Advanced QA/QC characterization
MS in QC : Multi Attribute Method

Global BioPharma Summit
A Complex Problem: Drug Safety and Quality

**Safety**
Is the product safe to use? (e.g. Immunogenic effects?)

**Potency**
Does the drug have the expected effect? (e.g. CDR complementation)

**Knowledge**
How do changes effect the therapeutic? (e.g. Oxidation)

**Quality**
How do changes in process effect the product? (e.g. Glucose concentration on glycoforms)
Drug Development Workflow: From Discovery to Production

**What?**

- Discovery
- Pre-Clinical Development
- Approval
- Release

**How much?**

- Unregulated
- GLP
- GCP / GMP

**BioPharma Finder**

**Chromeleon**

**MS in QC: Multi Attribute Method (MAM)**

- Molecular Assessment
- Quality Control
- Attribute Science Groups
- Analytical and Automation Process Technologies
- Protein Attribute Chemistry
- Manufacturing
- Manufacturing
## Advantages of MAM

<table>
<thead>
<tr>
<th>Required Characterizations</th>
<th>MAM Method</th>
<th>Conventional Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggregate Assessment</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Deamidation (Isomerization) Assessment</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Disulfide Isoform Assessment</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Glycation Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>High Mannose Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Methionine Oxidation Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Signal Peptide Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Unusual Glycosylation Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>CDR Tryptophan Degradation Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Non-consensus Glycosylation Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>N-terminal pyroGlutamate Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>C-terminal Lysine Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Galactosylation Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Dimer Assessment</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Fragmentation (peptide bond) Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Disulfide Reduction (DS Fragmentation) Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Host Cell Protein Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Mutations/Misincorporations Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hydroxylsine Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Thioether Assessment</td>
<td>Maybe</td>
<td>Yes</td>
</tr>
<tr>
<td>Trisulfide Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Non-glycosylated Heavy Chain</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>DNA Assessment</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cysteine Adducts Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>C-terminal Amidation Assessment</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CDR Conformers (HIC Isoform) Assessment</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>O-linked Glycans Assessment</td>
<td>Maybe</td>
<td>Yes</td>
</tr>
<tr>
<td>Fucosylation Assessment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Residual Protein A</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Identity</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### Multi Attribute Method

<table>
<thead>
<tr>
<th>Pep Map-MS</th>
<th>SEC</th>
<th>CEX</th>
<th>rCE-SDS</th>
<th>nrCE-SDS</th>
<th>HILIC</th>
<th>ID ELISA</th>
<th>HCP ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Yes</td>
<td>Indirect</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Maybe</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Maybe</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Maybe</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Maybe</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
Multi-Attribute Method for QC

**Current Release Method**
- 70% Potency
  - Attribute 1
  - Attribute 2
  - Attribute 3
  - Attribute 4
- 100% Potency
  - Main Peak
- 150% Potency
  - Attribute 5

**Product Knowledge**
- CEX separation
- 100% Potency
  - A1, A2
  - 2 x A3
  - A3, A4
- 150% Potency
  - A5

**Multi Attribute Method**
- CEX separation
- Sub-fraction Potency Assessment

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main peak</td>
<td>100%</td>
</tr>
<tr>
<td>A1</td>
<td>50%</td>
</tr>
<tr>
<td>A2</td>
<td>102%</td>
</tr>
<tr>
<td>A3</td>
<td>95%</td>
</tr>
<tr>
<td>A4</td>
<td>102%</td>
</tr>
<tr>
<td>A5</td>
<td>150%</td>
</tr>
</tbody>
</table>

Replacing conventional CEX monitoring of pre-peaks with more specific methods directly measuring attributes affecting safety and efficacy.
Key Tenets of MS in QC (aka MAM)

Multi Attribute Method

High Resolution
Using High Resolution/Accurate Mass MS instrumentation to directly measure CQA

Confirmation
Confirm process by evaluating release product against a gold reference standard

Knowledge
Eliminate the need of traditional lot release methodologies while increasing product knowledge

Quality by Design
Implement hybrid traditional (evaluation in QC) and QbD approach (monitoring of CQA and end-result of CPP space)
Materials

3 μg Trypsin Digested NIST mAb

Sample

Thermo Scientific™ Vanquish™ UHPLC
70 minute Gradient 250 μL/min
0.1% FA in H2O and MeCN
50°C Column Temp

Column

2.1 x 150 mm 1.5 μM
Thermo Scientific™ Accucore™
Vanquish C18+

uHPLC

Thermo Scientific™ Q Exactive™ HF
120k Resolution @ 200 m/z
300 to 1800 m/z

MS

Thermo Scientific™ Chromeleon™
CDS 7.2 SR5
CFR 21 Part 11 Compliant Data
Acquisition, Processing, and Reporting

Processing

Thermo Scientific™ BioPharma
Finder™ 2.0
Component Detection, Peptide Mapping, and CQA Selection

Discovery

3 μg Trypsin Digested NIST mAb
Methods

1. Product Denaturation with 7 M Guanidine HCl and reduction with 500 mM DTT (30 min)
2. Alkylation with 500 mM Iodoacetate (20 min)
3. Desalting with BioRad P-6 column
4. Trypsin Digestion at 37 C (30 min)
5. LC-MS/MS Analysis – Top 5
6. Peptide Mapping Selection of Attributes to Monitor
7. Targeted peak detection
8. Non-targeted peak (new features)

R. Rogers, et al. MAbs 2015, 7, 881-890

Method Evaluation and Advancements

nibrt

ThermoFisher SCIENTIFIC
Peptide Mapping

TIC
Intensity 3e9

Basepeak
Intensity 1.6e9
Peptide Mapping

**TIC**
Intensity 3e9

**Basepeak**
Intensity 1.6e9

**Light Chain**

**Heavy Chain**

**Sequence Coverage**
Complete sequence coverage of heavy chain and light chain

**Feature Chromatogram**
Detected features belonging to light chain, heavy chain, and unidentified species.
Establishing CQAs

**SELECT Component Used for Quantitation**

- Component
- Confidence
- Extracted Ion Chromatogram
- Observed Spectra
- Predicted

**Modification Summary Chart**

- Predicted/Observed RT shift

**<3 ppm mass error**
MS in QC – Late Stage Discovery to Process Dev / QC

- Analytical group is primary driver and is responsible for:
  - Identification and selection of target peptides / modifications
  - Generation of targeted component list (HR/AM MS1) for QC
  - Complete analytical method (processing and instrument methods, designing report templates, eWorkflows)
  - Development of peptide mapping assay SOPs (for QC)
  - MS list is transferred directly from the discovery experiment in BioPharma Finder 2.0 to Chromeleon
  - Checkboxes enable easy selection of components to transfer.
  - More can be added at anytime in the future
Data Analysis Processing Chromeleon

**Chromeleon 7.2**

- Set up injection sequence
- Build LC and MS methods
- Target. Confirm. Integrate.
- Look for new peptides/impurities/features
- Automatically generate reports outputs/formulas

**Simple. Logical.**

1. **Create Injection List** (eWorkflow)
2. **Define Instrument Method** (LC and MS, eWorkflow)
3. **Targeted MS Processing**
   - Verify all expected peptides
4. **Non-Targeted MS Processing**
   - Identify new peptides/impurities/modifications
   - Comparison to reference standard (A to B)
5. **Reporting**

---

[ThermoFisher Scientific Logo]
Building Targeted List of Critical Quality Attributes
Adjust Processing Settings as Desired

Chromeleon 7.2

MS Settings

high resolution enables accurate extraction and quantitation

no offline recalibration necessary
Adjust Processing Settings as Desired

MS Settings

Composite Scoring

No. of Conditions Satisfied for a Full Pass

No. of Conditions Satisfied for a Partial Pass

- Isotopic Ratios (Individual)
- Isotopic Correlation (Envelope)
- Mass Accuracy
- Retention Time Apex Alignment
Adjust Processing Settings as Desired

Chromeleon 7.2

- MS Settings
- Composite Scoring

Integration Settings

- RT Extraction Settings
- MS or MS/MS Filters
- Ion Ratio Settings
- MS Spectra
- Smoothing
Adjust Processing Settings as Desired

**Chromeleon 7.2**

- **MS Settings**
- **Composite Scoring**
- **Integration Settings**
- **Retention Time Settings**

- Set absolute or relative retention time
- Set absolute or relative RT window
- Setting on specific to individual component
- First, greatest, nearest match within window

**Retention**

- **Retention Time**
  - Retention Time: 35.24 min
  - Use this component as reference component

- **Interpretation**
  - Absolute Time
  - Time Distance
  - Time Ratio

- **Reference component:**
  - Requires Reference Peak

- **Retention Time Standard Peak Area Ratio**
  - Ratio Tolerance: 0.000

- **Window**
  - Detect peak within retention time +/- 0.100 min

- **Interpretation**
  - Absolute
  - Relative

- **Component Match**
  - First
  - Greatest
  - Nearest
  - Spectrum only
  - Spectrum and Time
Quantification of CQA: M255 Oxidation

Components
Targeted components and their detection and quantitation status

Experimental Stage
Current state of experiment from injection to reporting

Ion Chromatogram
5 ppm extraction based on scan filters and optional smoothing

Interactive Results
Continually updated results during processing and acquisition

Mass Spectra
Visualize Full MS spectra for selected component

Isotopic Correlation
Validation of peptide identity based on theoretical elemental composition
Separation by Resolution

Deamidation of N392
GFYPSDIA...[Deamid]NYK

Chromatographic Resolution
Temporal separation of many deamidated species
Deamidation of N62

HYN[Deamid]PSLK

Not Chromatographically Resolved
No temporal separation of this deamidated species

Main Peak
Deamidation
Separation by Resolution

Deamidation of N62
HYN[Deamid]PSLK

Not Chromatographically Resolved
No temporal separation of this deamidated species

Main Peak
Deamidation

30k Resolution
25 ppm Extraction
Completely incorrect quantitation
Deamidation of N62
HYN[Deamid]PSLK

Not Chromatographically Resolved
No temporal separation of this deamidated species

30k Resolution
25 ppm Extraction
Completely incorrect quantitation
Deamidation of N62

Not Chromatographically Resolved
No temporal separation of this deamidated species

Separation by Resolution

30k Resolution
25 ppm Extraction
Completely incorrect quantitation

HYN[Deamid]PSLK

Completely incorrect quantitation
Deamidation of N62
HYN[Deamid]PSLK

Not Chromatographically Resolved
No temporal separation of this deamidated species

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Ret Time min</th>
<th>Area counts*min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HYNPSLKD</td>
<td>7.928</td>
<td>96322458.682</td>
</tr>
<tr>
<td>HYN[Deamid]PSLKD</td>
<td>8.014</td>
<td>82470.684</td>
</tr>
</tbody>
</table>

TOTAL
Rel Abundance
3.09%

30k Resolution
5 ppm Extraction

Not enough true resolution, over extracts the data
Deamidation of N62

HYN[Deamid]PSLK

Not Chromatographically Resolved
No temporal separation of this deamidated species

Separation by Resolution

30k Resolution
5 ppm Extraction

Not enough true resolution, over extracts the data

Main Peak
Deamidation
Deamidation of N62
HYN[Deamid]PSLK

Not Chromatographically Resolved
No temporal separation of this deamidated species

Main Peak
Deamidation

120k Resolution
5 ppm Extraction
Spectrometrically resolve deamidated peptides
Deamidation of N62
HYN[Deamid]PSLK

Not Chromatographically Resolved
No temporal separation of this deamidated species

Peak Name
First Injection
MS Quantitation Peak
HYNPSLK
HYN[Deamid]PSLK

Ret.Time
min
7.930
8.022

NistAb02_MAM120k_1
MS Quantitation Peak

Peak Area
counts*min
83977247.099
202432.341

NistAb02_MAM120k_1
MS Quantitation Peak

TOTAL
84179679.44

Rel Abundance
0.24%

120k Resolution
5 ppm Extraction
Spectrometrically resolve deamidated peptides
Data Analysis Processing Chromeleon

**Chromeleon 7.2**

- Set up injection sequence
- Build LC and MS methods
- Target. Confirm. Integrate.
- Look for new peptides/impurities/features
- Automatically generate reports outputs/formulas

Simple. Logical.

1. Create Injection List
2. Define Instrument Method (LC and MS)
3. Targeted MS Processing
   - Verify all expected peptides
4. Non-Targeted MS Processing
   - Identify new peptides/impurities/modifications
   - Comparison to reference standard (A to B)
5. Reporting
Detection of New Features: Batch to Batch

- Assign a reference injection (A) and compare as many new injection (B) as desired
  - First Sample Prep (A)
  - Second Sample Prep (B)
- Set Alignment and Framing Settings
  - 0.1% Base Peak
  - 10 ppm
  - 0.5 min
- Automatic Alignment and Framing
- Reporting of Features
- Filtering of Detected Features
  - Monoisotopic
  - Multiple Isotopes
  - Charge between 2 and 4
  - More than 10-fold Change
- Validation of Results
Detection of New Features: Batch to Batch

- Assign a reference injection (A) and compare as many new injection (B) as desired
  - First Sample Prep (A)
  - Second Sample Prep (B)

- Set Alignment and Framing Settings
  - 0.1% Base Peak
  - 10 ppm
  - 0.5 min

- Automatic Alignment and Framing

- Reporting of Features

- Filtering of Detected Features
  - Monoisotopic
  - Multiple Isotopes
  - Charge between 2 and 4
  - More than 10-fold Change

- Validation of Results
Detection of New Features: Batch to Batch

- Assign a reference injection (A) and compare as many new injection (B) as desired
  - First Sample Prep (A)
  - Second Sample Prep (B)

- Set Alignment and Framing Settings
  - 0.1% Base Peak
  - 10 ppm
  - 0.5 min

- Automatic Alignment and Framing

- Reporting of Features

- Filtering of Detected Features
  - Monoisotopic
  - Multiple Isotopes
  - Charge between 2 and 4
  - More than 10-fold Change

- Validation of Results
Detection of New Features: Batch to Batch

- Assign a reference injection (A) and compare as many new injection (B) as desired
  - First Sample Prep (A)
  - Second Sample Prep (B)

- Set Alignment and Framing Settings
  - 0.1% Base Peak
  - 10 ppm
  - 0.5 min

- Automatic Alignment and Framing

- Reporting of Features

- Filtering of Detected Features
  - Monoisotopic
  - Multiple Isotopes
  - Charge between 2 and 4
  - More than 10-fold Change

Validation of Results

In Spec: No New Features
Detection of New Features: PRTC Syn Peptide Kit

Detection of new features in Nist Ab digest with 1% of Peptide RT Calibration peptides spiked in

Digest Only (A)
Digest + 500 fmol/ul PRTC (B)

Alignment and Framing Settings
1.0% Base Peak 10 ppm
Detection of New Features: Stress Study

Two Weeks at pH 8
Forced stress study versus sample immediately after preparation

849.0461 m/z @ 37.11 min
Detection of New Features: Stress Study

Two Weeks at pH 8
Forced stress study versus sample immediately after preparation

559.9413 m/z @ 24.62 min
CQA Profiling
## Glycan Profile

### Glycan Profile Table

<table>
<thead>
<tr>
<th>Sequence Details</th>
<th>Thermo Fisher Scientific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td></td>
</tr>
</tbody>
</table>

### EIC SIZE Glycopeptides

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>G0F</th>
<th>G1F</th>
<th>G0F</th>
<th>G1F</th>
<th>G0F</th>
<th>G1F</th>
<th>G0F</th>
<th>G1F</th>
<th>MS</th>
<th>MS</th>
<th>MS</th>
<th>MS</th>
<th>Debye</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### % Abundance

![Bar Chart of Glycan Profile]

Custom MS Report Template
eWorkflows automatizes the acquisition, data processing, and reporting processes.

- Ultimate goal of a QC implementation
Pack Everything Up

Chromeleon 7.2

Contains:
Sequence
Methods (Instrument and processing)
Report Formats
Studio Layouts
Raw Data
And more…
Enterprise Based Solution

Chromeleon 7.2

Remote:
- Data Vaults
- Data Processing
- Licensing
- Administration

Remote: Data Vaults
- Data Processing
- Licensing
- Administration

SQL Server
- Database

File Server
- Data Vault Service
- Domain Services
- Dongle

Laboratory
- Client (x1)
- Inst Controller (x1)
- Inst Operation (x1)
- MS Acquisition (x1)
- Class 3 Inst. (x1)
- Compliance (x1)

Data Proc
- Data Client (x1)
- Compliance (x1)
- Inst Operation (x1?)
- Non-target (x1 float)
- Rprt Design (x1 float)

Laboratory
- Client (x1)
- Inst Controller (x1)
- Inst Operation (x1)
- MS Acquisition (x1)
- Class 3 Inst. (x1)
- Compliance (x1)
Conclusions

Multi-Attribute Method

Not just about reducing the number of tests, but a better way to validate purity

**Insightful**
Use high quality deeper knowledge of product to improve from development to production through QbD

**Easy to Use**
Simple LCMS methods without the need for advanced chromatography or gas phase separations

**High Resolution**
Take advantage of another dimension of separation to see what is missed.

**Powerful**
Replace numerous conventional lot release techniques while providing greater knowledge of product attributes

**Previously Filed**
Software/hardware already used for several filings to begin clinical trials with the FDA

**Confident**
Composite peptide scoring and separation of isotopes/charge states helps eliminate false quantitation