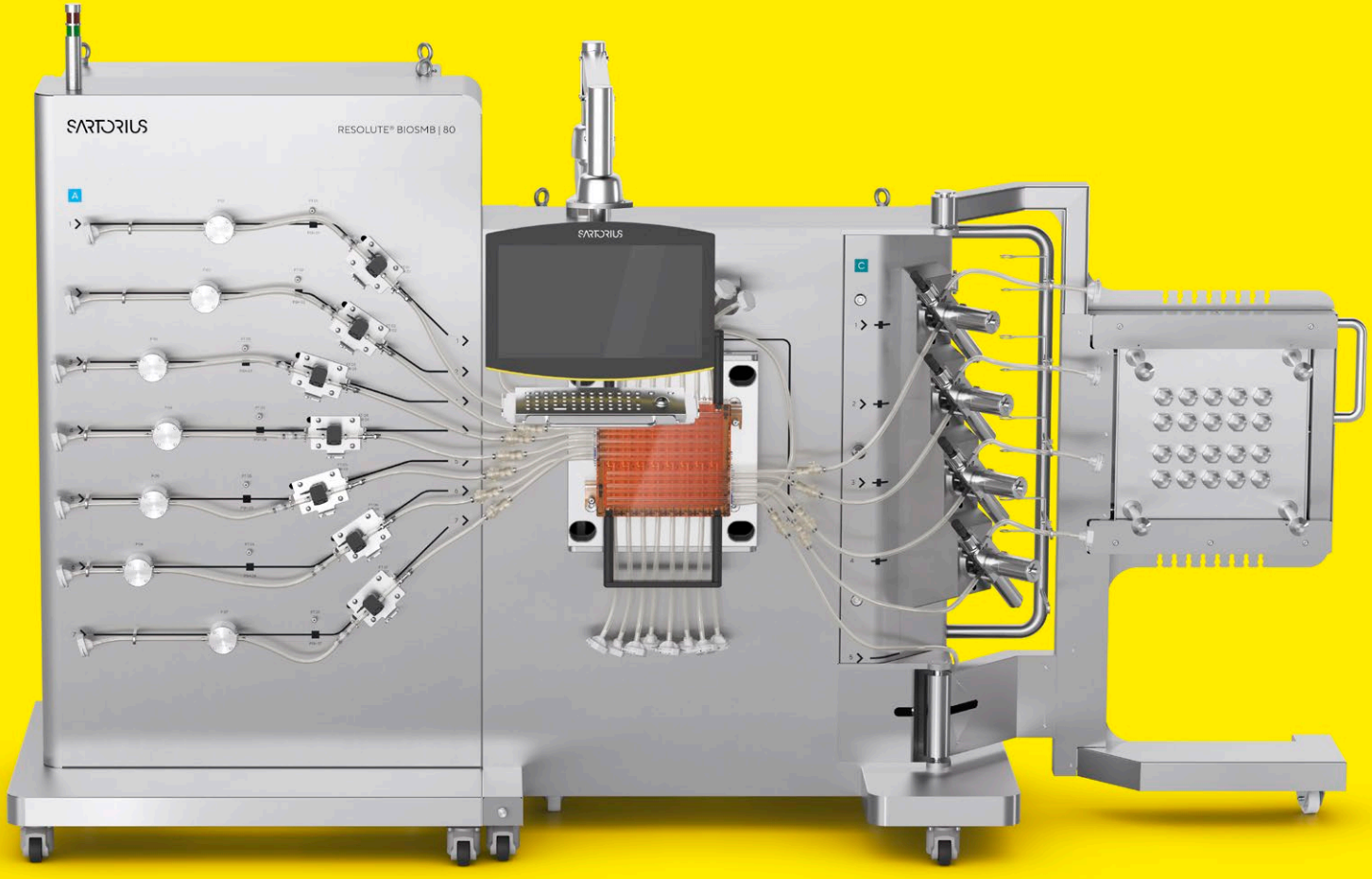

Simplifying Progress

Enhanced Process and Quality Control for Multi-Column Chromatography Using UPLC Technologies

June, 2023 | Karl Rogler, Geert Lissens, Jean-Luc Beulay, Keith Gillette

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Continuous downstream, Multi Column Chromatography (MCC), Critical Quality Attributes (CQA), process analytical technology (PAT), Quality by Design (QbD), At-line Analytic

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Introduction

Continuous processing has gained considerable interest and has been demonstrated to offer widespread improvements in biologic production and plant efficiency. The general approach involves running unit operations in a steady or cyclical state for extended periods, avoiding costly stop-start disruptions and downtime. Because production operations are operated in a continuous manner, there is a greater need to monitor the process in real-time to ensure that product quality does not deviate from the design space. Analytics that provide rapid, directly actionable, and integrated measurements for downstream process (DSP) teams can be used for more advanced process control, deviation management, and, ultimately, the real-time release of batches.

This white paper gives an overview of two disruptive technologies working together to address major purification challenges in DSPs:

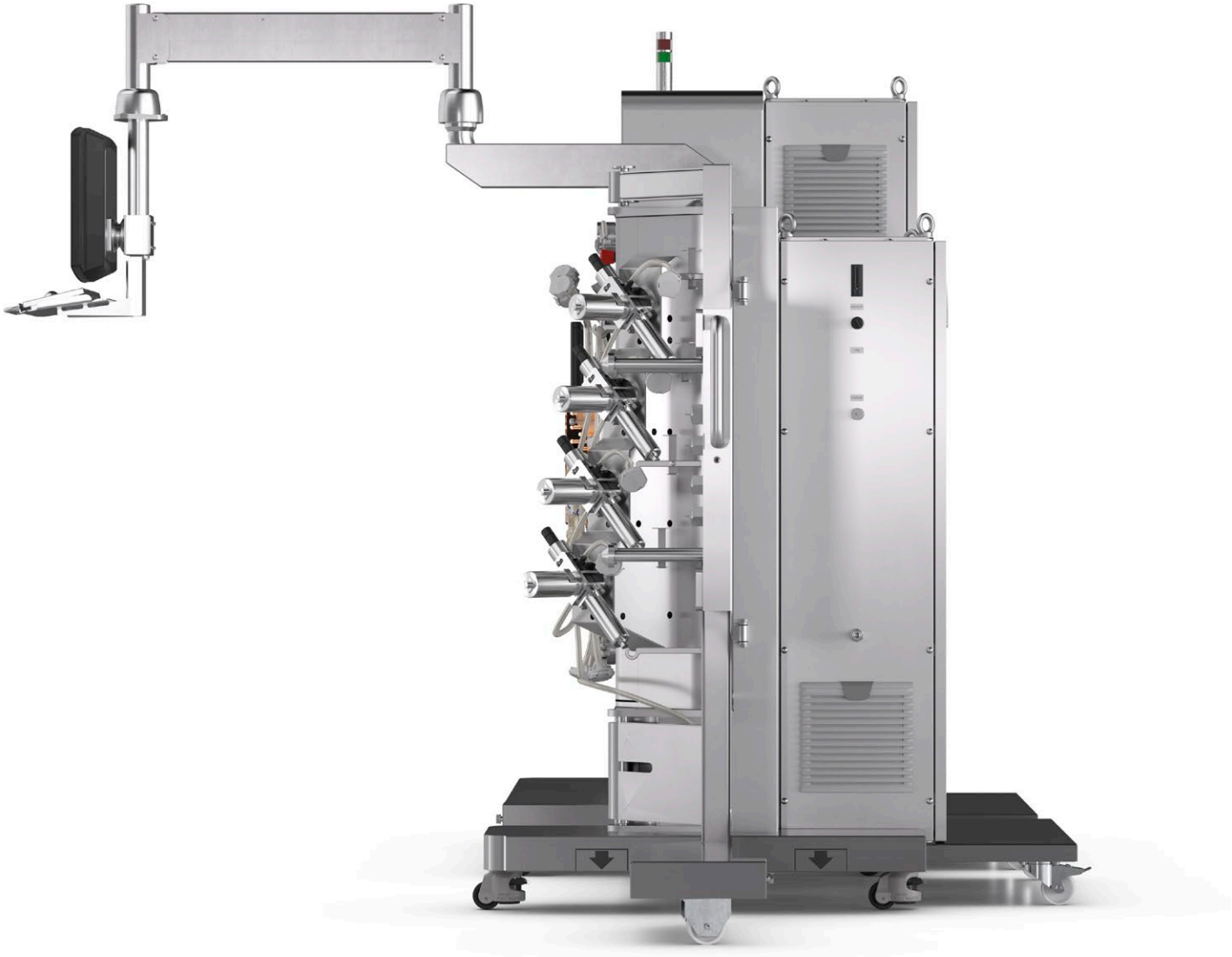
- Improving efficiencies for purification itself through continuous multi-column chromatography (MCC)
- The use of advanced chromatography (LC) or liquid chromatography-mass spectrometry (LC-MS) analytics for routine at-line monitoring

Protein A purification using the Resolute® BioSMB MCC platform (Sartorius) reduces operating and resin costs of purification during manufacturing. The scalable single-use architecture and GMP-ready automation of the Resolute® BioSMB system have made it the primary technology to enable the continuous manufacturing of biologics.

Direct LC measurement using the Waters™ PATROL UPLC Process Analysis System is a valuable tool for both upstream and downstream biological drug development and manufacturing. Coupling and embedding ultra-performance liquid chromatography-based (UPLC) analytics directly into the process empowers bioprocessing engineers with additional high-quality measurements that complement other analytics commonly employed in DSPs, such as the direct titer and attribute monitoring of Protein A purification processes capable using the Resolute® BioSMB system. For continuous approaches, measuring the product's concentration, titer, and critical quality attributes (CQAs) frequently in near real-time can assure the process engineer that quality is maintained and controlled throughout the process and during any potential holding steps.

Expanding on the joint agreement for upstream bioprocessing analytics, Waters Corporation and Sartorius will now develop integrated analytical solutions for downstream biomanufacturing. As a first step, Sartorius and Waters will partner to implement the PATROL UPLC Process Analysis System as a new at-line PAT with data connectivity to Sartorius Resolute® BioSMB PD system for multi-column chromatography to deliver additional CQA monitoring for more consistent drug substance quality. Combining additional at-line CQA information with the ability to intensify chromatography purification will enhance the process and quality control in the design space, reducing the need for critical and timely decisions while ensuring consistent drug substance quality and significant cost savings on out-of-spec product waste by early indication of quality deviations.

Herein, we describe the partnership of Sartorius and Waters Corporation to develop solutions for process development and manufacturing by integrating Waters™ PATROL UPLC Process Analysis System technology into the Resolute BioSMB platform.



Technologies Overview

Resolute® BioSMB Platform

In the past 20 years, the capture chromatography step has remained one of the most costly and time-consuming operations conducted in biopharmaceutical production suites. The Resolute® BioSMB platform has been designed to address this bottleneck and cost burden of chromatography resin by utilizing the simulated moving bed (SMB) principle.

The Resolute® BioSMB platform solves these problems by enabling greater utilization of chromatographic media via automated continuous column cycling. Additionally, the operation of the Resolute® BioSMB overcomes the diffusion limitations of typical chromatographic media, allowing higher utilization of the binding capacity. There is no risk of overloading columns due to secondary columns in the load zone, capturing breakthrough and allowing the primary column to be loaded as close as possible to the static binding capacity. This results in up to a 40% increase in resin utilization without risk to product quality or process yield.

Increasing resin utilization and capacity can reduce the total resin volume by up to 80%. This minimizes the total size of column hardware and eliminates the need to purchase large resin quantities, decreasing the overall manufacturing capital and operating expenditures. Smaller volumes and higher productivity also decrease buffer consumption and, ultimately, clean room floor space.

The Resolute® BioSMB platform facilitates real-time process control and monitoring through an industry-standard OPC-UA connection. Through a collaboration with Waters Corporation, the Resolute® BioSMB platform can be integrated with the PATROL UPLC Process Analysis System to monitor CQAs at-line and in near real time, allowing dynamic control of Resolute® BioSMB operations. By combining the Waters PATROL UPLC Process Analysis System as an at-line PAT tool with intensified bioprocessing operations like Resolute® BioSMB (Sartorius), continuous downstream operations can be optimized to reduce production expenses, and effectively monitored and controlled to manage product quality.

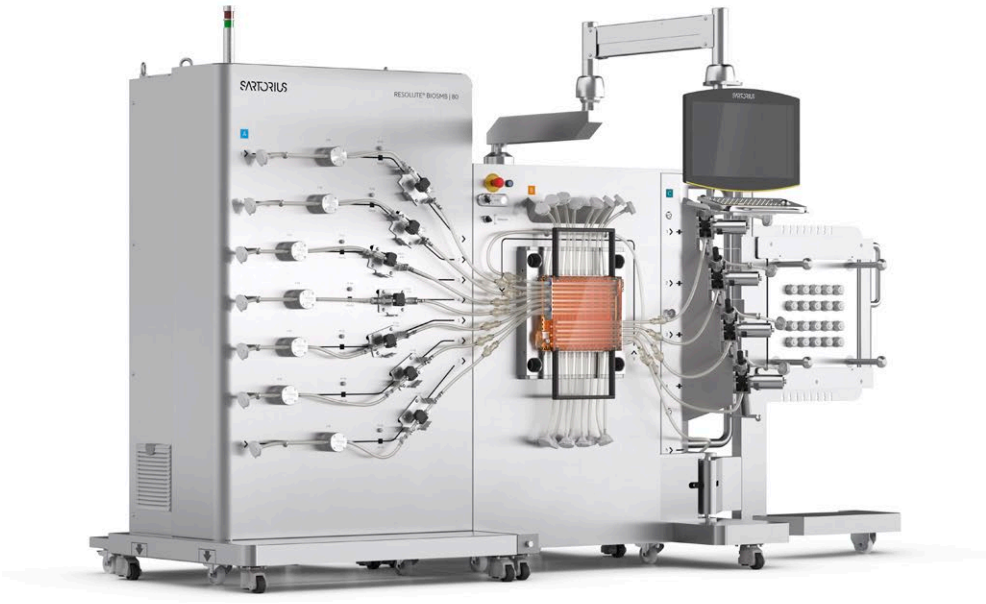
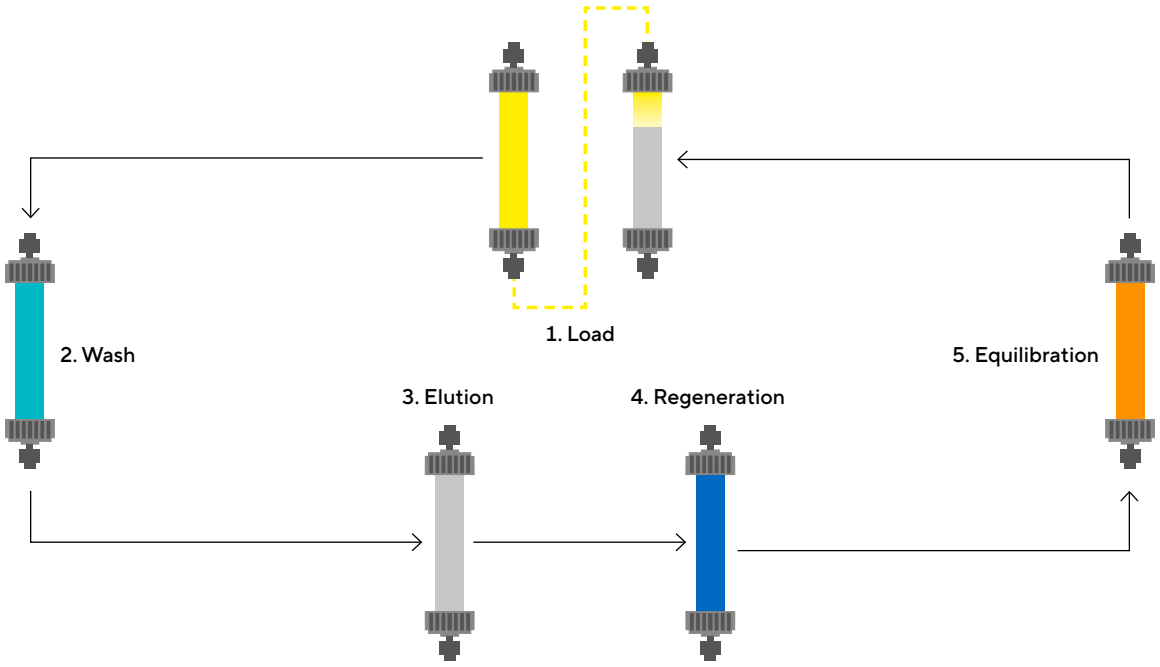


Figure 1: *Resolute® BioSMB 80 System for Clinical and Commercial Multi-Column Chromatography Operations.*

Figure 2: *Demonstration of the Multi-Column Chromatography Concept.*



Note. Multiple chromatography steps are operated on multiple columns simultaneously.

Waters™ PATROL UPLC Process Analysis System

The introduction of UPLC in 2004 made it possible to run faster methods and realize actionable data more quickly and frequently. For PAT applications, an on-line LC system equipped with on-board sample dilution can monitor multiple attributes over a wide dynamic range. In collaboration with leading biologics companies, the PATROL UPLC Process Analysis System was developed for robust operation in development and production environments.

The system combines established technologies used for analytical UPLC with unique capabilities like at-line and in-line sampling. Additionally, the system has dilution and aseptic sampling capabilities to better ensure robust and contamination-free measurements.

Figure 3: *Waters™ PATROL UPLC Process Analysis System*

Performance

CQA Monitoring – Measuring Aggregation with Rapid SEC

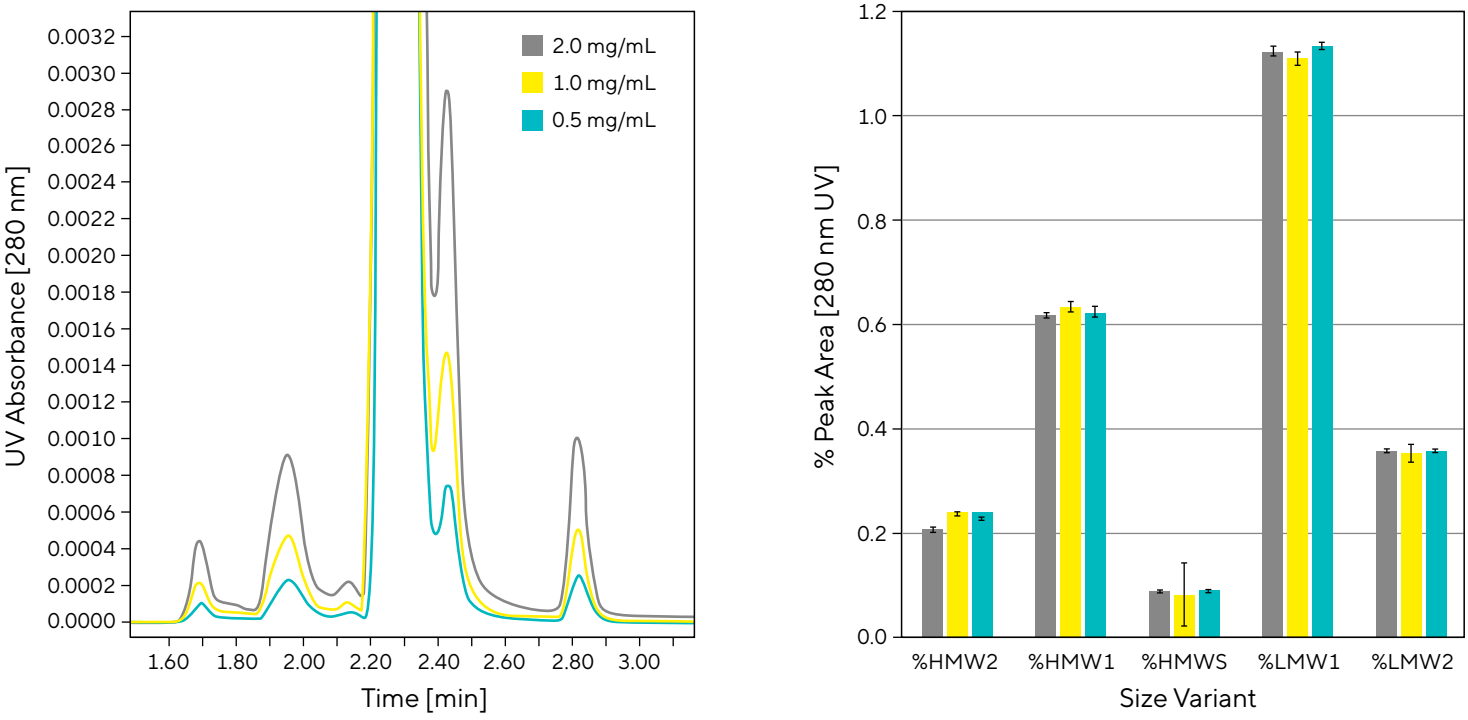
In-line or at-line measurements are increasingly employed for analytics that directly inform decisions about product quality. One assay performed by liquid chromatography is aggregation monitoring, where small but critical impurity levels that may significantly impact the final product quality or the quality of subsequent purification step outcomes are measured.

The formation of high molecular weight species (HMWS) by aggregation of therapeutic proteins is a complex phenomenon and is a CQA monitored and controlled by multiple process parameters at most bioprocess steps. Aggregation is affected by many processing conditions, including temperature, pH, air/surface interactions, and mixing speed. Aggregation is known to mediate undesirable immune responses, implicated in the formation of anti-drug antibodies (ADAs), which ultimately compromise patient safety.[1] Thus, ensuring aggregation levels are minimized throughout the process is critical to improving the overall quality of the product.

The most robust and widely accepted means of measuring aggregation in product samples is through size-exclusion chromatography (SEC) on analytical chromatography systems like the PATROL UPLC Process Analysis System. In a continuous processing application, frequent at-line sampling and measurements are required to generate a near real-time understanding of process performance and product quality. The PATROL UPLC Process Analysis System and method optimization enables SEC analysis of HMWS in minutes, resulting in rapid, high-throughput aggregate quantitation.

Figure 4 shows typical profiles obtained for SEC separations of mAbs (ACQUITY™ Premier Protein SEC, Waters). A three-minute method was used to resolve multiple HMWS and low molecular weight species (LMWS), giving a complete overview of aggregation and fragmentation in the product profile. The short duration of this automated analytical operation eliminates time-intensive operations like sample preparation and chromatogram interpretation, meaning SEC can be used in the production suite for rapid monitoring of aggregation.

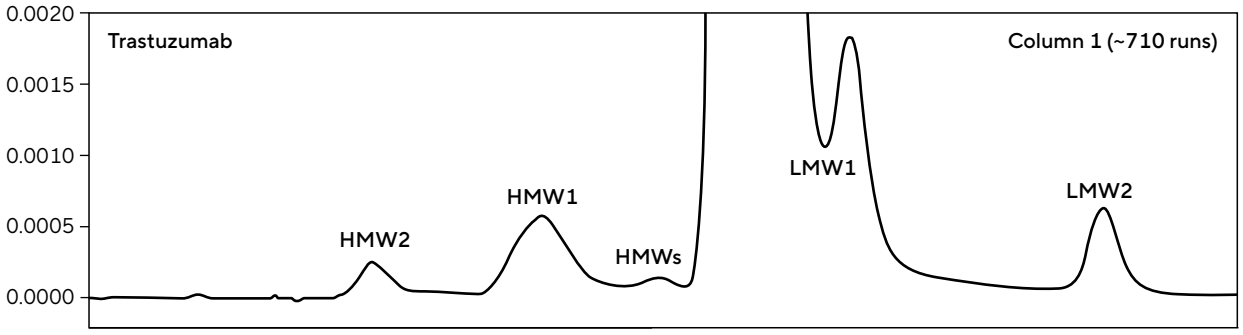
Figure 4: *SEC-UV Chromatograms and Quantitative Results for Trastuzumab*



Note. Evaluated at concentrations of 0.5, 1.0, ad 2.0 mg/mL.[2]

In addition to high-quality method performance, reproducibility is critical in moving any technology toward at-line approaches. Repeatable measurements (between columns and over time) is needed to ensure long-term assay performance throughout the purification process and across campaigns. Figure 5 shows the high reproducibility of SEC-based aggregation measurements over the course of hundreds of experiments, producing high-quality, detailed information about aggregated species.[2]

Figure 5: *SEC-UV Chromatograms for Trastuzumab Evaluated After 710 Runs*

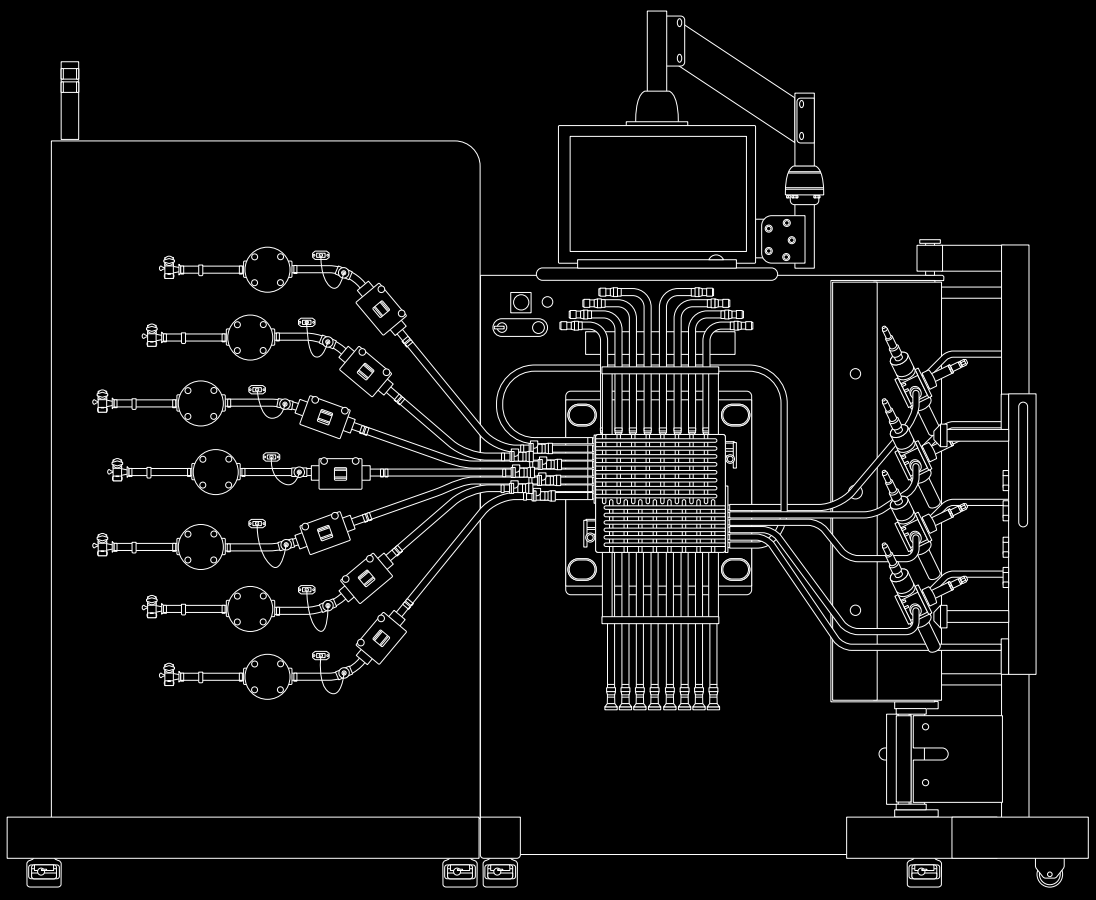


Case Study - Practical use of Waters™ PATROL UPLC Process Analysis System

The move of analytical technologies towards process-controlled integration and feedback loop monitoring has been championed and pioneered by groups such as the Biologics & Vaccines departments at Merck Research Labs.

Several CQA measurements are desirable for linking to and generating clear critical process parameter set points; Protein A measurements, aggregation, and charge variant analyses are often cited as particularly valuable in this regard. By combining the PATROL UPLC Process Analysis System as a PAT tool with continuous production approaches (like those possible in the Resolute® BioSMB), Merck could detect CQAs regularly and in near real-time.

The Biologics & Vaccines R&D team at Merck performed charge variant analysis; the acidic, main, and basic peak distribution was monitored over two weeks and no changes were observed.[3] A forced degradation experiment was also performed to show the ability to accurately detect changes to the CQA profile. This work shows how on-line PAT LC-based approaches (including the integration of the PATROL UPLC Process Analysis System) enable continuous monitoring of platforms such as the Resolute® BioSMB system. The findings reveal that implementing next-generation analytics can inform and accelerate process decision-making.



Conclusion

By combining the PATROL UPLC Process Analysis System as an at-line PAT tool with intensified bioprocessing operations provided by Sartorius Resolute® BioSMB, continuous downstream operations can be optimized to reduce production expenses and effectively monitored and controlled to manage product quality. The PATROL UPLC Process Analysis System can be integrated with the Resolute® BioSMB system to rapidly and continuously monitor CQAs like aggregation and charge variant profiles in product streams.

Thus, the implementation of at-line PAT tools enable more direct QBD approaches through direct CQA measurement in the production line. Consequently, traditional QC bottlenecks between unit operations is remediated. This integration brings the industry one step closer to real-time drug release. Furthermore, CQAs that otherwise could not be monitored in real time now have the opportunity to be assessed, placed in predictive models, and subsequently used for process control.

Waters and Sartorius are committed to providing biologics manufacturers with the best tools to ensure safe and efficient manufacturing by developing integrated production and analytical systems and forging partnerships with our customers.

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