1026 (2004) 101–108
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Resolution of a racemic pharmaceutical intermediate A comparison of preparative HPLC, steady state recycling, and simulated moving bed

Charles M. Grill*, Larry Miller, Tony Q. Yan[1]

Pharmacia, 4901 Searle Parkway, Skokie, IL 60077, USA

Received 14 March 2003; received in revised form 7 November 2003; accepted 12 November 2003

Abstract

Preparative HPLC and simulated moving bed (SMB) chromatography were used to resolve significant quantities of a racemic pharmaceutical intermediate. In addition, smaller scale studies using closed-loop recycling and steady state recycling (SSR) were performed so that a meaningful comparison of all these techniques could be made using the same real world separation. A highly optimized, six-column SMB process was clearly the superior technique and was used for the process-scale (247 kg of racemate) resolution. At the more moderate lab-scale (33 kg of racemate and 19 kg of racemate), a frequently used but less optimized eight-column SMB process was used. It was found that SSR was comparable to the lab-scale SMB process in productivity and solvent consumption. Thus, it appears that SSR can be a useful choice at such moderate scales. Finally, at moderate scales when neither SSR nor SMB is available, it was found that acceptable results were obtained with both closed-loop recycling and with a two-step preparative process.

Keywords: Steady state recycling; Simulated moving bed; Preparative chromatography; Enantiomer separation

1. Introduction

The use of chromatographic techniques to obtain significant quantities of enantiomerically pure drugs and drug intermediates is well established [1–11]. Closed-loop recycling has been shown to be a useful technique in chiral separations in which resolution is poor [12]. Simulated moving bed (SMB) chromatography in recent years has become a routine technique for the separation of enantiomers [13–22]. Closed-loop steady-state recycling (SSR), a relatively new technique [23,24], has shown promise in approaching the performance of SMB [25].

Although chromatographic purification is generally an expensive process, the economics of the pharmaceutical industry has made chromatography cost effective in the large scale separation of enantiomers [26]. This is especially true with SMB where several companies are using this technique to separate commercial scale quantities of enantiomers [27,28]. In this paper, we will compare several chromatographic techniques in the enantiomeric separation of a proprietary pharmaceutical intermediate with the aim of determining under what circumstances and at what scales each technique would be most useful.

2. Experimental

2.1. Racemate

The racemate to be separated was a proprietary pharmaceutical intermediate belonging to and synthesized at Pharmacia (Skokie, IL, USA). The compound contains an hydroxyl group at the asymmetric center.

2.2. Mobile phase and chiral stationary phase (CSP)

Acetonitrile, from various sources, was the mobile phase in each of the chromatographic techniques reported here. In each case, the CSP was Chiralpak AS, 20 μm particle size (Daicel Industries, Tokyo, Japan).

* Corresponding author. Tel.: +1-847-982-7499; fax: +1-847-982-4771.
E-mail address: charles.m.grill@pharmacia.com (C.M. Grill).
[1] Present address: Amgen, Thousand Oaks, CA, USA.

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.11.049

2.3. Preparative HPLC

The preparative HPLC run was performed at Pharmacia (Skokie, IL, USA) using a pre-packed column (10 cm i.d. × 50 cm, 2.35 kg CSP) obtained from Chiral Technologies (Exton, PA, USA). The preparative chromatograph was an SepTech ST/1000 XPS system (Varian, Wakefield, RI, USA) The flow rate was 500 ml/min. The sample solution was made by dissolving a sufficient quantity of racemate or enriched enantiomeric mixture in acetonitrile to give a final concentration of 30 mg/ml.

2.4. SSR and closed-loop recycling

The SSR and closed-loop recycling experiments were performed at the Pharmacia laboratories in Skokie, IL, USA using a NovaPrep® 200 preparative chromatograph (manufactured by Varian Inc., Wakefield, RI, USA) modified to perform the SSR process. This instrument has been described elsewhere [25]. The 10 ml injection loops used in the SSR processes were made of 1/8 in OD stainless steel tubing, three or four of which were connected in series to give the desired injection volume.

LC ReSponder control software (Varian, Wakefield, RI, USA) was used to automate the system in both the SSR and closed-loop recycling modes.

In the SSR experiments reported here, the relative time method [23,25] was used to determine the initiation times of collection and injection events. In this method, the leading edge of the profile was detected by setting a time window in which the control software looked for a detector signal ("ascending height") of 5–10% of full scale. When this condition was met, the collection valve for Fraction 1 was opened and a timer was started: the times of all other collection and injection events were then defined relative to the opening of the Fraction 1 collection valve (hence the term "relative time"). Detailed accounts of how the SSR technique is performed are given elsewhere [23,25].

The preparative column used for the SSR and the closed-loop recycling runs was a Prochrom 50 mm i.d. × 500 mm column (NovaSep, Nancy, France), packed with 240 g of CSP. The bed length was 20 cm.

The sample solution was made by dissolving a sufficient quantity of racemate in acetonitrile to give a final concentration of 30 mg/ml.

All fractions collected using preparative LC, closed-loop recycling, and SSR were analyzed by analytical HPLC. The mobile phase was HPLC grade acetonitrile, and the flow rate was 1.0 ml/min. The column, having dimensions 4.6 mm i.d. × 250 mm, was packed with Chiralpak AS, particle size 10 μm (Chiral Technologies, Exton, PA, USA). Detection varied from 290 to 300 nm.

2.5. Laboratory scale SMB

The laboratory scale SMB work was performed by contractors at two different sites (Contractors #1 and #2). Both groups used Licosep Lab 8-50 SMB systems (NovaSep, Nancy, France). The CSP was 20 μm Chiralpak AS (Daicel Industries, Tokyo, Japan) and was packed into eight Self Packer columns, each with 50 mm i.d. (Merck KGaA, Darmstadt, Germany). Each column was packed with 110 g of CSP to give an average cm bed length of 10.7 cm. The operating conditions used by each group are summarized in Table 1.

The collected products were analyzed by analytical HPLC. At Contractor #1 the column, having dimensions 4.6 mm i.d. × 250 mm, was packed with Chiralpak AS. Detection varied from 250 to 330 nm. At Contractor #2, the column, having dimensions 4.6 mm i.d. × 250 mm, was packed with Chiralpak AD. The mobile phase was ethanol/isohexane 5/95, and the flow rate was 1.0 ml/min. Detection was at 254 nm.

2.6. Process-scale SMB

The process-scale SMB work was performed at a third location by Contractor #3 using a Licosep 6-200 SMB system (NovaSep, Nancy, France). The CSP was packed into six dynamic axial compression columns, each with 202 mm i.d. (Merck KGaA, Darmstadt, Germany). Five of the columns were packed with 2.2 kg of CSP to give an average cm bed length of 10.9 cm; the sixth column was packed with 1.8 kg of CSP to give a bed length of 8.6 cm. The operating conditions are summarized in Table 1. The collected products were analyzed by chiral analytical HPLC.

2.7. Purity and yield specifications

The second-eluting enantiomer (Enantiomer 2) was the desired species. In the SMB runs, Enantiomer 2 was

Table 1
SMB operating conditions

Group	SMB system	Feed concentration (mg/ml)	Temperature (°C)	Flow rates[a,b]					Switch time (min)
				Feed	Eluent	Extract	Raffinate	Recycle	
Contractor #1	Licosep 8-50	36.1	30	24.67	128	98.38	54.29	374.19	0.62
Contractor #2	Licosep 8-50	30	30	22.5	84.75	66.0	41.25	376.5	0.51
Contractor #3	Licosep 6-200	40	25	55	182.2	129.4	107.8	480.3	0.66

[a] Flow rates expressed in ml/min for Licosep 8-50 systems.
[b] Flow rates expressed in l/h for Licosep 6-200 system.

collected in the extract. The project specifications called for an Enantiomer 2 optical purity of ≥99% (≥98% enantiomeric excess, ee) and an Enantiomer 2 recovery of ≥90%.

3. Results

3.1. Preparative HPLC

The preparative HPLC separation was carried out in two steps as shown in Fig. 1. In the first step, 7.0 g of racemate were injected onto the column. Three fractions were collected in the first step: most of the undesired, first-eluting enantiomer (Enantiomer 1) was collected in Fraction 1; a small overlap fraction was collected in Fraction 2; and the rest of the profile consisting of a small amount of Enantiomer 1 and all of the desired, second-eluting enantiomer (Enantiomer 2) was collected in Fraction 3. Fraction 2 was ultimately discarded.

Fraction 3 was dried using rotary evaporation and redissolved in acetonitrile to give a concentration of 30 mg/ml.

In the second step, 4.5 g of the Fraction 3 product, consisting of Enantiomer 1: Enantiomer 2 in the weight ratio of 3:97, were re-injected onto the column. The stacked injection technique was used in each step to maximize throughput. In this technique injections are made before the profile from the previous injection elutes. The timing of the injections is such that the profiles are as close together as possible without overlapping. In the first step, the cycle time was 5.5 min; in the second step, 4.5 min.

As shown in Table 2 the purity and recovery of Enantiomer 2 were within specifications at 98 and 93% ee, respectively. The solvent usage was 0.71 l/g of racemate, and the overall production rate for the two-step process was 435 g racemate per day/kg CSP. Because the evaporation process was faster than the chromatography process, the chromatography process never had to shut down to wait for product from the evaporator. Thus, the evaporation step did not adversely affect the production rate.

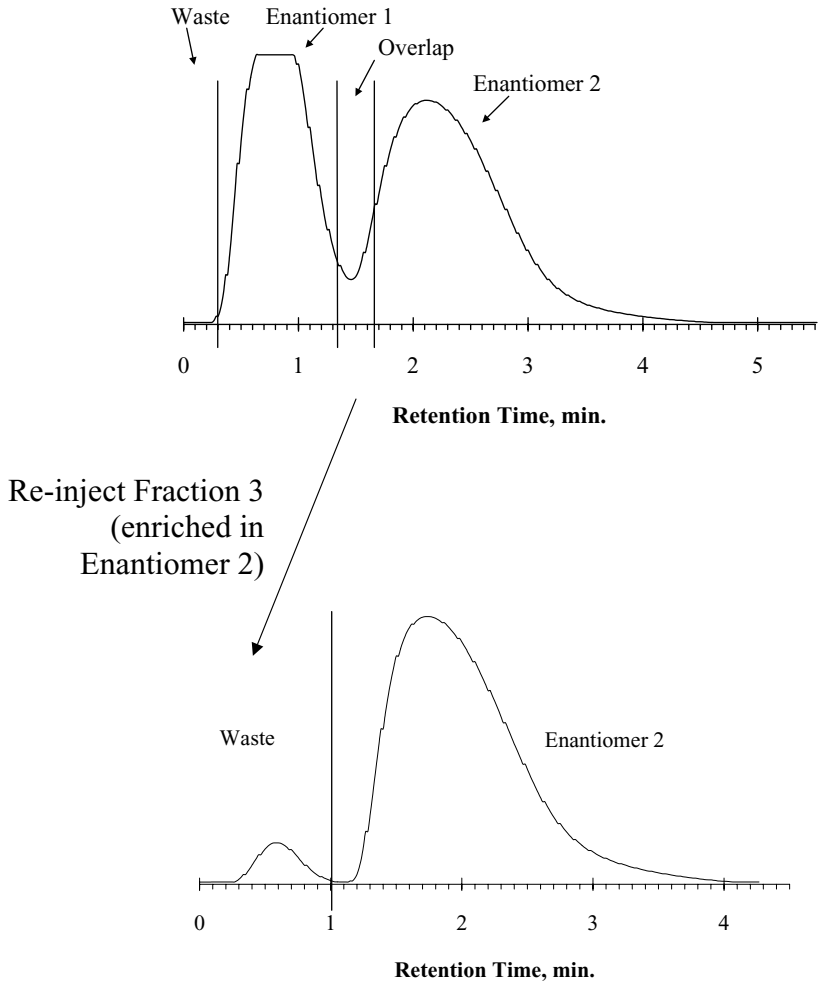

Fig. 1. The two-step preparative process. Column dimensions were 10 cm i.d. × 50 cm. In the first step, 7.0 g of racemate were injected. In the second step, 4.5 g of Fraction 3 from step 1, which was enriched in Enantiomer 2 (93% Enantiomer 2), was re-injected after an evaporation step.

Table 2
Comparison of results

Technique	Number of columns	Column size (cm i.d. × cm)	Amount processed (kg racemate)	Production rate (g racemate/kg CSP per day)	Purity of enantiomer 2 (% ee)	Recovery of enantiomer 2 (%)	Solvent usage (l/g racemate)
Batch chromatography (two-step process)	1	10 × 50	1.05	435	98.0	93.0	0.71
Closed-loop recycling	1	5 × 20	0.001[d]	514	98.4	>90	0.94
SSR (Method #1)	1	5 × 20	0.135[e]	1662	98.3	94.8	0.26
SSR (Method #2)[a]	1	5 × 20	0.036[f]	2057	97.8	89.6	0.21
SMB (Lab scale, Contractor #1)	8	4.8 × 10.7	33	1454	99.5	91	0.17
SMB (Lab scale, Contractor #2)	8	4.8 × 10.7	19	1105	>98	98.4	0.16
SMB (Process scale, Contractor #3)	6	20.2 × 10.9[b]; 20.2 × 8.6[c]	247	4100	98.4	93	0.11

[a] Purity and recovery values are slightly out of specification.
[b] Average length of columns 1–5.
[c] Length of column 6.
[d] Proof-of-principle study. Only one 1200 mg injection was performed. Production rate assumes a 14 min stacked injection cycle time.
[e] Proof-of-principle study. One hundred and fifty cycles performed. Steady state reached after about 10 cycles.
[f] Proof-of principle study. Thirty cycles performed. Steady state reached after about 10 cycles.

3.2. Closed-loop recycling

The two-step preparative process described above is rather labor-intensive in that two automated methods, one each for steps 1 and 2, must be developed. Also, from a chromatographic perspective, this process is an inefficient method of recycling because the partial separation accomplished in the first step is lost when the profile is collected: upon re-injection the separation of Enantiomers 1 and 2 must begin anew. Closed-loop recycling with peak shaving is generally considered to be a more efficient method of recycling [12]. In this technique, as the chromatographic profile elutes from the outlet of the column it is sent through the mobile phase pump directly to the inlet of the column. Thus, the partial separation accomplished during the previous trip(s) through the column is preserved. The leading and trailing edges of the profile will eventually be quite pure in Enantiomers 1 and 2, respectively. Thus, the leading and trailing edges can be shaved as needed during each cycle to achieve the desired purities of each enantiomer. The profile can be sent through the column as many times as necessary to achieve the desired purity and recovery.

An experiment was performed to ascertain whether an improvement in productivity could be expected if the racemic pharmaceutical intermediate were separated using closed loop recycling. As shown in Fig. 2, 1200 mg of racemate were injected on the 50 mm × 200 mm column. The resolution of the enantiomers increased each cycle, and the leading and trailing edges of the profile were shaved as needed to achieve the desired purity and recovery of Enantiomer 2. As shown in Table 2, the purity (98.4% ee) and recovery (>90%) of Enantiomer 2 were within the project specifications. The solvent usage was 0.94 l/g of racemate and was somewhat higher than that obtained using the two-step preparative process. The productivity (514 g racemate per day/kg CSP) was calculated assuming a stacked injection cycle time of 14 min and was somewhat higher than the two-step result.

3.3. Steady state recycling (SSR)

Two SSR methods, summarized in Table 3 were developed as proofs-of-principle in order to compare SSR to the other chromatography techniques. The purpose was not to produce significant quantities of the desired enantiomer, as sufficient quantities were already on hand from the SMB separations discussed below. Rather the purpose was to determine the relative usefulness of SSR and where it might reasonably be used in future projects.

In SSR Method #1, 900 mg of racemate were injected each cycle (see Fig. 3). With a cycle time of 3.25 min, this gave a production rate of 1662 g racemate/kg CSP per day. The purity and recovery of Enantiomer 2, as shown in Fig. 3 were 98.3% ee and 94.8%, respectively, and were well within project specifications. This SSR production rate represents an increase by a factor of 3.2 relative to closed-loop recycling and an increase by a factor of 3.8 relative to the two-step

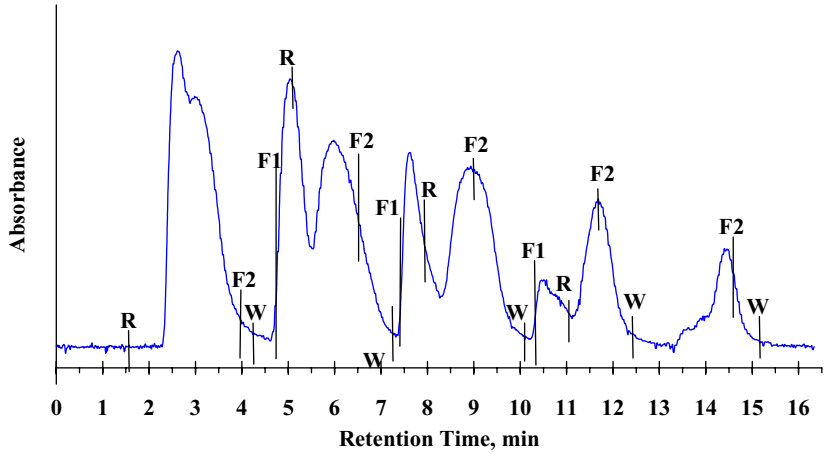

Fig. 2. Closed-loop recycling with peak shaving in which 1200 mg of racemate were injected.. Column dimensions were 5.0 cm i.d. × 20 cm. R: recycling, W: waste, F1: fraction 1, F2: fraction 2.

Table 3
Conditions and event times for the SSR processes shown in Figs. 3 and 4

Event	Description	Flow rate: 125 ml/min, cycle time: 3.25 min (Fig. 3) Time, min		Flow rate: 125 ml/min, cycle time: 3.50 min (Fig. 4) Time, min	
		Absolute	Relative	Absolute	Relative
1	Inject valve to load position, valve W opened	0.0	–	0.0	–
2	Load injection loop	0.17	–	0.17	–
3	Collection of Fraction 1, valve F1 opened	0.18	0.0	0.25	0.0
4	End of Fraction 1	0.78	0.60	0.90	0.65
5	Injection of sample	1.18	1.00	1.25	1.00
6	Collection of Fraction 2, valve F2 opened	2.26[a]	2.08[a]	2.33[a]	2.08[a]
8	Mobile phase pump switched off	3.10	2.92	3.17	2.92

[a] These are the values entered into the LC ReSponder control program. To determine the actual cut points of Fraction 2 on the chromatogram as shown in Fig. 3, subtract 0.24 min. This correction is necessary because for these events, the volume of the injection loop (30.0 ml) separates the detector and the collection valves. At a flow rate of 125 ml/min, this represents a lag time of 0.24 min between passage of the cut point through the detector and its arrival at the collection valve manifold. Similarly, for Fig. 4 (loop size 40 ml) subtract 0.32 min.

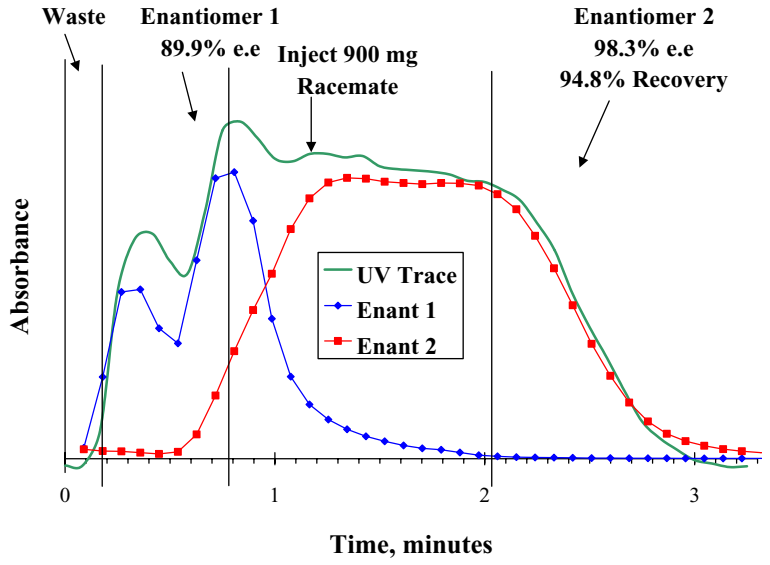

Fig. 3. SSR Method #1 in which 900 mg of racemate were injected each cycle. Column dimensions were 5.0 cm i.d. × 20 cm. The UV trace is indicated by the line with no symbols, the (◆) indicates the concentration profile of enantiomer 1, and the (■) indicates the concentration profile of enantiomer 2.

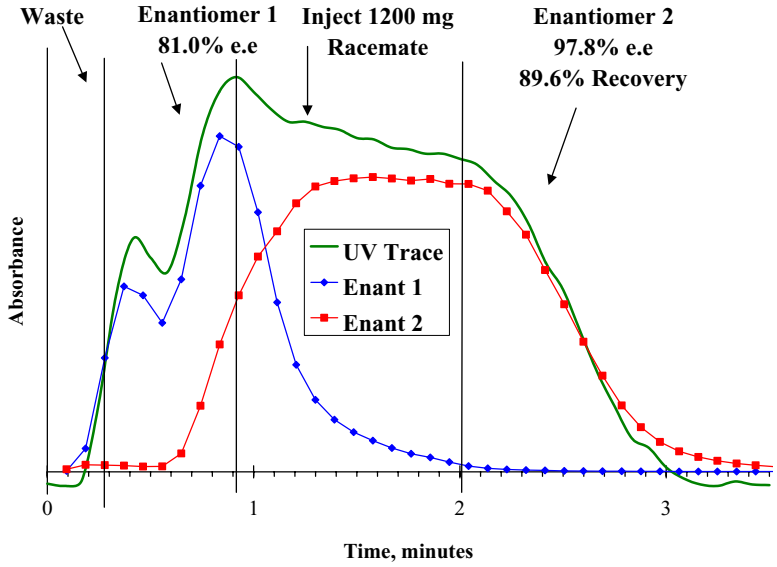

Fig. 4. SSR Method #2 in which 1200 mg of racemate were injected each cycle. Column dimensions were 5.0 cm i.d. × 20 cm. Note that the purity and recovery of enantiomer 2 are slightly outside project specification of >98% ee and 90%, respectively. The UV trace is indicated by the line with no symbols, the (◆) indicates the concentration profile of enantiomer 1, and the (■) indicate the concentration profile of enantiomer 2.

preparative process. Also, at 0.26 l/g of racemate, the SSR solvent usage was significantly lower than the those of the recycling techniques (by a factor of 2.7 compared to the two-step preparative process and by a factor of 3.6 compared to closed-loop recycling). These results are summarized in Table 2.

To ascertain the distribution of the enantiomers within the steady state profile, fractions were collected across the profile every 0.08 min. Each fraction was then analyzed by HPLC. The results are shown in Fig. 3. The data with diamonds represent Enantiomer 1; the data with squares represent Enantiomer 2; and the top curve is the UV trace of an earlier steady state profile. As shown, Fraction 1 contains some Enantiomer 2, but the recovery of Enantiomer 2 in Fraction 2 is greater than the required 90%. Fraction 2 is essentially pure in Enantiomer 2, the concentration of Enantiomer 1 having decreased nearly to zero before the Fraction 2 collection point.

In SSR Method #2 (Fig. 4), 1200 mg were injected every 3.5 min to give a production rate of 2057 g racemate/kg CSP per day. Solvent usage decreased to 0.21 l/g of racemate. As shown in Fig. 4, the purity and recovery of Enantiomer 2 (97.8% ee and 89.6%) were slightly outside project specifications. It is likely that minor adjustments, such as shifting the injection point slightly to the right and taking a slightly larger first fraction [25], would have improved the yield and purity of Enantiomer 2. Unfortunately, time constraints precluded such tweaking of the SSR parameters. However, assuming that such minor adjustments would have been successful, the SSR Method #2 gave a production rate 4.0 times greater than closed-loop recycling and 4.7 times greater than the two-step preparative process. These results are summarized in Table 2.

3.4. Lab-scale SMB

The lab-scale SMB techniques used in this study, whose operating conditions are shown in Table 1, can be described as conventional SMB in that in each case all eight columns had the same length. As summarized in Table 2, the lab-scale SMB procedure used at Contractor #1 gave a production rate of 1454 g racemate/kg CSP per day, a purity and recovery of the desired enantiomer of 99.5% ee and 91%, and a solvent usage of 0.17 l/g racemate. The lab-scale SMB procedure used at Contractor #2 gave a production rate of 1105 g racemate/kg CSP per day, a purity and recovery of the desired enantiomer of >98% ee and 98.4%, and a solvent usage of 0.16 l/g racemate. As shown in Table 2, these results are comparable to those obtained with SSR Method #1. The solvent usage obtained with the SSR Methods were higher than those obtained with the lab-scale SMB methods. We have reported similar results previously, i.e. lower SMB solvent usage when the SSR and SMB production rates are comparable [25].

3.5. Process-scale SMB

The process-scale SMB procedure used in this study is unconventional in that one column (column 6) is shorter than the other five. This shorter column corrects for dead volume in the system caused by the recycle pump and other components [29]. As fewer columns and less CSP are required, this SMB technique, when optimized, frequently results in higher production rates relative to conventional SMB.

The process-scale SMB operating conditions are given in Table 1, and the results of the separation are shown in Table 2. At 4100 g racemate/kg CSP per day, the

process-scale SMB separation gave by far the largest production rate. The solvent usage, 0.11 l/g racemate, was also the lowest of the techniques. The purity and recovery of the desired enantiomer, 98.4% ee and 93%, were comparable to the other techniques.

4. Discussion

As a general principle it is possible to obtain the required purity and recovery of the desired components regardless of which chromatographic technique is chosen for a given separation. This is reflected in Table 2 in which all of the techniques, with the exception of SSR Method #2, met the purity and recovery specifications of the separation. Thus, the most meaningful way to compare preparative- and process-scale techniques is to compare production rate and solvent consumption results. These quantities are the most important factors in determining operating costs. The higher the production rate, the shorter the time required to process a sample using a given amount of CSP. This translates into lower utility and labor costs. The lower the solvent consumption, the lower the amount of solvent that must be purchased, evaporated/distilled, and disposed of as hazardous waste.

Using the criteria of production rate and solvent usage, the six-column SMB technique used in the process-scale separation is clearly the superior technique. Relative to the scaled-up eight-column SMB method used in the lab-scale work, the six-column method would require significantly less CSP, processing time, and solvent recovery for a given throughput. Recently a new enhanced SMB process, the Varicol process, has been reported [27,30,31]. Preliminary modeling suggests that if the Varicol process were used, the productivity could be increased by 10%, and the solvent consumption could be reduced 7% relative to the six column SMB process.

A comparison of the SSR and lab-scale SMB results is interesting. As shown in Table 2, the two techniques had comparable production rates. Also, the SSR solvent usage was higher than those of the lab-scale SMB runs, but was significantly lower than the two-step preparative process and the closed-loop recycling process. Thus, the operating costs of a suitably scaled SSR system would have been somewhat higher to those encountered with the lab-scale SMB system, but significantly lower than those of the two-step preparative and the closed-loop recycling processes. Modeling work performed by Contractor #3 suggests that a fully optimized 8-column SMB process would give a production rate of 2500 g racemate/kg CSP per day with a solvent usage of 0.17 l/g of racemate (comparable to, but in both respects better than, SSR Method #2). And of course, the productivity of the lab-scale SMB run could have been improved still further by using the same six-column method used in the process-scale SMB run. However, at the time of this work, simulation software was not available for the lab scale systems that would predict the flow rates and switching time for either the six-column process or the fully optimized eight-column process. Thus, it was felt that the extra effort needed to develop an optimized SMB method empirically was not justified for this scale of separation (33 kg for Contractor #1 and 19 kg for Contractor #2). It appears, therefore, that at the scale of tens of kilograms of racemate where it might not be worth the effort to fully optimize an SMB separation, SSR could be a viable choice. Other factors are that for systems of comparable capacity, the complexity and capital cost of an SSR system are less than those of an SMB system.

Finally, as shown in Table 2, the production rate for closed-loop recycling was somewhat higher than that obtained with the two-step preparative process. However, the two-step preparative process used less solvent than the closed-loop recycling method. Thus, the results of this study show no clear advantage of closed-loop recycling over the two-step preparative process.

5. Conclusions

For the separation discussed in this study, the six-column SMB technique was the best choice for the process-scale process in which 247 kg of racemate were separated. However, for the moderate scale separation (33 kg for Contractor #1 and 19 kg for Contractor #2) where complete optimization of the SMB separation was not justified, productivity and solvent consumption obtained with the lab-scale SMB system appear to be comparable to those expected with an SSR system of similar capacity. Thus, SSR appears to have a place at moderate scales (tens of kg of racemate). Finally, when neither SSR nor SMB is available, both external and closed-loop recycling appear to give acceptable results for those separations in which complete resolution of the component(s) of interest is not possible using preparative sample loads.

References

[1] L. Miller, H. Bush, J. Chromatogr. 484 (1989) 337.
[2] L. Miller, C. Weyker, J. Chromatogr. 511 (1990) 97.
[3] L. Miller, R. Bergeron, J. Chromatogr. 648 (1994) 381.
[4] L. Miller, C. Weyker, J. Chromatogr. A 653 (1994) 219.
[5] L. Miller, D. Honda, R. Fronek, K. Howe, J. Chromatogr. A 658 (1994) 429.
[6] S.B. Thomas, B.W. Surber, J. Chromatogr. 586 (1991) 265.
[7] C.J. Shaw, P.J. Sanfilippo, J.J. McNally, S.A. Park, J.B. Press, J. Chromatogr. 631 (1993) 173.
[8] Katti, P. Erlandsson, R. Dappen, J. Chromatogr. 590 (1992) 127.
[9] E.R. Francotte, Switz. Chimia 51 (10) (1997) 717.
[10] E.R. Francotte, in: S. Ahuga (Ed.), Switz. Chiral Sep., American Chemical Society, 1997, p. 271.
[11] E. Kuesters, Switz. Chim. Oggi 14 (7–8) (1996) 39.

[12] J. Dingenen, J.N. Kinkel, J. Chromatogr. A 666 (1994) 627.
[13] R.M. Nicoud, G. Fuchs, P. Adam, M. Bailly, E. Kusters, F.D. Antia, R. Reuille, E. Schmid, Chirality 5 (1993) 267.
[14] E.R. Francotte, P. Richert, J. Chromatogr. A 769 (1997) 101.
[15] E. Cavoy, M.F. Deltent, S. Lehoucq, D. Miggiano, J. Chromatogr. A 769 (1997) 49.
[16] E. Francotte, P. Richert, M. Mazzotti, M. Morbidelli, J. Chromatogr. A 796 (1998) 239.
[17] S. Nagamatsu, K. Murazumi, S. Makino, J. Chromatogr. A 832 (1999) 55.
[18] J. Strube, A. Jupke, A. Epping, H. Schmidt-Traub, M. Schulte, R. Devant, Chirality 11 (1999) 440.
[19] L.S. Pais, J.M. Loureiro, A.E. Rodrigues, Sep. Purificat. Technol. 20 (2000) 67.
[20] J. Strube, S. Haumreisser, H. Schmidt-Traub, M. Schulte, R. Ditz, Org. Proc. Res. Dev. 2 (5) (1998) 305.
[21] E. Francotte, T. Leutert, L. La Vecchia, F. Ossola, P. Richert, A. Schmidt, Chirality 14 (2002) 313.
[22] D.W. Guest, J. Chromatogr. A 760 (1997) 159.
[23] C.M. Grill, J. Chromatogr. A 796 (1998) 101.
[24] I. Quiñones, C.M. Grill, L. Miller, G. Guiochon, J. Chromatogr. A 867 (2000) 1.
[25] C.M. Grill, L. Miller, J. Chromatogr. A 827 (1998) 359.
[26] G. Guiochon, J. Chromatogr. A 965 (2002) 129.
[27] J. Bléhaut, O. Ludemann-Hombourger, S. Perrin, Chim. OGGI Chem. Today 19 (2001) 24 September.
[28] M. McCoy, Chem. Eng. News, 19 June (2000) 17.
[29] G. Hotier, C. Cohen, R.M. Nicoud, US Patent 5,578,216 (1996).
[30] P. Adam, R.M. Nicoud, M Bailly, O. Ludemann-Hombourger, US Patent 56,136,198 (2000).
[31] O. Ludemann-Hombourger, G. Pigorini, R.M. Nicoud, D.S. Ross, G. Terfloth, J. Chromatogr. A 947 (2002) 59.