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Case Study

Selecting the Right Chromatography Strategy for Difficult-to-Purify Small Molecules

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From digital chromatography modeling to fraction mapping and preparative HPLC, our team applies the right purification strategy to overcome complex separation challenges while balancing purity, yield, manufacturability, and development speed.

When crystallization isn't an option, chromatography often becomes the appropriate strategy for achieving the purity required for small molecule clinical or commercial manufacturing. Every purification requires balancing competing priorities, from purity and yield to development timelines, fraction processing, facility capabilities, and manufacturing efficiency.

The challenge becomes even greater with complex molecules such as linker-payloads for antibody-drug conjugates (ADCs), where subtle structural differences can make impurities exceptionally difficult to separate and traditional development approaches can consume significant amounts of time, solvent, and valuable material.

We combine deep chromatography expertise with advanced analytical and digital modeling tools to identify the most effective purification strategy for each molecule. Every purification requires balancing multiple, often competing priorities (**Figure 1**), including purity, yield, analysis time, fraction processing, facility capabilities, and manufacturing efficiency. By understanding both the chemistry and the practical realities of manufacturing, our scientists optimize purification methods, improve process control, and determine when more advanced technologies are required to achieve demanding purity specifications.



The following case studies illustrate three different chromatography strategies used to solve distinct purification challenges. Each example demonstrates that successful purification is not about applying a single technique, it's about selecting the right approach for the specific molecule, process, and manufacturing objectives.

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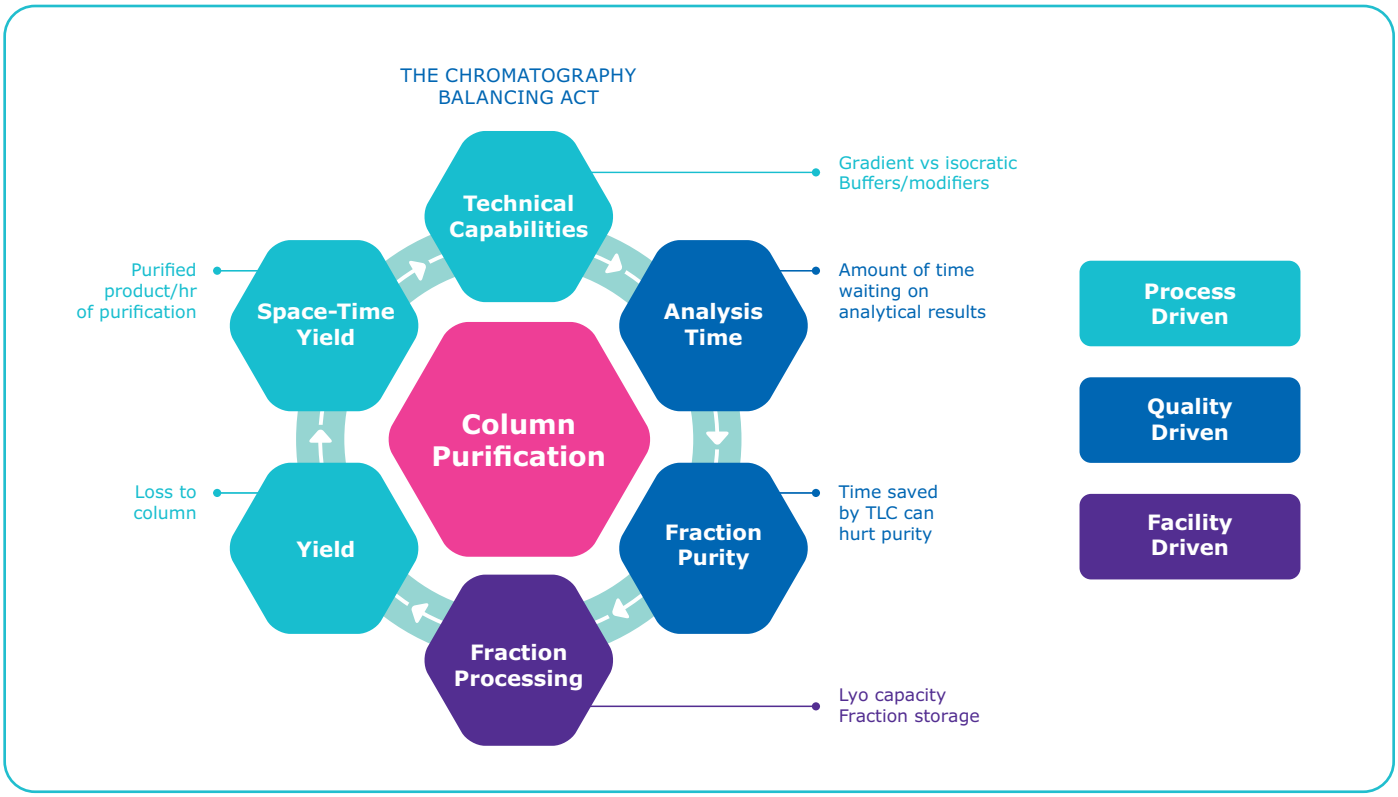


Figure 1.

Successful chromatography requires balancing multiple interdependent factors, including purity, yield, analysis time, fraction processing, facility capabilities, and manufacturing efficiency. Selecting the optimal purification strategy depends on understanding these tradeoffs for each unique molecule.

Case #1: Digital Modeling Addresses a Difficult Chromatography Separation

The Challenge: A Closely Eluting Impurity with a Stringent Specification

A client developing a complex linker-payload for an ADC had a particularly difficult separation challenge. The final product contained an impurity that eluted almost simultaneously with the target molecule, making it extremely difficult to separate during chromatography (**Figure 2**). At the same time, the impurity specification was exceptionally stringent, less than 0.1 area%.

The client's existing purification process relied on a traditional multi-step gradient, a common approach for production-scale flash chromatography. While the method reduced impurity levels significantly, it consistently plateaued at approximately 0.2 area%, well above the required specification. Meeting the specification would require more than incremental optimization.

As can be the case with complex linker-payloads, the impurity differed from the desired product by only a small structural change. Those subtle molecular differences translated into nearly identical chromatographic behavior, leaving very little separation between the two peaks. Conventional trial-and-error optimization would require extensive screening, consume valuable high-potency material, and extend development timelines without any guarantee of success.

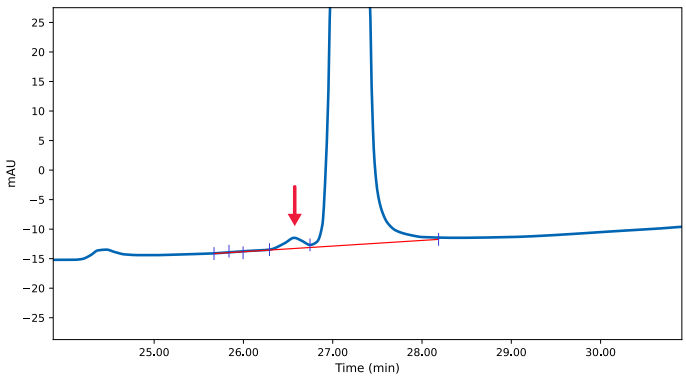


Figure 2.

The impurity eluted extremely close to the desired product, making it difficult to achieve the required purity specification using a traditional stair-step gradient.

The Solution: Replacing Trial-and-Error with Chromatography Modeling

Using analytical HPLC and chromatography modeling software, the team generated two analytical gradients (a 20-minute method and a 60-minute method) using the same solvent system as the client's existing process. Retention times, gradient conditions, and column parameters were then entered into the modeling software to predict how the separation would behave under different purification conditions.

Instead of evaluating one condition after another experimentally, the software allowed the team to rapidly explore multiple purification scenarios and visualize how subtle changes would affect the separation. Rather than recommending another multi-step gradient, the software predicted that an isocratic purification, using a constant mobile phase composition throughout the separation, would provide significantly better resolution between the product and the impurity. This approach was then verified experimentally using a 5-gram flash cartridge representative of the planned production process. The prediction proved correct, delivering complete impurity removal and achieving the required purity specification.

The optimized method created substantially greater separation between the product and impurity peaks, providing a much more robust operating window for manufacturing (**Figure 3**). The data-driven approach enabled the team to rapidly identify the optimal purification strategy while minimizing experimental screening. The result was a robust isocratic flash chromatography method that achieved complete impurity removal, met the demanding specification of less than 0.1 area%, conserved valuable high-potency material, shortened development timelines, and eliminated the need for a more solvent-intensive preparative HPLC process.

Beyond an Improved Separation

Beyond improving the separation itself, chromatography modeling also transformed the overall development process.

The value of chromatography modeling extended well beyond finding a better purification method. Traditional column development often requires screening numerous purification conditions, each consuming additional crude material and development time. For highly potent linker-payloads, where only grams, or sometimes less, of material may be available during early development, this approach can quickly become impractical.

By contrast, the modeling workflow required only small analytical HPLC injections to generate the data needed for optimization. Instead of repeatedly loading flash columns with valuable material, scientists could identify a highly promising purification strategy using only microgram quantities before confirming the method at laboratory scale.

Reducing material requirements also delivers important safety benefits. Because many linker-payloads are highly potent compounds, minimizing handling during development

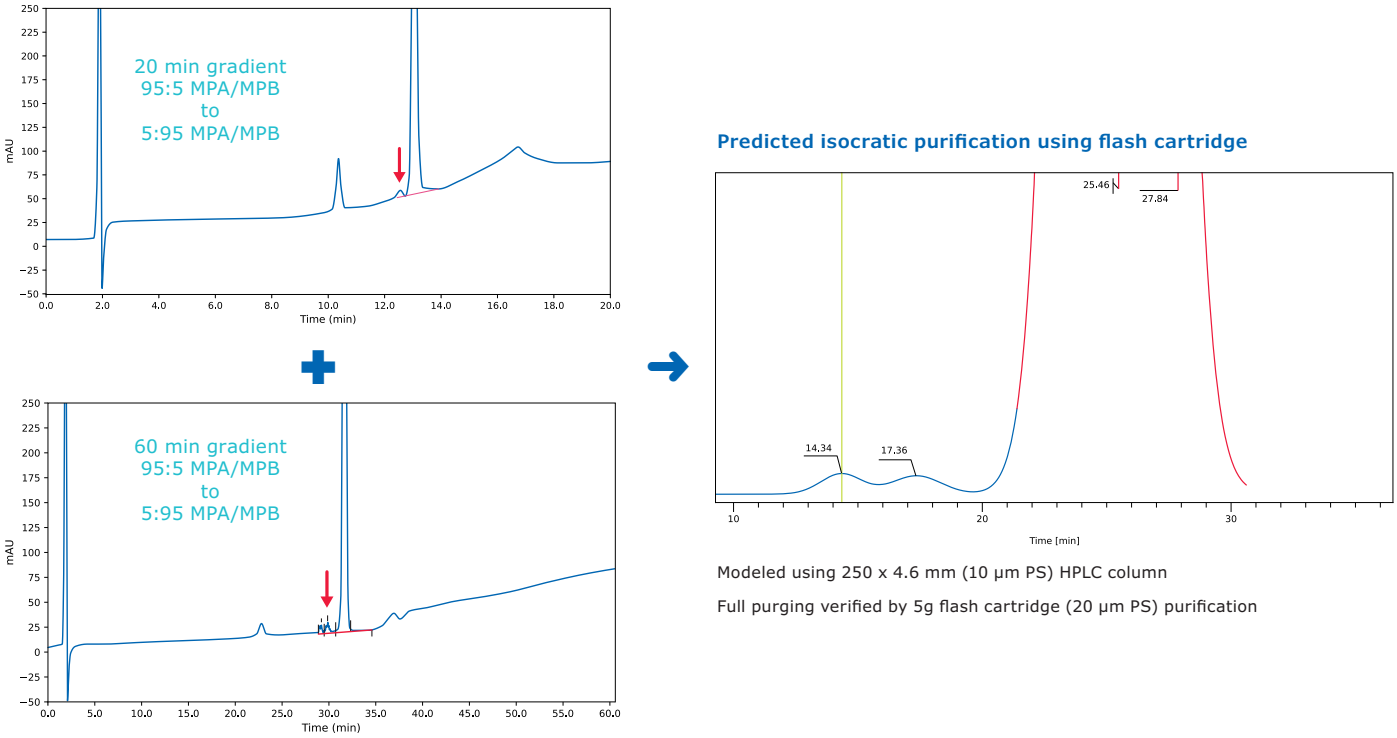


Figure 3.

Analytical HPLC data were used to model multiple purification conditions, predicting an isocratic flash chromatography method that delivered significantly improved separation and complete impurity removal.

reduces operator exposure risk while conserving expensive intermediates for process development and manufacturing.

The approach also shortened development timelines by replacing extensive experimental screening with predictive modeling. Rather than evaluating one purification condition after another, scientists began with a data-driven starting point that required minimal refinement before implementation.

The optimized purification strategy delivered another important advantage. The client's original process relied on preparative HPLC, a technique capable of achieving high-resolution separations but one that can consume significant volumes of solvent during production. By successfully optimizing the flash chromatography method instead, the need to transition to preparative HPLC for this purification was eliminated.

Maintaining the process on flash chromatography reduced solvent consumption, simplified manufacturing logistics, and avoided the additional operational complexity associated with large-scale preparative HPLC systems. While preparative HPLC remains an invaluable tool for particularly demanding separations, this project demonstrated that careful modeling can sometimes achieve the required purity using a simpler, more efficient approach.

Case Study 2: Maximizing Recovery Without Compromising Purity Through Fraction Mapping

The Challenge: Balancing Purity, Yield and Product Stability

For many small molecule purification processes, identifying which fractions to carry forward is a straightforward decision. But when a molecule is unstable and purification conditions are tightly constrained, fraction selection becomes a critical determinant of process success.

This challenge was encountered while developing a purification process for a complex linker-payload used in an ADC. The molecule presented multiple obstacles: it required reverse-phase chromatography, exhibited poor air stability, and began to decompose during conventional concentration steps. At the same time, the process had to maximize product recovery while achieving a minimum purity specification of 90%.

Complicating matters further, downstream processing imposed strict volumetric limitations. Because the available lyophilization equipment could accommodate only six fractions, simply collecting every fraction containing product was not an option. Every additional fraction increased processing volume and placed the molecule at greater risk of degradation. The challenge was determining exactly which fractions would deliver the optimal combination of purity, recovery and processability.

The Solution: Bringing Precision to Fraction Selection

Traditionally, production-scale flash chromatography relies on thin-layer chromatography (TLC) to determine which fractions contain product. While effective for many applications, TLC provides qualitative information and can introduce variability in interpretation between operators, particularly when closely related impurities are involved. For this project, our team implemented a more quantitative approach known as fraction mapping.

Rather than relying solely on TLC, every collected fraction was analyzed by HPLC (**Figure 4**). Those analytical results were then used to mathematically model different fraction combinations, allowing scientists to predict both the overall product purity and expected recovery before any fractions were combined.

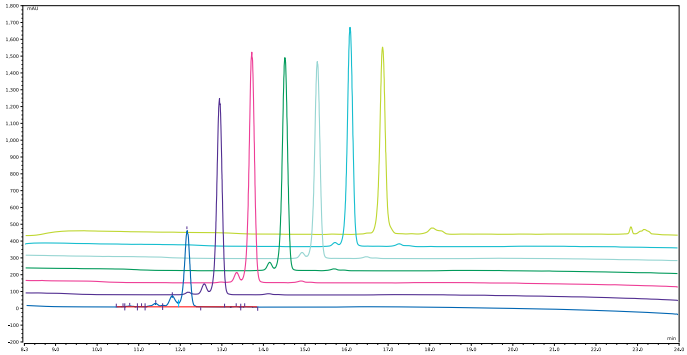


Figure 4.

HPLC analysis of individual fractions enabled quantitative fraction mapping, and prediction of purity and recovery for different collection windows before combining fractions for downstream processing.

The analytical insight provided by fraction mapping proved especially valuable because of the molecule's stability limitations.

Normally, large volumes collected during reverse-phase chromatography can be reduced through concentration or catch-and-release techniques before lyophilization. In this case, however, those approaches caused product decomposition. Every unnecessary fraction carried forward increased both processing complexity and the risk of product loss.

Using fraction mapping, the six-fraction window that maximized recovery while still meeting the required purity specification was identified (**Table 1**). Rather than estimating which fractions appeared acceptable based on TLC results, the team could confidently predict the outcome before committing material to downstream processing. The result was tighter process control at one of the most critical points in purification.

The approach also reduced the likelihood of discovering an out-of-specification batch only after final analytical testing, a situation that can require costly reprocessing or even result in loss of valuable material. By understanding the purity contribution

of every individual fraction, scientists could make informed decisions early in the process rather than reacting after purification was complete.

Turning Analytical Data into Manufacturing Confidence

Although fraction mapping requires additional analytical testing, that investment delivers significant advantages for complex purification processes. For molecules with difficult impurity profiles or tight specifications, the technique removes much of the ambiguity associated with traditional fraction selection. Predicted purities closely align with isolated purities, giving development and manufacturing teams greater confidence that the material moving downstream will ultimately meet release specifications.

Retention Time (minutes)	**12.159**
Relative Retention Time	**1.000**
Injection Name	**Area mAU*min (Product)**
Fraction 28	11.0929
Fraction 29	5.5509
Fraction 30	3.4565
Fraction 31	2.7576
Fraction 32	10.2740
Fraction 33	70.4786
Fraction 34	**180.2464**
Fraction 35	**211.6270**
Fraction 36	**195.7129**
Fraction 37	**180.3418**
Fraction 38	**201.0009**
Fraction 39	**178.8166**
Fraction 40	n.a.
Fraction 41	n.a.

Table 1.

Analytical evaluation of individual fractions enabled selection of the optimal six-fraction collection window, maximizing recovery while meeting purity specifications and remaining within available lyophilization capacity.

The approach is particularly valuable when purification decisions are influenced by more than chromatography alone. Facility constraints, equipment capacity, product stability and downstream processing requirements all become part of the optimization process. Rather than treating chromatography as an isolated unit operation, fraction mapping allows scientists to optimize the entire manufacturing workflow.

Case Study 3: When Preparative HPLC is the Right Tool for Complex Purification

The Challenge: When Complex Molecules Demand Greater Resolution

Flash chromatography is often the preferred choice for production-scale purification because it is efficient, scalable, and well suited for many small molecule separations. But as molecules become larger and more structurally complex, or when purity requirements become exceptionally demanding, even an optimized flash chromatography process may not provide sufficient resolution.

A client required an extremely high level of purity, and the separation challenge exceeded what conventional flash chromatography could reliably deliver. While the purification conditions had been successfully optimized during development, the process needed greater chromatographic resolution to consistently achieve the required product quality at manufacturing scale.

The Solution: Scaling to Preparative HPLC

The answer was preparative high-performance liquid chromatography (prep HPLC). Unlike flash chromatography, prep HPLC operates at significantly higher pressures, allowing the use of much smaller particle-size stationary phases. The increased surface area creates sharper peaks and substantially improved resolution, enabling separation of compounds that differ only slightly in their chromatographic behavior.

The technology also introduces a higher level of automation. Instead of manually preparing solvent mixtures and collecting fractions based primarily on operator observations, preparative HPLC automatically mixes gradients in real time and uses inline UV detection to collect fractions precisely as target peaks elute. This removes much of the variability associated with manual fraction collection while providing greater confidence that only the desired material moves forward for processing.

For particularly demanding separations, those advantages can make the difference between a workable purification process and one that consistently meets manufacturing specifications.

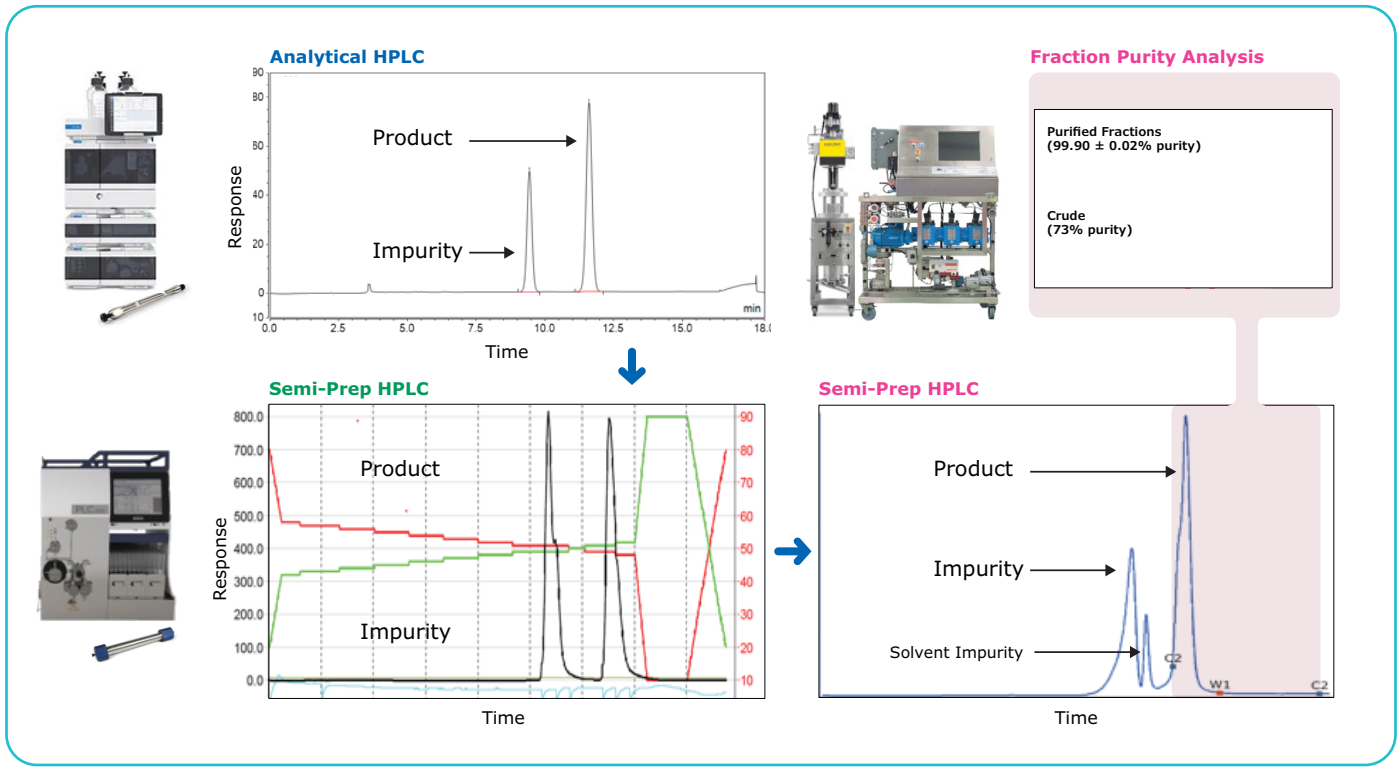


Figure 5.

The purification method translated predictably from analytical HPLC to semi-preparative development and ultimately to production-scale preparative HPLC while maintaining chromatographic performance.

Scaling with Confidence

One of the greatest strengths of preparative HPLC is how predictably it scales. The separation was first developed on an analytical HPLC system, where only small quantities of material were required to optimize chromatographic conditions. Once the desired separation was achieved, the method was transferred to a semi-preparative development system using straightforward scaling principles before ultimately being implemented on full production-scale preparative HPLC equipment.

Across each stage, the chromatography remained remarkably consistent. This predictable scale-up allowed the team to move confidently from laboratory development to manufacturing while minimizing redevelopment work and reducing technical risk.

Most importantly, the purification delivered dramatic improvements in product quality. Material entering the process at approximately 73% purity was purified to 99.9% purity, demonstrating the capability of preparative HPLC to resolve exceptionally challenging impurity profiles when simpler purification approaches are no longer sufficient (**Figure 5**).

Understanding the Tradeoffs

While prep HPLC is an exceptionally powerful technology, it is not the right answer for every purification. The increased resolution comes with additional operational complexity. Smaller particle sizes create higher back pressure, requiring specialized pumps and dynamic axial compression columns that must be carefully packed and maintained. Sample loading is also lower than flash chromatography, meaning additional injections are required to process the same quantity of material. Those additional injections increase solvent consumption and require facilities capable of managing significantly larger solvent volumes.

For these reasons, our team evaluates preparative HPLC within the broader context of the entire manufacturing process. If flash chromatography can achieve the required purity with lower cost and greater efficiency, it remains the preferred solution. But when molecules become larger, impurity profiles become more complex, or product specifications become exceptionally tight, preparative HPLC provides the resolving power needed to achieve consistent, high-quality purification. By understanding when advanced purification technologies provide meaningful value, and when simpler approaches are sufficient, we help clients achieve the purity they need while minimizing complexity, cost, and development risk.

The Right Expertise for Complex Molecules

Complex molecules can challenge conventional purification and downstream processing approaches in very different ways. Some demand advanced crystallization and particle engineering; others require sophisticated chromatography to achieve the necessary purity. In either case, the ability to understand the molecule, diagnose the underlying process challenge, and select the right technology is critical.

As a specialized CDMO, we bring together process understanding, advanced analytical capabilities, and digital tools to address these challenges. From solid-state characterization and particle engineering to chromatography modeling and process optimization, these capabilities help reduce development and scale-up risk while delivering robust, manufacturable processes.

Difficult-to-Purify Molecules

Complex purification challenges can add risk, cost, and time to small molecule development. We combine specialized process development expertise and high-throughput experimentation and AI-enabled scale-up modeling to identify and optimize purification strategies for challenging molecules. With a proven track record of achieving 99.5%+ purity APIs, our team helps streamline development and move robust purification processes toward manufacturing.

Difficult-to-Filter Molecules

When particle properties and filtration performance become manufacturing bottlenecks, the right crystallization strategy can make a significant difference. We apply crystallization expertise, particle engineering, high-throughput experimentation, and AI-enabled scale-up process modeling to address filtration challenges from process development through commercial scale. The result is greater process consistency, significant time savings, and reduced risk of costly batch failures.

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