Enabling Compliant Multiple Attribute Methodology for Assessment of Biopharmaceutical Product Quality Attributes

Application of the Multiple Attribute Methodology workflow in BioPharmaView™ Software 3.0 and SCIEX OS 1.5 Software

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Introduction

Development and production of biopharmaceuticals is complex. Even minor impurities, or changes in attributes such as glycosylation or charge heterogeneity, can have a profound impact on the safety and efficacy of the final product. Traditionally, multiple analytical techniques have been required to assess the full range of biopharmaceutical product attributes, which requires significant expenditure of time and resources.

The Multiple Attribute Methodology (MAM), an orthogonal approach based on peptide map separation coupled with high-resolution mass spectrometry, is rapidly emerging as a powerful tool for characterization and monitoring of biopharmaceutical attributes. The range of attributes that can be monitored using this approach is extensive. MAM can be used to assess, track, and provide detailed data on multiple, specific biologic product quality attributes at the peptide level. In addition to tracking the therapeutic molecule itself, MAM can be used to detect known impurities related to production of the biotherapeutic, as well as unknown impurities, or new peaks, present in samples but not in corresponding standards.

Essential to successful implementation of an MAM workflow is software that can manage all aspects of the workflow, including: product quality attribute (PQA) definition, tracking, and quantification; detection of known and unknown impurities; and reporting. This technical note describes the use of BioPharmaView Software 3.0 and SCIEX OS 1.5 Software for MAM workflow management. BioPharmaView software performs as a characterization tool while SCIEX OS manages all aspects of a routine MAM workflow from a single project. SCIEX OS 1.5 is 21 CFR Part 11 compliant ready and as such offers a full compliant package ready to implement in Quality Control labs.

Figure 1. SCIEX Solution for MAM featuring X500B and SCIEX OS.

Key Features

- BioPharmaView and SCIEX OS Software provide a complete and compliant software solution for MAM workflows
- Powerful product characterization, attribute definition and tracking, and quantitation
- Flexible custom calculations for attribute-level assessment based on specific user needs
- Reliable detection and monitoring of both specified and unspecified impurities
- Concise review and reporting of targeted attributes
- Accurate quantification tool for attribuite monitoring.

Method Creation

Creation of a characterization project in BioPharmaView 3.0 software is simple and streamlined. The assay starts with the definition of the target protein sequence. If multiple chains are required, each is defined separately as shown in Figure 2. Known disulfide linkages and any modifications that may be present are applied, entered, and positioned to specific amino acids within the sequence. Desired modifications not built into BioPharmaView software can easily be added. These custom modifications are then available for use across all BioPharmaView projects.

In addition to the target biotherapeutic, any known impurity sequences are entered separately as a targeted peptide or protein sequence. Impurity sequences are treated identically to the target molecule during in-silico digestion, using the parameters defined for the biotherapeutic. Impurity sequences are searched and presented separately throughout the workflow for easy distinction from the biotherapeutic.

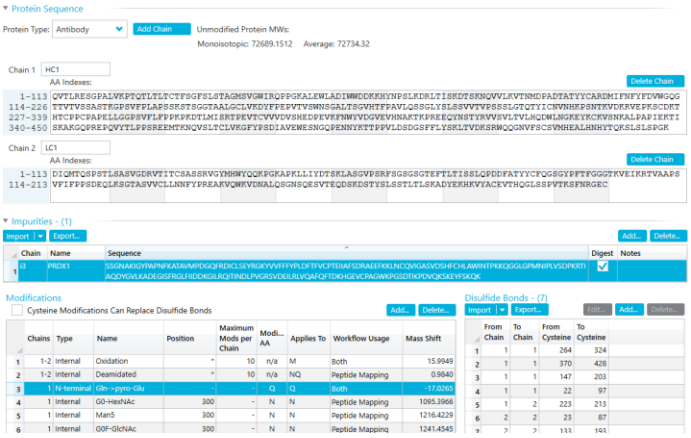

Figure 2. Definition of protein sequence, disulfide bonds, and modifications within BioPharmaView software

The assay information is completed by definition of digestion parameters using a range of built-in cysteine alkylation reagents and digestion enzymes. The maximum number of modifications and missed cleavages to search within the data is also defined, as shown in Figure 3.

Characterization

Using the defined assay information, acquired data is submitted for processing. Characterization of samples is accomplished automatically by the BioPharmaView software. Peptide assignments are based on defined search parameters by correlation of MS- and MS/MS-level data. After processing, peptide results are easily reviewed using a single interface. To expedite review, results are easily sorted using a wide range of available filters.

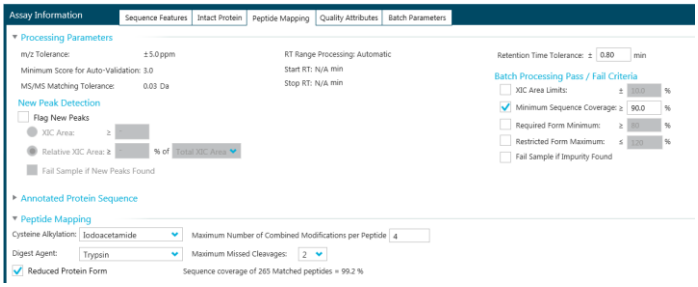

Figure 3. Definition of search parameters for batch analysis in BioPharmaView.

Peptide modifications defined in the assay are automatically annotated in the peptide results and are easily filtered. In cases where modifications are not automatically positioned, assignment of the position is guided using pre-populated scoring results from processed data. When a modification has been positioned, the position information is used in ongoing studies. After characterization is complete, the assay information is updated for use in batch analyses.

Attribute Definition

Targeted attributes are easily defined within BioPharmaView software as shown in Figure 4. Applying the same filter criteria used in characterization, targeted attributes can be compiled in peptide sets. Each attribute is captured within its own peptide set, which contains all of the data that matched the defined filter criteria. The attribute peptide sets are named and can be shared within and between projects. Sharing peptide sets reduces the overall time required to define assays and may reduce variability in set definition. After definition, the attributes defined in BioPharmaView are easily exported. The exported attributes may then be imported into SCIEX OS as shown in Figure 5. Three charge states of each component are automatically or manually defined in SCIEX OS for further data analysis.

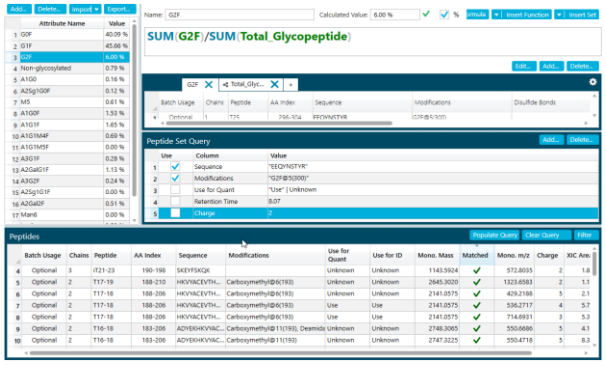

Figure 4. Definition of quality attributes and custom calculations within BioPharmaView software

Integration parameters for individual components can be adjusted to achieve the most accurate quantitation in SCIEX OS software. Peptides may be grouped by the user, attribute levels can be calculated for each group using flexible custom calculations (Figure 7). After processing, the calculated integration values are displayed within a results table. Data processing speed is rapid, and within minutes the results table is populated with the determined attribute levels. There is no defined limit to the number of attributes that can be defined or monitored as part of an assay.

For each attribute defined in an assay, the acceptance criteria for that attribute can also be defined. Ranges may be set based on a percentage deviation from a defined value, or as needed to be greater/less than a specific value. The defined range is then be used to determine the overall pass/fail status for a corresponding attribute, allowing users to easily identify attributes that don't meet the necessary criteria. For example in Figure 8, the reference abundance of G0F is 40% and a 15% tolerance is allowed for the assay to pass.

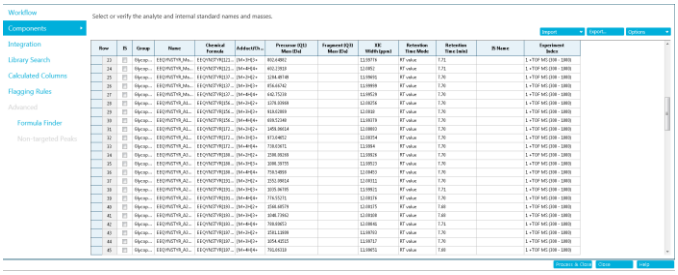

Figure 5 Attribute definition in SCIEX OS

Attribute Quantification

Sample batches are submitted for processing in SCIEX OS software, which tracks defined attributes across submitted sample sets. Batch analysis can be performed on TOF-MS, IDA, or SWATH® Acquisition data. SWATH and IDA acquisition provide greater detail by generating MS and MS/MS data for all detectable components in each sample. The additional fragment data provided by IDA and SWATH acquisition may be used to confirm defined attributes or to enable identification of detected new components without the need for reanalysis.

Comparing sample differences in PTMs, SCIEX OS can process a batch of two or more data files simultaneously to enable direct sample comparison. For each sample, a comparison summary table (Figure 9).is generated, which provides an overview of the calculated attribute levels and a pass/fail indication.

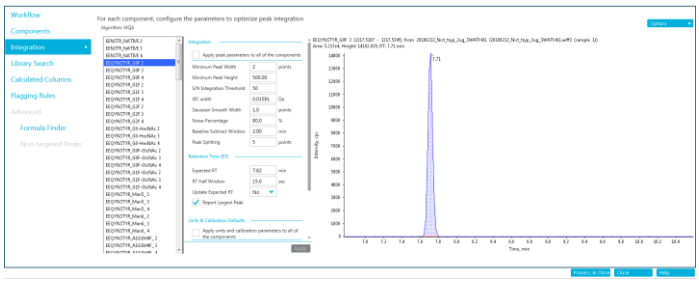

Figure 6. Define integration parameter for each component in SCIEX OS.

Attribute data can be investigated directly by viewing the underlying data used for each calculation in a table generated in SCIEX OS, which allows independent data validation within the same software interface (Figure10). This same table allows for the MS and, if acquired, MS/MS data to be viewed for the selected component, an important feature allowing users to perform attribute monitoring and data investigation within the same piece of software.

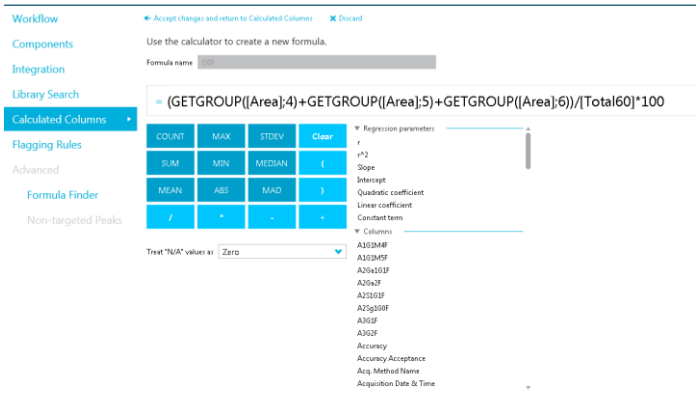

Figure 7 Example Custom calculation for percentage G0F.

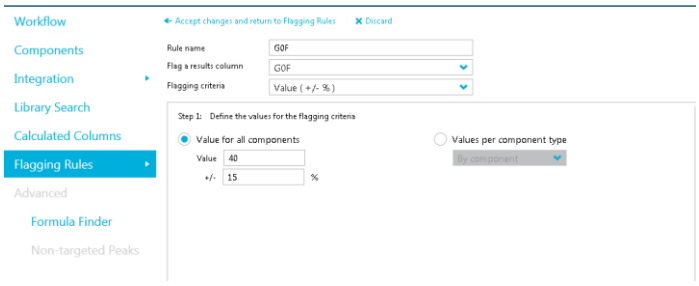

Figure 8 Example of definition of Pass/Fail criteria for G0F

Index	Sample Name	*Nat	*G0F	*G1F	*Total60	*G2F	*G0 -HexN...	*G0F -GlcNAc	*G1F -GlcNAc	*Man5	*Man6	*A1G1 ...	*A1G1 ...	*A3G1F	*A2Sg...	*A2Ga...	*A3G2F	*A2S1 ...	*A2Ga2F
4	tryp_2ug_IDA01	1.437	40.579	40.116	515068.0...	7.242	0.583	2.677	2.297	1.005	0.014	0.969	0.140	0.425	0.133	1.339	0.396	0.000	0.649
100	tryp_2ug_SWATH01	0.963	41.996	45.271	861638.5...	5.133	0.289	1.649	1.559	0.562	0.028	0.490	0.109	0.258	0.119	0.865	0.272	0.000	0.437
196	tryp_2ug_TOFMS01	1.276	37.237	44.681	575695.4...	6.761	0.623	2.533	2.149	0.974	0.026	0.942	0.151	0.427	0.111	1.194	0.361	0.000	0.554
292	tryp_2ug_TOFMS02	1.461	37.157	44.299	538269.9...	6.824	0.713	2.550	2.349	0.974	0.041	0.844	0.112	0.408	0.148	1.216	0.345	0.000	0.557

Figure 9 Review of batch analysis results in BioPharmaView software. Failed results are shown in blue.

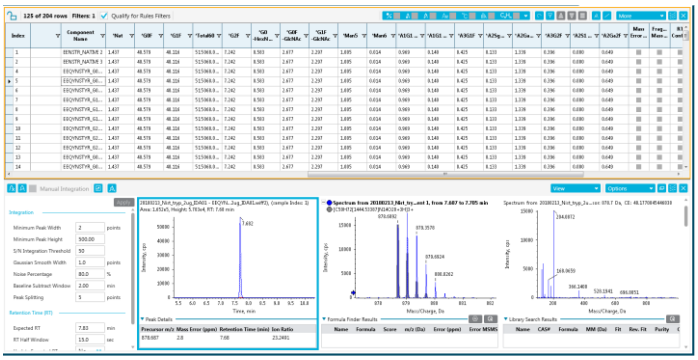

Figure 10 Review of batch analysis results and supporting data is easily accessed in the same window.

Reporting

Results from batch analyses are compiled in a full report which includes assay information, sample name, a concise summary table of attributes percentage, integration of all attributes defined in the assay and a detailed results table. The report template is a standard template within the software, which can also be customized based on individual customer request. Within the report, each attribute is flagged as to whether it has passed or failed. The number of impurities and new peaks detected is also summarized in new peak detection report.

New Peak Detection

SCIEX OS software also enables the detection of unknown impurities during batch analysis, highlighting new peaks that appear when comparing samples to a standard or quality control sample. Preparation and analysis of both the control and sample in the same study are needed to account for any variability in sample preparation. To execute new peak detection, the threshold for detection can be defined in the assay by using absolute signal intensity and fold changes (Figure 11), which is guided by ongoing characterization work,.

When executing new peak detection, one of the data files must be defined as the quality control. The control sample serves as the benchmark against which the other samples that are compared. Often, the control sample is one that has been previously characterized and is well understood.

New peak detection results are presented in a result table (Figure 12). The overall number of peptides for each sample is listed, as well as the new peaks detected.

If further interrogation of new peaks is required, peptide results are easily filtered to display only those components which are flagged as failed. For detailed information on new peaks, each new detected peak can be selected and the corresponding TIC, MS and MS/MS spectra viewed as well as the TIC at the same RT in control sample are shown for investigation.

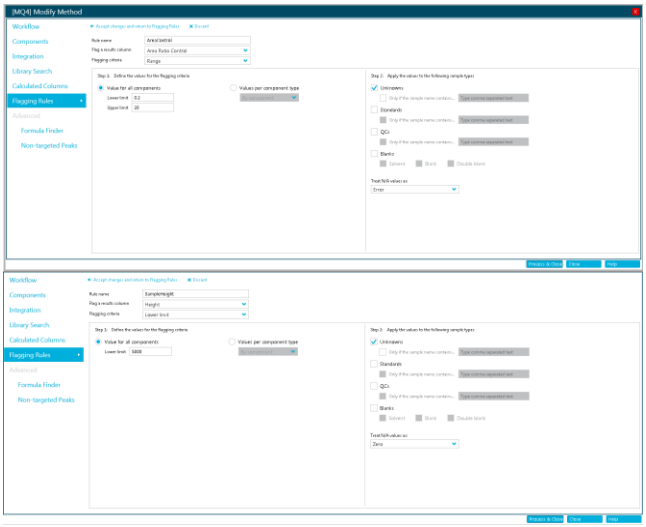

Figure 11 Definition of New Peak Detection criteria for batch analysis..

Conclusion

The BioPharmaView and SCIEX OS software provide a complete and compliant software package for creating the entire MAM workflow, including: characterization, attribute definition, custom calculations, known impurity detection, unknown impurity (new peak) detection, and reporting. The ability of SCIEX OS to offer advanced integration for individual component enables an accurate quantitation, which is essential for MAM assays. The full compliance package offered by SCIES OS realizes the possibility to implement MAM in QC environment. Taken together, BioPharmaView and SCIEX OS software provide a superior MAM solution which can fulfil the needs in both upstream discoveries and downstream development and quality control.

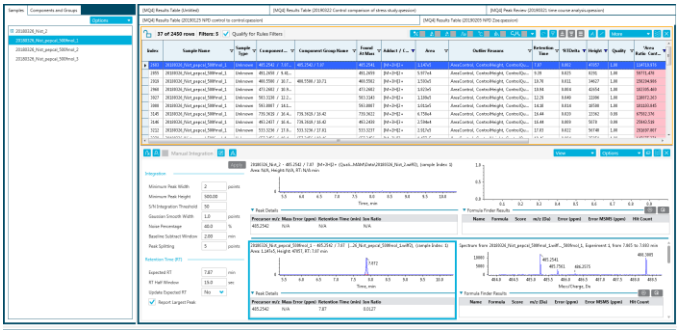

Figure 12 Results for new peak detection within SCIEX OS software. The number of new peaks, as well as the data supporting their detection, is easily displayed..

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