Table 3: Practical Considerations: Mass Spec Based Multi-attribute Method

**Session 1:**
**Facilitator:** Carly Daniels, *Pfizer, Inc.*  
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**Session 2:**
**Facilitator:** Liqiang (Lisa) Zhou, *AbbVie Inc.*  
**Scribe:** Tyler Carlage, *Biogen*

**SCOPE:**
This Table will discuss practical considerations for implementation of the mass spec-based, peptide mapping multi-attribute method for product development, QC release, and on-line monitoring with feedback control using process analytical technology (PAT). Table members will discuss strategies for the introduction of the multi-attribute method at appropriate stages, as well as potential challenges in implementing novel analytical tools in manufacturing and QC environment.

**QUESTIONS FOR DISCUSSION:**
1. What requirements do we need to meet to have MAM accepted by regulatory agencies?

2. Data processing challenges - what software tools have been useful, level of manual intervention required?

3. Is the multi-attribute method being employed during drug development, if so at what stages?

4. What are the criteria for selection of attributes being measured by MAM? (detectability, criticality, etc.)

5. What system suitability are we using in our multi-attribute method?

6. Is there an intention to consider replacement of typical release methods with MAM? What are the regulatory and/or technical hurdles involved?

7. What are the advantages/disadvantages of using MAM over other orthogonal methods i.e. charge profile or purity methods?

8. Where does MAM fit into the analytical strategy - early stage cell line screening, characterization of DS, release/in-process test, etc.?

9. Is MAM being used on-line or at-line for feedback control? If so, how is it working?

10. Are non-peptide mapping multi-attribute methods being employed? If so, how and where?
DISCUSSION NOTES:

Day 1:

MAM Acceptance by HAs:
- Characterization, not yet GMP
- No attendees have MAM in QC currently; MAM consortium reports MAM is ongoing validation – potentially in QC in 2019
- No attendees have filed MAM in marketing application

Technical Challenges:
- How to ensure reproducibility during transfer
- Need robustness, good system suitability criteria including for the mass spec (eg, standard mix of peptides)
- Manual sample prep is rate limiting – automation is goal
- MAM may not match absolute charge profile data (eg, deamidation vs acidic species); site-specific information more important than profile shift

MAM Usage:
- MAM run in parallel to traditional methods (routine peptide mapping and orthogonal methods)
- Most commonly used as process tool, especially for bioreactor testing (including ambr)
- MAM used for process support including cell line development and forced deg studies
- Platform MAM would be nice, but product-specific MAM may still be needed (eg, using different mix of multi-enzymes)
- In general, use multi-enzyme MAM to ensure better coverage of attributes
- Quantitation method – absolute vs trending. Most use for trending
- Real time release, or online, or at line? Not yet, this is the goal

MAM Software:
- Software is critical. Most use BioPharma Finder, Genedata, and Chromeleon (for QC)
- New peak detection – correlation already demonstrated with traditional separation methods
- New peak detection should look for all new peaks
- False positives, how to reduce? New peak round robin results: robust digest + robust LC + robust MS did not see false positives. May be issue if looking too low. False positives may be system related issue

MAM Validation:
- MAM criteria should mirror traditional process limits and method criteria
- How to set system suitability? Control samples, retention time, site specific RSDs, missed cleavage. Treat like any UPLC method validation

Miscellaneous:
- Better cleavage with GuHCl compared to urea; look for undigested peaks at end of chromatograms as indicator
- Glycation – relative (parent:missed cleavage)
- Stability of relative quant peptides can be demonstrated by dilution and monitor signal
- Non-peptide mapping MAM could be used for smaller proteins and peptides
- Can cIEF-MS be orthogonal to MAM? Possibly
- O-glycosylation? Software solution BioPharma Finder 3.1
- Characterization methods converting to routine methods – increasing trend?
Day 2:
Where does MAM fit into drug development?
- No clear consensus
- Challenges in QC based on method complexity and reproducibility
- Some reports of successful application in clone selection and process development studies
- “New Peak Detector” application of MAM cited as particularly useful, in one case was able to detect a sequence variant at ~3% abundance using MAM method which had been previously missed by regular peptide map
- Interest was expressed in applying MAMs to hotspot analysis, and in-process attribute monitoring (i.e. glycan distribution and glyco-occupancy)
- The potential interfacing of MAM systems with data management systems for process monitoring and modeling was also discussed as being an exciting opportunity in the future

Reproducibility and Robustness
- Some developers cited poor reproducibility in general for MAM methods
- An inter-lab study using NIST mAb cited great variations in results due to method performance across labs
- One lab reports typical CV’s at 10% or lower for most attributes, anything below 0.5% would have poorer reproducibility
- Most users are using high-res Orbitrap instruments for MAM, but moving to triple-quad may help to improve CV’s further
- System suitability samples are critical for optimal method performance over time to ensure sensitivity and resolution of the system before running samples
- Dedicated systems running formic acid only (no TFA) was also cited as an important factor for reproducibility

Replacement of traditional analytical methods with MAM
- Challenges cited with replacing charge methods such as CEX or icIEF with MAM methods based on the difficulty in achieving “mass balance” (sum of variants by MAM do not equal the values reported by CEX or icIEF)
- Differences between CEX/icIEF and MAM were discussed including difficulty in measuring low-level polydisperse variants by MAM, ionization efficiency differences between acidic and non-modified variants, low recovery of small hydrophilic peptides such as VSNK (deamidation), and a general lack of complete understanding of all of the species being separated by icIEF

Other general comments
- One key difference between regular peptide mapping, which focuses on providing high sequence coverage, and MAM which focuses on reporting out abundance of attributes, is the sample preparation which is optimized (typically via shorter digestion times) to reduce artifacts such as increased oxidation or deamidation.
- Most users are using Orbitrap-type instruments and data processing software. Genedata and Protein Metrics were also cited as powerful data processing solutions with high learning curves.