Compliant attribute monitoring for biopharmaceutical product quality attributes employing intact mass analysis

Application of intact mass attribute monitoring workflow in SCIEX OS Software 1.7

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Liquid chromatography mass spectrometry (LC-MS) is a widely adopted analytical technique used in biopharmaceutical research, as it enables detection and measurement of a variety of product quality attributes (PQAs). Intact mass and subunit analysis are among of the most common LC-MS assays employed in research and development due to the high degree of information provided and high sample throughput. They have been used for determination of molecular weight, glycoform distribution, other post translational modification (PTM) assessment, and domain specific information.

In recent years, multiple attribute methodology (MAM) has gained popularity in monitoring biologic PQAs of biotherapeutics within late-stage development and manufacturing laboratories.[1] The concept of MAM is to offer a single method capable of monitoring various PQAs simultaneously. This approach may increase efficiency and quality by replacing or supplementing conventional QC tests. Current MAM solutions require the generation of peptides from a protein digest, which increases the potential of inducing PTMs during sample manipulation and is time consuming. With the advantage of limited sample preparation and the potential for real time monitoring, the implementation of MAM for intact mass analysis (including subunit analysis) has attracted high interest in biopharmaceutical industry.

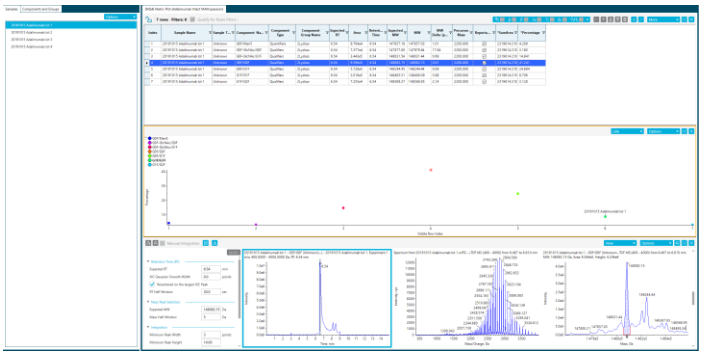


Figure 1. SCIEX OS Software results table. Results table showing extracted ion chromatogram (XIC), TOF-MS raw spectra, reconstructed spectra, percentage calculation and statistics graph in SCIEX OS Software 1.7.

Essential to successful implementation of a thorough and productive intact MAM is a software solution that can manage all aspects of the workflow, including: product quality attribute (PQA) definition, tracking, and quantification in a compliant environment. To date, there has not been a user friendly or streamlined software package capable of data acquisition, intact mass reconstruction and intact mass MAM analysis for compliant environments. With the launch of SCIEX OS Software 1.7 from SCIEX, an integrated software solution from data acquisition, data analysis to intact mass MAM is now available. In this technical note, the use of SCIEX OS Software 1.7 to perform an intact MAM analysis is demonstrated, highlighting a high throughput analysis of intact data as well as the capability to monitor and track quality attributes from different lots in a compliant environment.

Key features of the MAM Solution in SCIEX OS Software

- Complete and compliant software solution for intact MAM workflows with high resolution mass spectrometry
- Powerful product attribute definition, tracking, and quantitation with flexible custom calculations for attribute-level assessment based on specific user needs
- Reconstruction level quantification for high specificity analysis and comparison between raw and reconstructed data for rapid investigation of similarities and differences in samples

Methods

Sample preparation: Adalimumab antibody samples were diluted to a concentration of 0.1 mg/mL using deionized water for intact mass analysis.

Chromatography: Separation was accomplished using an ExionLC™ System fitted with an Agilent PLRP-S column (2.1 mm×50 mm, 300 Å, 5 µm) at 80°C using the gradient shown in Table 1. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile.

Table 1. LC conditions for intact mass analysis.

Time [min]	%A	%B	Flow Rate [ml/min]
Initial	*85*	*15*	*0.5*
5.0	*85*	*15*	*0.5*
9.0	*5*	*95*	*0.5*
11.4	*5*	*95*	*0.5*
11.5	*85*	*15*	*0.5*
15.0	*85*	*15*	*0.5*

Mass spectrometry: A SCIEX X500B QTOF System with a Turbo V™ Ion Source fitted with a Twin Sprayer ESI Probe was used for data acquisition. MS instrument conditions are listed in Table 2.

Data Processing: Data were processed using the Analytics tool in SCIEX OS Software 1.7.

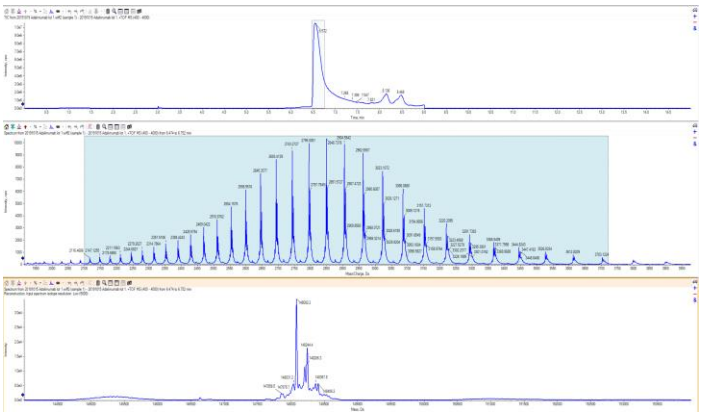


Figure 2. Reconstruction of intact mass analysis in SCIEX OS Software 1.7. (Top) Total ion chromatogram shows a single major peak, the TOF-MS raw spectra (middle) is extracted from the apex of that peak. Reconstruction of the TOF-MS raw spectra to a zero charge state (bottom).

Table 2. MS Parameters.

Parameter	Setting
Scan Mode	*Positive*
Gas 1	*60 psi*
Gas 2	*60 psi*
Curtain Gas	*30 psi*
Temperature	*550°C*
Ion Spray Voltage	*5500 V*
Time Bins to Sum	*80*
Accumulation Time	*0.5 sec*
TOF Start Mass	*m/z 400*
TOF Stop Mass	*m/z 4000*
Declustering Potential	*250 V*
Collision Energy	*10*

Attribute Definition

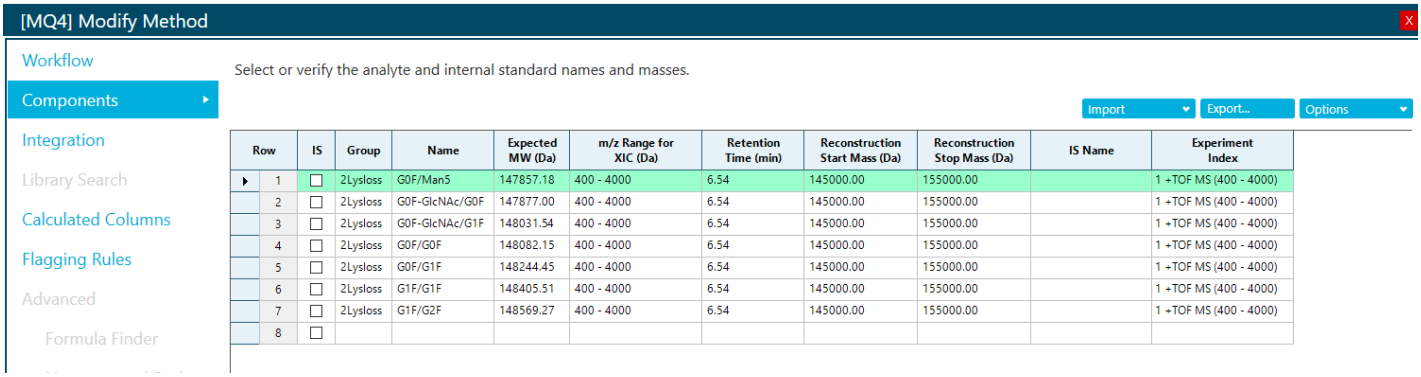
Data was acquired using SCIEX OS Software with a SCIEX X500B QTOF System. Acquired data was processed using the built in reconstruction algorithm without the need for definition of the target sequence (Figure 2). For studies in which target sequences may be refined or where the scientific question relates only to PTMs which are easily identified using mass shifts, such as glycosylation, this ability streamlines data analysis.

From the data, targeted attributes are easily defined within SCIEX OS Software 1.7 as shown in Figure 3. The expected molecular weight, *m/z* range, retention time (RT) and reconstruction range are entered based on the study. If the some relevant information is not known when developing the assay, such as RT and expected molecular weight, these may also be defined during integration by selecting peaks from chromatographic and reconstructed spectra.

Integration parameters for individual components can be adjusted to achieve accurate quantitation in SCIEX OS Software 1.7, providing the flexibility to refine processing parameters on a component by component level (Figure 4). Using the advanced reconstruction parameters, the user can define the number of iterations, S/N threshold, step mass size, and mass ranges for the assay.

Within each attribute group, the attribute level can be calculated using flexible custom calculations. Figure 5 and Figure 6 show examples of percentage calculation for glycoform distribution.

Figure 5 represents the equation defined to sum the peak areas of all targeted glycoforms, while Figure 6 shows the percentage calculation based on the summed data. After processing, the calculated values are displayed in a results table as shown in Figure 1. Within minutes the results are generated and available for review. There is no practical limit to the number of attributes that can be defined or monitored as part of an assay, highlighting that SCIEX OS Software 1.7 has the flexibility to monitor a large number of attributes simultaneously.



Row	IS	Group	Name	Expected MW (Da)	m/z Range for XIC (Da)	Retention Time (min)	Reconstruction Start Mass (Da)	Reconstruction Stop Mass (Da)	IS Name	Experiment Index
1	☐	2Lysloss	G0F/Man5	147857.18	400 - 4000	6.54	145000.00	155000.00		1 +TOF MS (400 - 4000)
2	☐	2Lysloss	G0F-GlcNAc/G0F	147877.00	400 - 4000	6.54	145000.00	155000.00		1 +TOF MS (400 - 4000)
3	☐	2Lysloss	G0F-GlcNAc/G1F	148031.54	400 - 4000	6.54	145000.00	155000.00		1 +TOF MS (400 - 4000)
4	☐	2Lysloss	G0F/G0F	148082.15	400 - 4000	6.54	145000.00	155000.00		1 +TOF MS (400 - 4000)
5	☐	2Lysloss	G0F/G1F	148244.45	400 - 4000	6.54	145000.00	155000.00		1 +TOF MS (400 - 4000)
6	☐	2Lysloss	G1F/G1F	148405.51	400 - 4000	6.54	145000.00	155000.00		1 +TOF MS (400 - 4000)
7	☐	2Lysloss	G1F/G2F	148569.27	400 - 4000	6.54	145000.00	155000.00		1 +TOF MS (400 - 4000)
8	☐									

Figure 3. Attribute definition for intact mass analysis in SCIEX OS Software.

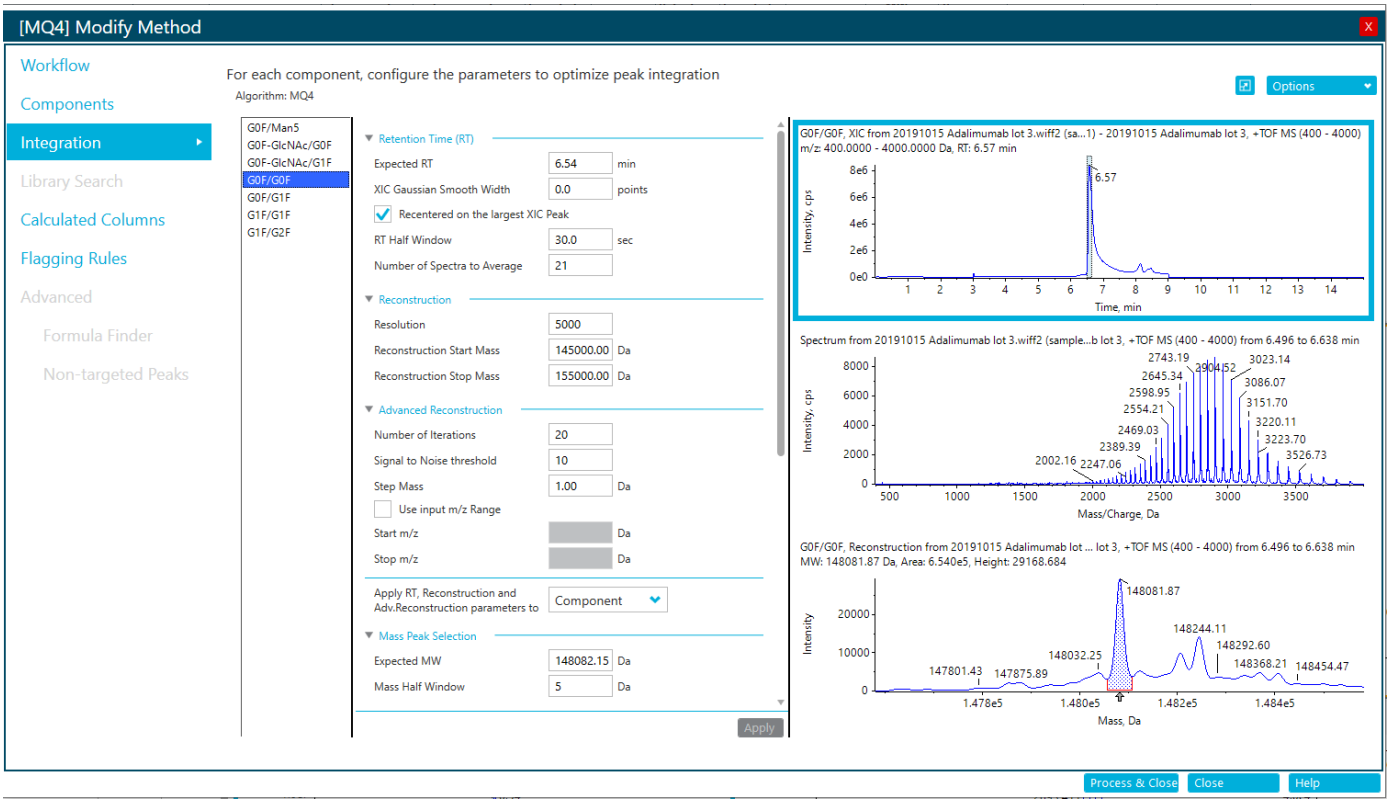


Figure 4. Integration parameter definitions in SCIEX OS Software.

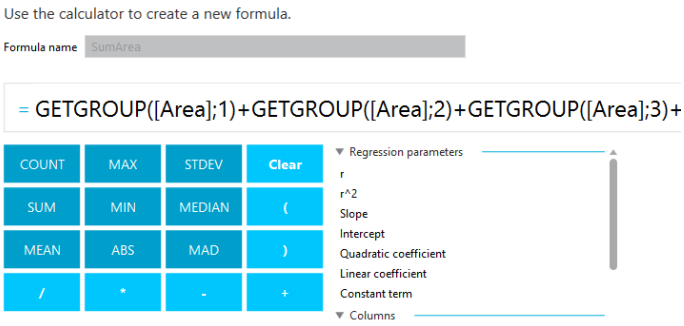


Figure 5. Custom calculations within attribute group. Calculation formula showing the summing peak areas from different attributes within the same attribute group.

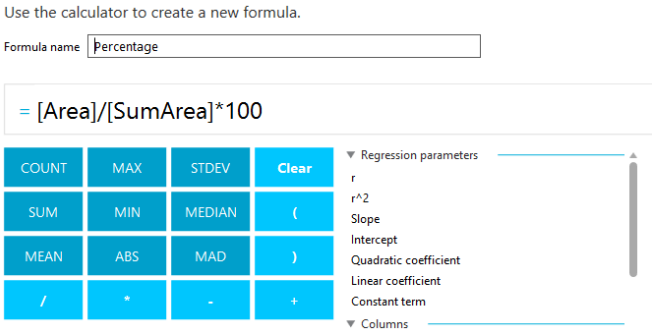


Figure 6. Custom calculations from summed data. Calculation of percentage in SCIEX OS Software.

For each attribute defined in an assay, the acceptance criteria for that attribute can also be defined. Each attribute acceptance level is defined independently providing flexibility for specific assays. To highlight outliers, flagging criteria may be set based on a percentage variation from a defined value or over a range of values. The pass/fail range for each attribute can be aggregated and used to determine the overall pass/fail status for a corresponding attribute, allowing users to screen values that don't meet the necessary criteria. For example, in the presented assay the acceptance range of G0F was set to a range of 37-45% with results falling outside of this range being flagged (Figure 7).

Attribute Quantification

Sample batches can be submitted for processing in SCIEX OS Software. During processing, the targeted attributes are quantified and tracked across each submitted sample. Sample differences related to the defined attributes are easily identified. By design, SCIEX OS Software can process batches of two or greater files simultaneously to enable direct sample comparison. For each sample and study, a summary table is generated (Figure 1). The summary table provides an overview of the calculated attribute levels and a pass/fail indication. The results may be viewed in totality or for each specific attribute defined among the samples. Failed attributes are highlighted in red, as demonstrated in Figure 7 and the results can be easily filtered to focus on only those responses which do not meet the defined acceptance criteria.

Attributes can be examined directly by viewing the underlying raw and reconstructed data used for each calculation by selected entries in the table generated in SCIEX OS Software, which allows independent data validation within the same software interface. This same table provides direct access to extracted ion chromatograms, raw MS spectra, and reconstructed spectra for the selected component, which enables users to perform attribute monitoring, data investigation, and assay refinement seamlessly within the same workspace. The optimized parameters can be applied to the entire assay within results table interface or to specific attributes or responses. Finally, a metric plot can be generated for the results as a visualization tool, so the review of attribute response across different samples is expedited.

Conclusions

The SCIEX OS Software 1.7 provides a breakthrough in intact MAM analysis by providing a streamlined and compliant software package, from data acquisition through data analysis. The entire intact mass MAM solution is provided including survey analysis, attribute definition, custom calculations, and method optimization. The ability of SCIEX OS Software 1.7 to offer advanced integration for individual components enables accurate quantitation, which is essential for MAM assays. The full compliance feature set offered by SCIEX OS Software provides the potential to implement intact mass MAM in regulated environments. SCIEX OS Software provides a superior MAM solution which can fulfill the needs in discovery, development, and quality control laboratories.

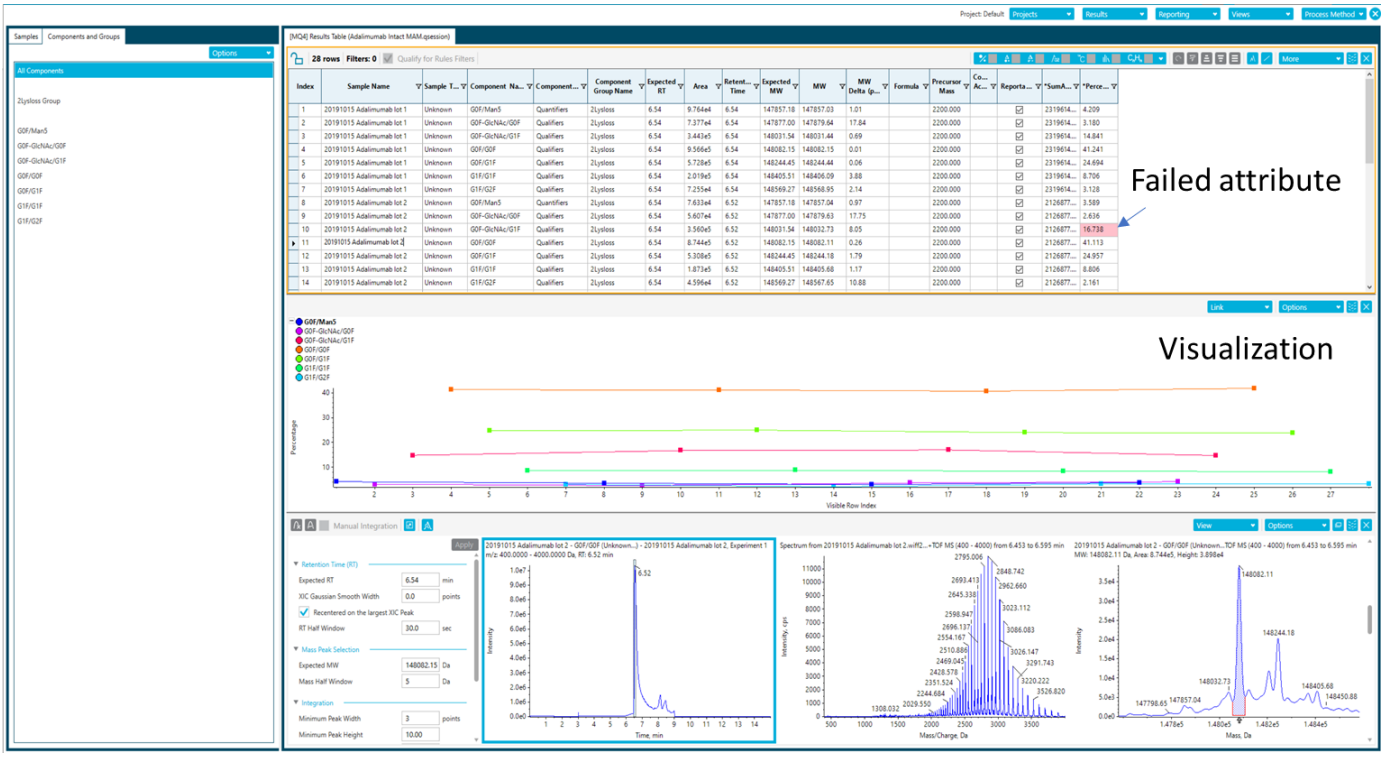


Figure 7. Attribute summary. Attributes are summarized in tables with each entry linking to underlying XIC, raw, and reconstructed data. The trend of each attribute is plotted as shown in the middle panel to expedite data review.

References

1. Rogers RS et al. (2018). A View on the Importance of "Multi-Attribute Method" for Measuring Purity of Biopharmaceuticals and Improving Overall Control Strategy. *The AAPS Journal*, **20**, Article 7.

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