Performing the biopharmaceutical multi-attribute method (MAM)

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Introduction

The Multi-Attribute Method (MAM) was introduced in 2015[1] and has since gained increasing recognition and adoption among biopharmaceutical manufacturers and regulatory agencies.[1-3] MAM enables characterization and monitoring of a wide range of critical quality attributes (CQAs) in a single high resolution accurate mass (HRAM)-based liquid chromatography mass spectrometry (LC-MS) assay. Additionally, MAM allows for detection of known and unknown impurities, a procedure referred to as new peak detection (NPD). MAM can be implemented at different phases of biopharmaceutical drug development, from research and process development to quality control (QC).

The analysis of HRAM MS data for MAM consists of three major components:

1) Peptide mapping for identification of product quality attributes (PQAs) and full sequence coverage of the therapeutic drug product
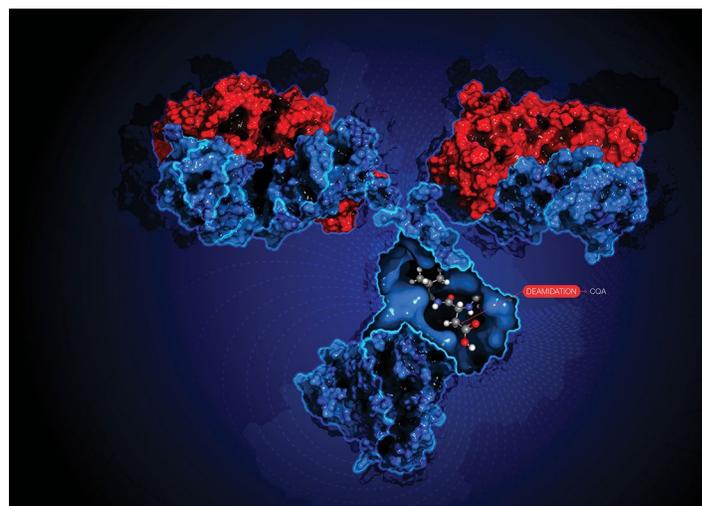
2) Monitoring of CQAs with targeted quantitation

3) NPD for detection of product- and process-related impurities

The successful implementation of MAM in a research laboratory and its subsequent transfer into the routine analytical department requires robust hardware to provide consistent data across labs as well as reliable, intelligible software that can manage all aspects of the workflow as described above. To meet this requirement, we have recently introduced the Thermo Scientific™ HR MAM workflow[4] providing highly reproducible peptide separation

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on the Thermo Scientific™ Vanquish™ Horizon UHPLC system, HRAM mass detection with Orbitrap™ technology, and streamlined data analysis using Thermo Scientific™ BioPharma Finder™ software and Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software.

In this technical note, we provide an overview of how HR MAM data are processed in BioPharma Finder software and Chromeleon CDS as well as how the handover between discovery and compliance-ready data analysis is best executed. In addition, we highlight specific software features important for MAM implementation, and briefly describe key compliance aspects of Chromeleon CDS.

HR MAM data flow in BioPharma Finder software and Chromeleon CDS

Successful implementation of MAM begins with attribute characterization in the discovery phase (Figure 1). This requires acquisition of LC-MS/MS data for performing peptide mapping in BioPharma Finder software. The aim here is to identify all peptides and their post-translational modifications (PTMs) to obtain a full list of PQAs. After risk-assessment studies, a subset of PQAs are identified as CQAs and are selected to build the target peptide workbook in BioPharma Finder software that can then be imported into compliance-ready Chromeleon CDS for quantitation and monitoring based on MS[1]-only data (Figure 1). This targeted monitoring strategy of defined CQAs is complemented with NPD, in which a comparative analysis of unknown samples *versus* a reference sample is carried out to detect new peaks ("impurities") in the sample.

In the following sections, we describe the MS data acquisition using Chromeleon CDS and the main steps of HR MAM data analysis.
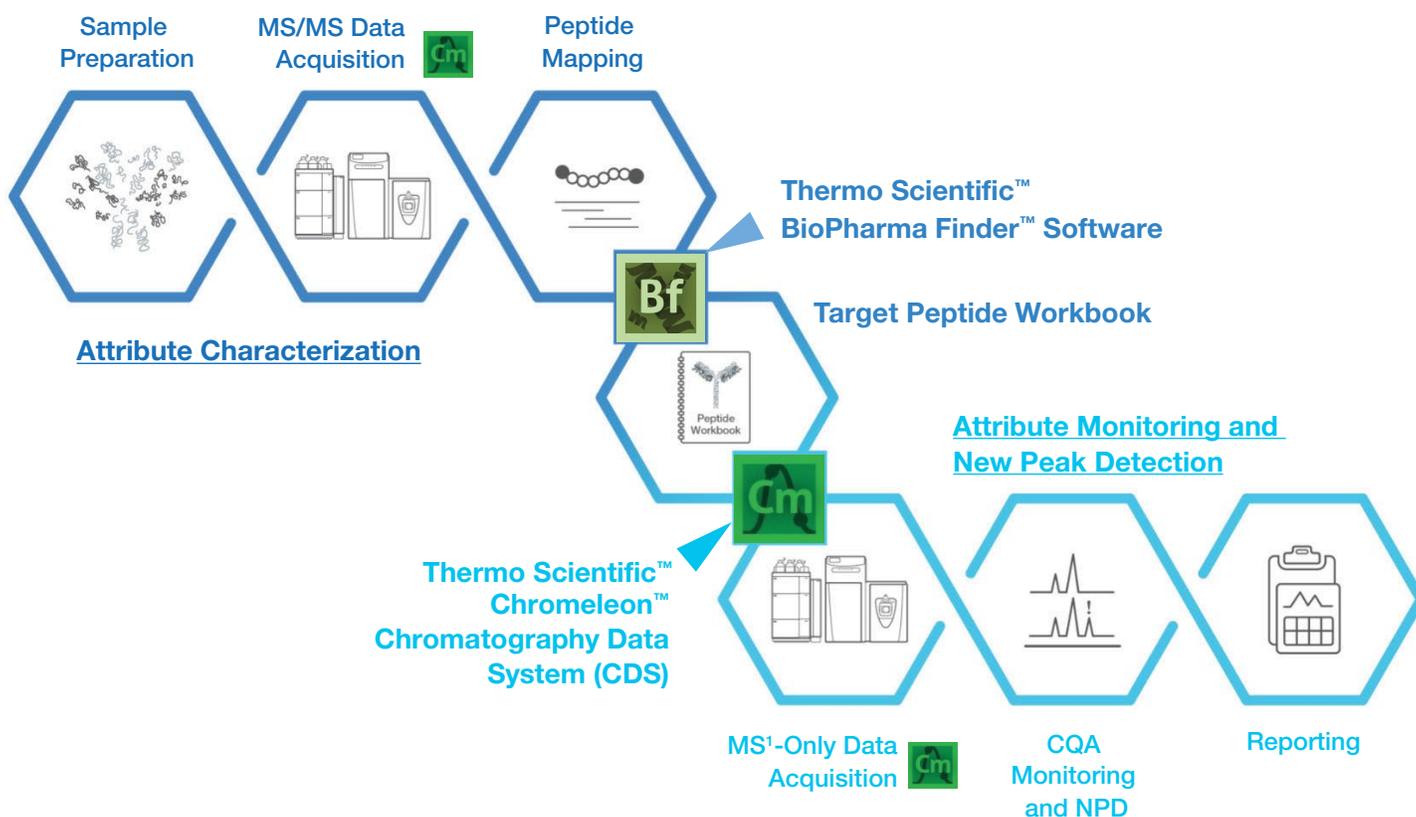

Figure 1. HR MAM data acquisition and processing flow in BioPharma Finder software and Chromeleon CDS

Data acquisition in Chromeleon CDS

Chromeleon software allows for 21 CFR Part 11 compliance-ready data acquisition and instrument control of the entire LC-MS system composed of the Vanquish Horizon UHPLC system and the Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ or Exactive™ Plus Orbitrap mass spectrometer. The acquired data are automatically saved to a data vault in a predefined database, with any changes to the data recorded in the data audit trail, thereby ensuring full data integrity. MS[1]-only data acquired for CQA relative quantitation and monitoring, plus NPD, have a specific processing method associated for data analysis within Chromeleon CDS.

Peptide mapping in BioPharma Finder software

The process of peptide mapping requires the amino acid sequence of the protein of interest as an entry in the Protein Sequence Manager. The protein sequence can be imported as a FASTA file followed by setting fixed and variable modifications.

The BioPharma Finder software processes the MS/MS raw data exported from a Chromeleon CDS sequence. The data file is mapped against the chosen protein sequence using an appropriate processing method, which can be built from the basic default method with minimal optimization.

The result of peptide mapping can be viewed in two tabs. The customizable Process and Review tab (Figure 2) contains a rich amount of information, such as base peak and selected ion chromatograms (Figure 2a), comparison of experimental versus predicted MS/MS spectra as well as comparison of modified versus non-modified (Figure 2b), and the results table containing all identified peptides (Figure 2d). If multiple raw files are compared, the ratio and absolute MS area of any peptide selected in the results table will also be displayed (Figure 2c). The location of the identified peptide in the protein sequence and the experimental full scan *versus* the deconvoluted scan spectra can also be viewed in this tab.
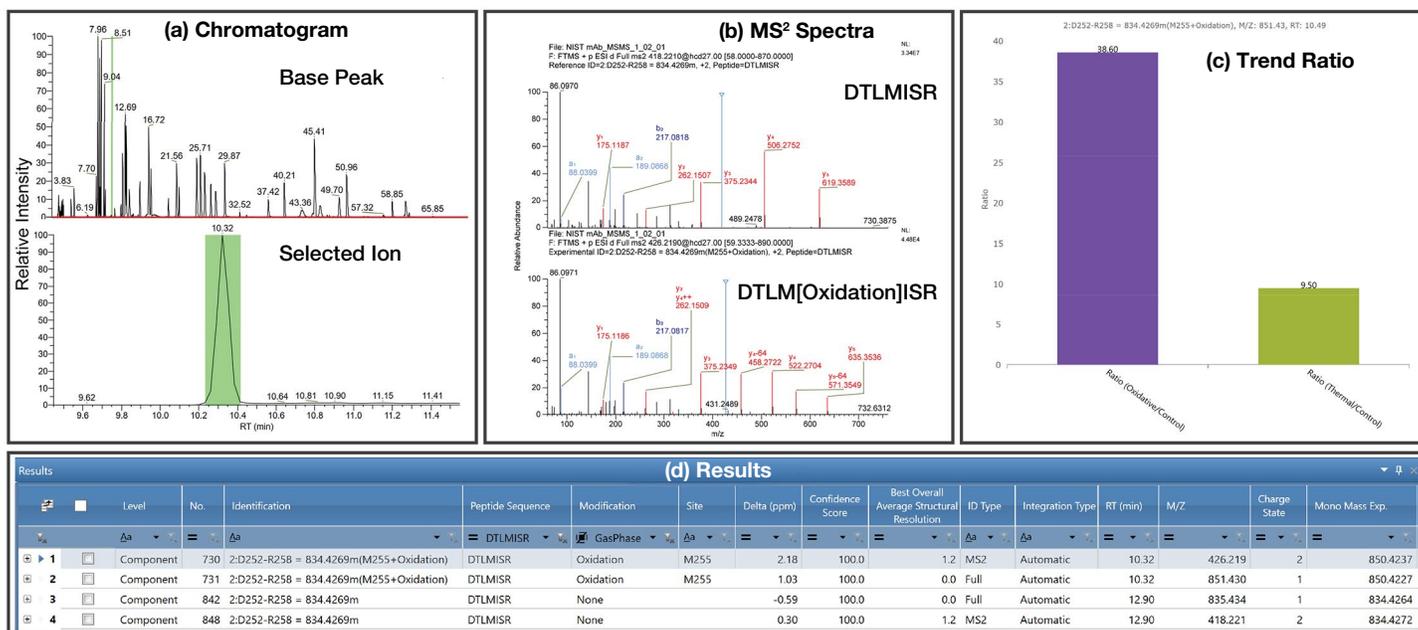

Figure 2. Example of customizable result view in Process and Review tab of BioPharma Finder software for peptide DTLMISR and its oxidized form. (a) Base peak and zoomed selected ion chromatogram, (b) experimental MS/MS spectra of non-modified (top) and oxidized (bottom) DTLMISR with assignment of *b*- and *y*-type fragment ions labeled in blue and red colors, respectively, (c) trend ratio when multiple files are compared, and (d) results table filtered for peptide DTLMISR and its oxidized form.

The Mapping tab in the peptide mapping result of BioPharma Finder software contains information related to sequence coverage and percent abundance for modifications (Figure 3). The quality of the peptide map can quickly be assessed from the chromatographic representation of identified peptides associated to the different subunits of the molecule (Figure 3a). A sequence coverage map (Figure 3b) displays the overall sequence coverage of each subunit. The intensities and retention times of identified peptides are represented by color code and numbers, respectively, as illustrated in the sequence below. The Modification Summary page lists percent abundance of modifications in a table, with the peptides used for ratio calculations highlighted. The relative abundance of specific modifications can also be visualized in a graphical form (Figure 3c).
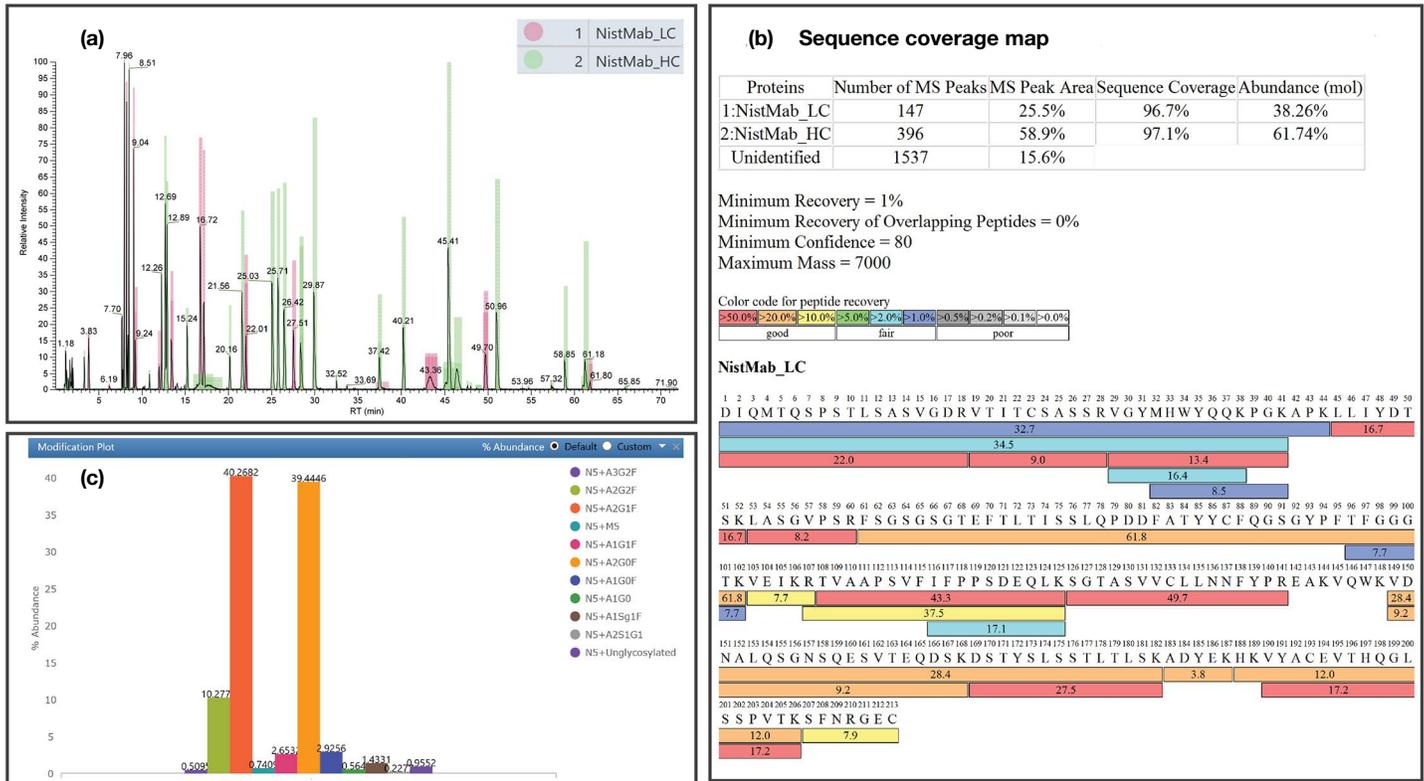

Figure 3. BioPharma Finder software Mapping tab. (a) Chromatographic representation of identified peaks for NISTmAb light and heavy chains (LC and HC), (b) sequence coverage map, including a summary table and a peptide heat map (heat map of NISTmAb_HC not shown here), and (c) bar chart of percent abundance of NISTmAb glycosylation shown in the modification summary

CQA selection and target peptide workbook creation

For creation of the target peptide workbook, the quality attributes determined to be critical for the molecule of interest are selected in the results table (Figure 4a) and exported to a new or existing target peptide workbook (Figure 4b). The selection of CQAs in the results table can be facilitated using multiple filtering options available in BioPharma Finder software, e.g., filtering by peptide sequence. As an example, "Peptide Sequence = EEQYNSTYR" was used to find this peptide and its associated glycoforms shown in Figure 4. The target peptide workbook can be created after all the CQAs are selected in the results table. Alternatively, it can be built in a stepwise fashion by filtering for specific peptide sequences and appending the filtered lists to the same workbook.

The target peptide workbook captures detailed information about each CQA, such as peptide sequence, modification, charge state, and theoretical mass. Within the Target Peptide Workbook tab (Figure 4b) modifications can be made to the saved CQAs, including redefining the charge state range and/or number of isotopes (typically 3–5) used for relative quantitation. It is also possible to add more CQAs relating to other species, such as peptides from host cell proteins (HCPs) or others with special modifications, to the existing peptide workbook using Theoretical Protein/ Peptide Manager in BioPharma Finder software. Upon completion, the target peptide workbook can be exported as a .wbpf file for import into a processing method of Chromeleon CDS for CQA quantitation, as described in the following sections.

(a) Results table

		Level	No.	Identification	Peptide Sequence	Modification	Site	Delta (ppm)
1	✓	Component	453	2:E296-R304 = 1188.5047m(N300+A1G0)	EEQYNSTYR	A1G0	N300	-1.43
2	✓	Component	454	2:E296-R304 = 1188.5047m(N300+A1G0)	EEQYNSTYR	A1G0	N300	-1.11
3	✓	Component	447	2:E296...	EEQYNSTYR	A1G0F	N300	-0.74
4	✓	Component	448	2:E296...	EEQYNSTYR	A1G0F	N300	-0.84
5	✓	Component	412	2:E296...	EEQYNSTYR	A1G1F	N300	-0.55
6	✓	Component	440	2:E296...	EEQYNSTYR	A1G1F	N300	-0.46
7	✓	Component	495	2:E296...	EEQYNSTYR	A1Sg1F	N300	-1.13
8	✓	Component	500	2:E296...	EEQYNSTYR	A1Sg1F	N300	-1.04
9	✓	Com...			EEQYNSTYR	A2G0F	N300	-0.87
10	✓	Com...		R304 = 1188.5047m(N300+A2G0F)	EEQYNSTYR	A2G0F	N300	-0.50
11	✓	Component	405	2:E296-R304 = 1188.5047m(N300+A2G1F)	EEQYNSTYR	A2G1F	N300	-0.43
12	✓	Component	408	2:E296-R304 = 1188.5047m(N300+A2G1F)	EEQYNSTYR	A2G1F	N300	-0.60
13	✓	Component	415	2:E296-R304 = 1188.5047m(N300+A2G1F)	EEQYNSTYR	A2G1F	N300	-0.05
14	✓	Component	393	2:E296-R304 = 1188.5047m(N300+A2G2F)	EEQYNSTYR	A2G2F	N300	2.69
15	✓	Component	401	2:E296-R304 = 1188.5047m(N300+A2G2F)	EEQYNSTYR	A2G2F	N300	-1.10
16	✓	Component	499	2:E296-R304 = 1188.5047m(N300+A2S1G1)	EEQYNSTYR	A2S1G1	N300	-1.17
17	✓	Component	399	2:E296-R304 = 1188.5047m(N300+A3G2F)	EEQYNSTYR	A3G2F	N300	1.21
18	✓	Component	416	2:E296-R304 = 1188.5047m(N300+M5)	EEQYNSTYR	M5	N300	-0.90
19	✓	Component	431	2:E296-R304 = 1188.5047m(N300+M5)	EEQYNSTYR	M5	N300	-1.00
20	✓	Component	504	2:E296-R304 = 1188.5047m(N300+Unglycosylated)	EEQYNSTYR	Unglycosylated	N300	2.57

Context menu:
- Filters ▸
- Export All Components ▸
- Export Checked Components ▸
- Create .mgf File
- Run De Novo Processing
- Show Component Information
- Save As Peptide Workbook ▸
  - All
  - Checked

(b) Target peptide workbook

Tabs: Home | Peptide Mapping Analysis | Load Results | Queue | **Target Peptide Workbook**
Workbook Manager | Workbook Editor

▼ Add or Edit Target m/z

○ Apply to All Peptides   Charge State Range [2] to [3]   Number of Isotopes [3]
● Apply to Selected Peptides   m/z Range [400.00] to [1,500.00]   (The recommended number of isotopes for a peptide is between 3 and 5)

Peptide Workbook

		Level	Identification	Normalized Id	Peptide Sequence	Modification	Site	Normalized Site	Relative Quantitation Group Number	Charge State Distribution
1	☐	Peptide	2:E296-R304 = 1188.50...	E1-R9 = 1188.5047m(N5+A2G2F)	EEQYNSTYR	A2G2F	N300	N5	1	2 - 3
2	☐	Peptide	2:E296-R304 = 1188.50...	E1-R9 = 1188.5047m(N5+A3G2F)	EEQYNSTYR	A3G2F	N300	N5	1	3 - 3
3	☐	Peptide	2:E296-R304 = 1188.50...	E1-R9 = 1188.5047m(N5+A2G1F)	EEQYNSTYR	A2G1F	N300	N5	1	2 - 3
4	☐	Peptide	2:E296-R304 = 1188.50...	E1-R9 = 1188.5047m(N5+A2G0F)	EEQYNSTYR	A2G0F	N300	N5	1	2 - 3
5	☐	Peptide	2:E296-R304 = 1188.50...	E1-R9 = 1188.5047m(N5+A1G1F)	EEQYNSTYR	A1G1F	N300	N5	1	2 - 3
6	☐	Peptide	2:E296-R304 = 1188.50...	E1-R9 = 1188.5047m(N5+M5)	EEQYNSTYR	M5	N300	N5	1	2 - 3
7	☐	Peptide	2:E296-R304 = 1188.50...	E1-R9 = 1188.5047m(N5+A1G0F)	EEQYNSTYR	A1G0F	N300	N5	1	2 - 3
8	☐	Peptide	2:E296-R304 = 1188.50...	E1-R9 = 1188.5047m(N5+A1G0)	EEQYNSTYR	A1G0	N300	N5	1	2 - 3
9	☐	Peptide	2:E296-R304 = 1188.50...	E1-R9 = 1188.5047m(N5+A1Sg1F)	EEQYNSTYR	A1Sg1F	N300	N5	1	2 - 3
10	☐	Peptide	2:E296-R304 = 1188.50...	E1-R9 = 1188.5047m(N5+A2S1G1)	EEQYNSTYR	A2S1G1	N300	N5	1	3 - 3
11	☐	Peptide	2:E296-R304 = 1188.50...	E1-R9 = 1188.5047m(N5+Unglycosylated)	EEQYNSTYR	Unglycosylated	N300	N5	1	2 - 2

Figure 4. Generation of target peptide workbook for N300 glycosylation in NISTmAb. (a) CQA selection and (b) target peptide workbook creation within BioPharma Finder software.

CQA quantitation and monitoring in Chromeleon CDS

In the HR MAM workflow, relative quantitation and monitoring of CQAs are performed within Chromeleon CDS (Figure 5), which includes advanced options and integration parameters, an array of compliance tools, and a powerful integrated reporting function.

CQA quantitation and monitoring in Chromeleon software includes three main steps:

1) Import of a target peptide workbook file (.wbpf) into the MS Component table (Figure 5d) of a Chromeleon Processing Method

2) Adjustment of MS detection and peak integration parameters within the Processing Method

3) Relative quantitation of CQAs using the integrated peak area within a predefined report template.

Defining the correct parameters for peak detection and integration during the research and development phase is critical to achieving accurate and consistent CQA quantitation, particularly for deamidation and other low abundant species. Global parameters, including the MS detection algorithm, mass accuracy settings, and composite scoring, as well as CQA-specific parameters, such as retention time (RT) and extracted ion chromatogram (XIC) settings can have a notable effect on accurate CQA quantitation.

Chromeleon CDS facilitates accurate and robust peak integration. The RT of a modified peptide can be defined either as a fixed value or time distance relative to the native reference peak. The time distance (relative RT) of the modified and reference peptide will remain consistent, even in the case of a systematic RT shift. Therefore, correct integration of the reference peak (native form) will lead to correct integration of the low abundant modified form(s).

Chromeleon CDS also provides alternatives to support correct integration of low abundant peptides, even with abundant interference peaks. The modified peptide can be selected based on RT or intensity order in the XIC. A small degree of smoothing (e.g., Gaussian, 5–7 points) will often help detection and integration of low abundant peaks. In addition, Chromeleon software provides solutions to resolve complex data when multiple peaks are present in an XIC. Parameters can be adjusted to integrate a specific peak or multiple neighboring peaks together. Some of these features are described in Application Note 72916.[4]

Figure 5 displays the commonly used panes for data processing in Chromeleon CDS. The type, number, and size of the active panes can be customized and then saved as default or a new View Setting. The MS Components pane displays XICs and integration of CQA isotopic peaks in an overlaid (shown in Figure 5a) or stacked view. The Processing Method pane (Figure 5d) is the central piece of MAM data analysis in Chromeleon CDS. It consists of the MS Component table containing detailed information about CQAs imported from the target peptide workbook, and multiple tabs where global parameters for MS detection, MS settings, and composite scoring are adjusted. The MS spectra (Figure 5c) and isotopic distribution chart within the integrative charts (Figure 5e) are useful for confirming correct integration of low abundant peaks such as deamidated species.
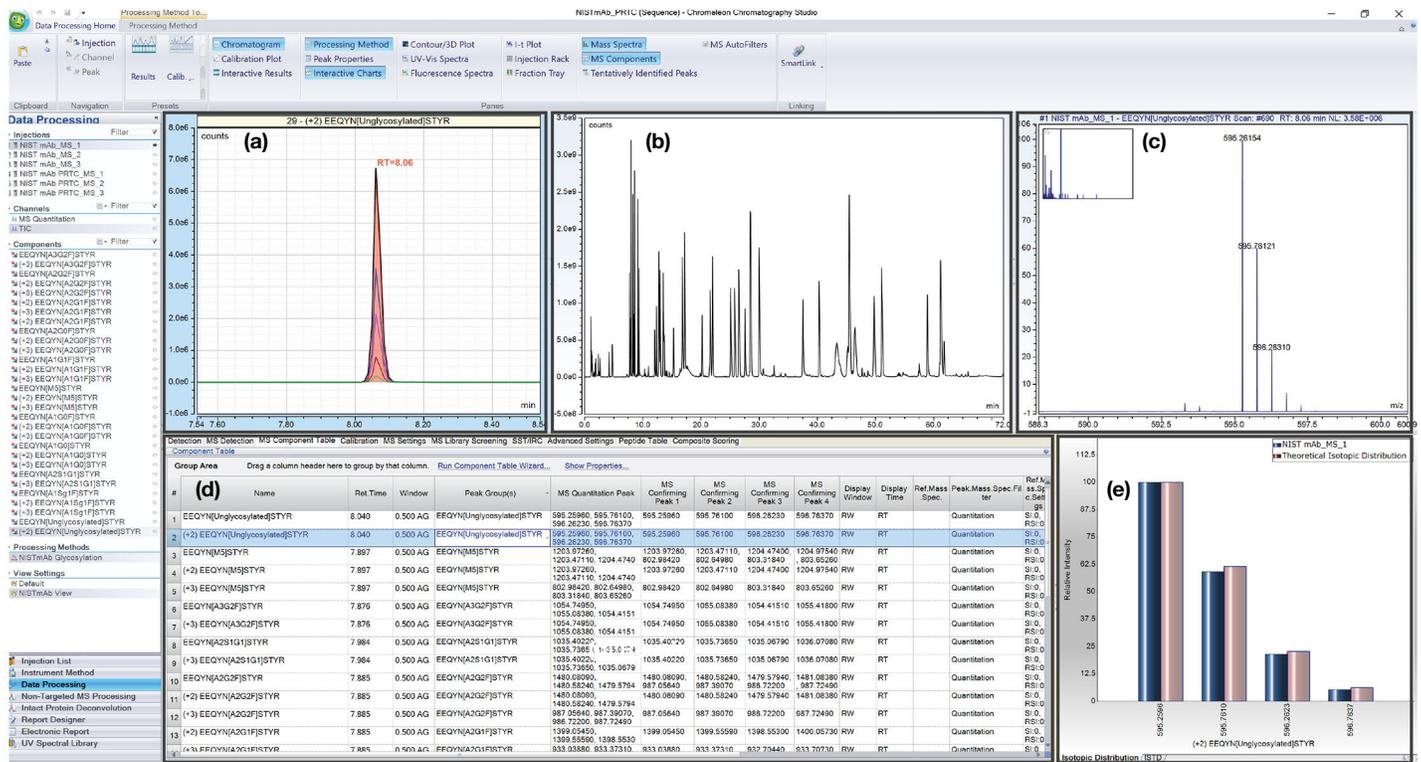

Figure 5. Overview of data processing in Chromeleon CDS. Five commonly used panes: (a) MS components, displaying integration of CQA isotopic peaks in an overlaid (shown) or stacked view, (b) total ion chromatogram (TIC) or base peak chromatogram (BPC) or XIC, depending on the selected channel; (c) mass spectra, (d) Processing Method, containing multiple tabs for global integration parameters; and (e) interactive charts to visualize theoretical *versus* observed isotopic distribution of specific charge states of a CQA.

Reporting in Chromeleon CDS

The Chromeleon software offers users great flexibility in building customized reports using a report template in a Microsoft® Excel®-like environment. The Chromeleon Report Template for MAM normally contains information regarding sequence, relative quantitation of CQAs, composite scoring, and NPD. The report template shown in Figure 6 provides a summary of the integrated peak area of all glycoforms (lower table) and their calculated percent abundances (top table and bar chart). A similar worksheet can be created in the same report for each CQA category, such as deamidation and oxidation. The result of composite scoring and NPD can be included in separate tabs in the report. Additional information, including experimental details and audit trails, may also be incorporated into the Report Template. When it is finalized for a specific molecule to contain all required information, it can be applied to any analysis of that molecule at any stage of development. This supports a smooth method transfer, e.g., from research and development to QC.
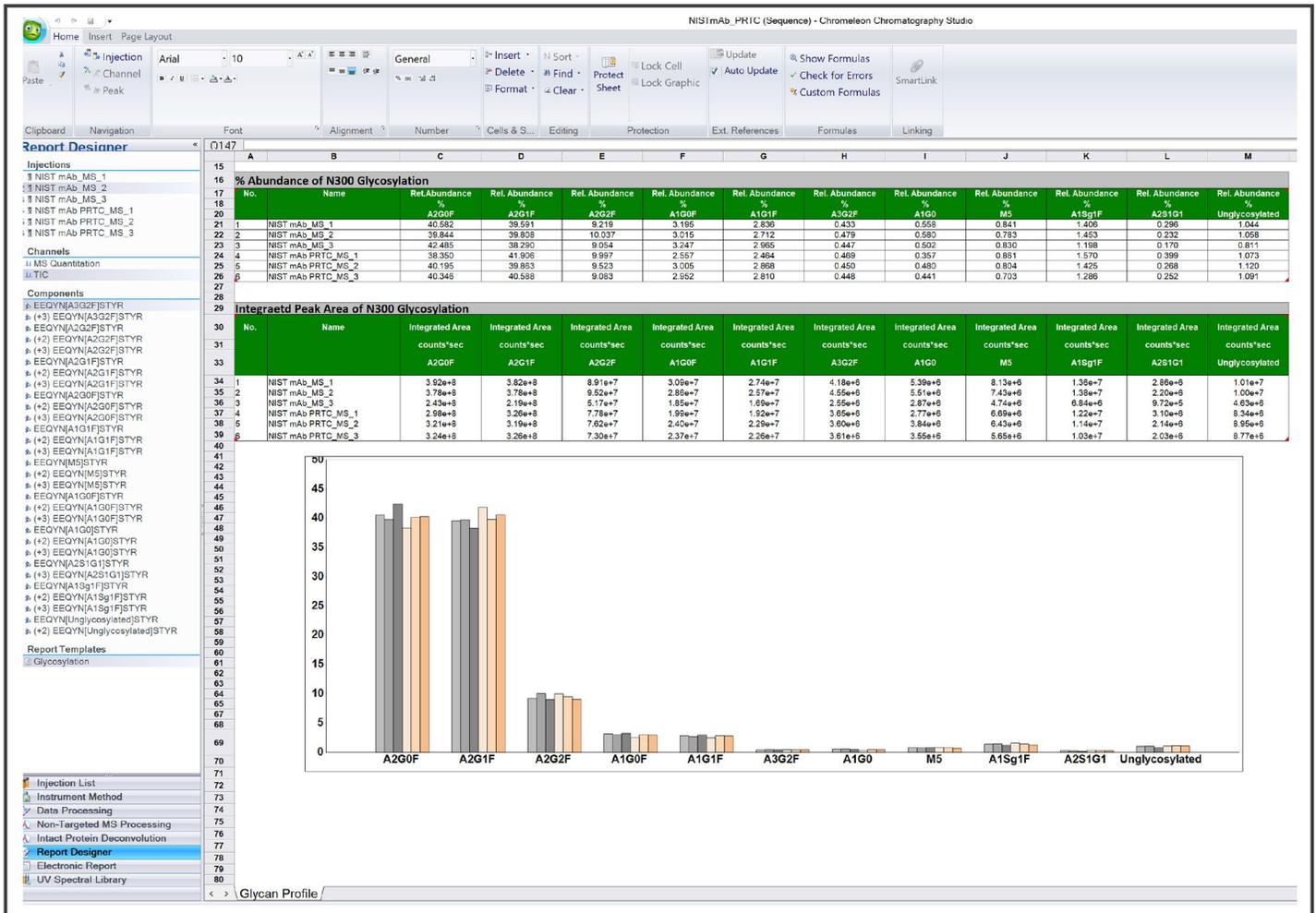

Figure 6. Example of a Chromeleon Report Template for N300 glycosylation in NISTmAb. This report shows the integrated peak areas (lower table) and percent abundances (top table and bar chart) of all N300 glycoforms identified in six injections

New Peak Detection (NPD)

NPD performs a comparative analysis of an unknown sample (e.g., the sample from a new process or a new batch) *versus* the control sample (e.g., reference material). In Chromeleon CDS, NPD is carried out based on full scan MS[1] data using the Non-Targeted MS Processing (NTMS) functionality. Data processing includes the following main steps (Figure 7):

1) Selection of a reference injection within the same sequence (Figure 7a).

2) Setting frame parameters for peak alignment and detection (Figure 7a). The key parameters are maximum number of frames and peak intensity threshold. These two parameters, particularly peak intensity threshold, need to be optimized to minimize false positives. The peak intensity threshold can be set as a fixed value or percentage of reference or sample TIC or BPC (e.g., 0.1% of sample TIC).

3) Application of filtering rules to the alignment result (Figure 7c). Typical rules include PR Element = 0 (monoisotopic peak), PR Size > 1 (more than one isotope), charge (e.g., 2–7), and ratio (e.g., not between 0.1 and 10).

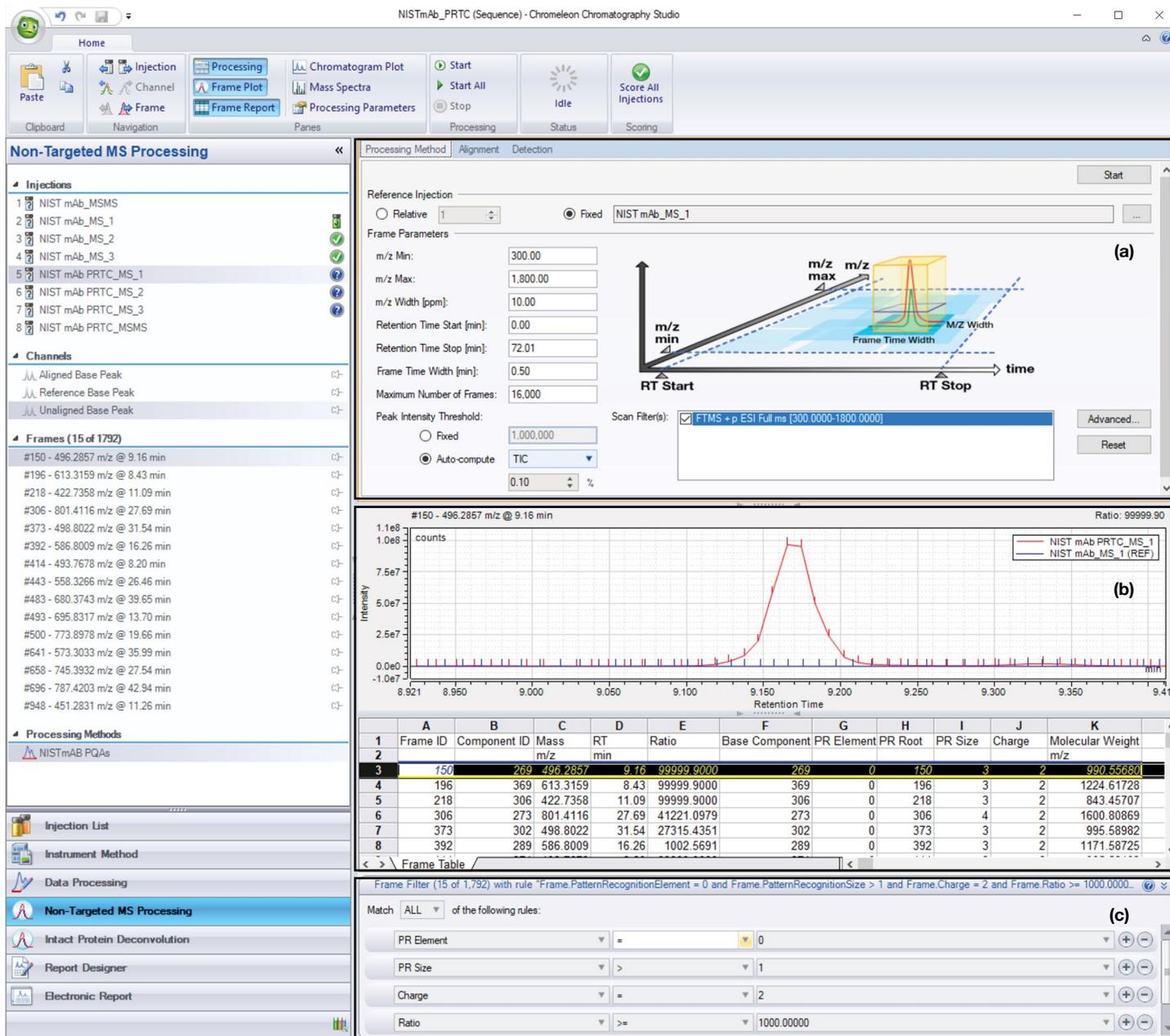4) Reporting of the NPD result (Figure 7b) in a frame table in the report.

Figure 7. Snapshot of non-targeted MS processing in Chromeleon CDS. (a) frame parameters, (b) NPD result, and (c) filtering rules.

Creating an eWorkflow procedure

To ensure smooth method transfer and consistency in data acquisition and analysis, Chromeleon software offers an option for sequence creation that incorporates sequence layout, acquisition method, processing method, view settings, and report template into a single eWorkflow™ procedure that can be shared across labs within a facility or across different sites. The execution of an eWorkflow procedure is straightforward and requires minimum training, and hence is particularly useful for transferring MAM from process development into QC.

Compliance tools in Chromeleon CDS

Chromeleon CDS is 21 CFR Part 11 compliance-ready software, providing the full set of compliance tools relating to user management with different user roles and privileges, data integrity and security throughout the data acquisition and analysis process. Chromeleon software maintains detailed audit trails for any activity that takes place within the software, such as modifications made to the data and sequence. In addition, Chromeleon software provides powerful tools for data organization, query of historic data, automated data archiving, electronic signature, and software/instrument qualification. These features support the successful implementation of MAM in a GMP compliant environment.

Conclusion

In this technical note, we describe an overview of data processing in the HR MAM workflow. While BioPharma Finder software offers rapid peptide mapping and easy CQA selection and management in a non-compliant environment, Chromeleon CDS provides powerful tools to perform CQA quantitation, NPD, and reporting in a GMP setting. This combination of software provides flexibility in different phases of drug development and manufacturing, while allowing smooth data processing and method transfer.

References

1. Rogers, R. *et al.* Development of a quantitative mass spectrometry multi-attribute method for characterization, quality control testing and disposition of biologics. *Mabs,* **2015,** *7,* 881.
2. Rogers, R. *et al.* A view on the importance of "multi-attribute method" for measuring purity of biopharmaceuticals and improving overall control strategy. *The AAPS Journal,* **2018,** *20,* 7.
3. Rogstad, S. *et al.* Multi-attribute method for quality control of therapeutic proteins. *Anal. Chem.,* **2019,** *91,* 14170.
4. Liu, HC. *et al.* A high-resolution accurate mass multi-attribute method for critical quality attribute monitoring and new peak detection. Thermo Scientific™ Application Note 72916. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-72916-lc-ms-multi-attribute-method-cqa-mab-an72916-en.pdf

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