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Research

The Multi-Attribute Method (MAM): A Paradigm Shift in the Characterization and Quality Control of Biopharmaceutical Drugs

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Abstract

Biopharmaceuticals have revolutionized the treatment of many diseases especially monoclonal antibodies (mAbs). Nevertheless, their very structural complexity poses great problems of analytical characterization and quality control (QC). The old fashion method, based on a big panel of single attribute measurements, is frequently both labour-intensive, time consuming and offers an oblique perspective of a product quality. Multi-Attribute Method (MAM) has become an innovative mass spectrometry (MS)-based platform, which can determine and measure dozens of care-giving quality attributes (CQAs) in an individual, centralized test. In this review I have described the fundamentals of MAM, preparation of the samples, analysis using LC-MS and data processing. We discuss the whole spectrum of CQAs that can be further thorough and comprehensively, such as product identity, post-translational changes (PTMs) such as deamidation, oxidation, and glycosylation and process related impurities. Moreover, we talk about the feasible aspects of the application and experimental validation of the MAM in a regulated cGMP facility and clarify its benefits in comparison to the traditional ones and the present-day view held by regulatory bodies. Lastly, we discuss potential opportunities and challenges that stand out and wait in the future, where automation, machine learning, and expansion into new modalities will reinforce MAM even more as a staple of current biopharmaceutical development and QC.

Keywords: Multi-Attribute Method (MAM), Biopharmaceuticals, Monoclonal Antibody (mAb), Mass Spectrometry, Quality Control (QC), Critical Quality Attributes (CQAs).

1. INTRODUCTION

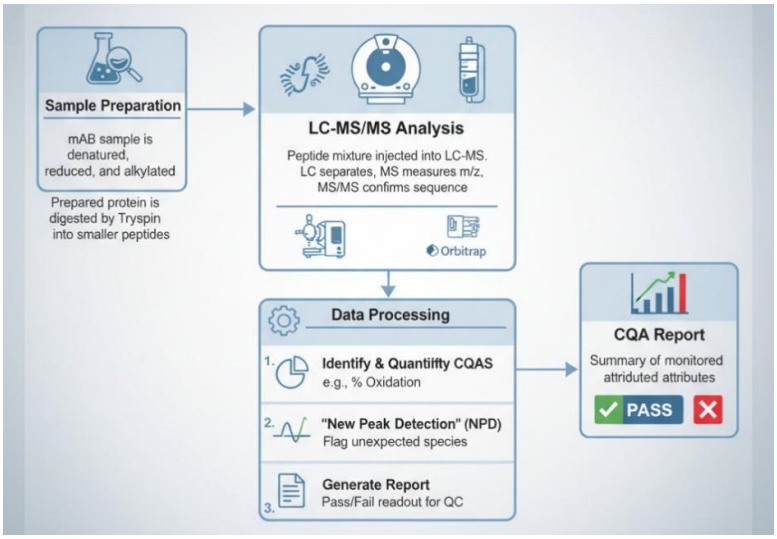
Monoclonal antibodies (mAbs) are gaining momentum in the pharmaceutical industry and currently dominate the biopharmaceuticals area of the pharmaceutical industry with respect to treatment of cancers and autoimmune disease. [1]. In contrast with small-molecule drugs, mAbs are large, heterogeneous proteins ([?]150 kDa) which are synthesized in cells. The issue and success of their DNA are their complex structure with complex and extensive post-translational modifications (PTMs) that are important in their safety and effectiveness [2]. It therefore follows that there is a lot of analytical characterization needed before the quality and consistency of such products established.

The classic model of quality control (QC) of biopharmaceuticals has taken the form of a single test at a time. According to [3], a high battery of assays, such as different types of chromatography, capillary electrophoresis, and ligand-binding assays, is used to measure the individual critical quality attributes (CQAs). This is an effective method, but it is laborious, large quantities of sample material are needed and may be slow in identifying deviations. Moreover, it gives you a sporadic impression of product quality, thus it becomes hard to correlate between various traits.

In order to overcome these shortcomings, a holistic, high-throughput area of analytical platform called the Multi-Attribute Method (MAM) has been created [4]. Making use of high-resolution mass spectrometry (HRMS) on its foundation, MAM provides the possibility to track the primary chain back, recognize and measure a great number of PTMs, as well as detect impurities all in single run of an analysis. This fits very well with FDA Quality by design (QbD) program as it gives more understanding of product and process [5]. This review will entail in-depth discussion of the MAM workflow, its usage in the monitoring of the CQAs, the implementation plan in the regulated environment, prospects, and futures.

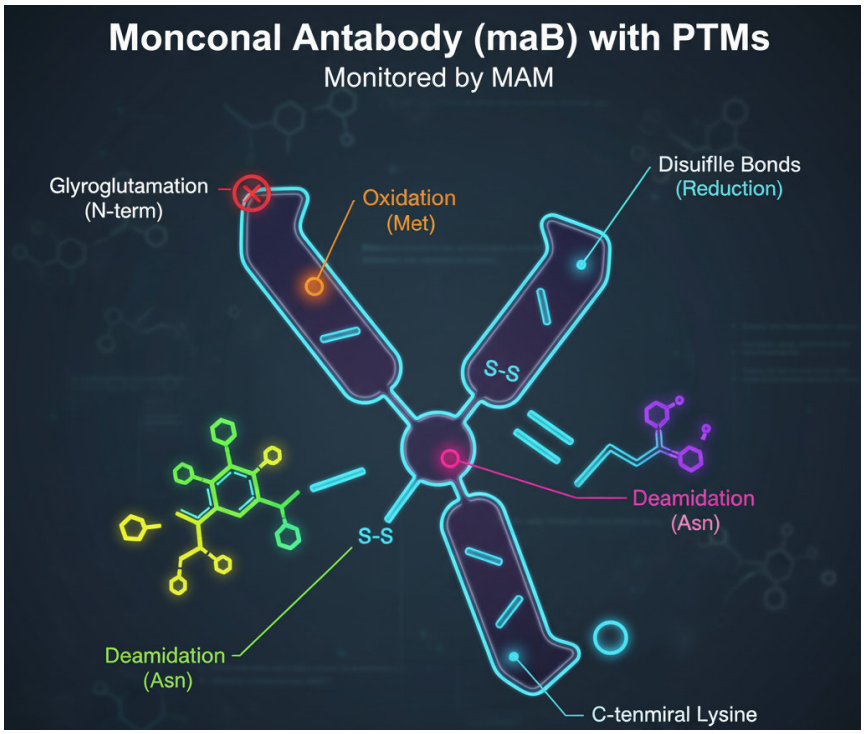
2. The Mam Workflow: From Sample To Data

- The MAM workflow is a standardized process designed to convert a complex protein into peptide data that can be mined for CQA information. The process can be broken down into four key stages, as flowchart below.



3. A Multi-Faceted Approach: CQAs Assessed by MAM

MAM's primary advantage is its ability to monitor a vast array of CQAs that traditionally require a suite of orthogonal methods. These attributes can be broadly categorized into identity, PTMs, and impurities.



3.1 Product Identity

In its most basic sense, MAM is an ultimate identity test. The method confirms the primary amino acid sequence without potentially purification products by forming a peptide map of the therapeutic protein and this ensures that the appropriate product is being analyzed. This traditional method of actually testing a peptide map is much more complete than traditional techniques.

3.2 Post-Translational Modifications (PTMs)

PTMs refer to chemical changes on amino acids that take place during of after protein synthesis (enzymatic or non-enzymatic). They are a significant cause of heterogeneity and may have a pernicious influence on stability, efficacy, and immunogenicity of a drug. MAM is only great in tracking such changes

- Deamidation: Typical alternative degradation of asparination and glutamine residues which cause a +1 Da mass change. MAM is able to identify the localisation of deamidation and measure the extent [10].
- Oxidation: Oxidation can cause changes in the residues such as methionine and tryptophan (+16 Da mass shift), thereby interfering with protein functionality. MAM is capable of quantifying oxidation specifically at the site.
- Glycosylation: Sugar moieties are acylated to the protein and are essential to its functioning and half-life. Using the glycopeptides, MAM would be able to heal the glycan profile, providing the relative abundance of the various glycoforms [11].

Additional alterations- MAM could measure scores of additional characteristics, such as N-terminal pyroglutamate formation, C-terminal lysine clipping, disulfide bond linkages.**3.3 Product- and Process-**

Related Impurities

MAM is a sensitive instrument of identifying impurities besides what is supposed to be changed. It has the ability to determine and measure the product-associated variants including truncations or aggregates and process-associated impurities including Host Cell Proteins (HCPs) that have the possibility of co-purification with the

drug product [12]. New Peak Detection feature stands especially when unexpected impurities might be detected due to the process changes.

4. Bridging Development and QC: Implementation and Validation

For MAM to be used as a QC release test in a current Good Manufacturing Practice (cGMP) environment, the method must be thoroughly validated and accepted by regulatory agencies.

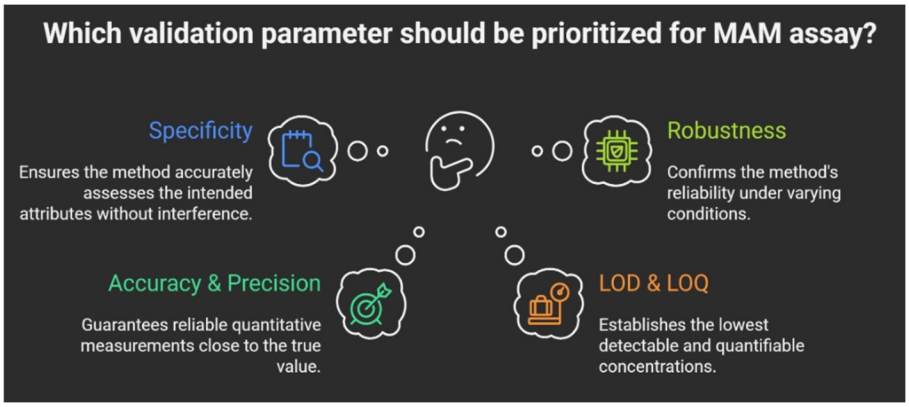
4.1 Implementation Pathway

The application of MAM is usually introduced in the development of the processes, and its strong analytical capability assists scientists to comprehend the influence that process parameters will bring to the quality of the products. As the process becomes mature and the product passes the clinical trial the process of the assay is advanced and made to be used on routine basis. Its eventual aim will be to scale up a proven MAM assay to a QC laboratory to substitute various traditional release tests, including ion-exchange chromatography (IEX) and capillary electrophoresis with SDS (CE-SDS) [13].

4.2 Method Validation

Validating a MAM assay is a highly complicated process and needs to comply with International Council for Harmonisation (ICH) requirements. The important validation parameters are:

- Specificity: To prove that the approach can definitively measure the desired characteristics when there are other components.
- Precision and Accuracy: In the case of quantitative measurements (e.g. 100 out of 100 oxidation), the smallness of the measured value to the actual value (accuracy) and the size of the measurements dispersion (precision).
- Limit of Detection (LOD) & Limit of Quantification (LOQ): Determining the lowest level in terms of concentration at which an attribute or impurity may be detected and percented.
- Robustness: Evaluation of the performance of the method in situations where the method parameters (e.g. pH, temperature) have been modified in minor and precise ways, to ascertain how trustworthy the method is during routine operation [14].



4.3 Regulatory Perspective

The regulatory bodies, such as the European Medicines Agency (EMA) and the U.S. FDA have become more accepting of MAM. Collaboration and consortiums throughout the industry has been critical in terms of best practice and dealing with the regulators to gain confidence in the platform [15]. Regulators posit MAM is more favourable because it offers a more comprehensive profile of the product, and is more resonant to the risk-based principles of QbD.

5. The MAM Landscape: Advantages, Hurdles, and the Road Ahead

5.1 Summary of Advantages

The use of MAM has obvious advantages:

- Specialisation and Efficiency: Eliminates a large number of assays, conserving time, effort and valuable sample material.
- Increased Understanding of the Product: Displays information about dozens of CQAs at once at a more detailed site level.
- Optimized Sensitivity: In many cases, it is a more sensitive and specific tool that can identify modifications than the older ones.
- Quick Deviation Notification: allows the development of out-of-spec or patterned results faster.

5.2 Current Challenges and Hurdles

Although the advantages are obvious, still there are a few challenges:

- High Start-up cost: The start-up capital is a lot of money and high-resolution mass spectrometers and advanced software demand huge capital investment.
- Expertise: the work of the tool and the interpretation of the complicated information needs extremely qualified analysts.
- Complexity of validation: As is explained, validation of a multi-attribute assay is more complicated than a multi- attribute test.
- Data Management: MAM generates massive amounts of data in need of a powerful IT infrastructure to store and process data.

5.3 Future Directions

The field of MAM is continuously evolving. The future will likely focus on:

- Full Automation: Adding robotic sample preparation to the LC-MS and data analysis pipeline and making it truly a walk-away system.
- Artificial Intelligence (AI) and Machine Learning: Trend optimization: Applied AI algorithms to interpret the data better, ensure prediction, and augment their ability to detect the new peaks [16].
- Scaling up to New Modalities: Scaling-up the MAM workflow to support still more complex biotherapeutics, including antibody-drug conjugates (ADCs), bispecific antibodies and cell and gene therapy products [17].
- Real-Time Release Testing (RTRT): The long-term vision is to directly to bring MAM to the manufacturing line, where it will be possible to monitor and release the product in real-time, eliminating the necessity of final batch testing.

6. CONCLUSION

Multi-Attribute Method is an expression of the critical paradigm shift that has occurred in the study of biopharmaceuticals. Through substituting a battery of unrelated, single quality, examinations with one information driven platform, MAM offers an unparalleled level of product insight and information mining effectiveness. Although implementation and validation challenges have not been resolved yet, obvious advantages in both development and Quality Control have established MAM as an essential instrument of providing the safety and effectiveness of modern biologic medicines. With the further maturation of the technology, MAM will become biopharmaceutical characterization and release test technology of the future, leading the industry into a more efficient and scientifically speculative future.

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