Table 4: Tips and Tricks for Automated MS Data Analysis

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Scope:
Mass spectrometry has been increasingly leveraged in all stages of protein therapeutic development, with applications ranging from target discovery (proteomics) to protein characterization (peptide map, intact mass) to monitoring of critical quality attributes for release testing (Multi-Attribute Method). While data analysis has been one of the more resource-intensive aspects of mass spectrometry-based workflows, the availability of several data processing platforms tailored toward the biotechnology industry have greatly streamlined many components of data analysis to the point that automated data analysis is becoming a reality. This round table will focus on how users have incorporated automated data analysis into their routine analysis, the limitations that still necessitate manual data analysis, and how these gaps can be closed.

Questions for Discussion:
1. Is automated data analysis routine practice for confirming peptide identity? How often is MSMS data being evaluated for manual confirmation of peptide and PTM identification? How often are quantitation levels of challenging PTMs such as iso-aspartic acid and deamidation being manually verified?
2. Is automated data analysis used for monitoring system suitability? Are analysts tracking method performance? What metrics are being monitored, and can this be automated?
3. What measures are being taken to ensure data integrity of (automatically) processed MS data?
4. For intact mass analysis, are vendor-supplied or vendor-neutral data processing platforms being utilized? Beyond confirming the expected molecular weight, what other components are being monitored during intact mass analysis (e.g. PTMs, fragmentation, etc.)? Can quantitative analysis be performed by automated data processing?

Discussion Notes:

1. Is automated data analysis routine practice for confirming peptide identity? How often is MSMS data being evaluated for manual confirmation of peptide and PTM identification? How often are quantitation levels of challenging PTMs such as iso-aspartic acid and deamidation being manually verified? Input from the round table participants was that these challenging modifications are still being manually verified following initial identification with data processing software such as BioPharmaFinder or Protein Metrics. The general consensus from the Round Table participants was that

automated data analysis hasn't progressed to the point that identification and quantitation of these attributes without analyst oversight.

2. Is automated data analysis used for monitoring system suitability? Are analysts tracking method performance? What metrics are being monitored, and can this be automated? Multiple participants are batching a pre-digested standard with the sample sequence in combination with digesting a standard protein when prepping a sample set. Analysts are monitoring alkylation levels, retention time shifts, mass accuracy, peak area of reference peptides, and on-column oxidation levels to assess system suitability and sample acceptance. Many of these parameters are being monitored using automated workflows with software such as Chromeleon or Protein Metrics.
3. What measures are being taken to ensure data integrity of (automatically) processed MS data? Discussion of data integrity focused on standardization of sample naming when building sequences in data acquisition software as well as cloud-based storage of raw and processed data using platforms such as Chromeleon or Protein Metrics, which both office compliant data archiving solutions.
4. For intact mass analysis, are vendor-supplied or vendor-neutral data processing platforms being utilized? Beyond confirming the expected molecular weight, what other components are being monitored during intact mass analysis (e.g. PTMs, fragmentation, etc.)? Can quantitative analysis be performed by automated data processing? Due to time constraints, discussion of intact mass data processing was limited, though the input from the Round Table participants suggested that most people are only using automated data processing to confirm identity of the molecule of interest. Evaluation of variants identified in intact mass data is generally still being assessed by the analyst.