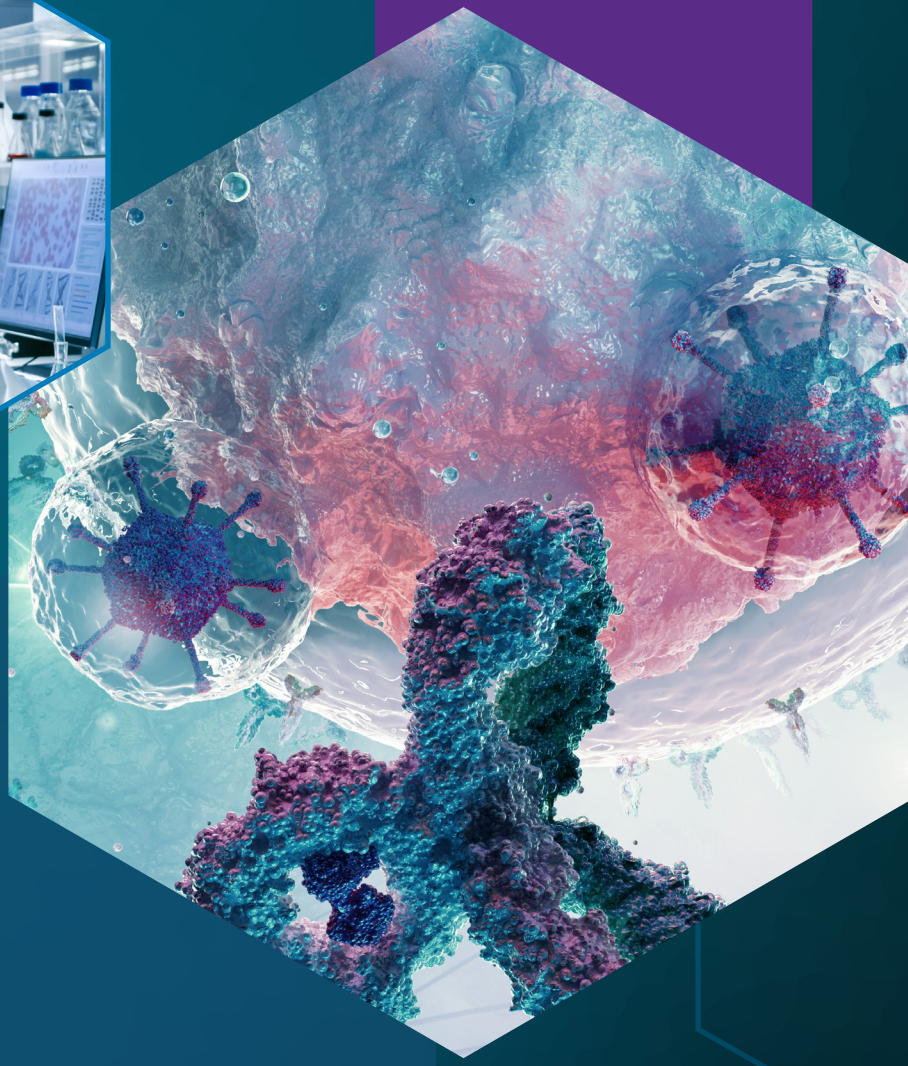
[eBOOK]

Multi-Attribute Methods for Biopharmaceutical Analysis

Perspectives on the adoption of LC-MS based attribute analysis for biopharmaceuticals



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Introduction

The adoption of LC-MS-based multi-attribute method (MAM) analysis for routine monitoring of biotherapeutic variation has progressed greatly over the last five years. The ability to directly assess the molecular attributes that contribute to efficacy, safety, stability, and process robustness is enabling analysts and their organizations to obtain more data without the ambiguity of traditional chromatographic and electrophoretic assays that measure product variation indirectly. In many cases, this data is generated with greater sensitivity and dynamic range than these legacy assays, furthering the discussion whether they complement or eventually replace these traditional analyses in development, manufacturing, and quality organizations.

This e-Book presents three perspectives on the capabilities and adoption of MAM workflows within the biopharmaceutical industry.

- The first article details the movement of LC-MS in biopharmaceutical development from a product characterization focus to one that combines attribute characterization and MAM based attribute monitoring. This is the area of greatest impact of MAM methodologies today, as key innovators have demonstrated the value of the approach, and the industry, in general, has begun embracing the technique with proof-of-principal studies and in some cases practical deployment of MAM assays spanning from clone screening to stability and formulations studies.
- The second article relates to the deployment of MAM assays within process science, manufacturing, and quality organizations. The flexibility of the MAM assay to respond to new product and process knowledge, and the efficiencies of multi-attribute analysis over many traditional assays are fueling the drive to deploy robust validatable assays that can be maintained over commercial lifecycle of a molecule. Several companies are using MAM assays for process monitoring, and a select few have progressed to their use for clinical lot analysis and release. The need for workflows and instrumentation that can be supported by organizations without extensive LC-MS experience has been both a source for technical innovation and a barrier to adoption in these regulated environments
- The last article collates the trends and feedback that Waters has encountered as the industry moves MAM analysis forward. The main focus is avoiding the challenges and pitfalls experienced by others when developing and deploying MAM analyses. We also provide insights into how partnering with Waters could help avoid these so-called “Traps” commonly reported for MAM analysis.

Along with highlighting additional MAM resources and examples from the peer-reviewed literature, we hope these articles inform the reader on the current state of attribute-based LC-MS analysis and encourages further discussions with your colleagues and the biopharmaceutical team at Waters.

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Scott received his B.S. (Biochemistry) degree from the Philadelphia College of Pharmacy and Science and was awarded a Ph.D. in Chemistry (Biochemistry) from the University of Colorado at Boulder. Following a post-doctoral fellowship with Dr. Richard D. Smith (Pacific Northwest National Laboratories), combining high-resolution separations and mass spectrometry for the analysis of complex protein mixtures, he joined the Waters Corporation as a Senior Scientist and proteomics group leader in 2001. Scott was promoted to Principal Scientist and moved to the newly established Biopharmaceutical Markets team in 2007. He is currently a Senior Manager, with a technical and business focus on the production of novel and robust workflows for analysis of biotherapeutic molecules. Scott has published scientific papers and presented posters in the areas of bioseparations, mass spectrometry, and biomolecule characterization, and has been an invited presenter at national and international conferences on these topics.

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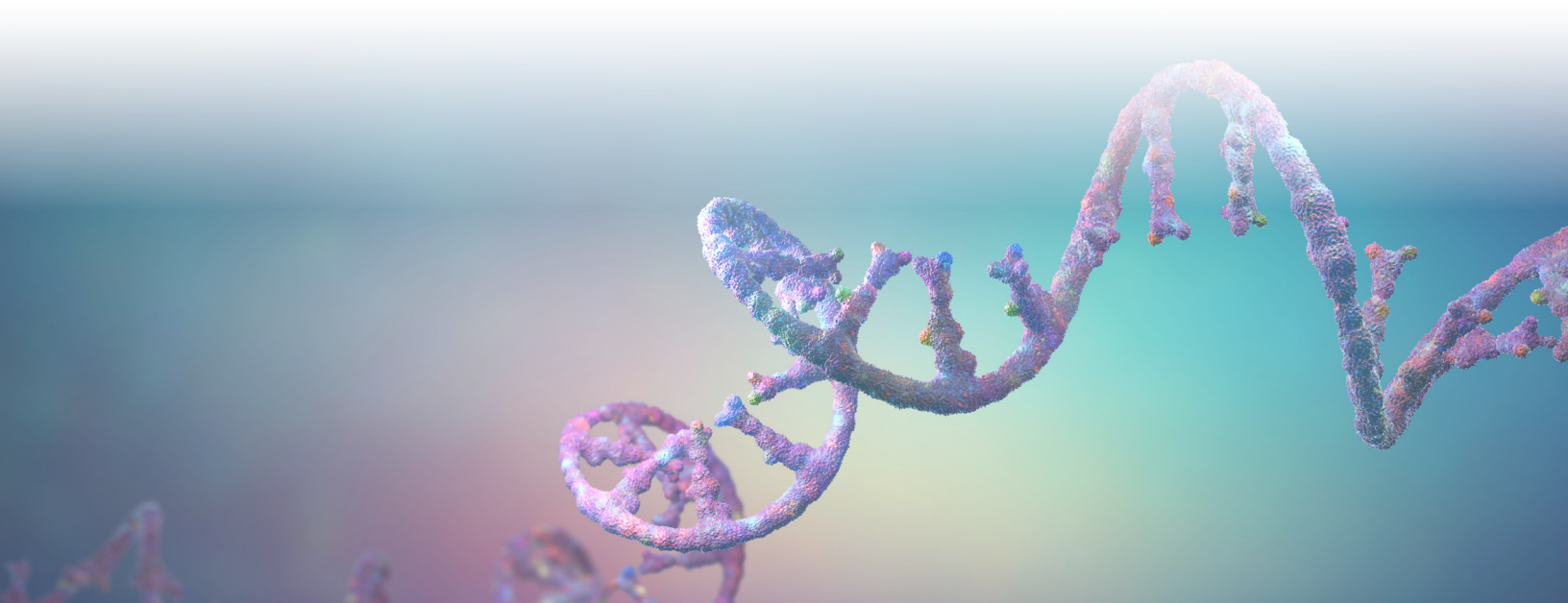

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Multi-Attribute Method Analysis: Moving Toward the Mainstream for Biotherapeutic Development

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The heterogeneity of biopharmaceuticals, both naturally occurring and the result of modifications such as oxidation, glycation, or deamidation, has created a need for robust monitoring tools that capture potential shifts in a drug product profile. Employing the Multi-Attribute Method (MAM) based on liquid chromatography-mass spectrometry (LC-MS) analysis of biotherapeutics represents a critical advancement in evaluating the increasing complexity of biotherapeutic molecules, as well as improving the product and process understanding underlying that complexity.

Often undertaken through LC-MS peptide mapping analysis, MAM offers operators more detailed insights into the specific attributes of a biotherapeutic protein. This can help drug developers achieve greater process and product understanding, as these assays enable a direct measurement of the critical quality attributes of a drug product when compared to traditional assays such as optical-based chromatography or electrophoresis, which measure the aggregate of these properties indirectly. Other levels of MAM analysis, either at the intact protein or subunit level, can also afford varying degrees of detailed understanding of the molecular features linked to a drug product's various safety, stability, and efficacy measures.

MAM analysis affords users both increased specificity and a greater range of attributes analyzed by way of a single technique; in doing so, it allows operators to establish a more streamlined laboratory workflow, supporting more robust Quality by Design (QbD) approaches and faster decision cycles. By pursuing MAM analysis for attribute characterization and monitoring, companies can accelerate their development activities, acquire more robust data faster, and further optimize their biotherapeutics for specific attributes linked to efficacy, stability, pharmacokinetics, and safety.

MAM: Exploring Levels of MAM Analysis

The detailed characterization of biotherapeutics enabled by LC-MS has provided biopharmaceutical companies the ability to produce "well-characterized" biomolecules more flexibly, providing crucial insights into the safety and efficacy of the advanced therapeutics they work to pioneer. The three primary structural levels of analysis undertaken with LC-MS analysis occur at the digested peptide, subunit (for mAbs), and intact protein levels. Analysis at the peptide level involves enzymatic digestion of a protein, followed by separation of the resulting peptides, which are then assessed using mass spectrometry. At the subunit level, this analysis occurs following antibody fragmentation via enzymatic digestion and/or reduction of Immunoglobulin G (IgG) antibody molecules. Finally, at the intact protein level, analytical tools assess a protein's total molecular weight without prior digestion or fragmentation.

While peptide-level analysis of the digested biotherapeutic protein has gotten the largest share of attention from industry for its ability to target multiple attributes independently, the comparative ease of sample preparation and ability to execute higher throughput analyses has made intact protein and mAb subunit level analysis attractive for many MAM applications not requiring this level of detail. These analyses are also more holistic, potentially revealing unexpected product or process changes that could be overlooked in more focused peptide-level MAM analyses. For many organizations, it can be challenging to generate consistently digested samples for the purpose of quantitative peptide mapping, and the sheer number of measurements inherent to peptide-level analysis can serve to elevate the potential for error and need for manual interventions.

Applying MAM Throughout Product Development

Despite the notion that MAM is largely useful for applications in mid to late-stage development, where a product has already been characterized, it is also finding utility in early development for clone screening, Where MAM can be used to identify clones for biosimilars that produce an attribute profile most similar to the targeted innovator molecule, so that less process development is required to achieve biosimilarity. It can also be used as part of a Quality by Design (QbD) effort to apply more analytics into purposeful design of an innovator biotherapeutic with optimized attributes for function, stability, and safety.

While the use of MAM approaches for attribute screening early in development is an area of emerging interest, the more typical application is within mid-to-late-stage product and process development. As the knowledge of product attributes is assembled from product characterization studies, accelerated stability, and early process development, MAM assays can be readily assembled to monitor attributes of potential interest/concern and identify new variants/contaminants. The ability to readily modified these multiplexed assays for new targeted attributes can accelerate these processes, and provide better information to drive development decisions. When used in areas where new attributes are expected, such as stability and formulation studies, the requirements of MAM as a purity assay (new peak detection) become more pronounced. In these areas, throughput expectations may also be a key factor in MAM experimental design and favor use of automation in sample preparation.

How Waters is Simplifying MAM Workflows and Improving MAM Data Quality

Despite the advantages conferred by MAM analysis, many biotherapeutic companies are still reluctant to move forward in integrating MAM into their existing laboratory workflows. This is largely due to the perceived costs and technical complexity of deploying LC-MS based analysis as routine assays – many companies, particularly smaller ones, may assume that to do so requires the addition of highly technical personnel, as well as an overhaul of the existing processes for their organization. However, advancements in the supporting technologies that enable this analysis, coupled with a growing level of expertise among vendors, have paved the way for attribute monitoring that can be adopted by existing personnel and organizational structures.

When evaluating the complexity of an MAM workflow, there are several elements that operators must address: the preparation of samples, the acquisition of data, and the subsequent interpretation of results. An increased interest in sample prep automation has arisen, not just from the need for higher throughput analysis, but more often because of the consistency provided by removing manual sample prep from the workflow. This allows labs to gain consistent results for sample digestion and processing between analysts and transfer methods between labs more efficiently. Waters has developed capabilities for automating protein digestion and sample processing on an automated pipetting robotic system called the Andrew+ that enables simple transfers of a user's existing methods or prepared methods from Waters to an automated format.

Great strides have also been made in increasing the usability LC-MS, such that non-LC-MS experts can acquire high-quality, reproducible data. Technologies such as the ACQUITY Premier System, a biocompatible UPLC system, and associated Premier chemistries that features MaxPeak High Performance Surfaces (HPS), are capable of improved separations, reducing the need for system conditioning, and resulting in unbiased detection of product variants studied by MAM analysis.

The first generation of MAM data was often produced using the more complex "research" LC-MS systems typically utilized for biotherapeutic characterization. As a result, the high user burden associated with these characterization technologies was also ascribed to MAM analysis. Mass detection has experienced huge gains in the automation of both system functionality and instrument setup; technologies such as the BioAccord LC-MS System have been engineered to include one-button automation for its setup and SmartMS system monitoring tools that ensure that the user is aware when the system is not capable of generating quality data, with specific guidance on any remedial actions required.

With advances in increased automation and real-time diagnostics, technologies such as ACQUITY and BioAccord combine robust analytics with intuitive, simplified user informatics interfaces, creating a platform that can be operated effectively by analysts without previous MS expertise. These systems still require users with knowledge of both the molecule and an understanding of the quality characteristics of the data being generated, but with appropriate applications training and support from vendors such as Waters, companies are readily integrating these platforms into their existing laboratory workflows.

Despite this simplification and automation of sample generation and data collection,, effective and efficient data processing and interpretation can ultimately determine the ultimate utility of MAM analysis by non-MS experts. Additionally, organizations must ensure that they have well-defined system suitability samples and criteria in place that cover every aspect of an MAM assay, so that any deviations in a set of results can be readily identified, diagnosed, and rectified.

Integrating MAM Analysis for More Comprehensive, Streamlined Monitoring

MAM possesses great potential in advancing the state-of-the-art for biopharmaceutical analysis. In automating as much of the process as possible and streamlining data collection with the key analytical technologies, a few key vendors in the space are positioning MAM technologies for the future. As MAM assays transition from informational insights to decision-making tools, and as regulators take more notice of these technologies, the importance of transitioning these technologies from evaluating their potential for existing and nascent biopharmaceutical applications to deploying them as robust assays will be crucial in moving toward what is likely to be the next generation of biopharmaceutical analytics.

Informatics platforms such as waters_connect offers users scalable, comprehensive application-driven workflows that allow for greater reproducibility and laboratory efficiency by integrating data collection, processing, review, and reporting, accelerating sample processing and reducing manual errors. With tailored workflows designed for biopharma LC-MS applications, intact mass (proteins, subunits, ADCs,), peptide mapping, peptide multi-attribute monitoring, and released N-glycan analysis, waters_connect, in synergy with technologies like the BioAccord System or Xevo G3 QTof, can afford operators a complete laboratory workflow that can be integrated alongside their existing analytics. Peptide MAM is based on the same LC/MS data acquired for peptide mapping studies but processed with a fundamentally different logic. The peptide mapping data processing workflows in waters_connect and other packages looks at each new data set as a de novo analysis, in practice discovering each peptide anew, and justifying those assignments every time the sample is analyzed. The need for a dedicated Peptide MAM application in waters_connect was driven by the need for a streamlined workflow for targeted interrogation of the data for specified attributes, and the need to directly and efficiently answer specific questions of attribute quantitation, product identity and product purity. This demarcation between the mapping and monitoring data workflows facilitates routine use of MAM by less experienced analysts, while maintaining the capability for a more flexible and detailed study of the same data by traditional peptide map processing when questions arise about specific results or overall data quality. By adding this layer of data processing automation to the laboratory workflow, companies can create a data ecosystem wherein their data collection, processing, review, and reporting occur across a common platform for both attribute characterization and monitoring studies.

For those considering integrating MAM into their existing product and process characterization workflows, understanding the ultimate goal of the assay or assays is key to avoiding undue analytical burden and unnecessary complexity. In particular, the potential for incorporating MAM as a purity assay has seen a great deal of interest, taking the MAM assay beyond the semi-quantitative monitoring of targeted molecular attributes, by incorporating new peak detection in an effort to pinpoint the emergence of potential new attributes or impurities in a drug product.

To integrate new peak detection functionality represents a new level of complexity in MAM analysis, as new peak detection is the aspect of MAM analysis arguably most prone to potential false positives and negatives, and most demanding in the need for manual review and interventions. This has been validated in a cross-lab study conducted under the auspices of the MAM Consortium, where labs were challenged to identify spiked peptides in a common analyzed mAb sample [1, 2]. Still, many see promise in utilizing MAM for purity-based assays, and Waters has invested significant research producing the Peptide MAM application to address this challenge. The application has used a robust non-linear alignment and co-detection approach to ensuring proper sample-to-sample- comparisons, even when signal intensity for specific components are too weak for independent peak detection. The use of isotopic match and quality filtering criteria further facilitate the elimination of false positives, and requisite time spent validating the actual changes between samples.

A Positive Outlook for MAM Adoption

Ultimately, the potential for MAM analysis to complement and eventually supplant more traditional single attribute assays with more informative, robust, and sensitive analyses represents an important opportunity for the biopharmaceutical industry. While widespread adoption of these technologies in product development is not a reality for many today, early adopters of MAM are reporting greater insight and familiarity into their molecules and processes, poising them to readily adapt alongside regulatory expectations and evolving industry standards. The partnership between the pharmaceutical industry, the vendors supporting them, and the regulators overseeing these activities is advancing the deployment of the robust technologies and best practices that are continuing to drive adoption of these powerful approaches.

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Application Notebook | *Multi-Attribute Methods for Biopharmaceutical Analysis*

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Multi Attribute Method (MAM) Solutions for Process and Product Control of Biotherapeutic Proteins

Nick Pittman, Magnus Wetterhall, and Scott Berger,
Waters Corporation

One of the biggest challenges to manufacturing a complex biotherapeutic at commercial scale is in achieving the same tightly controlled product profile established in the clinical phase. Continuing to tighten the specifications of a biopharmaceutical to achieve better product quality and improved patient outcomes is core to enabling continuous improvement and compounding efficiencies in manufacturing. Doing so can require additional insights into a process that traditional assays, though well codified during development, cannot offer with the efficiency of more modern approaches.

Multi-Attribute Method (MAM) analysis using liquid chromatography – mass spectrometry (LC-MS) has emerged as a valuable tool for drug discovery, development, process monitoring and quality control. By affording operators greater insights into the critical quality attributes (CQAs) of a biotherapeutic protein, MAM LC-MS can help improve a drug's safety profile and optimize its commercial viability over time. By measuring the product variation directly as specific levels of targeted product attributes, process science teams can achieve an unprecedented amount of process knowledge and control.

Despite the advantages conferred by MAM LC-MS analysis for process monitoring and quality control (QC), many bioprocess organizations are reluctant to incorporate these technologies into existing laboratory workflows. This is largely due to the perception that both running the LC-MS, as well as performing MAM analysis, require a depth of expertise that necessitates additional, highly trained personnel and a burdensome amount of work to integrate within existing monitoring paradigms. Additionally, the perceived costs of deploying these assays, as well as concerns surrounding regulatory compliance, robustness, and reproducibility have likewise served to hinder their adoption. Recent advances in the technology and automation supporting MAM LC-MS have mitigated or even eliminated the issues surrounding the complexity of these systems, facilitating robust, reliable, and automated data acquisition and processing even for novice MS users.

Adopting MAM LC-MS for Greater Process and Product Insights

MAM analysis affords users both increased specificity and a greater range of targetable attributes analyzed with greater efficiency than single attribute analytical techniques. This enhanced specificity can offer detailed insights into a molecule, otherwise impossible to achieve by way of traditional process monitoring or quality control techniques. For example, an operator may examine the intact mass of a drug product and determine that it experiences between five and 10 percent oxidation consistently. In contrast, MAM can allow operators to pinpoint specific sites or regions of a molecule capable of generating amino acid oxidation, affording them crucial understanding of the specific attributes contributing to a product's function, stability, or safety profile. Furthermore, the MAM approach at the peptide digest level enables the structural elucidation and confirmation of small mass differences that are not readily detectable on the subunit or intact protein level, such as specific amino acid substitutions, disulfide heterogeneity and deamidation.

The potential for first supplementing then supplanting traditional process monitoring and QC assays to achieve greater understanding with fewer required tests is a key financial and operational factor in favor of adopting MAM analysis. While the increased specificity afforded by MAM analysis is core to this value proposition, the elimination of extraneous tests represents a distinct advantage for operators, as every additional test conducted increases the risk of an "out-of-spec" result.

The concerns of adopting new technology into manufacturing and quality organizations – the potential for unexpected attribute monitoring results, or a false positive new peak detection when used as a purity assay – is another concern surrounding MAM adoption for use as a single integrated attribute monitoring and purity assay. But many in the industry see promise in utilizing MAM for purity assays, and targeted development efforts have been made to make its use more robust and reproducible.

Waters Corporation has invested significantly in its automated software to address this, facilitating sensitive new peak detection while excluding false positive and false negative results that can lead to extended investigations and delays in getting the product to market. This new peak detection capability not only enhances the possibility of uncovering new modifications on the drug molecule; it also offers greater certainty in the process monitoring and QC settings that the drug product profile has remained consistent during the manufacturing and storage process. Newly detected peaks would indicate a change in the drug product that can be traced back to either a change in the manufacturing process or storage of the drug product. The ability to monitor this both enhances the manufacturing and stability knowledge available to an organization, but ultimately increases patient safety and outcomes. This is especially important when the patient is dependent on lifelong medication for a chronic disease, and the need for product consistency and avoidance of immunogenicity is of higher concern.

Just as valuable as detecting unwanted modifications to a product is the ability to more accurately maintain the profile of desired modifications. MS is a particularly useful tool for this type of monitoring; manufacturers looking to prove a biosimilar is the same as the molecule it has been modeled after, or that a process change is not affecting product quality, can more accurately do so with MAM approaches. Likewise, new innovator drugs can be better protected by this additional specificity, as the additional product and process knowledge enabled by MAM LC-MS can serve as the basis of additional intellectual property and as a quality challenge to potential biosimilar competition.

Reproducible Separations Produce Better MAM Results

The quality of MAM analysis is inherently related to the quality reproducibility of the underlying separations of the analytes of interest, as data is typically evaluated by showing consistent values, trends, and in the case of purity analysis – direct run-run comparisons. Consistent analyte retention, recovery, resolution, and peak shape all contribute to a high-quality MAM outcome.

With separations solutions combining the ACQUITY Premier UPLC System and Premier Chemistries based on organosilica hybrid particle technologies, analysts can achieve increased loading capacity for ultimate sensitivity, improved separation and detection of acidic and basic peptides, better run-run and system-system reproducibility in evaluating product modifications, more accurate glycopeptide profiles, and greater system throughput due to the need for less system and column conditioning. The ACQUITY Premier System features the new, innovative solution of MaxPeak High Performance Surfaces (HPS) Technology, which effectively reduces non-specific adsorption due to metal interaction — without complicated mobile phases or laborious methods for system stabilization. The underlying particle technology in BEH and CSH UPLC columns ensures that analytes with positive charge (such as the typical tryptic peptide) exhibit consistent retention under long term use and superior peak shape from minimized silanol and particle interactions. These elements synergize, so that analysts can:

- Support meeting compliance standards with consistent higher-quality data
- Achieve faster, more reproducible, more accurate data integration with less need for manual intervention
  Shorten time from sample to result by eliminating the need for system conditioning and sample reanalysis

Implementing Integrated, Interconnected Systems for Improved CMC and QC Responsiveness

Traditionally, the bulk of LC-MS expertise within the industry resides in drug development organizations. For process development, manufacturing, and quality control, the use of MAM and LC-MS is far less common, creating a reluctance by many to be an early adopter of these systems for CMC or QC. A decade ago, the idea of integrating mass spec into process monitoring and QC/Release was radical; today, systems like the Waters ACQUITY QDa Mass Detector, and the BioAccord LC-MS System, have emerged to simplify the user experience to such a degree that bioprocess engineers are able to run assays independent of an MS expert and garner consistent, reproducible results. LC-MS and product expertise may however be required for method development, and for investigations of unexpected results. Automation of data processing, as enabled by the waters_connect informatics platform, with its integrated suite of workflow solutions for a range of MAM analyses at the protein, subunit, or digested peptide level, offers users a streamlined architecture that can serve to greatly reduce the potential for manual processing errors and reduce training challenges.

Moreover, because the drug products for these applications have already been characterized to a large degree and key attributes defined during the development phase, the relative complexity of transferring this knowledge to more targeted LC-MS based MAM assays is reduced. For process monitoring and quality control, MAM LC-MS can provide analysts three core analytical capabilities: the first is identity testing, which ensures that the substance in the vial or bottle matches the label claim and has not been cross-contaminated during manufacture. The second is targeted attribute monitoring, wherein operators select a specific set of product attributes to monitor quantitatively, to track a process, affirm a profile that supports a product's efficacy, and ultimately, ensure its safety and efficacy. The third aspect of MAM is elucidating the unknown – namely, detecting "new peaks" that flag unexpected variations in a product.

These three primary uses of MAM analysis, combined with the relative specificity and power of mass spectrometric detection, result in a wide array of analytical flexibility that can be implemented in phases depending on the level of monitoring required. The QDa mass detector under control of Empower CDS has been utilized by several pharmaceutical companies as a platform for peptide MAM based identity tests and targeted attribute quantification.[1,2] The BioAccord LC-MS System, that adds the additional selectivity of accurate mass, has been designed specifically for ease of use, with intelligent self-monitoring, user-guided system troubleshooting and push-button start-up and calibration. While still feasible for non-expert users, the Xevo G3 QTof system – a high-resolution, quadrupole time-of-flight (QTof) mass spectrometer – has greater capability for characterizing any new peaks that arise during MAM analysis with more extensive MS/MS functionality. The BioAccord LC-MS System, and Xevo G3 QTof under the waters_connect informatics platform, achieve a harmonized, intuitive laboratory workflow for connecting attribute characterization and attribute monitoring that transcends development, and can be readily deployed for manufacturing process monitoring, and quality control.

The waters_connect informatics platform supports multiple integrated application workflows to streamline biotherapeutic analyses, from intact and subunit mass analysis to released glycan profiling and peptide attribute characterization and monitoring. This software, combined with systems like the BioAccord LC-

MS System and Xevo G3 QTof, affords operators the flexibility to deploy attribute characterization and attribute monitoring in development and scale those investments as project move into the clinic and commercial operations. Additionally, the incorporation of prepared calibrants, system check standards, and default methods that are easily deployed by operators has further simplified system operation, enabling platform methods to be transferred more readily between these organizations. The availability of Waters Professional Services training on the applications as well as system operations ensures that both systems and their operators can be made fit for purpose.

Any discussion of MAM informatics deployed for process monitoring and quality cannot ignore the need for operation within a regulated laboratory environment. Data used for quality decisions is subject to direct regulatory scrutiny, including the provisions of 21 CFR 11 and Annex 11 requirements for data security and traceability. Data used to support CMC regulatory filings may not be as readily auditable by regulators but demands similar data integrity treatment to ensure the quality and security of the information relied upon for submission. While involved procedures can be adopted for the use of software that is not fully regulatory compliant, this is far from ideal in terms of workflow efficiency and adds to data risk.

The ability to do data acquisition, processing, review, and reporting within a single compliant-ready informatics platform provides the most efficient route to both result generation and maximized data integrity. Both Empower and waters_connect informatics platforms are foundationally designed with data integrity and regulatory compliance in mind, and common system configurations and methods can be utilized across development and later deployed to process monitoring or quality organizations on these same platforms. Examples of validated MAM methods developed on these platforms for QC application can be found in the literature. 1, 2, 3

Moving Forward with Waters for MAM in Process Science and Quality

The potential for MAM analysis to supplement or replace more traditional biotherapeutic monitoring assays with more comprehensive, robust, and sensitive analyses represents an important opportunity for the biopharmaceutical industry. With Empower and waters_connect based informatics platforms for multi attribute characterization, monitoring, and data integrity – enabling audit trails, user administration, automated data processing, electronic signatures, data backup and recovery, and LC-MS system validation – users can achieve compliance with 21CFR part 11 requirements while securing more detailed, streamlined data insights into a process or molecule. Additionally, the availability of these scalable, networked informatics solutions to support manufacturing and QC release, and its long-established expertise make Waters a premier partner for developing your MAM LC-MS capabilities across the development and manufacturing continuum.

In a manufacturing landscape typified by high-value, high-cost biotherapeutics, the importance of maximizing uptime and minimize disruption requires solutions that prioritize compliance, robustness, and functionality. In the landscape of biosimilars and in-class competitors, manufacturers also need to maximize their reputation for quality and consistency. By incorporating LC-MS based MAM analysis, organizations can greatly reduce the potential for human error, rationalize the number of assays needed for quality determinations, and achieve greater process and quality control. As the molecules moving on from development continue to increase in complexity, flexible assays that afford greater insight directly into the key features of a drug product will help manufacturers stay ahead of both regulation and competition.

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Multi-Attribute Method Analysis: Avoiding Potential Traps During Assay Development and Deployment

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Multi-Attribute Method (MAM) analysis using liquid chromatography – mass spectrometry (LC-MS) has emerged as an invaluable tool for drug discovery and development, now extending into process monitoring and quality control. In offering operators superior understanding of the product and critical quality attributes (PQAs/CQAs) of a biotherapeutic protein, MAM can help improve a drug's safety profile, maximize efficacy, and optimize its commercial viability over time. By measuring product variation directly, as specific levels of targeted product attributes, and enabling non-targeted searches for potential new impurities, organizations can achieve an unprecedented amount of actionable process knowledge.

The implementation of attribute-based analysis of biopharmaceuticals by LC-MS is now largely accepted by development organizations as a natural extension of the use of LC-MS for product characterization. Broader acceptance is hampered by concerns surrounding the cost of scaled implementation of LC-MS-based assays, as well as the challenge inherent to developing streamlined workflows and platform-based methods for implementing these capabilities without requiring significant organizational changes.

These concerns are balanced by increasing regulatory encouragement to adopt a more modernized and precise approach to biopharmaceutical analytics 1,2. Still, the challenge of justifying the capital and operational costs of LC-MS analysis can be difficult, and many organizations are hoping that the replacement of multiple conventional/traditional assays by MAM will provide not only better data to bolster their decisions, but to realize cost savings in the long run. This transition also requires organizations to carefully vet these technologies and techniques in order to determine the right partners, workflows, and personnel needed to ensure an MAM LC-MS platform is fit for their products, sites, and teams. Much of this work involves understanding the potential pitfalls that have been experienced by innovators and early adopters of MAM, as well as the rationale on how to avoid them.

Waters Corporation, a long-time partner and innovator for the biopharmaceutical industry, has focused on the development and commercialization of high-value analytical and informatics technologies specifically intended for biopharmaceutical analysis. With industry-leading scientific expertise and customer support, Waters has pioneered solutions to some of the most pressing challenges facing customers interested in adopting MAM into their organizations. In doing so, it has identified a number of common traps, those that challenge the performance and robustness of MAM workflows, and those that organizations may encounter as they transition MAM analysis out of feasibility testing and into product development, manufacturing, and quality organizations.

MAM Workflow Traps and How to Avoid Them

Trap 1: Irreproducible Sample Preparation

The Problem: The quality of a sample is perhaps the single most important variable in any testing paradigm. Sample quality for MAM analysis determines the quality of the data and results that decisions on progressing a product and process are based on; poor quality or irreproducible samples will fundamentally undermine an organization's ability to gain this actionable information from an MAM study, which inherently relies on the comparison and trending of multiple samples within a data set. The movement of MAM assays from product development to commercial operations extends these requirements to assays capable of long-term stability and consistent results across labs and locations.

The Waters Solution: Automation of sample preparation is useful for more than attaining higher throughput – robust automation platforms like the Andrew+ pipetting robot allow for sustained repeatability and full traceability by enabling consistent liquid handling and sample processing. This automation, coupled with system suitability standards that allow for close diagnostics of each step of a workflow, facilitate standardized and consistent analysis over time. Waters has commercialized its NIST mAb-based system check samples for antibody analysis at the digest, subunit, and intact levels, as well as for released glycan analysis.

Trap 2: Inconsistent Separations – System Conditioning and Analysis Stability

The Problem: Once operators have established robust sample preparation, the next challenge to address is developing a separation that offers reproducible chromatographic elution profile of peptides, subunits, or intact mass peaks for MAM data processing. In order to achieve this, operators must ensure that assays are run on a well-controlled system and with the same amount of material, so that the first injection of a series results in the same response as the last.

The Waters Solution: In order to achieve consistent separations, operators should start with a system that is bioinert in order to avoid bias within an injection or across a sample set. Waters' ACQUITY Premier System, a robust biocompatible system with a proprietary covalent coating technology, represents the next level of biocompatibility and inertness for MAM analysis. Having this unbiased analysis means that the various peptide levels, independent of their individual properties, will yield the same analyte profiles and analyte recovery for all analytes from the first injection to the last, eliminating the time and monetary costs of analyzing throwaway samples to achieve a stable system state, or the need to rerun experiments due to system performance drift.

Trap 3: Separations Chemistries that Vary from Batch to Batch and Over Time

The Problem: Chemistries are consumables, produced in batch processes, that need to perform for the lifetime of a development program and potentially the commercial lifetime of a product. As such, variations in separation analyte retention, recovery, resolution, or selectivity over a period of time can serve to derail automation of MAM analysis, requiring manual interventions in data processing or potentially more impactful the need for method re-optimization.

The Waters Solution: Waters has addressed this challenge in several ways. Decades of experience producing large molecule separations media, and foundational technologies for robust long-lifetime bioseparations columns ensure batch after batch of consistent product data. This is, in part, owing to a higher quality standard for batch release– rather than using small molecules as a quality check on each batch of material, Waters tests with standards relevant to a specific biotherapeutic assay. The availability of such system check standards enables customers to continue to demonstrate fitness of a column and system for analysis. For peptide mapping, for example, this means peptide maps run with acceptance criteria for each batch of material, to guarantee consistency over the decades-long span clients may utilize these separation chemistries. This robustness is provided by foundational technologies such as the Bridged Ethyl Hybrid (BEH) and Charged Surface Hybrid (CSH) Organosilica particles in these columns that resist physical damage under high pressures and over broad array of system conditions, and that minimize silanol functionality that distort peaks of analytes with positive charges such as a typical tryptic peptide. The more recent innovations included in the Max Peak Premier System and Column Solutions address column variability associated with metal-sensitive analytes (those containing negatively charged sites), improving chromatographic performance for these analytes, and reducing the need for time-consuming tasks such as system and column passivation. It is the combination of column robustness, consistency, and the quality of chromatographic performance that ultimately meets the challenge of deploying high-quality MAM assays over the lifecycle of a biotherapeutic molecule.

Trap 4: System Troubleshooting Woes – Skipping Optical Detection

The Problem: During MAM analysis, questionable results can generate complex investigations, as operators work to determine whether sample preparation, separations, detection, or data processing has contributed to the unexpected findings. Any ability to diagnose problems with separations independent mass detection hinges on the availability of optical detection data. Despite this, many technology vendors espouse workflows that neglect to incorporate inline optical detection in their LC-MS system configurations for MAM analysis.

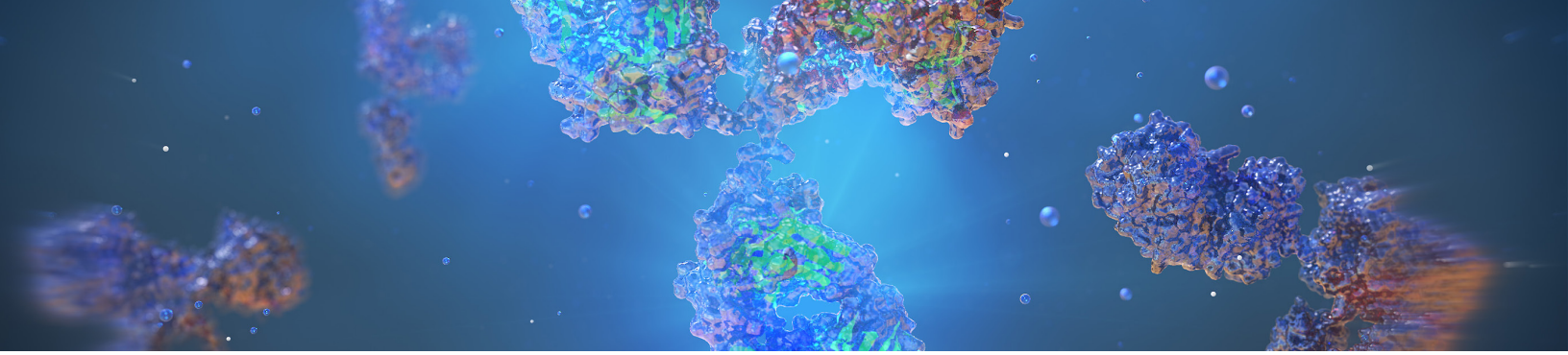
The Waters Solution: Having an optical detector within the flow path of an LC-MS system affords operators an insight into the workflow elements occurring prior to mass detection. Consistent optical chromatographic profiles can confirm sample quality, injection load, and separation quality, enabling MS data quality and response to be assessed independently. This distinction can help avoid an investigation that may prevent the use of an instrument for a significant amount of time or delay product moving to the next stage of manufacture or release. The use of a selectable wavelength detector exposes the LC effluent to a narrow band of UV energy, preventing potential photo-oxidation artefacts that can occur with more intense full spectrum exposure in a diode array UV/Vis detection. Employing appropriate system check standards and SOPs, pre-detection issues with sample prep or separation quality can be readily identified using data from the MAM experiment itself.

Trap 5: Mass Detector Robustness and Assay Variability

The Problem: Once an organization has tackled sample preparation and separations, it must turn its attention to the next potential pain point of an MAM workflow: mass detection. While MS detection setup is semi-automated in many modern systems, mass spectrometers used for research tend to afford users significant latitude to adjust parameters for analyte class and assay optimization. For more routine analysis, this flexibility can represent a double-edged sword – the expertise needed to operate many LC-MS technologies often limits their use to more highly trained personnel, and requires significant efforts from operators to optimize multiple systems, particularly when distributed across multiple sites of operation.

The Waters Solution: Targeted innovation in the areas of intelligent system diagnostics, coupled with "Smart MS" capabilities that allow for automated startup and calibration, can open the door for operators to perform MS more easily while reducing the number of manual operations. Systems like Waters' ACQUITY QDa Mass Detector and the BioAccord LC-MS System have emerged to simplify the user experience to such a degree that bioprocess engineers and QC analysts are able to run assays independent of an MS expert and garner consistent, reproducible results. The BioAccord has been designed specifically for ease of use, with push-button start-up operation and proactive intelligent, user-guided troubleshooting when issues could threaten data quality. The use of more flexible but complex systems such as the Xevo G3 QTof for MAM analysis may be appropriate when organizations desire to transition from MAM analysis to the characterization of "new peaks" detected by MAM with a single system.

The common use of the waters_connect informatics platform for both the BioAccord and Xevo systems enables both to be deployed on a common network, using a common set of applications, including the Intact Mass and Peptide MAM Applications used for Subunit and Peptide MAM.



Trap 6: Relying on Manual Data Analysis/Reporting

The Problem: As with any modern biopharmaceutical application, the reliance on manual data analysis has the potential to create inconsistencies between analysts and over time, distorting results drawn from the data. Manual data interventions create additional risk of regulatory scrutiny, and require oversight and documentation that significantly slows down time to results. Successfully automating data processing and reporting can serve to mitigate this potential for human error and bias, helping companies obtain more consistent results and scale MAM deployments across systems, labs, and organizations.

The Waters Solution: The waters_connect software system, with its integrated suite of workflow solutions for a range of biopharmaceutical LC-MS applications, offers users a highly automated, intuitive interface that can serve to greatly reduce the potential for manual error. The waters_connect platform is foundationally designed for data integrity, compliance-readiness, data security, and controlled data accessibility and sharing, and allows for the creation of a scalable ecosystem with the ability to automate workflow driven biopharmaceutical analysis for greater organizational efficiency. Possessing a full toolset for manual review and interrogation of the data for method development and troubleshooting can help organizations quickly develop methods that streamline assays for routine use by those with lesser expertise with LC-MS.

Deployment and Implementation Traps and How to Avoid Them

Trap 7: Investing in Hard-to-Train-and-Maintain Solutions

The Problem: Like any other analytical technology, MAM LC-MS represents a significant investment for organizations, one that requires an understanding of its utility and adaptability over the long term. Organizations must ask themselves a number of important questions – how long will it take to get these systems up and running? Who in the organization can operate these methods? Can the organization plan for widescale and long-ranging implementation?

The Waters Solution: The deployment of fit-for-purpose LC-MS technologies for attribute-based analysis enables organizations to realize the return on investment more rapidly, and do so without changing the composition of their organization to do so. Both MS detection technologies were developed to be operated by non-MS experts, possessing a core reliability and robustness to be deployed in challenging functions like product quality and manufacturing, and a level of automated functionality to be operated by those without previous LC-MS experience. Turnover continues to be a challenge for the biopharmaceutical space, and organizations realize the benefits of this operational simplicity by having the flexibility to redeploy existing staff, and more readily recruit new personnel Informatics platforms like waters_connect and Empower, that fully integrate and automate data acquisition, processing, review and reporting can help organizations cultivate a wider network of personnel capable of performing routine MAM assays and improve the results by minimizing the need for manual intervention. Additionally, the capability of implementing assays that can directly transition from development to process monitoring and QC demands the foundational robustness of all aspects of the workflow, and the support of a technology partner that can offer ongoing support to organizations.

Trap 8: Not Recognizing The Added Risks Of Deploying Purity Assays Using MAM

The Problem: While MAM LC-MS has made considerable headway in characterization, identification, and attribute-centric quality control and process monitoring, its feasibility for deployment as purity assays may be limited by the occurrence of false positives and negatives. Whether it is false positives/negatives as a result of incomplete characterization of the reference material, or limitations of the algorithms employed to detect new peaks in the experimental samples, these errors will force operators to investigate individual new peaks manually, torpedoing the efficiency of assay execution and creating the potential for increased regulatory attention.

In many of the industry discussions on MAM held at meetings sponsored by the CASSS organization, ASMS, and USP, this New Peak Detection functionality has been identified as the most challenging aspect of MAM method development and validation. A round-robin study produced by the MAM Consortium [3,4] revealed the practical challenge of robust new peak detection in MAM analysis.

The Waters Solution: Waters has recognized the algorithmic challenges of new peak detection in MAM and implemented several automated processing steps to avoid the false positive/negative challenges seen with software from other vendors. This includes filters that avoid chemical matrix noise peaks to report only peaks with appropriate mass errors and peptide-like isotopic profiles, and the use of advanced non-linear alignment algorithms employing the concept of "codetection" that ensure datasets are properly compared, even with run-run elution profile variation in peptide maps. These quality tools combine to yield true new peaks, while minimizing false detections requiring user investigations.

When these peaks are reported, our typical peptide MAM acquisition methods on QTof and BioAccord Systems enable the collection of data independent fragmentation data, so that ion fragmentation can be collected on all eluting species. This allows for troubleshooting, giving operators insight into new peaks quickly, avoiding the need for sample reanalysis and enabling faster validation of potential new attributes and impurities.

Trap 9: Delaying Assay Validation and System Qualification Strategies

The Problem: Any discussion of MAM informatics deployed for process monitoring and quality cannot ignore the need for operation within a regulated laboratory environment. Data used for quality decisions is subject to direct regulatory scrutiny, including the provisions of 21 CFR 11 and Annex 11 requirements for data security and traceability. Data used to support CMC regulatory filings may not be as readily auditable by regulators, but demands similar data integrity treatment to ensure the quality and security of the information relied upon for submission. While involved procedures can be adopted for the use of software that is not fully regulatory compliant, this is far from ideal in terms of workflow efficiency and adds to risk register of deploying these capabilities.

The Waters Solution: The ability to perform data acquisition, processing, review, and reporting within a single compliant-ready informatics platform provides the most efficient route to both result generation and maximized data integrity. Both Empower and waters_connect platforms are foundationally designed with data integrity and regulatory compliance in mind, and common system configurations and methods can be utilized across development and later deployed to process monitoring or quality organizations on these platforms. The worldwide capability of the Waters Professional Services organization to fulfill the documented training, installation, service, and validation-planning needs of our customers allows organizations to realize all the benefits of these platforms more quickly after purchase, manage staff turnover, and maintain those capabilities as they scale or replicate.

Trap 10: Investing in an Informatics Platform that is Not Scalable or Economical

The Problem: Selecting a technology platform that is robust enough to support analytics from sample to report is important. Equally important is ensuring a platform is transferrable and scalable, so that it can be replicated as sample throughput needs increase or when attribute-based analysis capabilities are transferred to additional sites. Failing to do so can force organizations into a piecemeal strategy or, more likely, into investing in replacement technologies that can adequately scale.

The Waters Solution: Waters' scalable, networked-based deployment of the waters_connect platform allows organizations to easily add additional instruments to a common network, and the commonality of configuring mixed networks for Xevo QTof-based attribute characterization and BioAccord-based attribute monitoring facilitates simple and controlled transfer of methods and information across organizations. The added challenges presented by transferring methods from development to QC and manufacturing include requirements for system redundancy in these time sensitive functions, and organizations should also plan for the potential for system downtime or outages. Waters' centralized method, data, and result management in a network-based deployment can help support organizations long term plans to adapt its technologies to scale or for various functions. Fit for purpose attribute monitoring platforms such as the QDa and BioAccord system enable companies to attain this scalability and redundancy with a cost effectiveness not achievable with the use of research LC/MS platforms.

Trap 11: Failing to Plan for Technology Upgrade Cycles or Continued Vendor Support

The Problem: Even the transition from development to clinic represents years of work – the assays established in development will need to survive the years it often takes to move through clinical phases and establish a commercial manufacturing strategy. Failing to consider additional the years (or decades) an analytical specification will be tied to the lifecycle of a commercialized biotherapeutic can create the potential for necessary upgrades in analytical capability and instrumentation, equaling additional capital expenditures and extensive crossover studies as organizations evaluate alternatives.

The Waters Solution: Establishing a long-term analytics strategy for the life cycle of a product is best executed in conjunction with a vendor that has demonstrated forward-thinking innovation and a history of comprehensive customer support. Waters long-term commitment to the pharmaceutical industry is exemplified by the continued production support of µBondapak columns, which have been continually used in QC/release methods since 1973, the commitment to controlled incremental innovations on the Alliance HPLC platform since 1996, and commitment to maintaining the consistent performance of the QDa mass detector since its initial release in 2013. With its long-term dedication to being a partner in the biopharma industry, embedded industry expertise, and global reach, Waters is devoted to supporting its partners for the lifecycle of a product and beyond.

Waters as your MAM Partner

Ultimately, the importance of maximizing uptime and minimizing disruption in the biopharmaceutical space over the lifetime of a molecule requires solutions that prioritize robustness, functionality, flexibility, and regulatory compliance. Building the capacity for validated, streamlined MAM LC_MS assays from the foundational development studies onward, organizations can greatly reduce the potential for human error, minimize the number of discrete assays needed for the same attributes, and achieve greater process understanding and quality control capacity. Waters' capabilities for sample preparation, separations, and a range of scalable, integrated, networked LC-MS solutions complement its long-standing commitment to remain a premier partner for the biopharmaceutical industry and reflects our investments in these MAM LC-MS capabilities from early development to commercial manufacturing and QC release.

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