

# The Plight of Purity

A multi-detector approach to chromatography can help ensure that “pure” is pure enough for compounds in drug discovery.

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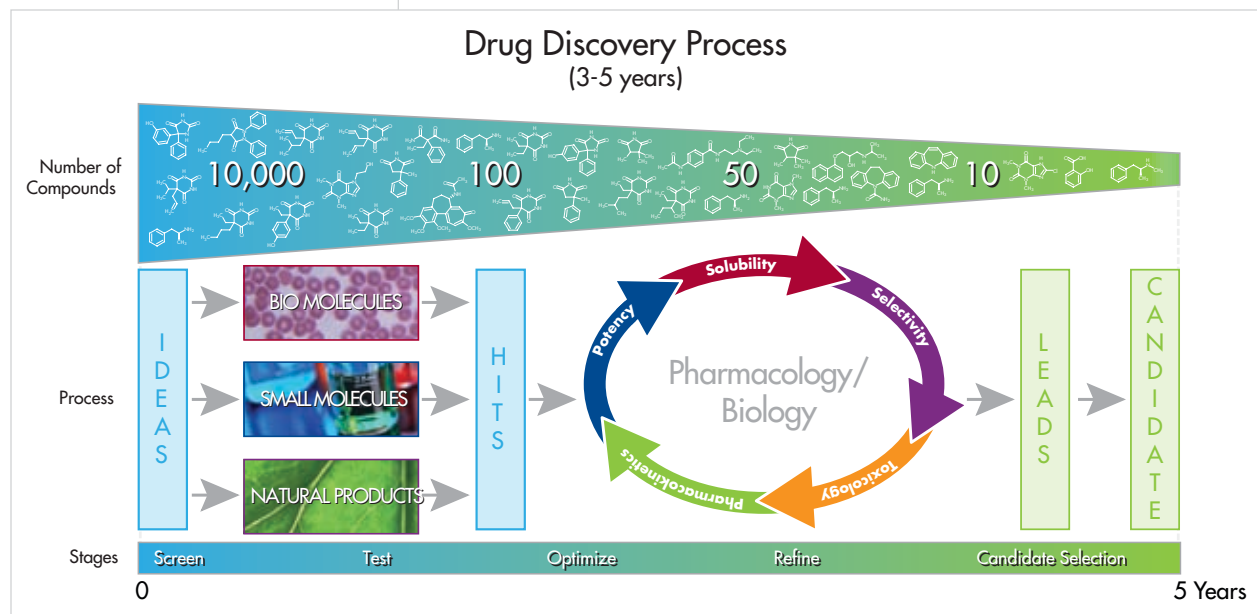
Thousands of compounds are synthesized and purified, then passed along for bioassay screening where the most promising compounds become “leads” that could graduate to a new chemical entity. (Source: Grace Davison)

For decades, liquid chromatography has been the primary tool to purify synthesized compounds in drug discovery. A target compound’s successful progression from hit to lead status not only depends upon the structure-based biological activity, it also hinges on compound purity. The medicinal chemist who created and purified the target compound relies on biologists to verify and test for biological activity.

In the past, invisible contaminants and inadequate purification of the target compound has led

to finger pointing and tension between chemists and biologists. Each group blamed the other for irreproducible and confusing results. Additionally, the standard acceptable purity for new target submissions has risen over the last decade from 80% to 85% to a minimum of 95%.

Drug discovery chemists need to be confident that hits are valid and deserving of lead status, and that bioassay results are reliable. Testing target molecules of suspect purity not only results in continuing an optimization unjust



tifiably, but can also lead to time wasted on tangent projects resulting from contaminant bioactivities.

Because it takes 10 years and \$1.2 billion to bring new therapies to market, pharmaceutical compa-

nies cannot afford to lose time and money to impurities.

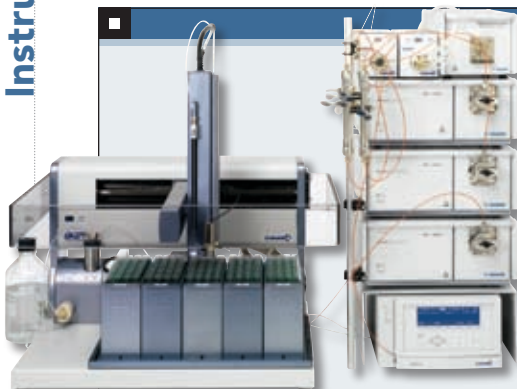
### Quantity vs. quality

Historically, the purity and stability of entire compound collections

has worried the pharmaceutical industry and has been the focus of discussion and debate among analytical chemists. Since the early 1990s, the industry has been plagued with compound purity concerns following the failed attempt to turbo-boost their pipelines with high-throughput synthesis efforts. This lead generation strategy, originally known as “combinatorial” chemistry, was criticized for producing highly impure targets and false hits.

Combinatorial chemistry was, and in many ways still is, a valid concept. A molecule with three points of diversity (R1, R2, and R3) can generate many possible structures. In order to handle the vast number of possibilities, researchers create a “virtual library,” a computational enumeration of all possible structures of a given pharmacophore with all available reactants.<sup>1</sup> Combinatorial libraries could include thousands, or even millions, of virtual compounds as synthesis options. Advances in automation led to an industrial approach to combinatorial synthesis, enabling chemists to routinely produce over 100,000 new and unique compounds per year. However, because there was no consensus on either the definition or the impact of purity, making sense of the bioactivity associated with library compounds resulted in chaos.

Even though combinatorial chemistry has been an essential part of early drug discovery for more than two decades, so far only one *de novo* combinatorial chemistry-synthesized molecule has been approved for clinical use by the **US Food and Drug Administration (FDA)**—sorafenib, a multikinase inhibitor indicated for advanced renal cancer).<sup>2</sup> In light of this unimpressive track record, the question must be asked whether poor purity was the downfall of the combinatorial chemistry approach.



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## “Pure” may not be pure enough

What has led to this plight of purity? Why do the medicinal chemist and the biologist get two different results for the same compound, especially when the chemist has resynthesized a new lot of compound? The answer may lie in detection during purification.

Flash purification today relies almost exclusively on ultraviolet (UV) detection. However, UV detectors cannot see compounds that lack chromophores, and the response relates to the strength of the chromophore rather than the quantity of material. Non-chromophoric impurities are not detected, and therefore increase the apparent quantity of target compound collected. Purity analysis using high-performance liquid chromatography (HPLC) systems with multiple detection schemes later reveals the true quantity of target

versus impurity. Additionally, false “hits” in bioassays can be a result of the impurity, rather than the target.

Exploiting the benefits of different detection options for early stage purifications could result in better quantified and higher purity compounds entering the screening pipeline. For the pharmaceutical company, this means less time wasted on false hits and rework, and more time spent on truly promising targets.

### Building better libraries

Feher and Schmidt noted that combinatorial chemistry libraries suffer from a lack of chirality in particular, as well as structural rigidity, both of which are widely regarded as drug-like properties.<sup>3</sup> A better approach to synthesis and purification requires the use of diversity-oriented library design,

commonly referred to today as “focused” libraries.

Assessing purity and removing contaminants sounds easy enough, but many chemists agree it is completely dependent on the ability to correctly detect target compounds as well as all contaminants present in a sample at submission.

**OpAns** (Optimized Analytical Solutions) is a contract analytical services company serving the pre-clinical therapeutic drug discovery market. Their co-founder and CEO, Dr. Ken Lewis, discussed some of these concerns in a recent interview.<sup>4</sup>

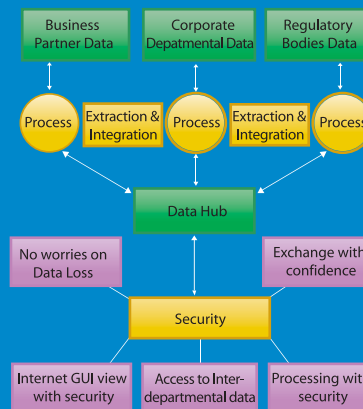
“For general collections as a whole, the focus is on determining whether samples are of reasonable purity. Purity is the primary driver, and identity is secondary. If a compound is not the correct molecular weight, and if it shows up as active in a screen, then the company will

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invest the time and resources to figure out the identity of the compound. So purity is the primary goal of QA/QC, followed by identity, and then concentration.

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... We use four on-line detectors. The foundation of the system is good chromatography. Without it we cannot perform accurate purity or quantity assessments. The mass spectrometer is used for molecular weight confirmation. The [chemoluminescent nitrogen detector] CLND and evaporative light scattering detectors] ELSD are used for quantitative assessment. ... Each type of detector will look at a sample differently and come up with a different purity number. The most accurate approach is to come up with a potency number—for example, if a powder is weighed out to create a 10 mM solution but when that solution is assayed it is quantified to be 8 mM, then the sample is only 80% pure. However, no one in discovery works off of potency. It only comes into play in active pharmaceutical ingredient manufacturing, not in early discovery."

Applying a multi-detector approach early in the process—at the flash purification stage—could significantly improve the quality and quantity of promising targets submitted by medicinal chemists for screening.

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## Breaking the cycle

The old cycle of synthesizing, purifying, and assaying only to be left with bioactivity that cannot be reproduced and work that must be redone has to stop. Time, money, and maybe even a potential cure may be lost in the clutter. But what can be done about it?

New detection advancements allow familiar techniques like flash chromatography to benefit from multiple detectors. Multiple-signal processing and triggering is now available for flash systems. The critical issues associated with producing target compounds with compromised purities can now be addressed early in the process—at the point of synthesis—instead of needlessly wasting screening resources.

When purity analysis is completed with only UV detection, hidden contaminants can lead to questionable purity, and worse, skewed concentrations in bioassays. Compound progression in discovery is dependent on activity reported from bioassays that assume a consistent concentration for all samples. If chemists are able to detect and trigger collection from ELSD in addition to UV, they can submit "cleaner" target compounds.

Chemists using UV/ELSD during flash chromatography can benefit from detecting more, collecting smarter, affirming compound identity, and getting a relative mass balance simultaneously. Medicinal chemists can benefit from knowing relative reaction yields at the time of synthesis and be confident targets and contaminants are detected. The plight of purity can and must be solved, because only truly pure targets will lead to reliable, reproducible bioassay data and eliminate rework, streamlining the drug discovery process. ■

*With an extensive background as a medicinal chemist, Ms. Lawrence previously worked as a purifications specialist for major instrumentation manufacturers. She currently manages the instrumentation marketing programs for Grace Davison Discovery Sciences.*