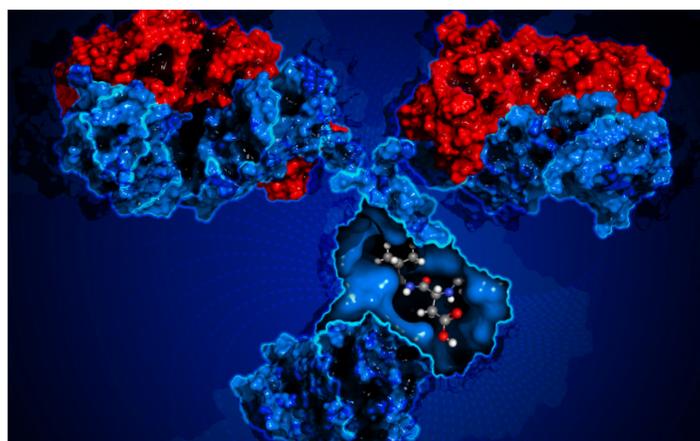


Deploying the multi-attribute method (MAM) across sites at Pfizer

Pfizer is building the MAM lab-of-the-future

Pfizer is a leading research-based biopharmaceutical company. At Pfizer, scientific principles, innovation, and the company's global resources are applied to bring therapies to people that extend and significantly improve their lives. Pfizer strives to set the standard for quality, safety and value in the discovery, development and manufacture of healthcare products. Its global portfolio includes many well known, life-saving medicines and vaccines. As one of the world's premier innovative biopharmaceutical companies, they collaborate with healthcare providers, governments and local communities to support and expand access to reliable, affordable healthcare around the world.

To support Pfizer in reaching its goals within the company, the Biotherapeutics Pharmaceutical Sciences (BioTx PS) organization in Worldwide Research, Development and Medical has a mission to develop quality, compliant manufacturing processes and deliver safe and efficacious products with industry leading cycle times and robust Chemistry, Manufacturing and Controls (CMC) regulatory submissions. Equally important, BioTx PS also needs to advance and innovate the scientific capabilities to deliver one of the most complex and diverse portfolios in the industry comprised of monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs), Fc fusion proteins, recombinant proteins, vaccines, biosimilars, and gene therapy modalities. BioTx PS is sectioned into three major scientific research and development (R&D) disciplines: Analytical R&D (for routine testing and heightened characterization), Bioprocess R&D (for cell line and process development) and Pharmaceutical R&D (for formulation development and devices).



Within Analytical R&D, there are three Project Progression Lines (PPL) who develop, qualify and implement informative, high quality methods for process support and product characterization/comparability throughout the lifecycle of each therapeutic molecule—from pre-clinical development to commercialization. They implement analytical techniques, including chromatographic and electrophoretic methodologies, and more recently they have expanded into mass spectrometry. In parallel, the Mass Spectrometry and Biophysical Characterization (MSBC) group has the mission of heightened characterization, which is to elucidate the molecular structure of biotherapeutics in an open, collaborative, and timely manner using leading characterization strategies, state-of-the-art instrumentation, and innovative analytical methods, that ultimately contributes to the development and commercialization of well-characterized medicines.

In the development of biotherapeutics, a thorough understanding of a molecule's product quality attributes (PQAs) as well as their effect on various structure–function relationships and long-term stability is essential for ensuring product quality, safety and efficacy. At present, numerous routine profile-based chromatographic and electrophoretic assays are used to characterize and monitor individual PQAs for batch release, stability time-points, and process/formulation development support. Execution of multiple routine methods becomes time and resource intensive, and often provides an indirect measure of biologically relevant PQAs.

The multi-attribute method (MAM)¹, based on liquid chromatography-mass spectrometry (LC-MS) peptide mapping and automation principles, provides

simultaneous, site-specific detection, identification, quantitation, and monitoring of PQAs. As compared to profile-based assays, where individual PQAs may not be resolved, MAM provides direct quantitative assessment of an expanded repertoire of PQAs, leading to enhanced process and product understanding. At Pfizer, the goal is to implement MAM at the start of new developmental projects following initial characterization activities such that PQA monitoring of various sample streams by MAM can guide process development, structure-function studies, and eventually control strategy and specification setting. Consequently, a dedicated team at Pfizer has developed and implemented a multi-site MAM “Lab-of-the-Future” for streamlined analyses of PQAs.



Jason Rouse manages the Mass Spectrometry and Biophysical Characterization (MSBC) laboratory at Pfizer. MSBC works collaboratively with research, development, and manufacturing groups across Pfizer to carry out product characterization and comparability/biosimilarity exercises, as well as time-sensitive investigations and bioprocess support.



Andrew Dawdy is a principal scientist in the MSBC laboratory at the Pfizer St. Louis site and a member of the Pfizer MAM team. He champions the Pfizer-Thermo Fisher partnership that focuses on maximizing the utility of Biopharma Finder and Chromeleon CDS software for MAM and other MS-based characterization.



Carly Daniels is a principal scientist and group leader in Project Progression Line 2 (PPL2) at the Pfizer St. Louis site, and she co-leads the Pfizer MAM team.



Thomas Powers is a principal scientist in the MSBC laboratory at the Pfizer St. Louis site and a member of the Pfizer MAM team.



Keith Johnson is a senior principal scientist and group leader in Project Progression Line 3 (PPL3) at the Pfizer Andover site, and he co-leads the Pfizer MAM team.



Kristin Boggio is a principal scientist in the MSBC laboratory at the Pfizer Andover site and a member of the Pfizer MAM team.

Pfizer's journey with MAM

Pfizer's MAM team initially incorporated MAM into their pre-IND (Investigational New Drug application), early stage development timelines for antibody biotherapeutics. At the start of every antibody project, following the official hand-off from the Discovery and Molecular Design groups, the core mass spectrometry group performs an in-depth analysis of molecular attributes including both expected and unexpected posttranslational and chemical modifications. In this exercise, they analytically compare drug substance (unstressed) with accelerated stressed samples that independently underwent thermal incubation and light exposure to better understand degradation pathways and potential impacts to structure-function relationships. Using Thermo Scientific LC-MS instrumentation and software, a comprehensive workbook of key PQAs is created by MSBC for subsequent monitoring by the PPL biochemistry/separation science groups, who already support product and process development with release, stability and identity assays, and quantify attributes like high molecular mass species, charge variants, size variants, N-glycans, etc. PPL is directly involved with the finalization of the manufacturing process and with product formulation for each therapeutic program, in addition to providing further understanding for product stability under accelerated stress and real-time stability. Therefore, MAM is expected to provide PPL with additional capability to monitor a broader array of attributes in a site-specific manner, as well as potentially consolidate release/stability/identity assays and/or streamline workflows for increased efficiency.

The Pfizer MAM team has also implemented MAM for comparability exercises of manufacturing process improvements and site changes. MAM analytically provides more rigorous site-specific attribute information/quantitation for pre-change and post-change batches. Additionally, MAM is being applied for novel modalities such as gene therapy, ADCs, fusion proteins, and recombinant protein assets, specifically at the start of these programs when batch release and stability assays do not exist, as a means to rapidly characterize/monitor key attributes and guide method development, as well as the larger process and formulation development effort. MAM training and method transfer within MSBC and PPL groups are short-term goals. Full integration of MAM into quality control (QC) laboratories, formulation teams, and GMP (good manufacturing practices) manufacturing is a longer term goal as the instrumentation and software mature and become more intuitive for non-classically trained mass spectrometrists.

The key benefits on why Pfizer invests in bringing MAM into their analytical processes are:

1. MAM is a single LC-MS-based method for simultaneous analysis of multiple PQAs with site-specificity that effectively complements routine identity, release and stability assays (Figure 1).
2. MAM allows for automated monitoring and quantitation of known PQAs and detection of new peaks during process and product development that contributes to building product-specific, institutional, and holistic scientific knowledge.

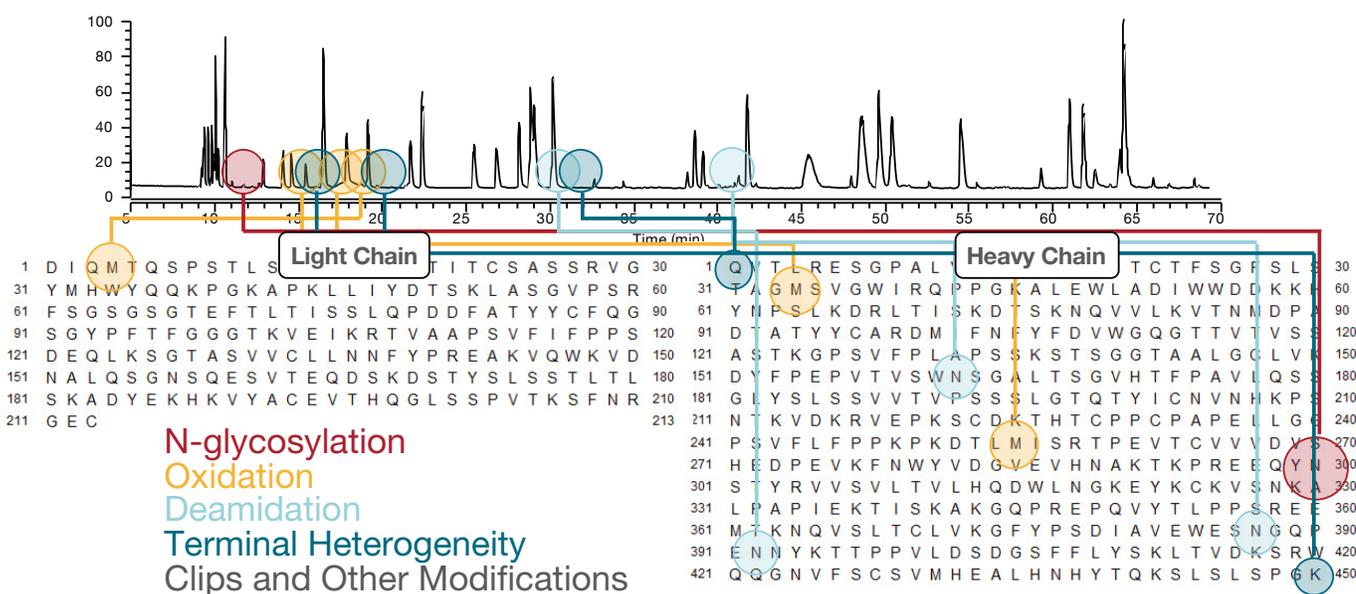


Figure 1. Visualization of how MAM, a single (LC-MS)-based method, is utilized at Pfizer for simultaneous analysis of multiple PQAs with the benefit of site-specific information.

Development of the MAM workflow

From the seminal 2018 industry publication entitled “A View on the Importance of ‘Multi-Attribute Method’ for Measuring Purity of Biopharmaceuticals and Improving Overall Control Strategy”,⁴ MAM involves simultaneous detection, identification, quantitation, and quality control (monitoring) of molecular attributes. A primary motivation behind MAM is to provide more comprehensive information content in a more efficient manner. With companies trying to address more unmet medical needs via more program starts, in addition to streamlining product and process development practices and timelines to deliver high quality medicines to patients, MAM is viewed as a new analytical platform that can better guide product and process development. Additionally, it can serve as a platform in the disposition of clinical materials from the QC lab.

As shown in Figure 2, there are a few essential components of MAM. It starts with a rapid proteolytic digestion step (usually by trypsin) to maintain the integrity of labile posttranslational modifications (PTMs) (i.e., N-/O-glycosylation, sialylation, etc.) and minimize method-induced chemical modifications (i.e., some proteolytic peptides are susceptible to further deamidation, oxidation, etc.). Using LC-MS/MS—trypsin peptide mapping on a Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer,

the PQAs comprising the biotherapeutic molecule are characterized in a traditional, comprehensive, site-specific manner, perhaps requiring additional endopeptidases and/or electron-transfer dissociation (on a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer), in addition to the standard higher-energy collisional dissociation (HCD), to pinpoint modifications in challenging sequence motifs/stretchers. Then, using a combination of manual and automated data interrogation via the Thermo Scientific™ BioPharma™ Finder software, the array of PQAs comprising posttranslational and chemical modifications is detected, identified, and recorded in a target peptide workbook that enables routine attribute monitoring via LC-MS. Once the biotherapeutic is designated as “well characterized” and the workbook is finalized, the list of essential and/or diagnostic PQAs are transferred into a method within Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), which is employed for routine attribute monitoring using a Thermo Scientific™ Exactive™ Plus mass spectrometer. The Exactive Plus instruments are used in full MS mode only for the routine monitoring, which takes advantage of chromatographic retention time, high resolution/accurate mass, and isotopic distribution to automatically identify and quantitate the PQAs in the library.

PFIZER PLATFORM MAM WORKFLOW

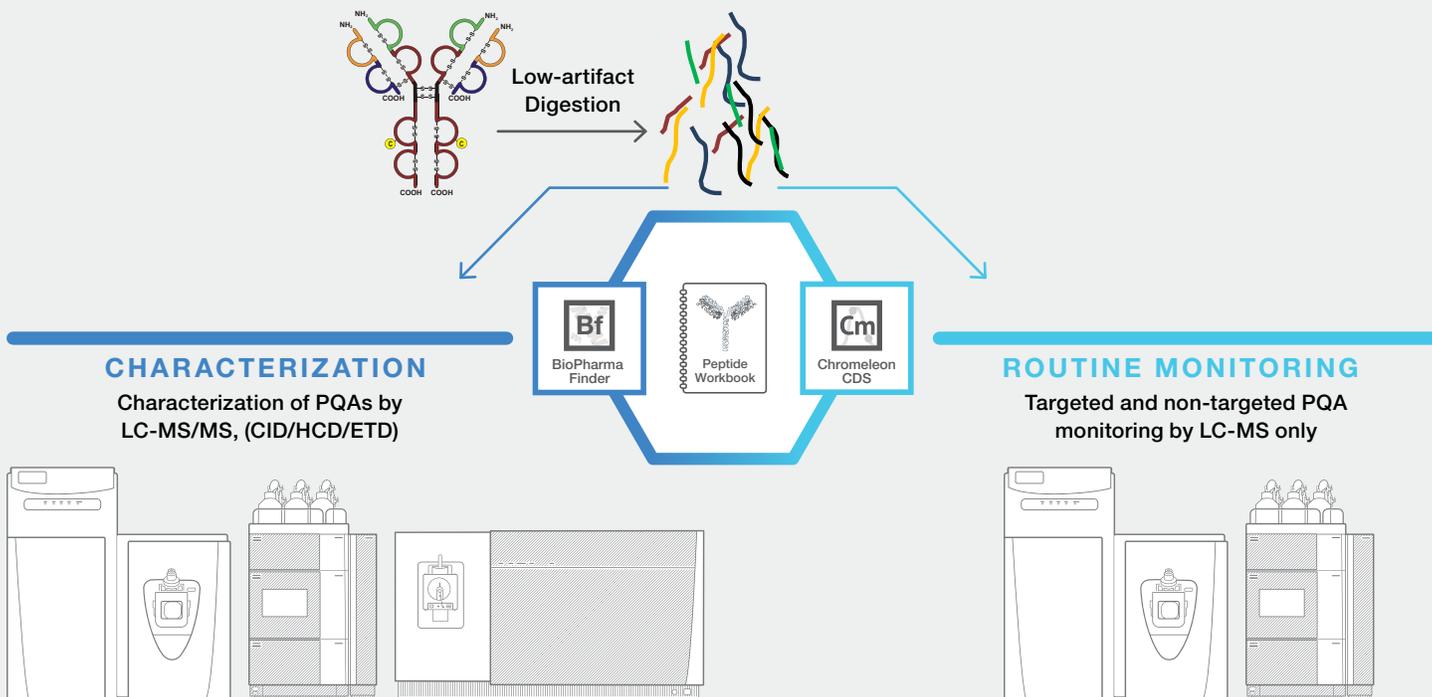


Figure 2. Pfizer MAM Workflow. The initial characterization step involving LC-MS/MS is performed in MSBC to map site-specific PQAs. Subsequently, PQAs are routinely monitored via LC-MS in PPL to build process and product information.

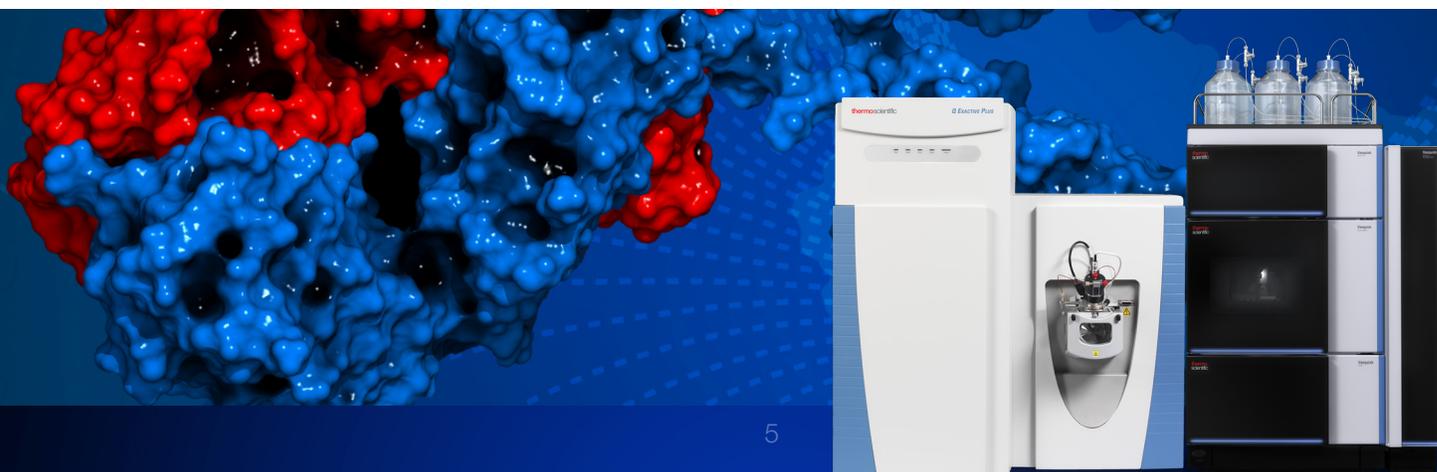
Lastly, while it would be ideal to monitor all potential Met and Trp oxidation sites in the protein, for example, it is also important to balance the number of PQAs in the primary workbook to ensure fast, reliable monitoring by MAM. Thus, the primary workbook for general screening may contain key PQAs pertaining to N-glycosylation, N-/C-terminal heterogeneity and other PTMs, in addition to a series of diagnostic PQAs for the more abundant, known sites of Asn deamidation, Asp isomerization, and Met/Trp oxidation to monitor potential degradation. As the project progresses through development and more product heterogeneity and structure-function knowledge is gained, additional PQAs can be added to the primary workbook as required to ensure comprehensive monitoring of product consistency. Additionally, focused workbooks can be created for bioprocess optimization or forced degradation purposes where more N-glycoforms or oxidation/deamidation sites can be monitored, respectively. Fortunately, with Chromeleon CDS and the enterprise data server, one is easily able to retrospectively interrogate historical MAM data, together with recently acquired MAM data, using new/ revised workbooks to trend PQAs over the duration of the project for more insight.

Additionally, an appropriate system suitability test (SST) is critical for the long-term success of MAM. Thermo Scientific™ Pierce™ BSA Protein Digest standard offers a comprehensive system suitability sample, unlike conventional system suitability samples for MAM which rely on simple synthetic peptide mixtures. While synthetic peptide standards ensure suitability for several key components of MAM, including UHPLC performance (i.e., retention time (RT) reproducibility and peak width/shape) and mass spectrometry performance (i.e., mass accuracy and peak area), the BSA Protein Digest enables additional attributes to be monitored, including % abundance of method-induced chemical modifications (i.e., asparagine/glutamine deamidation), system-induced oxidation (currently not used for our SST) and in-source fragmentation. Furthermore, by implementing BSA Protein Digest as an SST standard, system performance can be

monitored from installation onwards, as the same standard sample will be analyzed by Thermo Fisher engineers at the time of installation. This continuity in system suitability will enable an easy assessment long-term for system performance and instrument suitability.

Pfizer has elected to use a mAb standard developed in-house for monitoring day-to-day system suitability for MAM applications, while the Thermo Scientific™ Pierce™ LC-MS/MS System Suitability Standard (or the Thermo Scientific™ Pierce™ BSA Protein Digest standard) is used to re-evaluate LC/MS system performance following preventive maintenance and instrument repairs by Thermo engineers. Leveraging this internal mAb enables the fusion of system suitability and initial assay acceptance criteria for early stage programs. The resulting mAb system suitability/assay acceptance test is able to monitor UHPLC performance, MS performance, method-induced artifacts (i.e., deamidation), system-induced oxidation, and in-source fragmentation similar to the BSA Protein Digest standard. In addition, the Pfizer mAb standard allows evaluation of trypsin digestion robustness (i.e., digestion and alkylation efficiency), as well as examination of expected levels of site-specific deamidation, oxidation, N-/C-terminal heterogeneity, N-linked glycan ratios, etc. across the mAb standard in a targeted, diagnostic manner to ensure a reference point for assay acceptance in early phase projects.

MAM has been recognized throughout industry as value-added. It is acknowledged as a time saver in process and product development and characterization; and, it provides more in-depth monitoring of molecular PQAs. Its utility has especially been emphasized as biotherapeutics have increased in complexity, including gene therapies, bi-specifics, and tri-specifics. Understanding individual attributes is a new focus area in biotherapeutics development, and MAM allows Pfizer to perform site specific attribute analysis to help better understand attributes and their impact on the product quality.



Working with Thermo Fisher Scientific and utilizing Thermo Scientific products

The Pfizer MAM team has been working closely with Thermo Fisher Scientific for the past four years on their MAM development. This collaboration has made it possible for them to work and provide feedback on all aspects of the workflow including sample preparation, hardware, and software, as well as performance and fit-for-purpose aspects. Delegates of both companies have met in St. Louis, MO and in Andover, MA to learn more about MAM developments. In those meetings, Pfizer has given updates on progress and implementation of the method while providing a read out on difficulties that they have encountered with data analysis. “One of the most important actions that have come from these meetings is Thermo Fisher Scientific’s readiness to incorporate changes in their software to make the method easier for the end user.”— says Jason Rouse from the Pfizer team.

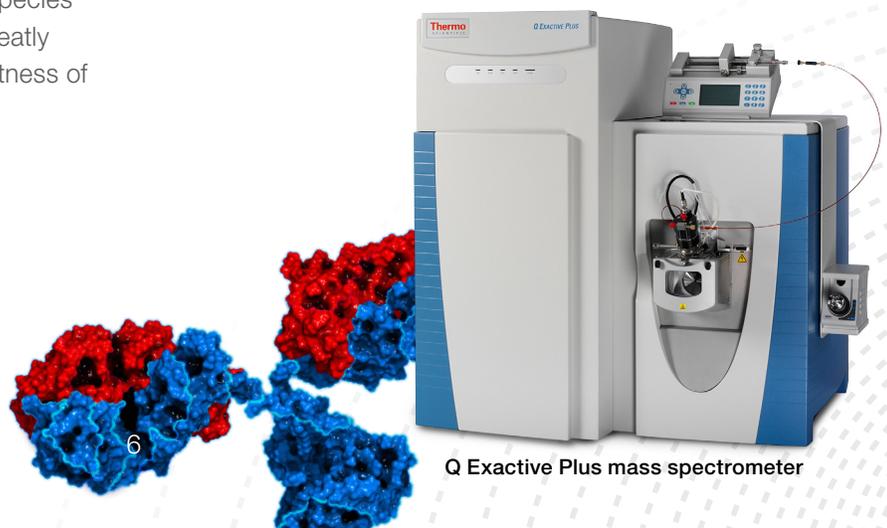
As Carly Daniels explains “Thermo Fisher’s single-vendor solution for MAM was very useful as there is seamless integration of hardware, software, and ultimately the data readout. Thermo Fisher was the first to introduce a ‘MAM workflow package’, an LC-MS system and software combination for MAM. Communication between various Thermo Scientific software packages for characterization and monitoring, as well as ease of transfer of methods between similar LC-MS systems in the same Thermo Scientific family of instruments allowed for a seamless transition from our characterization group to our routine testing groups.”

Orbitrap’s capability of high-resolution accurate mass (HRAM) is extremely beneficial and needed for MAM to provide high confidence in identifying peptides for the monitoring step without the need for MS/MS.

Each peptide map used for MAM is a highly complex dataset with thousands upon thousands of signals covering the m/z spectrum. The ability to highly resolve the correct species of interest and obtain sub-5 ppm accurate mass greatly increases the Pfizer’s team confidence in the robustness of

their MAM method. One common and clear example of this is the ability to resolve the isotopes of a deamidated peptide from the M+1, M+2, etc. isotopes of the unmodified species, which differ very little in mass from each other.

In order to harmonize instrumentation and software, the Pfizer team chose Thermo Scientific™ Vanquish™ UHPLC systems for their MAM LC-MS platform. Though they had no previous experience operating Vanquish LCs, the support and assistance provided by the Thermo Fisher team helped them overcome the initial learning curves faced when using new hardware and software. This enabled the Pfizer team to become familiar with Vanquish UHPLC operation and maintenance and work independently in the shortest time possible. Run times may be shortened by 50% or more as compared to the time typically required on an HPLC system. They specifically chose the Thermo Scientific™ Vanquish™ Duo UHPLC system in order to achieve maximum MAM throughput. The efficiency gained by washing one analytical column while acquiring data with the other has proven invaluable as their MAM operations have increased to the point where instruments are running constantly. The Vanquish Duo UHPLC system enables further efficiency by producing superior chromatographic peak capacity relative to standard HPLC systems. To maintain this performance and maximize harmony between the Pfizer MAM platforms, great care was taken in selecting and harmonizing all the plumbing for these systems. The MAM team also found huge benefit in harmonizing analytical column lots when running a Vanquish Duo system as this can maintain replicate retention times between columns within 6–12 seconds. The Vanquish Duo UHPLC has indeed proven to be a key asset for Pfizer’s MAM workflow, and the MAM team is pleased with their decision, as Thomas Powers explains “The Vanquish UHPLC systems offer unprecedented retention time consistency and reproducibility. This significantly aids in the ability to easily implement MAM.”



Q Exactive Plus mass spectrometer

BioPharma Finder software offers the ability to efficiently characterize, quantify, and build a library of PQAs for monitoring by MAM. Chromeleon CDS provides a means of monitoring these attributes on a regular basis in a manner which does not require the user to be a mass spectrometry expert. The team has developed Chromeleon CDS workflows for MAM which allow them to operate the LC-MS system, track system suitability metadata, automatically process the LC-MS data and report quantitation of the attributes from the data. “In a longitudinal test of our entire MAM workflow, we have demonstrated the ability to obtain consistent quantitation of a variety of PQAs for an internal mAb standard across multiple analysts, digestions, instruments, and laboratories, over a period of 18 months, without any need to modify the processing method within Chromeleon CDS” says Andrew Dawdy, who has been focusing on the software aspects of their workflow development. And then he adds: “Thermo Scientific’s MAM software capabilities have grown by leaps and bounds in the last few years, largely due to their willingness to take direct feedback and make continual improvements”.

Thomas Powers explains “Thermo Scientific offers a comprehensive data solution for MAM, from data acquisition to automated reporting. While Chromeleon CDS is the primary software for MAM, the Chromeleon software interfaces nicely with BioPharma Finder for workbook creation. Furthermore, the Chromeleon software offers several advanced features that enable success with MAM, including the ability to record all metadata associated with an experiment, query specific data files, graph the abundance of modifications, ensure system suitability prior to analysis, and visualize all extracted ion chromatograms

associated with the report method. Additionally, future implementation of eWorkflow™ procedures will enable MAM to be rolled out to non-MS experts, a crucial component of MAM and a critical need for it to reach its full potential. While we have just scratched the surface of using Chromeleon CDS for MAM, I have no doubt that Chromeleon software will enable successful implementation of MAM at Pfizer.”

A standardized, commercialized MAM workflow from hardware to software, consumables, training, service, and technical support has allowed for a more seamless integration of MAM into Pfizer’s lines. Communication between hardware and software, as well as the availability of expert support from engineers familiar with the MAM workflow has allowed for quick answering of questions and faster resolution of issues when they arise.

Examples of how MAM can help for real-life applications in Pfizer

In a recent Webinar (Understanding Biotherapeutic Product Quality Attributes through a Multi-Attribute Method (MAM) Lab-of-the-Future, [available on-demand](#))²⁻³ Andrew Dawdy presented examples for the applications of their MAM Workflow.

MAM is being piloted as a non-GMP assay in active Pfizer projects to evaluate the workflow, compare data to traditional assays, and gain enhanced PQA understanding. N-glycoform quantitation by MAM was compared to released, 2-AB-labeled N-glycan assays for samples from three different bioreactors. Shown in Figure 3, the MAM results in blue match the traditional assay data (black/gray) for major, minor, and trace-level glycoforms.

3 bioreactor samples run by: Traditional Released N-glycan Assay (2-AB) and MAM

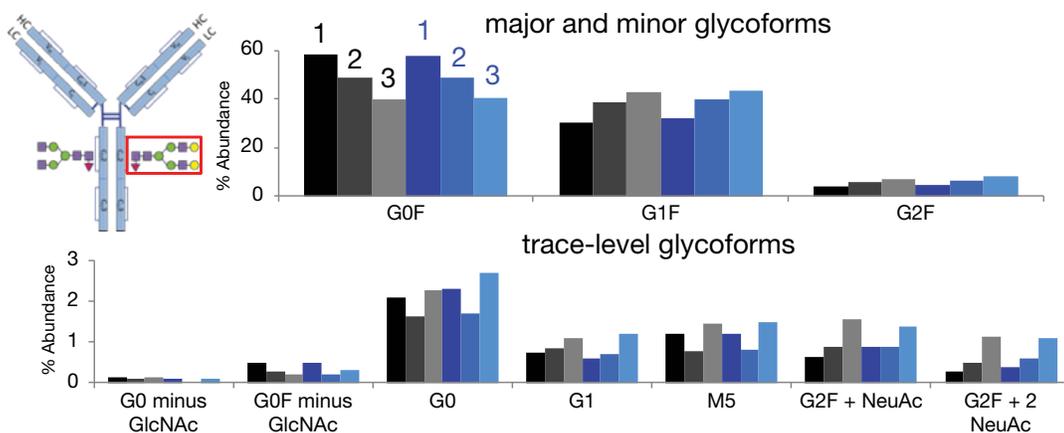


Figure 3. N-Glycoform quantitation by MAM vs. the conventional PNGaseF-released, 2-AB-labeled N-glycan HILIC-FLR assay for 3 bioreactor samples. Results from MAM (blue bars) match 2-AB-labeled N-glycan assay results (black/grey bars) for major and minor glycoforms and for trace-level glycoforms very well.

In a forced degradation study a mAb sample was stressed for 18 weeks at 40 °C. Subsequent charge variant analysis by imaged capillary electrophoresis (iCE) compared with MAM proved excellent agreement between the two techniques. However, the iCE data only yielded global information, with no details on identification or location of attributes. In contrast, MAM provided identification and site-specificity for each acidic PQA, which led to the understanding of their individual contributions to the degradation profile. This was observed and shown in the color-coded MAM data in Figure 4. In addition to specificity, MAM's capabilities often provided more comprehensive information. For example, traditional methionine oxidation

assays target one specific site in the Fc-region, yet MAM could quantitate multiple sites and types of oxidation in a single assay.

In the same thermal stress study, MAM was used to identify the effect of formulation on non-enzymatic lysine glycation. Site-specific levels of lysine glycation were measured and compared for a sucrose-containing formulation and a formulation with trehalose. The graph in Figure 5 illustrates that the drug substance in the formulation with sucrose is at much higher risk for lysine glycation. Moreover, the locations of lysine glycation hotspots could be easily identified.

The increase in acidic species observed independently by iCE and MAM correlate well together. MAM provides the added benefit of ID and site-specificity for each acidic PQA.

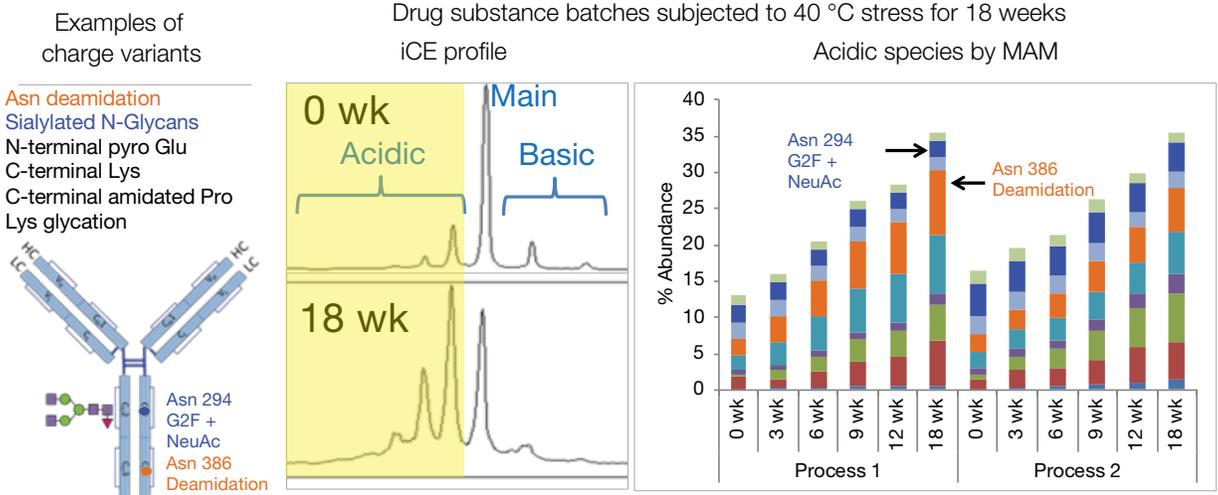


Figure 4. Charge variants identified and site-localized by MAM. MAM provides enhanced charge variant understanding vs. traditional assays like iCE and CEX, because MAM site-specifically identifies and quantifies each individual charge variant. This information from MAM can be converted to a percentage value which is representative of their probable presence in the intact 4-chain mAb, and also summed, if global numbers are needed (e.g., total sialylation, total glycation, etc.).

MAM reveals the location and degree of lysine glycation risk in formulation studies

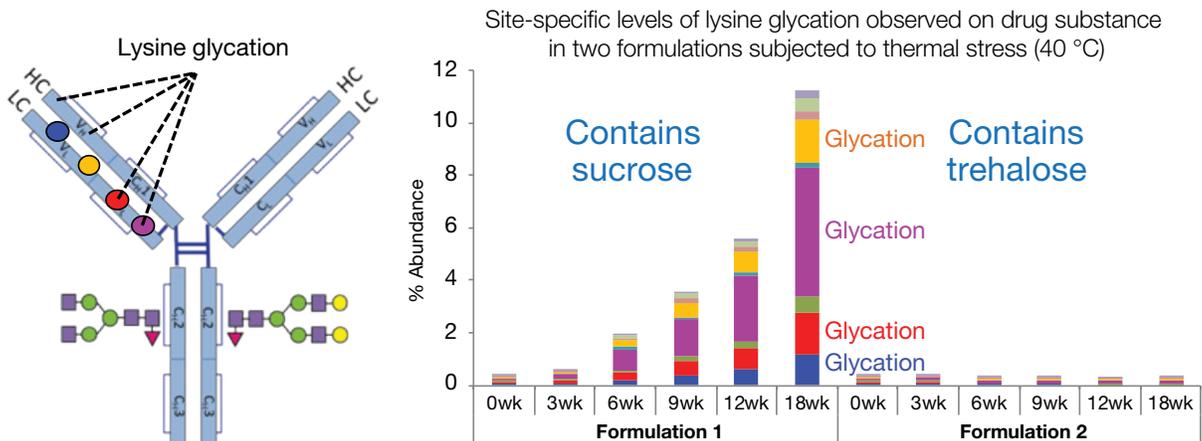


Figure 5. Effect of formulation on non-enzymatic lysine glycation identified by MAM. MAM reveals that drug substance in Formulation 1 is at much higher risk for lysine glycation.

Non-targeted differential analysis of the same stress study detected an unknown peak that was increasing with stress. The unknown was routed back through the workflow for in-depth characterization and was identified as a D/P clip within the Fc region resulting from hydrolysis. It was then added to the attribute library for monitoring. After running multiple drug substance batches, the comparison of fragmentation of this D/P clipping by MAM correlated very well with the abundance of clipped species observed by reducing capillary gel electrophoresis.

Outlook of wider adoption of MAM in industry and further requirements

At Pfizer, the team continues to build on their platform development processes as new technologies are demonstrated to be ready for implementation. They would like to balance increase in efficiency of their processes with the need to assure that new instrumentation and/or new methods provide the same robustness, precision, and accuracy of the conventional methods that they have been relying on to make informed decisions and to guarantee biopharmaceutical products are at the highest quality. Consistent optimization of technology is important as regulatory requirements continue to evolve. Increased product understanding requires not only better knowledge of attributes but also information about their quantitative levels. The heterogeneity of biotherapeutics makes collecting this information challenging even with today's advanced technologies.

For MAM to be successful in both biotherapeutics development and QC labs, the LC-MS system needs to become easier to operate, calibrate, and maintain, more robust, with less instrument-to-instrument variability, and more user-friendly software interface. Automation, or the possibility for increased automation is desired, and in the ideal future the software tools will allow LC-MS data processing without significant user intervention.

While today's mass spectrometers likely require expert users, due to the intricacy of the instrumentation and the complexity of the data interpretation, the goal remains to make this workflow more accessible to the general analyst by developing more intuitive software and fail proof operation. This is especially true for the QC lab, where problems during operation or data analysis could cause investigations or false positives that lead to batch

failures or delays during product release. Having backup instrumentation is not always a cost-effective possibility, especially when considering the global drive for making biotherapeutics more widely accessible. Laboratory bench space also comes at a price, and smaller footprint instruments, as well as decreasing the cost and complexity of these instruments, are seen as imperative for widespread adoption of MAM in QC laboratories. Full automation of MAM is also seen as valuable direction as it would reduce potential for variability introduced by human factor as well as the hands-on time needed for preparing samples or setting up experiments.

At Pfizer, MAM has the most immediate potential to replace peptide mapping for product identity, released N-linked glycan profiling, protein fragmentation profiling/monitoring, and charge variant analysis (for mAbs). For gene therapy or more complex biotherapeutics, MAM has the potential for incorporation as a routine assay for site specific monitoring of critical PTMs.

Summary

- Pfizer, in partnership with Thermo Fisher Scientific, has successfully developed a robust multi-site "Lab-of-the-Future" to implement MAM for biotherapeutic development
- MAM enables direct measurement of multiple PQAs in a single assay, providing enhanced product understanding with increased efficiency
- Pfizer is currently implementing MAM in active developmental projects to support non-GMP monitoring of PQAs and to conduct "real-world" evaluations of their MAM workflow for late-stage development activities
- MAM data to date agree well with the results from traditional assays, and for several developmental projects, MAM has yielded key information for specific PQAs either in real time or via retrospective analysis of current versus previous datasets to help solve critical structure-function investigations
- Pfizer plans to implement MAM in QC laboratories within the next 3–5 years after satisfactory completion of method validation and requisite compliance and risk assessments

“One of the most important actions that have come from these meetings is Thermo Fisher Scientific’s readiness to incorporate changes in their software to make the method easier for the end user”

– Jason Rouse

Mass spectrometry has been increasingly influential within Pfizer for biotherapeutic development by providing integral information regarding product quality and informing key decision-making for projects. This is seen as a result of advancements in mass spectrometry-based sequence variant analysis, orthogonal host cell protein analysis, online charge variant analysis, trisulfide analysis, and degradation hotspot characterization. Pfizer is a very forward-looking company, and they have recognized the power of mass spectrometry in these areas and have been enthusiastically endorsing the widespread deployment of MAM across all stages of biotherapeutic development. They feel they have the opportunity to inspire and actively take part in an industry-wide paradigm shift by enabling efficient and comprehensive process and product understanding in a manner they have not routinely experienced before, and that is truly exciting for both the pharmaceutical industry and for the analytical scientists working towards higher quality lifesaving medicines. All these efforts have been in line with Quality by Design principles advocated by regulators and industry with an aim to build better product and process knowledge from the earliest stages of development and keeping that guiding principle all the way through manufacturing.

The Pfizer team currently aspires to transfer and implement their MAM method into QC laboratories by 2025, but in order to reach these early days in QC, MAM needs to be already well-established in the non-GMP development lines (process support and stability) by then.

Author’s Note

Sincere thanks and appreciation go to the Pfizer MAM team and respective project teams for their valuable contributions, helpful discussions, and critical review of this work. Additionally, we are grateful for the generous support from the Pfizer Biotherapeutics Pharmaceutical Sciences organization.

The scientists on Pfizer’s MAM team

Jason C. Rouse—Director-Mass Spectrometry and Biophysical Characterization (MSBC)

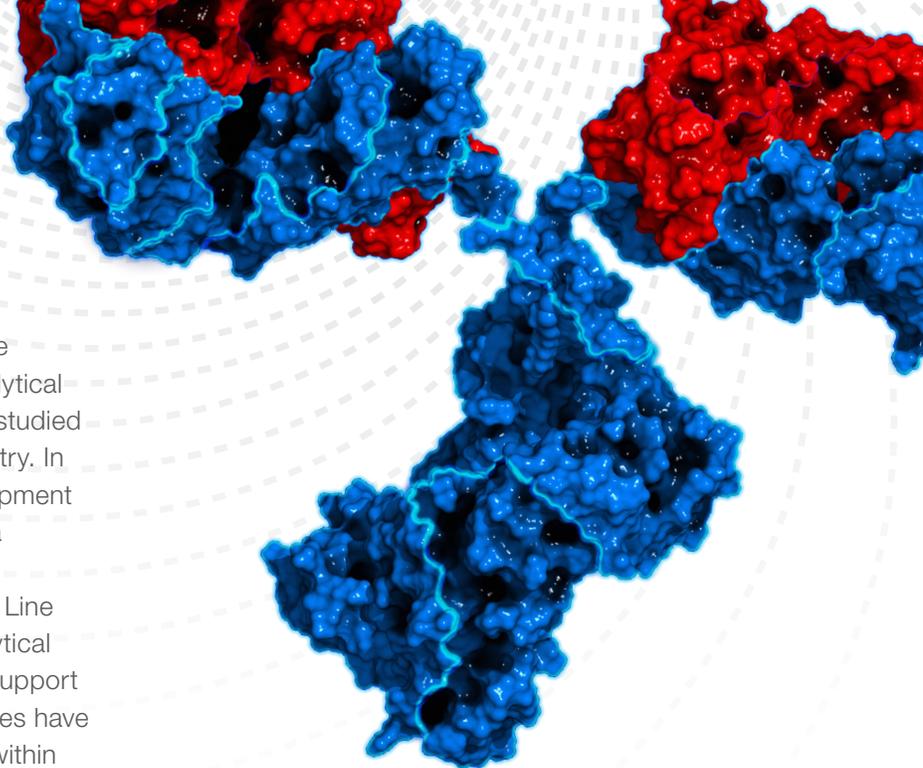
Jason received his Ph.D. degree in Analytical Chemistry from Michigan State University in 1993 and joined Genetics Institute (GI) as a post-doctoral research fellow. Jason was promoted into positions of greater responsibility over the years in GI, Wyeth, and Pfizer, and currently he is the director of the MSBC group. Jason’s scientific interests include the elucidation and analysis of released N- and O-linked glycans by on-line and off-line MS techniques, as well as the top-down characterization of intact glycoprotein therapeutics by ultrahigh-resolution MS approaches. Jason is an active program committee member for the annual CASSS “Mass Spec” conference.

Andrew Dawdy—Principal Scientist

Andrew received his Ph.D. degree in Chemistry from the University of Virginia in 2013 where he performed research in protein MS. Andrew has spent the last seven years working in pharmaceutical development, with the last five of those developing biotherapeutics at the Pfizer St. Louis, MO site. As a principal scientist in the MSBC laboratory, Andrew supports development of a variety of modalities, including mAbs, ADCs, fusion proteins, and recombinant proteins. Andrew is on the front line in the development and implementation of online CEX-MS and MAM at Pfizer, having made respective presentations at ASMS 2018 and 2019, as well as co-leading evening workshops on the topics.

Carly N. Daniels—Principal Scientist and Group Leader

Carly joined Pfizer in 2015 in Analytical Research and Development’s Project Progression Line and is a principal scientist and group leader within this group. She received her B.S. degree in biochemistry from the University of Florida, her Ph.D. in biochemistry and molecular biology from the University of California Los Angeles and worked as a postdoctoral fellow for the US Food and Drug Administration prior to joining Pfizer. At Pfizer, she has focused on analytical method development for the diverse biotherapeutics pipeline, with an emphasis on glycan profiling, peptide mapping, and LC-MS technologies. She currently sits on the board of directors for the MAM Consortium.



Keith A. Johnson—Senior Principal Scientist and Group Leader

Keith received his B.S. degree in Chemistry from the University of Colorado, and his Ph.D. degree in Analytical Chemistry from the University of Georgia where he studied noncovalent protein complexes by mass spectrometry. In 2001, he joined the Analytical Research and Development group at Wyeth, now Pfizer. At Pfizer, he has been a member of the Mass Spectrometry and Biophysical Characterization group and the Project Progression Line group studying structural characterization and analytical method development of protein biotherapeutics in support of Pfizer's biotherapeutics portfolio. Keith's other roles have included leading technology and innovation teams within the analytical groups, and he represents his department on product development teams.

Thomas W. Powers—Principal Scientist

Thomas received his Ph.D. degree in the Biomedical Sciences from the Medical University of South Carolina, where he developed mass spectrometry imaging methods for N-linked glycans and applied these, and other characterization approaches, to biomarker discovery. After his Ph.D., Thomas joined Pfizer's Analytical Research and Development group in St. Louis, where he has been for the last five years. Thomas works in the MSBC lab and has experience working with a variety of modalities including mAbs, ADCs, recombinant proteins, and gene therapy products.

Kristin J. Boggio—Principal Scientist

Kristin received her Ph.D. degree in Physical Chemistry from Brandeis University in 2012 where she developed mass spectrometric methods to analyze biological molecules. After her Ph.D., Kristin completed a postdoctoral appointment in neuroscience, utilizing TMT reagents and bottom-up mass spectrometry to quantitate differences in a cellular model of ALS. Kristin joined the Mass Spectrometry and Biophysical Characterization group at Pfizer in 2015 and focuses on development of MS methods and characterization for Pfizer's diverse biotherapeutic portfolio, including vaccines and fusion proteins. She currently sits on the board of the Greater Boston Mass Spectrometry Discussion Group (GBMSDG).

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