Table 9: Best Practices for Reporting MS Data in Regulatory Filings

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Scope:

Mass spectrometry (MS) is integral to the characterization sections of biologics regulatory filings. These characterization sections typically include peptide mapping (reduced and non-reduced), intact protein mass analysis, N-glycan analysis and charge variant analysis. The use of MS has empowered scientists to examine many product quality attributes for protein characterization (amino acid sequence, post translational modifications) as well as understanding protein degradation behavior (oxidation, deamidation, isomerization, etc) and profiling process-related impurities (host cell proteins, genetic sequence variants, misincorporations) with increasing detail, depth and speed, thereby providing an unprecedented understanding of our molecules. This roundtable discussion will focus on the best practices for reporting MS data in regulatory filings including: the location of MS data, data typically included in an IND, BLA, comparability assessment, use of quantitative data, description of methods and qualification/validation of methods.

Questions for Discussion:

1. What is the overall level of MS detail that your company incorporates into INDs, comparability sections, and BLAs?
2. How are companies incorporating MAM data? What section in filing? How are MAM results being compared to traditional purity assays? How are potential discrepancies handled?
3. How much MS data and information are included in forced degradation sections?
4. Are you filing additional MS data for multi-specifics (bispecifics, trispecifics etc)? If yes, what?
5. What strategies are employed to report characterization of trace level species (such as low abundance charge variants) where routine methods may not be sufficient?

Rogstad S, Faustino A, et al. A retrospective evaluation of the use of mass spectrometry in FDA biologics license applications. JASMS. 2017; 28(5):786-94.

Discussion Notes:

1. What is the overall level of MS detail that your company incorporates into INDs, comparability sections, and BLAs?

- Typically, low level of MS incorporation in INDs, but level increases with program maturity. Extensive understanding of all peaks in any method is expected by the time BLA is written.
- For late stage filings, each visible peak in the UV profile of a peptide map is analyzed and its accurate mass determined with the goal of obtaining high amino acid sequence coverage.
- Primary sequence coverage is included along with intact mass.

2. How are companies incorporating MAM data? What section in filing? How are MAM results being compared to traditional purity assays? How are potential discrepancies handled?

- MAM is employed and shared internally for testing and evaluating potential impacts on release SPEC.
- MAM used during forced degradation, comparability, can be used to tie forced deg to potency drop (structure function connection).
- Attribute groups still rely on additional untargeted peptide map(s) for complete characterization especially for forced deg where potential attribute impacts are unknown and outside the typical ranges observed during manufacture.
- Sensitivity is frequently lower than we need to evaluate, therefore it is important to avoid ask binary questions (ie “is x detected?”). Instead, it is preferable to establish threshold before reporting, (ie all attributes above x%). There is often no value in reporting 0.002% misincorporation for instance.

3. How much MS data and information are included in forced degradation sections?

- No discussion on this topic, may be a topic to revisit next year.

4. Are you filing additional MS data for multi-specifics (bispecifics, trispecifics etc)? If yes, what?

- No discussion of bispecific or trispecific, but ADCs (antibody drug conjugates) rely heavily on established mAb platforms and prior knowledge. It is common to compare antibody and ADC during filings. However, the antibody is considered an intermediate – not final product or final DS. Therefore, the antibody is treated almost like a critical reagent that still needs to establish good quality.
- mAb and ADC analytical groups may differ in terms of instrumentation, methodology, but this leads to different results from the two different groups. One group may be

responsible for the data generation for filing, therefore it may make sense to defer to this group's finding in the case of minor discrepancies between methods.

5. What strategies are employed to report characterization of trace level species (such as low abundance charge variants) where routine methods may not be sufficient?

- No discussion on this topic, may be a topic to revisit next year.

Other discussion topics and considerations:

- No single method or map is sufficient or comprehensive. Complementary maps, orthogonal methods are essential to draw holistic conclusions.
- Intact mass in particular is a powerful and informative assay to supplement peptide maps and MAM.
- Especially important for ADC where DAR (drug-to-antibody ratio) is critical. Intact is valuable to get the DAR profile/distribution, but peptide map can complement the intact mass DAR info with site-specificity.
- UV profile in particular can be misleading and is susceptible to hiding coelution in peptide maps. It is important to rely on MS data to understand what components are contributing to each UV peak.