Some Tips for Achieving Greener, Safer Flash Chromatography

A White Paper from Biotage

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Introduction

As environmentally responsible chemists we all want to reduce the chemical waste volume we generate in our daily research activities. While the chemicals we use are necessary for what we do, there are ways to minimize not only the volume of organic solvents used but also to replace hazardous chemicals with safer solvents.

The part of the synthesis workflow that is most solvent intensive, of course, is flash chromatography. This technique typically consumes hundreds of milliliters or more per chromatographic run, even for low milligram level purification, Table 1.

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Table 1. Linear gradient method volumes for 5-gram to 350-gram columns

Column Size (NP)	CV (mL)	Method length (CV)	Equil length (CV)	Method volume (CV)	Method volume (mL)
5	9	13	2	15	135
10	15	13	2	15	225
25	42	13	2	15	630
50	80	13	2	15	1200
100	150	13	2	15	2250
200	310	13	2	15	4650
350	530	13	2	15	7950

Flash chromatography methods are often developed using thin-layer chromatography (TLC) which may not always supply the desired results. This is because of silica property differences, both physical and chemical, between the TLC plate and column or even the differences in mass transfer kinetics between the techniques. These differences can result in purifications that either do not provide enough separation, requiring re-purification, or too much separation, with both situations wasting solvent.

Typical solvents used with straight-phase (normal-phase) flash chromatography include hexane or heptane, ethyl acetate, dichloromethane, and methanol. These solvents can be hazardous, especially dichloromethane, which is now proposed to be banned in the US by the EPA[1], but they are also expensive to buy and dispose. In fact, solvent disposal costs can be 5 to 10x higher than their purchase price[2], Table 2. Organic waste also adds CO2 and other pollutants to the atmosphere when they are incinerated[3] which is the most common disposal method by chemical waste companies.

Table 2. Organic solvent costs

	Volume (L)	Cost ($)	Cost/mL($)
Hexane	20	$371.57	$0.02
Heptane	20	$354.34	$0.02
Ethyl acetate	20	$568.11	$0.03
Dichloromethane	20	$894.40	$0.04
MeOH	20	$236.10	$0.01

Reducing Waste

Fortunately, there are several ways to reduce the volume of organic solvents used for flash purification.

Step Gradients

There are three typically used purification strategies as part of a synthesis workflow – isocratic elution, linear gradient elution, and step gradient elution. Each purification method has its benefits, but the step gradient has been shown to supply more value than the others. When correctly created, step gradient benefits can include…

- Reduced solvent use and waste generation
- Increased loading capacity
- Increased product purity
- Faster purification

A common technique used to create step gradients begins with thin-layer chromatography (TLC). When the column and TLC silica have comparable properties, TLC retardation factors (Rf) can be used to create isocratic flash chromatography methods[4] and linear gradients[5]. What is less well known is that Rf data from two plates, using the same solvents at different ratios, can be used to create a step gradient[6]. Since step gradients are designed to isolate a specific compound in a mixture, they are very efficient at maximizing purification throughput – maximum load, minimal solvent per time unit.

As an example, the reaction product of isatoic anhydride, α-methylbenzylamine, and benzaldehyde synthesized with a Biotage® Initiator+ microwave synthesizer was purified using TLC-based linear and step gradients.

One of the keys to creating a successful step gradient is ensuring there is a separation between the target product and the reaction by-products. In this example, the product is well separated from the leading and trailing by-products, Table 3.

Table 3. TLC data for isatoic anhydride reaction

	10%	20%
Rf2	0.70	0.74
Rf product	0.09	0.32
Rf1	0.00	0.10

Flash systems, such as the Biotage® Selekt, convert Rf values and solvent % from a single TLC plate into a linear gradient. While linear gradients typically work well for mixture purification, they can waste solvents.

In this example, TLC data was generated from 10% and 20% EtOAc in hexane solvent systems. The data from each TLC separation were used to create a linear gradient method. The 10% EtOAc TLC data generated a 2-20% linear gradient over 10 column volumes (CV) while the 20% EtOAc TLC data created a 5-40% linear gradient over 10 CV. The column used was a 5-gram Biotage® Sfär HC silica column.

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Both methods fully separated the product from unwanted compounds, as predicted by the TLC plates, but with an exceptionally large gap between them. Though this situation is ideal for performing high-load purifications, the solvent between the peaks is wasted if the column is underloaded to maximize purity, Figure 1.

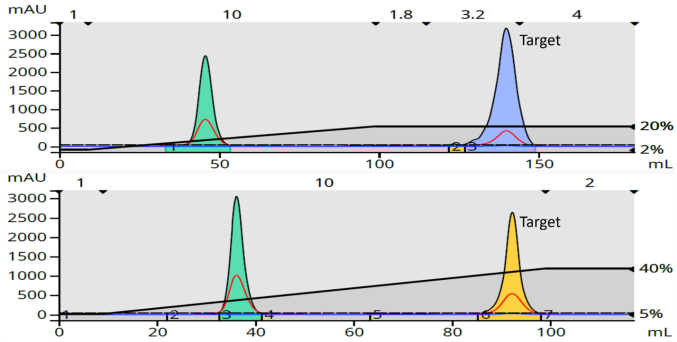

Figure 1. Linear gradient results based on TLC data. Top – 2-20% ethyl acetate in hexane (from 10% TLC). Bottom – 5-40% ethyl acetate in hexane based on 20% TLC.

Gradient length also contributes to solvent consumption and waste. The 2-20% linear gradient consumed 162 mL while the 5-40% method needed 117 mL to complete their respective purifications. While the volumes are not excessive, larger columns will consume proportionately more solvent and, with multiple purifications per day per chemist, the total solvent throughput can be several liters per day.

Step gradients can often reduce the total required solvent for purification without compromising the separation. By using a step gradient created from the 10% and 20% EtOAc/hexane TLC data, a far more solvent-efficient method was generated, Figure 2. The step gradient not only reduced the solvent requirement by 46% (117 mL down to 63 mL), but also partially separated a compound that was co-eluting with the high Rf compound.

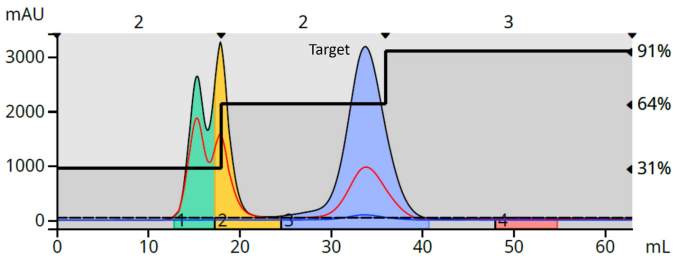

Figure 2. Step gradient results from 10% and 20% TLC data decreased solvent consumption vs the linear gradient while partially separating another by-product.

Step gradients are also effective in reversed-phase flash chromatography.

Smaller Columns

The primary reason chemists use flash chromatography is to maximize the purity of their synthetic product. Simple separations where the product is well resolved from all other impurities allow high sample loads with minimal solvent volume and column size. Unfortunately, this situation rarely occurs, and gradient elution, as mentioned above, is usually needed.

> For challenging purifications, where there is minimal separation between the product and by-products, chemists will often compensate by using columns much larger than needed hoping to maximize the separation and therefore target product purity. While this approach is often successful, this technique requires more solvent than an optimized method using a smaller column.

Particle size impacts band broadening or peak width. Smaller particles reduce band broadening which, in turn, increases the resolution between peaks. This increased resolution increases both loading capacity and product purity. Surface area also directly affects loading capacity, the higher the surface area, the greater the load.

Modern flash chromatography silica is available in particle sizes between 20 and 30 µm, which helps increase loading capacity. However, these silicas typically have the same 500 m^2/g surface area as their larger particle counterparts which moderates the capacity increase. That is because when particle size is cut in half, there is only a 42%, or so, increase in resolution (loading capacity).

To improve on this, some flash column manufacturers, such as Biotage, now use small particle, high surface area silica (20 µm, 700+ m^2/g). This combination of small particle size and high surface area fully maximizes loading capacity and therefore provides the opportunity to use smaller columns to achieve the same purification results as larger columns, but with less solvent.

This is clear with the purification of 100 mg of a natural product extract oil. A traditional column packed with 50 µm, 500 m^2/g of silica (12-gram) and a 5-gram Biotage® Sfär silica HC column (20 µm, 730 m^2/g) were used with the same gradient and sample load. The chromatographic results showed a similar separation, but the 5-gram column used 47% less solvent in the process, Figure 3.

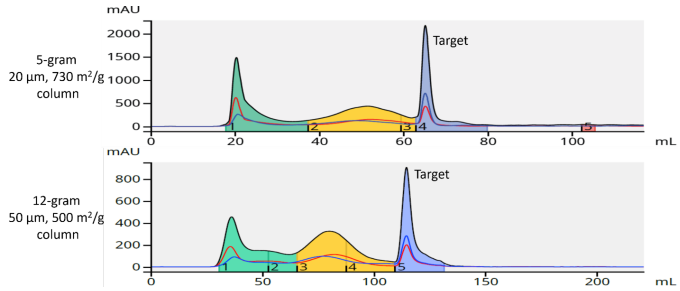

Figure 3. A 5-gram column packed with smaller particle size, higher surface area silica (20 µm, 730 m2/g), top, provides a nearly identical separation as a larger, 50 µm, 500 m2/g, 12-gram silica column, bottom, but uses only half of the solvent.

Dry Loading

There are two ways to load a flash column with a crude mixture, liquid loading, and dry loading. While liquid loading is easier, the choice of dissolution solvent is critical. Ideally, the crude sample solvent needs to be of equal or lesser eluting strength as the mobile phase to minimize undesired bandspreading in the column which can diminish separation effectiveness. When this occurs, larger columns are used to compensate for the strong solvent, which, of course, wastes solvent and adds more solid organic waste into the environment.

Dry loading involves combining a fully solvated crude mixture (in a volatile organic solvent) with a solid media (silica, alumina, C18, diatomaceous earth, ion exchange) and drying the resultant slurry. The dried sample is then packed into a second column or cartridge. The dry load column/cartridge is inserted either into the main purification column (e.g., Biotage® Samplet® cartridge) or attached to the top of the primary flash column in the form of a dry load vessel, Figure 4.

Figure 4. External dry loading uses dried mixture of crude with a solid support media packed into a column mounted on top of the primary purification column.

With dry loading, the crude is concentrated on a small amount of sorbent which improves the separation because there is no sample solvent to carry the sample components down the column. The results include higher loads and better separations, Figure 5.

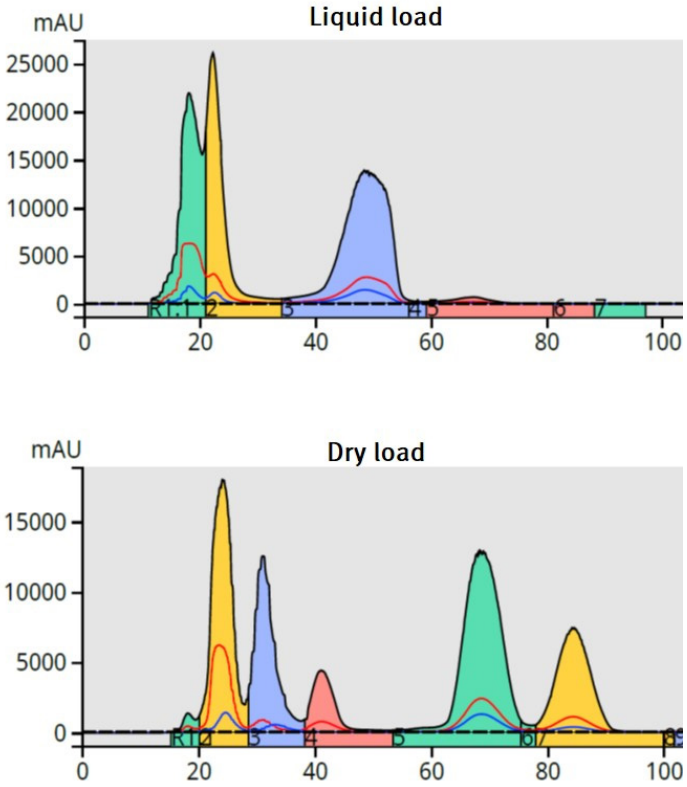

Figure 5. The benefits of dry loading include using smaller columns and improved separations.

Dry loading can also be used to remove excess starting material and some unwanted by-products such as excess amines. In this situation, the dry load media can be a cation exchanger which can scavenge the excess base and simplify the purification. A simpler purification typically supplies higher loads on smaller columns, Figure 6.

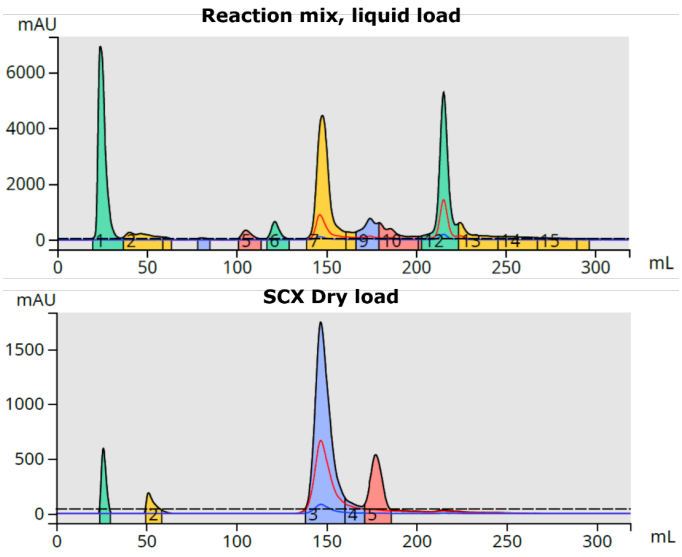

Figure 6. Dry loading with an ion exchange media can help reduce unwanted excess starting materials and by-products. Top – non-scavenged reaction mixture crude liquid load. Bottom – dry load scavenged reaction mixture.

Dry loading improves purification effectiveness and allows use of a smaller column which reduces solvent consumption.

Safer Solvents

While we need to reduce overall organic solvent waste generation, there are some solvents considered more hazardous than others. As mentioned earlier, dichloromethane (DCM) is on the EPA's short list for banishment for commercial use. Other targeted solvents include chloroform and other halogenated solvents, which have been declared possible carcinogens[7].

Chlorinated Solvent Replacement

Both DCM and chloroform are popular in normal-phase flash chromatography as sample diluents and as part of a mobile phase, usually paired with methanol for the purification of polar organic compounds. Not only is their disposal problematic, as they do not incinerate well on their own, purification methods do not always provide the desired results.

Some pharmaceutical companies have teams dedicated to finding alternatives to hazardous, chlorinated chemicals. In fact, chemists at Amgen in 2012 published a paper showing the results of their research using heptane with a 3:1 mix of ethyl acetate (EtOAc) and ethanol as a methylene chloride/methanol replacement[8].

Other variations of the Amgen solvent blend are useful as well. We have found a solvent blend of 2:1 acetone and 2-propanol paired with heptane provides excellent separations of polar reaction mixtures while increasing UV detection of compounds absorbing at the lower end of the UV spectrum (200-240 nm).

Why do these alternative solvent systems work as replacements of chlorinated solvents? Well, much of the reason is due to solvent strength, a particular solvent's eluting power relative to the column media, typically silica. Methanol has a very high solvent strength[9] (0.7) while DCM is moderately weak (0.3) creating potential proportioning issues, Table 4. Too weak a mixture and compounds elute later than desired, too strong a mix and compounds elute too quickly. This is true even if the TLC data shows a good separation with a specific ratio.

Table 4. Solvent strength table

Solvent	Strength
Acetone	0.53
Acetonitrile	0.52
Cyclohexane	0.00
Dichloromethane	0.30
Ethanol	0.65
Ethyl acetate	0.48
Ethyl ether	0.40
Heptane	0.00
Hexane	0.00
Isopropanol	0.60
Methanol	0.70
Methyl T-Butyl ether	0.48
Tetrahydrofuran	0.53
Toluene	0.22

Snyder and Kirkland

Another reason alternative solvent systems can work as replacements to DCM/MeOH and $CHCl_3$/MeOH is solvent selectivity differences, Table 5. Solvent selectivity is the effect that a particular solvent has on the separation of any pair of compounds. This includes elution order and the spacing between those eluting compounds. Dichloromethane falls in selectivity class V while chloroform is class VIII and methanol is part of selectivity class II, as outlined by Kirkland and Snyder[8]. No one selectivity class works for all compound mixture separations so evaluating solvents from different selectivity classes can alter and often improve the separation of reaction mixtures or natural product extracts.

Table 5. Solvent selectivity table

Solvent	Selectivity
Acetone	VI
Acetonitrile	VI
Cyclohexane	0
Dichloromethane	V
Ethanol	II
Ethyl acetate	VI
Ethyl ether	I
Heptane	0
Hexane	0
Isopropanol	II
Methanol	II
Methyl T-Butyl ether	I
Tetrahydrofuran	III
Toluene	VII
Water	VIII

In the case of using 3:1 EtOAc/EtOH with heptane or 2:1 acetone/IPA with heptane instead of DCM/MeOH, these solvent blends often provide similar selectivity (elution order) to DCM/MeOH but can provide a separation with better resolution (spacing between eluting compounds). This is very desirable in flash purification as it maximizes loading capacity on a column which then reduces solvent use.

To highlight the utility one of these safer, alternative solvent systems, the synthetic products of a hippuric acid and benzylamine reaction and a nicotinuric acid and benzylamine reaction were evaluated by TLC with various methanol/DCM and (2:1 acetone/IPA) in heptane mixtures and purified using various solvent systems, Table 6.

Table 6. TLC data of the hippuric acid and nicotinuric acid reactions

	Hippuric acid RxN		Nicotinuric acid RxN	
	10% MeOH/ DCM	30% ((2:1) Acetone/ IPA)/ Heptane	15% MeOH/ DCM	40% ((2:1) Acetone/ IPA)/ Heptane
Rf 1	.73	.51	.63	.45
Rf Product	.10	.40	.28	.21
Rf 2	.00	.20	.10	.07

The products of both reactions were not soluble in solvents weaker than acetonitrile. They were soluble in MeOH making them candidates for both DCM/MeOH and (2:1) acetone/IPA in heptane purification.

TLC was used to find the appropriate gradients. Blends of 5, 10, and 15% MeOH in DCM were tried as well as 20, 30, and 40% (2:1) acetone/IPA in heptane. The data showed the hippuric product was best separated using the 10% MeOH/DCM method and the 30% (2:1) acetone/IPA in heptane method. The nicotinuric acid product was more polar and was best resolved from its by-products using the 15% MeOH in DCM and 40% (2:1) acetone/IPA in heptane methods.

With both reaction mixtures, the DCM/MeOH TLC showed a large separation between the product and its by-products while the acetone/IPA/heptane TLC solvent blends also provided good separation data but with not as much resolution, Table 7.

Table 7. TLC data used to create step gradients for the hippuric acid and nicotinuric acid reaction mixtures.

	Hippuric acid RxN		Nicotinuric acid RxN	
	20% ((2:1) Acetone/ IPA)/ Heptane	30% ((2:1) Acetone/ IPA)/ Heptane	30% ((2:1) Acetone/ IPA)/ Heptane	40% ((2:1) Acetone/ IPA)/ Heptane
Rf 1	.31	.51	.20	.45
Rf Product	.20	.40	.07	.21
Rf 2	.08	.20	.00	.07

However, one of the fundamental issues with DCM/MeOH is that small amounts of methanol can drastically alter elution and separation results often requiring long, shallow gradient methods to effectively perform the separation. Even then, the TLC results often do not reflect what will happen in a flash column.

In the flash chromatography of both reaction mixtures, when the DCM/MeOH TLC data were converted to flash methods on a Biotage® Selekt, they provided little separation of the major hippuric acid reaction mix by-product from the product while providing a moderate separation of the nicotinuric acid product from its major by-product, Figure 7.

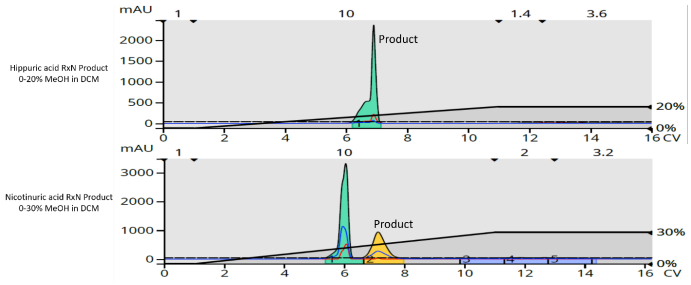

Figure 7. Hippuric acid and nicotinuric acid reaction mix DCM/MeOH flash chromatography results. Though the TLC results for these two reaction mixtures showed a large separation between the product and by-product, the actual results were much poorer.

The (2:1) acetone/IPA + heptane TLC methods, when converted to flash, changed the selectivity of the separation. The major impurities that were poorly resolved using the DCM/MeOH method were fully resolved maximizing the separation and providing an opportunity for further optimizing the purification with a step gradient, Figure 8.

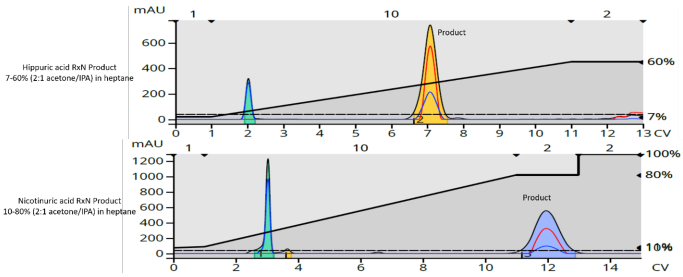

Figure 8. Linear gradient comparison using 2:1 acetone/IPA with heptane solvent systems. Top - hippuric acid reaction mixture. Bottom - nicotinuric acid reaction mixture.

The selectivity change was due to the use of solvents from different selectivity classes. While DCM and methanol are from classes V and II, respectively, acetone is from class VI and IPA from class II; heptane is in class 0. Solvents from other classes will impart other differences in selectivity.

Not only did the alternative solvent system improve each reaction mixture's separation but it improved the purifications' safety by replacing a carcinogenic solvent (DCM) with less toxic substitutes.

Can more be done?

As mentioned above, the increased separation between each synthetic product and its major by-products created the opportunity to further optimize each purification through use of a step-gradient. The benefits of doing this add onto the health benefits achieved through replacing DCM with acetone/IPA.

As seen in the previous example, Rf data from two TLC plates can create a solvent-saving step gradient purification method. For the hippuric acid reaction a step gradient was created from the 20% and 30% 2:1 acetone/IPA in heptane data while the nicotinuric acid step gradient was created using 30% and 40% blends of the same solvents, Table 7.

The combined TLC data generated an 18-31-54% step gradient for the hippuric acid reaction and a 46-100% step gradient for the nicotinuric acid reaction, Figure 9.

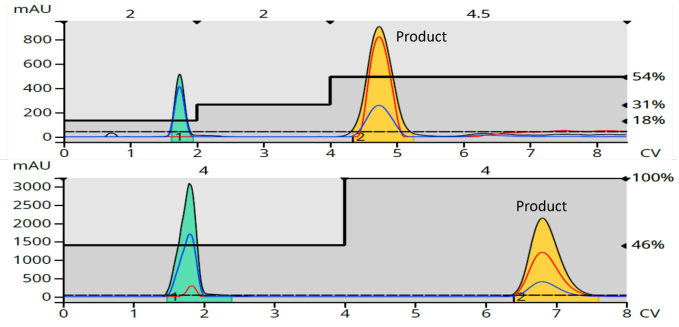

Figure 9. TLC-based hippuric acid (top) and nicotinuric acid (bottom) reaction mixture step gradients reduced solvent consumption 35% or more vs each reaction mixture's linear gradient.

The step gradients not only kept the separation efficiency but reduced solvent use by 35% with the hippuric acid reaction and 46% with the nicotinuric acid reaction. With resolution between the product and by-products maintained, loading capacity can be optimized to supply maximum product purification throughput (mg product/mL solvent), Figure 10.

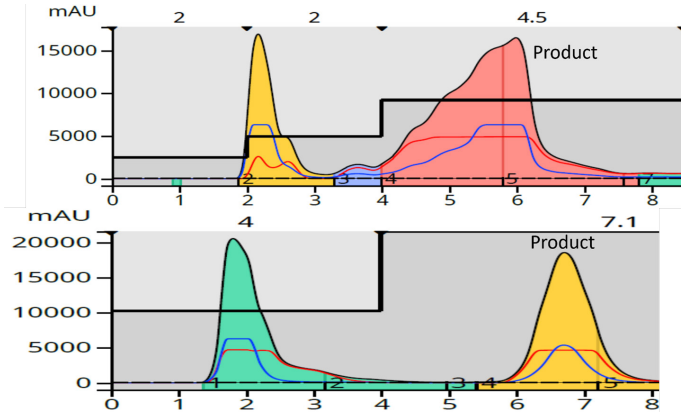

Figure 10. Step gradient scale-up purifications using 10-gram Biotage® Sfär HC columns showing high loading capacity. Top – Hippuric acid reaction mixture (~500 mg). Bottom – Nicotinuric acid reaction mixture (~700 mg).

HILIC

HILIC, or hydrophilic interaction liquid chromatography, is yet another safer possibility for polar compound purification. With HILIC, polar stationary phases are used such as silica, amino, diol, nitrile, etc. with water and acetonitrile gradients. With HILIC, water is the strong solvent while acetonitrile is the weak solvent.

Some chemists call this technique reversed reverse phase, but it is more accurately aqueous normal-phase chromatography. The basic idea behind this is that polar compounds will be attracted to a polar surface (the principle behind traditional normal phase chromatography) and that water (a strong polar solvent) can be used to displace the compounds from the silica. Since chromatographic methods typically need a non-polar solvent to help regulate compound elution, earlier research has found acetonitrile to work best because it not only is water miscible but aprotic[10].

An example of what HILIC can provide is shown with the nicotinuric acid and benzylamine reaction mixture shown above, Figure 11. With HILIC, the lipophilic compounds elute first while the more polar compounds elute later. Another green benefit is the columns often can be reused, which is not the case when using other organic solvents.

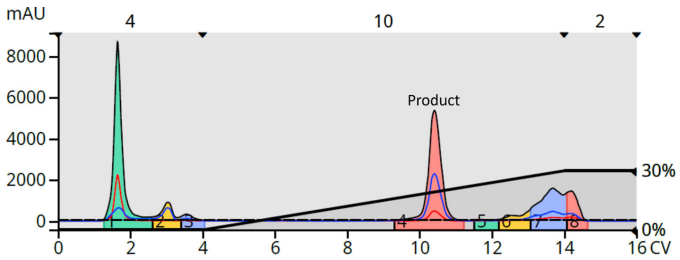

Figure 11. HILIC purification of the nicotinuric acid plus benzylamine reaction mixture provided a complete separation of the product and by-products using water and acetonitrile as solvents.

Reversed-phase

A purification choice for many chemists is reversed-phase flash chromatography. Like HILIC, the solvents are safer to use and dispose of. Reversed-phase methods, like normal-phase methods, are directly scalable and can often be based on analytical HPLC methods used for reaction monitoring and purity analysis.

In the following example, a reaction mixture (hippuric acid + α-methylbenzylamine) was first purified using a 5-gram Biotage® Sfär HC silica column and a 0-10% MeOH in DCM gradient (7 mg load), Figure 12. The column and method are only able to partially separate the two peaks, an inefficient purification method.

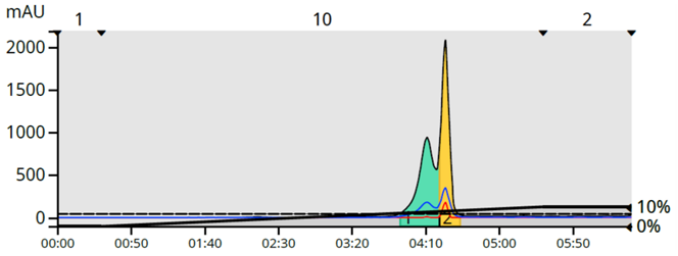

Figure 12. Reaction mixture purification (7 mg load) using a 0-10% MeOH in DCM mobile phase and 5-g silica column provides a marginal separation.

Reversed-phase flash was also used with this reaction mixture. Using a 6-gram Biotage® Sfär C18 column, 35 mg of the same sample (5 x more) was easily purified with the product eluting considerably later than the more polar by-products, Figure 13.

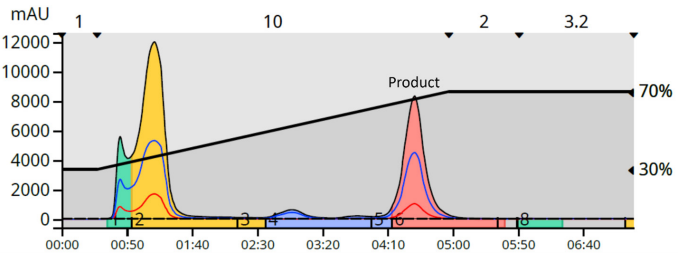

Figure 13. Reversed-phase flash purification of the reaction mixture (35 mg) using a 6-gram C18 column fully separates the product from by-products.

This method was then used to purify 175 mg of the reaction mixture with a 30-gram Biotage® Sfär C18 column proving direct scalability, Figure 14.

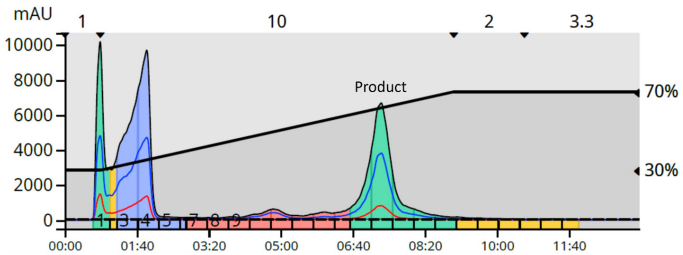

Figure 14. Direct, 5x scale up of reaction mixture using a 30-gram C18 produced a virtually identical purification.

Another major benefit that makes reversed phase the greenest option is column reusability. Silica columns can usually be used only once or twice while reversed-phase columns can be cleaned and reused dozens of times dramatically reducing plastics in the landfills.

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Summary

Greener flash chromatography is easily achievable by making relatively minor changes to your purification process.

1. Step gradients instead of isocratic or linear gradients
2. Smaller columns packed with small particle size, high surface area silica
3. Safer solvents replacing chlorinated solvents
4. Reversed-phase and HILIC for organic solvent and plastic waste reduction

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