Defining and Tracking Biopharmaceutical Attributes Using the BioPharmaView™ Software 3.0 MAM Workflow

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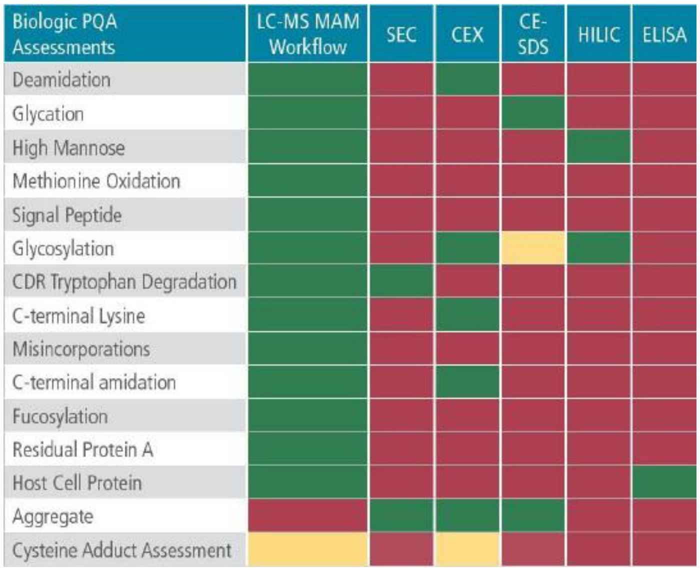
The concept of a multiple attribute method (MAM) was introduced several years ago and represents the formalization of a drive within the pharmaceutical industry to supplement, and in some cases replace, existing assays [1]. Overall MAM enables a single peptide mapping MS method to assess a wide range of molecular attributes. While the initial concept was for MAM implementation within a quality control setting, it is becoming clear that the functionality afforded by MAM is broadly applicable whenever researchers seek to monitor multiple aspects of a molecule simultaneously.

Presented here is the use of BioPharmaView™ software 3.0 for defining and tracking multiple attributes of a biotherapeutic following characterization. Within a single interface it is possible to select and define attributes using powerful filtering criteria and built in custom calculations to calculate the abundance of a range of attributes simultaneously without the need for additional external software.

Figure 1. Selection of attributes that may be assessed using MAM assay.



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	🟨	🟥	🟨	🟥	🟥	🟥

The MAM workflow in BioPharmaView™ coupled with high resolution mass spectrometers including the X500B QTOF System and the TripleTOF® 6600 System and the ExionLC™ System, provides a streamlined and simple solution for characterizing, defining, and tracking quality attributes of biotherapeutics.

Key Features of BioPharmaView™ 3.0 Software

- 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

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. The optimized parameters for data acquisition, specific to each platform, are described in detail in respective application notes.

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

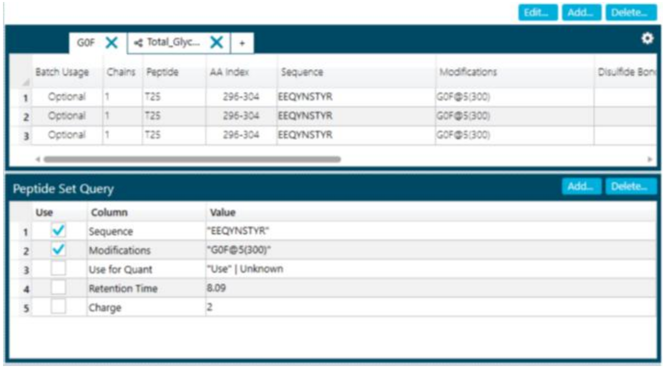
Attribute Definition

Previously the use of BioPharmaView™ Software 3.0 was discussed for characterization of a biotherapeutic to verify sequence coverage, identify attributes, and define targeted modifications within an assay. Presented in this work is the workflow for defining and tracking attributes for ongoing assays. To this end, assay information from characterization efforts is easily updated and applied to the BioPharmaView™ Software 3.0 project to enable the use of matched or unmatched components to define attributes.

The attributes are defined based on the characterization of the Gold standard. For each attribute, characterization data is filtered using a range of filter criteria including the peptide sequence, modification, retention time, and/or charge state and others as shown in Figure 2. The use of these filter criteria results in a list of desired peptides for which attributes needs to be defined. Each filtered set of peptides is then saved for use in future calculations. In addition, saved sets of peptides can be shared within or between projects to reduce the overall time for defining future assays. Importantly, the filter criteria defined for a peptide set are quite flexible and user friendly. Any additional experimental evidence that is found to fit in the filter criteria will be included in the calculations.

Table 1. LC Gradient

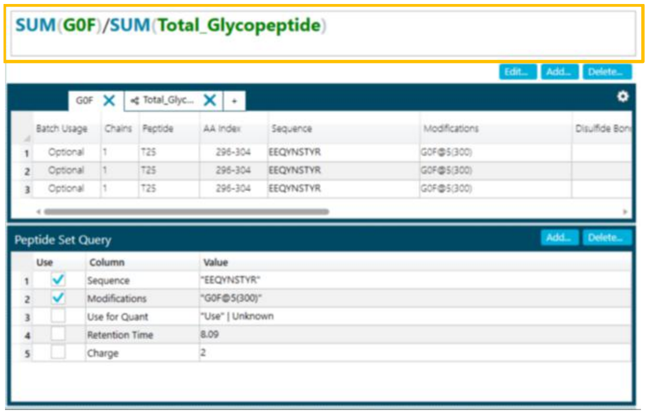
Time (min)	Flow Rate (ml/min)	%A	%B
Initial	0.3	99	1
5.0	0.3	99	*1*
6.0	0.3	90	10
50.0	0.3	65	35
55.0	0.3	40	60
56.0	0.3	10	90
60.0	0.3	10	90
62.0	0.3	99	1
64.0	0.3	99	1
66.0	0.3	10	90
70.0	0.3	10	90
72.0	0.3	99	1
74.0	0.3	99	1
76.0	0.3	10	90
80.0	0.3	10	90
82.0	0.3	99	1
95.0	0.3	99	1



	Batch Usage	Chains	Peptide	AA Index	Sequence	Modifications	Disulfide Bon
1	Optional	1	T25	296-304	EEQYNSTYR	G0F@5(300)	
2	Optional	1	T25	296-304	EEQYNSTYR	G0F@5(300)	
3	Optional	1	T25	296-304	EEQYNSTYR	G0F@5(300)	

	Use	Column	Value
1	☑	Sequence	"EEQYNSTYR"
2	☑	Modifications	"G0F@5(300)"
3	☐	Use for Quant	"Use" | Unknown
4	☐	Retention Time	8.09
5	☐	Charge	2

Figure 2. Definition of Peptide Sets using Filter Criteria. Each peptide set may be saved and shared across projects.



SUM(G0F)/SUM(Total_Glycopeptide)

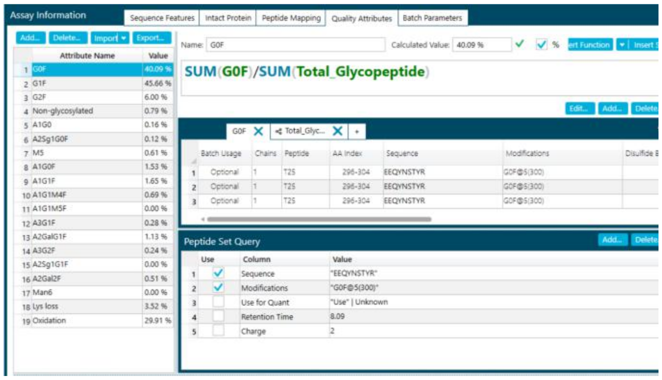
	Batch Usage	Chains	Peptide	AA Index	Sequence	Modifications	Disulfide Bon
1	Optional	1	T25	296-304	EEQYNSTYR	G0F@5(300)	
2	Optional	1	T25	296-304	EEQYNSTYR	G0F@5(300)	
3	Optional	1	T25	296-304	EEQYNSTYR	G0F@5(300)	

	Use	Column	Value
1	☑	Sequence	"EEQYNSTYR"
2	☑	Modifications	"G0F@5(300)"
3	☐	Use for Quant	"Use" | Unknown
4	☐	Retention Time	8.09
5	☐	Charge	2

Figure 3. Custom Calculations.

Once peptide sets are defined, they may be used in the creation of custom calculations for defining attributes. Calculations may use any combination of defined peptide sets containing sum total of all the peptide forms present including modified or non-modified forms. The software has a wide range of built in mathematical operators which provide flexibility in how attribute responses are determined. As an example, Figure 3 shows a calculation for the relative response of the G0F as a ratio of all glycopeptides found within the sample. The resulting value may be expressed as an absolute value or as a percentage.

Additional attributes may be defined in an identical manner to create the list of all the desired attributes for the assay. The list of targeted attributes along with peptide sets and custom calculations are presented in a single user interface to streamline the processes of defining assays (Figure 4). Defined attribute and peptide sets can be exported or imported between projects which can reduce the time required for defining assays across projects.



	Attribute Name	Value
1	G0F	40.09 %
2	G1F	45.66 %
3	G2F	6.00 %
4	Non-glycosylated	0.79 %
5	A1G0	0.16 %
6	A2Sg1G0F	0.12 %
7	M5	0.61 %
8	A1G0F	1.53 %
9	A1G1F	1.65 %
10	A1G1M4F	0.69 %
11	A1G1M5F	0.00 %
12	A3G1F	0.28 %
13	A2GalG1F	1.13 %
14	A3G2F	0.24 %
15	A2Sg1G1F	0.00 %
16	A2Gal2F	0.51 %
17	Man6	0.00 %
18	Lys loss	3.52 %
19	Oxidation	29.91 %

Name: G0F
Calculated Value: 40.09 % ☑ %

SUM(G0F)/SUM(Total_Glycopeptide)

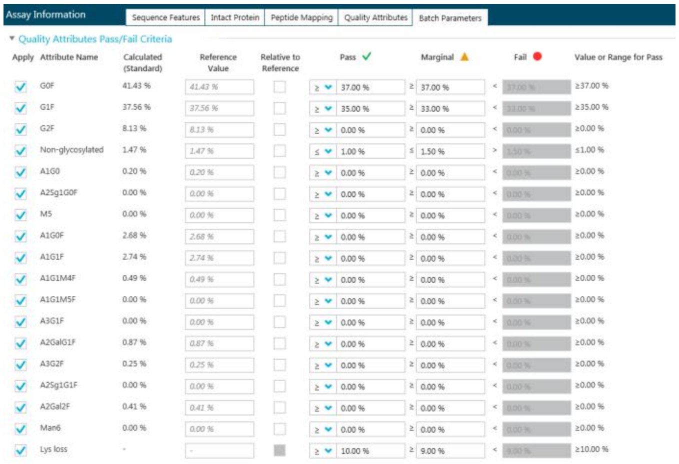
	Batch Usage	Chains	Peptide	AA Index	Sequence	Modifications	Disulfide E
1	Optional	1	T25	296-304	EEQYNSTYR	G0F@5(300)	
2	Optional	1	T25	296-304	EEQYNSTYR	G0F@5(300)	
3	Optional	1	T25	296-304	EEQYNSTYR	G0F@5(300)	

	Use	Column	Value
1	☑	Sequence	"EEQYNSTYR"
2	☑	Modifications	"G0F@5(300)"
3	☐	Use for Quant	"Use" | Unknown
4	☐	Retention Time	8.09
5	☐	Charge	2

Figure 4. Single Interface for Defining Peptide Sets, Custom Calculations, and Targeted Attributes.

The next step after defining attributes is to define the acceptance levels for each attribute. The acceptance levels for each attribute can be defined separately in multiple ways depending on the requirements for the assay. The software provides flexibility to use either the calculated value from the characterized standard or define a custom value from previous studies as the reference for defining attribute pass/fail criteria. Ranges are easily set as greater than or less than specified values or needing to be a specific value. It is also possible to set a range based on a percent difference from a specified value. Separate ranges for pass and marginal acceptance values are defined independently with any value falling out of the defined range being classified as a failing result. Within the project, any specific attribute may be selected or de-selected for processing, depending upon the assay requirement.

Once attributes and their acceptance levels have been defined, samples may be submitted for batch analysis. After analysis is complete the results are reviewed in a concise list of each attribute, its determined level within each sample, and an easy to read indicator for the attributes pass/fail status. Selection of any attribute in the list provides an immediate view of the experimental evidence used in the calculation of the attribute response in the assay to expedite review of the underlying MS and MS/MS data.



Apply	Attribute Name	Calculated (Standard)	Reference Value	Relative to Reference	Pass ✓		Marginal ▲		Fail ●		Value or Range for Pass
☑	G0F	41.43 %	41.43 %	☐	≥	37.00 %	≥	37.00 %	<	37.00 %	≥37.00 %
☑	G1F	37.56 %	37.56 %	☐	≥	35.00 %	≥	33.00 %	<	33.00 %	≥35.00 %
☑	G2F	8.13 %	8.13 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	Non-glycosylated	1.47 %	1.47 %	☐	≤	1.00 %	≤	1.50 %	>	1.50 %	≤1.00 %
☑	A1G0	0.20 %	0.20 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	A2Sg1G0F	0.00 %	0.00 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	M5	0.00 %	0.00 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	A1G0F	2.68 %	2.68 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	A1G1F	2.74 %	2.74 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	A1G1M4F	0.49 %	0.49 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	A1G1M5F	0.00 %	0.00 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	A3G1F	0.00 %	0.00 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	A2GalG1F	0.87 %	0.87 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	A3G2F	0.25 %	0.25 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	A2Sg1G1F	0.00 %	0.00 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	A2Gal2F	0.41 %	0.41 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	Man6	0.00 %	0.00 %	☐	≥	0.00 %	≥	0.00 %	<	0.00 %	≥0.00 %
☑	Lys loss	-	-	■	≥	10.00 %	≥	9.00 %	<	9.00 %	≥10.00 %

Figure 5. Definition of Pass/Marginal/Fail Criteria for each Attribute.

Conclusions

- BioPharmaView™ software 3.0 provides a single software solution for MAM workflows
- Definition and tracking of quality attributes is achieved in a single software package
- Calculation of targeted attributes is executed easily using built in functions
- A wide range of number of attributes may be defined and tracked simultaneously
- Batch analysis of data sets results in a concise and easy to understand result for each attribute across all samples.
- Traffic light indicators for batch pass/marginal/fail criteria for highlighting the outliers.

References

1. A View on the Importance of "Multi-Attribute Method" for Measuring Purity of Biopharmaceuticals and Improving Overall Control Strategy. *The AAPS Journal (# 2017) DOI: 10.1208/s12248-017-0168-3,* Richard S. Rogers, Michael Abernathy, and etc

	View	Quality Attributes	20180213_Nist_...	20180213_Ni...	Reference Value	Value or Range for Pass
4	☐	Non-glycosylated	0.79 % ✓	0.84 % ✓	0.79 % ✓	0.67 % - 0.91 %
5	☐	A1G0	0.16 % ✓	0.17 % ✓	0.16 % ✓	0.13 % - 0.18 %
6	☐	A2Sg1G0F	0.12 % ✓	0.10 % ✓	0.12 % ✓	0.10 % - 0.13 %
7	☐	M5	0.61 % ✓	0.63 % ✓	0.61 % ✓	0.52 % - 0.70 %
8	☐	A1G0F	1.53 % ✓	1.65 % ✓	1.53 % ✓	1.30 % - 1.76 %
9	☐	A1G1F	1.65 % ✓	1.69 % ✓	1.65 % ✓	1.40 % - 1.90 %
10	☐	A1G1M4F	0.69 % ✓	0.76 % ✓	0.69 % ✓	0.58 % - 0.79 %
11	☐	A1G1M5F	0.00 % ✓	0.00 % ✓	0.00 % ✓	0.00 % - 0.00 %
12	☐	A3G1F	0.28 % ✓	0.32 % ✓	0.28 % ✓	0.24 % - 0.33 %
13	☐	A2GalG1F	1.13 % ✓	1.29 % ✓	1.13 % ✓	0.96 % - 1.30 %
14	☐	A3G2F	0.24 % ✓	0.19 % ▲	0.24 % ✓	0.20 % - 0.27 %
15	☐	A2Sg1G1F	0.00 % ✓	0.03 % ●	0.00 % ✓	0.00 % - 0.00 %
16	☐	A2Gal2F	0.51 % ✓	0.54 % ✓	0.51 % ✓	0.43 % - 0.58 %
17	☐	Man6	0.00 % ✓	0.00 % ✓	0.00 % ✓	0.00 % - 0.00 %
18	☑	Lys loss	3.52 % ✓	3.77 % ✓	3.52 % ✓	2.99 % - 4.05 %
19	☐	Oxidation	29.91 % ✓	0.00 % ●	29.91 % ✓	25.43 % - 34.40 %

	Quality Attribute	Set	#	RT	Theoretical Mono m/z	Observed Mono m/z	Charge	XIC Area	Sequ
1	Lys loss	Lys_Loss	1	10.71	394.7293	394.7295	2	3.43e5	SLSLS
2	Lys loss	Lys_Loss	1	10.71	788.4512	788.4522	1	2.89e4	SLSLS
3	Lys loss	Total C Te	1	10.71	394.7293	394.7295	2	3.43e5	SLSLS
4	Lys loss	Total C Te	1	10.71	788.4512	788.4522	1	2.89e4	SLSLS
5	Lys loss	Total C Te	1	13.03	330.6818	330.6819	2	6.05e5	SLSLS
6	Lys loss	Total C Te	1	13.04	660.3563	660.3553	1	9.59e6	SLSLS

Figure 6. Attribute Summary for Batch Analyzed Samples. Attribute levels and pass/fail status are clearly indicated to expedite data review .

Document number: RUO-MKT-02-7508-A