Table 33: Multi-Attribute Method - Challenges in Using MAM for QC of Therapeutic Proteins

Facilitators –

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Scope:

Multi-attribute method (MAM), a liquid chromatography-mass spectrometry (LC-MS) based peptide map method, consists of a targeted quantitation function and a non-targeted feature known as new peak detection (NPD). The targeted function provides relative quantitation of multiple PQAs in a single analysis, while NPD is a data processing approach performing differential analysis of LC-MS chromatograms, which can be used to detect unexpected peaks in clinical batches compared to reference standard.

MAM has been discussed with FDA's Emerging Technology Team, which works with drug developers to facilitate the adoption and implementation of novel technologies. It is suggested that MAM could be used as a replacement for at least several conventional QC methods, including hydrophilic interaction liquid chromatography for glycan profiling, ion exchange chromatography for charge variant analysis, reduced capillary electrophoresis-sodium dodecyl sulfate for clipped variant analysis, and identity test as well. There has been broad interest in the implementation of MAM for QC testing of therapeutic proteins in the biopharmaceutical industry.

This roundtable focuses on the scientific and regulatory considerations and challenges when implementing MAM for QC of therapeutic proteins.

Questions for Discussion:

This roundtable will begin with an introduction by participants on the current status of MAM in their organizations. The following are topics for discussion:

1. Method development
    a. Targeted MS: Precision, Robustness, etc.
    b. New Peak Detection: false positive detections, thresholds and fold changes that constitute a new species
2. Method validation
    a. System suitability
    b. Operating MAM in QC lab

3. Setting product release specifications: MAM vs conventional method
4. Regulatory considerations
    a. Risks assessment of using MAM to replace conventional QC method
    b. Best practices for incorporating MAM data and information in regulatory submissions
5. New and unique MAM applications, and MS in QC beyond MAM.

Discussion Notes:

January 26 and 28 –

Table 33 had a nice balance from users and managers from industry, mixed with attendees from the regulatory and vendors. MAM was discussed from both a protein-based as well as GT perspective.

MAM was defined as the replacement of traditional analytical technologies with LC-MS as a single replacement technology. MAM has been used for a number of years, and biopharma organizations are now looking at taking MAM all the way to QC.

Two cases drive this development: traditional technologies do not work for certain molecules, but probably more important is the need to replace a number of technologies providing complimentary data with one technology that preferably with one analysis provides all the information.

The case of charge heterogeneity was discussed as it is one of the more challenging ones to correlate. Traditional methods such as iCIEF, CZE and ion exchange chromatography provide rich data and for example group all acidic peaks into one category under a single specification. The point was made that not all these peaks are CQAs – something that can be decided upon using the knowledge of the molecule as well as stress test results. As long as trends are the same, though granted that unknowns may be harder to catch with LC-MS than knowns.

Glycan analysis was assumed to have easier correlation between traditional methods and LC-MS. Intact and attribute monitoring have been described in the literature and subunit based attribute analysis is known to be used as well.

Challenges that still need to be resolved that the team seemed to agree on are as follows:

- Overall transfer to QC

o Currently biopharma organizations looking at Phase I, but what about commercialization a few years from now, where a method might be tied to a molecule for many years?

- Regulatory submission requirements for a MAM method important. Will this be case by case or will there be standards?

- Working with the very small available quantities of gene therapeutics

- In short, a fair amount of things still to be learned

There are some other considerations worth noting:

- Advanced mass spectrometry instrumentation will continue to be required for research and development

- For QC however, robustness, size (desktop) and ease of use are paramount, which includes the software with locked down methods. Initial work seems to indicate that with the proper software, analysts who are new to MS can perform routine assays.

- New peak detection is challenging and important

- Targeted quantification also of importance

- Working with large nucleic acids can be a challenge, but consult your MS provider

- Though currently LC-MS is the standard for MAM, CE-MS developments could be considered going forward.

February 1 and 3 –

Method Development:

- One company claims that Lys-C offers advantages over Trypsin for MAM. These include a more complete digest, less small hydrophilic peptide that are easy to miss, and less susceptibility to enzyme inhibitors (Gu-HCl for example)
- Key features (CQAs) to track with MAM include: Glycans, PTM Hotspots (glycation, ox, deam, sialylation etc.), sequence variants, CDR peptides (for identity test)
- Validation of new peak detection is the biggest challenge: Determine the right thresholds and fold changes that constitute a new species; Avoid treating false positive identifications as new peaks; Potential use of routine MS2 (data-independent fragmentation) for new peak detection; Importance of providing development data about e.g. degradation peaks to QC to reference, so that not treated as new peaks.
- Measuring peak areas of native and modified peptide can be used for relative quantitative assessment; Quantitation is based on MS, not UV; Prefer to use high resolution mass spectrometer; The entire workflow, including quantitation, should be as automated as possible.

Method Validation:

- One company uses a reference sample as a System Suitability standard, tracking key suitability attributes including mass accuracy, retention time, and peak area; The peak area

for MAM can vary quite a bit, due to issues like dirty source of the mass spectrometer, generally, suggests ensuring Peak Areas are at least ca. 80% of expected.

- Data integrity consideration: As much of automation of the QC MAM process as possible to limit potential user error and keep assay suitable for QC; It can drive selection of instrument platform, software, and method; Consider using system suitability for the entire workflow not limited to the LC-MS system: include sample preparation and data processing etc.
- What instruments are best suited for QC MAM? Several manufactures are working on instruments that are designed for MAM QC analysis. Instruments designed for characterization work in research and development are likely too complex for QC applications.

Product Release Specification:

- No definite specifications were mentioned during discussion. Studies would be necessary to determine suitable MAM specifications, including what CQA's to monitor and what specific limits to use.
- Challenges reconciling results of traditional methods (e.g. overall modification level) vs MAM (site-specific).

Regulatory Considerations:

- MAM is not ideal for replacing all conventional QC tests. For example, it is much more practical to use Size Exclusion Chromatography to assess aggregation.
- MAM peptide mapping for QC could replace some conventional assays. For example, released glycan analysis using a HILIC column. It would be important to include bridging studies demonstrating the ability of MAM to detect similar changes compared to the conventional method it could replace.
- When submitting MAM it is important to include how you validated the assay, why you picked certain peptides to monitor, and how you determined the acceptance criteria.
- A risk to using MAM for QC work is the discovery of new peaks at the stage of QC. For this reason, it is important to carry out detailed characterization work to identify as many potential species and adducts as possible; Analyzing data from forced degradation studies can help supplement the targeted library with common drug modifications; Detailed upfront work to build a complete list of potential target masses reduces the risk of new species showing up at the QC targeted MS analysis stage.
- Risk of creating frequent deviations or OOSs (comparing to conventional methods)
- Application of MAM may be product-specific decision.

- Best practices include: Importance of early engagement with regulators; Provide complete validation information and development info; Provide comparison to results from other methods.

New applications:

- Intact, reduced, and subunit MAM could be performed in addition to peptide mapping. Reduced MAb MAM is useful for assessing glycosylation.
- HCP methods: Conventionally ELISA for HCP is used for release. MAM has good potential, however, not sure if the performance can catch up with proteomics-based LC-MS method for HCP analysis, since detection of low-level HCPs might need sample overloading and targeted data acquisition workflow.