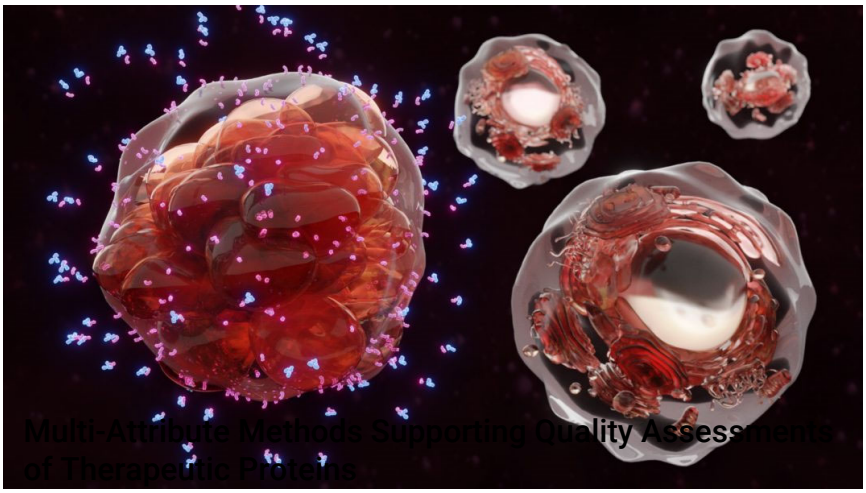

(https://www.genengnews.com/wp-content/uploads/2020/11/GettyImages-1250270684-scaled.jpg)

By Diane McCarthy, PhD - November 25, 2020

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Biotherapeutics require extensive characterization and quality control (QC) testing prior to product release. These tests are resource intensive, time-consuming, and require updating as manufacturing processes are improved or new analytical instruments with greater sensitivity reveal previously undetected impurities.

Replacing multiple QC tests provides an opportunity to streamline lab work and decrease development and post-approval costs. Given the high costs associated with drug development (https://jamanetwork.com/journals/jama/article-abstract/2762311), both patients and developers stand to benefit from more rapid development and release of biologics.

In the strictest sense of the word, a multi-attribute method (MAM) could use any technology that allows a scientist to investigate multiple quality attributes at the same time. As high-resolution mass spectrometry (MS) instruments evolved and new versions became available that could more easily be implemented in a quality control laboratory, this tool has become a workhorse for biopharmaceutical analytical laboratories.

Increases in mass accuracy, resolution, and data processing power, coupled with the large lab footprint of traditional methods and the molecular complexity of biologics, have created an opportunity for this new technology to emerge. While MAM is a relatively new application of MS, it has the potential to replace multiple assays that are currently used in routine QC testing, including capillary electrophoresis, capillary isoelectric focusing, peptide mapping, glycan analysis, as well as others.

Platform has gained traction

In 2015, Rogers et al., published the first paper (https://www.ncbi.nlm.nih.gov/pubmed/26186204) on an LC-MS-based MAM method intended to be used as a platform method for monoclonal antibody therapeutics. Over the last five years, MAM has gained traction throughout pharmaceutical development and QC labs, with several developers implementing some form of MAM in characterization or release. While MAM holds promise for deployment in the QC lab setting, several challenges remain. USP is actively exploring potential areas where best practices can be developed, described, and presented to harmonize MAM practices across the industry.

While even mass spectrometry-based MAM can include multiple approaches—including top-down, middle-down, and peptide-based workflows—it provides developers with a single platform that is capable of simultaneously monitoring both expected product quality attributes as well as unexpected impurities. Peptide-based MAM conducted with MS detection provides very specific and accurate measurements with much more information than traditional procedures. It is also the most mature MAM and is, therefore, the focus of this article.

MS-based measurements typically involve the following processes: sample preparation, chromatography or sample introduction, ionization, mass analysis, and detection. From the many types of ionization methods to the variety of MS instruments, there is substantial diversity in the deployment of MS. USP's General Chapter <1736> *Applications of Mass Spectrometry* provides an overview of

various types of mass spectrometers and their uses for both small molecules as well as larger, complex macromolecules like some biologics. It includes additional guidance for measurement of glycopeptides, peptide mapping, sequencing, and other applications in either qualitative or quantitative modes. Chapter <736> *Mass Spectrometry* provides development and validation expectations for these methods.

Four areas of consideration

In a recent review of the application of MAM for the QC of therapeutic proteins (https://pubs.acs.org/doi/10.1021/acs.analchem.9b03808), Rogstad and others employed by the FDA identified the following four key areas of consideration:

- risk assessment
- method validation
- new peak detection capability and specificity
- performance versus conventional methods

Developers interested in replacing traditional QC tests with MAM should take a risk-based approach to the monitoring of post-translational modifications. An important question to answer is: Is this a critical quality attribute? That is, does the attribute impact safety, efficacy, or quality? Comparison assessments via spiking studies can assess the ability of a MAM to replace a traditional QC assay.

Replacing multiple QC tests provides an opportunity to streamline lab work and decrease development and post-approval costs. A multi-attribute method (MAM) could use any technology that allows a scientist to investigate multiple quality attributes at the same time. [Witthaya Prasongsin/Getty Images]

For example, developers can analyze charge variants by first using traditional methods like CEX or cIEF and then comparing them to MAM, which monitors individual post-translational modifications that impact charge.

In order to obtain feedback from industry and the FDA regarding current applications, challenges, and quality considerations for MAM, USP held a Biologics Stakeholder Forum focused on this topic in early 2020. Attendees and speakers shared that some of the most significant challenges relate to the application of MAM in a QC setting due to the complexity of the instrument and interpretation of complex data sets.

While some developers have already advanced the methodology to the QC lab, others are still evaluating MAM and building data to support implementation in QC by adding it as an additional characterization tool and doing comparative analyses. A phased approach allows labs to assess the robustness and sensitivity of methods across laboratories, understand the frequency of any issues, and build an extensive set of comparative data.

Standard operating procedures that delineate what to do in different scenarios are essential to develop in order to help QC scientists make decisions regarding release versus triggering an investigation.

Another challenge is the novelty of the technology, which can draw additional scrutiny from regulators. Applicants must demonstrate to regulators that the method is fit for its intended purpose; therefore, engaging and collaborating with regulators early on provides an important mechanism for developers to provide a comprehensive data package that will be approved.

The group also discussed opportunities for standardization and development of best practices to improve consistency of MAM across multiple sites and organizations. Differences in instrumentation, sample preparation, and data analysis were cited as key factors that can lead to variability. Sample preparation is particularly important and requires both method optimization and adequate training to minimize discrepancies across labs and analysts.

The use of different software and data analysis parameters also impacts the results; therefore, best practices for data analysis, including new peak detection, would be useful. Participants indicated that industry may benefit from additional considerations and best practices for MAM, such as setting threshold limits, establishing system suitability, and validating assays.

“Implementing MAM in the pharmaceutical industry is a good example of analytical method modernization, and MAM fits very well in the QbD framework,” states Da Ren, process development scientific director at Amgen. He remarks, “MAM enables amino acid level monitoring and control of product quality attributes, which ensures higher quality of pharmaceutical products.”

In addition to its promise in the QC laboratory, MAM may also provide a valuable tool for future data mining. The MS dataset provides a rich source of information. Data gathered on a portfolio over years of testing has the potential to yield additional insights

during future analyses, which may lead to a better understanding of structure-function relationships as well as informing process development.

One day, this method could be a key test for manufacturers and control laboratories to use throughout the product lifecycle, from development through post-market analysis.

Given all these questions and the stage of maturity, the attendees recommended that USP bring together subject matter experts to help develop best practices, training, and standards that may help facilitate uptake and consistency across the industry. USP has since formed an Expert Panel to begin this work and will hold an open webinar in late 2020 to provide an update on USP's efforts toward developing MAM standards. Stakeholders are encouraged to submit additional recommendations to USPBiologics@USP.org (mailto:USPBiologics@USP.org) and explore opportunities to collaborate with USP.

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