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Sustainable Chromatography (an oxymoron?)

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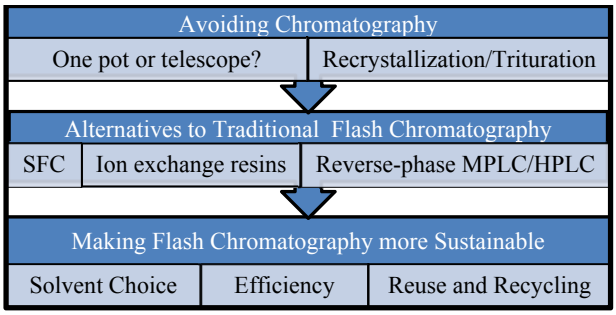
Graphical Abstract



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This perspective details practical techniques for reducing the waste generated from chromatography within pharmaceutical drug discovery labs and additionally provides sustainable alternatives to flash chromatography.

Abstract

Chromatography is routinely used in drug discovery as a means to isolate intermediates and final compounds. From a sustainability perspective, it is one of the largest contributors of solvent waste in the drug discovery process. The medicinal chemistry subgroup within the American Chemical Society's Green Chemistry Institute Pharmaceutical Roundtable (ACS GCI PR) offers a perspective aimed at providing chemists with practical tools and easily implemented techniques to improve the sustainability of drug discovery through reduction of the waste generated during chromatography. This perspective also offers alternatives to traditional, silica gel-based chromatography as well as information on how to avoid chromatography completely through use of crystallization and reaction telescoping.

Key words

Chromatography

SFC

Waste reduction

Crystallization

Reaction Telescoping

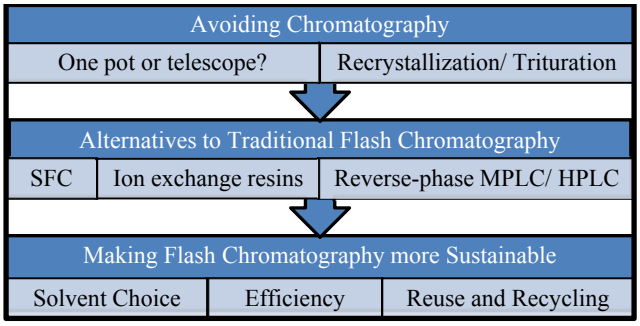
Introduction

Flash chromatography is a significant source of solvent waste generated in drug discovery labs. Recent advances in flash chromatography from hand-packed glass columns to fully automated machines with disposable cartridges have made compound purification by silica gel flash chromatography more effective and time efficient. Due to the implementation of automated systems, a chemist can now set up a purification and walk away. While this has reduced the amount of time invested by the chemist, it has perhaps not improved the use of resources from the standpoint of solvent usage. Furthermore, the ease with which a compound can be purified by chromatography means that less incentive exists to explore alternatives to chromatography that produce less waste. The goal of this perspective is to offer a practical

assessment of the implementation of chromatography within pharmaceutical drug discovery laboratories and to provide methods to reduce the waste produced in obtaining pure compounds without sacrificing the speed of synthesis. Additionally, the topic of avoiding chromatography will be briefly discussed with the aim of providing a set of ideas with which to reduce the generation of waste during synthesis.

Figure 1 is a schematic representation of how a chemist might evaluate their compound isolation plan from a sustainability viewpoint, with the least wasteful techniques listed at the top. Using Figure 1 as a decision tree allows the consideration of several means by which to isolate a compound without the use of silica gel flash chromatography. Ideally, during the execution of a synthesis, intermediates could be carried through multiple steps without purification (telescoping), or isolated through crystallization techniques. If this is not feasible, then alternatives to flash chromatography could be employed, such as super-critical fluid chromatography (SFC), ion exchange resins or reverse-phase high-pressure or medium-pressure liquid chromatography (HPLC/MPLC). Finally, if a silica gel flash column is necessary, there are aspects of flash chromatography that can be altered to reduce waste generation. Since the focus of this paper is on how to perform chromatography in a more sustainable manner, the perspective will focus on the topics shown in the flow scheme in reverse order, starting with techniques and tips on how to minimize the waste impact of silica gel flash chromatography.

Figure 1. Compound Isolation Decision Tree



As stated previously, avoiding chromatography can lead to significant reduction in waste; however, chromatography is sometimes unavoidable due to the reaction scale, product profile and the interests of time efficiency. The section below outlines several practical steps that can be taken to mitigate the amount and the content of the waste generated from chromatographic

purification. The techniques discussed have already been demonstrated to be successful in drug discovery programs. Due to the diversity of chemistry performed in discovery research, the practicing chemist will have to make a critical assessment to whether the recommendations below can be practically incorporated into a particular synthetic route.

Overall reduction of organic solvent use during silica gel chromatography. If a flash column is necessary, there are several means by which to reduce overall solvent consumption during routine silica gel flash chromatography. Column selection at the outset is critical, and using the smallest column that will provide adequate separation, or using smaller columns packed with higher quality silica (smaller particle and/or spherical silica gel), which provide superior separation, will lead to a reduction in the amount of solvent used and the required separation time. Although these columns can be more expensive, they can be reused without significant effort (*vide infra)*. Ideally, the MPLC machine would be configured to collect only the peaks and the chemist would assay the collected peaks in real-time to determine if the compound of interest has eluted from the column so that the run may be terminated. This action will conserve fraction tubes, solvent and time. Another means by which to reduce solvent use is to forego column equilibration. A report comparing the performance of pre-packed silica-gel flash cartridges from several suppliers has indicated that for pre-packed columns, the separation performance is not significantly altered by eliminating the pre-equilibration step for columns less than 120 g size.[1]

Gradient development is another area where separation efficiency can be improved and solvent waste can be reduced. Often, chemists that have not assayed their reaction by thin-layer chromatography (TLC) will run a gradient from 100% non-polar eluent to 100% polar eluent (*e.g.* 100% heptanes→100% ethyl acetate (EtOAc)) to ensure that the desired compound elutes. This is generally unnecessary and wastes solvent at both extremes of the gradient. Judicious use of TLC will enable optimization of the gradient (or isocratic solvent mixture) for efficient isolation the desired compound. A shallower gradient can often provide superior separation to a steep gradient that goes from one polarity extreme to the other, which can cause streaking or co-elution of impurities with the desired product. A rough guideline for initial gradient development has been previously reported and can be summarized by the following example.[1] Ideally, the TLC of the reaction mixture would show that the desired compound has an Rf between 0.1 and 0.5 in the chosen solvent mixture with X% of the polar solvent. The MPLC would then be

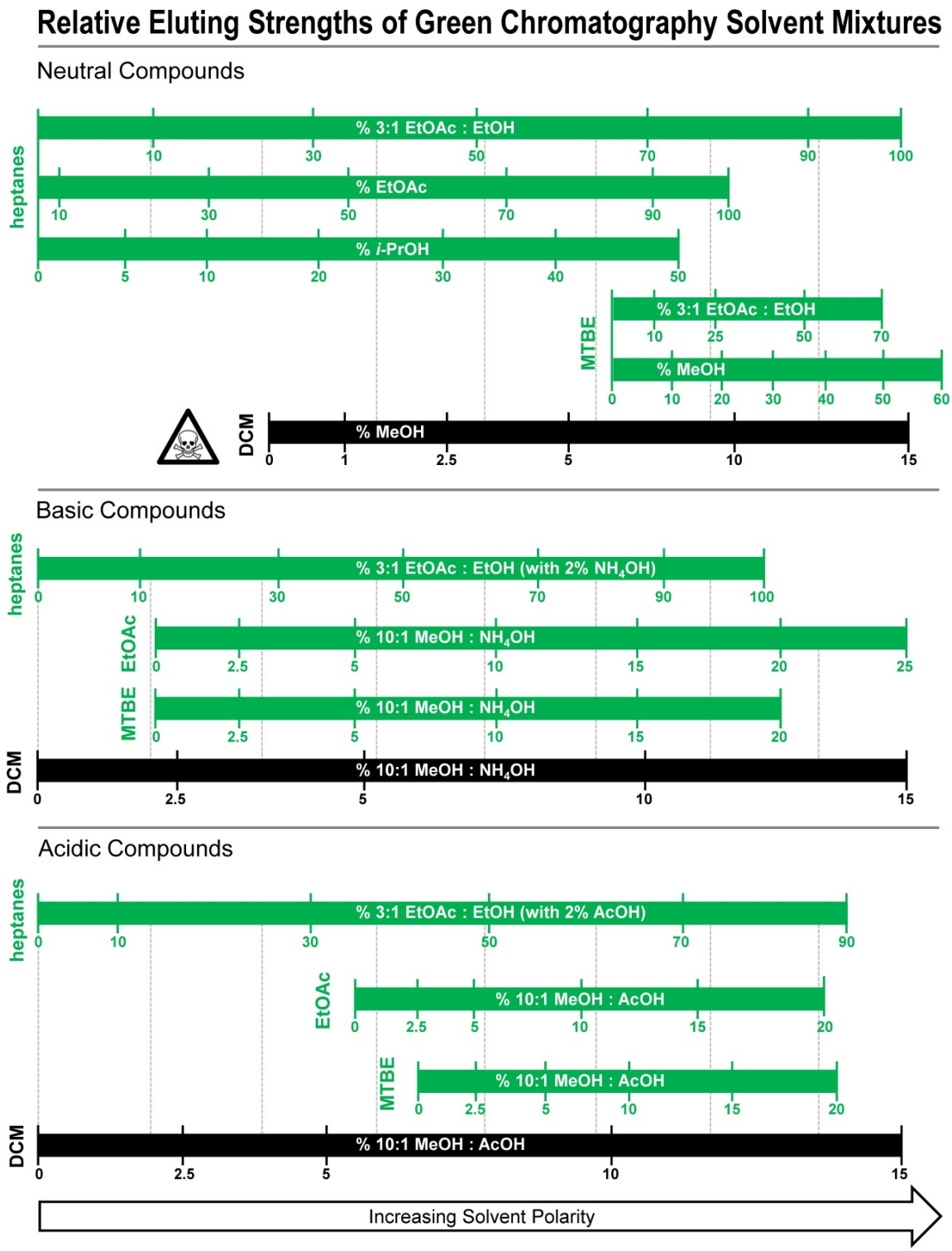
programmed to start the gradient at X/4, increasing the % of polar eluent to 2X over 10 column volumes (CV). The run would then finish with holding at 2X for 1 CV. For example, if a compound had an Rf = 0.2 in 20% EtOAc in heptanes, the gradient would start at 5% EtOAc and increase to 40% EtOAc over 10 CV, then hold at 40% EtOAc for 1 CV. From this starting point, the gradient can be further optimized and once efficient separation conditions have been developed for one analog in a series, the method can be saved on the machine for use on subsequent analogs, since medicinal chemists frequently employ a single reaction type to prepare a series of related analogs. Furthermore, recent advances in MPLC machine software packages include tools to analyze and optimize separation conditions on previously performed runs so that a more optimal method may be applied to subsequent separations. Note that the suggestions above take advantage of automation not only to reduce solvent waste, but to optimize performance and therefore improve efficiency.

Solvent Choice. Several guides have been published regarding selection of greener solvents for use in organic chemistry that can provide a useful starting point for solvent selection in reactions and purifications.[2] The following section will focus on the reduction of dichloromethane (DCM) use in chromatography, since flash chromatography is also a large source of chlorinated solvent waste in academic and drug discovery labs. DCM is widely employed for chromatography due to its properties as a non-polar eluent that dissolves a wide variety of chemical compounds and is commonly used in conjunction with methanol (MeOH), with or without acidic or basic modifiers to purify heterocyclic compounds. DCM is toxic to humans, poses risk to the environment and is challenging to dispose of due to the fact that its lack of flammability hinders incineration.[3] Due to the toxicity of DCM, as well as the increasing disposal costs, reduction of DCM use is a main directive of many green chemistry programs within pharmaceutical drug discovery.[4]

The routine use of DCM/MeOH for heterocycle purification presents an obstacle with regard to reducing DCM consumption. The challenge is familiarizing chemists with an alternative solvent system that can, in most cases, actually provide superior separation than the traditional DCM/MeOH solvent system. Toward this end, solvent guides have been previously published aimed at helping chemists end their dependence upon DCM and move toward greener solvents for chromatography.[5] Figure 2 is a reproduction of an empirically derived solvent guide specifically tailored at converting a DCM/MeOH solvent system to greener alternative solvents.[6]

The most promising solvent combination to emerge from these efforts is the use of a 3:1 EtOAc:EtOH blend as a polar eluent.[7] This can be combined with non-polar eluents such as heptanes and MBTE to provide a range of solvent strengths which can be used to purify a wide variety of compounds. The 3:1 EtOAc:EtOH solvent blend can also be used with basic and acidic additives and is now commercially available from several suppliers.[5a,8] The alternatives to DCM mentioned above can provide adequate, if not superior alternatives to DCM. The DCM/MeOH solvent system can often lead to streaking of polar compounds from silica gel and is often less effective at separating homogenous catalysts such as $Pd(dppf)Cl_2$ from polar compounds than EtOAc combined with MeOH or EtOH.[9] If solubility problems arise, many automated MPLC machines allow the use of a third additive solvent in addition to the polar/non-polar eluents used for a gradient. If DCM is required to solubilize the compound, using as little as 5% DCM as an additive can be sufficient to improve solubility. Employing this technique still leads to a significant reduction in DCM use. To encourage adoption of greener solvent blends such as 3:1 EtOAc:EtOH, it is recommended that pre-mixed ready-to-use 4-L bottles are provided in research laboratories. While a 3:1 EtOAc:EtOH solvent blend is now commercially available from select vendors, it is also possible to recycle clean 4-L bottles and prepare the blend periodically in-house.

Figure 2. TLC Solvent Guide to Aid in Replacing DCM in Chromatography[5a]

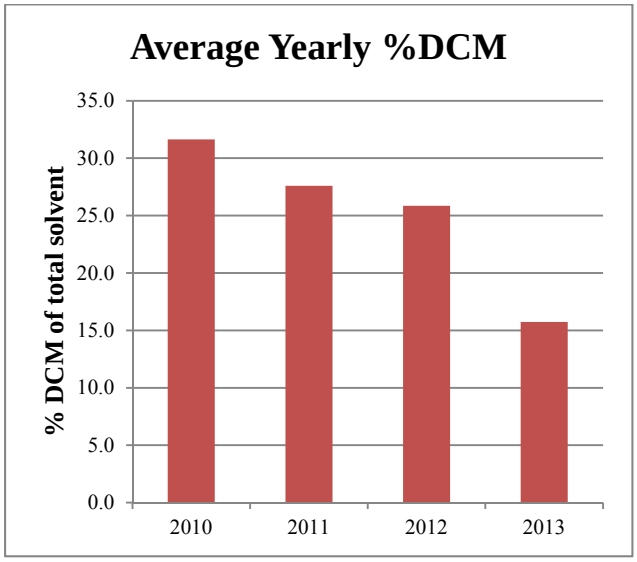


Metrics. Metrics are the key to understanding if efforts to introduce greener practices in the laboratory have been effective. For chemists in manufacturing or process disciplines, this is more straightforward because of the documentation requirements for establishing Good Laboratory/Manufacturing Practices (GLP/GMP). In drug discovery, there are few metrics that can be reliably tracked due to the diversity in both reaction types and scales that are performed on any given day. However, one metric that can be consistently measured is solvent consumption. Depending on how a company or university stocks solvent for use by chemists, there are several methods by which to measure solvent consumption: 1) solvent ordered, 2) solvent consumed, and 3) solvent waste. Each of these methods has advantages and disadvantages. Perhaps the easiest metric to collect is the amount of *solvent ordered*. While this metric is the easiest to collect, it is frequently not very accurate when considering that solvents are often ordered in bulk and may remain in storage for lengthy periods before consumption. It also leads to large fluctuations in the measurements, making it difficult to make timely comparisons between consumption of different solvents at any specific time. Measuring the *solvent consumed* by tracking solvent bottles as they are removed for use from a common store room by the chemist is the most accurate means to measure solvent consumption. The biggest challenge to this method is determining how to capture in real-time what is replenished or being taken from the stocking area over a given period. The easiest way to overcome this challenge is through an electronic inventory that tracks when bottles are added and removed from a particular location. Many companies and universities have switched to barcoded electronic inventory systems to maintain accurate inventory for safety, purchasing and fire codes. This method also allows for facile collection of consumption metrics. The key to successful implementation of this practice is chemist compliance in scanning out bottles from the stocking area. Finally, the last metric that could be collected is *solvent waste*. While it would seem that this method is consistent with the spirit of determining actual waste as well as ensuring "cradle to grave" waste management, the inherent accuracy of this practice is questionable at the discovery level due to evaporation considerations as well as contamination with organic compounds, aqueous wastes and inorganic salts. Furthermore, this method also provides the least amount of information, since the combination of solvents in the waste stream precludes the measurement of any particular solvent used (such as DCM).

With the above considerations in mind, measuring real-time solvent consumption is the ideal method for metrics collection. Once the solvent metrics are gathered, the data can be analyzed and represented by several different methods. Considering the case of DCM consumption, the absolute amount of DCM consumed over a particular time period is the most obvious measurement and allows the determination of the scale and impact of any reductions that occur. Another very useful metric is measuring the DCM consumption as a percentage of total solvent used. While this method requires more solvents to be tracked and extra analysis time, it also normalizes for personnel changes as well as productivity. Furthermore, if a company stocks solvent in several different locations, the %DCM consumption can be measured for each location and can provide a means for determining trends across the chemistry of different project teams, which can be utilized to identify further opportunities for improvement.

Employing the methods described above, the chemists in the Drug Discovery Department at the Amgen Massachusetts site have reduced their absolute consumption of DCM by over 60% between 2010 and 2013. Knowing the absolute consumption numbers revealed that this reduction was on the order of thousands of liters of DCM. Comparison with the alternative (and recommended) metric of DCM as a percent of total solvent, the discovery chemists at the Massachusetts site reduced their DCM use from 32% of total solvent (2010) to 16% (2013)—a 50% reduction in the percent DCM used (Figure 3). The slight differences in these reported outcomes (60% absolute reduction vs. 50% reduction of DCM normalized to total solvent) highlights how analyzing the data several ways can be beneficial for understanding whether the reductions are occurring through productivity/personnel changes or implementation of green chemistry practices. In this case, the significant reduction in %DCM gives confidence that chemists are choosing alternative solvents to DCM in addition to reducing the overall amount of solvent consumed in an absolute sense. Although it was expected that the magnitude of reduction would decrease each year as the easiest behavioral changes were implemented at the outset, there was still a significant additional decrease in DCM consumption in 2013. This was likely a result of stocking ready-made solutions of 3:1 EtOAc:EtOH in the stockrooms and the visible posting of the chromatography TLC guide shown in Figure 2.

Figure 3. DCM Consumption as a Percentage of Total Solvent Used



Reuse and Recycling to Mitigate Chromatography Waste

The widely promoted three R's within the waste reduction hierarchy are "Reduce, Reuse, and Recycle," with a fourth emerging as "Recovery" (usually referring to energy).[10] The most desirable of these practices is waste reduction, and practices aimed at preventing waste are highlighted throughout this perspective. Second to reduction of waste is reuse, where materials are used repeatedly for the purpose for which they were intended with minimal or no cleaning or processing between uses. Following reuse is the practice of recycling, where materials are converted through processing into raw materials for manufacturing. Recovery usually involves incineration to reclaim energy from disposed substances. If a material cannot be converted to energy through incineration then it is generally disposed of in a landfill. The following section will focus on methods for improving reuse and recycling practices within the context of chromatography.

Solvent Recycling. Solvent recycling in chromatography can be a difficult endeavor given that mixtures of solvents are typically used to elute and isolate desired compounds. Often the individual components of these solvent mixtures have similar boiling points such that a simple distillation will not provide a reasonable purity for the solvents to be reused. When considering in-house solvent recycling, safety and regulatory concerns also need to be considered. Special permits and the proper set up are often required in order to comply with safety regulations and

this often makes the cost of this option prohibitive. As a result, it is usually not feasible to employ recycling of solvents in a medicinal chemistry environment, although it has been demonstrated in process chemistry, and is often standard practice manufacturing.[11] This is an area of potential improvement for the drug discovery industry, in which the obstacles presented by solvent mixtures and safety regulations could be overcome through the use of specialized solvent recycling vendors. The difficulties presented for recycling solvent also highlight the importance of solvent use reduction efforts.

Silica Reuse. An often overlooked and easily employed opportunity for reducing waste is the reuse of silica gel cartridges in flash chromatography. With the advances in available chromatography equipment, the need to self-pack columns has been eliminated and pre-packed columns are now standard; high performance pre-packed columns allow for higher flow rates and better separation while reducing silica and solvent amounts required. While higher performance columns (uniform silica particle size and spherical silica) are becoming more widespread, it is common for users to view these as a "single-use" item to be disposed of after one use. Given the high cost of these columns and the corresponding high cost of their disposal as chemical waste, any decrease in the amount used can yield significant cost savings and waste reduction. The quality of performance is usually quite similar to the initial use, but can vary based on the factors discussed below.

In order to reuse a column effectively there are some simple steps that can be taken to ensure optimal results. Most important is the use of TLC, which allows the user to determine the proper gradient and flush solvents to confirm that the majority of material is eluted from the column. Often this is simply an increase in the percentage of the polar eluent for one to two column volumes and then re-equilibration with the desired gradient for one column volume. This procedure is similar to that employed for preparative HPLC, an application where columns are reused for a multitude of separation runs. The use of pre-columns (also known as loading columns) is also a convenient way to clean up a sample by removal of the majority of baseline material, thus preventing accumulation or in the worst case, elution from the reused column in subsequent runs. Optimal results are achieved when preparing similar intermediates, since the purifications and impurity profiles are likely similar. In this case a column can be flushed and reused multiple times. If the column is not to be reused right away it can be flushed with air and

re-equilibrated at a later time. However, this can lead to gaps and air pockets if the column is not packed well, leading to decreased performance. Even if multiple reuses are not possible, it should be remembered that even a single reuse of each column represents a reduction of solid waste and the cost of purchase and disposal by half.

Fraction Tubes. Another often overlooked area of waste reduction is reuse of fraction tubes. Frequently, test tubes used to collect fractions are used only once before disposal. With the advent of new chromatography systems that can collect by UV threshold (at a selected or over a variety of wavelengths) or by an evaporative light-scattering detector (ELSD), fraction collection is much easier to control such that fewer tubes are used to capture the desired product from purification. However, this still leads to a significant amount of glass waste. Fraction tubes can be easily rinsed/washed and reused, thereby significantly reducing the solid waste that can be generated from purification. This simple step can reduce solid waste generated from test tubes by >90%. While it seems that this process would appear to create more waste due to rinsing with solvents, one must consider the life cycle of a test tube: manufacture, transportation to user, usage/disposal, transportation to treatment facility and then incineration or transportation to landfill. Most glass test tubes that are used in chemistry labs are made of borosilicate glass in order to increase their durability and for chemical and heat resistance for a variety of lab uses. Currently, borosilicate glass is not readily recyclable due to the high temperatures required for this process, and as a result it cannot be combined with regular glass for recycling and typically ends up as slag in a landfill. Although rinsing the tubes and reusing them may seem to create more solvent waste, solvent waste can be incinerated as fuel for other processes; and reusing the test tube significantly reduces the need for production and disposal and dramatically reduces solid waste generation. At Pfizer's La Jolla site, a program was adopted where tubes were rinsed and included in the normal glass washing process. The baskets of tubes that were washed required no additional water usage as they filled the gaps between the other types of glassware. As a result of this process, over 115,000 tubes were reused, eliminating 25 cubic yards of solid waste per year while reducing associated costs of purchase and disposal.[12] Despite this analysis, the rinsing and reuse of fraction tubes has not been widely implemented in pharmaceutical research, likely due to the perception that increased solvent usage and chemist time invested in rinsing the tubes is a higher cost than the increased waste from disposal of tubes. As a compromise to rinsing and reusing *all* tubes from a chromatography run, a convenient

compromise is to simply reuse the tubes that contained the desired material, which were likely rinsed clean in collecting the desired compound. While this isn't a complete mitigation of tube waste, it could reduce tube use if combined with glass wash procedures as described above. Diversion of uncontaminated borosilicate glass waste from the landfill is a potential area for improvement within the industry.

Reuse of 4L bottles. While empty brown 4-L bottles can be recycled using standard glass recycling, as described above, reuse is a more sustainable practice than recycling and can provide an opportunity for cost savings on the purchase of custom blends. In-house mixing of aqueous HPLC solvents can reduce expense and waste from 4-L bottles. Many companies purchase pre-blended mixes for use with analytical and preparative HPLC. Combining a small amount of acid additive with water to make aqueous HPLC blends is relatively trivial and can save both money and waste. An additional use for empty 4-L bottles is pre-mixing greener chromatography blends such as 3:1 EtOAc:EtOH. Having these blends readily available encourages their use, saves on purchasing cost and mitigates waste from 4-L bottles.

Alternatives to Normal-Phase Silica Gel Chromatography

Reverse Phase HPLC and MPLC. For very polar compounds, alternatives to silica gel flash chromatography are available. If one is purifying a small amount of material, a reverse-phase HPLC is often the best option for obtaining pure product. Although drying the fractions obtained from HPLC can be tedious and requires increased energy consumption to remove the aqueous portion of the eluent, the amount of waste generated from HPLC purification is often less than that for normal-phase chromatography for highly-polar compounds. It should also be noted that HPLC columns are often reused for upwards of 1500 injections, which minimizes column disposal waste. There are several methods for minimizing the waste generated from HPLC. A technique known as "at column dilution" can be used to increase sample loadings and improve peak shape.[13] Similar to normal-phase chromatography, using a focused gradient that aims to have the desired compound elute at the midpoint of an 8 min run (19 x 100 mm column) can reduce solvent use when compared to the traditional 5–95% gradient used in longer runs (typically 20–30 min).

Reverse-phase HPLC is not the most practical way to purify polar synthetic intermediates synthesized in larger amounts because the scale of most preparative HPLCs employed within discovery would require multiple injections to purify the requisite amount of material. Ideally, such and intermediate could be isolated using crystallization or telescoped into a subsequent reaction. If these solutions are not feasible, the chemist is left with the choice of multiple HPLC injections or a very long silica gel column where the product streaks off after significant volumes of solvent have been used, often with an acidic or basic modifier. A compromise to this situation is the use of reverse-phase MPLC, which has numerous advantages. First, the columns can be reused many times without excessive flushing, in a similar manner to HPLC columns, however, they are affordable enough to dispose of if they become too dirty. The C-18 columns are available in a variety of sizes and the compound to be purified can be loaded in a similar manner to a traditional normal-phase silica gel column, which means that the sample does not have to be homogeneous as it would for use with an auto-sampler. Columns are available for use with either acidic aqueous eluents (usually paired with MeCN or MeOH) or basic modifier (NH_4OH). Larger amounts of material can be concentrated onto Celite® (instead of silica) and packed into a loading cartridge which can then be placed in line with the reverse-phase column. Finally, it is not necessary to purchase a dedicated MPLC system for use with reverse-phase columns. Most of the newer MPLC systems have pumps that can handle the slightly higher pressures required for reverse-phase, and changing between normal-phase purification and reverse-phase is as simple as flushing the lines with *i*-PrOH, attaching a new column to the machine and switching eluent bottles.

Super-critical fluid Chromatography (SFC). Super-critical fluid chromatography has established itself as not only an analytical technique, but as an attractive and green alternative in preparative chromatography. Non-toxic and non-flammable pressurized carbon dioxide is the most favorable fluid for SFC due its moderate critical pressure and temperature (74 bars, 31 °C) and is most commonly paired with methanol (5–30%) or other alcohols to increase the polarity and elution strength of the supercritical CO_2.[14] The addition of small amounts (0.1–0.5%) of acidic (trifluoroacetic acid, formic acid) or basic additives (diethylamine, ammonium hydroxide) may also be needed to improve peak shape and facilitate efficient elution of ionizable compounds. While first impressions of SFC and its use of the green-house gas CO_2 may not directly convince one of its green characteristics, when utilized and applied thoughtfully, SFC can be the best

alternative when preparative chromatography is unavoidable. It is important to note that the delayed addition of CO_2 back to the environment from preparative SFC is not a generator of carbon dioxide, since CO_2 for this process is captured waste from industrial processes such as fertilizers, alcohol fermentation, or natural gas refinement.[15] The addition of CO_2 to the atmosphere can be additionally mitigated if the instrument is capable of on-line CO_2 recycling.[16] In recent years, the increase in popularity of SFC is not only due to instrument advances and a subsequent resurgence of manufacturers, but also because of the internal push within companies to become more sustainable.

Preparative SFC has distinct advantages over preparative HPLC and conforms to the principles of green chemistry through reduced solvent consumption, reduced waste generation, and increased overall productivity. The intrinsic properties of supercritical CO_2 include low viscosity and high diffusivity which contribute to SFC's ability to employ increased flow rates with low pressure drop across the column. An SFC system can flow at linear velocities of at least twice those seen in HPLC while maintaining comparable efficiencies.[14] This feature translates into shorter separation times, and subsequently greater compound throughput relative to HPLC. The importance of solvent reduction can be further realized on larger scale quantities involving grams of compound for which SFC typically consumes 2-10 times less solvent than seen in preparative HPLC.[14,17] When the need to use chromatography arises, SFC can offer a more sustainable alternative to traditional chromatography or HPLC separation through reduction of organic solvent consumption and the ensuing waste generated. In the following section, a general overview of SFC is provided to help guide the medicinal chemist toward incorporation of this technique as an alternative to traditional chromatography.

Chiral separation using SFC. Due to the complexity of biological targets, medicinal chemists frequently synthesize compounds with at least one chiral center; in 2006 nearly 80% of small molecule drugs approved by the United States Food and Drug Administration (FDA) contained a chiral center, 75% of which were sold as single enantiomers.[18] Consequently, the FDA requires investigators to evaluate the safety and effectiveness of therapeutic drugs in their enantiomerically pure state due to variations in pharmacological and/or toxicological effects of individual enantiomers.[19] There are numerous ways (asymmetric synthesis, classical resolution,

chiral separation) to obtain a single enantiomer, all of which have limitations with regard to time investment and waste production (*vide infra*).

SFC is a widely used approach for stereochemical analysis and preparative separation of enantiomers.[20] There are a number of commercially available chiral stationary phases (CSPs) that have been widely adopted, however, prediction of which column and modifier combination will provide the best separation using SFC is complicated.[21] Optimal conditions vary greatly and are compound-specific. It is not uncommon that a slight change in a single functional moiety of a molecule, such as changing a methyl to an ethyl group, leads to the requirement for different columns and/or modifiers to achieve the desired resolution of the respective enantiomers. Most commercially available analytical SFC equipment offers column and modifier switching valves to automate method development.[22]

Achiral SFC. In addition to its use in chiral analysis and purification, SFC is also frequently employed for achiral separations.[23] Medicinal chemists often rely upon walk-up or open-access HPLC-MS systems in order to monitor reactions, identify compounds and determine purity.[24] The introduction of integrated SFC-MS instrumentation has opened the door to greater acceptance among medicinal and analytical laboratories, however, the absence of commercially available SFC-MS instruments has limited the technique to centralized in-house chromatography groups. Although recent advances in integrated SFC-MS have enabled the possibility for faster separations and comparative selectivity to HPLC-MS, currently the main constraint of utilizing achiral SFC as a means for open-access sample analysis is the lack of a general column comparable to the C-18 column most commonly used in HPLC-MS. Due to the wide range of available achiral column chemistries with differing selectivity, a preliminary screening of columns is typically performed prior to purification. Until a more universal column is developed, efforts of the individual research laboratories must therefore be put toward identifying best-in-class columns to meet their own diverse range of compounds.

Ion exchange chromatography. Ion exchange chromatography involves the use of silica, modified by bonds to a variety of functional groups.[25] This purification technique is only appropriate when ionizable and non-ionizable components require separation. The advantages of ion-exchange chromatography must always be carefully weighed against the costs, both financial and environmental, of using a highly processed resource. However, this method can sometimes

offer advantages where a rapid work-up is essential. Crude reaction mixtures can be applied directly to a pre-conditioned column, and this can be advantageous in instances where decomposition is suspected to be a risk over the time scale of other purification techniques.

Ionizable compounds can be quickly separated from non-ionizable compounds in the so called "catch-and-release" approach.[25] This can result in a quicker and less sorbent- and solvent-intensive purification than flash chromatography. For complex reaction mixtures, it can also partially clean the desired product before other purification techniques are applied, which results in the downstream benefits of reduced purification times and solvent usage. Ion exchange columns (and resins) have also been used as a convenient means for desalting compounds before a reaction, or in removing acidic modifiers after reverse-phase HPLC. The most commonly used ion exchange adsorbents include sulfonic acid modified silica (which retains basic compounds), and aminopropyl modified silica, and macroporous carbonate (which retain acidic compounds). The mixture to be purified can be added in any solvent, though MeOH solutions tend to be used most frequently. Acidic or basic modifiers can be used as necessary for the "catch-and-release" approach (Table 1).

Table 1. Techniques for product isolation using ion exchange columns

Mixture Target + Impurity	Cartridge Type	Elution steps
Neutral + Acidic	Aminopropyl (NH_2)	Product elutes in MeOH wash
Acidic + Neutral	Aminopropyl (NH_2)	i) MeOH wash ii) Product elutes in MeOH/HCl
Neutral + Basic	Sulfonic acid (SCX-2)	Product elutes in MeOH wash
Basic + Neutral	Sulfonic acid (SCX-2)	i) MeOH wash ii) Product elutes in MeOH / NH_3

Practical considerations include the use of slow flow rates, as time should be allowed for the ionic interactions to establish an equilibrium; for this reason the sample should be loaded without the aid of external air pressure. Cartridges should be pre-conditioned by wetting with MeOH prior to loading, and care should be taken not to let the sorbent dry out between conditioning, sample loading, and elution steps. The pH should be adjusted to 2 units different from the pKa of

the target to ensure elution.[26] Overall, the use of ion exchange cartridges can provide a quick and easy method for obtaining clean compound when the appropriate functionality is present. Although these columns can be expensive and are typically single-use items due to the intensive process to re-condition them, when used in the appropriate context to avoid a longer chromatography, they can lead to an overall reduction in waste.

Avoiding Chromatography

If applicable, the most straightforward way to reduce the impact of chromatographic purification is to avoid the chromatography altogether. This statement assumes that additional synthetic steps are not required to avoid chromatography. Avoiding chromatography not only reduces the carbon footprint of the individual reaction sequence, but also streamlines the overall sequence in the form of time savings. In this section, a number of commonly employed strategies toward avoiding chromatography are briefly discussed.

Crystallization. An important technique to consider when trying to avoid chromatography is crystallization or trituration, methods which when performed correctly, are fast and efficient. While crystallization is widely used on-scale within process chemistry, the technique remains under-utilized within medicinal chemistry. This is likely a result of the small scale of reactions and the fact that the vast majority of compounds are often synthesized only once in drug discovery. Attempting to develop crystallization conditions on less than 100 mg of material is often an unrealistic endeavor, unless the compound in question is very insoluble and can be isolated cleanly through trituration. Despite these challenges, crystallization can be a powerful tool for the isolation of both intermediates and final compounds that are synthesized on a larger scale. Furthermore, crystallization can be employed in the challenging task of avoiding the chromatographic separation of enantiomers, a requirement faced by the majority of drug discovery and development programs today in which chiral drug candidates predominate (*vide infra*).[18]

Crystallization is a multifaceted technique with many aspects that cannot be discussed in the confines of this overview, but many specialized articles for further reading are available.[27] The following section is intended as an introduction to the methods of crystallization as they might be employed by a medicinal chemist, keeping in mind the considerations that the

technique will likely be performed on a small amount of compound with less emphasis on crystal form. While the process chemist may be focused on cooling cycles, seeding, and strict criteria for the composition of the active pharmaceutical ingredient (API), these requirements can sometimes seem supplementary to the primary goal of quick compound isolation with less regard for the polymorph obtained for the medicinal chemist. Since organic solvents are often used for crystallization, it is important to consider the use of greener solvents (see published solvent selection guides) where possible for this procedure.[2]

The greenest and simplest option for crystallization is *via* a "direct drop" process, whereby the starting materials are soluble in the reaction solvent but the product is insoluble resulting in precipitation during the reaction and allowing for collection by direct filtration. Additionally, as the starting materials are soluble, a small wash is commonly sufficient to ensure any residues are fully removed from the product cake. This procedure can sometimes be predicted by measuring relative solubilities of the starting materials and product in a range of solvents prior to repeating the reaction. The availability and use of pre-prepared product to perform the solubility trial does make this process less plausible for one-off isolations.

The drown-out/anti-solvent crash is a technique whereby a solvent of which the product has minimal solubility is added to the reaction mixture resulting in precipitation; common solvent combinations include either adding hexanes/heptanes to an EtOAc mixture, or water to an alcoholic solution. Problems that can arise include formation of a gum, oil, or emulsion. Although sometimes unavoidable, there are some actions that may work in favor of precipitation. If a gum results, cooling with a longer stirring period sometimes affords precipitation. Likewise, if a solid sample of the product is available, seeding of either the gum or emulsion can facilitate precipitation. Reverse addition, whereby the reaction mixture is added slowly to the stirred neat anti-solvent can also influence precipitation as opposed to oiling/gumming. The drown-out technique is simple to try, and if successful is often a more efficient alternative to chromatography (and ultimately is not a destructive technique, so if unsuccessful, the mixture can be concentrated back to dryness). Drawbacks to the method are that it lacks robustness on scale and can cause further complications such as small particles and/or amorphous material which can ultimately lead to slow filtration times. Other common issues include the simultaneous

precipitation of impurities and inorganic materials that often impede later stage synthesis of intermediates, and unacceptable quality for final products.

The solvent swap/exchange is another common technique for influencing precipitation. The quick (small-scale) method is to concentrate to dryness (or near dryness) and then add a solvent in which the product has poor solubility. A more controlled method is to distill off the original solvent while slowly topping up with a second solvent, eventually removing all of the original solvent. Commonly, the second solvent has a higher boiling point to speed up the exchange, however if an azeotrope exists this is not essential. The advantage of the controlled method is that the gradual change in conditions generally allows for more successful precipitation as opposed to oiling. In addition, if a gum results using the former method of concentrating to dryness, the residual amounts of original solvent retained within the mixture can often complicate and even prohibit precipitation upon addition of the anti-solvent, whereas the gradual process will remove all the original solvent, and result in fewer issues.

Overall, isolation of the desired compound through precipitation can result in significant waste reduction as well as time savings for the chemist. These savings are magnified upon scale-up and will aid in additional time and waste savings as a particular series advances toward development.

Avoiding Chromatographic Separation of Enantiomers. The majority of pharmaceutically active molecules under development today contain at least one stereogenic center.[18] One of the major challenges within discovery is to probe for differences both in activity and deleterious side effects between a pair of enantiomers. Typically, in the early stages of a program, analogs are generated in a racemic form and separated by chromatographic means to give the pair of enantiomers. Although these separations will be on small scale, often it is necessary to implement a chiral column screen for each pair of analogs to determine the optimal separation conditions (see SFC section). Biological testing is then utilized to distinguish between the activity of the enantiomers. From a resource standpoint, this is clearly not sustainable, and trends should rapidly emerge as to a preferred enantiomer.[28] To alleviate the strain on the chiral chromatography resource, teams will attempt to identify a late-stage chiral intermediate that will enable access to their key analogs. Often this intermediate will still be separated by a chiral chromatographic technique. Although this method has a high chance of success, there are a

number of drawbacks with such an approach. From a resource standpoint, although separation of an intermediate does reduce the onus on the purification team in terms of the number of separations required, separation of an intermediate will be required on a larger scale, and as such will be time consuming. From a waste perspective, the separation of an intermediate is certainly less wasteful with regard to both time and materials, but unless a strategy is developed to invert the chiral center by an additional chemical step, the undesired enantiomer is discarded, and as such, 50% of a potentially valuable intermediate is lost unless a racemization step can be employed. Furthermore, it is possible that the lower molecular weight intermediate could lack a UV handle, thus requiring extra derivatization steps to enable effective separation.

The primary drivers within a discovery program are speed and successful separation of the enantiomers. Approaches to accessing chiral compounds within a discovery program are summarized in Table 2. The subjective nature of the assessments in Table 2 are intended to provide the reader with an indication on how likely the method is to succeed in providing the compound with the desired enantiopurity and the anticipated timescale for that method. While it is expected that significant variations exist in the subjective designations shown in Table 2, they provide a useful general comparison. Evaluation of the methods outlined in Table 2 illustrates why, despite the drawbacks listed above, chromatographic separations such as SFC are so often relied upon within medicinal chemistry. As evident from Table 2, chromatography offers both a rapid turnaround (though this will depend on amount of method development required and scale), and with a variety of stationary phases available, a high probability of success. However, there are various other approaches available, which are attractive from a sustainability and scale standpoint. The main drawbacks for early adoption of these alternative approaches are the time and resource required to develop these solutions within a discovery environment.

Classical Resolution. Classical resolutions are well-established for the isolation of enantiopure compounds.[29] The technique relies on the formation of a pair of diastereomeric salts, which display different solubilities, thereby enabling their separation by techniques such as recrystallization. Classical resolutions are commonly carried out within process chemistry, and are usually placed at the early stage of a synthesis because, like chiral chromatography, 50% of the material is discarded. The key to investigating this technique is the presence of an ionizable functional handle within the molecule of interest such as a carboxylic acid or a basic amine,

which can react with a chiral base or acid respectively to generate the desired diastereomeric salt. This approach is facilitated by the wide variety of readily available chiral acids and bases available, many of which are inexpensive and available as both enantiomers. Additionally, the chiral resolving agent is easy to recover after the separation through a salt-break and extraction process. Furthermore, the widespread application of high throughput screening technology can also enable the search for a resolving agent for classical resolution.[30]

Biotransformation. The significant increase in the application of biotransformations in drug discovery over the past decade has been linked to the realization that these reactions can be carried out in typical lab glassware using readily-available enzyme reagents.[31] In addition, numerous screening kits of different enzyme classes have become commercially available, enabling a chemist to adopt high-throughput screening to determine the viability of this particular approach in an expeditious manner. Kits of esterases, hydrolases, ketoreductases and transaminases are available from a range of vendors with detailed instructions on how to perform the screen for which the enzymes are intended. Advantages of the use of enzymes have been well documented in terms of often working in an aqueous environment, and the ability to obtain highly enantioselective transformations. Drawbacks with this approach include the inherent bias of many enzymes to give a particular enantiomer, and limitations in terms of substrate scope. Many of these transformations are resolutions, which does enable access to both enantiomers (though loss of 50% of material is an issue), and there are many examples now of optimizing an enzyme for a particular substrate/transformation through mutagenesis.[32] Finally, it should be noted that there are numerous subtleties involved in scaling such transformations even to gram scale (co-factor recycling, pH control, co-solvents for optimal concentrations), though these are becoming increasingly well-documented to enable widespread uptake of the methodology in an efficient manner.[33]

Chemical Methods. An asymmetric synthesis of a key intermediate is a highly sought after approach to access a single enantiomer of product. It is important to recognize that although highly desirable, any proposed asymmetric synthesis should be carefully evaluated in terms of literature precedent, as this endeavor can rapidly become a major investment in terms of both time and resources expended. Although high-throughput experimentation can expedite these efforts, a deeper analysis and subsequent optimization is typically required. In the worst case

scenario, modest levels of observed enantioselectivity could lead to extensive optimization or even an approach in which an asymmetric synthesis is employed followed by recrystallization or even chromatography to obtain highly enantioenriched product. A robust asymmetric synthesis of a compound is a significant asset to a discovery program, and teams must carefully decide when to invest in its development. One driver for early adoption is for cases in which the transformation is well precedented and predictable in terms of the outcome, and has a high chance of success with only modest optimization. Examples of transformations that meet these criteria are the Sharpless asymmetric epoxidation[34] or dihydroxylation,[35] asymmetric hydrogenation,[36] or the hydrolytic kinetic resolution.[37]

Strategic use of chiral pool materials can also be a rich source for the introduction of asymmetry into a molecule, and this method has been widely exploited.[38] The key advantage of chiral pool starting materials is that the natural materials are both cheap and readily available, one disadvantage is that often only one enantiomer is readily available (or if both are, the unnatural enantiomer tends to be very expensive). An important consideration of this approach is the resource and number of steps required to elaborate the natural material to the desired intermediate. In some cases, the additional steps required to convert the chiral pool starting material to the requisite intermediate may erode the sustainability advantage over chiral separation or chemical resolution methods.

Table 2. Methods for Obtaining Enantioenriched Compounds

Method	Limitation	Turnaround	Resource	Probability of Success
SFC	Gram scale – lose 50% of material	Days	Medium	High
Synthetic Methods	High resource	Months	High	Medium
Classical Resolution	Requires salt handle – lose 50% of material	Weeks	Medium	Medium
Enzyme	Requires functionality	Weeks	Medium	Medium
Chiral Pool Starting Materials	Availability of both enantiomers. Often extra synthetic steps required	Weeks	Medium	High

*Reaction Telescoping***.** Reaction telescoping is broadly defined as the execution of more than one reaction step in a single operational sequence. Related to telescoping are one-pot reactions, multi-component couplings, and cascade/tandem reactions, which may require

additional synthetic planning. For the purposes of this perspective, a telescoped reaction is defined as any chemical transformation in which multiple synthetic steps are accomplished without the need for traditional purification (chromatography, crystallization, distillation), thereby reducing the overall number of unit operations.[39] This can be accomplished by (a) traditional reaction work-up (removal of solvent, aqueous extraction, filtration) followed by use of the unpurified intermediate in the subsequent step (telescoped reaction), (b) addition of reagents directly to the initial reaction mixture to initiate the second reaction step (one-pot reaction), or (c) employing reaction conditions in which multiple consecutive transformations take place sequentially as a result of an intended reaction mechanism (multi-component coupling, tandem/cascade reaction).[40] While reaction telescoping is straightforward in concept and execution, telescoped reactions are put into practice much less frequently than possible. This is due, in part, to the ease of use and ubiquity of automated purification systems, as well as the common notion that proceeding through a synthetic sequence with pure isolated intermediates is always optimal.

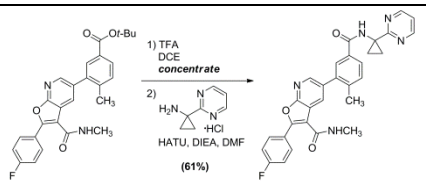
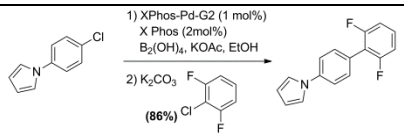
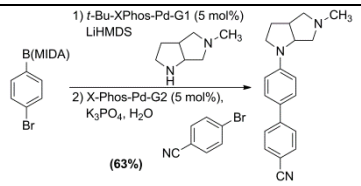
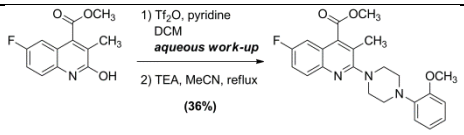
When employing a telescoped reaction, the time savings are realized immediately, as the purification step can often take as long as the reaction itself. However, reaction telescoping affords numerous additional advantages that warrant its application in the early discovery space, as the benefits are compounded as a reaction sequence increases in scale as it moves into development. From a Green Chemistry perspective, there are significant environmental cost-savings that are gained by avoiding the waste associated with traditional chromatographic separations. Eliminating the solvent waste, solid waste, and energy use of automated or manual purification systems renders the overall synthetic process inherently more sustainable. Along with the environmental savings, compounded cost savings in the form of unused goods, and increased chemist efficiency adds to the benefits of reaction telescoping.

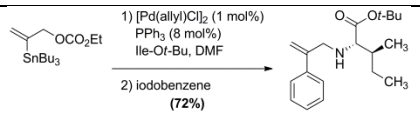
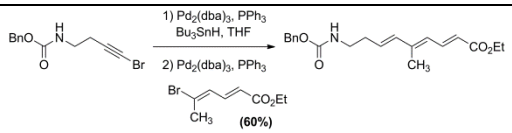
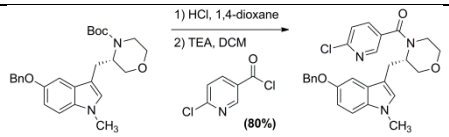
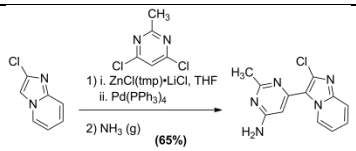
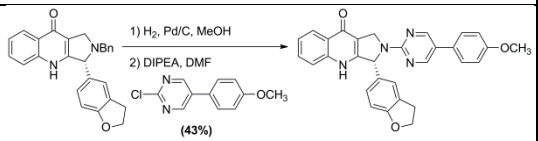
Reaction telescoping bears advantages from a safety perspective as well, often allowing the synthetic chemist to avoid the isolation of hazardous or toxic intermediates.[41] Examples of this practice include Stille reactions initiated without isolation of the intermediate stannane, alkylations performed without isolation of the intermediate alkyl halide, and cycloadditions or reduction reactions executed without isolation of the intermediate azide. Again, while this might

seem insignificant on the milligram-scale, if the same process were to advance into development, the safety benefits would be compounded.

Telescoped sequences can simplify the execution of a reaction.[40] As reaction scale increases, difficulties associated with the isolation of reaction intermediates can become increasingly challenging. These difficulties arise in the form of work-up emulsions, incompatibility with chromatography solvents, and gummy, semi-solid, or flocculent forms that complicate material transfer. Moreover, the yield may be compromised due to the inherent instability of an isolated intermediate to air, light, moisture, or temperature. These described challenges associated with multi-step reactions can often be addressed through the successful execution of a telescoped reaction sequence. Examples of commonly telescoped reactions are shown in Table 3. While some of these examples are performed in undesirable solvents from a sustainability perspective, they serve the purpose of demonstrating reactions that are amenable to telescoping and it is presumed that appropriate substitutions of greener solvents are possible.

Table 3. Examples of Telescoped Sequences from Drug Discovery

Reaction 1	Reaction 2	Intervening manipulation	Example	Reference
Ester hydrolysis	Amide coupling	concentration; solvent change		42
Borylation	Suzuki cross-coupling	none		43
C-N cross coupling	Suzuki cross-coupling	none		44
Triflation	S_NAr	aqueous work-up		45

Allylic amination	Stille cross-coupling	none		46
Hydro-stannylation	Stille cross-coupling	none		47
Boc-deprotection	Acylation	concentration; solvent change		48
Negishi Cross-Coupling	Amination	none		49
Hydrogenolysis	S_NAr	filtration		50

Executing Telescoped and One-pot reactions. A classic one-pot reaction sequence involves the addition of reagent(s) to an ongoing reaction in order to initiate the second reaction step in a single vessel. Recently, Hayashi and co-workers published an elegant example of the power of one-pot synthesis in practice.[51] Their synthesis of (–)-oseltamivir (Tamiflu®) employed three sequential one-pot sequences, required only a single instance of column chromatography, and provided (–)-oseltamivir in 57% overall yield (not shown). While the authors do not quantify the overall energy, waste, time, and cost savings of this synthesis compared to a conventional single pot/single column route, the benefits afforded by employing one-pot chemistry are evident, and the route compares favorably to established methods for the production-scale synthesis of (–)-oseltamivir.[52]

When considering a one-pot synthetic protocol, probing the subsequent reaction with a pilot aliquot is a simple first step. On occasion, an aqueous workup or a change in solvent may be required, which would classify the combined reactions as a telescoped sequence. However, several additional considerations should be made at the outset to maximize the chances of success. Solvent compatibility with any consecutive reaction operation is critical, as even trace quantities of an interfering solvent may compromise a telescoped reaction. Complications arise

when the second step of a telescoped sequence introduces conditions that react with both substrate and solvent. This occurs commonly either with trace protic solvents (MeOH, EtOH, *i*-PrOH) telescoped into a second reaction containing a base, or with polar carbonyl-containing solvents (acetone, DMF, EtOAc) telescoped into a second reaction containing a nucleophile or reducing agent. Similarly, reagent compatibility is important. Care must be taken to either employ orthogonal reagents that will not interfere with one another in consecutive reaction steps, or identify *in situ* quench conditions that ensure effective elimination of the first reagent or interfering by-product prior to the introduction of the second reagent. Problems can arise in telescoped sequences in which consecutive steps involve an oxidation/reduction protocol, acidic/basic transformations, or a metal-catalyzed reaction preceded by introduction of a possible catalyst poison. In many cases, the addition of a sacrificial reagent to ensure removal of an offending reagent or by-product is sufficient to overcome this potential issue.

Multi-Component Couplings. Multi-component couplings (MCC) are a class of organic reaction in which three or more reacting partners come together in a single pot to form one product, with most of the atoms of the coupling partners incorporated into the final product. Examples of MCCs are the Mannich, Biginelli, Ugi, Passerini and Gewald reactions.[53] MCCs typically function under thermodynamic control, in which a single irreversible step governs product formation. Like classic one-pot reactions, the environmental benefits of employing MCCs are obvious, as multiple bond-forming events can be completed in a controlled manner without the need for chromatographic purification of intermediates. Furthermore, the application of MCCs in library format within the drug discovery space facilitates rapid exploration of structure-activity-relationships (SAR) due to the multi-dimensional nature of MCC products and the growing availability of common MCC building blocks.[54] A potential limitation of any MCC is its applicability to a specific target product, sometimes limiting general applicability. Typically, reaction development and optimization are required in order to access the specific structural class, scaffold, or pharmacophore of interest. Nevertheless, MCCs have seen tremendous growth over the past decade, especially within the pharmaceutical industry.

Overall, telescoped sequences are straightforward to develop and execute, and while successes are rarely highlighted in the chemical literature, minimal investment is typically required in order to gauge feasibility. If applicable to a synthetic sequence, it is strongly

recommended that the practicing organic chemist attempt to employ this simple technique in their synthetic arsenal in order to reduce overall waste as well as benefit from the cost and time savings that are immediately realized.

Conclusion

As the pharmaceutical industry strives to incorporate Green Chemistry into earlier stages of the pipeline, an increased focus will be placed on improving the sustainability of drug discovery while maintaining efficiency. With chromatography identified as a major contributor of solvent waste within the medicinal chemistry discipline, innovative solutions will be required to decrease its contribution to the waste output of discovery labs. Chromatography is an integral part of the current drug discovery processes. The aim of this perspective is to familiarize the reader with methods that may mitigate the significant waste generated by chromatography as it is commonly practiced. The suggestions aim not only to make the practice of chromatography more sustainable and less time consuming for chemists, but also to recommend practical alternatives to chromatography that are more sustainable while not slowing down the pace of research. A significant portion of the perspective is also dedicated to avoiding chromatography altogether, which can result in substantial reduction of solvent and solid waste. While the practice of chromatography has seen dramatic improvement with the development of automated machines, further innovations in chromatographic technology and practices are necessary to in order to improve the sustainability of compound isolation.

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