Challenges and opportunities for the greening of separation science in the pharmaceutical industry

Opportunities for reducing solvent consumption and waste generation in separation sciences used to support industrial pharmaceutical discovery and development are presented and discussed. Several approaches are considered for 'greening' analytical chromatography, where small solvent savings multiplied across the hundreds or thousands of users found in a typical pharma company can add up to significant waste reduction. In addition, replacement of petrochemical-derived hydrocarbon chromatography solvents with supercritical carbon dioxide, and the use of selective adsorbents and reactive resins, are presented as examples of greener approaches to the removal of impurities from pharmaceutical intermediates.

By Dr Christopher J. Welch

Separation technologies in the pharmaceutical industry offer an attractive target for green chemistry improvements[1-3]. Somewhat ironically, separation technologies and other cleaning techniques are often significant generators of waste. This is true in industries such as the refining of metal ores[4] or isolation of paper pulp[5], but is also true in the pharmaceutical industry[6,7], where contaminated solvent waste streams may be generated during the purification of intermediates and final drug products. In this article we focus on challenges and opportunities for greening chromatography, adsorption and other separation technologies currently used in the pharmaceutical industry.

The technique of chromatography was invented by botanist Mikael Tswett in 1903, and originally used for the separation of plant pigments[8,9]. Chromatography relies on the selective partition of different molecules between a stationary phase and a mobile phase, as depicted in **Figure 1**. Liquid chromatography, most commonly used for purification in the pharmaceutical industry[10], typically employs modified silica or polymer particles as a solid phase, with water, organic solvents, or a mixture of the two as a mobile phase. It is these waste mobile phase solvents that offer a significant target for green chemistry and engineering improvements. Solvent reduction, substitution or

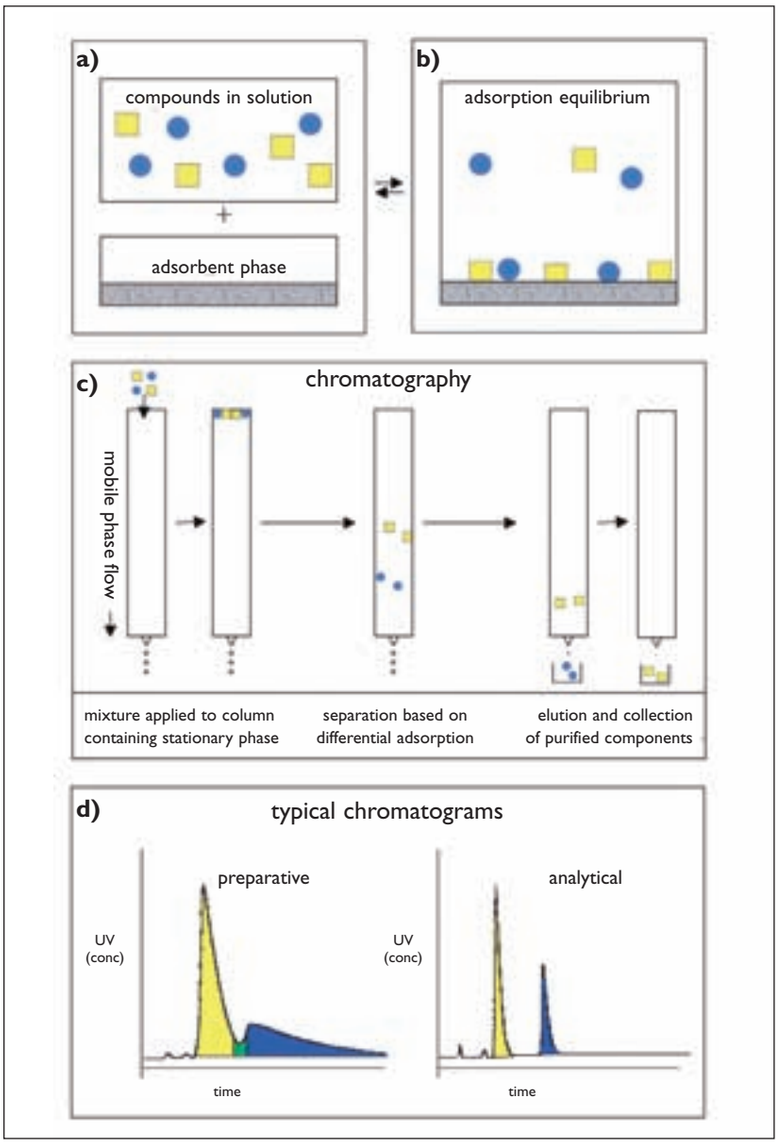

Figure 1: Illustration of the principle of chromatographic separation. Compounds in solution (a) when allowed to contact an adsorbent stationary phase and come to equilibrium (b) may have differing adsorption equilibria for different components – in this example, adsorption of red squares is favoured (3 adsorbed, 1 free) relative to adsorption of blue circles (2 adsorbed, 2 free). When applied to a column containing the same stationary phase and eluted with mobile phase, compounds with differing adsorption equilibria can be chromatographically resolved and isolated (c). Detection of compounds eluting from a chromatography column affords a chromatogram, a record of the separation (d)

elimination has long been a major focus for many areas of green chemistry research[11], including dry cleaning, extractions and organic reactions and processing[12,13], and many of the approaches used in those areas are also useful in reducing solvent waste from chromatography.

Although originally invented by Tswett as a purification technique, chromatography has evolved into one of the most widely used analytical technologies in the pharmaceutical industry. Analytical chromatography is typically performed using a stationary phase column of 4.6mm diameter and 25cm length at a mobile phase flow rate of about 1mL/min. In contrast, preparative chromatography is carried out using much larger columns, sometimes in excess of 1 metre diameter, with mobile phase flow rates of many litres per minute. Given this huge amount of solvent, preparative chromatography must clearly be a major focus for green chemistry efforts. However, given the sheer number of analytical chromatography instruments in use throughout the pharma industry, the greening of analytical chromatography remains an important focus as well. In this article, we treat both of these subjects, and also survey recent developments in purification using batch adsorption techniques.

Four options for greening analytical chromatography: solvent savings one drop at a time

High performance liquid chromatography (HPLC) is one of the most commonly used analytical techniques in pharma today[10]. It is a workhorse analytical tool that allows many different types of scientists to measure the outcomes of their chemical experiments – letting them know if the correct compound has been made, if the purity has changed, if a compound has been metabolised, etc. Despite the relatively small amount of solvent used for a typical analytical HPLC instrument (~1mL/min) the fact that there may be hundreds or even thousands of these instruments operating within a single pharmaceutical company makes the cumulative use of solvent for analytical HPLC a significant green chemistry concern. In addition, recent developments in more fully automated HPLC systems means that these instruments are now often running overnight and even 24/7, making for more significant accumulation of waste solvent. We describe here several different options for reduction of analytical HPLC solvent waste.

Option 1: Solvent recycling by distillation

Recycling of waste solvents by distillation is only rarely used to support analytical HPLC operations[14], although the approach is more widely used in larger scale preparative chromatography operations. In order to be practical, distillation requires easily accessible pools of solvent mixtures that can be easily recovered. For example, distillation was the method of choice for recycling of 2-propanol/hexane mixtures 25 years ago in the

Pirkle laboratories at University of Illinois, when I began my career in chromatographic research[15]. In this instance, IPA/hexane solvent mixtures provided a very general solvent system for carrying out analytical chiral chromatographic investigations, and in addition, the existence of an azeotropic mixture at about 22% IPA/hexane made recovery by distillation relatively straightforward. Although not typically performed at this time, modern distillation or membrane separation technologies could conceivably be used for solvent recovery in the industrial pharmaceutical analysis laboratory, the recovery of acetonitrile from aqueous waste streams being an obvious target. While clearly not worth the trouble on an individual scale, such an approach may begin to look more attractive when hundreds of users within a building or thousands of users within a research site are considered, or when applied by a contract service organisation to the waste generated by multiple laboratories.

Option 2: Recycle of 'clean' eluent

Another option for reducing analytical HPLC solvent waste takes advantage of the fact that significant portions of the analytical chromatogram often contain no eluted compounds (see **Figure 2**). In these instances, an assumption can be made that the solvent in these 'dead zones' is clean, and a switching valve can be used to reroute this waste solvent back to the eluent reservoir for this period of the chromatogram. Switching can be performed on the basis of time, or 'clean' portions of the chromatogram can be identified based on a UV response below a certain threshold value. Several commercial devices to carry out such switching have been available for several years[16], and have received some limited use, although one general problem with this approach is that sections of the chromatogram that appear to be clean can actually contain poor UV absorbing impurities that, when accumulated, can alter chromatographic performance.

Option 3: 'Downsizing' chromatography

Another approach for reducing HPLC solvent waste focuses on reducing the overall scale of the HPLC experiment. When considering the reasons behind the standardisation of HPLC columns size at 4.6mm i.d. and flow rate at about 1mL/min, the answer has little to do with optimal performance, and much to do with the state of the art for packing chromatography columns and for robust and precise pulseless pumping technologies in the early 1970s, when the first HPLC instruments were commercialised[17]. Microflow HPLC has existed for many years, and a number of researchers have recognised the important solvent savings that can be gained when columns are reduced to submillimetre diameters and flow rates are reduced to only a few microlitres per minute[18].

We have recently described a new microflow multiparallel HPLC instrument utilising 300 micron i.d. columns that was developed with Eksigent Technologies for carrying out high throughput analysis in support of high throughput experimentation initiatives in pharmaceutical process research[19-21]. Such high throughput experimentation routinely generates hundreds or even thousands of samples, which places a severe constraint on the analytical tools that must be used in order to acquire and interpret the experimental results. Our initial interest in microflow HPLC came about primarily as a consequence of trying to fit eight independent HPLC devices within a laboratory instrument of a reasonable size. However, with increased usage in the past few years, we have become very impressed with the substantial solvent savings that can be realised with this equipment and with microflow HPLC in general. One example will suffice to illustrate this point – when faced with the challenge of analysing a 96-well plate to support catalyst screening, conventional HPLC took 16 hours and utilised more than a litre of solvent. In contrast, the new multiparallel microfluidic HPLC instrument was able to complete the analysis in one hour with use of only 4ml of solvent. This ~250x reduction in solvent usage is typical (**Figure 3**), and while the net solvent savings are not so impressive for a single experiment, when one considers the hundreds or thousands of microplates analysed in a single laboratory each year, and then considers the number of similar laboratories within any given pharma company, the potential accumulated savings become quite

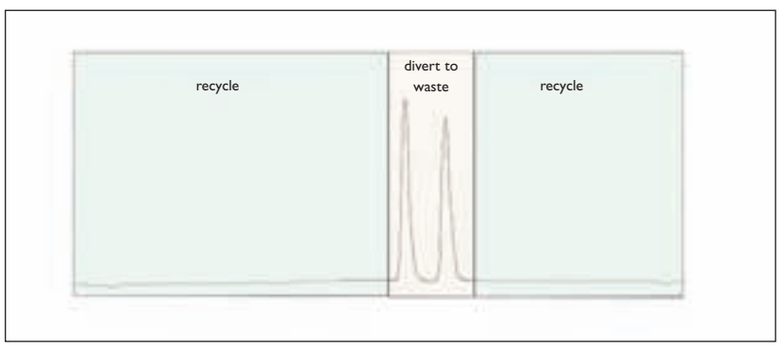

Figure 2: Solvent savings by recycle of 'clean' eluent. A switching valve can be used to recycle effluent from regions of the chromatogram containing no peaks (green areas – about 85% of total solvent)

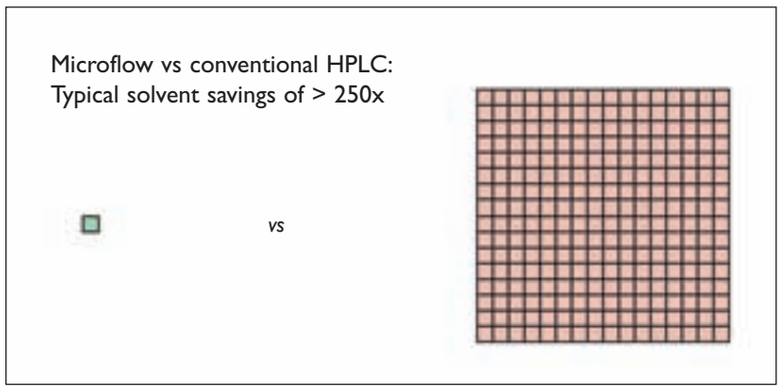

Figure 3
Microflow vs conventional HPLC typically affords a reduction in solvent usage of greater than 250x

impressive. Although our initial experiences focused mostly on the multiplex microflow HPLC instrument (Express 800), the microflow solvent savings can be realised with more conventional single channel instruments available from Eksigent or a variety of other vendors including Agilent[22], Waters[23], etc.

We have recently worked with Eksigent on the development of a new reaction sampling HPLC instrument[24] based on its single channel microflow HPLC instrument. This instrument periodically withdraws reaction aliquots, performs precise dilution and carries out HPLC analysis, with graphical representation of the results. The instrument operates in a completely automated and unattended fashion, and generates less than 10mL of waste per day when operating continuously. In contrast, the use of conventional HPLC (which requires manual sampling, dilution and analysis) generates well over a litre of waste per day, which not only has serious green chemistry implications, but is also a concern for the relative safety of the unattended operation of the system.

Option 4: Replacing hydrocarbons with CO_2

Another approach for reducing the use of organic solvents in analytical chromatography is the switch to supercritical fluid chromatography (SFC) where pressurised carbon dioxide (CO_2) is used as a chromatographic eluent, along with a small amount of an organic solvent such as methanol[25,26]. SFC is often a suitable or superior replacement for normal phase HPLC, especially for chiral HPLC where mirror image enantiomers are separated. Much has been written on SFC using pressurised carbon dioxide, but the important thing to remember is that the very inexpensive gas, carbon dioxide, when pressurised above 74 bar and 31°C, becomes a supercritical fluid – with properties similar to an alkane hydrocarbon, making it a useful chromatographic eluent (**Figure 4**). An SFC instrument is very much like an HPLC instrument, the exception being that the system is maintained under pressure using an outlet pressure regulator that serves to keep the carbon dioxide in the system in a liquid or supercritical fluid state. Clearly, replacing expensive, toxic and flammable hydrocarbons with inexpensive carbon dioxide is financially attractive, especially when one considers that not only are organic solvents expensive, but their disposal is also quite costly.

Not all HPLC is amenable to replacement with SFC. In particular, the most widely used form of analytical HPLC, reversed phase HPLC – where compounds are separated based on bulk hydrophobicity properties using aqueous mobile phases, is not easily translated to SFC. In contrast, normal phase HPLC, in which organic solvents are used as eluents, is often readily translated to SFC, with equivalent or superior performance. This is especially true for the chromatographic separation of enantiomers, analytical chiral SFC having become the preferred method for carrying out these analyses over the past decade[27-29].

With the proven ability to reduce solvent consumption 250x through miniaturisation of equipment (option 3) or 10x through the use of SFC, where pressurised carbon dioxide replaces organic solvents (option 4) it is natural to wonder if the combined approach of miniaturised microflow SFC will afford the expected 2500x reduction in solvent. Microflow SFC has a long and interesting history[30], but has been slow to cross the threshold from use by academic experts to routine use in pharmaceutical analysis. However, there remains little doubt that this technology could offer even greater solvent savings, and could become an important future technique for pharmaceutical analysis.

Greening preparative chromatography

It is in the preparative chromatography arena where the most solvent is used, and where the most impressive green chemistry solvent reductions can be realised. In the past few years preparative chromatography has evolved to become an indispensable tool to support drug discovery, development and manufacturing, with the scale of chromatography, and the amount of solvent usage increasing with each stage[31,32]. In particular, the preparative chromatographic separation of enantiomers has had a profound effect on the way that chiral drug molecules are discovered and developed[33]. In order to be economically viable, most industrial-scale chromatography operations incorporate solvent

recycling and other technologies that optimise and reduce the overall use of solvent and generation of waste, such as simulated moving bed[34] (SMB) or steady state recycle[35] (SSR) chromatography. Owing to issues of speed, convenience and reliability, recycling of waste solvent from preparative chromatography operations is often neglected by the discovery and early development areas within the pharmaceutical industry, presenting a significant green chemistry opportunity.

Automated mass-directed reversed phase preparative chromatography purification of a few milligrams of investigational compounds has become commonplace in drug discovery[36,37] with a single worker/instrument combination being able to carry out several thousand such separations per year. The corresponding amount of waste (typically a combination of acetonitrile and water) generated by these approaches is substantial – on the order of half a litre or more per sample. Technologies for simple and clean recovery of acetonitrile while generating sewerable water would be a great advance, but are not readily available or utilised at the present time. Instead, there has been growing recent interest in carrying out these small scale purifications using supercritical fluid chromatography (SFC)[35,38], where pressurised carbon dioxide (CO_2) is used as a chromatographic eluent, along with a small amount of an organic solvent such as methanol. While the replacement of reversed phase automated library purification with SFC is still a work in progress, with an as yet uncertain outcome, the use of preparative SFC for carrying out chiral separations is much better established, with a documented solvent savings that is quite impressive[39]. Interestingly, carbon dioxide is easily recycled, and is itself a recovered industrial waste product, both important green chemistry considerations.

Greening separation science with selective adsorbents and reactive resins

While chromatography is a very powerful tool for removing impurities from pharmaceutical compounds, it is somewhat mechanically intensive and can be costly to scale up. In contrast, batch adsorption purification treatments using process adsorbents such as activated carbon, ion exchange resins, etc are often preferred for industrial manufacturing, owing to general low cost, ease of scale up and sparing use of solvents, an important green chemistry consideration. In this approach, illustrated in **Figure 5**, a solution containing the compound of interest, with accompanying impurities, is treated with an adsorbent or reactive resin that specifically adsorbs or reacts with the undesired impurity. Simple filtration then allows recovery of the desired component, with effective removal of the impurity. In some cases, the impurity can then be purged from the adsorbent, which can be reused in another clean-up cycle. Of course, in order to be economically viable for use in purifying pharmaceutical intermediates, the adsorbents must be both selective and affordable. It should be pointed out that this technology has, in principle, the potential to impact the general green chemistry problem of remediation of large volumes of slightly contaminated waste by removal and concentration of the offending species, which can then be safely and efficiently disposed of.

With the wide variety of possible adsorbent materials and treatment conditions, developing a method for batch adsorption has historically been a difficult and time-consuming task. We have reported a systematic approach to this problem, employing miniaturised assays carried out in 96-well plate or small reaction tubes[40], that has been used to successfully develop solvent-sparing clean-ups of a number of impurity problems, including removal of metal residues resulting from the use of organometallic catalysts[41], removal of coloured impurities[42], and removal of reactive impurities using specific reactive resins[43,44].

Conclusion

There are a number of opportunities for reducing solvent consumption and waste generation in the separation sciences used to support drug discovery and development in the pharmaceutical industry. We have pointed out a number of approaches for 'greening' analytical chromatography, where small solvent savings multiplied across the hundreds or thousands of users found in a typical pharma company can add up to significant waste reduction. In addition, we have illustrated how replacing petrochemical-derived hydrocarbon solvents for preparative chromatography with supercritical carbon

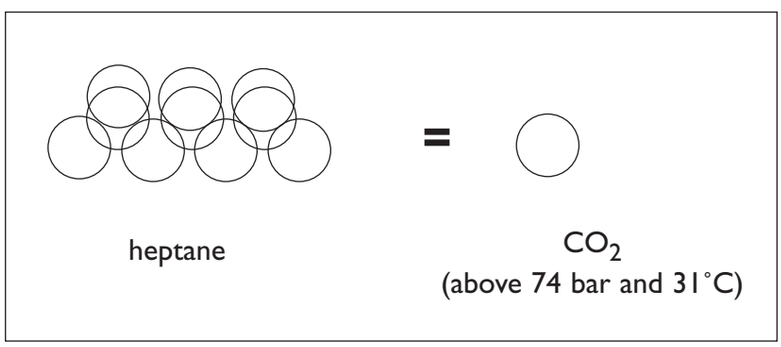

Figure 4
Supercritical carbon dioxide has solvent properties that are similar to petrochemical-derived hydrocarbons such as heptane

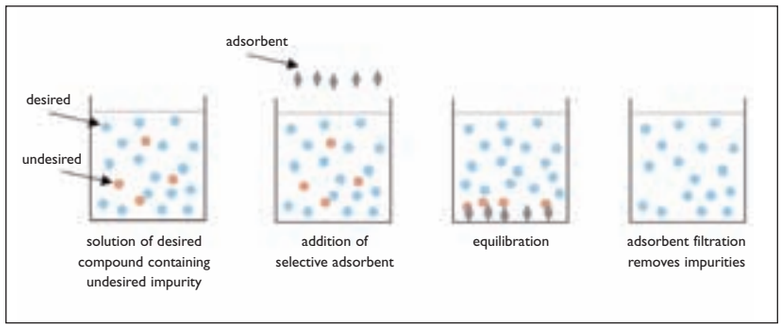

Figure 5
Removal of undesired impurities using selective adsorbents can be an inexpensive and readily scalable approach for purifying pharmaceutical compounds

dioxide can afford dramatic decreases in the generation of waste solvent, and how the use of selective adsorbents and reactive resins can afford significant green chemistry advantages for the removal of impurities from pharmaceutical intermediates. Finally, we have pointed out several opportunities where the intelligent application of technology to existing problems may lead to improved, greener processes for the discovery and development of new pharmaceuticals. DDW

Dr Christopher J. Welch *leads the Separation and Analysis Technologies (SAT) group in the Department of Process Research at Merck & Co in Rahway, New Jersey. He is also the scientific head of the Merck Center of Excellence for Separation Science. Current research focuses on fast method development, high throughput analysis, chromatographic purification from milligrammes to kilogrammes, adsorbent screening and mass spectrometry. Dr Welch has worked in a variety of fields within the chemical industry, including discovery synthesis of agrochemicals (Velsicol-Sandoz), development of reagents for improved immunodiagnostic assays (Abbott Laboratories) and development and commercialisation of chromatographic stationary phases, reagents and enantioselective catalysts within a small chemical business environment (Regis Technologies). Since joining Merck Process Research in 1999, he has focused on developing and applying improved methods and equipment for purification and analysis of pharmaceutical intermediates, and is particularly interested in new paradigms for integrating preparative chromatography with organic synthesis. Dr Welch has authored more than 100 scientific publications and 15 patents, is co-founder of the journal,* Enantiomer, *and a member of the editorial board of the journal* Chirality, *a member of the editorial advisory board of* Organic & Biomolecular Chemistry, *and a member of the executive committee of the American Chemical Society, serving as a councilor for the Organic Division. He is the recipient of several awards in the separation science area, including the NJCG 2004 Award for Excellence in Chromatography and the 2007 PACS Activated Carbon Hall of Fame award.*

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