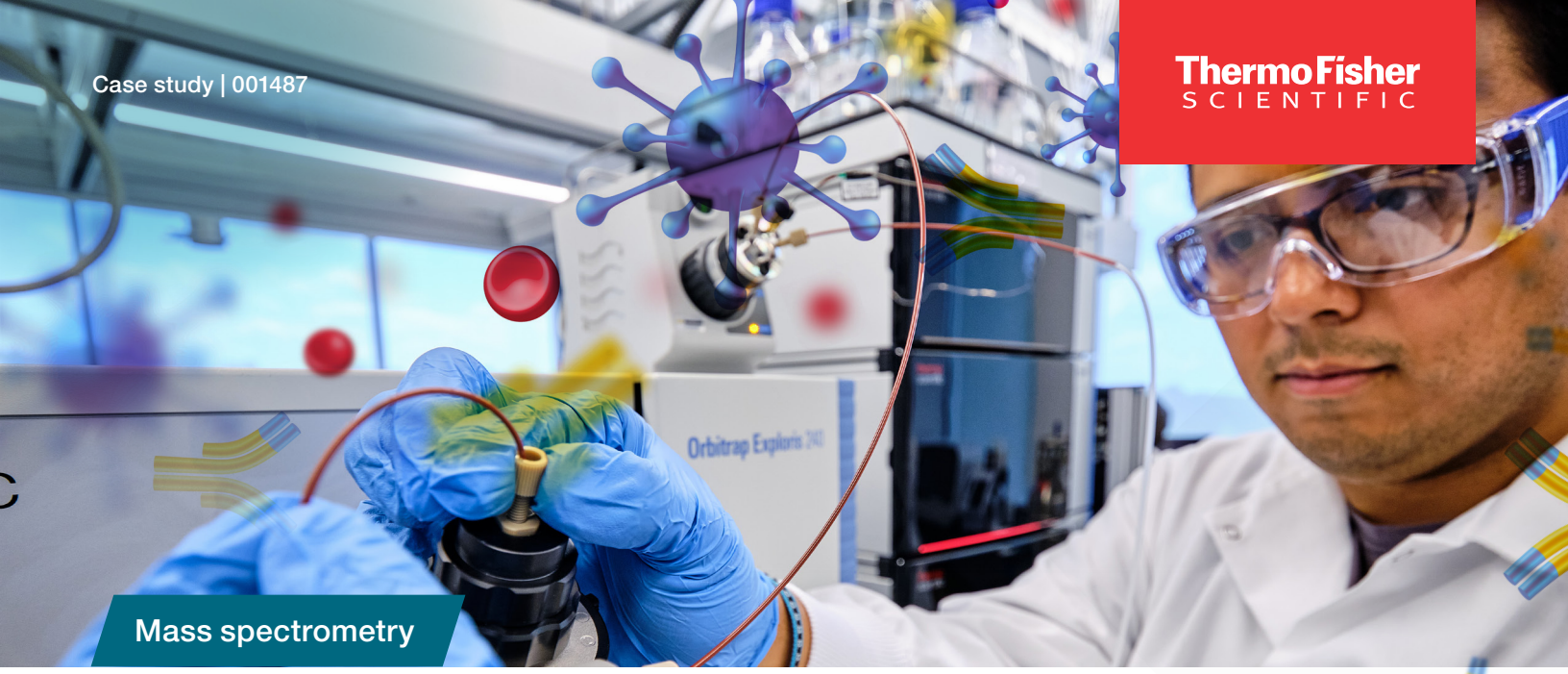


Multi-Attribute Method (MAM) offers an ideal solution for development and release of safe and effective biotherapeutics

Northeastern University Biopharmaceutical Analysis and Training Laboratory (BATL)

"I approach drug development with a quality lens. Understanding the critical process parameters used to manufacture drugs and monitoring the critical quality attributes of a product boils down to streamlined solutions that give you as much reproducible and high-sensitivity data as possible. The Thermo Scientific™ MAM workflow does that."

—Dr. Jared R. Auclair, Vice Provost Research, Economic Development and Director of Bioinnovation, Northeastern University Biopharmaceutical Analysis and Training Laboratory (BATL)

Introduction

Biopharmaceuticals represent a billion-dollar industry. Though monoclonal antibodies (mAbs) are the most common biopharmaceuticals, there are a growing number of more complex molecules such as antibody-drug conjugates and gene and cell therapies now in development and commercialization. Biopharmaceuticals are unique in that they are produced in living cells, resulting in variability that must be characterized and controlled. In research and development, comprehensive characterization of a biopharmaceutical and its product quality attributes (PQAs) is an essential first step in a potential drug's lifecycle. During process development, PQAs are monitored to understand how process parameters affect product yield and quality. Additionally, comprehensive characterization of the biopharmaceutical, including peptide mapping and post-translation modification (PTM) analysis, is requisite for regulatory filings. Downstream in manufacturing and quality control (QC), accurate and consistent critical quality attribute (CQA) monitoring and new peak detection are imperatives.

thermo scientific

"Thermo Fisher Scientific probably has the best solution on the market. One of the things that is appealing in working with the company is that it's a one-stop shop for everything. When they approach an application, the solution comes with a global perspective for both the company and the industry. The MAM workflow from Thermo Scientific capitalizes on their strengths in sample preparation and MS analysis. I don't know who else you would go to that has that amount of technology and the application expertise."

—Jared R. Auclair

MAM connects research, development, and QC

Liquid chromatography-mass spectrometry (LC-MS) methods are commonly used for biopharmaceutical characterization, including intact-mass analysis, peptide mapping and PTM analysis, and released-glycan analysis. The LC-MS-based multi-attribute method (MAM) is becoming broadly embraced by the biotherapeutic industry and regulatory agencies as the ideal approach to characterizing and monitoring large numbers of PQAs and CQAs throughout the product lifecycle. The MAM offers unique quality, time, and cost advantages compared to other methods because it monitors several attributes in the same experiment. Characterization and QC of biotherapeutics without using the MAM typically requires multiple labor-intensive or time-consuming analytical techniques; for example, cation-exchange chromatography, imaging capillary isoelectric focusing, and capillary electrophoresis. Adopting the MAM across all stages of development and manufacturing offers deeper scientific insight, speeds up process development and manufacturing, and improves confidence in the safety and quality of released batches.

The MAM is integral to BATL's focus on quality, compliance, and patient-driven science.

The Northeastern University Biopharmaceutical Analysis and Training Laboratory (BATL) exists to enable innovative biopharmaceutical education, global regulatory convergence, industry partnerships, and user-inspired research to benefit patients worldwide. BATL's state-of-the-art facility offers unique hands-on training for the pharmaceutical industry that is affordable, practical, and comprehensive. Recognizing the importance of the MAM in ensuring that quality, safe, and effective medicines get to patients on accelerated timelines, BATL is advancing the approach as part of its mission. "From a product development perspective," said Dr. Jared R. Auclair, Vice Provost Research, Economic Development and Director of Bioinnovation at Northeastern University BATL, "quality by design—which means that quality is considered from the very beginning—is one of the most important concepts I promote. In the research and development stage, you have to characterize the product and understand it. We do that using the Thermo Scientific MAM workflow."

Thermo Scientific MAM 2.0 solution overcomes implementation barriers

Due to its quality, time, and cost-saving advantages, major biopharmaceutical companies are making significant investments in establishing MAM workflows. Historically, however, MAM implementation hasn't been easy or fast due to a lack of a complete commercial solution. Scientists have had to piece together multiple hardware and software components. Such solutions often require expert MS users and are difficult to deploy in QC environments.

As the leading biotherapeutic analysis solution provider and a trusted partner, Thermo Fisher Scientific has worked closely with industry and academic leaders like BATL to offer a purpose-built MAM solution (Figure 1). A Thermo Scientific MAM solution accelerates decision-making with high-confidence data for PQAs and CQAs acquired by industry-proven Thermo Scientific™ Orbitrap™ mass analyzer technology. Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software adds GMP-supporting data acquisition and processing, along with seamless knowledge sharing and method transfer across instruments, departments, and sites. The solution also offers maximum productivity through a dedicated global support team of Thermo Fisher Scientific MAM experts, who provide application-specific training and service.

"Having our postdoc Liang Xue, who had MS experience analyzing small molecules, learn the biologics space to develop her skills in the MAM workflow at the research and development stage was great. Then, as we transitioned into the quality space, we were able to execute the analytical process in place with no variability in how it was carried out."

—Jared R. Auclair

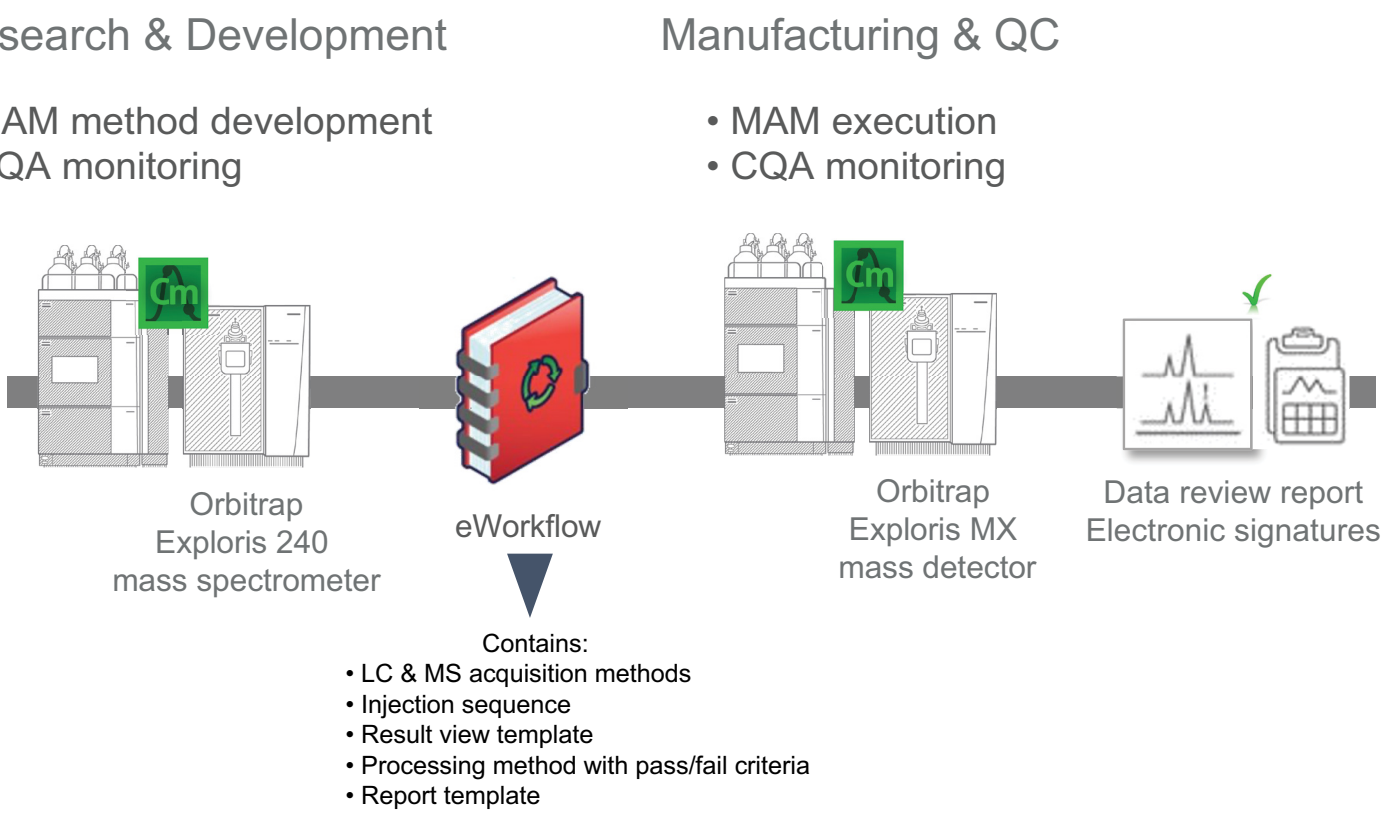
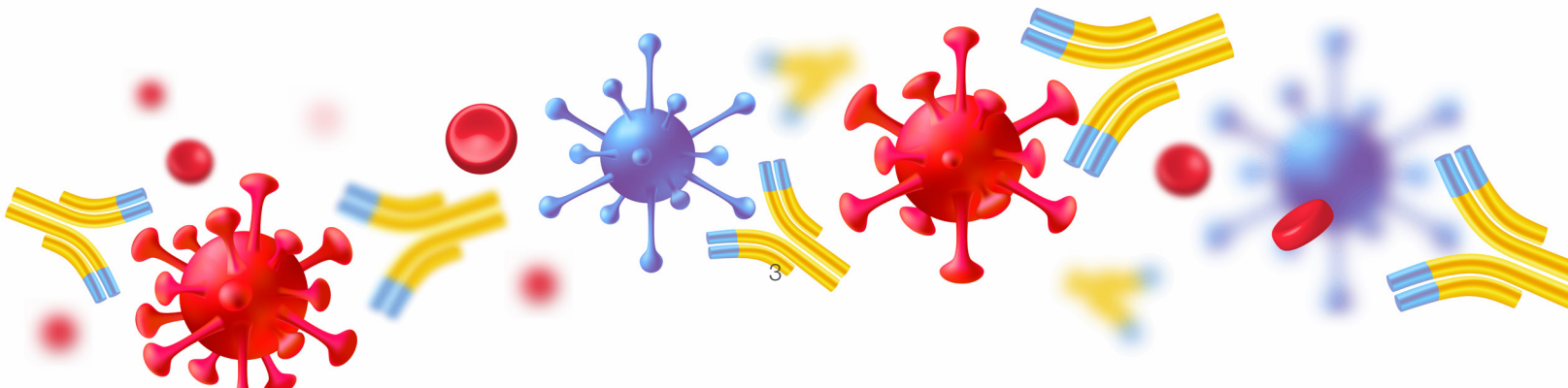


Figure 1. The Thermo Scientific™ MAM 2.0 solution connects research and development to manufacturing and QC with seamless method transfer using an eWorkflow. The eWorkflow contains all the associated methods and the reporting template required to set up the injection sequence, acquire and process the data in the Chromeleon CDS, and summarize the results in a report.



"A Thermo Scientific MAM solution is not only robust, but also easily implemented in any mass spectrometry laboratory. For those that have mass spectrometry—or even better, large-molecule mass spectrometry—experience, becoming a proficient operator is relatively straightforward."

—Dr. Liang Xue, Post-Doctoral Fellow.

Thermo Scientific MAM 2.0 solution addresses workflow needs from research and development to QC

Research, process development, and QC have different workflow objectives and needs. Auclair explained, "In research and development, you not only characterize the product but define what the criteria and method will be in QC. You need an operator who has the experience to adjust the method parameters and sample preparation, and to do method troubleshooting. There are more degrees of freedom the researcher uses to understand the product characteristics and to improve the process. As you transition to the quality space," continued Auclair, "you need a method that is locked down and performs exactly the same every time, so when regulators look at the data, it's from a well-defined process. That means that you need a solution that's "plug and play" so the operator can put the sample in the autosampler, hit a button, get the data out automatically, and then either the analyst or the analyst's manager looks at it and immediately and unequivocally knows if it matches the established criteria. As you go down the product lifecycle pipeline from research and development to commercial manufacturing, it's beneficial if the methods can be directly transferred downstream and generate the same high-quality results for the CQAs. We know the use of similar methods and technologies (i.e., instrumentation) throughout the product life cycle, with quality in mind, from research to commercialization, increases the likelihood that a successful product gets to market."

The MAM 2.0 solution addresses workflow needs at every step of the product lifecycle. For research and development, the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer combines analytical flexibility with quantitative precision and accuracy needed to facilitate comprehensive characterization and robust monitoring of PQAs. For QC, robustness and reliability with minimal instrument-to-instrument variability and operational simplicity with predefined method templates, ensure productivity.

For routine testing environments, a MAM developed on the Orbitrap Exploris 240 mass spectrometer is easily transferred to the Thermo Scientific™ Orbitrap Exploris™ MX mass detector with highly comparable results using a Chromeleon eWorkflow procedure. Robust and easy to operate, this purpose-built mass detector delivers consistent quantitation of quality attributes and new peak detection. Automatic workflow execution simplifies the operation of either mass spectrometer for users of all skill levels.

Auclair described the aspects of the MAM 2.0 workflow that streamline its deployment: "For sample preparation, there are well-defined protocols—for example for protein digestion—that are easy to perform reproducibly. Front-end sample handling automation also makes the workflow easy—and importantly consistent. One of my philosophies in ensuring that quality products get to patients, is that the less human interaction with the analysis the better. Automation reduces the variability that can occur during manual tasks like pipetting. The Orbitrap Exploris 240 mass spectrometer and the LC-MS control software is easy to use and looks and feels like other Thermo Scientific systems. Once you have been trained on the data analysis workflow it's very intuitive and straightforward, taking the guesswork out of data analysis. That's critical because we don't want any guesswork in ensuring quality medicines get to patients," noted Auclair.

"The solution that Thermo Scientific has for the MAM 2.0 workflow is well thought out, and easy to be trained on and implemented by someone with no experience in biologics. Liang went through the Themo Fisher Scientific training programs, and in a short amount of time, was able to get up to speed and do the analysis."

—Jared R. Auclair

Approachable, to overcome challenges that novice MS users face

"Generally speaking, MS and LC-MS are intimidating technologies for people with no experience," said Auclair. "A lot of what we do at BATL in terms of training is confidence building and making sure people understand the technologies and how to use them. That translates to needing solutions that are approachable, easy to implement and use, and robust, even for someone with no MS experience. The workflow that Thermo Fisher Scientific has put together makes the MAM more approachable and is the easiest for novices to engage with. We used all the preset methods. I would never buy an instrument that doesn't have those, especially at BATL where we have people with and without experience."

A global dedicated Thermo Fisher Scientific MAM team of application scientists, service engineers, and software experts supports customers to ensure maximum workflow productivity and confidence. The team provides installation, instrument and application specific training, and ongoing service with fast response to maximize uptime. A combination of on-site and remote trainings are conducted by the MAM application experts and are customizable to meet customer needs.

Thermo Fisher Scientific's training for BATL covered three general areas: (1) Orbitrap mass spectrometer use, including maintenance and troubleshooting for large-molecule analyses, (2) how to perform the MAM workflow, and (3) software operation and data analysis. According to Auclair, "The training Thermo Fisher Scientific provided was well thought out and holistic, from sample preparation, instrument operation through to data analysis and data analytics. In my experience, the level of training provided by Thermo Fisher Scientific is not typical of other instrument manufacturers. Often times you might get trained on the operation of the instrument, but that is about it. Thermo Fisher Scientific has a fantastic team with tremendous expertise, able to tailor the training to the learner whether they are a novice or more advanced. They are very good at explaining the MAM process in simple and understandable terms."

"Learning and understanding the Thermo Scientific MAM workflow from sample preparation to instrument analysis was straightforward. With very little protein mass spectrometry experience, I was able to learn how to perform the MAM workflow—including peptide mapping, peptide quantification, and new peak detection—in only a few weeks' time."

—Dr. Liang Xue

Training overview

Instrument system: After system installation, the training began with three days of onsite Orbitrap Exploris 240 mass spectrometer training, which covered Orbitrap mass analyzer theory, hardware, calibration, maintenance, and direct-infusion analysis of proteins. Hands-on training on intact-mass analysis and peptide mapping of standard protein samples, and mAb analysis was next. Also covered was use of the instrument control and data analytics software: Chromeleon CDS software, and Thermo Scientific™ BioPharma Finder™ software.

MAM: MAM workflow training began with sample-preparation tips and tricks. Using samples obtained from the digests, operators learned how to run a MAM on the LC-MS system, including monitoring sequence coverage and optimization of the digestion method, LC gradient, and MS parameters. Users also learned to troubleshoot problems they encountered when running real protein samples. This phase of training covered system suitability testing, monitoring reproducibility performance, evaluating chromatograms, and analyzing MS^1- and MS^2-level data using BioPharma Finder software (Figures 2 and 3). The outcome of this step was a robust and reproducible digestion method, and LC-MS parameters for BATL's MAM workflow.

Chromeleon CDS software for MAM analyses: The MAM training was followed by three and half days of remote and on-site training covering CQA analysis and new peak detection.

Protein	Sequence coverage	Number of MS peaks
BSA digested #1	100%	4062
BSA digested #2	100%	4227
BSA digested #3	100%	4230

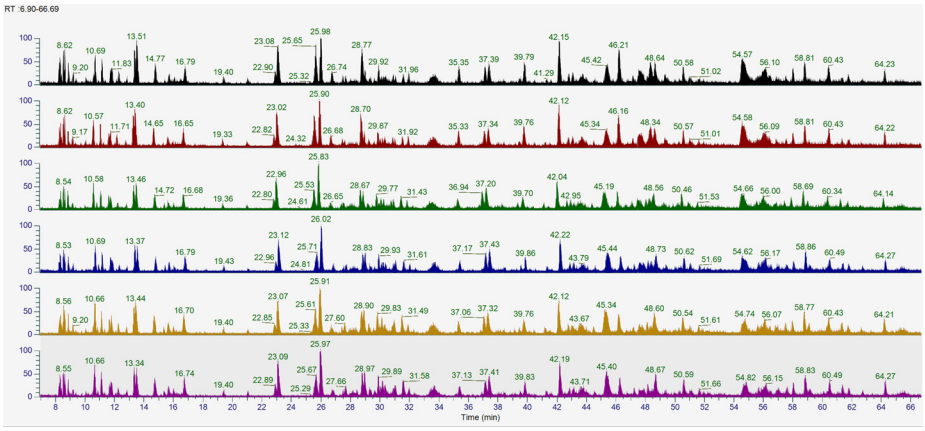


Figure 2. Reproducibility obtained for analysis of replicate Bovine Serum Albumin (BSA) digest peptides and sequence coverage. After only a few trainings, BATL staff were able to perform system suitability and reproducibility studies using BSA. Across three replicates 100% sequence coverage using MS^1 and MS^2 data, and nearly identical spectra, were obtained

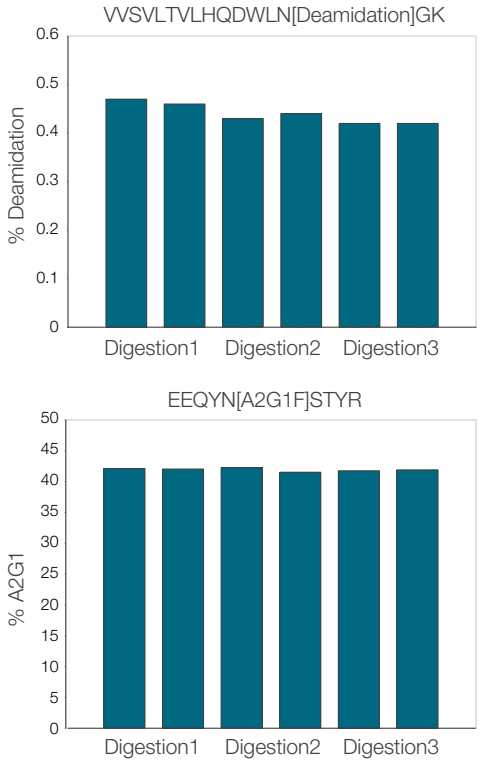


Figure 3. Chromeleon CDS software-generated results from a MAM-based analysis of deamidation in the peptide VVSVLTVLHQDWLN[Deamidation]GK and glycosylation in the peptide of EEQYN[A2G1F] STYR for three separate BSA digests with two replicate runs highlighting data reproducibility.

"Because the MAM provides more information from one experiment that is reproducible and sensitive, and the process is controlled, regulators appreciate the approach. The compliance features in Chromeleon software are particularly crucial and of high value to regulators and the companies implementing MAM workflows."

—Jared R. Auclair

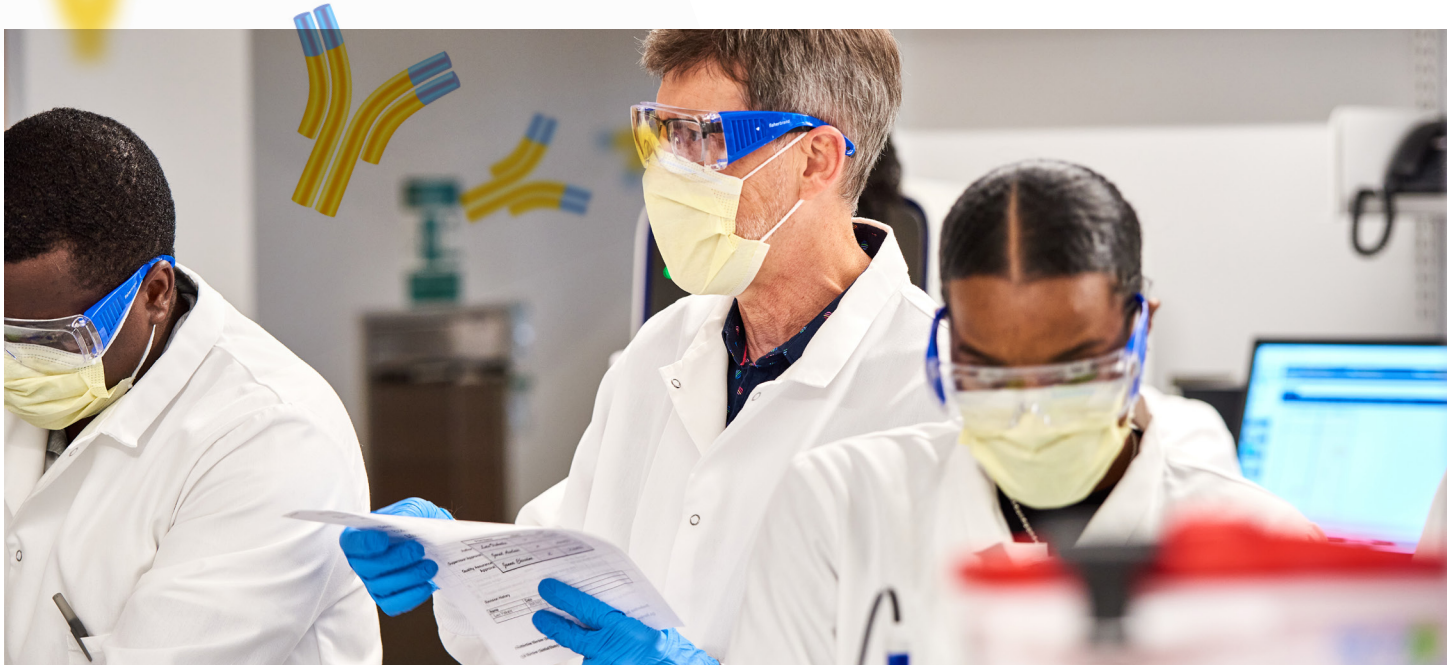
Appeals to regulators

BATL provides training to regulators working in various agencies around the world. Much of this training is focused on communicating what the MAM is and its value in regulatory filings. Auclair commented, "I started training on the idea of the MAM in the context of quality by design to regulators about five years ago and it was a very vast array of unwieldy information. Thermo Scientific's work in the MAM space really streamlined it, so even now it's an accepted practice in a regulatory dossier and perhaps even preferred."

Many of the compliance capabilities in Chromeleon CDS software are leveraged when BATL trains regulators, including security tools for data integrity and traceability, eReports and eSignatures, and automated software and instrument qualification and monitoring.

Conclusion

Embraced by academia, industry, and regulatory leaders concerned with the development and release of safe and effective biotherapeutics, the MAM brings research, development and quality control together to accelerate time-to-market of safe and effective therapeutics. As the leading biotherapeutic analysis solution provider and a trusted partner, Thermo Fisher Scientific has worked closely with BATL and other leaders in academia and industry to offer a purpose-built, end-to-end workflow that overcomes historic barriers to MAM implementation. For BATL, the Thermo Scientific MAM solution provides an ideal platform with capabilities and performance that facilitate all stages of drug development from research and process development to manufacturing QC, while seamlessly linking PQA and CQA characterization and monitoring methods from one phase to the next. To address BATL's training objectives, the Thermo Scientific MAM 2.0 solution is easy for users of varied expertise to learn and use with pre-built protocols and methods, automation, and training and support supplied by Thermo Scientific's dedicated team of MAM application scientists and software experts.



About Jared R. Auclair, Ph.D.

Dr. Jared R. Auclair is the Vice Provost Research Economic Development and Director of Bioinnovation at Northeastern University. In addition to this role, Dr. Auclair directs the BATL. This latter appointment allows Dr. Auclair to collaborate with both academic researchers and industry in the area of biopharmaceutical development and analysis. He has expertise in molecular biology, protein biochemistry, analytical chemistry, protein crystallography, and biological MS, and is interested in understanding the molecular mechanisms of neurodegenerative diseases in order to develop novel therapeutic approaches.

About Liang Xue, Ph.D.

Previously Postdoctoral Research Associate at BATL collaborating with peers in biopharmaceutical analysis, Dr. Liang Xue is now a scientist at Sanofi. Dr. Xue specializes in analytical chemistry (e.g., LC-MS), and received a Master's and Doctorate Degree in Chemistry from the University of Massachusetts, Dartmouth. She has been an active member in the school community, serving as a senator in the UMass Dartmouth graduate student senate. In addition, she was a recipient of the Withycombe-Charalambous Graduate Award based on oral presentation in the August 2020 ACS National Meeting AGFD Division Graduate Student Symposium.

About Northeastern University BATL

Established in 2014, BATL enables innovative biopharmaceutical education, global regulatory convergence, industry partnerships, and use-inspired research to benefit patients worldwide. BATL's state-of-the-art facility offers unique hands-on training for the pharmaceutical industry in the form of affordable, practical, and comprehensive courses. Its diverse, innovative, and globally recognized experiential learning and credentialing programs offer a neutral platform to train the industry's workforce while converging the intricacies of global regulatory considerations for pharmaceutical and biopharmaceutical products. These efforts increase the potential for accelerated drug approvals with quality, safety, and efficacy at the forefront.

BATL provides one-place partnership focused on collaborations that unite various scientific and medical stakeholders across the globe. Projects in analytical, operational, and process improvements, and standard validation are at the core of its research programs. Its research collaborations include the United States Pharmacopeia (USP) for standards validation, Thermo Fisher for MS application validation, and others. BATL is recognized by the Asia-Pacific Economic Cooperation (APEC), the International Council for Harmonisation (ICH), the Pharmaceutical Research and Manufacturers of America (PhRMA), and the World Health Organization (WHO) as a neutral, global facilitator of collaboration between academia, industry, and government toward regulatory convergence.

Learn more at **thermofisher.com/mam**