CASE STUDY 73479

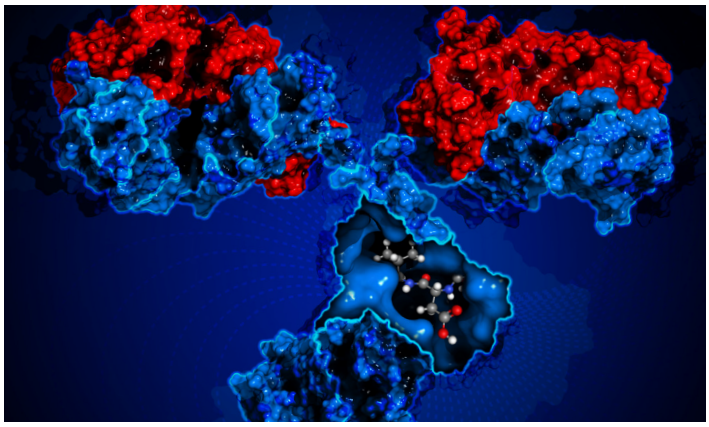
Meet the expert

Rich Rogers discusses his vision of what the future may hold for the multi-attribute method (MAM) in the biopharmaceutical industry

Introduction

In 2015, the scientific article titled, “Development of a quantitative mass spectrometry multi-attribute method for characterization, quality control testing and disposition of biologics,” caught the attention of the biopharmaceutical world. Since then MAM has been a focus of many biopharmaceutical strategic conversations at scientific conferences and other forums around the world.

At the basic level, MAM is a version of peptide mapping; however, by providing highly accurate relative quantitation of post-translational modifications in a sample when compared to a reference standard, we can use mass spectrometry to monitor product attributes to further understand and improve biological manufacturing processes. The real added benefit of mass spectrometry is allowing for the examination of relevant peaks by extracted-ion chromatograms and, in the case of high-resolution mass spectrometry, being able to get accurate mass information aiding identification, removing the need for full chromatographic separation necessary in traditional LC-UV methods. This attribute-specific MS trace quantitation leads to a more comprehensive biomolecule process understanding, allowing for better process control for manufacturing.



The MAM Consortium was established to enable the biopharmaceutical industry to leverage MAM as a process development tool for better process understanding. This effort will pave the way to the ultimate goal: Introducing MAM in a quality control laboratory as a product release method, replacing select traditional analytical methods with a single MAM assay. The MAM Consortium is a forum where representatives of biopharmaceutical companies, instrument and software vendors, contract manufacturing organizations, contract research organizations, and regulatory agencies connect to discuss ways to leverage MAM in the industry, what is working well with method adoption, what are the key challenges and areas of development, and ways to be successful with MAM. The MAM Consortium benefits from these discussions by collecting industry views and addressing the challenges collectively to build a strong case for MAM to be adopted in quality control laboratories.

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"The MAM Consortium is still in the early stages of educating the biopharma industry but with the growing membership of the MAM Consortium and increased interest in how MAM can be applied, we feel energized in our efforts."

– Rich Rogers, President, MAM Consortium

From the perspective of the MAM Consortium, it is important for those agencies who evaluate regulatory submissions and support approval of biotherapeutics to see MAM as a viable release assay. It was clear to the founders of the MAM Consortium that one company alone would have major hurdles in achieving acceptance of MAM in quality control laboratories; a MAM movement was needed. The MAM Consortium aims to align companies on the topic of MAM and provide a united front when presenting work performed with MAM to regulatory agencies. The MAM Consortium is a great forum to educate the community, highlighting the tools (mass spectrometry (MS) technologies, software, etc) and empowering them to utilize MAM in biotherapeutic process development through to quality control.

Rich Rogers is the president of the MAM Consortium and is regarded as an analytical pioneer and leader in the MAM community. Rogers has worked closely with his former colleague, Da Ren from Amgen, as co-founders of the MAM Consortium and as advocates for MAM adoption in the biopharmaceutical industry. Together they have navigated the hurdles of implementing MAM and celebrated a recent noteworthy success. In 2019, results generated by MAM was accepted by the FDA as part of supporting documentation for the first time. Rogers describes the moment he received this news from Ren as the most exciting moment of his MAM efforts to date. In this interview, Rogers provides his vision of what the future may hold for MAM in the biopharmaceutical industry.

Defining the term "MAM" within the analytical community

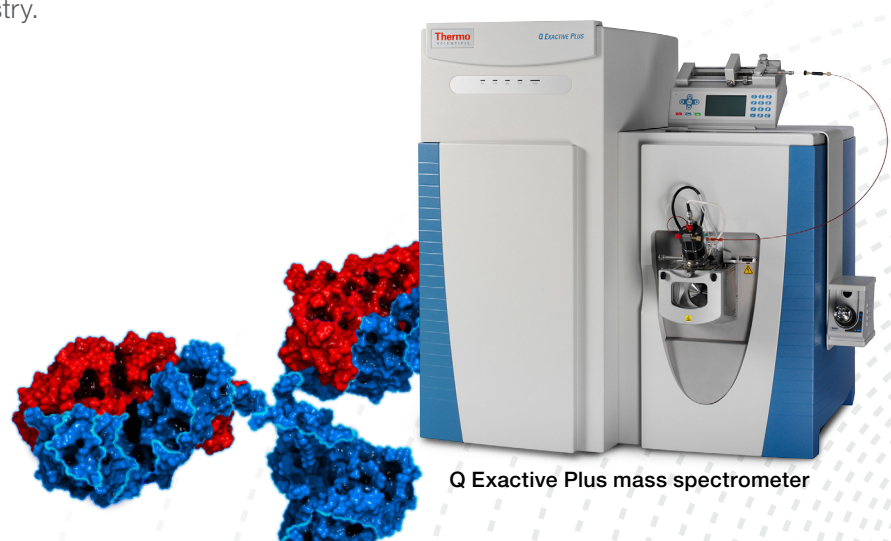
Biopharmaceutical laboratories are exploring the use of MAM to monitor critical quality attributes of biologics during product development. As MAM has been growing in interest, the strategies for use have broadened and so has the use of the term "MAM". The MAM Consortium has focused on defining the term for clarity and to support a unified strategy for streamlined adoption. According to Rogers, MAM must contain the following components: attribute analytics, high-resolution mass spectrometry, and New Peak Detection (NPD).

Attribute analytics

The attribute analytics component is a targeted LC-MS analysis used to monitor a pre-defined set of post-translational modifications, degradations, and impurities.

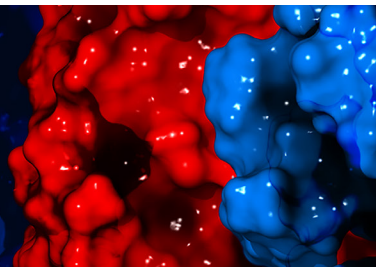
High-resolution mass spectrometry

High-resolution accurate mass (HRAM) mass spectrometers with superior detection sensitivity and resolving power, such as the Thermo Scientific™ Q Exactive™ Plus mass spectrometer, offer the key to specificity in accurate mass determinations of intact and fragmented proteolytic peptides. The mass accuracy that the Q Exactive Plus MS provides allows for identification of most product variants and impurities with unambiguity. Using HRAM, a comprehensive product characterization can be performed earlier in the product development cycle and throughout the product development pipeline.



Q Exactive Plus mass spectrometer

"By adding MAM throughout process development, one establishes a deep understanding of the method, leading to higher confidence in the method's ability to replace traditional release assays once the molecule reaches the quality control laboratory."



New Peak Detection (NPD)

NPD leverages differential analysis to identify new or changing peaks in a test sample compared to a reference standard. It is a key component of MAM because it allows for the potential replacement of purity testing methods in the quality control environment with the analytical confidence necessary in this regulated space.

There are many ongoing discussions surrounding the true benefits of NPD within the MAM community. Because of the importance of NPD from the perspective of the MAM Consortium, they are heavily focused on educating the biopharmaceutical community on the value and necessity of analyzing data and utilizing NPD in this way.

MAM workflow

The MAM workflow consists of the following steps:

- First, fully characterize the molecule of interest for full product understanding.
- Once fully characterized, define attributes to monitor and set attribute criteria using LC-MS/MS (targeted MS^1 list of the product quality attributes (PQA)).
- Next, leverage the targeted MS^1 list of the PQA to monitor known attributes and apply NPD to detect new attributes.
  - Inject the sample onto an LC-MS system, followed by an injection of a well-optimized batch of the product used as reference standard.
  - Report the relative abundance of the known PQAs.
  - Then perform a base peak alignment and look for relative quantitative differences between the two injections using a set threshold.
- Once these PQA differences are understood, MAM can be used to develop and monitor processes, as well as to build in manufacturing process controls.

MAM is positioned to replace the combined use of traditional release assays such as reduced CE-SDS, charge-based assays, released glycan assays, identity tests, and HCP ELISA assays. In the short-term, MAM will be complementary, as it is used to develop process understanding. In the long-term, MAM has the potential to displace these conventional assays in quality control laboratories.

The power of MAM is realized when used throughout product development

Rogers states, "A benefit of MAM lies in the industry's ability to use the method throughout the whole development journey of a biomolecule. This powerful benefit of MAM would be lost if the method was only applied to a molecule as it is moving into the QC space." MAM was created with Quality by Design (QbD) principles in mind, allowing for the development of a thorough product and process knowledge from early stages of development through manufacturing.

Overcoming challenges of MAM through collaborations

There are several challenges for the industry to overcome. There needs to be a focus on ensuring quality data is acquired which requires methods that are highly robust and reproducible to provide the level of confidence needed. It is extremely important to start with a robust and well-established system suitability test (SST). Rogers has been visiting labs for the past 4 years and often finds that a suboptimal system suitability test is the roadblock to advancing MAM workflows into the quality control space. To increase robustness and repeatability, a thorough system suitability test procedure must be established to facilitate the performance evaluation of the chromatography system and the mass spectrometer. Having a robust digestion method that is reproducible across analysts and laboratories is also essential in the success of a system suitability test. A proper SST will reveal how the overall assay and all individual components are working, allowing to resolve any potential issues prior to study sample analysis. Another important tip from Rogers: Be sure to add a series of blank injections before injecting the SST and study samples to ensure the system is prepared, conditioned, and primed to obtain the most reproducible results.

One of the largest challenges for the MAM Consortium is identifying easy-to-use software solutions. There needs to be optimization of how molecules are characterized, and target lists created for a simple transfer into the Thermo Scientific™ Chromeleon™ Chromatography Data System, or other suitable data systems. Once software is implemented, upgrades become a challenge. Upgrades to software in the regulated space create validation hurdles that add extra layers of complexity to MAM in a quality control laboratory. Vendors work to support this process as much as they can. For example, Chromeleon Chromatography Data System has differentiated pathways depending on a customer's risk level and the chosen pathway determines the upgrade tasks.

Many potential MAM users in the biopharmaceutical industry have reached out to Thermo Fisher Scientific for support in developing their MAM capabilities. The Thermo Fisher team has helped Rogers to identify key software tools in building his MAM capabilities. Rogers said, "The team listens, they are accessible, and they are always available to answer questions. The present success of MAM could not have been achieved in a vacuum." Thermo Fisher has active collaborations focused on creating a well-defined MAM workflow solution that delivers value to the entire biopharmaceutical industry.

Next steps in Rogers' Thermo Fisher collaboration to drive further success of MAM

When discussing how he envisions collaborating with Thermo Fisher going forward, Rogers mentioned three opportunities to further develop MAM capabilities. First, the MAM community needs vendors to create simple starting templates for MAM workflows. Rogers describes, "We need vendors to create a template that include constant domain amino acids in monoclonal antibodies that are hotspots for modifications such as: deamidation on PENNYK peptide, oxidation of the methionine on the MISR peptide, and N-linked glycosylation sites with common glycans. "These templates containing constant domain amino acids and modifications would save the community considerable time when developing target lists.

Second, Rogers says, "The future of MAM is automation from start to finish. Soon samples will be taken directly from bioreactors, moved to a sample preparation system with automated digestion capabilities, and then delivered to the LC-MS where analysis and report generation will occur without human intervention."

The third developmental need Rogers notes is improving NPD. "We need to work with Thermo Fisher to further develop NPD with the changing regulatory landscape and QbD principles in mind. Adding in the ability for QC laboratories to collect and store MS^2 data for a separate expert review in cases where a new peak is detected would be a powerful step in the regulatory acceptance of MAM," Rogers said.

Summary

The biopharmaceutical industry is experiencing unprecedented innovation, both with established protein therapeutics as well as novel therapies. This product development pace and innovation requires development of analytical technologies, such as MAM, that provide enhanced product and process knowledge in a short timeframe. The success of MAM relies on strong collaborations between vendors and the biopharmaceutical industry to quickly address challenges and offer solutions, with a focus on efficient and successful implementation of MAM throughout the biotherapeutic development process. Thermo Fisher is well positioned to partner with industry to build a robust, accepted MAM solution, as a world leader in serving science and offering a full workflow solution from protein digestion through to analysis, data processing and reporting.

For more information please visit **thermofisher.com/mam** and **mamconsortium.org**

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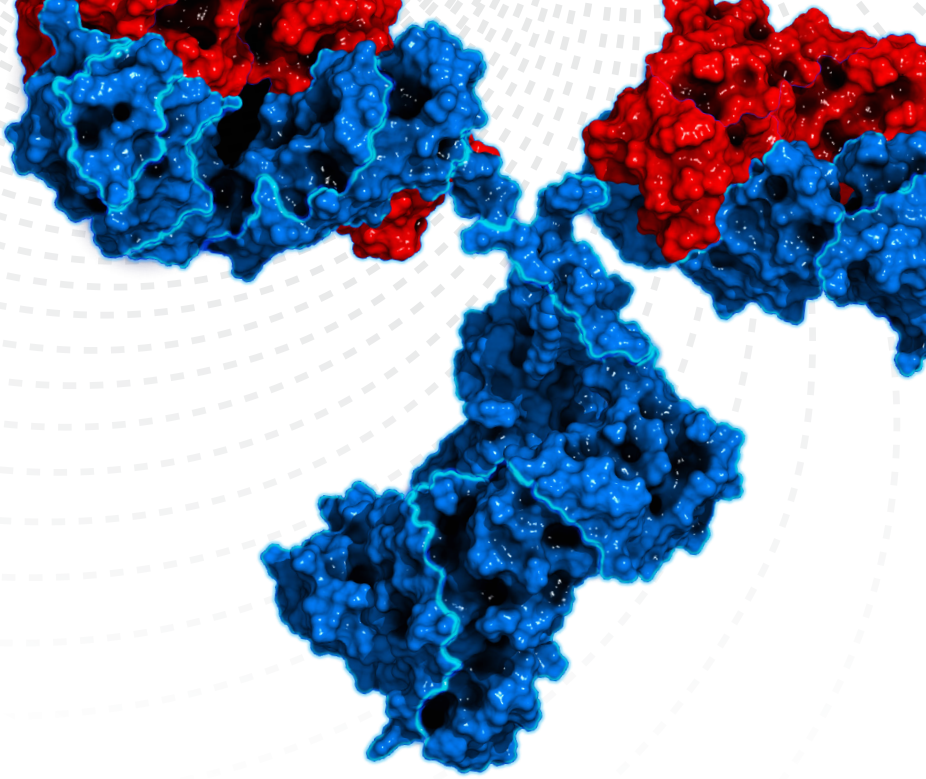

Find out more at **thermofisher.com/mam**

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