A Four Part Approach to Reducing the Environmental Impact of Purification Chromatography

White Paper

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Traditional approaches to compound purification involving chromatography utilize large volumes of relatively toxic and expensive solvents, and significantly contribute to the environmental footprint of organizations involved in molecular research. Current directives for greener chemistry have put pressure on organizations to reduce the environmental impact of their work. Through an understanding of the workflow of such organizations and new developments in flash purification systems and columns, it is possible to significantly reduce the consumption and toxicity of solvents used in purifications and therefore improve the environmental impact of such research. In this paper, we present a four-part approach that delivers reductions in solvent consumption of over 50%, as well as the use of substituted solvents of less environmental impact such as acetone.

Introduction

Liquid chromatography (LC) remains one of the most important tools for the purification of molecules from complex mixtures, such as those created as part of a drug discovery process. For those compounds that remain in solution but are unsuitable for recrystallization, LC is the single most important methodology for achieving the high compound purities required for further analysis. However, the drawback of LC techniques is that large volumes of solvent are required which must be purchased, stored, manipulated and disposed of.

Furthermore, increased environmental awareness has led to greater scrutiny of the use of potentially harmful chemicals. The lack of suitable alternative methodologies has kept LC in the laboratory, but pressure is mounting to find cheaper and greener ways of purifying compounds in drug discovery.

Greener Purifications

In a typical drug discovery R&D workflow, some degree of purification is required for every step of the synthesis, with only the highest level of purification required in the 'polishing' step at the end of the process. LC for compound purification is typically performed on a preparative scale, with amounts to be purified ranging from a few hundred milligrams to grams. flash purification is often used for the intermediate purification steps, and preparative HPLC for the final polishing step. Flash purification is most often performed in the normal phase mode, whereas it is common for the final step to involve reverse phase chromatography as the resulting drug compound is often highly water soluble. Given that most synthetic routes are multi step, improvements to the environmental performance of flash chromatography will have the greatest impact on the overall workflow.

Flash purification traditionally enables the purification of up to grams of material, employing solvents such as hexane and ethyl acetate (although dichloromethane and methanol are common). Flow rates vary with column size but tend to be around 10–30 mL/min, with run times of on average 10–30 minutes, using liters of solvent. Preparative HPLC uses lower flow rates but due to the lower loading capacity of the columns, more runs are needed, again using liters of solvent.

The large quantities of solvent employed in compound purification must be purchased and stored, manipulated in the laboratory, removed from the final compound by evaporation, and finally disposed of safely and responsibly. Furthermore, the environmental impact is very high, contributing significantly to the green footprint of a research organization. This is a huge cost in the overall budget of small molecule drug development, and under increasing scrutiny as environmental concerns increase.

A Four-part Approach to Greener Purification

LC remains a key methodology for small molecule purification, however to meet with new environmental concerns and to reduce the cost, modifications to the LC experiment can be made through the design of the instrumentation and consumables used in the purification, and the design of experiments. Environmental concerns in flash chromatography can be approached in four ways:

1. Reducing the amount of solvent used in flash purification by the use of columns with increased loading capacity, allowing the same purifications to be performed on smaller columns.
2. Developing software to automate the adoption of step-gradients, with reduced solvent consumption compared to traditional linear gradients.
3. Increasing the resolution of flash purification columns, particularly reverse phase, enabling flash purification to replace preparative HPLC in the polishing step, which results in much higher column loadings and reduced solvent consumption.
4. Investigating the use of substituted solvents such as acetone for flash purifications, reducing the toxicity and therefore storage, manipulation and disposal costs of solvents.

Together these factors form part of a greener approach to molecular synthesis and purification, and will be discussed in detail below.

High Capacity Flash Columns for Reduced Solvent Usage

Standard flash purification columns are chosen on the basis of the mass of silica they contain, either unmodified for normal phase or typically C18 for reverse phase. They are selected on the basis of the amount of material that may be loaded onto the column, with flow rates chosen on the basis of back-pressure generated by the column. Many of the materials chosen for traditional flash purification columns are irregular as this is a cheaper form of silica.

Recently, spherical column media have been developed with significantly higher surface area than older style media. A comparison between traditional media and the newer high surface area material is shown in Figure 1.

Increased surface area of the modern columns (approximately 700 m^2/g compared to 500 m^2/g) results in higher loading on a column of the same dimensions. As a result, the same separation can be performed using columns of approximately half the

Standard Silica

» Good Resolution

» Intermediate Cost per Column

High Surface Area Silica

» Ultra Resolution

» Lowest Cost per Gram Purified

Figure 1. Performance of standard vs. high surface-area silica.

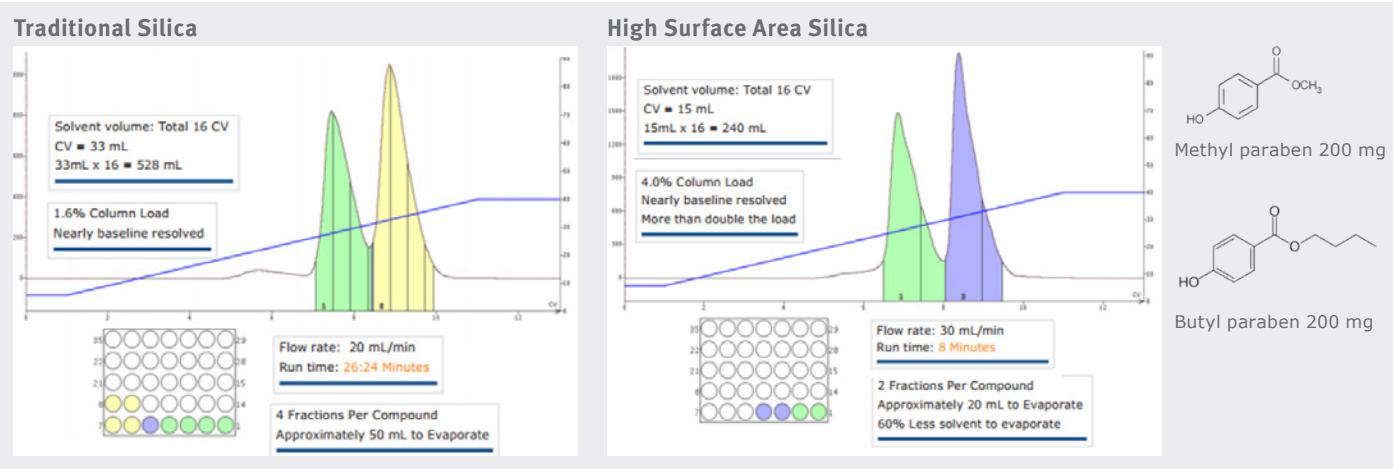


Figure 2. Separation of methyl paraben and butyl paraben using traditional and high surface-area silica.

size. This is illustrated with the separation of two parabens (Figure 2).

The use of high surface area columns results in an identical purification in less than half the time using less than half the amount of solvent compared to traditional silica. For the user, this results in less fractions requiring evaporation and faster separation, and for the organization separation improvements of this kind reduce the consumption of solvents by over 50%.

Step Gradients to Reduce Time and Solvent Consumption

Most flash purifications are performed using gradients where the mobile phase composition changes throughout the run. Linear gradients, where the composition changes in a linear fashion between start and end compositions are common in LC, resulting in tighter separation bands and improved peak shapes over generally longer isocratic (single solvent) methods. Step gradients involve a step-wise change in the solvent composition. These gradients have the advantage of being faster than linear gradients, but they are not widely used in analytical separations due to the risk of losing peak resolution during the step change.

However, step gradients offer significant advantages in flash chromatography, where typically we are looking to isolate a single product molecule from a mixture and are uninterested in the side products or starting materials. Algorithms that enable step gradients to be created from scratch based on results from multiple thin layer chromatography plates can be introduced into software for method development in flash purifications. This enables large amounts of product to be purified by first running a small scale purification before converting the method to a step gradient.

The result of converting a linear gradient to a step gradient for the paraben mixture presented above is shown in Figure 3.

The use of the step gradient results in halving the run time and solvent consumption. The fractions are also more concentrated, which reduces the evaporation time. Note that these improvements are in comparison to a linear gradient run on high capacity columns. The benefits compared to flash chromatography columns based on traditional irregular silica are even greater.

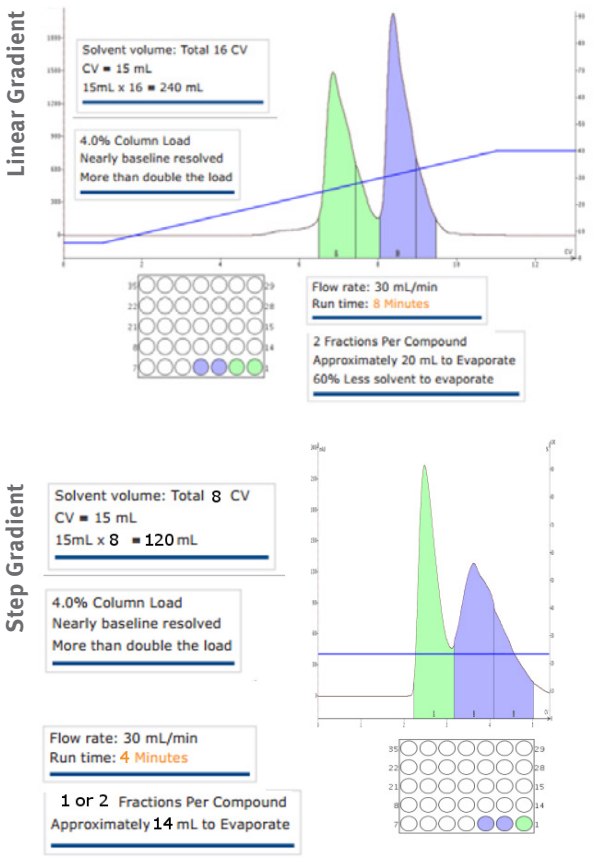
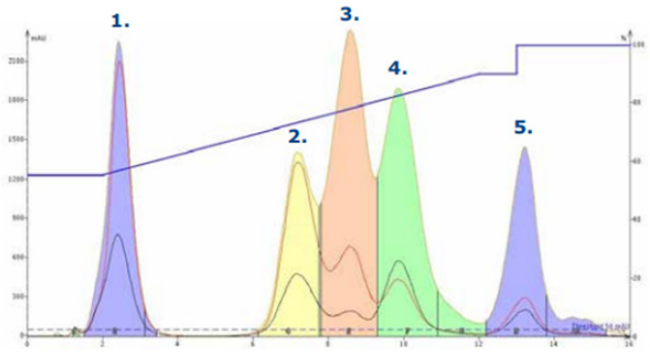


Figure 3. Comparison of linear and step gradients.

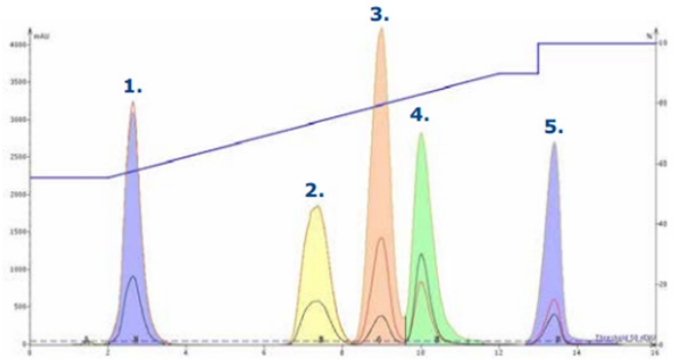
C18



1. Methyl paraben **2.** Butyl paraben **3.** 1-Nitronapthalene

4. Napthalene **5.** 3,5-Dibenzyloxyacetophenone.

High Surface Area C18



1. Methyl paraben **2.** Butyl paraben **3.** 1-Nitronapthalene

4. Napthalene **5.** 3,5-Dibenzyloxyacetophenone.

Figure 4. Comparison of the performance of standard and high surface area C18 columns.

Increased Resolution in Flash Chromatography as an Alternative to Preparative HPLC

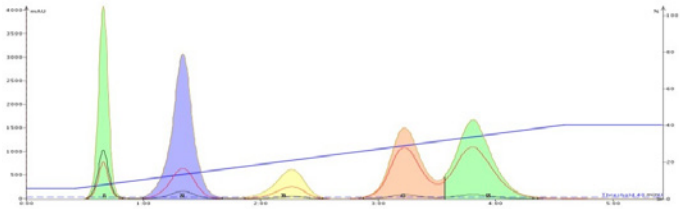
Traditionally, preparative HPLC has been used for the final 'polishing step' in a synthetic route before delivery of the final compound for further development and testing. Preparative HPLC has the advantage of being a high-resolution technique, but has much lower loading capacity than flash purification. Even at the lower flow rates used in preparative HPLC compared to flash purification, solvent usage tends to be much greater due to the longer run times and multiple injections required.

Recent efforts to increase the resolution of flash chromatography to the level of preparative HPLC have included the use of spherical particles and lower particle sizes, particularly for reverse phase chromatography. Comparisons of traditional flash purifications using an irregular C18 medium and equivalent high surface area C18 medium are shown in Figure 4. The resolution of the smaller spherical material is much higher, making flash chromatography an alternative choice to preparative HPLC in some instances, which leads to a further reduction in the solvent usage during the final purification step.

Facilitating the Use of Alternative Solvents in Flash Chromatography to Reduce Costs

Some of the solvents employed in flash purification have been employed widely due to their low cost and high chromatographic performance. However, they may not be the best choice from a cost or environmental viewpoint, and alternatives may be available from the common stock of laboratory chemicals. One of the most commonly employed solvents, ethyl acetate, is generally selected as it is a good solvent for many organic compounds, evaporates easily, and has low UV absorbance above 250 nm where many organic compounds have their strongest UV absorption. However, it is a costly solvent, which has implications for storage and disposal. An alternative polar modifier would be acetone. There is one attribute that keeps chemists from using acetone in chromatography – its strong UV absorbance above 250 nm that can mask compound detection and make UV-triggered peak fractionation a challenge, especially for aromatic compounds.

Hexane/Ethyl Acetate Gradient



Hexane/Acetone Gradient

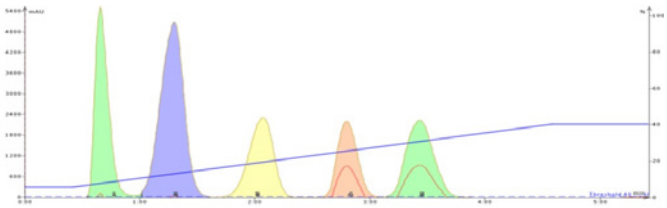


Figure 5. Peak shape improvement by replacing ethyl acetate with acetone.

However, with some state-of-the-art flash systems, mobile phase UV absorption is zeroed in real time, which enables the use of UV absorbing solvents such as acetone. This gives chemists flexibility to explore alternative solvent options.

Acetone and ethyl acetate are in the same solvent selectivity class which means they are essentially interchangeable from a chromatography perspective. Figure 5 shows a representative gradient separation using hexane and ethyl acetate. The chromatogram shows a good separation but not a complete separation, especially for the last two compounds. By simply replacing ethyl acetate with acetone and running the same gradient, each peak sharpens, which in turn improves the separation.

The gradient does not show any UV absorption from acetone because a UV absorption correcting algorithm subtracts any solvent UV absorption during the gradient in real time. It is believed that acetone positively impacts the mass transfer of the more polar compounds, which slightly decreases retention while reducing band-broadening. Reduced band broadening improves detection and minimizes fraction volume.

This greener approach to flash purification encourages the creative use of solvents through the development of features such as UV baseline correction. Changing from ethyl acetate to acetone effectively reduces the cost of a typical separation by around 20%, and reduces the cost of solvent disposal.

Business Benefits from a Greener Approach

The adoption of a greener approach to purification will result in a substantial reduction in solvent usage of over 50% compared to traditional purification. The concurrent reduction in purchase and storage capacity, manipulation time in the laboratory and disposal costs will have a substantial impact on a research organization. This four-part approach aims to minimize the environmental impact of chemistry and lower costs by reducing overall solvent usage. The benefits of this approach are outlined in Table 1 for the same purification using traditional and high capacity columns with a step gradient. This example does not include the benefits of changing to alternative solvents.

	Typical Flash Purification	High Capacity Column	High Capacity and Step Gradient
	Irregular Silica	Spherical Silica	Spherical Silica
Operator Time: Instrument Setup (min)	5	5	5
Total Operator Time (min)	5	5	5
Column Size	25 g	10 g	10 g
Flow Rate (mL/min)	25	36	36
Total Volume/Run (mL)	564	325	257
Total Purification Time and Operator (min)	28	14	12
Reduction in Time to Purify	-	50%	57%
Reduction is Solvent Usage	-	42%	54%

Table 1. Time and solvent reductions from the Biotage workflow approach to flash purification.

Summary

The approach to purification described in this article makes use of developments in instrumentation and consumables around flash purification to reduce the typical solvent consumption levels of a typical drug discovery workflow by 50% or more. This is achieved by making use of high capacity columns and step gradients to reduce solvent use and high resolution columns that act as a viable alternative to preparative HPLC. Also, new detector technologies open the application space to the use of less harmful or expensive solvents. Adopting this approach gives an immediate return in the laboratory, with time savings and reduced costs compared with traditional approaches.

To learn more about getting the best result from flash purification, visit the Biotage blog at http://www.flash-purification.com

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