

Roundtable Session 1 – Table 1 – MS of Antibody-Drug Conjugate and Antibody-Peptide Conjugate

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Abstract:

The biopharmaceutical landscape is rapidly evolving beyond established monoclonal antibodies (mAbs) toward increasingly complex therapeutic modalities, including multispecific antibodies and oligonucleotide-based therapeutics. At the same time, growing interest has emerged in mixed modalities such as antibody-drug conjugates (ADCs), antibody-oligonucleotide conjugates (AOCs), and antibody-peptide conjugates (APCs). These conjugated formats are designed to combine the benefits of different molecular classes, but they also introduce new analytical challenges.

Mass spectrometry is the analytical technology of choice for the detailed characterization of conjugated and mixed-modality therapeutics. However, combining different molecular classes within a single product introduces challenges throughout the analytical workflow. Sample preparation can affect labile conjugates, leading to degradation, deconjugation, or other preparation-induced artefacts. Furthermore, the diverse physicochemical properties can result in atypical chromatographic behavior, altered ionization efficiencies and complex mass spectrometric response. Data analysis presents another emerging challenge, that includes the confident assignment of conjugation sites, accurate quantification of product-related variants, differentiation of true molecular heterogeneity from preparation-induced artefacts, and generation of results that are reproducible, interpretable, and actionable throughout development.

Discussion Questions:

- 1) Where do you primarily see bottlenecks in the LC-MS characterization of ADCs, APCs, and other mixed-modality therapeutics: sample preparation, data acquisition, data analysis, or interpretation?
- 2) Have characterization methods kept pace with the increasing complexity of conjugated therapeutics or are there still important analytical blind spots?
- 3) Is DAR still the most informative metric for ADC quality, or should a broader selection of quality attributes be considered? Which attributes would you prioritize?

- 4) What are the biggest challenges in studying biotransformation products of complex conjugated therapeutics?
- 5) As therapeutic modalities and analytical data sets continue to increase in complexity, how can LC-MS characterization workflows be scaled to meet future development needs?

Notes:

The group agreed that overall analytical workflows are generally similar across antibody-drug conjugates (ADCs), antibody-oligonucleotide conjugates (AOCs), and antibody-peptide conjugates (APCs). However, data analysis continues to be one of the biggest challenges, especially when it comes to determining conjugation sites. This adds another layer of complexity to characterization efforts. Across all modalities, participants emphasized the ongoing challenge of identifying and characterizing product-related impurities and other unknown species.

For ADCs, monitoring payload release and understanding biotransformation were highlighted as particularly difficult areas. Participants noted that obtaining enough material for biotransformation studies can be challenging, and unexpected post-translational modifications or other biotransformation products are often difficult to identify. Additional complications can arise from linker or payload modifications, such as the 2 Da mass loss observed during biotransformation studies. Interpreting small-molecule fragmentation data also remains challenging, in part because current software tools are not always well suited for these analyses. The limited amount of published literature and reference information available further adds to the difficulty of data interpretation.

As molecule complexity increases, so does the complexity of data analysis. This is especially true for modalities such as antibody-peptide conjugates, where both the antibody and peptide components need to be considered during characterization. Antibody-cyclic peptide conjugate was brought up as an example as complex modality. Participants also discussed the challenges associated with impurity characterization, noting that while some product-related species are expected and can be anticipated, unexpected species can also emerge and significantly complicate analytical investigations.

Drug-to-antibody ratio (DAR) determination remains a key focus area, particularly for biotransformation studies. Participants described challenges related to coelution of species with different DAR values, which can make accurate quantitation difficult. The use

of difluoroacetic acid (DFA) was mentioned as a potential approach to improve reversed-phase separations. These challenges can become even more pronounced for bispecific ADCs, where longer chromatographic gradients may lead to broader peaks and lower mass spectrometry signal intensity. Ion mobility-mass spectrometry (IM-MS) was viewed as a useful tool for species identification, although limitations remain for quantitative applications and instrument accessibility. Native mass spectrometry was also discussed, but its routine use in biotransformation studies is often limited by low sample concentrations.

For release testing, DAR determination was generally considered more straightforward. Participants reported good agreement between results obtained using RPLC-UV and HIC-MS methods, and vendor-provided software is commonly used for DAR calculations and data processing. For dual-payload ADCs, baseline chromatographic separation was viewed as important for accurately quantifying each payload distribution. UV-based methods continue to play an important role for quality control testing and regulatory submissions. The group also discussed whether removing one or both payloads during sample preparation could help reduce analytical complexity and simplify characterization of dual-payload ADCs.