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White Paper

Accelerate Process Development with Automated Aseptic Sampling

Bioprocess development is iterative. Ideally, each development cycle is short and generates data that contributes to optimization of the process. While increasing the number of data points can accelerate this workflow, it requires process samples to be collected and analyzed throughout the course of an experiment, regardless of day or time. The goal of PAT is to build quality into biopharmaceutical processes through identification and control of critical process parameters (CPP) and critical quality attributes (CQA) within a specified design space. Hence, access to a larger quantity of near real-time data is especially important when leveraging process analytical technology (PAT).

Implementing frequent manual sampling procedures for PAT slows process development, consumes valuable resources due to extensive time and labor demands, poses a risk to sterility, and is typically constrained by staff scheduling limitations.

Low sampling frequency coupled with batch rather than continuous testing generates datasets at low rates and delays critical process decisions by days or even weeks from the start of a run. The extended time to results forces a step-by-step control of parameters as opposed to comprehensive control. This delays, or in the case of bacterial cell culture, prevents corrective action and process optimization.

Automated aseptic sampling and reliable delivery of materials to on-line preparation and analytical instruments augment the throughput and time coverage of PAT. When implemented correctly, automated sampling shortens experiment run time and frees staff to focus on value-generating tasks, such as output evaluation and implementing optimization steps. Increased data density and immediacy is also possible, effectively condensing development cycle times and informing decisions in rapid data feedback loops.

This whitepaper describes evaluation of the MAST® Autosampling Solution as part of an automated PAT system implemented by Takeda Pharmaceuticals. Takeda sought to reduce their bioprocess development cycles with rapid, data-backed decisions, but faced a lengthy data delivery pipeline. To address this bottleneck, three MAST® Autosampling Solutions were deployed for daily bioreactor sampling and on-line, “right time” data acquisition to characterize cell culture performance and product quality.

The following studies summarize how the automated sampling systems facilitated resource efficiency and accelerated turnaround times, while generating data that were comparable to manual results. By implementing the MAST® Autosampling Solution, Takeda was able to achieve new efficiencies in process development including:

- 10X reduction in Full Time Equivalent (FTE) time
- 10X reduction in experiment run time
- 10X faster turnaround time for analytical results
- 60% reduction in number of analysts needed

Autosampling Technology and Experimental Design

The MAST® Autosampling Solution collects, directs, and reliably transfers samples to analytical devices automatically and aseptically.

Each component of the modular and scalable MAST® Autosampling Solution was designed for continuous, long-term operation. MAST® Sample Pilots draw samples into an aseptic zone using a patented valve system to ensure sterility and avoid the contamination risk of other sampling systems such as automated devices, syringes, and probes. Positive displacement pumps then push samples over long distances rather than pulling samples with a weaker vacuum force. Upon delivery of the sample, a sanitation and drying step clears the lines for the next draw and prevents clogging. Every sample is recorded with metadata for complete traceability as to the origin, analytical purpose, and operator. To date, more than sixty MAST® Autosampling Solutions installed at customer sites have collected more than 150,000 samples with no recorded instances of sterility issues caused by the system.

In this study, eight bench-scale bioreactors were equipped with MAST® Sample Pilots and Controllers that delivered 1–2 samples per day to bioprocessing analyzers and an on-line UHPLC (**Figure 1**). Samples were delivered to UHPLC following cell removal using the MAST® Cell Removal System and a Gilson® GX-271 Liquid Handler.

A key performance criterion for Takeda's automated system was more efficient use of personnel to accomplish the same monitoring as with manual methods. As such, data acquisition via manual sampling and off-line analytics were compared to automated sampling and on-line analysis. Takeda's manual and automated experimental runs collected and analyzed samples in the following processing streams:

- Purification and titer concentration determination via Protein A-based chromatography
- Aggregate analysis via size-exclusion chromatography (SEC)
- Charge variant analysis via cation exchange chromatography (CEX)
- A multi-attribute method (MAM) measuring 15 parameters via liquid chromatography-mass spectroscopy (LC-MS)

The resources and time needed to complete experiment runs, cell culture performance, and product quality attributes over the course of 14 days were also evaluated.

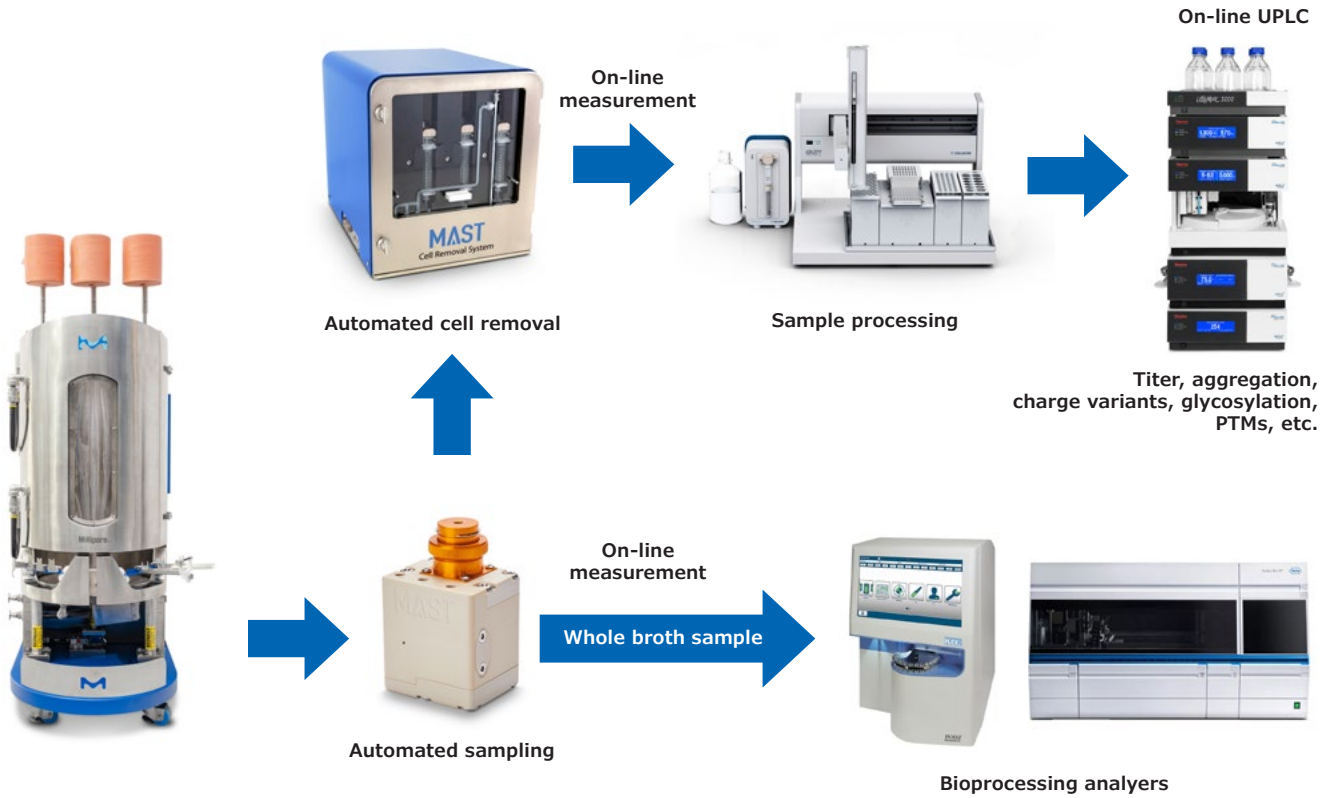


Figure 1: Example workflow for a non-GMP laboratory using the MAST® Autosampling Solution for automated on-line sampling and analysis to support rapid process development.

Improving Resource Efficiency and Multiparametric Insight

Manual sampling and testing required five full-time analysts while the same experimental runs were performed by two analysts supervising the automated sampling and on-line purification and testing (**Table 1**). By eliminating strict manual sampling schedules and tedious testing activities, team members could instead focus on optimizing process development and refining methodologies. Takeda realized a 60% reduction in the number of analysts required per testing panel, a result of streamlining experimental preparation and data analysis from 48 hours to 4 hours.

Activities	Traditional Purification & Off-Line Testing (Days 5-14)		On-Line Purification and On-Line Testing (Days 5-14)	
	Experiment Prep/Data Analysis (hrs)	# Analysts	Experiment Prep/ Data Analysis (hrs)	# Analysts
ProA Purification	9	-	1	-
Titer	9	-	1‡	-
SEC	11	-	1	-
CEX	14	-	1	-
MAM	5	-	1	-
Totals	48	5	4	2

‡ Titer and ProA Purification are same assay

Table 1: Comparison of staff and time required for manual sampling and off-line analysis with automated sampling and on-line analysis of daily samples from eight bioreactors and four analytical streams.

By eliminating bottlenecks caused by manual sampling, automated sample collection, processing, and delivery created greater opportunity for concurrent analyses and use of multiparametric insights. Collected samples each generated a set of parameter values that yielded a more comprehensive characterization of each timepoint. As such, the experiments provided a deeper understanding of bioreactor conditions and the overall comprehensive profiling resulted in fewer and more informative development cycles.

Accelerating Process Development with Data Immediacy and Near Real-time Analyses

Activities	Traditional Purification & Off-Line Testing (Days 5-14)		On-line Purification and On-Line Testing (Days 5-14)	
	Total Run Time (hrs)	Results TAT (Days)†	Total Run Time (hrs)	Results TAT (Days)
ProA Purification	52	10	0.5	0.04
Titer	12	3	0.5‡	0.04‡
SEC	36	10	4	0.04
CEX	96	10	12	0.16
MAM	0.5	3	4	0.16
Totals	196.5	10	20.5	0.16

† Traditional batch testing **‡ Titer and ProA Purification are same assay**

Table 2: Comparison of time investment to carry out manual sampling and off-line batch testing versus automated sampling coupled to on-line testing.

Comparability of Data from Automated and Manual Sampling Processes

The following studies demonstrate comparability of data derived from manual and automated sampling.

Measures of titer concentration once or twice per day over the course of the 14-day evaluation period were similar for manual sampling with off-line analysis and automated sampling with on-line analysis (**Figure 2**). Autosampling performed by the MAST® Autosampling Solution did not require the presence of staff and was operated independent of staffing schedules. As shown in **Figure 2**, coupling the MAST® Autosampling Solution to bioreactors had no negative impact on cell titer. Importantly, no contamination was observed across 25 evaluations using the automated sampling process.

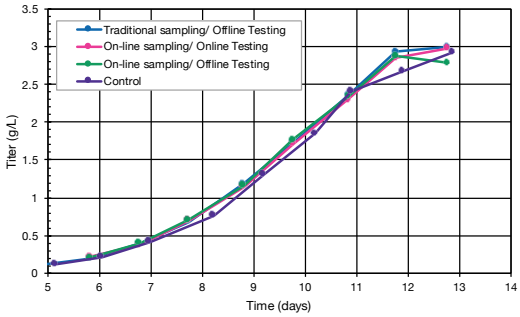
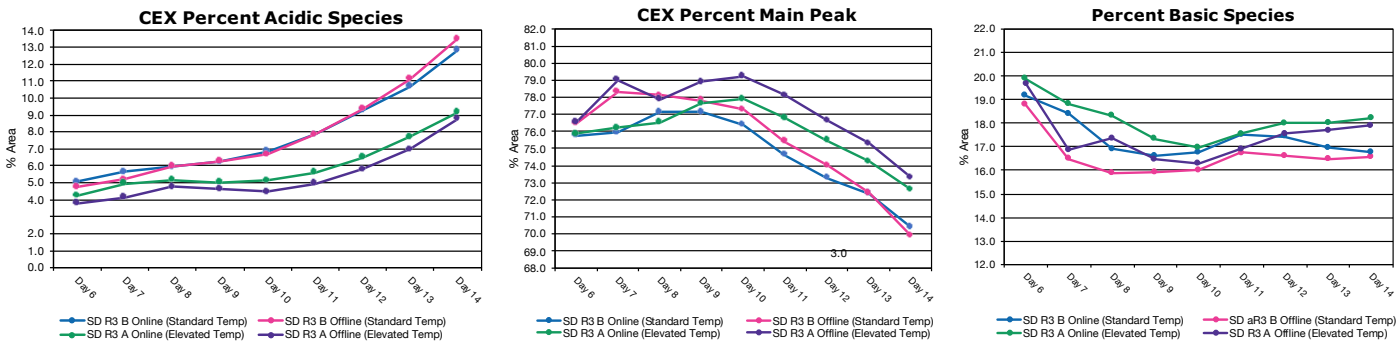


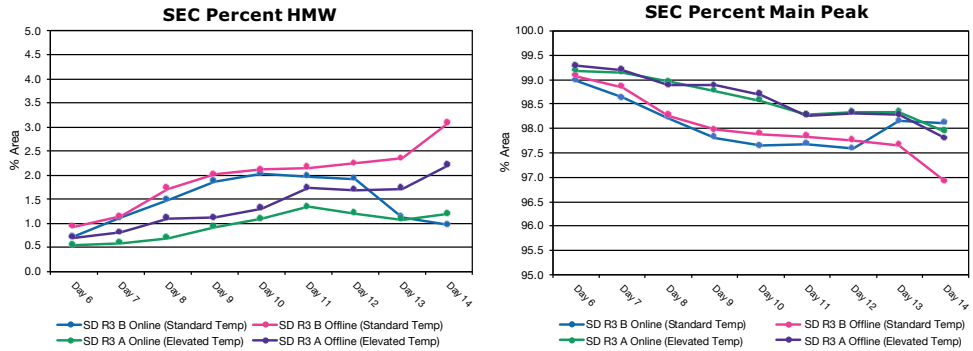
Figure 2: Bioreactor titer sampled manually or with the MAST® Autosampling Solution.

CEX, SEC, and MAM by LC-MS results were also comparable for off-line and on-line methods (**Figure 3**). Both methods were also able to differentiate product quality of process runs under different bioreactor conditions (standard and elevated run temperatures). The differences in percent high-molecular weight species between off-line and on-line methods were attributable to scale differences between the purification methods.

CEX



SEC



MAM by LC/MS

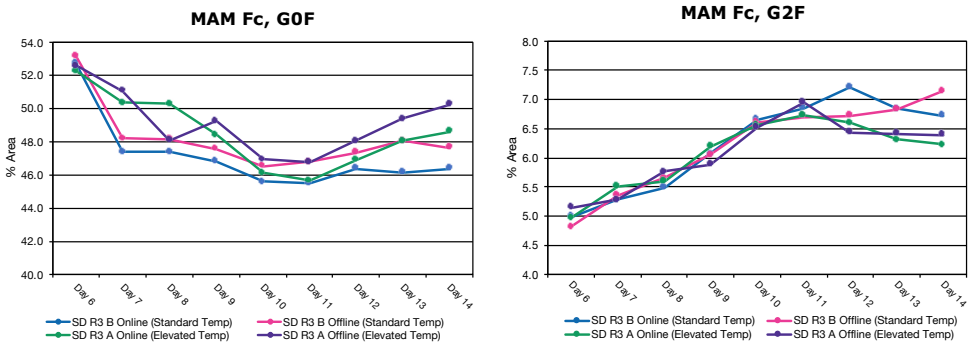


Figure 3: Automated sampling with on-line analysis generated CEX, SEC and MAM datasets compared to results from manual sampling and off-line analysis.

Increasing Data Resolution

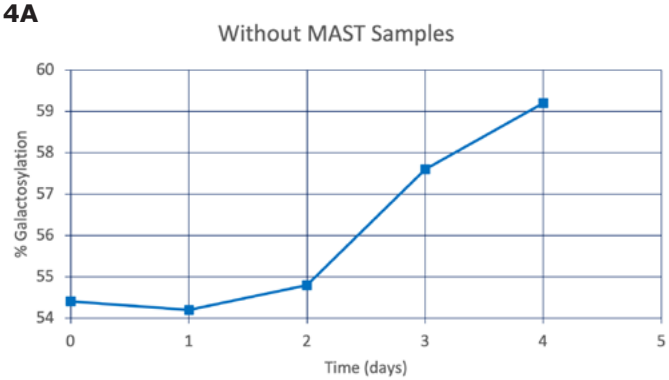
Bioprocesses can run for several days and critical time points (e.g., peak cell growth) can occur at any time of day. Managing a manual sampling regime in such cases can be limited by staff scheduling, and time-course data may be interrupted when on-site staffing is low (e.g., on weekends). Automated sample collection does not have those limitations; representative parameters from an experimental run can be collected from start to finish, more consistently and frequently throughout the process, improving overall process understanding by capturing progressive variation with greater granularity.

For example, analyzing bioreactor contents on a more frequent basis can reveal details about the trigger, course, and impact of detrimental or beneficial changes to culture conditions. The additional information allows researchers to identify more impactful manipulations that improve efficiency and better-targeted measures to correct deviations.

Figure 4 shows the changes in percent product galactosylation measured in samples drawn from a bioreactor subjected to a step-change in galactose concentration. Galactosylation of protein biologics may strongly influence their function and is impacted by bioreactor conditions; changes can occur rather abruptly, and manual sampling is often insufficient to accurately reconstruct response dynamics. Samples drawn manually or via the MAST® Autosampling Solution from a perfusion reactor subject to a change in galactose concentration from 1 to 10 mM were analyzed by UHPLC to measure response in percent galactosylation.

Daily manual sampling showed an increase in galactosylation that appeared to occur evenly over 2 or more days (**Figure 4A**). More frequent Autosampling (4x/day) with the MAST® Autosampling Solution showed that most of the response occurred 12–24 hours after the boost in galactose with subsequent slowing (**Figure 4B**). This level of understanding of when and how process parameters trigger changes is essential to define critical product quality attributes.

4A



4B

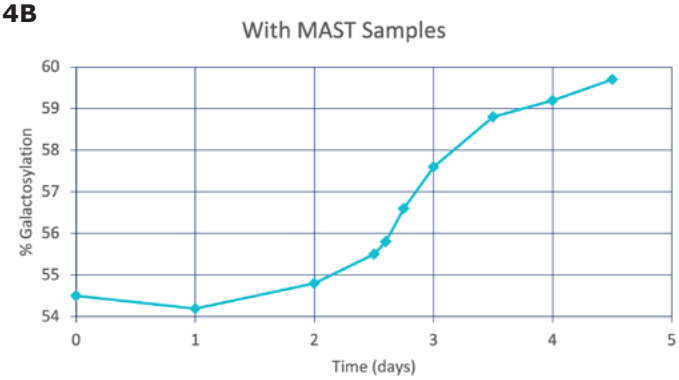


Figure 4: Comparison of manual **(4A)** and automated high-frequency sampling **(4B)** to characterize increased galactosylation in response to step changes in galactose.

Conclusion

Given the demand for more efficient and agile biopharmaceutical manufacturing capabilities, PAT is essential to optimize bioprocesses and enable rapid, data-driven decisions. Manual sampling approaches, however, are insufficient to leverage the full potential of PAT. Slow, labor-intensive manual sampling and analytics increase the possibility of handling errors and consume valuable time and team resources that are better invested in translating empirical insights into actions.

The evaluation described in this whitepaper underscores the accuracy and comparability of data generated with PAT automated through the MAST® Autosampling Solution. This system provides robust, near real-time and on-line results to accelerate process development. A mature, time-tested technology, the system ensures sterility, eliminates handling variation, shortens development cycles, and augments data generation capabilities. Automated sample collection can occur on the day and time programmed or on-demand.

It ensures that an experimental run is recorded in its entirety, at predefined sampling points regardless of time of day. The MAST® Autosampling Solution thus guarantees a uniform and consistent stream of data, providing a clear and uninterrupted picture of the bioprocess. In addition to the dramatic reduction in turnaround time for results and the reduced sampling burden placed on staff, the MAST® Autosampling Solution enables integration into existing process development routines and can be rapidly implemented in an existing bioprocess.

Start your journey in optimized bioprocess development. Learn how to generate a rich and continuous stream of data and track your sample source in near real-time. For more information on the MAST® Autosampling Solution and ideas on how to get started, visit: **sigmaaldrich.com/PAT**

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MK_WP13189EN Ver. 1.0
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04/2024