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 has recently become widely used for providing rapid access to enantiopure materials to support pharmaceutical development. Here we provide 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.

**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 performing more traditional chemical syntheses, thereby saving considerable labour 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 classic preparative chromatography. (Please see the sidebar that follows.)

**Chirality and the Pharmaceutical Industry**

Most pharmaceuticals are chiral; that is, they can exist as either of two non-superimposable 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 towards 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 development 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...
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. Chemists have long been concerned with the topics of waste prevention, atom economy, and the use of inherently safe solvents and auxiliaries (principles 1, 2 and 12). Also, preparative chromatography is concerned especially with the issue of safer solvents and auxiliaries (principle 12).

Elimination of waste is always a key green chemistry concern and an important factor in the separation of enantiomers (i.e., 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, racemization and recycling of the undesired enantiomer is sometimes possible, enabling higher yield and reduction of waste. Coupling of such resolution and isomerization approaches can lead to truly impressive processes for generating enantiopurity, 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 hour 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 hours 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 is not typically 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 enantioprocessation has enjoyed considerable recent attention 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 labour, 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 biodiversity of preparative SFC separations relative to HPLC methods. Cumulatively, these advantages make preparative SFC enantioprocessation an attractive and potentially broader 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 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 is typically 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 parts of the system at the critical point) would be in the order of 2 kW, whereas power requirements to keep pace with solvent evaporation for a comparable HPLC unit would be in the order of 20 kW. Thus, it can be seen that even in terms of energy utilization, SFC is more efficient than HPLC, because of the lower 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 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 improvements it brings.

When desorbing with carbon dioxide (a known greenhouse gas) as a green solvent alternative, the cost of the base that preparative SFC is not a net generator of carbon dioxide. Instead, it takes carbon dioxide that is condensed from the atmosphere or industrial waste plumes, ships it to the chromatography installation and then later returns it to the atmosphere. In this respect, carbon dioxide is a renewable resource and non-recoverable 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 performing 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 is often 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
When discussing carbon dioxide as a green solvent alternative, it is important to note that preparative SFC is not a net generator of carbon dioxide.
dimensions. Prototype SFC-SMB systems have been described by Novasep27 and Johannsen and colleagues28,29 and show 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.

References

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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.