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ENHANCING QUALITY ASSURANCE: LEVERAGING MULTI-ATTRIBUTE METHODS TO ENSURE PURITY IN BIOPHARMACEUTICALS

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Abstract:

The pharmaceutical industry is undergoing an era of unprecedented growth and innovation, with novel therapeutic modalities such as bispecific T cell engagers (BiTEs), gene therapies, and chimeric antigen T cell receptors (CARTs) revolutionizing medical treatments. In parallel, advancements in analytical technologies have given rise to Liquid Chromatography-Mass Spectrometry Multi-Attribute Methods (LC-MS MAMs). This paper explores how LC-MS MAMs, based on peptide mapping principles, have transformed product characterization, formulation development, stability testing, and manufacturing processes in the context of evolving biopharmaceuticals.

Keywords: *Quality control, Biopharmaceuticals, Pharmaceutical industry, Quality assurance, Patient safety*

Introduction

The application of Multi-Attribute Method (MAM) has heralded a profound transformation in the realm of biopharmaceutical quality control. Its remarkable success story spans across early-stage clinical programs, encompassing a diverse array of protein therapeutics. The distinguishing hallmark of MAM lies in its remarkable versatility, rooted in its capacity to furnish highly precise and quantitative insights. This, in turn, has become an invaluable pillar of support during the labyrinthine journey of process development and the intricacies of molecular characterization.

Furthermore, MAM's seamless alignment with the hallowed principles of Quality by Design (QbD) has been substantiated through a wealth of compelling data. This technology has proven its mettle, not merely as an experimental endeavor but as a robust and practical solution for evaluating release and stability in the biopharmaceutical arena. Recent leaps in technological innovation have added yet another layer of fortitude to the already persuasive argument for adopting MAM in the context of current Good Manufacturing Practice (cGMP) environments.

This article endeavors to encapsulate the profound advantages that MAM bestows upon biopharmaceutical quality control when juxtaposed with conventional product testing paradigms. It ardently champions the unique attributes of MAM, setting it apart from traditional purity assessment methods. MAM's unparalleled capacity to monitor and quantify the molecular attributes that underpin product quality, as well as product/process-related impurities, represents a paradigm shift in the pursuit of precision. This heightened attribute specificity, irrevocably tethered to the paramount concerns of safety and efficacy, promises to usher in a new era of enlightenment. Through

MAM, biopharmaceutical stakeholders can aspire to attain an elevated understanding of their products and processes, expedite development timelines, and orchestrate control strategies of unparalleled finesse by amplifying the precision of their measurements.

In essence, MAM has transcended its status as a mere method; it has become a beacon guiding the biopharmaceutical industry toward a future where quality control is an art and a science, where product understanding is paramount, and where safety and efficacy reign supreme.

Overview of Multi-Attribute Method (MAM) in Biopharmaceutical Quality Control

Multi-Attribute Method (MAM) stands as a revolutionary analytical approach that has redefined the landscape of quality control within the biopharmaceutical sector. This comprehensive and sophisticated method is primarily employed for the evaluation of quality and attributes in biopharmaceutical products, with a particular focus on protein therapeutics. Its widespread acclaim arises from its remarkable capacity to furnish highly precise, specific, and quantitative insights, thereby offering a comprehensive perspective on product quality. Below is a concise summary of the essential facets of MAM:

1. Purpose and Scope: MAM is intricately designed to concurrently assess multiple attributes inherent to biopharmaceutical products. These encompass structural, chemical, and physical characteristics. The overarching objective of MAM is to safeguard the safety, efficacy, and uniformity of biopharmaceuticals by detecting impurities, variants, and modifications that can exert pivotal influence over product quality.

2. Versatility: MAM stands as an adaptable solution, capable of accommodating various categories of protein therapeutics, ranging from monoclonal antibodies to fusion proteins, bispecific antibodies, gene therapies, and beyond. Its utility extends across diverse phases of biopharmaceutical development, seamlessly transitioning from early-stage research and development to pivotal roles in clinical trials and commercial-scale manufacturing.

3. Key Components: At its core, MAM commonly integrates the combined powers of liquid chromatography (LC) and mass spectrometry (MS) to meticulously scrutinize biopharmaceutical samples. LC efficiently segregates the constituent components of the sample, while MS undertakes the crucial role of identification and quantification.

4. Benefits of MAM: Comprehensive Assessment: MAM stands as a paradigm shift, offering an all-encompassing view of product quality, diverging markedly from traditional single-attribute methods. Real-Time Monitoring: MAM's real-time capabilities empower it to scrutinize product quality throughout the manufacturing process, thereby catalyzing process optimization.

Efficiency: This methodology drastically reduces testing duration and resource outlays by simultaneously evaluating multiple attributes.

Specificity: MAM distinguishes itself through its heightened specificity, enabling precise measurement of product quality attributes and impurities.

Alignment with QbD: MAM's synergistic alignment with the principles of Quality by Design (QbD) reinforces its role as a cornerstone of risk-based approaches to product development and quality control.

5. Applications of MAM: MAM finds utility across various domains of biopharmaceutical development, spanning product characterization, formulation refinement, stability assessment, and the optimization of production processes. It assumes a pivotal role in quality control, ensuring the fulfillment of stringent regulatory standards for every batch of biopharmaceutical products.

6. Future Outlook: Ongoing research efforts and technological advancements continue to augment MAM's capabilities, firmly establishing it as an integral component of the ever-evolving biopharmaceutical landscape. MAM is poised to play a pivotal role in safeguarding the safety and efficacy of next-generation biopharmaceuticals, including the realm of personalized medicine therapies.

In essence, Multi-Attribute Method (MAM) has transcended its status as a mere analytical tool; it has become an indispensable driver of progress within biopharmaceutical quality control. Its capacity to deliver precision, specificity, and comprehensiveness positions it as a linchpin for ensuring the safety and efficacy of biopharmaceutical products at every stage, from inception to commercialization. As the biopharmaceutical industry continues its dynamic evolution, MAM is destined to remain at the forefront of endeavors focused on quality assurance and comprehensive product understanding.

The Regulatory Environment and Mass Spectrometry-Based Multiple Attribute Monitoring (MAM)

Over the past two decades, regulatory agencies have consistently encouraged manufacturers to adopt Quality by Design (QbD) and Design Space Models to enhance operational flexibility and reduce the complexity of managing product life cycles. An essential component of QbD is the creation of a Quality Target Product Profile (QTPP) and the development of advanced process control strategies to ensure product quality. While these principles have been extensively discussed, tested, and incorporated into regulatory guidance documents, their practical implementation and full realization have proven challenging.

Another pivotal concept introduced in ICH Q8 and Q11 is that of Critical Quality Attributes (CQAs). These are defined as specific physical, chemical, biological, or microbiological properties or characteristics that must meet defined limits, ranges, or distributions to guarantee the desired product quality. For the purposes of our discussion, we can consider any product attribute, whether critical or noncritical, as a Product Quality Attribute (PQA). CQAs are typically associated with the drug substance, excipients, intermediates, and the final drug product. In biopharmaceuticals, CQAs may encompass aspects like the nature and quantity of product-related substances, product-related impurities, and process-related impurities.

As per ICH Q6B, product-related impurities are defined as molecular variants of the desired product, including precursors and certain degradation products that may arise during manufacturing or storage. These impurities do not possess properties comparable to the desired product concerning activity, efficacy, and safety. CQAs falling under the category of product-related impurities can be related to post-translational modifications (PTMs),

chemical modifications of amino acid residues, amino acid misincorporations, or cleavage of the primary sequence due to hydrolysis during production and storage.

Conventional purity assays like CEX-HPLC, cIEF, and CE-SDS, commonly used for lot release and stability testing, are profile-based and may have limitations in identifying and quantifying residue-specific CQAs. Each conventional assay is typically designed to monitor a specific type of CQA and may have limited resolution and specificity. Consequently, complementary methods like Mass Spectrometry-based Multiple Attribute Monitoring (MAM) should be incorporated to address these limitations effectively. MAM offers the sensitivity and precision needed to pinpoint the location of PTMs or cleavages, leading to a deeper understanding and more effective monitoring of these critical attributes. If deemed essential, MAM can facilitate their specific monitoring during release and stability testing in a cGMP (current Good Manufacturing Practice) laboratory environment, contributing to comprehensive control over these crucial attributes.

In summary, LC-MS-based MAM represents a significant opportunity for the pharmaceutical industry to effectively embrace and leverage the QbD principles outlined above:

a) MAM enables the identification of residue-specific attributes, direct quantitative monitoring, and a more comprehensive understanding of PTMs and chemical modifications compared to conventional profile-based purity methods.
b) MAM is a crucial analytical tool for distinguishing between CQAs and PQAs, along with structure-function studies, aiding in identifying the relationship between individual attributes and the product's profile and performance.
c) The specificity and quantitative capabilities of MAM enable precise control over CQAs at the appropriate stage in the manufacturing process, streamlining testing methods and eliminating redundancy.
d) MAM can simplify life cycle management activities by providing regulatory relief through the use of a single method instead of multiple conventional purity methods.

Fig 1.

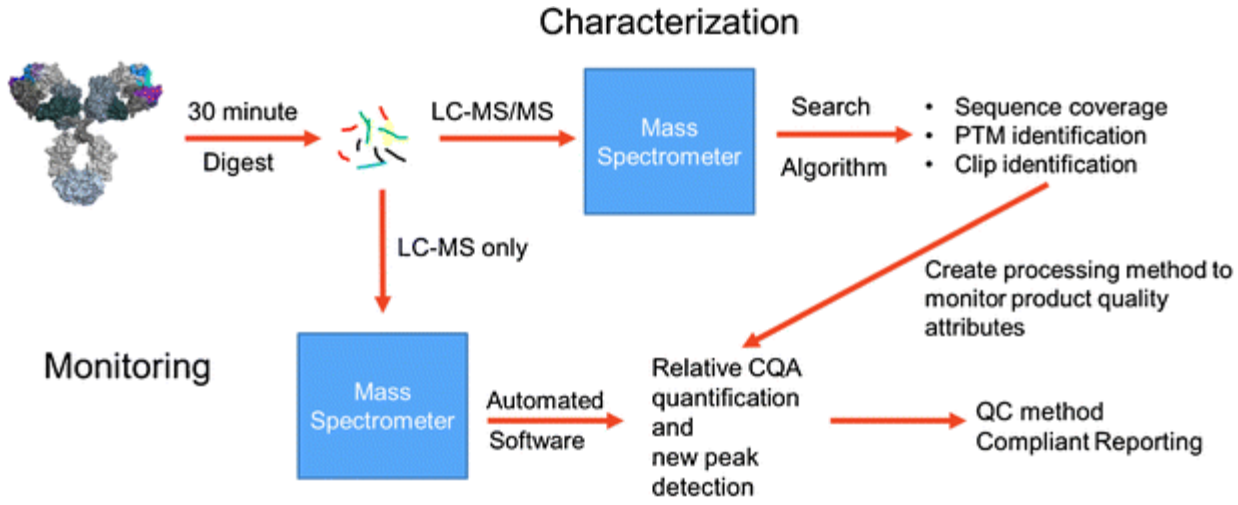

Fig 1. The Mass Spectrometry-Based Multiple Attribute Monitoring (MAM) workflow commences with a controlled digestion process to minimize artificial PTMs, followed by comprehensive biotherapeutic characterization using LC-MS/MS analysis. An automated search algorithm extracts essential data, including sequence coverage, PTM identifications, and clip sites, enabling the development of a processing method for monitoring Product Quality Attributes (PQAs) through relative quantification. The monitoring phase, relying on LC/MS data, involves comparing experimental samples to a well-characterized reference standard, quantifying PQAs, and identifying impurities and PTM changes. In the event of new species, an MS/MS capable mass spectrometer is employed for precise identification. Finally, the results are exported for either developmental insights or compliance reports to facilitate the release of biotherapeutics from Quality Control (QC) laboratories.

"Historical Approaches to Assessing Product and Process Understanding

"Historically, the assessment of product-related purity and control in the biotechnology industry has been closely tied to the level of process understanding and the analytical technologies available at the time. For instance, early monoclonal antibody (mAb) products like OKT3, commercialized in 1985, relied on quality control methods such as SDS-PAGE (reduced and non-reduced), gel permeation chromatography, and ion-exchange chromatography. As technology and process knowledge advanced, quality control practices evolved, as seen in the case of A-mAb, where proposed QC testing for product-related impurities shifted to primarily include HPSEC.

Recent years have witnessed significant advancements in analytical technologies. Traditional slab-gel SDS-PAGE methods for assessing biologic purity and impurities have largely been replaced by capillary or chip-based electrophoretic separation techniques. Gel isoelectric focusing methods have given way to more user-friendly imaging capillary isoelectric focusing (iCIEF) methods in QC laboratories. These technological strides have also facilitated a deeper understanding of overall product quality. Conventional analytical techniques, such as various chromatography or electrophoresis methods, when applied to heterogeneous biologics, typically report Critical Quality Attributes (CQAs) as acidic and basic charge isoforms or total fragmented impurities, respectively. While these CQAs serve as valuable indicators of biologic stability, any changes in charge or size distributions and peak

profiles suggest alterations in the product. However, these methods often fall short in providing a clear understanding of the underlying causes of such changes.

To address these limitations, the biotechnology industry has historically employed conventional assays like capillary electrophoresis (CE), ion exchange chromatography, reversed phase high-performance liquid chromatography (RP-HPLC), and normal phase (NP) or hydrophilic interaction chromatography (HILIC) to monitor modifications such as clipping, N-terminal signal peptides, C-terminal lysine, deamidation, isomerization, oxidation, and glycosylation. While these methods remain crucial for early development and characterization, they offer limited information about specific product-related species and impurities. For instance, reducing CE can detect polypeptide clips but does not pinpoint the location within the protein sequence. Ion exchange chromatography reveals charge distribution but lacks site specificity for modifications. The HILIC glycan assay provides an overall glycan distribution but does not offer details about occupancy at specific glycosylation sites. Historically, in the absence of Mass Spectrometry-Based Multiple Attribute Monitoring (MAM), these advanced methods, prior to characterization with orthogonal assays, have often served as surrogate measures, typically assessing global rather than site-specific chemical modifications at the amino acid level.

MAM Implementation

A significant breakthrough in analytical technology has revolutionized the assessment and control testing of complex biopharmaceutical products—mass spectrometry. The integration of peptide mapping with mass spectrometric analysis has emerged as a powerful approach for identifying numerous Product Quality Attributes (PQAs), including critical attributes (CQAs) in monoclonal antibody (mAb) therapeutics. Peptide mapping is widely recognized as a cornerstone identity test in Quality Control (QC) laboratories. Recent advancements, including the adoption of reversed-phase Ultra/High-Performance Liquid Chromatography (U/HPLC) and improved detection sensitivity, have significantly enhanced its precision. However, the key to its exceptional specificity lies in accurate mass determinations of intact and fragmented proteolytic peptides. This advancement allows for comprehensive product characterization earlier in the development cycle, aligning with bioprocess and formulation advancements.

The strategic amalgamation of chromatography with mass spectrometry, exemplified by Mass Spectrometry-Based Multiple Attribute Monitoring (MAM) and advanced data processing software, empowers the biopharmaceutical industry. MAM, transcending traditional purity tests, acts as an "identity" test by identifying unique product-specific sequences like Complementarity Determining Region (CDR) sequences in mAbs. Unlike conventional purity tests like ion exchange chromatography and reducing Capillary Electrophoresis (CE), which rely on automated integration and visual inspection, MAM utilizes software to scrutinize samples. This eliminates the challenges posed by co-eluting impurities and significantly boosts sensitivity. MAM also offers direct impurity characterization when coupled with MS/MS data, a capability missing in conventional methods.

MAM's scope extends to monitoring post-translational modifications (PTMs), sequence variants, and host cell proteins (HCPs), making it a versatile tool. It enhances early decision-making regarding cell lines with sequence variants and aids in optimizing purification processes. Additionally, MAM is invaluable for monitoring specific

events like oxidation, deamidation, isomerization, and succinimide formation, which can impact product potency and expiry dates.

"Addressing Challenges and Facilitating Wider Adoption of MAM"

The broader implementation of Mass Spectrometry-Based Multiple Attribute Monitoring (MAM) for cGMP testing of biotherapeutics and advanced control strategies faces several challenges that span technical, regulatory/compliance, its potential role as a replacement rather than an addition to release tests, and the diverse global regulatory landscape.

Technical challenges encompass concerns related to critical reagents for sample preparation, hardware consistency between mass spectrometers, method transferability, and software robustness for automated data analysis. Regulatory and compliance concerns involve the management of large data volumes, method validation, the possibility of increased out-of-specification results and investigations, parallel testing alongside conventional assays, and the need for comprehensive technical understanding within quality control labs.

Additionally, there are varying perspectives on MAM's capability to fully replace conventional assays in cGMP settings. Demonstrating strong correlation between MAM results and conventional purity/impurity assays has been recommended to support the replacement of traditional release assays with MAM. However, this correlation may depend on the specific protein biopharmaceutical, introducing complexity.

Moreover, the diverse regulatory environments globally present additional challenges, as different regulatory agencies may hold varying opinions on adopting MAM as a release test, especially in lieu of conventional tests. Efforts to gain industry and regulatory acceptance for MAM involve ongoing research and development to address technical and compliance-related challenges. Solutions include optimizing critical reagents, enhancing liquid handling robotics, improving instrument hardware, and advancing software for MAM data analysis.

In conclusion, while challenges exist in implementing MAM more broadly, ongoing efforts, collaborative initiatives, and increased understanding within the industry and regulatory agencies are paving the way for leveraging the benefits of MAM as an advanced technology across the entire product and process development life cycle.

"Exploring the Advantages, Current Limitations, and Future Application of MAM"

While conventional assays remain essential for assessing certain product characteristics, the adoption of Mass Spectrometry-Based Multiple Attribute Monitoring (MAM) represents a significant opportunity to align critical quality attributes (CQAs) with overall product structure-function relationships, control, and evaluation, thereby enhancing our understanding of how CQAs relate to product performance. Advances in mass spectrometry technology, coupled with sophisticated data processing and interpretation tools, have revolutionized the analysis of biologics. The introduction of MAM technology aims to harness this progress by enabling a comprehensive analysis of all molecular attributes in an automated manner while ensuring compliance with regulatory standards, including those defined by ICH and regional authorities.

Looking ahead, the goal is to gradually replace conventional assays in Quality Control (QC) settings, encompassing release specifications and stability testing protocols, with MAM that adheres to appropriate acceptance criteria. Furthermore, in future clinical trial applications, MAM's capabilities as a characterization tool, release assay, and integral part of stability assessments can be leveraged, provided it is substantiated with data and scientific rationale. To maximize the potential of this emerging technology, MAM should be included in submissions to regulatory authorities as a characterization assay, a release method, and a stability-indicating test for both new market applications and established commercial products. Successful integration of MAM into these environments will establish it as a pioneering advancement in testing methodologies.

Presently, MAM has some limitations, such as its inability to assess disulfide bond linkages, disulfide isoform ratios, trisulfide levels, cysteine adducts, and succinimide under certain conditions. Additionally, it cannot measure non-proteinaceous molecules like DNA or address aspects related to self-association, aggregation, higher-order structure, and potential conformers. However, ongoing developments in structure-based mass spectrometry techniques may provide solutions for some of these challenges.

Looking to the future, potential advancements in MAM could involve:

a) Expanding the range of quantifiable and controllable Product Quality Attributes (PQAs).
b) Standardizing data analysis across various instrument and software platforms.
c) Enabling real-time on-the-floor testing, both in-process and for release, and enhancing product attribute control.
d) Reducing instrument size, enhancing automation, and accelerating analysis times.
e) Streamlining regulatory submissions and life cycle management processes.
f) Facilitating the submission of raw MAM data to regulatory agencies for evaluation.

Conclusion

Mass Spectrometry-Based Multiple Attribute Monitoring (MAM) represents a cutting-edge product testing approach that is highly sensitive and offers exceptional resolution while minimizing any undesirable artifacts. This technique holds the promise of reshaping the future of biopharmaceutical products by enabling precise measurements and facilitating control over multiple Critical Quality Attributes (CQAs) and Product Quality Attributes (PQAs). MAM aligns seamlessly with the principles of Quality by Design (QbD), offering a comprehensive understanding of both product and process intricacies, all while streamlining the product testing process by potentially consolidating or replacing various profile-based release and stability assays.

In light of the growing recognition of attribute criticality within the biopharmaceutical industry and scientific literature, MAM is well-positioned to significantly enhance our comprehension of processes and products. Its enhanced detection capabilities empower the development of processes that consistently deliver high-quality products, thereby ensuring patient safety and the efficacy of medications. By improving product characterization, MAM contributes to a deeper understanding of processes, enabling control over specific post-translational modifications, size variants, and impurities. This, in conjunction with real-time, user-friendly, on-the-floor

monitoring of PQAs, gives rise to advanced control mechanisms that can be seamlessly integrated into a comprehensive control strategy for biologics.

The potential substitution of conventional methods with MAM, combined with the incorporation of process control mechanisms, holds the potential to facilitate Product Attribute Control (PAC) for biologics. Additionally, MAM aids in root cause analysis for variations in raw materials or deviations in manufacturing processes, fostering improved product knowledge and a heightened understanding of the production process, which aligns perfectly with the tenets of Quality by Design (QbD) in the realm of product development and manufacturing.

As the biotechnology industry continues to experience unprecedented growth and innovation, it becomes increasingly important to embrace advanced, modality-independent analytical technologies and seek regulatory approval for their use. Emerging therapeutic modalities, including novel protein-based treatments such as recombinant human proteins, monoclonal antibodies (mAbs), BiTEs, CARTs, antibody-drug conjugates, and fusion proteins, demand more sophisticated techniques for product and process characterization, CQA assessment, structure-function analyses, and stability testing. MAM stands as a solution capable of establishing a global release strategy that elevates the safety and efficacy of these therapeutics. Its implementation not only advances control strategies but also strengthens the connection between CQAs and the clinical performance of therapeutic products, ultimately enhancing patient safety and treatment efficacy.

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