Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1156 (2007) 220–227
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Rapid and high throughput separation technologies—Steady state recycling and supercritical fluid chromatography for chiral resolution of pharmaceutical intermediates

Tony Q. Yan *, Carlos Orihuela

Separation Group, Process Analytical Science, Department of Chemical Process Development and Research, Amgen, Inc., Amgen One Center Dr., Thousand Oaks, CA 91320, USA

Available online 6 April 2007

Abstract

The SSR and SFC techniques were used for the enantiomeric resolution of three pharmaceutical intermediates at various sample scales. The separation conditions, the sample purities and yields, the productivities and the solvent consumptions were discussed in three case studies in this paper. In case (I), the SSR process was used for a low selectivity resolution of 2.0 kg of pharmaceutical intermediate. By using this separation process, a productivity of 750 g racemate/kg stationary phase/day was achieved, while solvent usage was minimized (∼200 l/kg racemate). Case (II) pertained to the effectiveness of the SSR process. Productivity using SSR techniques increased by a factor of 4.5, while solvent usage decreased by a factor of 4.1 when compared to the productivity and solvent usage of batch HPLC. Case (III) compared SFC purification to HPLC purification. The SFC process was more effective in terms of an increase in productivity and a reduction in solvent usage. Based on these results, it appears that SSR and SFC are very useful choices at the early stage of the drug development for a high throughput and a rapid turn around of samples.

Keywords: Preparative chromatography; Chiral separation; Steady state recycling; Supercritical fluid chromatography

1. Introduction

Most important drugs in the market are chiral. The enantiomers exist as either right-handed or left-handed molecules. One enantiomer is effective as a drug, the other is ineffective at best and occasionally toxic. Therefore, the chiral separation has been a very important aspect in the pharmaceutical industry. Chromatographic resolution of pharmaceutical racemate has been proven to be the most efficient approach for the generation of small quantities of enantiomers during early pharmaceutical research and development [1–5]. The development timeline for a drug candidate can be significantly shortened by using this technique. The use of chromatographic resolution allows more time for synthetic chemists to optimize their process. Hence, the chiral separation of racemate by using preparative chromatography has rapidly become a standard approach for the generation of enantiomers in pharmaceutical research and development in recent years [6–8]. Requirements for chromatographic resolutions include the ability to develop analytical chiral separation methods rapidly and the subsequent ability to scale up separations to the preparative columns. These requirements are critical for meeting challenge timelines.

The most frequently used preparative chromatography techniques for chiral resolution include batch high pressure liquid chromatography (HPLC), simulated moving bed (SMB), steady state recycling (SSR), and supercritical fluid chromatography (SFC). These techniques can be used to resolute pharmaceutical racemates at various scales [5,9–11]. Batch HPLC requires good selectivity and uses a large quantity of solvent [12,13]. This process has been widely used for providing rapid access to enantiopure materials because it can easily scale up from analytical to preparative conditions. SMB has become a routine technique for large scale chiral separation [14–16]. SMB and other multicolumn chromatography approaches predominate at an industrial scale (greater than hundreds of kilogram) due to a high efficiency. However, the initial capital and facility cost for SMB can be substantial. Packing and testing of the

* Corresponding author. Tel.: +1 8054470356.
E-mail address: qiy@amgen.com (T.Q. Yan).

0021-9673/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2007.03.124

multicolumns can be time consuming as well. A relatively new technique, SSR, shows promise in approaching the performance of SMB for the large-scale separations, and its simplistic system design (only use one column) makes it low in cost and high in efficiency [17]. SSR is a chromatographic technique used mainly for the separation of binary components. The pure fractions are collected at either end of the elution profile, while the overlap portion is circulated onto the column, and fresh feed is injected in the middle of the profile. At a steady state, the amounts of sample collected and injected are equal. The method development and process optimization for SSR normally takes less than a day. The solvent usage of SSR is, in general, less than half the solvent usage of batch HPLC. The instrument cost is only ∼25% of a SMB system. Thus, SSR is "phase appropriate" technology for the early development support of the chiral separation of samples ranging from 100 g to a few kilograms [18]. However, the efficiency of the SSR process is lower than the efficiency of the SMB process, and SSR can only be compared to a nonoptimized SMB process. Another relatively new technique, SFC, has also been proven to be a very efficient tool for small quantity chiral separations [19,20]. Due to a high mass transfer of CO_2, the run time for SFC is much shorter than the run time for HPLC. In the pharmaceutical industry, SFC has become a routine technique for purification support during the discovery stage. Recently, SFC has also been used more frequently for the purification support in the development stage. SFC uses supercritical CO_2 as a main solvent. This usage conserves the organic solvent. It also reduces the certain down stream working times such as rotovaping and waste disposal. Supercritical CO_2 also has a high mass transfer and a low viscosity. These properties allow SFC to flow much faster than HPLC and it also allows for the use of more efficient media, such as a small particle chiral stationary phase (5 μm) for tight separations. Morever, SFC has a unique selectivity which often offers an alternative method to the HPLC purification. However, for large scale chiral separation, the capital and facility cost for SFC can still be substantial.

Examples of a few chiral separations for three pharmaceutical intermediates using SSR, SFC and batch HPLC were discussed in this paper. These intermediates were used for the animal toxicity studies in the early stage of the drug development. These animal toxicity studies ranged from 4 days to 28 days of testing. Final sample testing amounts ranged from ∼5 g (4 day test) to a few hundred grams (28 day test). A significantly high amount of intermediates were needed to make these final testing compounds. A >98%ee (enantiomer excess) purity was required and a >90% yield was also required for the chiral separation of these intermediates in general. A rapid turn around time was crucial for meeting scheduled animal testing dates and shortening the drug development times. Therefore, the time allowed for each project was very limited. This time limitation prevented purification scientists from fully optimizing their purification conditions. Detailed information, such as the "pareto curves" for each compound, was not studied in this paper. However, the separation results, the sample turn around time, the productivity, and the solvent cost are discussed and compared using our laboratory results.

2. Experimental

2.1. Material

The racemates separated in this paper are the pharmaceutical intermediates belonging to and synthesized at Amgen (Thousand Oaks, CA, USA).

The mobile phases used in all case studies were varied, and were all reagent grades from a variety of sources. The chiral stationary phases were obtained from Chiral Technologies (Exton, PA, USA) as 20 μm bulk packing materials. These bulk materials were packed into MODCOL spring load columns from Grace (Hesperia, CA, USA) at a packing pressure of 50 bar. The prepacked columns were also used for HPLC and SFC separations. These prepacked columns were purchased from Chiral Technologies (Exton, PA, USA). The particle size of prepacked column for HPLC separation was 20 μm; and for SFC separation, it was 5 μm.

2.2. Batch preparative HPLC

The batch preparative chromatographic system, used in case (II), was a Pro-star system from Varian (Wakefield, RI, USA). In this study, the preparative HPLC run was performed using a prepacked column from Chiral Technologies (Exton, PA, USA) with an inner diameter of 5.0 cm; the column was packed to a length of approximately 50 cm with 0.5 kg of chiral stationary phase of ChiralPAK-AS. The mobile phase was 5% isopropyl alcohol (IPA) in heptane. The flow rate was 200 ml/min. The column was jacketed, and the mobile phase passed through a heat exchanger before entering the column. The heat exchange fluid was thermostated at 30 °C before entering the column jackets and the heat exchanger. The racemate was only able to dissolve in 20% IPA in heptane at a concentration of 20 mg racemate/ml. The sample solution was then filtered through a 30–50 μm sintered glass funnel before injecting into the system.

2.3. SSR

The SSR experiments were performed at Amgen in case (I), (II), and (III) using a steady cycle system from Hitachi (San Jose, CA, USA). The instrument consists of four 20 ml injection loops which were connected in series to give the desired injection volume. Conventional recycling methods were used to develop the SSR process. Recycled data from the first cycle and the second cycle were graphically overlaid using Microsoft Excel in order to determine approximate position for isomer fraction cuts and midpoint of the profile for injection of racemate. The detailed description of SSR method development was addressed by Grill and Miller [21]. In case (I), the SSR run was performed using a MODCOL spring column with an inner diameter of 10.3 cm, and was packed to a length of approximately 40 cm with 2.0 kg of chiral stationary phase of ChiralPAK-AD. The column and the mobile phase were also thermostated at 30 °C. The mobile phase was 100% methanol. The racemate was dissolved in methanol at a concentration of 100 mg racemate/ml. The sample solution was then filtered through a 30–50 μm sin-

tered glass funnel before injecting into the system. In case (II), SSR purification was performed on the same column as the batch HPLC (column information was given in Section 2.2). The mobile phase for the SSR separation in the case (II) study was 20% IPA in heptane. The flow rate was 200 ml/min. The sample was dissolved in 20% IPA in heptane at a concentration of 20 mg racemate/ml. The sample solution was also filtered before injecting into the system. In case (III), the Chiralcel-OD material was packed using a MODCOL spring column with an inner diameter of 10.3 cm. It was also packed to a length of approximately 40 cm with 2.0 kg of chiral stationary phase. The column and the mobile phase were also thermostated at 30 °C. The mobile phase was 100% acetonitrile. The flow rate was 400 ml/min. The racemate was dissolved in acetonitrile at a concentration of 20 mg racemate/ml. The sample solution was filtered through a 30–50 μm sintered glass funnel before injecting into the system.

2.4. Preparative SFC

The preparative SFC system used in case (III) was Supersep 30/50 from Novasep (Boothwyn, PA, USA). The flow rate was 170 g CO_2/min. The operating pressure was 100 bars. The SFC run was performed using a pre-packed column (3 cm I.D. × 25 cm length, 100 g of chiralcel-OD-H, 5 μm) from Chiral Technology (Exton, PA, USA). The column was placed in an oven and thermostated at 40 °C. Methanol was used as a co-solvent. The percentage of the methanol was about 20% in supercritical fluid CO_2. The racemate was dissolved in methanol. The concentration was 20 mg racemate/ml. The sample solution was filtered through a 30–50 μm sintered glass funnel before injecting into the system.

3. Results and discussion

3.1. Case (I): SSR separation of 2.0 kg pharmaceutical intermediate

The racemate was a proprietary pharmaceutical intermediate. This intermediate was used for a 28-day animal toxicity study. The compound contained a hydroxyl group and an amine group at the asymmetric center. The timeline was short. Thus, a week turn around time was highly desirable. The sample had good solubility in polar organic solvents such as methanol and acetonitrile. However, the sample had much lower solubility in the presence of nonpolar solvent such as the mixture of IPA and heptane. The sample solubility was estimated by weighting small amount of sample into the vials. A certain amount of selected solvents were added into the vials to make concentrations of solution at 10 mg/ml, 20 mg/ml, 30 mg/ml, 50 mg/ml, 100 mg/ml and 150 mg/ml. The contents in the vials were subsequently stirred for at least 30 min at a temperature of 30 °C. The sample solubility in case (I) was estimated to be >100 mg/ml in methanol. The temperature of 30 °C was chosen by considering sample stability and solubility. Sample stability was not fully investigated because the compound was at an early stage of the development. Synthetic chemist gave us a precaution to avoid

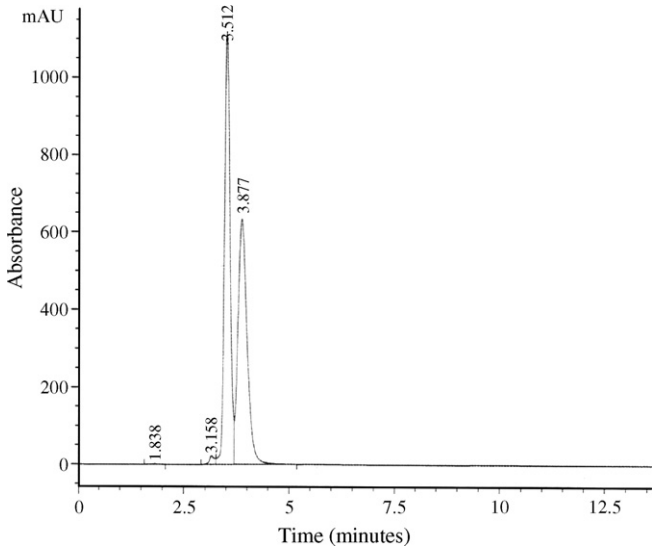

Fig. 1. Analytical HPLC separation. Analysis conducted on ChiralPAK-AD column (250 mm × 4.6 mm I.D.), detection at 230 nm. Mobile phase: 100% methanol, flow rate 1 ml/min.

any prolong operations at an elevated temperature of 35 °C or higher. For analytical method development, various chiral stationary phases (ChiralPAK-AD, ChiralPAK-AS, Chiralcel-OD, Chiralcel-OJ, Whelk-O, RR, Kromasil TBB and Whelk-O, SS) and solvents (methanol, acetonitrile, ethanol, IPA and mixture of IPA and heptane) were screened for the best conditions. The ChiralPAK-AD column in 100% methanol was chosen based upon selectivity, retention time, solubility and viscosity considerations. The analytical HPLC chromatogram is shown in Fig. 1. In this figure, the resolution of two enantiomers is tight and retention time is short. SSR method was selected for this separation because SSR is beneficial for low-resolution separations with short capacity factors. SSR method development starts with conventional recycling. The sample loading tests were performed on the three conventional recycle process. Three cycles were normally performed in order to obtain the best estimation for the cycle time. The best sample loading can be estimated by observing a ∼2/3 peak valley on the first cycle and a close to baseline separation in the third cycle. The three conventional cycle processes are described in Fig. 2, and the overlay graphic from the first cycle to the second cycle is described in Fig. 3. From Figs. 2 and 3, the cycle time, the peak collection time, and the injection time were estimated. Once these time events were obtained, the steady state recycling process was then started. It took ∼5–10 cycles to reach the steady state. The material, generated before a steady state, is often below the specification. We normally recombined it into the crude racemate for reprocessing. The steady state recycle profile is shown in Fig. 4. A detector signal (ascending height) of ∼3% of the full scale was used as a trigger point to start a timer. Peak-1 and peak-2 collection time and injection time events were then defined relative to this trigger time. In this separation, approximately 52 s of clean fraction of peak-1 was collected and followed by ∼3.5 min of recycling the middle overlap profile back to the column. Then, approximately 2 min of clean fraction of peak-2 was collected at the end of the

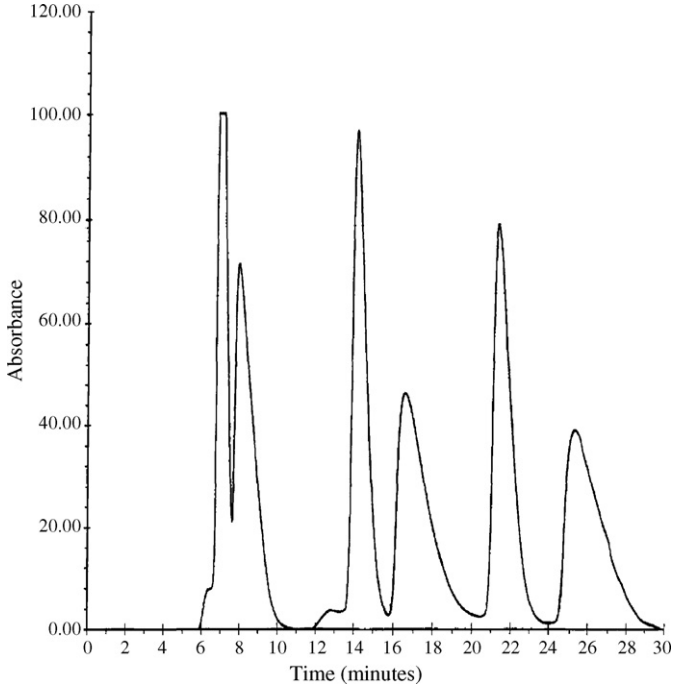

Fig. 2. SSR method development—three initial cycles in case (I) study, preparative column: ChiralPAK-AD; column size: 4 in. × 40 cm; mobile phase: methanol; flow rate: 400 ml/min.

profile. The cycle time was ∼7.2 min. Approximately, ∼7.5 g of racemate was loaded into the column during each cycle. The peak-1 and peak-2 were collected into the 20 liter of plastic carboy. The carboy of peak-1 and peak-2 were then analyzed using HPLC to determine the purities. If the purity met the requirement, the carboy was rotovaped to dryness. At the end of the separation, the fractions were collected across the profile vary for ∼0.3 min in order to understand the distribution of the enantiomers within the steady state profile. Each fraction was then

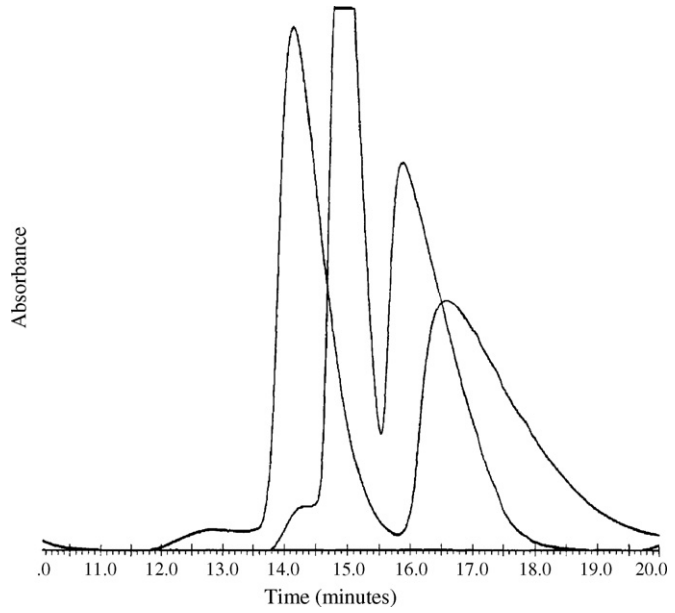

Fig. 3. SSR method development—overlay profile in case (I) study.

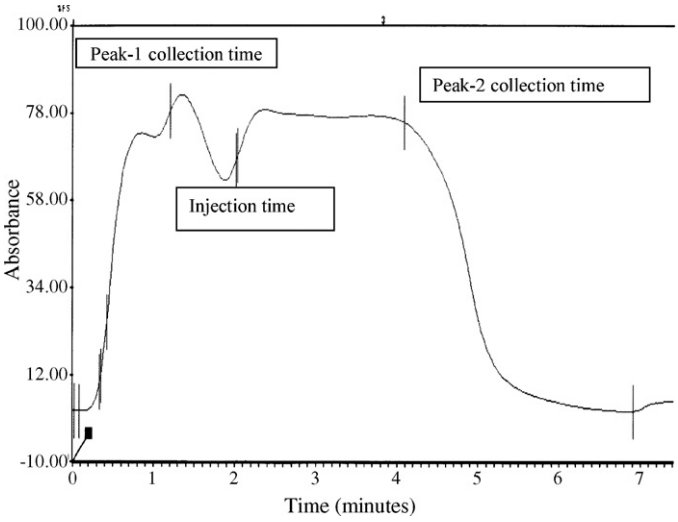

Fig. 4. SSR steady state profile in case (I) study.

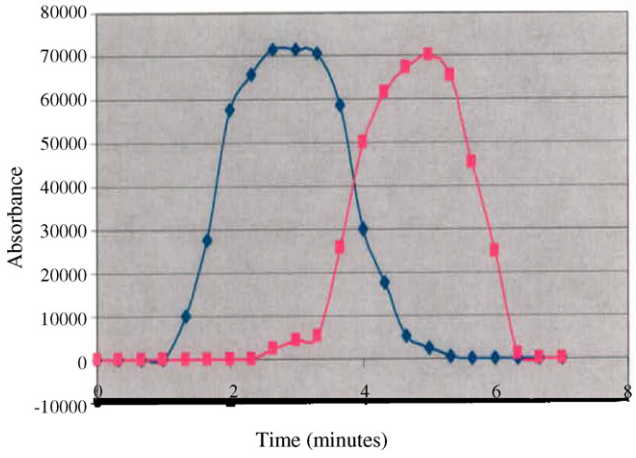

Fig. 5. SSR internal profile in case (I) study.

analyzed by HPLC as shown in Fig. 5. In this figure, the fractions collected for peak-1 and peak-2 are essentially pure, the peak-1 was collected before the enantiomer-2 appears and the concentration of enantiomer-2 having decreased nearly to zero before the fraction-2 collection event. ∼250 cycles were performed on the separation of 2.0 kg racemate. The total purification time for the 2.0 kg racemate was less than 30 h. The solvent usage was ∼200 l/kg racemate. The purification results are listed in Table 1. The purity of the desired enantiomer was 98.2%ee and the purity of the undesired enantiomer was 97.6%ee. A total of ∼968 g of the desired enantiomer was recovered, thus, the yield was estimated to be ∼96.8%. This yield was defined as the desired enantiomer yield [(the amount of the desired enan-

Table 1
Productivity, solvent usage, purity and yield of SSR process in case (I) study

	Productivity (g racemate/kg CSP/day)	Solvent consumption (l/g racemate)	Purity (%ee)	Yield (%)
SSR	750	0.20	98.2	96.8

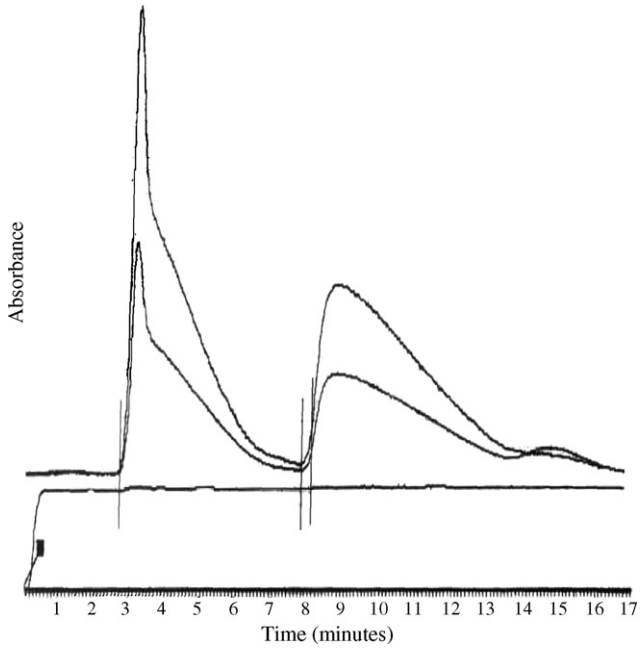

Fig. 6. Preparative batch HPLC separation in case (II) study, preparative column: ChiralPAK-AS; column size: 2 in. × 50 cm; mobile phase: 5% IPA in heptane; flow rate: 200 ml/min.

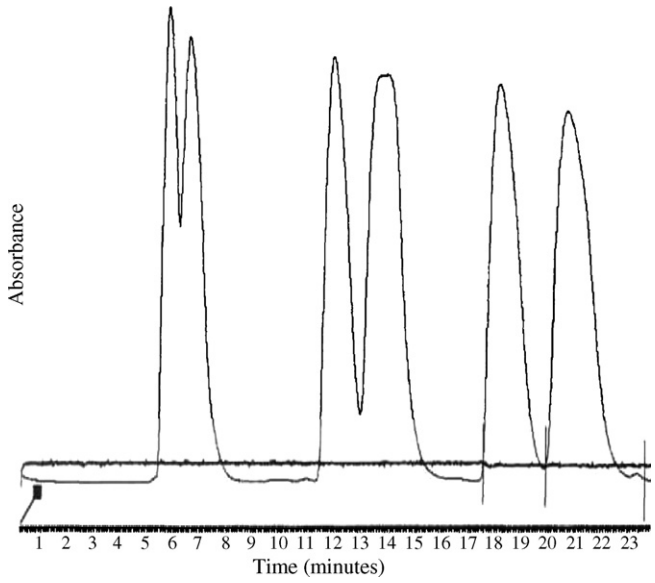

Fig. 7. SSR method development—three initial cycles in case (II) study, Ppreparative column: ChiralPAK-AS; column size: 2 in. × 50 cm; mobile phase: 20% IPA in heptane; flow rate: 200 ml/min.

tiomer recovered after the purification/the amount of the desired enantiomer presented in the origin) × 100%]. The high productivity on this sample can also be attributed to the high sample solubility in the mobile phase

3.2. Case (II): batch HPLC and SSR separations of a 200 g pharmaceutical intermediate

The reacemate was also an intermediate for the 14-day toxicity test. The compound contained an aromatic group and a hydroxyl group at the asymmetric center. The synthetic chemist needed ∼10 g of each isolated enantiomer immediately in order to test the experiment conditions for the next reaction. The rest of material could be separated within the next 2 weeks. Therefore, the first 20 g of racemate were isolated on batch HPLC because the SSR system was not immediately available at that moment. The various analytical columns (ChiralPAK-AD, ChiralPAK-AS, Chiralcel-OD, Chiralcel-OJ, Kromasil TBB and Whelk-O, SS) and mobile phases (methanol, acetonitrile, ethanol, IPA and mixture of IPA and heptane) were screened. No chiral resolution was apparent on these columns with only the polar organic solvents. The best resolution for the analytical column was found on the ChiralPAK-AS column. A baseline resolution was obtained on ChiralPAK-AS column with a mobile phase of 5% IPA in heptane. However, the sample had low solubility in this solvent. The sample solution was able to be prepared in 20% IPA in heptane at a concentration of 20 mg/ml. But, the sample solution was not able to be prepared in the mobile phase (5% IPA in heptane) at this concentration. The purification was then scaled up to a prepacked column (column information is described in Section 2.2). The preparative HPLC chromatogram is shown in Fig. 6. A baseline resolution was achieved with a 0.5 g sample loading per injection at a cycle time of ∼16 min. A total of 20 injections were made at this condition and ∼10 g of each enantiomer was delivered to the chemist. In Fig. 6, both peak tailing and peak fronting are observed from the chromatogram. The peak tailing is attributed to the low solubility of the sample in the mobile phase. The peak fronting is attributed to a difference between solvents used to dissolve the sample (20% IPA in heptane) with a mobile phase (5% IPA in heptane). It was clear that the batch HPLC was not an efficient method for the separation of the rest of the material. Thus, a more efficient SSR method was used for the rest of 180 g racemate separation. The three conventional cycles for SSR method development are shown in Fig. 7. From this figure, a better peak shape can be observed because using a stronger mobile phase increased sample solubility. The mobile phase of 20% IPA in heptane was used as the mobile phase for SSR separation because SSR separation does not require a baseline separation in order to achieve high purity levels in both peaks. The three conventional cycle processes and the overlay graphic from the first cycle to the second cycle were performed. The cycle time, the peak collection time, and the injection time of SSR were estimated. The steady state recycle profile is described in Fig. 8. Approximately 0.8 g racemate was loaded for each injection at a cycle time of ∼6.1 min. On comparing SSR to batch HPLC, it was found that the cycle time for SSR reduced to ∼2.8 fold, while sample loading increased by ∼1.6 fold. A total of a 4.5 fold productivity increase was evident by using SSR method, and the solvent usage was also significantly reduced. The results of the productivity, the solvent usage, the purity, and the yield are shown in Table 2.

3.3. Case (III): SSR and SFC separations of 770 g of a pharmaceutical intermediate

Our laboratory received two batches of racemate (700 g and 70 g). The racemate was also an intermediate for the 14-

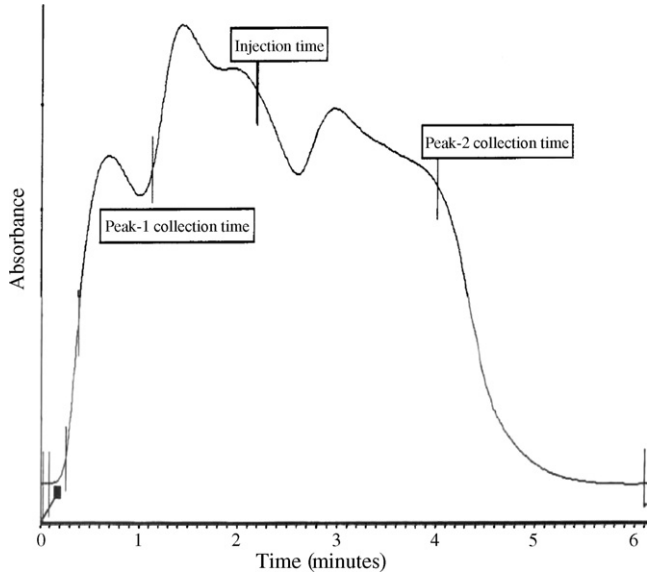

Fig. 8. SSR steady state profile in case (II) study.

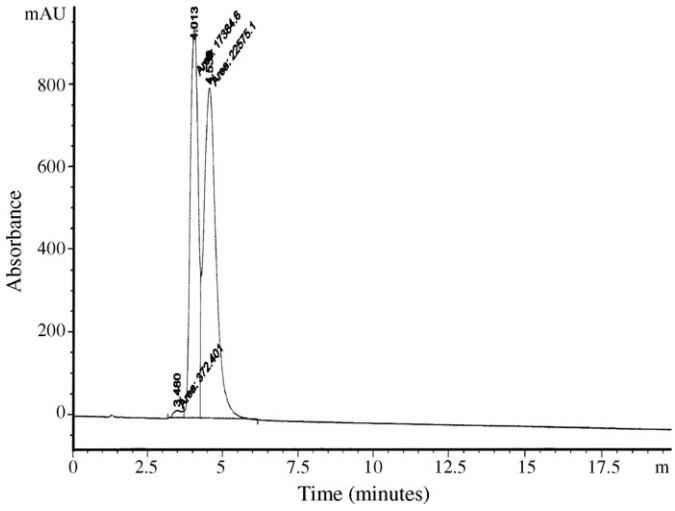

Fig. 9. Analytical HPLC separation. Analysis conducted on Chiralcel-OD column (250 mm × 4.6 mm I.D.), detection at 230 nm. Mobile phase: 100% acetonitrile; flow rate: 1 ml/min.

day toxicity test. The compound contained an amine group and a chlorobenzene group at the asymmetric center. Various analytical HPLC columns (ChiralPAK-AD, ChiralPAK-AS, Chiralcel-OD, Chiralcel-OJ, Whelk-O, RR, Kromasil TBB and Whelk-O, SS) and solvents (methanol, acetonitrile, ethanol, IPA and mixture of IPA and heptane) were screened. Only a limited HPLC resolution was achieved on the Chiralcel-OD with a mobile phase of 100% acetonitrile. The sample solubility was ∼20 mg/ml in this solvent. The analytical HPLC chromatogram is shown in Fig. 9. However, analytical screening on SFC columns showed a very promising separation on Chiralcel-OD-H with a mobile phase of 20% methanol as a co-solvent. The analytical SFC chromatogram is shown in Fig. 10. The solubility of the sample was also found to be ∼20 mg/ml in methanol. Due to a limited SFC column size, [3 cm × 25 cm (I.D. × length)], the timeline to process 700 g racemate would not be acceptable. Therefore, the first batch of 700 g racemate was separated on the HPLC with a SSR method. The second batch of 70 g was separated with SFC. For SSR method development, two initial cycles were performed instead of three cycles due to peak tailing (the peak-2 of the second cycle was tailing into the peak-1 of the third cycle). Therefore, only one data point was obtained in order to estimate the cycle time instead of using an average value from the three cycle process. The second cycle was then overlaid with the first cycle to get the overlay profile. The results of the initial two cycles and overlay profile are shown in Figs. 11 and 12. The cycle time, the injection time, and the peak collection time of SSR process were then estimated from these two figures. The steady state recycle profile is shown in Fig. 13. ∼1.8 g of racemate was loaded on the column, each injection at a cycle time of ∼11.2 min. Due to the peak tailing, the separation efficiency of the SSR process was limited. In contrast, as shown in Fig. 10, SFC offers much better selectivity with a co-solvent of 20% methanol on the chiralcel-OD-H (5 μm particle size) column. By using a more efficient 5 μm column, the peak tailing was minimized. The preparative SFC purification

Table 2
Comparison of productivity and solvent usage of SSR process to batch HPLC in case (II) study

	Productivity (g racemate/kg CSP/day)	Solvent consumption (l/g racemate)	Purity (%ee)	Yield (%)
SSR	378	1.5	98.4	96.2
Batch	86	6.3	98.6	97.0

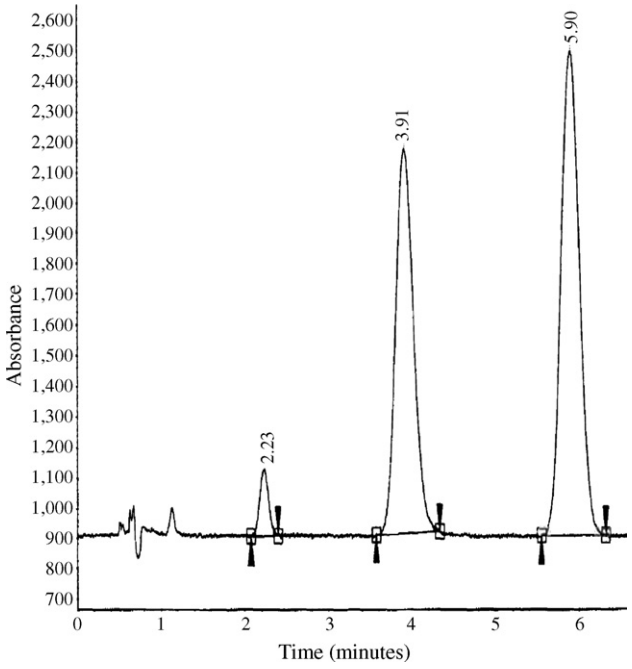

Fig. 10. Analytical SFC separation. Analysis conducted on Chiralcel-OD-H column (250 mm × 4.6 mm I.D.), detection at 230 nm. Mobile phase: 20% methanol as co-solvent; flow rate 5 g CO_2/min; pressure: 100 bar; temperature: 40 °C.

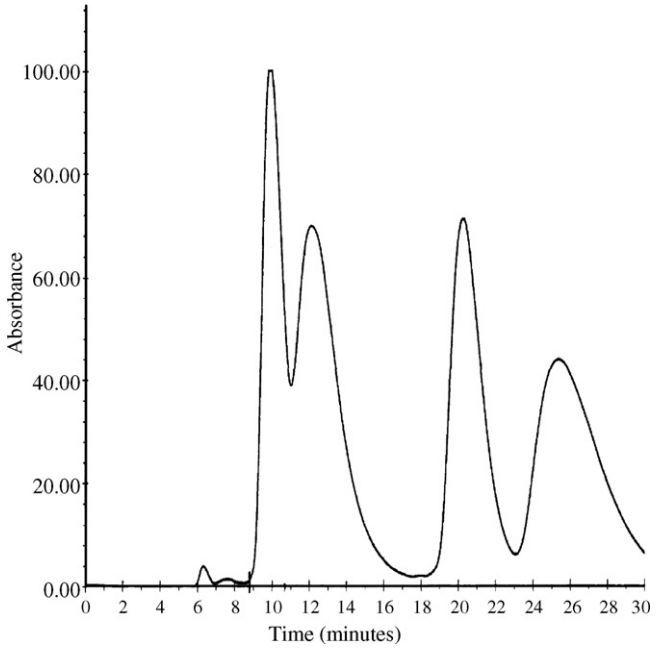

Fig. 11. SSR method development—two initial cycle in case (III) study, preparative column: Chiralcel-OD; column size: 4 in. × 40 cm; mobile phase: 100% acetonitrile; flow rate: 400 ml/min.

for second batch of 70 g racemate is described in Fig. 14. The second batch racemate also contained an impurity peak at ∼5% besides the two enantiomers. The impurity peak also required removal. This impurity was a starting material due to the incompletion of the reaction. ∼70 mg of racemate was loaded on the SFC column each cycle at a cycle time of ∼7 min. As shown in Fig. 14, the impurity peak along with two enantiomer peaks are well separated. The results of the HPLC and SFC purifications are listed in Table 3. From this table, it is clear that the SFC process was more effective in terms of an increase in productivity and a reduction in solvent usage.

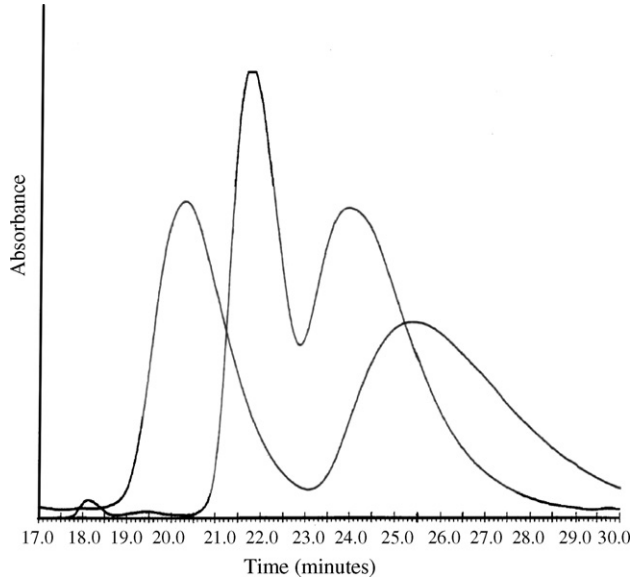

Fig. 12. SSR method development—overlay profile in case (III) study.

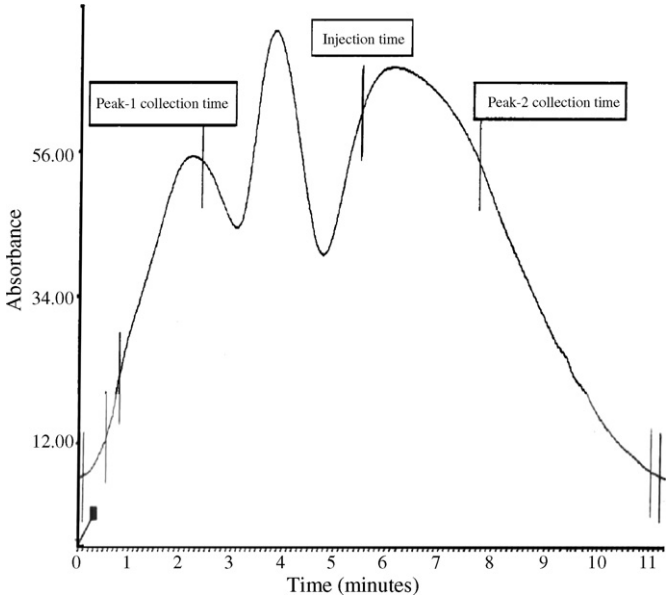

Fig. 13. SSR steady state profile in case (III) study.

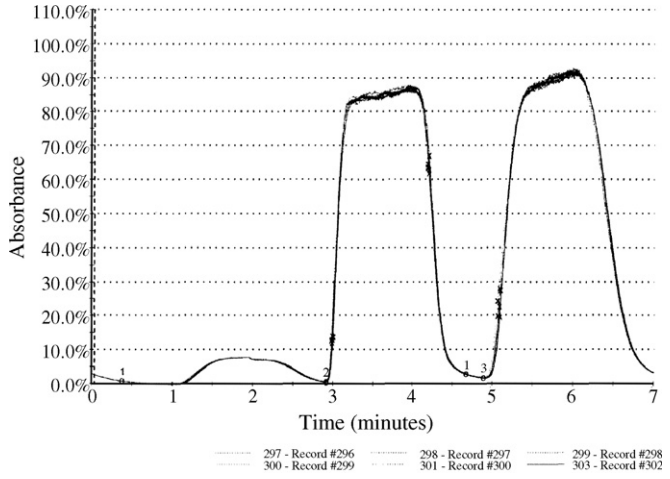

Fig. 14. Preparative SFC separation in case (III) study, preparative column: Chiralcel-OD; column size: 3 × 25 cm; mobile phase: 20% methanol in supercritical CO_2; flow rate: 170 g CO_2/min.

Table 3
Comparison of productivity and solvent usage of SFC process to HPLC process in case (III) study

	Productivity (g racemate/kg CSP/day)	Solvent consumption (l/g racemate)	Purity (%ee)	Yield (%)
SSR	116	2.49	97.6	92.4
SFC	246	2.25	99.4	98.6

4. Conclusions

Batch HPLC, SSR and SFC utilized for the enatiomer separation of three pharmaceutical intermediates. Higher productivities and lower solvent usage were observed with SSR and SFC relative to batch HPLC. Even though each process was not optimized, the advantages of SSR and SFC processes were obvi-

ous from our results. In case (I), SSR was able to turn around a low-resolution separation of 2.0 kg racemates in a short time. In case (II), SSR increased productivity and reduced solvent consumption by improving the sample solubility and peak shape. In case (III), SFC improved separation efficiency by using smaller particle size and unique selectivity. Thus, we can conclude that SSR is a reliable process, especially dealing with close eluting peaks. SSR and SFC allow us to scale up purification and turn around the sample more quickly. The integration of SSR and SFC in the discovery/early development interface is essential for rapid delivery of pharmaceutical intermediates and toxicology supplies.

Acknowledgements

The authors would like to thank LC support's Victor Riviera for his assistance and Oilver Thiel, Seb Caille, and Johann Chan for supplying all of the racemates.

References

[1] L. Miller, C.M. Grill, T.Q. Yan, O. Dapremont, E. Huthmann, M. Juza, J. Chromatogr. A 1006 (2003) 267.
[2] E.R. Francotte, Switz. Chimia 51 (1997) 717.
[3] L. Miller, D. Honda, R. Fronek, K. Howe, J. Chromatogr. A 658 (1994) 429.
[4] L. Miller, H. Bush, J. Chromatogr. 484 (1989) 337.
[5] S.B. Thomas, B.W. Surber, J. Chromatogr. 586 (1991) 265.
[6] S.C. Stinson, Chem. Eng. News 73 (1995) 44.
[7] J. Dingenen, J.N. Kinkel, J. Chromatogr. A 666 (1994) 627.
[8] E.R. Francotte, P. Richert, J. Chromatogr. A 769 (1997) 101.
[9] T.A. Berger, J. Smith, K. Fogelman, K. Kruluts, Am. Lab. 34 (2002) 14.
[10] C.M. Grill, J. Chromatogr. A 796 (1998) 101.
[11] D.W. Guest, J. Chromatogr. A 760 (1997) 159.
[12] L. Miller, R. Bergeron, J. Chromatogr. 648 (1994) 381.
[13] L. Miller, C. Weyker, J. Chromatogr. A 653 (1994) 219.
[14] G. Terflorth, J. Chromatogr. A 906 (2001) 301.
[15] E.R. Francotte, J. Chromatogr. A 906 (2001) 379.
[16] S. Andersson, S.G. Allenmark, J. Biochem. Biophys. Meth. 54 (2002) 11.
[17] C.M. Grill, L. Miller, T.Q. Yan, J. Chromatogr. A 1026 (2004) 101.
[18] J.H. Kennedy, M.D. Bevo, V.S. Sharp, J.D. Williams, J. Chromatogr. 1026 (2004) 101.
[19] C.J. Welch, W.R. Leonard Jr., J.O. Dasilva, M. Biba, J. ALbaneze-Walker, D.W. Headerson, B. Liang, D.J. Mathre, LC–GC N. Am. 23 (1) (2005).
[20] B.V. Karanam, C.J. Welch, V.G. Reddy, J. Chllenski, M. Biba, S. Vincert, Drug Metab. Dispos. 22 (2004) 1061.
[21] C.M. Grill, L. Miller, J. Chromatogr. A 827 (1998) 359.