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Chromatographic resolution of the enantiomers of a pharmaceutical intermediate from the milligram to the kilogram scale

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Abstract

The preparative chromatographic resolution of racemic mixtures is rapidly becoming a standard approach for the generation of enantiomers in pharmaceutical R&D. This paper will discuss the optical resolution of a pharmaceutical intermediate as the separation is scaled up from the milligram to the kilogram scale. Difficulties encountered and their solutions at each scale will be discussed. In addition, the exploration of Simulated Moving Bed (SMB) for the separation will also be discussed. Finally, a comparison of the productivities and solvent consumption for each method and scale will be presented.

Keywords: Enantiomer separation; Simulated moving bed chromatography; Preparative chromatography; Pharmaceutical intermediate

1. Introduction

The necessity to generate individual enantiomers for testing has become a growing priority in pharmaceutical R&D. This necessity is directly related to the increased knowledge of the effect differing enantiomers have in biological systems. There are two approaches to obtaining enantiomerically pure chemicals. These are: (1) asymmetric synthesis of the desired enantiomer; and (2) resolution of a racemic mixture into individual enantiomers. While asymmetric synthesis is useful when larger quantities of enantiomers are required, the time required to develop the synthesis can make this approach impractical when small quantities (<1 kg) are needed. An additional disadvantage of asymmetric synthesis is the generation of only one of the enantiomers Resolution of a racemic mixture has the advantage of producing both enantiomers, essential for testing. Resolution methods include recrystallization, enzymatic resolution, indirect chromatographic resolution and direct chromatographic resolution. The time required to develop a recrystallization method or an enzymatic resolution can also make this an inefficient approach for the generation of small quantities of enantiomers. Indirect chromatographic resolution, involving derivatization of the enantiomers to form a pair of diastereomers, followed by separation on an achiral stationary phase is useful for the generation of individual enantiomers [1]. While the separation of diastereomers is usually easier than the separation of enantiomers, and has the advantage of being easier to sale up, there are limitations to this approach. First, high enantiomeric purity of the de-

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rivatizing agent is essential. Also, removal of the derivative after chromatographic separation should not cause racemization of the desired enantiomer. Finally, the use of indirect chromatographic separation adds two steps to the synthesis, the reaction to form the derivatives prior to chromatography and the reaction to remove the derivatives after chromatography. The Separations Group at Searle has found the use of preparative chromatography using chiral stationary phases (CSP) to be the most efficient approach for generation of small (<1 kg) quantities of enantiomers [2–6]. In past years the preparative resolution of racemic mixtures has rapidly become a standard approach for the generation of enantiomers in pharmaceutical R&D [7–12].

Simulated Moving Bed (SMB) chromatography has been utilized in the food and petrochemical areas for over thirty years. SMB is often less expensive than batch chromatography. Higher productivities and decreased solvent consumption have been observed for SMB vs. batch operations. Only in the past five years has the use of SMB for enantiomeric separations been investigated [12–16].

This paper reports on the preparative chromatographic resolution of the enantiomers of a pharmaceutical intermediate from the mg to the kg scale. Difficulties encountered upon scale up are presented and the solutions discussed. The use of SMB for the large scale separation of this compound is also discussed. Finally, the productivities and solvent usage for each of the approaches used for the separation are compared.

2. Experimental

2.1. Equipment

The analytical chromatograph consisted of a Hewlett-Packard 1050 pump (San Fernando, CA, USA), a Waters Intelligent Sample Processor (Milford, MA, USA), a Kratos 757 variable wavelength detector (Ramsey, NJ, USA), a Kipp and Zonen BD41 two channel recorder (Delft, The Netherlands) and Digital Equipment Corporation VAX 11/785 computer with Searle chromatography data system.

Two preparative chromatographs were used for this work. The first consisted of two Rainin (Woburn, MA USA) SD-1 pumps, a Model UV-M detector and a Kipp and Zonen (Delft, The Netherlands) BD41 two channel recorder. A Rheodyne (Cotati, CA USA) Model 7125 syringe loading sample injector equipped with a 10 ml loop (Valvco, Houston, TX USA) or a Gilson Model 401 Dilutor (Middleton, WI, USA) in combination with a Rheodyne electrically actuated Model 7010 injector was used for sample injection. The column effluent was fractionated using a Gilson Model 202 fraction collector. This system was used with the prepacked 20 mm I.D. column obtained from Chiral Technologies, Inc. (Exton, PA USA) The second preparative chromatograph consisted of two Rainin (Woburn, MA USA) SD-1 pumps, a Model UV-M detector and a Kipp and Zonen (Delft, The Netherlands) BD41 two channel recorder. A separate Rainin SD-1 pump was used for sample injection. The CSP (1.5 kg) was packed into a 8 cm I.D. Prochrom (Indianapolis, IN USA) dynamic axial compression column, and the final bed length was 53 cm.

The simulated moving bed (SMB) system was a Licosep 12–26 (NovaSep, Nancy, France). The CSP was 20 μm Chiralpak AS and was packed into eight Superformance glass columns (Merck KgaA, Darmstadt, Germany). The ID of the columns was 26 mm and the bed length of each column was 10 cm. The mobile phase was HPLC grade acetonitrile. The following flow-rates were used: feed, 4.5 ml/min; eluent, 17.9 ml/min; extract, 14 ml/min; raffinate, 8.4 ml/min; and recycle, 54.0 ml/min. The concentration of the racemate in the feed was 5.82 mg/ml. The cycle time was 1.43 min.

2.2. Materials

The chiral stationary phases were obtained from Chiral Technologies, Inc. (Exton, PA, USA) as prepacked 10 μm analytical (250 mm×4.6 mm) and preparative columns (250 mm×20 mm) or as 20 μm bulk packing. All chemicals for purification were synthesized in the laboratories of Searle (Skokie, IL and St. Louis, MO USA). The solvents and reagents were reagent grade or better and obtained from a variety of sources.

3. Results and discussion

3.1. Milligram scale separation

The Separations Group provides preparative chromatography support to the chemists throughout Searle. Numerous different (~50) racemates are submitted each year for chromatographic resolution. Often the first time a compound is separated, the quantities are less than one gram. It is only after additional testing of the individual enantiomers that larger quantities are resolved. The compound to be separated was a proprietary intermediate to a compound being tested in our Discovery laboratories. While no information regarding the structure of the compound being separated is presented, the approach for separation discussed in this paper is generic and could be used for the separation of any small (<500) molecular weight racemate. It is preferable to perform the preparative enantioseparation of the final compound to be tested. This minimizes the number of chemical steps that are required. In this case, the final compound to be tested was extremely polar and had poor solubility in solvents commonly used for preparative enantioseparations. In addition, the enantioseparation of the final compound was poor ($\alpha <$ 1.1). Therefore the separation was performed on an intermediate which had better solubility and a better enantioseparation. The first step in any preparative separation is to perform analytical HPLC method development to screen stationary phases and mobile phases. The result of this work is summarized in Table 1. Both amylose based (Chiralpak) and cellulose based (Chiralcel) as well as a Pirkle type CSP were investigated. In developing an analytical separation for scale up to preparative loadings one wants a k' for the second enantiomer of less than five, as well as a maximum alpha. The low k' reduces the run time for the separation and helps to maximize the preparative throughput. The maximum alpha allows larger quantities of racemate to be loaded onto the column. Based on these criteria it was decided to use the Chiralpak AS CSP with an ethanol–heptane mobile phase for the preparative separation. The analytical separation using these conditions is shown in Fig. 1. The data in Table 1 shows that Chiralpak AS CSP with isopropanol–heptane gave a larger alpha than the ethanol–heptane mobile phase. Although it is preferable to scale up an analytical method with the largest alpha, it was not used because of the longer retention obtained with this mobile phase. This translates to a longer run time and decreased throughput when scaled to preparative loadings. The run time could be decreased if the isopropanol percentage was increased to 70 or 80%. While this would decrease the k', there would be a large increase in the viscosity of the mobile phase. Since the maximum operating pressure for this CSP is 500 p.s.i., the flow-rate would have to be reduced, eliminating the reduction in separation time.

The analytical HPLC method shown in Fig. 1 was scaled up to preparative loadings. Upon scale up it was discovered that the solubility of the racemate was extremely poor (~1 mg/ml) in ethanol–heptane. To apply the racemate to the column a technique developed in our laboratories called solid injection was used [17]. This technique involves mixing the racemate with stationary phase and packing this

Table 1
Chromatographic results

Column	Mobile phase	k_1'[a]	k_2'[b]	α
Chiralpak AS	60:40 (v/v) ethanol–heptane	1.37	2.85	2.08
Chiralpak AS	50:50 (v/v) ethanol–heptane	1.67	3.48	2.08
Chiralpak AS	50:50 (v/v) isopropanol–heptane	3.75	8.70	2.32
Chiralpak AD	50:50 (v/v) ethanol–heptane	1.57	1.57	1.00
Chiralpak AD	20:80 (v/v) isopropanol–heptane	1.91	1.91	1.00
Chiralcel OD	10:90 (v/v) ethanol–heptane	6.53	7.34	1.12
Chiralcel OD	30:70 (v/v) isopropanol–heptane	3.66	4.01	1.10
Whelk-O	30:70 (v/v) ethanol–heptane	2.30	2.47	1.07

[a] Capacity factor for first eluting enantiomer.
[b] Capacity factor for second eluting enantiomer.

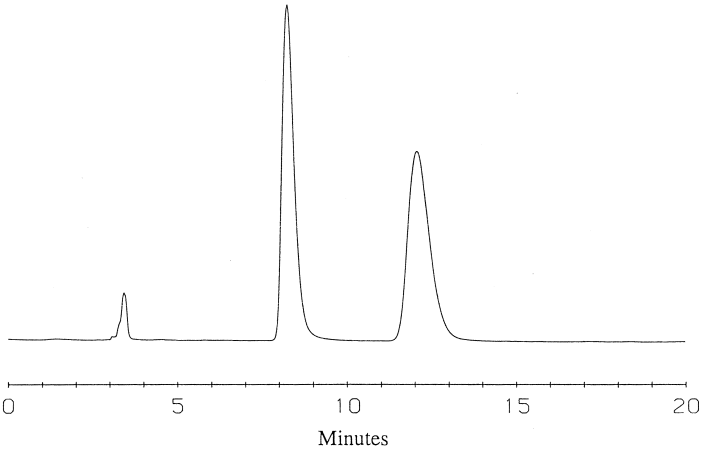

Fig. 1. Analytical HPLC separation. Analysis conducted on Chiralpak AS column (250 mm×4.6 mm I.D.), detection at 220 nm, 0.1 AUFS. Mobile phase: 50:50 (v/v) ethanol–heptane, flow-rate 1 ml/min.

mixture into a pre column. Since the separation of the enantiomers was large, a loading of 180 mg of racemate was chosen. For solid injection, three to five times by weight of packing is mixed with the sample. For this separation we mixed 180 mg of racemate with 700 mg of silica. Ideally the packing used for solid injection should be identical to the packing in the main column. In this case 40 micron silica was used instead of Chiralpak AS packing, due to unavailability of bulk AS packing. This separation is shown in Fig. 2. Good separation of the enantiomers was obtained. Purities of >96% and yields of ~90% were obtained for both enantiomers. A total of 600 mg of racemate was separated in four separate injections using this method. While this method was suitable for the separation of low quantities of racemates, the use of solid injection reduced the automation possibilities, resulting in high manpower requirements. In addition, the use of a precolumn required a lower flow-rate than desired, resulting in a run time of nearly 60 min.

3.2. Gram scale separation

Soon after the separation was performed at the milligram scale, the separation of 20 g of racemate was requested. Using the same method as above, the separation would require two weeks operating time. Additional work was performed to increase the throughput of the purification. The limiting factor for the preparative separation was the solubility of the racemate in ethanol–heptane. If a separation could be developed with a mobile phase that had better

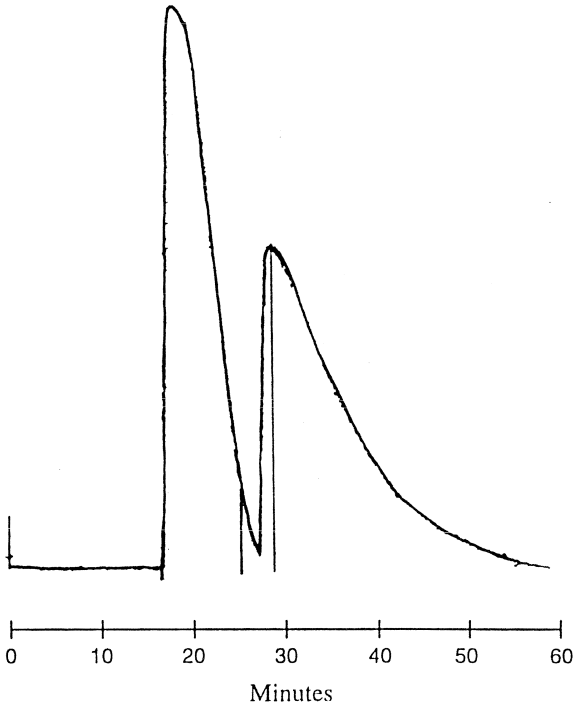

Fig. 2. Preparative HPLC separation. The purification was conducted on Chiralpak AS column (250 mm×20 mm I.D.), with a mobile phase of 50:50 (v/v) ethanol–heptane. A flow-rate of 6 ml/min, detection at 220 nm and a loading of 180 mg were used.

solubility of the racemate, the separation could be automated to allow 24 h operation. At this time a sample of the racemate was sent to Chiral Technologies, Inc. (Exton, PA) to be investigated in their screening service. From this work a mobile phase of acetonitrile with the Chiralpak AS CSP was recommended. The analytical separation using these conditions is shown in Fig. 3. This method had a lower alpha than the ethanol–heptane method (1.75 vs. 2.08) but had the advantage of increased solubility. An additional advantage of the acetonitrile mobile phase was decreased viscosity relative to the ethanol–heptane mobile phase. This allowed the flow-rate to be increased while still remaining below the pressure limitation of the CSP. A flow-rate of 20 ml/min was used for the separation. This is the flow-rate required to maintain a constant linear velocity with 1 ml/min. on a 4.6 mm analytical column. This method was scaled up to preparative loadings. The racemate has a maximum solubility of 10 mg/ml in acetonitrile. While this was satisfactory for the preparative work, there was concern that during the unattended overnight purification, the sample might come out of solution, clogging the injection equipment and causing the system to shut down. To avoid this problem ten percent (v/v) methanol was added to the diluting solvent. The racemate has a solubility of 20 mg/ml in a 90:10 acetonitrile–methanol mixture. Loading experiments showed that at 40 mg, touching bands were obtained. The preparative separation of 40 mg of racemate is shown in Fig. 4. Baseline resolution of the enantiomers was achieved in less than seven min. To maximize the throughput, the technique of overlapping injections was used. In overlapping injections (also called boxcar injections) the second injection is made prior to all the chemical eluting from the first injection. Overlapping injections allowed an injection to be made every four min instead of every 7–8 min. Automation was used to make injections and perform automated peak collection. Sixteen grams of racemate was resolved in 26 h. A purity of >98% and a yield of ~93% was obtained for both enantiomers.

3.3. Kilogram scale separation

The next step in this project occurred when the separation of one kilogram of racemate was requested. Using a 20 mm I.D. column, approximately 1600 h of operating time would be required. To meet project timeframes, the throughput of the separation needed to be increased. This was accomplished by using a larger column for the separation. An 8 cm I.D. dynamic axial compression column was packed with 1.5 kg of 20 μm CSP. The bed length for this column was 53 cm. Preparative method development involved increasing the load until separation was

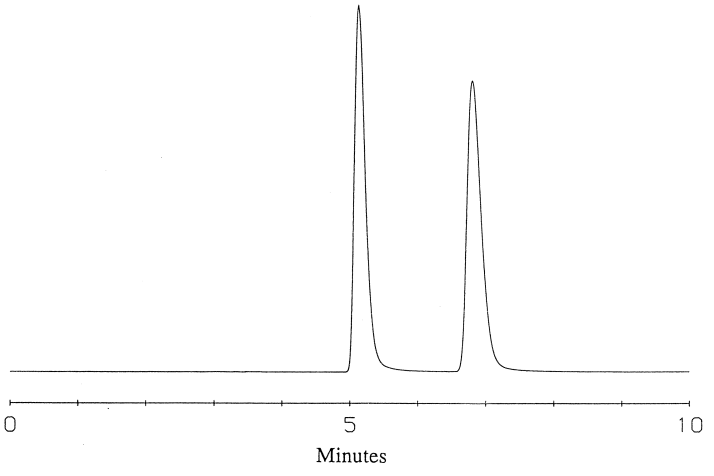

Fig. 3. Analytical HPLC separation. Analysis conducted on Chiralpak AS column (250 mm×4.6 mm I.D.), detection at 220 nm, 0.1 AUFS. Mobile phase: acetonitrile, flow-rate 1 ml/min.

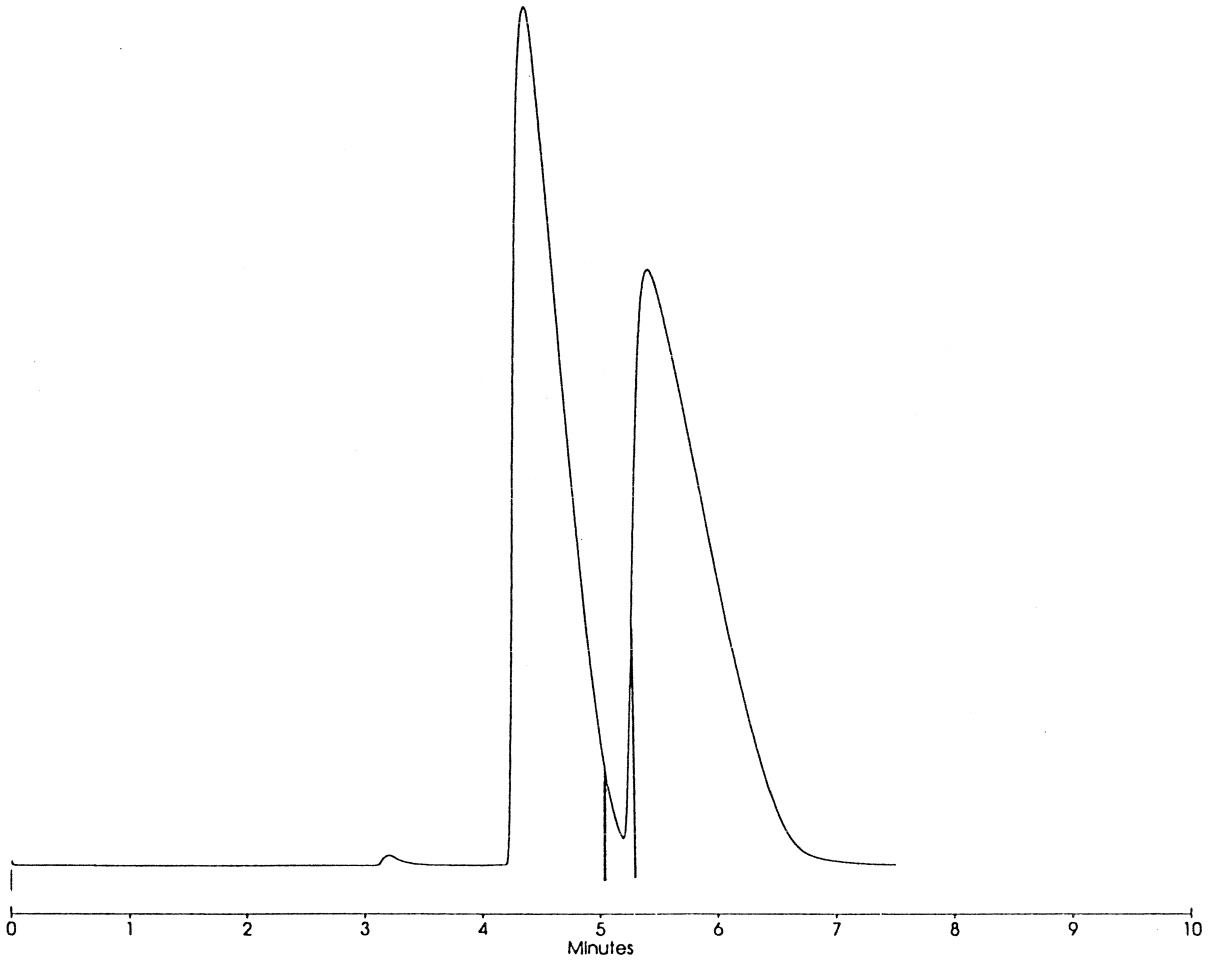

Fig. 4. Preparative HPLC separation. The purification was conducted on Chiralpak AS column (250 mm×20 mm I.D.), with a mobile phase of acetonitrile. A flow-rate of 20 ml/min, detection at 220 nm and a loading of 40 mg were used.

lost. By the time of this work it was known that the enantiomer with the desired biological activity eluted second in this chromatographic method. Loads of 500 mg, 1, 1.5 and 2 g of racemate were investigated. At a load of 2 g, slight overlap of the peaks was observed. Since the yield for this separation needed to be maximized, a load of 2 grams was used to process the material. The separation of 2 g of racemate is shown in Fig. 5. Overlapping injections were used with an injection being made every 10 min. One kilogram of racemate was processed in three and a half 24 hour days. A purity of >99% and a yield of 98% was obtained for the first eluting enantiomer. A purity of >98% and a yield of 93% was obtained for the second eluting enantiomer.

3.4. Simulated moving bed separation

While the use of batch chromatography is useful for the separation of enantiomers, at large scale the cost can be prohibitive due to large stationary phase and mobile phase requirements. SMB holds the promise to make chromatography a more cost effective alternative at large scale. SMB for the separation of this compound was investigated by Chiral Tech-

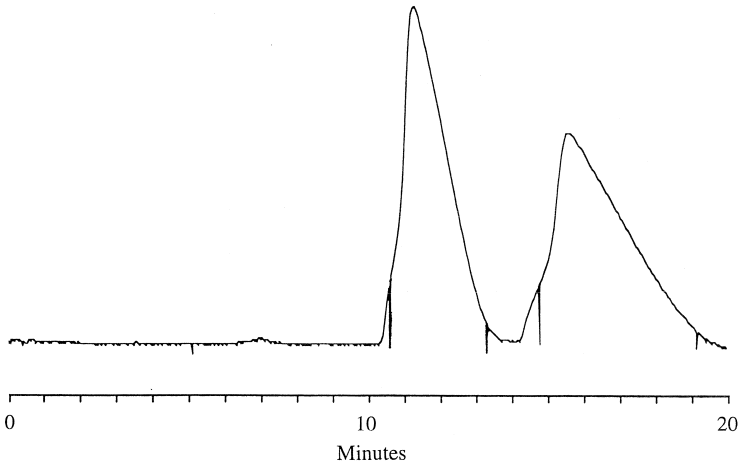

Fig. 5. Preparative HPLC separation. The purification was conducted on Chiralpak AS column (530 cm×8 cm I.D.), with a mobile phase of acetonitrile. A flow-rate of 300 ml/min, detection at 220 nm and a loading of 2 grams were used.

nologies (Strasbourg, France). This work was a preliminary analysis and was not optimized to maximize productivity. HELP software (NovaSep, Nancy, France) was used to develop operating conditions for the SMB. An eight column SMB (2 columns/zone) containing 240 g of CSP was prepared. The ID of the columns was 26 mm and the bed length of each column was 10 cm. The flow-rates are detailed in the experimental section of this paper. Purities of >98% and yields of >99% were obtained for both enantiomers. A total of 18.7 g of each enantiomer could be produced per 24 hour day under these non-optimized conditions. The average pressure drop in the system was only 20 bar which is far below the maximum pressure available (50 bars).

3.5. Comparison of chromatographic methods

Both batch and continuous chromatographic (SMB) methods were utilized to resolve this racemate. These methods can be compared by calculating the productivities and solvent consumption achieved with each separation. The productivities are summarized in Table 2. The solvent consumption is summarized in Table 3. The productivity for each separation is calculated assuming a column containing one kg of CSP and a system operating 24 h/day. Comparing the productivities we see the lowest productivity obtained with the ethanol–heptane mobile phase. This is not unexpected due to the long run time associated with this method. The maximum prod-

Table 2
Productivities for resolution methods

Solvent	d_p	g racemate/ kg CSP/day	g #1 produced/ kg CSP/day	g #2 produced/ kg CSP/day
Ethanol–Heptane	10 μ	118	51	54
Acetonitrile	10 μ	327	152	152
Acetonitrile	20 μ	192	94	89
Acetonitrile(SMB)	20 μ	240	120	120
Acetonitrile (SMB) Expected after Optimization	20 μ	314	155.6	155.6

Table 3
Solvent Consumption for resolution methods

Solvent	d_p	l solvent/ g racemate	l Solvent/ g #1 produced	l Solvent/ g #2 produced
Ethanol–Heptane	10 μ	1.6	3.8	3.7
Acetonitrile	10 μ	2.0	4.3	4.3
Acetonitrile	20 μ	1.5	3.1	3.1
Acetonitrile (SMB)	20 μ	0.85	1.7	1.7

uctivity is obtained with acetonitrile and the 10 μm CSP. As expected the productivity decreases when the particle size of the CSP is increased to 20 μm. While an increase in productivity is seen with 10 μm CSP vs. 20 μm CSP, keep in mind that this calculation does not take into account the additional cost of 10 μm CSP relative to 20 μm CSP nor does it include the added cost that occurs when operating at higher pressures. Productivity is only one way to compare purification methods. To ultimately decide on a method, it is necessary to consider the total cost of the separation including CSP, equipment, labor, etc. Over-reliance on the productivity number can lead one away from the real optimum for the separation. In addition, care should be taken when using productivities numbers to compare separations using CSP of differing particle diameters. The usual advantage of SMB to batch chromatography in term of productivity is not apparent here (rows 3 and 4 in Table 2) but one has to keep in mind that the system was not used at the maximum pressure available. Mazzotti et al. [18] explained that the productivity can be optimized by reducing the cycle time to a certain extent assuming enough theoretical plate per column in the system which is the case here. Hence it is reasonable to expect an increase in productivity by simply decreasing the cycle time and increasing all the flow-rates by the same factor. The system will reach a higher value of the operating pressure without affecting the quality of the enantiomer produced. A factor of two will increase the pressure in the system to 40 bar and the productivity to the value shown in row 5 of Table 2. Even not completely optimized, the SMB is using far less solvent than any other methods as can be seen in Table 3. However the optimization of the separation as recommended above will not affect the values displayed in row 4 in Table 3 because the ratio of product introduced in the system to the volume of solvent used stays constant. With SMB a decrease in solvent consumption of approximately 45% is observed. The improved utilization of the CSP and reduced solvent consumption is making SMB a technology that could go a long way towards making chromatographic resolution of enantiomers a technique that can be cost effective on the manufacturing scale.

4. Conclusions

Chromatographic separation of enantiomers can be used to support early Discovery and Development activities where 1 kg and smaller quantities need to be resolved. For Discovery activities it may be the method of choice for preparing individual enantiomer, surpassing asymmetric synthesis due to the ease of developing and scaling up a chromatographic separation. Extensive analytical method development, exploring various chiral stationary phases and mobile phases should be conducted prior to any preparative chiral separation. Particular attention should be paid to developing a separation using a mobile phase in which the racemate has good solubility. The use of SMB for resolution of enantiomers results in decreases in stationary phase and mobile phase requirements. This technology promises to make chromatographic resolution a viable alternative at the metric ton scale needed for pharmaceutical manufacturing.

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