Drug Discovery and Development

Detection and Definition of Attributes in a Multiple Attribute Method with BioPharmaView™ Software 3.0

Characterization and Attribute Identification

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Introduction

During the development of a biopharmaceutical it is critical to complete extensive characterization. As part of this work, the use of mass spectrometry provides unparalleled insight into many aspects of a biotherapeutics which can be challenging to determine using orthogonal assays. In recent years, the concept of a multiple attribute method (MAM) has been introduced which is expanding the use of mass spectrometry by providing an orthogonal, or in some cases displacing, assay to assess potential product quality attributes (Figure 1).[1] A benefit of a mass spectrometric approach is the ability to directly monitor attributes due to the additional specificity provided by this technology. In short, the concept of MAM comprises a range of stages to enable characterization, definition of attributes and their acceptance ranges, tracking of known impurities, and the ability to perform a purity assessment using new peak detection.

Presented here is the use of BioPharmaView™ Software 3.0 for characterization of a biotherapeutic. This discussion demonstrates characterization for the purpose of defining the key attributes to be tracked. For discussion on other steps within this workflow please refer to the other technical notes in this series.

Key Feature of BioPharmaView™ Software 3.0

- Single software solution for MAM workflows
- Easily characterize molecules for execution of MAM assays
- Streamlined definition of quality attributes and definition of custom calculations for tracking
- Define and track known protein and peptide based impurities within the same workflow with the use of additional software
- Ensure product purity with new peak detection capabilities
- Streamlined reporting for defined attributes

Biologic PQA Assessments	LC-MS MAM Workflow	SEC	CEX	CE-SDS	HILIC	ELISA
Deamidation						
Glycation						
High Mannose						
Methionine Oxidation						
Signal Peptide						
Glycosylation						
CDR Tryptophan Degradation						
C-terminal Lysine						
Misincorporations						
C-terminal amidation						
Fucosylation						
Residual Protein A						
Host Cell Protein						
Aggregate						
Cysteine Adduct Assessment						

Figure 1. Selection of Attributes that may be Assessed using MAM Assay/

Methods

Sample Preparation: NIST mAb standard (#RM8671) was purchased from NIST. An aliquot of 10 µl was taken and subjected to denaturation and reduction with 10 mM DTT at room temperature for 30 minutes. The sample was then alkylated with iodoacetic acid at 20 mM for 20 minutes in the dark at room temperature. After desalting, it was then digested with trypsin (Roche, sequence grade) for 30 min at 37 °C followed by quenching with TFA and used directly.

Chromatography: Separation was accomplished using an ExionLC™ system fitted with a 2.1×150 mm Agilent ZORBAX 300 SB-C18 , 1.8 µm column at 50°C with 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.

Mass Spectrometry: Experiments were executed on both TripleTOF® 6600 and X500B platforms. Instrument conditions for each specific platform are presented in dedicated application notes for each platform.

Data Processing: The complete data processing was performed using BioPharmaView Software 3.0.

Table 1. LC Gradient.

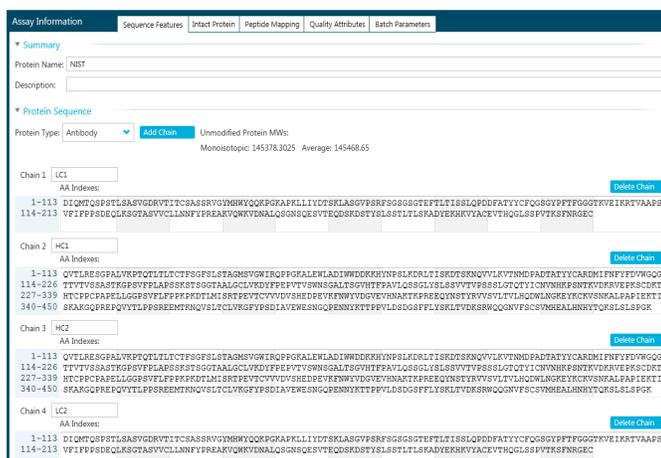| Time (min)	Flow Rate (ml/min)	%A	%B
Initial	0.3	99	1
5.0	0.3	99	1
6.0	0.3	90	10
50.0	0.3	65	35
55.0	0.3	40	60
56.0	0.3	10	90
60.0	0.3	10	90
62.0	0.3	99	1
64.0	0.3	99	1
66.0	0.3	10	90
70.0	0.3	10	90
72.0	0.3	99	1
74.0	0.3	99	1
76.0	0.3	10	90
80.0	0.3	10	90
82.0	0.3	99	1
95.0	0.3	99	1

Figure 2. Definition of Protein Sequence. Each chain of the protein can be entered separately and additional chains are easily entered. Impurity sequences are entered identically, however are captured in a discretely as impurities.

Assay Definition

Characterization of a biotherapeutic requires extensive analysis often spanning a wide range of experiments to understand molecular heterogeneity and identification of critical quality attributes (CQAs). For characterization with BioPharmaView 3.0 software detailed characterization data is leveraged to develop a routine peptide mapping assay to focus on target attributes as part of ongoing characterization efforts.

At the start of development of an assay the sequence for the therapeutic is defined. If there are multiple chains within the molecule they are defined separately to provide the flexibility for a range of molecular entities. As shown in Figure 2, the protein name as well as the name for each chain may be defined separately and the sequence provided for each. If additional chains are needed they may be added to the assay information providing flexibility in assay definition.

Modifications for each protein are defined using a wide range of built in modifications including C-terminal and N-terminal modifications as well as a range of internal modifications. Each modification may be applied to a type of amino acid or to particular residue within each peptide chain, such as glycosylation, as required, Figure 3.

If a particular modification is needed but is not pre-defined in BioPharmaView Software, they may be added easily. Each new modification is named and the particular residue(s) to which it applies are defined, Figure 4. Once the elemental composition is entered the molecular weight is automatically calculated. Each defined modification is available across all BioPharmaView Software projects to reduce the effort in definition in ongoing studies.

The defined sequence and associated modifications are submitted for in-silico digestion. This is accomplished using a range of built in reduction/ alkylation conditions and proteolytic enzymes to generate the library of components to search when processing data.

For characterization, information dependent analysis (IDA) and SWATH® acquisition data sets are easily submitted for processing. BioPharmaView™ software 3.0 automatically interrogates the submitted data set to match mass spectrometric data with expected peptides based on the parameters defined in the assay. Peptide matches are scored based on user defined mass accuracy requirements and overall match of observed MS/MS data to theoretical fragment spectra. In addition to peptide identification, modifications are automatically positioned and their relative abundance calculated.

In some cases, positioning of modifications requires manual review to ensure accurate assignment. In these cases, the modifications are presented with an asterisk. Using the powerful filtering capabilities in BioPharmaView Software it is easy to filter peptide results to focus on specific characteristics based on a wide range of criteria. As shown in Figure 5, filtering to deamidated species is readily accomplished by using the modification filter. Once filtered, modifications which were not automatically positioned may be manually positioned by selecting the modification in the result table. Users are presented with a prepopulated list of potential modification sites. Each potential site is annotated with a score which guides positioning. Once modifications are positioned, the position is saved and is available for future assays.

Figure 3. Definition of Modifications and Application of Modification to Workflows. Modifications can be searched easily to expedite assay definition.

Overall peptide mapping results are presented in comprehensive tables of results of both matched and unmatched data with an easily accessed coverage map based on matched MS and MS/MS data for each protein chain. Matching parameters may be adjusted based on user input using an interactive interface which presents the impact of matching parameters on the false discovery rate (FDR) of the assay.

After characterization and review are complete, the assay may be saved and used for ongoing studies including definition of attributes for a downstream MAM workflow.
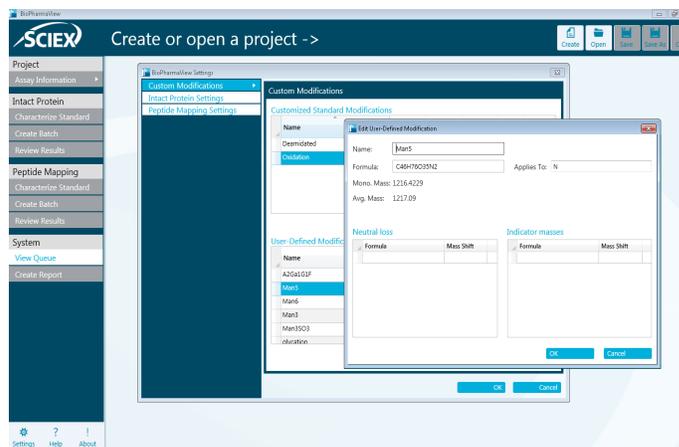

Figure 4. Definition of Custom Modifications in BioPharmaView Software. Once modifications are defined they are available across all projects.
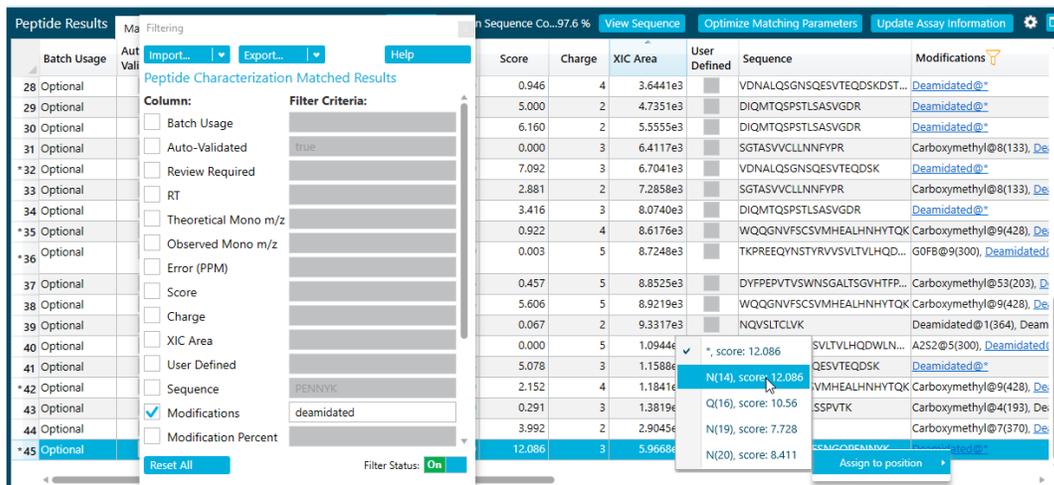
Figure 5. Filtering Experimental Results Based on a Range of Criteria, in this case by Modifications. In addition, modifications which are not automatically positioned may be done manually. A score for each potential position of the specific modification is displayed and can be correlated with supporting data.

Conclusions

- Characterization of biotherapeutics is flexible and easy using BioPharmaView™ Software

- Definition of modifications is flexible for emerging analytical challenges

- Review peptide map results using powerful filtering capabilities to focus on the scientific question at hand

- Position modifications using built in scoring to guide localization

- Easily view overall protein coverage of MS and MS/MS data

- Easily built up a MAM assay for potential high throughput analysis

References

Richard S Rogers, Nancy S Nightlinger, Brittney Livingston, Phil Campbell, Robert Bailey & Alain Balland(2015) Development of a quantitative mass spectrometry multi-attribute method for characterization, quality control testing and disposition of biologics, mAbs, 7:5, 881-890, DOI: 10.1080/19420862.2015.1069454

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