Watch

Preparative Chiral SFC as a Green Technology for Rapid Access to Enantiopurity in Pharmaceutical Process Research

Guest Authors

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This month's installment of "Column Watch" provides an overview of green chemistry considerations for the kilogram-scale preparative chromatographic separation of enantiomers, focusing especially on recent results from the authors' laboratories, illustrating solvent and time savings realized with preparative supercritical fluid chromatography (SFC). They compare optimized high performance liquid chromatography and SFC in terms of energy efficiency and throughput.

Ronald E. Majors
Column Watch Editor

Recently, the subject of "green chemistry" has received considerable attention from academic and industrial researchers alike (1). While the central tenets of waste reduction, process economy, and elimination of risks and hazards have long been embraced by industrial process chemists, the current focus on green chemistry concerns has led to renewed appreciation for the importance of these topics. The analysis of the "greenness" of any given process, operation, or methodology is a useful exercise that can lead to process improvement and refinements. This exercise can be particularly valuable for new technologies that might not yet have a long track record of practical utilization. Preparative chromatographic resolution of enantiomers is one such emerging technology that recently has become widely used for providing rapid access to enantiopure materials to support pharmaceutical development. Here we provide an overview of green chemistry issues relating to preparative chromatography, concentrating especially on the green advantages of preparative supercritical fluid chromatography (SFC), in which carbon dioxide replaces the flammable and toxic petrochemical-derived hydrocarbon solvents typically used in preparative liquid chromatography.

Can Preparative Chromatography Be Green?

First impressions might suggest that preparative chromatography, with its intensive use of solvent, is decidedly "un-green." However, economic factors usually dictate that industrial-scale chromatographic processes employ solvent recycling (2). In addition, the smaller scale preparative chromatography performed in support of developmental research can sometimes eliminate the need for developing and carrying out more traditional chemical syntheses, thereby saving considerable labor and time (3), and sometimes even resulting in a net decrease in waste generation. Thus, the use of preparative chromatography can sometimes be a "greener" approach to conventional development. Furthermore, newer forms of preparative chromatography, such as SFC, can be viewed as an even greener alternative to classical preparative chromatography. (Please see the sidebar on the following page.)

Chirality and the Pharmaceutical Industry

Most pharmaceuticals are chiral; that is, they can exist as either of two nonsuperimposable mirror image forms, termed enantiomers. Although the importance of chirality has been appreciated and addressed by the pharmaceutical industry for decades, it is only within the past few years that a shift toward development of most chiral pharmaceutical candidates as single enantiomers has occurred. As technologies for measuring and making enantiopure materials have improved, the production of enantiopure pharmaceuticals has become commonplace, with many of the top-selling drugs in the world now being sold in enantiopure form. Consequently, the subject of chirality and the pharmaceutical industry is a topic of considerable recent interest and importance (4–6).

Preparative Chromatography in Organic Synthesis: Realizing the Woodward Vision

Preparative chiral chromatography is being used increasingly in pharmaceutical devel-

12 Principles of Green Chemistry

(From reference 1)

1.Prevention: It is better to prevent waste than to treat or clean up waste after it has been created.

2. Atom Economy: Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.

3. Less Hazardous Chemical Syntheses: Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

4. Designing Safer Chemicals: Chemical products should be designed to effect their desired function while minimizing their toxicity.

5. Safer Solvents and Auxiliaries: The use of auxiliary substances (for example, solvents, separation agents, and so forth) should be made unnecessary wherever possible and innocuous when used.

6. Design for Energy Efficiency: Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.

7. Use of Renewable Feedstocks: A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.

8. Reduce Derivatives: Unnecessary derivatization (use of blocking groups, protection–deprotection, temporary modification of physical–chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.

9. Catalysis: Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

10. Design for Degradation: Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

11. Real-time Analysis for Pollution Prevention: Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control before the formation of hazardous substances.

12. Inherently Safer Chemistry for Accident Prevention: Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents.

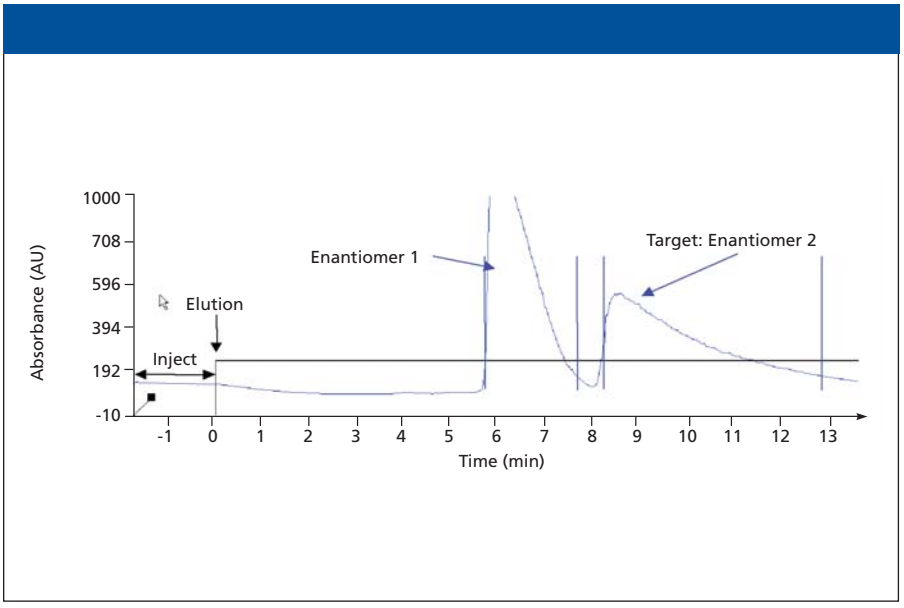

Figure 1: A typical case illustrating the preparative chromatographic enantioseparation of a development intermediate on the kilogram scale. Column: 25 cm × 11 cm DAC Chiralpak AD; flow rate: 600 mL/min; mobile phase: 25% ethanol–heptane; detection: UV absorbance at 285 nm; injection: 150 mL racemate at 83 mg/mL in mobile phase.

opment for rapidly accessing enantiomerically pure materials on the kilogram or even larger scale (7). Preparative high performance liquid chromatography (HPLC) is used most frequently for the kilogram-scale separations needed to support development, with simulated moving bed chromatography (SMB) or other multicolumn chromatography approaches predominating at industrial scale (greater than hundreds of kilograms) (8–12).The integration of preparative chiral chromatography into organic synthesis remains a rapidly evolving field, and new stratagems for merging the two fields are being created. Nevertheless, the incorporation of preparative HPLC into organic synthesis is by no means a new phenomenon. R.B. Woodward, perhaps the most famous synthetic chemist of the twentieth century, was an early advocate of preparative HPLC, declaring in 1973 (2) that "The power of these high pressure liquid chromatographic methods hardly can be imagined by the chemist who has not had experience with them; they represent relatively simple instrumentation and I am certain that they will be indispensable in the laboratory of every organic chemist in the near future." (13)

Interestingly, it is only within the last few years that Woodward's prediction of the widespread adoption of preparative HPLC as an enabling technique for organic synthesis has begun to be borne out. In recent years, there has been a growing appreciation of the value that preparative chromatography can bring to organic synthesis, and the technique is now used broadly within the pharmaceutical and fine chemical industries.

Green Aspects of Preparative Chromatography: Waste Reduction

Several of the 12 principles of green chemistry, outlined in the sidebar, are especially applicable to the field of preparative chromatography. Process chemists have long been concerned with the topics of waste prevention, atom economy, and the use of inherently safe chemistry for accident prevention (principles 1, 2, and 12). Also, preparative chromatography is concerned especially with the issue of safer solvents and auxiliaries (principle 5).

Elimination of waste is always a key green chemistry concern, and an important factor in any separation of enantiomers (that is, resolution). Whether using classical resolution via diastereomeric salt formation, enzymatic kinetic resolution, preferential crystallization, or chromatography, all resolutions suffer, in theory, from the fundamental drawback of being inherently wasteful, as at most, only half of the material is recovered (the half corresponding the desired enantiomer, the other half being "waste"). Despite this seemingly gross violation of the principles of atom economy (14), racemization and recycling of the undesired enantiomer sometimes is possi-

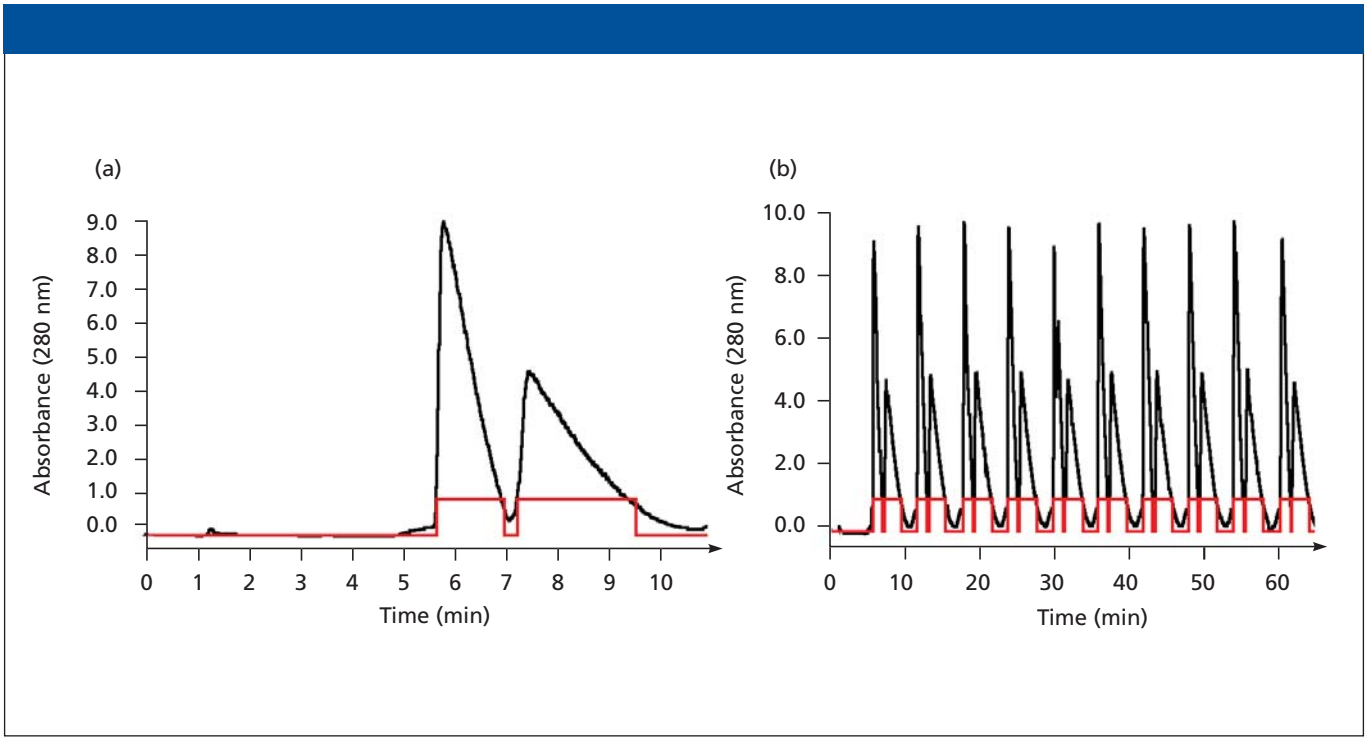

Figure 2: Representative chromatograms illustrating (a) a single injection and (b) repetitive overlapped injections for the semipreparative SFC resolution of the enantiomers of a developmental compound. Column: 25 cm × 2 cm Chiralcel OD; mobile phase: 32% (25 mM isobutylamine in methanol)–carbon dioxide; flow rate: 50 mL/min; outlet pressure: 100 bar; detection: UV absorbance at 280 nm; injection: 2 mL at 160 mg/mL.

ble, enabling higher yield and reduction of waste. Coupling of such resolution and isomerization approaches can lead to truly impressive processes for generating enantiopurity (15), and such approaches have long been a mainstay of successful industrial-scale synthesis of enantiopure materials. The possibility of racemizing and recycling the undesired enantiomer from a chromatographic resolution is becoming a routine consideration in development, and when possible, such racemization–recycling approaches can have a significant impact on process economy. Therefore, investigation of racemization is an important consideration when deciding where in a synthesis a resolution should be placed.

Green Aspects of Preparative Chromatography: Safer Solvents

The green chemistry principle of using safer solvents and auxiliaries is critically important to the area of preparative chromatography. A typical preparative HPLC resolution is illustrated in Figure 1. In this separation, the desired component is the second eluted enantiomer, which is collected with > 98% enantiomeric excess and 80% recovery. Repetitive injection under these conditions was used to obtain 1.1 kg of desired enantiomer with 55 h of instrument time and with the utilization of about 2000 L of solvent, 840 L of which was evaporated for an overall productivity of about 0.3 kkd (kilograms of purified enantiomer per kilogram of stationary phase per 24-h day). Although the conditions of this preparative separation could be improved to afford improved productivity and recovery, it is important to realize that accessing more than a kilogram of enantiopure product in only 55 h means the chromatographic approach can be performed with only a small fraction of the cost that would be required to obtain this same result by conventional methods. It is this economic reality that underlies the widespread adoption of preparative chromatography within the pharmaceutical and fine chemical industries.

Productivity is the key metric for preparative chiral chromatography and is given with units of kkd. In early development, chromatographic productivity is often poor (0.1 kkd or even lower) with a good separation having a productivity in the range of 1 kkd. A truly remarkable separation might have a productivity greater than 10 kkd. From the example shown in Figure 1, it can be appreciated that a large amount of solvent (2000 L) is required for performing this relatively unproductive separation, and while this solvent can in principle be recycled, this typically is not done during early development research. In addition to being somewhat wasteful of resources, the use of so much solvent requires specialized equipment and work environment, and dictates that a very large volume of solvent must be evaporated to recover the desired material.

The use of SFC for preparative enantioseparation has enjoyed considerable recent attention (16–19) and is the method of first choice in our own laboratories. In this technique, supercritical or subcritical carbon dioxide replaces flammable and toxic petrochemical-derived hydrocarbons, resulting in reduction in solvent utilization by as much as 90% or more. The resulting decrease in solvent use and waste generation offers a green advantage with an economic bonus that makes preparative SFC especially attractive. Furthermore, with preparative SFC, the product is recovered in a more concentrated form relative to HPLC, greatly reducing the amount of solvent that must be evaporated and resulting in considerable savings in labor, time, and energy costs. Finally, because of the low viscosity of the supercritical fluid eluent, separations can be conducted at flow rates that would be impossible with liquid solvents, an advantage that can contribute to the often higher productivity of preparative SFC enantioseparations relative to HPLC methods. Cumulatively, these advantages make preparative SFC enantioseparation an

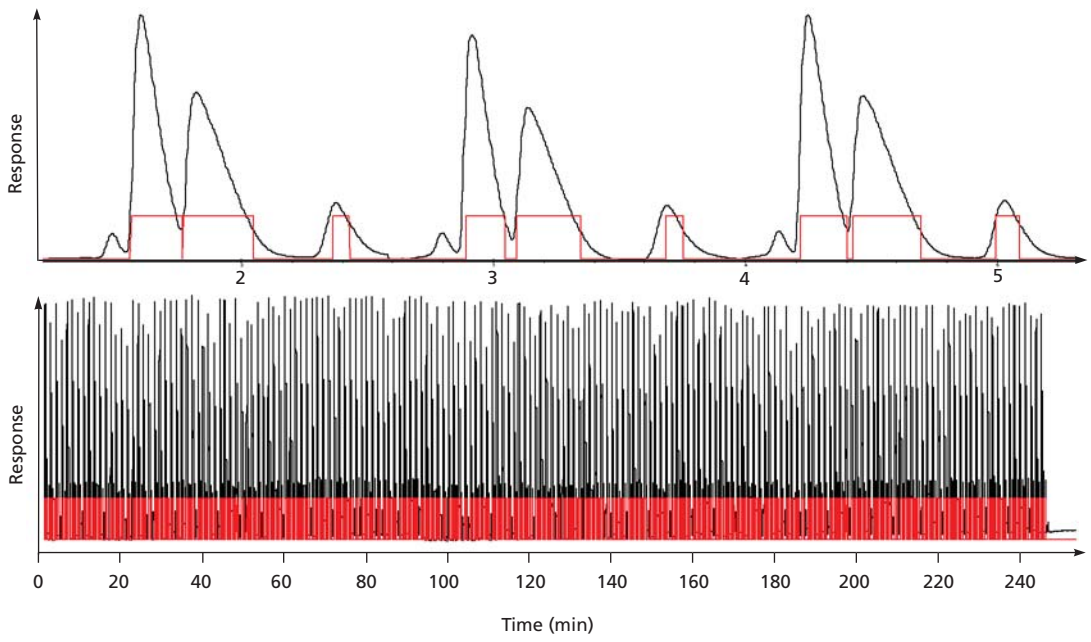

Figure 3: Semipreparative SFC used for preparative separation of enantiomers. More than 200 g of a racemate was resolved in ~2700 injections, with ~60 h of instrument time. Chiralcel OF (Chiral Technologies) chromatograms showing (top) three repeat injection cycles and (bottom) a block of 99 injections. Productivity = 0.8 kkd. Column: 25 cm × 2 cm Chiralcel OF; 15% isopropanol–carbon dioxide; flow rate: 50 mL/min; outlet pressure: 100 bar; detection: UV absorbance at 270 nm; injection: 500 mL at 150 mg/mL in isopropanol.

attractive and potentially greener addition to conventional HPLC approaches, and a technique with a promising future.

Green Aspects of Preparative Chromatography: Energy Efficiency

Energy efficiency is another matter of considerable importance in large-scale chromatographic separations. Preparative liquid chromatography using automated fraction collection and solvent recycling via continuous distillation was known by the early 1970s (20) and is increasingly used today. Solvent evaporation can be highly energy intensive, to the point that the energy requirements for evaporation can become the dominant cost in an industrial-scale chromatographic process. An SFC system requires additional heating and cooling not required for HPLC systems, and thus, additional energy demands. Incoming carbon dioxide eluent must be cooled to a liquid so as to allow effective pumping, and immediately following pumping, the temperature of the carbon dioxide stream typically is raised to about 35 °C before it enters the column. Evaporative cooling resulting from depressurization at the postcolumn outlet stream of the SFC instrument must be counteracted by more heating, which can be fairly energy intensive. Finally, recycling carbon dioxide requires additional cooling of carbon dioxide gas from the outlet stream so as to condense into a liquid for reuse. In total, an operating SFC unit is heated and cooled simultaneously at several locations, and although in principle some opportunities exist for reduction of energy consumption via heat exchange, this typically is not done on laboratory-scale instruments. In comparison, HPLC requires no heating and cooling for operation, raising the question of the energy utilization of SFC relative to HPLC. The power demand is less than 5 kW for a preparative SFC system pumping 350 g/min of a 10% methanol–carbon dioxide eluent mixture at a temperature of 35 °C and an outlet pressure of 100 bar with carbon dioxide recycling. Power requirements for continuous solvent evaporation (heating and cooling) to keep pace with such a unit would be on the order of 2 kW, whereas power requirements to keep pace with solvent evaporation for a comparable HPLC unit (assuming 10× solvent utilization) would be on the order of 20 kW. Thus, it can be seen that even in terms of energy utilization, SFC is greener than HPLC, because of the high energy cost of solvent evaporation. As later examples will show, this SFC advantage can be even more pronounced because of additional solvent reductions afforded by the oftentimes improved productivity of SFC versus HPLC.

Green Aspects of Preparative Chromatography: Temperature, Pressure, and Carbon Dioxide as a Greenhouse Gas

In addition to the fundamental point of designing for energy efficiency, green chemistry principle 6 (design for energy efficiency) clearly states, "If possible, synthetic methods should be conducted at ambient temperature and pressure." While it can be argued that a separation method is not a synthesis method, the intent of using extremes of temperature and pressure only when warranted is clearly implied. Thus, no analysis of the greenness of SFC would be complete without addressing the issue of the high pressure and temperature control

Thar Technologies
350 g/min

Novasep
1 kg/min

Figure 4: Commercial preparative SFC instruments currently being used in the authors' laboratories.

required with the use of supercritical carbon dioxide.

The critical point for pure carbon dioxide is 31.1°C and 73.8 bar, meaning that at temperatures and pressures beyond these values, carbon dioxide exists as a supercritical fluid. Clearly, any use of SFC must resort to high pressure, although only marginally higher than those addressable via standard preparative HPLC pumping equipment. The major difference between SFC and HPLC instrumentation is the need for a back-pressure regulator in SFC, whose function is to restrict outlet flow so as to create a back pressure on the system, thereby maintaining the carbon dioxide eluent in a supercritical or subcritical state. Thus, the need to operate carbon dioxide–based preparative chiral SFC at high pressure does introduce some undesirable but unavoidable engineering and safety concerns that are more than adequately compensated for by the performance of the technique.

When discussing carbon dioxide (a known greenhouse gas) as a green solvent alternative, it is important to note that preparative SFC is not a *net* generator of carbon dioxide. Instead it uses carbon dioxide that is condensed from the atmosphere

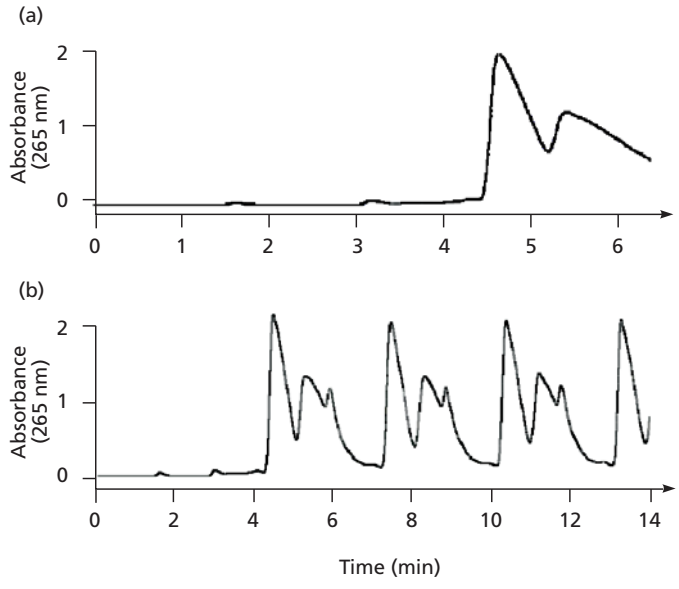

Figure 5: Representative chromatograms showing (a) a single injection and (b) repetitive stacked injections in kilogram-scale preparative SFC resolution of a diester intermediate. Columns: two 270 mm × 51 mm, 20-μm Chiralpak AD columns in series; mobile phase: 15% (mL/g) ethanol–carbon dioxide; flow rate: 350 g/min, pressure: 100 bar; temperature: 35 °C; detection wavelength: 265 nm; racemate concentration: ~325 mg/mL; injection volume: 5 mL; cycle time: 235 s.

or industrial waste plumes, shipped to the chromatography installation, and then later returned to the atmosphere. In this respect, carbon dioxide is a renewable resource, and is a recovered industrial waste byproduct, both important green chemistry features. In contrast, incineration of waste organic solvents resulting from preparative HPLC operations does result in the net generation of carbon dioxide.

Analytical Chiral SFC in Pharmaceutical Process Development

We have long relied on SFC for carrying out analytical chiral separations, having found this technique typically to be faster and more convenient than HPLC separations (21–25). We employ an automated overnight column screening protocol using a standard gradient method for analytical method development, which usually affords a usable method the next morning. Clearly, this approach offers a considerable advantage over a manual one-at-a-time evaluation of columns. When only one or a few analyses are required, the standard gradient method itself can be used without further modification. When analysis of more samples is required, conversion to an isocratic method often is performed to afford a shorter analysis time. For a single analytical instrument, the green advantages of analytical SFC over analytical HPLC are slight in terms of waste solvent generation, although when one considers a large number of analytical instruments, the waste reduction is more substantial.

Semipreparative Chiral SFC in Pharmaceutical Process Development

During the past few years, we have routinely used semipreparative SFC as our method of choice for rapid purification of small amounts of enantiopure materials, from a few milligrams up to about 20 g (11,25,26). Perhaps the most profound advantage of semipreparative SFC purification comes from the fact that information obtained from the analytical column screening can be translated immediately into a workable preparative SFC separation. This linking of analytical and preparative techniques requires matched pairs of analytical and preparative columns, but this investment allows one to resolve gram amounts of completely unknown compounds within a day or two of receipt. The general advantage of SFC results in a considerable saving of time and materials, a savings that is advantageous from an economic standpoint, but also from a green chemistry perspective.

The example depicted in Figure 2 illustrates a typical use of semipreparative SFC in pharmaceutical development. Analytical SFC screening suggested Chiralcel OD (Chiral Technologies, Inc., West Chester, Pennsylvania) was the best stationary phase for resolving the enantiomers of a developmental compound. Immediate translation to a semipreparative SFC separation in isocratic mode was performed, and the sample load was increased to the point at which baseline resolution of enantiomers was just maintained. Repeated injections were then performed using an overlapping injection strategy to maximize the use of the column, ensuring that some compound was being eluted from the column at nearly all times. Separations of this type are very convenient for preparing a few milligrams or a few grams of compound, and early access to such material using the fast and labor-efficient tool of preparative SFC can revolutionize the way that pharmaceutical research is performed. In addition to providing material for testing synthetic routes or conducting preliminary biological evaluations, the highly pure material obtained using semipreparative SFC can allow early research into the critically important crystallization experiments that often are used ultimately to effect purifications in pharmaceutical manufacturing at the industrial scale.

Kilogram-Scale Preparative Chiral SFC in Pharmaceutical Process Development

Until recently, chromatographic purification of amounts greater than about 20 g in these laboratories involved the use of preparative HPLC, which requires developing a new chromatographic method or at least translation of an existing SFC method into an HPLC method. This translation process is not always straightforward. In some instances, developing a comparable HPLC separation for scale-up can prove difficult or impossible, and occasionally we found ourselves in the position of using a semipreparative SFC instrument for carrying out a preparative-scale separation. An example is shown in Figure 3, which illustrates a resolution of enantiomers carried out at the >200-g scale that required thousands of injections and days of instrument time to complete. Nevertheless, automated sample injection and fraction-collection capability does make such separations relatively straightforward, although they are not as fast as one would like.

It is at the kilogram scale that preparative SFC begins to result in more dramatic savings in solvent, evaporation, and labor. Recently, we acquired two large-scale preparative SFC units (pictured in Figure 4), a 350-g/min system from Thar Technologies (Pittsburgh, Pennsylvania), and a 1-kg/min system from Novasep (Pompey, France). We have found this equipment to be quite helpful for performing the kilogram-scale purifications that are an essential component of modern pharmaceutical development.

An example aptly illustrates the value of the SFC approach. We were called upon recently to resolve the enantiomers of a pharmaceutical intermediate not readily accessible via enantioselective synthesis. Initial screening followed by method optimization using loading studies led to optimized methods in both the SFC and HPLC modes. Although optimized SFC methods for chiral separation often are more productive than the corresponding optimized HPLC method, the SFC advantage can be quite profound. Such was the case in the current example, where we observed a roughly 10-fold greater productivity for the SFC method relative to the HPLC method. It should be pointed out that both methods were fully optimized to the best of our abilities, and although the SFC advantage in productivity might more typically be in the range of twofold, much larger productivity advantages are by no means uncommon. In this particular instance, we were presented with the choice of performing a separation using either a 30-cm i.d. HPLC column (36,000 L of solvent, 10,000 L evaporated) or a 5-cm i.d. SFC column (900 L solvent, 215 L evaporated), with instrument time projected to be about 120 h for either approach. The choice in this instance was obvious, and SFC was used to carry out the separation. A representative chromatogram (Figure 5) illustrates a single injection of 1.5 g of racemate and a series of overlapping injections representative of the actual campaign.

A second case is illustrated by an example in which there was a need to purify at least 1.5 kg of a single diastereomer of an investigational compound. In this instance, differences between the optimized HPLC

and SFC methods were even more profound, as no suitable preparative HPLC separation could be discovered. The separation was performed using preparative SFC, and 1.725 kg of purified diastereomer (99% diasteromeric excess) was obtained in about 72 h using a 5-cm i.d. Chiralpak AS column (Chiral Technologies) with an eluent of 30% isopropanol in carbon dioxide (100 bar outlet pressure, 350 g/min) and using about 830 L of solvent (275 L evaporated). These results illustrate the utility of preparative SFC for generating kilogram amounts of development compounds and emphasize the tremendous saving in organic solvent use that can be possible with preparative chiral SFC.

Preparative SFC in Pharmaceutical Process Development: Future Prospects

Separation of amounts greatly in excess of a few kilograms would require the need for even larger preparative SFC units than those pictured in Figure 4. Interestingly, the relationship between pressure and volume places some constraints for the scale-up of preparative SFC columns. As column diameter increases, the need for ever-increasing wall thickness can place a practical and economic limit on the scalability of a stainless steel column construction approach. However, industrial scale supercritical carbon dioxide extraction vessels (see, for example, www.uhde-hpt.com) in the 1-m i.d. range and operating at much higher pressures, temperatures, and flow rates (for example, 600 bar, 80 °C, 20 kg/min) than those typically used in preparative chiral SFC, suggest that construction of the next size increment of preparative SFC units should be feasible technologically. An interesting approach to overcoming the pressure/volume problem at larger scale is the use of a multicolumn SFC approach, in which the adsorbent is spread between a number of columns of manageable dimensions. Prototype SFC-SMB systems have been described by Novasep (27) and Johannsen and colleagues (28,29) and show some promise for separations at industrial scale. Interestingly, the SFC-SMB approach offers the promise of more efficient desorption of the more retained enantiomer simply by increasing the pressure in the desorption zone of the SMB, an approach that could further improve performance.

Conclusion

In summary, we have found SFC to be an important tool with many green chemistry advantages for supporting preclinical development in the pharmaceutical industry. Our previous experience had shown that preparative SFC was the method of choice for rapidly accessing pure and enantiopure materials on a scale up to a few grams. Recent experience with larger scale SFC systems has shown that the SFC advantage can be quite useful for providing purified materials on the kilogram scale, where the green chemistry advantages of solvent and waste reduction make the approach attractive from both an economic and environmental standpoint.

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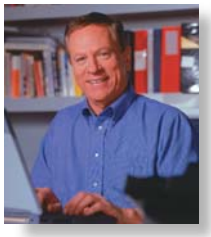

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