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Perspective

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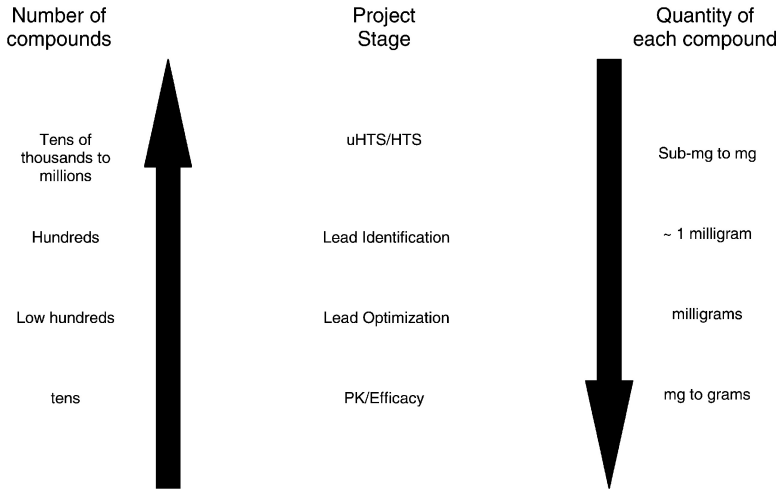

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Changing Requirements of Purification as Drug Discovery Programs Evolve from Hit Discovery

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Many pharmaceutical companies have produced and/or purchased libraries to augment their classically synthesized collection in an effort to enhance the likelihood of finding active compounds (hits) through high-throughput screening[1] that will lead to new drugs.[2–4] The automated synthesis techniques employed for generating large compound libraries facilitate the production of smaller, more focused libraries of compounds that typically are necessary for careful interrogation of structure–activity relationships and for the generation of lead compounds.[5] The automated synthesis tools increased the number of compounds a chemist could synthesize, thereby making it proportionately more difficult to purify all the compounds produced. Early collections produced using high-throughput organic synthesis (HTOS) were screened as crude reaction mixtures that may have been purified somewhat through the use of scavenger resins,[6] liquid–liquid extractions[7] or solid phase extractions.[5,8] While these techniques are readily automatable and often may remove a large amount of the excess reagents or salts, they generally do not remove all the unwanted reagents or intermediates from the reaction mixture.[9]

In an effort to improve the quality of the compounds tested, some groups set a minimum purity threshold as determined by the relative peak areas in UV and/or evaporative light scattering (ELS) chromatograms. Yan[10] discussed the importance of not assuming that the relative purity of unpurified crude products is a measure of the absolute purity of the product, pointing out that the reagents used to add diversity to libraries often are smaller and less likely to absorb at 254 nm. There are other "invisible impurities"[11,12] such as salts, and, in the case of solid-phase organic synthesis, extractables/leachables that are not detected readily. The relative purities of the crude products often are 20–40% higher than the absolute purities. Once the crude products are purified, the relative purities of the purified compounds determined by LC/UV/MS are approximately equal to the relative purities determined by NMR.

Purification reports often state that the isolation of the expected products from crude reaction mixtures results in higher quality compounds, which translates into fewer false positives and false negatives[6,13] because the activity observed would be more likely due to the expected compounds and not to some impurity from the reaction.[14] While this seems self-evident, there was only anecdotal evidence supporting these claims that typically were used to explain the reduction of positives following purification. Since purification is not an inexpensive endeavor, Guintu et al.[15] examined the impact of purification on screening results in order to assess its added value. It was of particular interest to evaluate if it were possible to reserve purification only for compounds found to be active[16] thereby reducing the overall costs associated with producing a reasonable-quality collection for screening.

Using a set of commercial compounds, the authors prepared two sets of screening solutions: neat DMSO solutions to represent the "pure" solutions and DMSO solutions from unsuccessful HT chemistry spiked with pure compounds to represent "crude" solutions which were ~85% pure. A third set of solutions was made by purifying a portion of the 85% pure solutions to create a set of "purified" solutions. When the three solutions were tested in the Gal-SXR assay, it was found that the results from the purified compounds more closely matched those obtained with the pure solutions and that several of the simulated crude mixtures showed higher activity than the pure or purified solutions. These results indicated that purification of compounds could reduce the false positive hit rate but did not address the potential of reducing false negatives.

A comparison of pre- and postpurification data for active compounds in the HIV-1 assay, summarized in Figure 1, showed a significant reduction in the number of active compound solutions after purification and a small number that demonstrated 5-fold greater activity. While the number of compounds used in these studies was small, it demonstrates that just knowing the compound concentration is not enough and that it is important to screen purified compounds, particularly when triaging hits and before medicinal chemists have begun synthesis to examine structure–activity relationships.

Visionaries saw a need for automating purification. Weller[17] described a custom HPLC system capable of successfully purifying up to 200 compounds per day. Kassel

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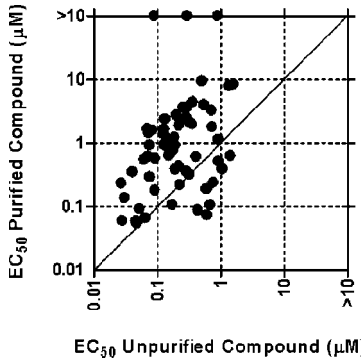

Figure 1. Comparison of compound activities before and after purification. Reprinted with permission from ref 15. Copyright 2006 Sage Publications, Inc.

revolutionized HT purification by coupling a single-quadrupole mass spectrometer to a HPLC and triggering fraction collection based on detection of the expected *m*/*z*.[18] This enabled chemists to purify milligram quantities of compounds without needing to optimize the separations. When compared to UV-triggered fraction collection, PrepLCMS could be used to reduce the number of fractions collected to as few as one fraction per sample injected, thereby expediting the isolation of compounds from crude reaction mixtures relative to UV-triggered purification.

Many publications in automated purification discuss throughput, purities and recoveries.[19–22] While these are important for measuring success in purification and the production of pure compounds, these metrics may not reflect the true impact of the technology on the pragmatic production of pure compounds in a format that is ready-to-screen. Likewise, rapid purification, in and of itself, may not be appropriate at all stages in the drug discovery process. This perspective will cover automated HPLC (/MS) purification and its role at different stages in the drug discovery process. It also will highlight important concerns regarding the long-term stability of purified compounds and the potential problems associated with TFA salts.

Project Evolution and the Role of Purification

HTS/uHTS and Lead Identification. HT-purification papers often deal with libraries on the scale of hundreds to thousands of compounds.[23–25] Such libraries produced by high-throughput organic synthesis can be quite useful for finding hits or for synthesizing analogs. As illustrated in Figure 2, when promising lead compounds are identified for a target, the progression of the project often requires the production of a smaller number of compounds, each in a larger quantity. During the hit finding stage of HTS, it is not uncommon for pharmaceutical companies to screen a collection of one million or more compounds;[1] however, once interesting active series are identified, often fewer than 2000 compounds are synthesized in the course of identifying the best candidate(s) for efficacy studies. As projects evolve, the requirements of purification change. For example, during the early stage of HTS, there usually are a number of interesting compounds in several chemical classes. Since additional chemical optimization will be required, there is more tolerance for unsuccessful purifications, i.e., those purification attempts which result in a loss of the compound. Typically, such HT purifications are accomplished using dedicated instrumentation operated by experts, with the success rate somewhat tempered by the throughput needs.

Lead Identification/Lead Optimization. As projects progress into lead optimization, the successful isolation of the desired compounds takes priority to throughput. There is a reduced tolerance for compound loss because each compound is important for establishing structure–activity relationships, thus the instrumentation used should have a proven history of reliability. Such instrumentation also should accommodate a reasonably high throughput and have the ability to purify up to ~100 mg of crude product per injection in order to accommodate not only the range of testing done at early stages of lead optimization,[26] such as determination of cell-based or biochemical activity and ADMET properties,[27] but also the testing done at later stages,[28] such as more in-depth determination of physical properties[29,30] or initial pharmacokinetics.[31,32] Such purifications may be done by a dedicated group or by individual medicinal chemists.

It is important to realize that recoveries obtained with synthesized compounds are often lower than expected, even when using instrumentation shown to provide >90% recoveries with standard test mixtures. For example, using a set of 744 compounds produced by 16 libraries, Searle et al.[33] compared purities and yields obtained using preparative HPLC or supercritical-fluid chromatography (SFC) to purify 8–10.5 mg of material. The mean yield reported for the sixteen libraries was 35% ± 13%. Likewise, Irving et al.[34] used a modified version of the accelerated retention window (ARW) technique to purify diastereomeric 4-amidopyrrolidones after a four-step synthesis and reported a mean yield of 36.6% for single diastereomers. These results help set realistic expectations and highlight the importance of designing parallel syntheses to target 2–4× the final amount required. The quality of the initial reaction mixture and the ease of isolation of the desired material play an important role in the success of purification. As many chemists are responsible for purifying their own compounds, it is in their best interests to purify intermediates and to design synthetic schemes that will result in a well-resolved separation of the product from the impurities.

Efficacy. As the project advances and very large amounts of each compound are needed for efficacy studies, custom methods are developed for each compound and the main concern is the timely delivery of material sufficient for the in vivo studies. With some rodent efficacy studies able to consume 1 g or more of compound/study, optimized methods capable of isolating this quantity of material are of paramount importance. The production of gram quantities of purified material usually is not too difficult when the compound needed for the efficacy study is achiral or is a chiral compound that may be dosed as a racemate.

Selecting the Appropriate Method of Chromatographic Purification

For most chromatographic separations, there are several solutions that may be employed successfully. The selection needs to be made based upon quantity required, capacity,

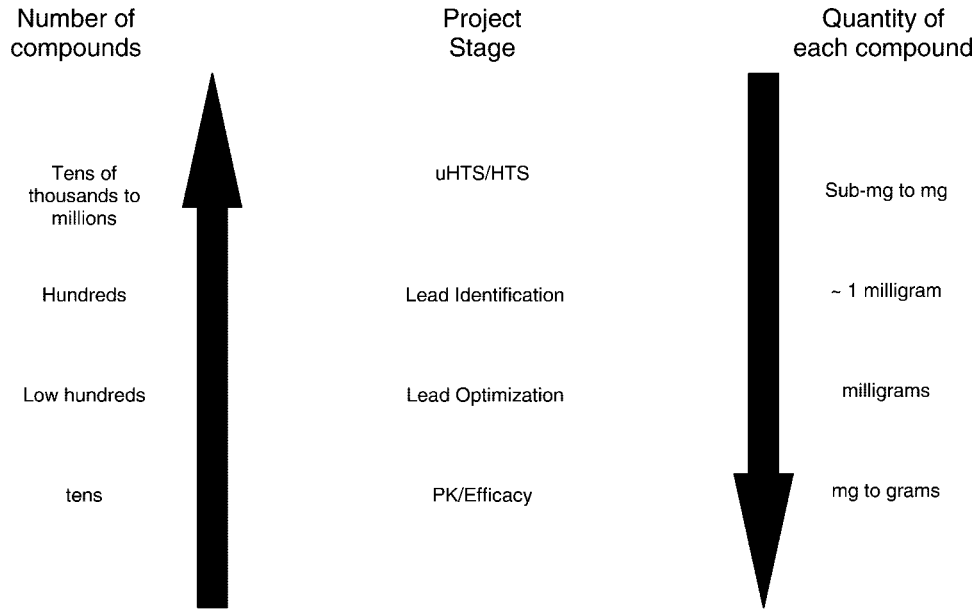

Figure 2. Number of compounds and the quantity required of each evolving with the project, thus changing the requirements of purification.

what instrumentation is available, and ease of obtaining the purified material.

Reversed-Phase HPLC (RP-HPLC). While there have been no recent significant breakthroughs in preparative HPLC hardware, there have been marked improvements in preparative column technology that have extended column lifetimes by 3-fold or more in the author's laboratories. Neue[35] reported that the wide bore, short length columns preferred for HT purification are less stable than analytical columns. Using a transparent HPLC column, it was discovered that a void formed at the head of the column and, if the column were used properly, that this was a significant determinant of column lifetime. This knowledge was used to engineer so-called "optimal bed density" (OBD) columns. These and the "dynamic axial compression" columns are the only two "new technology" columns tested in the author′s laboratory, and both have performed well.

Many groups continue to use reversed-phase HPLC for purification because of the generally facile creation of concentrated sample solutions in DMSO, DMF, dimethylacetamide, or methanol and because method development is relatively straightforward for a range of compounds with varying lipophilicities. Reversed-phase HPLC has been used in all stages of drug discovery, isolating small quantities of large numbers of compounds in lead discovery and large quantities of single compounds for efficacy studies.

Several reports were published concerning the purification of large numbers of compounds in support of lead discovery achieved using custom[21,25] or commercial reversed-phase parallel purification systems. Perhaps the most well-known commercially available parallel HPLC purification system was the Parallex, a four-channel UV-triggered fraction collection system capable of purifying up to 40 samples per hour.[20] In one example, this system was used with great success in isolating 99% of the 1440 compounds purified, with a mean of 3.6 fractions collected per sample injected. The major shortcoming of UV-guided purification is the potential for generating a very large number of fractions for each sample injected, as was shown for another purification of 1440 compounds that resulted in 15 740 fractions. While the success rate for final compound submission was quite good at 86.7%, the potential to generate so many fractions per injection would be a concern for continual purification of large numbers of compounds.

Mass-directed purification uses a mass spectrometer to trigger fraction collection. Although this detector is more expensive than a UV detector, its greater specificity allows the collection of fewer fractions. The Purification Factory reduced the cost per channel by introducing four flow streams into a multiplexed LC/MS system.[22] The peak throughput of this system was comparable to that of the Parallex and typically produced one or two fractions per sample injected, greatly reducing fraction handling.

Although parallel purification systems currently are the highest throughput instrumentation on a per-instrument basis, they require expert users to maintain their performance level and are most appropriately used for the purification of large numbers of compounds. Fast serial purification using high flow rates would be a more flexible alternative to parallel purification that would permit purification of large numbers of compounds yet remain adaptable to the lower throughput sometimes encountered by projects in lead optimization. After implementing the 10 μm columns and 100 mL/min flow rates reported by Goetzinger,[36,37] the author's laboratory realized >50% increase in instrument capacity. While these conditions may not be universal, the cost of implementation is small and the return on investment can be high.

Increasing Column Loading on RP-HPLC. A major shortcoming of RP-HPLC is sample loading. Neue[38] and Blom[39] reported using at-column dilution with gradient separations to increase loading on reversed-phase columns by 14× or more. Unlike a typical HPLC configuration that has the mobile phase mixed prior to the injector, the at-column dilution configuration directs a stream of aceto-

nitrile through the injector and combines this flow with that of the HPLC using a tee placed as close as possible to the HPLC column. By using acetonitrile to transport the sample from the injector to the tee, precipitation of the sample in the injector is expected to be minimized while the mixing with the aqueous component just before the column should allow for compound retention comparable to that obtained with the standard configuration. Since the injection solvent and sample is being introduced to the aqueous mobile phase and diluted in a more gradual fashion than typically occurs in HPLC purification, the pressure increase associated with injections of concentrated DMSO solutions should be less pronounced and the chromatography may be improved. Although several groups encountered excessive pressures when attempting to routinely increase of sample loadings by a factor of 10 or more, the at-column dilution technique is useful even if no attempt is made to increase sample loading, as it reduces sample precipitation in the injector. When coupled with 10 μm particle columns operating at flow rates of 100 mL per minute or more, the slight time added for sample loading is made up by the diminished down time.

Since most drug discovery programs produce basic compounds, enhanced loading of "druglike" compounds may be achieved using high pH modifiers in solvents instead of solvents modified with trifluoroacetic acid.[40] The use of NH_4HCO_3 as a modifier with the pH adjusted between pH 8–10 improved loading and sample retention. The increase in retention of the desired product requires a higher organic concentration for elution and thus reduces the time needed for solvent evaporation. Purification using higher pH mobile phases seems to be gaining more acceptance, particularly now that there are commercially available columns that can tolerate high pH mobile phases. Its widespread adoption seems to be limited mainly by the concern that the compounds produced in drug discovery programs may not be stable at higher pH.

Normal Phase HPLC (NP-HPLC). Normal phase HPLC has 5−10× the loading of typical reversed-phase HPLC and most often is used for large scale purification of intermediates or final products. Method development is straightforward using the thin-layer chromatography (TLC) procedure reported by Renold.[41] Briefly, the reaction mixture is analyzed by TLC and developed in a 4:1 mixture of hexanes:ethyl acetate. On the basis of the R_F of the desired product and the impurities, the scientist can select the appropriate exponential normal phase gradient for purification of up to 10 g of material. While other publications use linear gradients with normal phase chromatography,[42,43] this approach uses exponential gradients and has relatively short separation times of fifteen minutes. Unlike reversed phase HPLC, normal phase HPLC requires much longer column re-equilibration times. The throughput may be increased by incorporating switching valves to allow re-equilibration while purifying the next sample on another column, but, with the lower throughput needs associated with purification of intermediates,[43] the additional complexity may not be warranted.

Normal phase HPLC has been coupled with APCI-MS to purify larger numbers of compounds on a scale of up to 100 mg per injection.[42] In fact, DMSO solutions were injected onto a 100 mm × 20 mm i.d. cyano column, and the product was isolated using 20-min linear gradients. There are two obvious benefits to using normal phase HPLC instead of RP-HPLC for purification: NP-HPLC is orthogonal to RP-HPLC and, for some compounds, notably lipophilic ones, NP-HPLC may be superior; and the normal phase solvents in the collected fractions may be evaporated more quickly than the aqueous fractions collected by RP-HPLC. In the author's experience, normal phase HPLC seems to be better than reversed-phase HPLC at removing residual metal from metal-catalyzed reactions. One potential issue to bear in mind is that the HPLC pump may encounter problems with accurately maintaining the desired flow and may require a cosolvent be added to the weak solvent.

Supercritical Fluid Chromatography. SFC is a normal phase purification technique that has been reported to be an attractive alternative to HPLC purification. Carbon dioxide has a lower viscosity than HPLC solvents, so higher flow rates may be used with longer columns in order to improve resolution without sacrificing throughput. Re-equilibration times are shorter with SFC relative to normal phase chromatography. The use of CO_2 as a mobile phase reduces not only waste generation but also the amount of time needed for fraction evaporation, as the CO_2 evaporates on collection, leaving a small volume of the strong solvent to evaporate.

During fraction collection, the CO_2 expands as it exits the pressurized flow path resulting in an aerosol. To decrease the sample loss during this expansion, pressurized vessels[44] or custom "collection shoes"[45] were devised and used successfully. These engineering efforts to control/contain the aerosol, while effective, used specialized equipment or devices that may be difficult to implement. Zhang et al.[46] reported straightforward modifications to a standard 2757 fraction collector that permitted high recoveries without the need for specialized collection vessels or devices. They were able to reduce the aerosol formation and enhance recoveries by replacing the collection needle with a piece of Teflon tubing having a diameter of 3.2 mm.

SFC purification often has used a UV detector to trigger collection. As with HPLC purification, the use of a UV detector to trigger collection increases the likelihood of collecting multiple fractions for each sample injected. One group overcame this problem and purified many libraries and thousands of compounds by using the analytical SFC/MS data in conjunction with custom software to determine the preparative SFC retention time for the desired material.[47] As with HPLC purification, some groups have used a mass spectrometer to trigger fraction collection,[46,48] but this precludes the use of amines additives to improve the peak shape of basic compounds. Ethylpyridine SFC columns are reported to be an acceptable alternative for separating basic compounds.

Bottlenecks in Purification and Effect on Throughput

While purification usually is cited as the slow step in the production of pure compounds, a study comparing the throughput achievable with single and parallel LC/MS purification systems demonstrated that the time required for

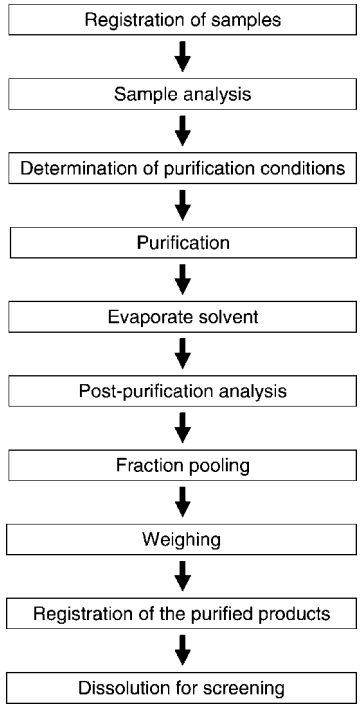

Figure 3. Typical HT-purification process.

the production of final compounds for screening was not decreased proportionally to the number of columns.[49]

Several publications have addressed the impact of HT purification on the overall process of producing purified screening solutions from crude reaction products.[49–51] In general, the same process, shown in Figure 3, is followed: sample registration, prepurification sample analysis, determination of appropriate purification conditions, purification, fraction evaporation, postpurification analysis, fraction pooling, weighing, registration of the purified products, and generation of plates for screening.

The Value of Prepurification Sample analysis. Earlier work in HT purification demonstrated success when creating "universal" gradients for a library based on prepurification analysis of a subset of the compounds. In that work, no attempt was made to exclude reaction mixtures that lacked the expected product. Prepurification analysis of all samples prior to purification is necessary for determining the most appropriate technique (HPLC/UV, HPLC/MS, SFC/UV) to maximize purification success,[47] for eliminating the added cost of purifying reactions that failed to yield enough of the desired product[23] or for automatically creating custom purification methods and/or triggering thresholds.[52,53] With the current availability of integrated vendor solutions that create optimized purification gradients based on the prepurification data, the value of such analyses is enhanced and strengthens the case for analysis of all samples before purification.

Purification. There are several techniques that have been used successfully to purify compounds generated using HTOS. It is important to realize, however, that parallel purification, while increasing the production of purified compounds approximately linearly with the number of columns in parallel, simply pushes the bottleneck to fraction evaporation[49] and pooling/compound transfers. In addition, parallel technologies may require additional expertise to maintain their performance. For example, the Purification Factory, a parallel, four-channel multiplexed purification system, was reported to have a peak throughput of 704–768 samples per day,[22] with a mean throughput of 528 samples per day for a 20-workday month.[24] The routine throughput achieved on the Purification Factory was approximately 70% of the maximum achievable, with most of the "idle time" used for performance checks and maintenance of the four-channel sprayer to obtain acceptable recoveries of at least 85%. Although parallel purification systems are considered state-of the art, operating and maintaining them requires more specialized training and expertise relative to single-channel purification systems, so the benefit of using parallel purification systems must be weighed against using 2–3 single-channel systems configured for fast serial purifications.

Postpurification Analysis. It is important that the collected fractions either be evaporated to dryness and redissolved or shaken to homogeneity prior to performing postpurification analysis, as the collected fractions are not homogeneous and may not become so after standing for 24 h.[54] Evaporation prior to LC/MS analysis is preferred, as some compounds may degrade in a centrifugal evaporator, thus the pre-evaporation purity assessment may not agree with the actual purity of the compound solutions sent for screening.

Cleaning the compound Collection: Is It Practical to Purify Everything before Screening?

Ideally, all HTS assays would be done using highly pure compounds. However, compound collections often are very large and contain historical or purchased compounds of unknown quality as well as compounds produced using HTOS technologies. With some collections exceeding 1 000 000 compounds and with the reported monthly purification throughput of 10 000–15 000 compounds/month,[24,55] it would take in excess of 5 years to purify such a collection. It may be impractical to undertake such a large scale effort, as HTS groups are unlikely to wait that long to complete a screen. Given the low hit rate of 0.1% typically found in HTS,[12] unless a strategic decision is made to generate focused libraries through purification of subsets of the collection, it may be more practical instead to purify all newly synthesized compounds and all interesting hits from HTS and to remove compounds found to have poor integrity[56] or stability from the corporate collection.[57] The latter is a cost-effective strategy for improving the collection and justifies the use of dedicated purification resources for the immediate needs of the active drug discovery programs.

Purification of libraries which produced on a scale of <2 μmol of each compound may not yield sufficient quantities after purification to justify the time and expense. Submilligram quantities are acceptable and useful for HTS, but any work beyond hit confirmation often would require resynthesis. Thus, before embarking on a large scale purification

effort of older combinatorial libraries, it would be prudent to evaluate a small subset and perform a cost/benefit analysis.

Compound Degradation and the Stability of Compound Solutions

Purification specialists focus on the rapid purification of compounds and seem to give less attention to the long-term stability of pure or purified compounds. There have been several compound solution stability publications in the past few years, with the most in-depth examination by Cheng et al.[58] Using a set of 644 compounds, the authors compared the stability of 10 mM DMSO solutions stored under a number of different conditions. A comparison of compound concentrations of DMSO solutions stored for five months at room temperature in polypropylene or glass plates showed no significant difference by LC/UV. However, they reported NMR evidence demonstrating that something leached from the polypropylene plates into the DMSO, again raising the issue of LC/UV/MS-invisible impurities.

While there had been discussion as to the importance of keeping the DMSO solutions very dry and even storing the solutions under an inert atmosphere such as nitrogen or argon, it was this report that provided the first careful experiments to address these questions. In order to accelerate their investigation into the long-term stability of compound solutions stored in dry or wet DMSO under air or under nitrogen, the authors examined stability at 40 °C for 26 weeks. There was a small difference observed between wet and dry DMSO and no difference when the solutions were stored in an air or nitrogen environment. Given that a plate containing anhydrous DMSO will absorb ~5% water by weight after being open for two hours in a laboratory environment, the costs associated with maintaining very dry DMSO make this an impractical endeavor. At least one group adds water to the DMSO so the compound solutions may be stored as liquids at 4 °C to allow for automated liquid handling without subjecting the solution to freeze–thaw cycles.[59] While the author is unaware of a definitive study on freeze–thaw cycles, the impact seems to be dependent on compound[60,61] and concentration.[58,62] Given the limited solubility of many compounds in water, it would seem prudent to try to minimize freeze–thaw cycles and to thaw compounds in a low-humidity environment to help reduce water absorption and compound precipitation.

Influence of Salt Forms on Long-Term Stability and on Bioassays

The salts of some modifiers, notably TFA, can yield nonadducted residue in excess of that expected. Hochlowski et al.[63] purified 48 compounds, half of the material by SFC and half-by HPLC using solvents modified with 0.1% TFA. After removing the solvent from the fractions using a centrifugal evaporator, the authors determined the amount of TFA present in the dried sample. According to fluorine NMR measurements, the amount of residual TFA ranged from approximately 0.2–0.6 equiv in excess of that predicted based on forming TFA adducts. Without knowing the equivalents of excess TFA, dissolution of the purified compound based on its gravimetric determination would result in a lower-than-expected concentration. In addition, a subset of the purified compounds showed poorer stability when stored as the TFA salt than when stored as the non-TFA salt.

Salts are not necessarily passive counterions, and they can have a large impact on assay results. For example, trifluoroacetic acid was reported to have a negative impact on cultures of osteoblasts and calvariae at concentrations below 100 nM.[64] Since the cells were unaffected when dosed with 300 nM of HCl, it seems that it was the TFA and not the presence of a strong acid that influenced the assay results. Given the observation that compounds purified using TFA as a modifier may contain 0.2–0.6 equiv of excess TFA, basic compounds screened at 10 μM may contain 12–16 μM TFA and potentially more if the compound has more than one basic site. While it may be possible to remove TFA salts using resins,[65] it would be more efficient if the purification method would either generate no salt, as may be achieved with separations done using normal phase HPLC or SFC, or reversed-phase HPLC at high pH. Although numerous basic compounds like those typically generated in drug discovery demonstrate excellent resolution when purified at pH 9–10, the stability of compounds at these pH values has been a concern that has slowed its adoption. Since some compounds may degrade in basic solutions and since low pH separations are commonly used, a low pH replacement for TFA that generated biologically acceptable salts[66] would be useful. Unfortunately, such modifiers may not provide adequate chromatographic resolution, damage the equipment/column, or generate ash during elemental analysis.

Different salt forms may affect the solubility of a compound and these differences can impact the results from in vitro assays, with low-solubility compounds contributing to inaccurate IC_{50} values or poor SAR correlations.[67] This also can be a significant problem during lead optimization when a project is trying to optimize favorable ADME properties[68] or to identify possible liabilities, such as herg binding or inhibition of cytochrome P450. Since different salt forms may have different dissolution rates or may dissolve in different regions of the gastrointestinal tract, the use of different salts may result in different pharmacokinetic and/or toxicity results.[69] As discussed earlier, TFA salts may contain a significant amount of excess TFA, so such salts should be avoided in PK or efficacy studies.

Conclusions

During the hit- and lead-finding stages of a drug discovery project, HTOS and HT purification play significant roles. The needs change as a project matures into the lead optimization stages and beyond. As the chemistry becomes more focused, fewer compounds are produced and the successful isolation of each is necessary for their use in understanding the SAR. Thus, the emphasis on purification changes from a high-throughput method producing acceptable purity products with a good success rate to a potentially lower-throughput, more successful one that produces highly purified compounds. The parallel, multichannel purification systems used for large-scale and focused libraries are less fully utilized for projects that progress into lead optimization,

so multiple single-channel systems should be considered for improved flexibility.

Prior publications on purification processes have discussed the length of time needed to transform crude reaction products into purified compound solutions ready for screening. A 2002 report[49] estimated the throughput achievable with one scientist using a custom two-channel parallel LC/MS and performing all the processing steps serially. With an estimated time of 45 days to process a 4400-member library, the mean daily output would be 44 samples per channel per day. Simply doubling the FTE and instrumentation would be expected to approximately double mean daily output. In fact, due to technological advances and enhancements in integration, the output tripled, to a sustained 132 samples per channel per day.[24] Given the shorter purification cycle times and the shorter evaporation times associated with SFC, one might expect a robust SFC system to provide higher throughput relative to HPLC. Unfortunately, there have been no such comparisons reported for large numbers of compounds purified using the same process with these two techniques. The results of such side-by-side testing would provide information to useful in assessing the robustness of SFC under typical usage.

Many medicinal chemists purify final products using open access LC/MS systems with reversed-phase columns and TFA-modified eluents. In spite of the known problems associated with TFA adducts and residual TFA salts, it remains popular because it generally provides superior resolution relative to other acids. High pH modifers such as NH_4OH or NH_4HCO_3 often provide sharp peaks and achieve higher compound loading than low pH modifiers; however, their use is not as widespread. On the basis of the numbers of compound purified with each technique, orthogonal alternatives to RP-LC/MS, NP-LC, and SFC, seem to be used less frequently than RP-LC in open-access environments. As described by Schaffrath,[43] both NP-LC and RP-LC chromatography are necessary in any chemistry laboratory. Given the faster separation and solvent evaporation achievable with SFC relative to NP-LC, it would be useful to have SFC and SFC/MS purification systems reliable enough to be used in an open-access setting.

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(65) For example, see the PL-SO3H MP and PL-SO3H resins from Varian. (http://www.polymerlabs.com/assets/userfiles/pdfs/spe/tbs/002050.pdf). Similar resins are available from other vendors.
(66) A partial list of salt forms used for known drugs in the FDA orange book include the following: acetate, arginine, chloride, citrate, fluoride, fumarate, hydrobromide, hydrochloride, iodide, lactate, malate, maleate, mesylate, nitrate, palmitate, phosphate, potassium, proprionate, sodium, succinate, sulfate, and tartrate. (source Select Committee on GRAS Substances (SCOGS) Database, available at http://www.cfsan.fda.gov/~dms/opascogs.html).
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