Validation of LC–MS Multi-Attribute Method Supporting Biopharma Process Characterization

Applying flexible automation to help drive multi-attribute method productivity in biopharmaceutical quality monitoring

Cédric Mesmin, PhD
Mass Spectrometry Specialist
Innovation Coordinator
Microbial Unit
Merck Biodevelopment SAS
Martillac, France

Lucie Manache-Alberici, PharmD
Analytical Development
Supervisor, Microbial Unit
Merck Biodevelopment SAS
Martillac, France

Jonathan Jones, PhD
Business Development
Expressionist
Genedata

OVERVIEW

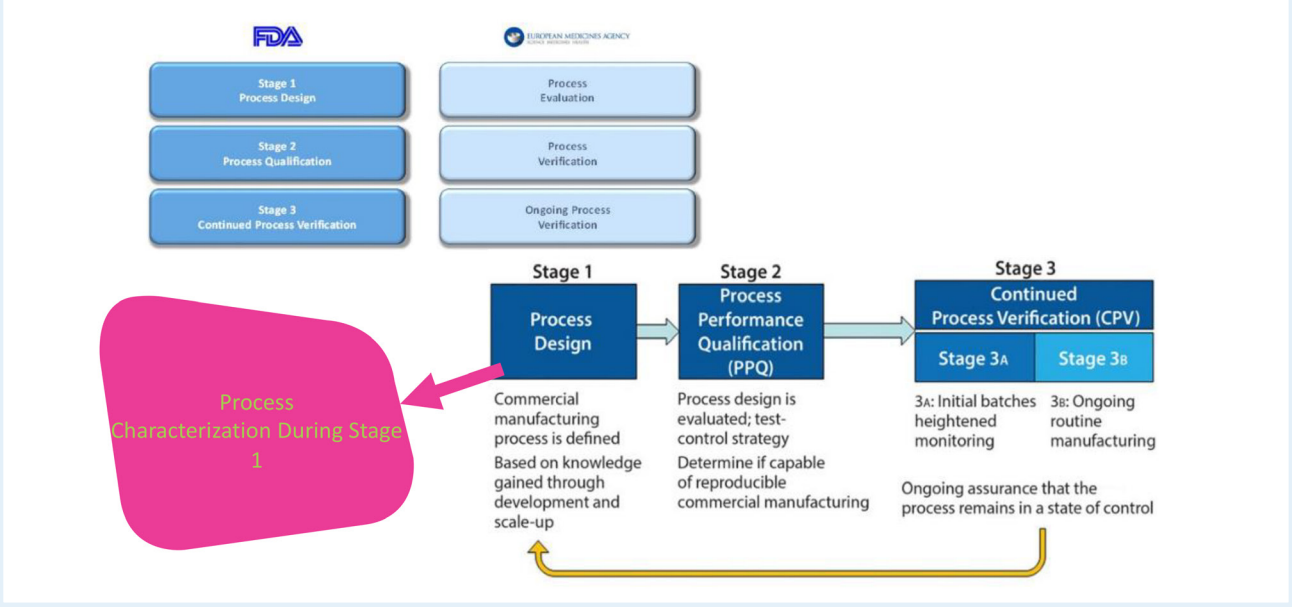
Multi-attribute method (MAM) approaches to characterizing and monitoring the production of biopharmaceuticals offer the ability to replace multiple analytical technologies with a single mass spectrometry-based (MS) analysis. However, routine use of MAM in this environment means overcoming various scientific, technological, and methodological challenges. These challenges include managing large amounts of data, producing unbiased audited results, and meeting process validation requirements. This article describes how scientists at one major biopharmaceuticals manufacturer have implemented a flexible software solution with automated workflows that has enabled them to address these challenges and reap the benefits of using MAM analyses routinely.

RECOMBINANT PROTEIN DEVELOPMENT

The Microbial Process Development Group at Merck KGaA (Martillac, France) is tasked with developing and producing recombinant proteins expressed in *Escherichia coli* and *Pichia pastoris*. The group includes scientists and experts in microbial processing analytics. They use MS as a routine tool for supporting process development in recombinant protein production and are involved in developing original MS-based approaches that enable better understanding of protein expression in microorganisms for biopharmaceutical development. Once a process is developed, it can be transferred to a manufacturing unit, where GMP specialists manage production, from pre-clinical stages through to commercial batch manufacturing of a drug substance.

Validation of the manufacturing process is a requirement and regulatory authorities in the United States and Europe have set out three main stages. Each involves the collection

Figure 1: The definition of process validation.



and evaluation of data, from process design through to commercial production (see FIGURE 1). Process validation demonstrates the product and process understanding necessary to deliver a product with consistent efficacy and quality. The process characterization conducted as part of Stage 1 validation falls increasingly within the remit of the process development group.

THE NEED FOR PROCESS CHARACTERIZATION

The overall production process for recombinant proteins involves multiple processing steps that are driven by defined process parameters. The characteristics of the resulting protein product must be such that the final drug substance is both safe and efficient. Studying the desired protein characteristics, then defining, monitoring, and managing critical quality attributes (CQAs) is key to success. Assessing multiple CQAs at the molecular level delivers a comprehensive understanding of the end product, which ultimately enables a true quality-by-design (QbD) approach to biotherapeutic development.

The goal of process characterization is to experimentally assess the impact of potential critical process parameters (CPPs) on the defined CQAs. This assessment involves multiple experiments and is performed at qualified small scale using a design of experiments (DoE) approach. At Merck Biodevelopment, since robotics is used in both upstream and downstream processes, they are also qualified for process development and the performance of Stage 1 process validation. With robotics platforms generating large numbers of samples, subsequent analytics must also be capable of high throughput. This has required the development of a fit-for-purpose MAM that operates in a highly automated manner.

CASE STUDY: DEVELOPING AN MAM APPROACH FOR PROTEIN X PRODUCED IN *E. COLI*

Protein X is a 20-kDa protein produced in *E. coli* as inclusion bodies that are then solubilized and refolded. The need for an efficient MAM methodology to support the large amount of work required for Stage 1 process validation led to the development of a peptide matching

Figure 2: FDA recommendations about MAM initiatives.

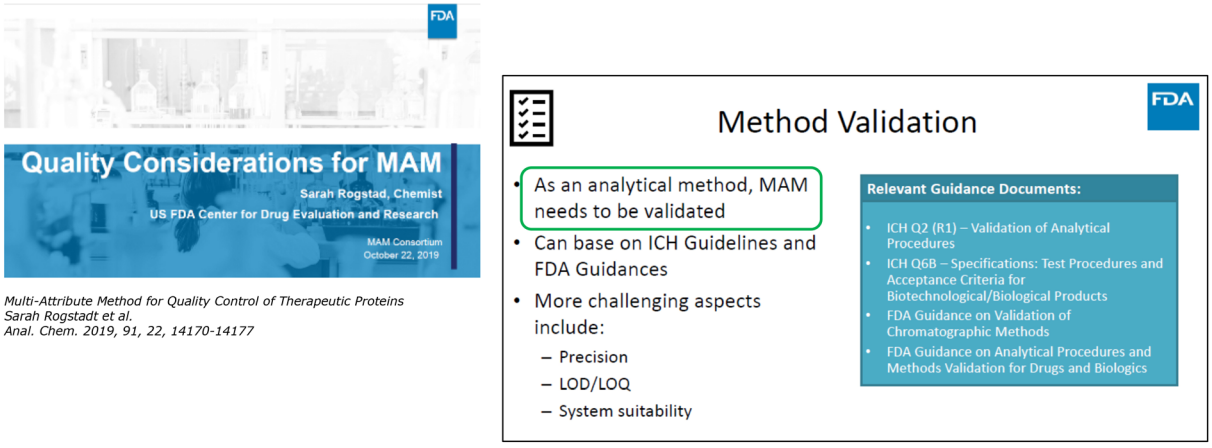
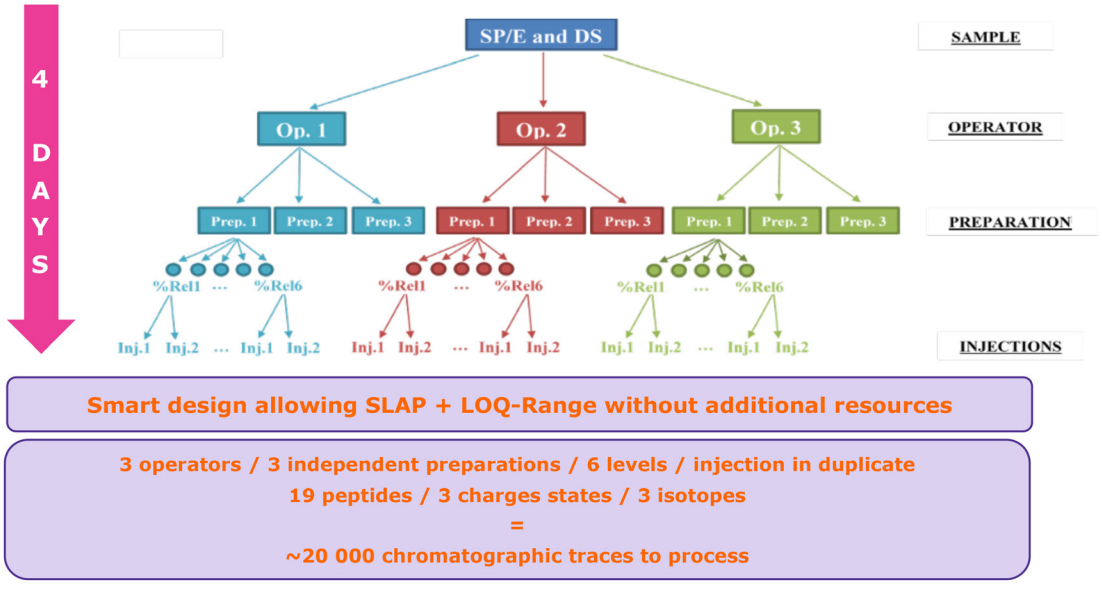


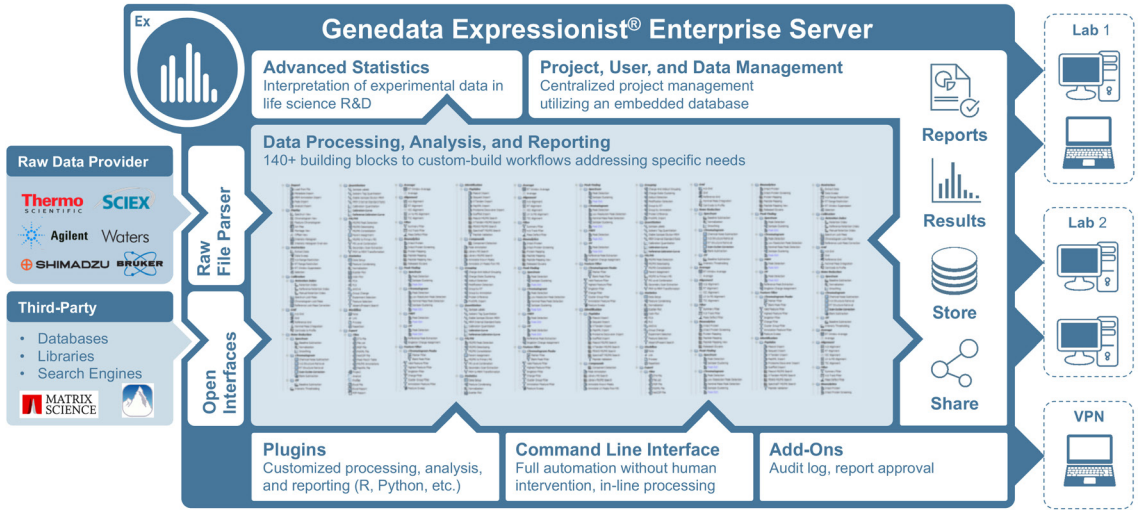
Figure 3: Non-GMP validation experimental design.



LC–MS method capable of monitoring four CQAs—oxidation, deamidation, gluconoylation, and truncation. Key challenges in the analytical method development were the sample preparation, the LC–MS itself, and data processing.

The starting point for developing the high-throughput LC–MS method for Protein X was the conventional (low throughput) ultra-high-performance liquid chromatography ultraviolet detector (UHPLC–UV) method used for analysis in pre-clinical and Phase-I stages of the molecule's development. UV detection was replaced by MS and the method was optimized to monitor the four identified CQAs, which reduced the run time from 120 minutes to just 30 minutes, quadrupling sample throughput. Comparison of this new method with the conventional UHPLC–UV approach showed

Figure 4: An open and modular enterprise software architecture enables the implementation of customized solutions.



good correlation across a wide range of CQA levels. Method robustness was confirmed by testing the impact of selected method parameters (identified by risk assessment) on the results generated.

With advances in MS driving the use of MS-based methods for quality control (QC) testing, FDA has made recommendations on *Quality Considerations for MAM* (see FIGURE 2). To validate MAM for use in process validation tests, the development group first designed a non-GMP validation package which involved testing samples containing different percentages of the CQA modifications produced by artificial and synthetic modification. This strategy helped to generate a wide linear dynamic range of CQAs and experimental LOQ of each. FIGURE 3 shows the experimental design (where SLAP stands for specificity, linearity, accuracy, and precision), which in this approach resulted in the need to process more than 20,000 chromatograms for the validation study. This led to the need for a more powerful and flexible data processing, analysis and reporting platform, and the subsequent adoption of an enterprise software solution (Genedata Expressionist, Genedata AG).

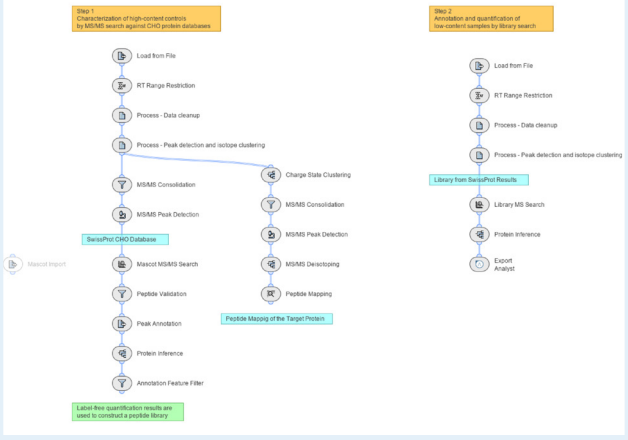
ENTERPRISE SOFTWARE BENEFITS

Genedata Expressionist is built on an open and flexible client server architecture and is suited to handle large and complex experimental MS datasets from all major MS vendors and technology platforms (see FIGURE 4). Once raw data is imported, more than 140 analytical building blocks, spanning the whole range of analytical processing and reporting functions, can be combined to develop automated data workflows. Developed workflows may be implemented in various ways to suit the needs of the user. Data can be visualized flowing from one activity to the next (See FIGURE 5), as can the actions that have been performed, from data processing and analysis through to reporting. Once data is processed, a stepwise approach allows full transparency throughout, with the ability to review results or export to final reports.

IMPLEMENTING THE AUTOMATED SOFTWARE SOLUTION

The overriding requirement for the development team was to build a robust, reproducible, and potentially QC-compatible system. Conventional data approaches that

Figure 5: An example illustrating how Genedata Expressionist's activities can be linked to create automated data workflows.



involve automated peak picking can be affected by batch-to-batch differences in peak shape co-elutions, which may result in erroneous peak integration. To avoid this, the team instead adopted a peak-mask strategy, regarding this as fundamental to achieving high specificity when monitoring just a few sets of attributes. This strategy involves systematic integration between two defined points.

Implementation using the Genedata Expressionist solution resulted in three sequential workflows that complement one another: the peak-mask workflow, the system suitability test workflow, and a workflow linked to CQA monitoring (see FIGURE 6).

The peak-mask builder is used to create the peak mask whereby targeted peaks are defined—a task that is performed only once per project. The target peptide is known, together with the charge state and which isotope to follow, and these are indicated to the software. Data is cleaned by the software and automated peak picking is used initially in drawing the integration zone. This zone is then refined manually to select only the part of the peak that is of interest. Once the peak mask is fully defined it can be applied for different purposes.

The first application of the peak mask is in the evaluation of system suitability. Here the goal is to evaluate the suitability of both the chromatography and MS systems to perform the analytics. This means checking the calibration of the MS and examining the chromatographic behavior of the peptide, especially retention time reproducibility. Since sample preparation efficiency is also an important part of the MAM workflow, that too is

Figure 6: Automated integration by Genedata Expressionist.

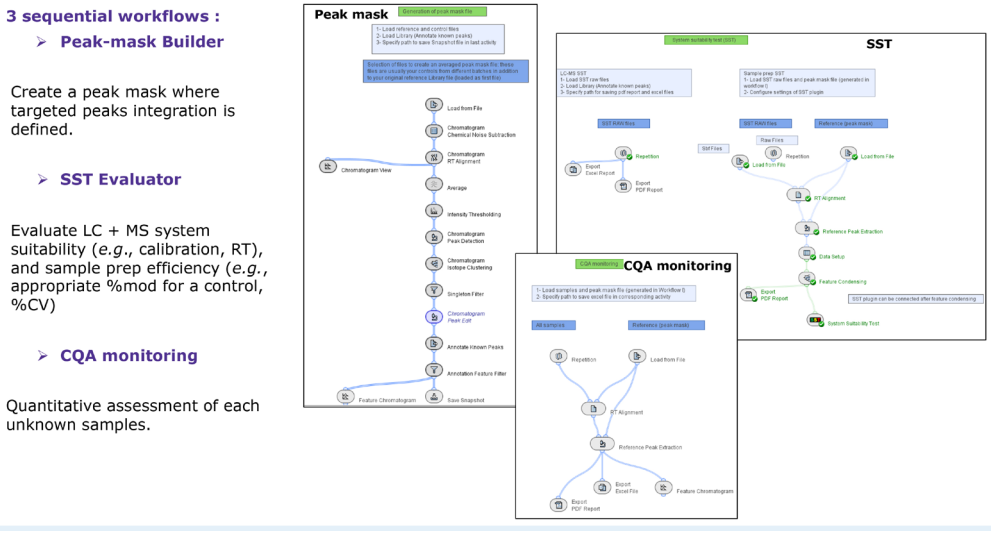
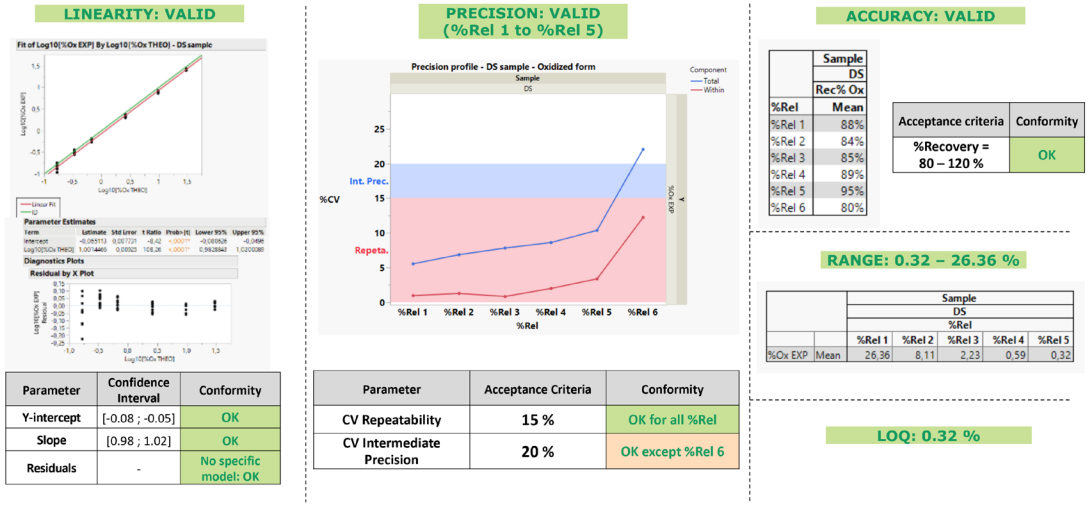


Figure 7: Validation results – Example of oxidized forms.



checked against several different specifications. One is the amount of modified material in a control. If a control is digested 25 times, for example, there is an expectation of X percent modification +/- the variability of the method. This measurement also serves to verify the reproducibility of results along the run.

The system suitability step—developed in collaboration with Genedata—has enabled the automated presentation of meaningful, user-friendly reports that provide easy assessment of a pass or fail outcome. This means the development team can quickly assess whether a test is working or if it needs to be repeated.

Finally, the CQA workflow drives the quantitative assessment of different unknown samples to determine CQA levels, applying the already-defined peak mask. Again, collaborative development has enabled reporting that provides an average percentage of the modification present and assessment of injection repeatability. Applying thresholds on the repeatability allows flagging of valid or failed outcomes, so it is easy to determine whether an analysis batch is acceptable simply by looking at the results for the samples and the controls.

THE IMPORTANCE OF AUTOMATED REPORTING

Development of the peak-mask approach for LC–MS and the use of customized aspects of the software platform has enabled fully automated data management and reporting. This means that all operators, whether or not they have experience in MS, can routinely analyze samples while maintaining the compliance level expected for process characterization.

VALIDATION STUDY RESULTS

Returning to the MAM validation study described earlier (see FIGURE 3), the results for the oxidation CQA (shown in FIGURE 7) typify the overall outcomes. Here linearity was very good with no pattern observed in the residuals, and the slopes and Y intercepts all fell within the pre-defined confidence interval. The precision profile indicates excellent repeatability and good intermediate precision. The last point of the dilution was, as expected, outside the limits initially designed. This was done to determine the experimental limit of quantification. Accuracy was always between 80–120% and

within the criteria initially defined and the dynamic range was also good. The method was therefore fully validated.

MAM OUTCOMES

The MAM approach developed was targeted to be high throughput. Simply being able to monitor four CQAs in one analytical run proved to be timesaving, but by using appropriate sample preparation and optimizing LC–MS gradients and analyses the team achieved a throughput of almost 300 samples per week. Using Genedata Expressionist to completely automate the data processing allowed for a reduction in total data analysis time to less than 1 hour. In addition, both the method and the data processing are user friendly, so the MAM approach can be run by operators with no prior MS experience. Furthermore, this MAM approach is validated and compliant within the 21 *Code of Federal Regulations* Part 11 elements of the software platform and as such, it is ready for QC testing to support GMP batch intermediate monitoring.

Ongoing work to redevelop sample preparation for implementation on a robotic platform is likely to further increase throughput and reduce end-to-end time, so that all the challenges originally identified in implementing MAM will have been addressed.

CONCLUSION

Scientists in the Microbial Process Development Group at Merck KGaA are routinely performing MS-based MAM analyses to study the impact of CPPs on CQAs in the production of recombinant proteins. Their novel MAM approach correlates well with existing analytical methods and has enabled the development team to significantly improve analytical throughput. Implementation of an enterprise software solution with flexible workflows for processing, analyzing, and reporting of MS data enabled this process validation strategy. Customization of the software and automated workflows enabled use by inexperienced users, and is helping bring this MS methodology closer to biopharmaceutical QC by providing the compliance level expected for process validation.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