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Biopharma

The MAM 2.0 workflow enables seamless transition from research and development to quality control

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Keywords

Liquid chromatography high-resolution accurate-mass mass spectrometry (LC-HRAM-MS), high resolution multi-attribute method (HR MAM), Orbitrap Exploris 240, Orbitrap Exploris MX, Vanquish Horizon UHPLC, Vanquish Flex UHPLC, method transfer, monoclonal antibody (mAb), product quality attribute (PQA), new peak detection (NPD), biopharmaceutical characterization and quantitation, BioPharma Finder, Chromeleon CDS, eWorkflow procedure

Application benefits

- A MAM method developed on the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer can be easily transferred to and automatically executed on the Thermo Scientific™ Orbitrap Exploris™ MX mass detector using a Thermo Scientific™ Chromeleon™ eWorkflow™ procedure and achieve highly comparable results.

- The robust, easy to operate, fit for purpose Orbitrap Exploris MX mass detector, operated under compliance-ready Chromeleon CDS Enterprise, delivers consistent quantitation of product quality attributes with minimal instrument-to-instrument variation to meet global QC deployment requirements for MAM.

Goal

Demonstrate seamless method transfer from research and development to quality control enabled by the end-to-end MAM 2.0 workflow

Introduction

Regulatory agencies have been strongly encouraging the biopharmaceutical industry to apply risk-based approaches and adoption of quality by design (QbD) principles with PQA and critical quality attribute (CQA)-centric control strategies in drug product development, manufacturing, and lifecycle management.[1] Full product characterization, product and process development, and CQAs monitoring are the foundation to support this journey of establishing robust bioprocesses with the goal of shortening the drug development timeline. Thus, many biopharma companies have been investing in

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establishing "factory-of-the-future" initiatives to adopt LC-HRAM-MS based Multi-Attribute Method (MAM) for direct monitoring quality attributes in product and process development, and for quality control (QC) lot release applications to improve operational productivity and ensure product quality and safety.[2–4]

Biopharmaceutical developers and manufacturers are working to overcome deployment challenges when moving MAM from research and development organizations into QC environments. Some of these challenges were discussed in a recent publication from Rogstad et al. "Multi-Attribute Method for Quality Control of Therapeutic Proteins".[5] To facilitate this technology transfer, the following aspects need to be taken into consideration: 1) compliance-ready software for MAM data acquisition, processing, and report generation and approval; 2) cost effective, robust, and simple-to-operate MS instruments that can provide comparable results; 3) consistent data generation, day to day, system to system, site to site; 4) scalability; and 5) methods/information/knowledge sharing intra-company and with their CxO partners.

Herein, we demonstrate how the Thermo Scientific™ MAM 2.0 workflow can help to overcome these deployment challenges to enable seamless method transfer from research and development into a QC environment (Figure 1). The newly introduced Orbitrap Exploris MX mass detector was designed to deliver high-quality full MS HRAM data consistently day after day and system to system with operational simplicity. This detector is well suited for deploying MAM in QC as it is fully operated under compliance-ready Chromeleon CDS software allowing for enterprise deployment. In this study, we developed and optimized a MAM assay using tryptic NISTmAb digest on a Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer coupled with a Thermo Scientific™ Vanquish™ Horizon UHPLC system. A Chromeleon eWorkflow™ procedure was created for this MAM assay and used for the subsequent method transfer and execution on two Thermo Scientific™ Orbitrap Exploris™ MX mass detectors, each coupled with a Vanquish Flex UHPLC system. Not only were consistent results achieved on the two Orbitrap Exploris MX mass detectors, but they also showed excellent correlation when compared to the results obtained on the Orbitrap Exploris 240 system.
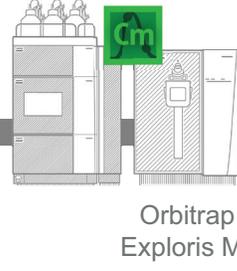

Research & Development

- MAM method development
- PQA monitoring

Orbitrap Exploris 240

eWorkflow
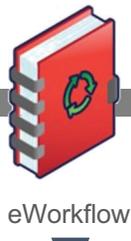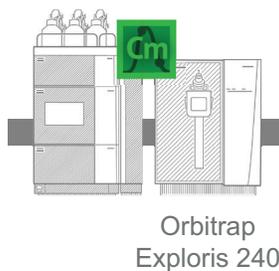
Contains:
- LC & MS acquisition methods
- Injection sequence
- Result view template
- Processing method with pass/fail criteria
- Report template

Manufacturing & QC

- MAM execution
- CQA monitoring

Orbitrap Exploris MX

Data review report Electronic signatures

Figure 1. Schematic overview of MAM method transfer from research and development to manufacturing and quality control using an eWorkflow procedure that 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 with a visual pass/fail representation.

Experimental

Reagents and consumables

- Water (H_2O), UHPLC-MS grade, Thermo Scientific (P/N W8-1)

- Methanol (MeOH), UHPLC-MS grade, Thermo Scientific (P/N A458-1)

- Isopropanol (IPA), Optima™ LC-MS grade, Thermo Scientific (P/N A461-1)

- Acetonitrile (ACN), UHPLC-MS grade, Thermo Scientific (P/N A956-1)

- Formic acid (FA), Pierce™ LC/MS grade (P/N TS-28905)

- NISTmAb humanized IgG1κ monoclonal antibody (NIST, RM 8671, Lot 14HB-D-002)

- 9 mm Autosampler inserts, Thermo Scientific (P/N 033753A)

- 9 mm Clear glass screw thread vials, Thermo Scientific (P/N C5000-1)

Sample preparation

Tryptic digestion of NISTmAb: 1 µg/µL tryptic digest of NISTmAb was prepared using a previously described procedure.[6] Twenty aliquots of digested NISTmAb were prepared by transferring 40 µL each to 9 mm autosampler inserts. The vials were screw-capped and stored at -80 °C until used. Prior to analysis, fresh aliquots were thawed in the Vanquish Flex/Horizon autosampler set at 6 °C for 1 hour. The vials were then vortexed for 10 s to achieve homogeneity before sample injections.

Chromatography

For peptide separation, either the Vanquish Flex or Vanquish Horizon UHPLC system can be used, as both systems exhibit similar performance for the applied gradient detailed in Table 1. The modules present in each UHPLC system are listed in Table 2. For all analyses, 2 µL containing a total of 2 µg digest samples were injected onto a Thermo Scientific™ Hypersil GOLD™ VANQUISH™ C18 UHPLC column using the LC gradient and conditions outlined in Table 1.

Mass spectrometry

Peptide mapping analysis of NISTmAb was first performed using data-dependent Top 5 MS^2 (ddMS^2) method on the Orbitrap Exploris 240 system. Raw ddMS^2 data were processed in Thermo Scientific™ BioPharma Finder™ software 5.0 (detailed processing parameters are shown in the Software section), and selected group of PQAs were monitored using a full MS scan method on

Table 1. LC and autosampler conditions

Setting	Parameter	
UHPLC column	Hypersil GOLD VANQUISH C18 UHPLC column, 150 × 2.1 mm, 1.9 µm (P/N 25002-152130-V)	
Column temp.	25 °C	
Flow rate	0.25 mL/min	
Solvent A	H_2O + 0.1% FA	
Solvent B	ACN + 0.1% FA	
Gradient	Time (min)	%B
	0	1
	5	1
	6	10
	70	35
	72	90
	77	90
	79	1
	81	1
	83.5	10
	91.5	45
	93	90
	99	90
	101	1
	115	1
Injection volume	2 µL	
Needle wash solution	10% MeOH in water (v/v) with 0.1% FA	
Seal rinse solution	10% MeOH in water (v/v) (Vanquish Flex) 75% IPA in water (v/v) with 0.1% FA (Vanquish Horizon)	
Autosampler temp.	6 °C	
Thermostatting mode	Still Air	
Needle wash option	Before and after injection	
Wash speed and time	34 µL/s for 10 s	

Table 2. Vanquish UHPLC system modules and part numbers

Modules	Vanquish Flex (P/N)	Vanquish Horizon (P/N)
System Base F/H	VF-S01-A-02	VF-S01-A-02
Binary Pump F/H	VF-P10-A-01	VH-P10-A
Split Sampler FT/HT	VF-A10-A-02	VH-A10-A-02
Column Compartment H	VH-C10-A-03	VH-C10-A-03

both Orbitrap Exploris 240 and Orbitrap Exploris MX systems. Detailed instrument methods and source parameters for both systems are summarized in Table 3.

Table 3. Instrument method parameters for the Orbitrap Exploris 240 and Orbitrap Exploris MX systems (Note: Unless otherwise indicated, default parameters were used.)

Instrument	Orbitrap Exploris 240	Orbitrap Exploris MX
MS source parameters		
Spray voltage (V)	3,500	
Sheath gas (Arb)	30	
Aux gas (Arb)	10	
Sweep gas (Arb)	1	Same as Orbitrap Exploris 240
Ion transfer tube temperature (ºC)	225	
Vaporizer temperature (ºC)	200	
Full scan parameters		
Expected LC peak width (s)	6	
Resolution	120,000	
Scan range (*m/z*)	200–2,000	Same as Orbitrap Exploris 240
Time range (min)	0–70 minutes	
AGC target	1E6	
Maximum injection time (ms)	100	
Data dependent MS² scan parameters		
MIPS filter		
Monoisotopic peak determination	Peptide	
Intensity filter		
Intensity threshold (counts)	5.0e3	
Charge states filter		
Include charge state (s)	2–8	
Dynamic exclusion filter		Not applicable
Dynamic exclusion mode	Custom	
Exclusion duration (s)	3	
ddMS² scan parameters		
Isolation window (*m/z*)	1.2	
HCD collision energy (%)	27	
Resolution	15,000	
AGC target	1E5	
Maximum injection time (ms)	250	

Peptide mapping and PQA selection in BioPharma Finder software

For peptide mapping, raw data obtained from the Orbitrap Exploris 240 mass spectrometer comprising Full MS and ddMS² spectra were processed against the NISTmAb protein sequence in BioPharma Finder software v. 5.0. Peptide mapping method settings are listed in Table 4. For this workflow demonstration, the selected NISTmAb PQAs are deamidation (e.g., N387), glycation (e.g., K189), glycosylation (e.g., A2G0F/A2G1F/A2G2F/A1G0F/A1G1F/A2Ga1G1F/M5/unglycosylated forms of N300), isomerization (e.g., D283), oxidation (e.g., M87 and M255), succinimide formation (e.g., N289 and N387), and C-terminal lysine truncation (e.g., G449). For each PQA, both native and modified components are selected, and four isotopes of the identified charge states are exported into a target peptide workbook.

Table 4. BioPharma Finder 5.0 processing method settings for peptide mapping of NISTmAb (Note: Unless otherwise indicated, default parameters were used.)

Sequence parameters	
Protein sequence	NISTmAb
Category	Peptide mapping
Protease name	Trypsin (C-term KR)
Specificity	High
Static modification	Carboxymethylation (C)
Variable modification	Deamidation (N, Q) Gln→Pyro-Glu (N-term) Glycation (K) Lys (C-term) NH$_3$ loss (N, Q) Oxidation (MW)
Glycosylation	CHO
Component detection parameters	
Task to perform	Final all ions in the run
MS noise level	
S/N threshold	
Typical chromatographic peak width (min)	Automatic detection by software
Maximum chromatographic peak width (min)	
Maximum retention time shift (min)	
Identification parameters	
Search by full ms only	No
Use MS/MS	Use all MS/MS
Maximum peptide mass	11,000
Mass accuracy (ppm)	8

Targeted processing method for evaluation of PQAs in Chromeleon CDS

A NISTmAb PQA processing method was created based on the MS quantitative template. The processing method applies the following settings: MS detection algorithm Genesis, manually defined mass tolerance 8 ppm, inhibit integration for TIC channel, and Gaussian smoothing 5 points. Composite scoring using isotopic dot product, mass accuracy, and peak apex alignment options were applied with scoring results to pass only if three criteria are met. The target peptide workbook was imported to the MS component table of the processing method, retention times of individual components were adjusted, and peak integration parameters were optimized to ensure accurate component detection and consistent peak integration across datasets.

View settings

The view settings window contains the same set of panes as described previously:[6]

- MS component pane showing the overlay of XICs of the quantitation peak and the four most abundant isotopes or confirming ion peaks

- The MS spectrum of the precursor at the peak apex

- A component table showing the master peptide and its charge states

- An interactive chart comparing the observed and theoretical isotope distribution for the selected component

Report template

A customized report was built to show an evaluation summary of NISTmAb PQAs across 10 replicate injections. The report provides a modification summary for % *N*-Glycans, % N387 deamidation, % K189 glycation, % D283 isomerization, % M87 and % M255 oxidation, % N289 and % N387 succinimide formation, and % G449 C-terminal lysine truncation with a pass/fail status. It also provides a graphical representation showing the reproducibility for the monitored PQAs.

eWorkflow procedure

MAM method transfer is enabled using the Chromeleon eWorkflow procedure. This electronic procedure consists of all the associated items (LC and MS methods, targeted processing method, view setting, report template, and sequence layout) required for setting up the injection sequence, through processing the data, to reporting of the results. Here, an eWorkflow procedure for NISTmAb PQA monitoring was created using the Orbitrap Exploris 240 mass spectrometer and then transferred to and deployed on the Orbitrap Exploris MX mass detector. The injections sequence contains a total of 12 injections. The first two injections are set up to condition the column, injections 3 to 12 are replicate injections of NISTmAb digest samples. The targeted processing method was only applied to replicate injections of NISTmAb digest samples for the analysis of NISTmAb PQAs.

Software

Chromeleon 7.3.1 CDS software was used for all data acquisition, MAM data processing, and reporting. BioPharma Finder 5.0 software was used for peptide mapping and target peptide workbook creation.

Results and discussion

Peptide mapping analysis and MAM method development on the Orbitrap Exploris 240 system

Peptide mapping was performed to identify the NISTmAb PQAs using the described ddMS^2 method on the Orbitrap Exploris 240 mass spectrometer. ddMS^2 data were acquired using Chromeleon CDS and automatically uploaded to a secured server-based data sharing center after each injection was completed. Multiple users with access privileges can process the raw data for peptide mapping using BioPharma Finder 5.0 software, enabling accelerated method optimization and determination of PQAs. A target peptide workbook, containing the selected PQAs, was first created from the characterization step and saved in the data sharing center. Users can access this target peptide workbook via a secure sign on, and import the target peptide list to the component table of their MAM processing methods in Chromeleon CDS. For robust integration, the MAM processing method was optimized by adjusting the retention time window and peak detection algorithm for each component such that every component is confirmed and properly integrated across all injections. An example report outputting the % modification summary table for the monitored PQAs is shown in Figure 4. In addition to the summary table, a graphic representation of the modification summary is also provided to demonstrate the reproducibility of the analysis.

Seamless method transfers from the Orbitrap Exploris 240 mass spectrometer to the Orbitrap Exploris MX mass detector using the eWorkflow procedure

Once the MAM processing method and report template have been optimized, a Chromeleon eWorkflow procedure was created. Since Chromeleon CDS is an enterprise software, the eWorkflow procedure can be launched using any active instruments that are connected to the enterprise network. No physical transfer of any components is required. To demonstrate the ease of use, an eWorkflow procedure was deployed in parallel on three instruments, one Orbitrap Exploris 240 mass spectrometer and two Orbitrap Exploris MX mass detectors,

for the analysis of NISTmAb PQAs. The same batch of tryptic NISTmAb digest samples was used to reduce quantitative variations. Ten replicate injections were analyzed on each system and their average % modification was plotted against the selected NISTmAb PQAs. As shown in Figure 2, not only do the two Orbitrap Exploris MX mass detectors exhibit very closely maching quantitative performance for the monitored PQAs, the obtained results are comparable to the ones obtained on the Orbitrap Exploris 240 mass spectrometer with very low instrument-to-instrument variation below 10% RSD even for the low abundant species..

(a)

(b)
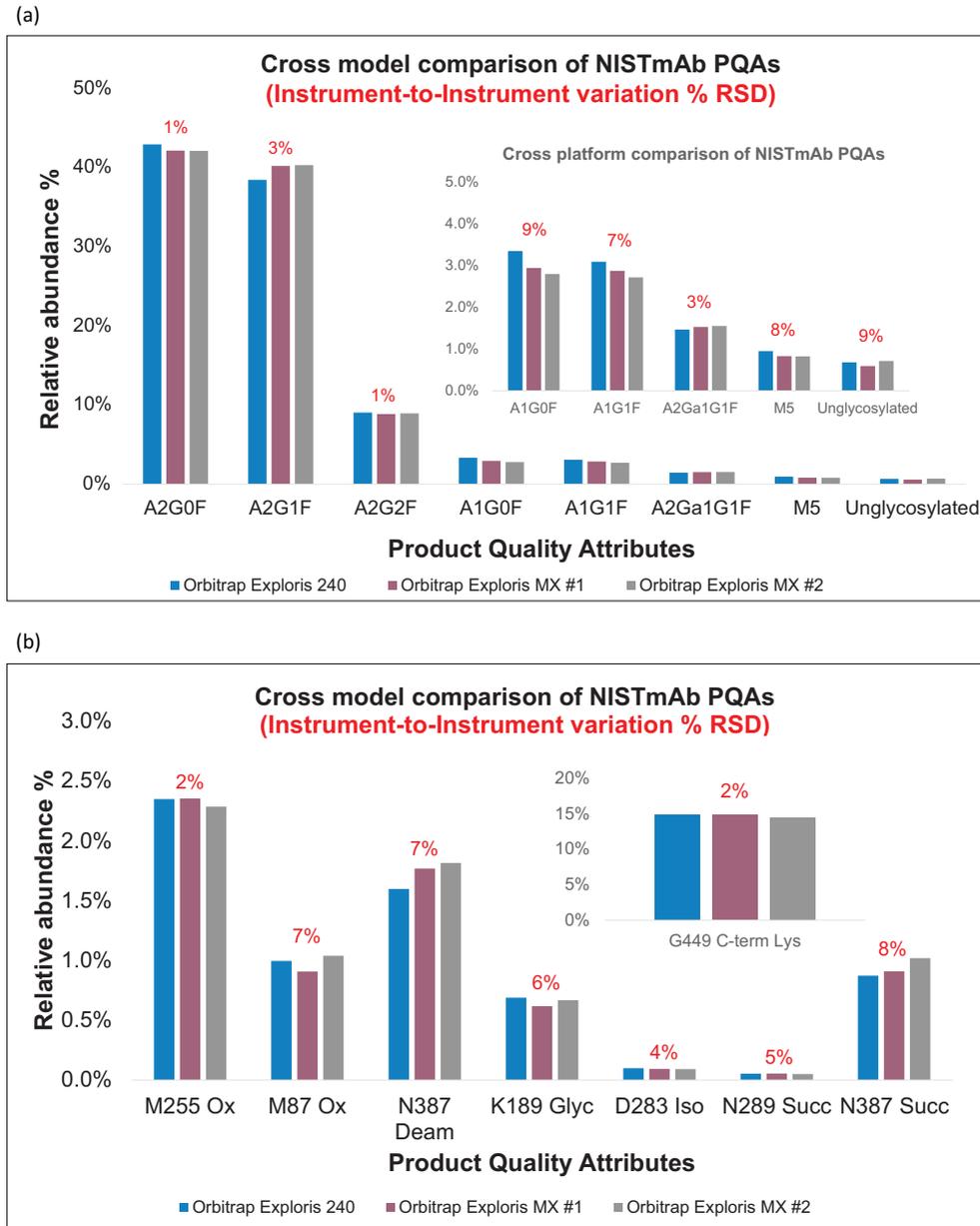
Figure 2. Cross model evaluation of NISTmAb PQAs. (a) Quantitation results obtained for selected PQAs across three instrument units. Average relative abundance % of the monitored PQAs were plotted, data were averaged from 10 replicate injections, and the averaged results were compared across three instruments. (b) Quantitation results of *N*-glycosylation across three instruments. Average relative abundances % (N=10) of the monitored glycoforms were plotted.

Fit-for-purpose, robust, easy-to-use Orbitrap Exploris MX mass detector for routine MAM analysis in QC

The Orbitrap Exploris MX mass detector was purposefully designed to deliver consistent unit-to-unit performance and to meet the needs for routine HRAM mass monitoring in regulated environments. This Orbitrap mass detector is a new member of the Orbitrap Exploris platform, sharing key hardware components including but not limited to the Thermo Scientific™ OptaMax™ NG ion source with H-ESI II sprayer and operating under Orbitrap Exploris instrument control software implemented in Chromeleon CDS. Thus, instrument methods are directly deployable across these two platforms. This harmonization of the hardware platform facilitates seamless method migration from research and development to manufacturing and quality control without additional re-development of transferred methods. In addition, a new internal mass calibration option was introduced, further simplifying the MS operation by reducing the frequency for mass calibration while ensuring less than 3 ppm root mean square

mass drift and stability over at least four weeks.[7] This feature utilizes an internal calibrant that is applied post source for single point mass calibration that is applied to the full mass range, thus eliminating the need to change the ion source or to supply calibration solution.

To demonstrate the exceptional reproducibility that the Orbitrap Exploris MX mass detector provides for MAM analyses, 10 replicate injections of tryptic NISTmAb digest samples were analyzed and % modification calculated by taking the peak area sum of the modified form and dividing it by the peak area sum of both modified and native forms. These ratio measurements across ten replicates were plotted against the selected NISTmAb PQAs. As shown in Figure 3a, less than 5% relative standard deviation (% RSD) was achieved for the evaluation of selected PQAs. Moreover, exceptional precision was obtained for quantitation of various glycoforms that ranged in relative abundance from as low as sub 1% (M5 and unglycosylated) to as high as 40% (A2G0F and A2G1F) as shown in Figure 3b.

(a)

(b)
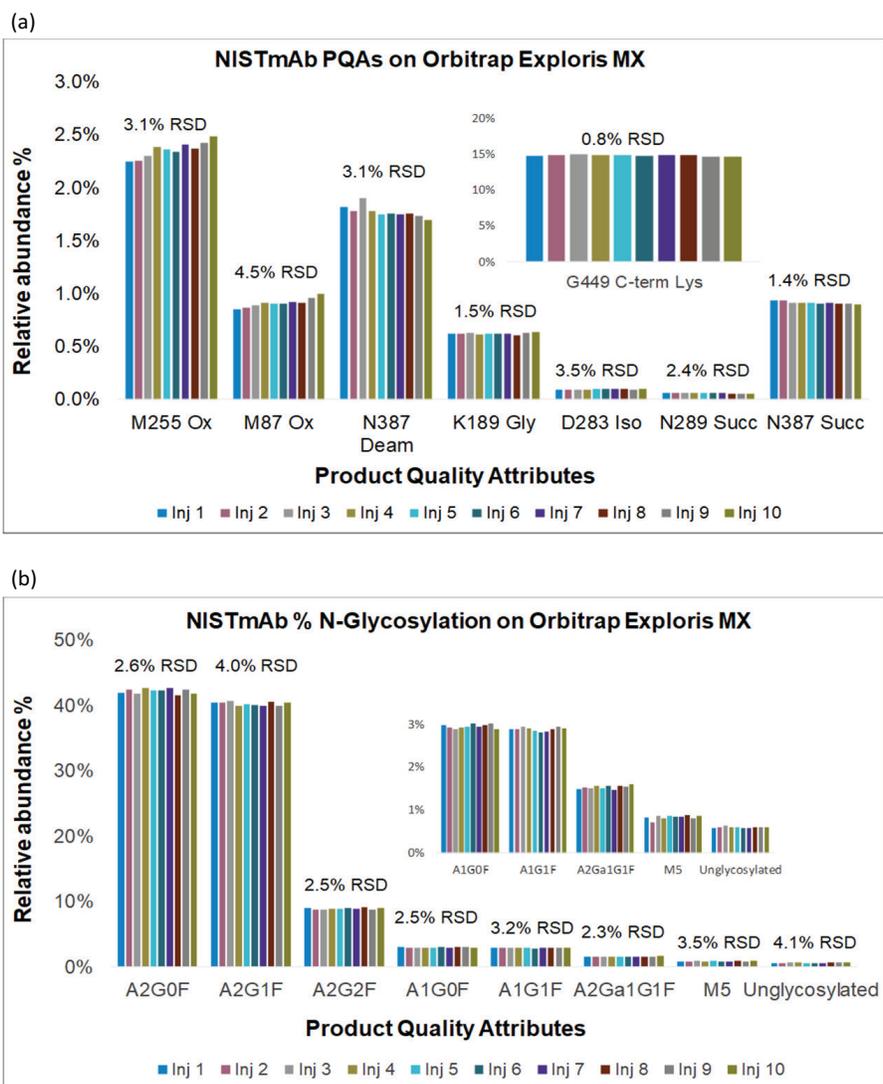
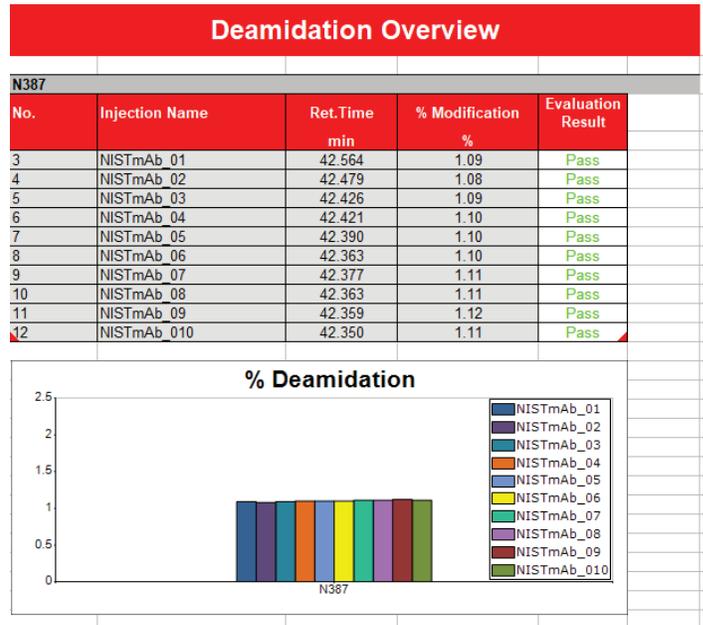**Figure 3. Monitoring of selected NISTmAb PQAs on the Orbitrap Exploris MX mass detector across 10 replicate injections.**
(a) Quantitation results obtained from M255 and M87 oxidation, N387 deamidation, K189 glycation, D283 isomerization, N289 and N387 succinimide profile. (b) Quantitation results obtained for the *N*-glycosylation profile for tryptic NISTmAb digest across 10 replicate injections.
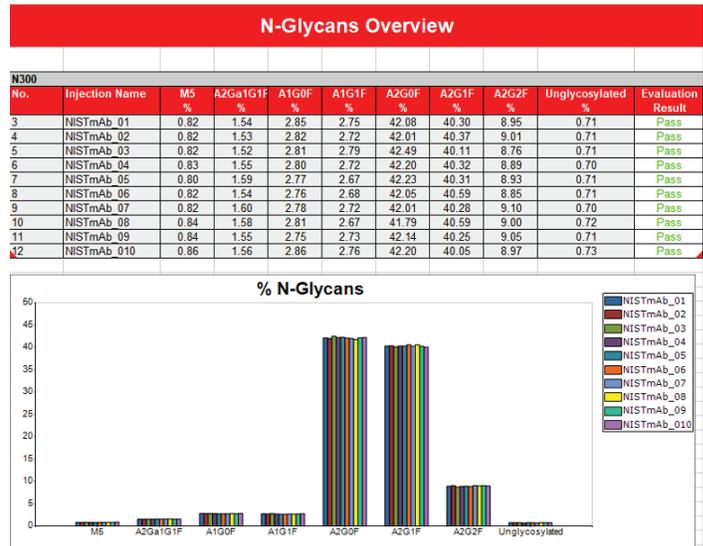
Customizable report

A customizable report for the MAM assay was built to show the relative quantitation of NISTmAb CQAs and a pass/fail status to evaluate whether the CQAs are within certain acceptable limits. For actual biotherapeutic drug products, these thresholds are determined through rigorous risk assessment studies that involve a systematic evaluation of each PQA considering the potential impact on safety and efficacy of the tested product. However, as a proof of demonstration, a tentative threshold was set for each monitored NISTmAb CQA, and the sample injection passes only if the quantified result is below the set threshold. For instance, as shown in Figure 4a, all 10 NISTmAb digest samples had passed as the measured % N387 is less than 2% set threshold. Similarly, the amount of *N*-glycans measured across all injections were also within the acceptable limits as shown in Figure 4b. In addition to the summary table, a custom bar graph plot showing the quantified CQA across all injections is also included in the report.

Chromeleon CDS also provides eSignature support to sign off electronic reports, offering pharmaceutical companies a secure way to verify the authenticity of these critical documents for record keeping. This built-in feature enables quality management systems for streamlining documentation, validation, and approval processes to meet the stringent compliance and quality standards.

(a)

Deamidation Overview

N387

No.	Injection Name	Ret.Time min	% Modification %	Evaluation Result
3	NISTmAb_01	42.564	1.09	Pass
4	NISTmAb_02	42.479	1.08	Pass
5	NISTmAb_03	42.426	1.09	Pass
6	NISTmAb_04	42.421	1.10	Pass
7	NISTmAb_05	42.390	1.10	Pass
8	NISTmAb_06	42.363	1.10	Pass
9	NISTmAb_07	42.377	1.11	Pass
10	NISTmAb_08	42.363	1.11	Pass
11	NISTmAb_09	42.359	1.12	Pass
12	NISTmAb_010	42.350	1.11	Pass

(b)

N-Glycans Overview

N300

No.	Injection Name	M5 %	A2Ga1G1F %	A1G0F %	A1G1F %	A2G0F %	A2G1F %	A2G2F %	Unglycosylated %	Evaluation Result
3	NISTmAb_01	0.82	1.54	2.85	2.75	42.08	40.30	8.95	0.71	Pass
4	NISTmAb_02	0.82	1.53	2.82	2.72	42.01	40.37	9.01	0.71	Pass
5	NISTmAb_03	0.82	1.52	2.81	2.79	42.49	40.11	8.76	0.71	Pass
6	NISTmAb_04	0.83	1.55	2.80	2.72	42.20	40.32	8.89	0.70	Pass
7	NISTmAb_05	0.80	1.59	2.77	2.67	42.23	40.31	8.93	0.71	Pass
8	NISTmAb_06	0.82	1.54	2.76	2.68	42.05	40.59	8.85	0.71	Pass
9	NISTmAb_07	0.82	1.60	2.78	2.72	42.01	40.28	9.10	0.70	Pass
10	NISTmAb_08	0.84	1.58	2.81	2.67	41.79	40.59	9.00	0.72	Pass
11	NISTmAb_09	0.84	1.55	2.75	2.73	42.14	40.25	9.05	0.71	Pass
12	NISTmAb_010	0.86	1.56	2.86	2.76	42.20	40.05	8.97	0.73	Pass

Figure 4. Sample report showing a summary of NISTmAb a) N387 deamidation and b) N-glycosylation across 10 replicate injections with a pass/fail evaluation result

Conclusion

Here, we have described the deployment of an eWorkflow procedure developed for the analysis of NISTmAb digest. Data generated on three instruments were compared and assessed regarding reproducibility and repeatability.

- The MAM eWorkflow procedure was developed on an Orbitrap Exploris 240 mass spectrometer and executed seamlessly on two Orbitrap Exploris MX mass detectors with all three instruments connected to the Chromeleon CDS enterprise network.

- The instrument method developed on an Orbitrap Exploris 240 mass spectrometer was included in the eWorkflow procedure and directly transferred to an Orbitrap Exploris MX mass detector without any manual adaptations required.

- The Orbitrap Exploris MX mass detector and the Orbitrap Exploris 240 mass spectrometer provided quantitative performance with very similar results for the evaluation of NISTmAb PQAs.

- The Orbitrap Exploris MX mass detector demonstrated excellent reproducibility for the analysis of NISTmAb PQAs.

- The new one-point calibration feature available on the Orbitrap Exploris MX mass detector ensures minimal mass deviation for long periods of time without performing a full mass calibration.

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