Roundtable Session 1 – Table 1 – New Mass Spec Methods to Tackle New Biotherapeutic Challenges

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Abstract:

Discovery and development of modern biotherapeutics relies heavily on mass spectrometry-based techniques. Successful scientific teams can maximize analytical power to optimize drug development and therapeutic outcomes while avoiding pitfalls. As new formats emerge, MS-based applications must be improved or newly replaced to accurately guide drug development campaigns of increasingly complex formats.

Specific chemical liabilities vary broadly across modalities. While advanced protein engineering efforts have rapidly propelled the success of antibody-related molecular formats, including ADCs, multispecific, and fusion proteins, new designs have changed the landscape of analytical risks. More complex formats such as engineered antigens, AAV samples, CAR T cells, and oligonucleotide-based formats, may require that scientists develop specialized analytical methods, novel reagents, or customized software tools.

Analytical objectives further vary dramatically depending on stage, spanning the entire breadth of discovery and development pipelines, including discovery biology, high throughput, lead characterization, clinical, process development, and manufacturing.

To address this vast set of analytical challenges facing the biopharmaceutical industry, analytical technology vendors push for higher performing consumables, instrumentation, and software tools. Which techniques (or combinations) are working or seem promising? Where are limitations remaining?

Discussion Questions:

At what stages are platform technology shifts happening? *Which emerging formats are presenting challenges to conventional LC-MS platforms? Which new technologies are showing promise to resolve bottlenecks? How fast does high throughput need to be? How deep must characterization efforts go?*

What are the emerging challenges for characterizing antibody related formats (mAb, ADC, multispecifics, fusion proteins)? *Which types of sample heterogeneity are proving to be challenging? Glycoprotein heterogeneity? Conjugation-related challenges? Which technologies are needed vs. nice to have? Chromatography or electrophoretic separation*

techniques? Top-down (MS/MS)? Charge reduction? Native (LC-)MS? Charge Detection MS? Ultra-high mass range? New peptide mapping or fragmentation techniques?

What are the emerging challenges for characterizing high complexity formats such as AAVs and engineered CAR-T cells? *What about discovery biology / proteomic applications? Which methods are finding success? For bottom-up analysis, which new technologies are transformative? Deep/fast proteome profiling?*

Which new HOS methods are changing lead assessment? *HDX? F-POP? Covalent labeling?*

What are the opportunities for MS-adjacent technologies in LC-MS labs? *Single molecule protein sequencing? Mass photometry? Other optical techniques?*

Where should vendors focus R&D efforts for the years ahead? *Sample prep? LC? MS? Software?*

Notes:

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We began by introducing the attendees, which included representatives from biopharma and software vendors. The discussion centered on which stages of molecule development would benefit from platform methods. There was consensus on the importance of seamless information flow between discovery and development, though discovery poses challenges due to issues like glycosylation and heavily glycosylated antigens.

We explored difficulties some molecules face during ionization, leading to a discussion of new techniques such as Native SEC-MS with PCD (post-column denaturation), similar to denaturing SEC but involving organic T-in for denaturation before the source. Other techniques considered for complex glycosylation include cIEF-UV-MS and CDMS. Sample preparation options like sialic acid removal, subunit analysis with Genovis enzymes, and buffer exchange methods such as DynaChip, Sample Stream, or ZipChip were also discussed.

New molecule formats, such as antibody-oligo conjugates (e.g., siRNA), present additional challenges. For these ADCs, developing robust methods is crucial, especially considering hydrophobicity and charge. Removing salt is essential, and software should support a single database containing both amino acid and oligo sequences.

We then discussed the speed requirements for high-throughput methods. There was alignment on using fast 3-minute SEC runs with OBE at 0.2 mL/min, though data analysis remains the slowest step. Groups are processing hundreds of samples weekly using tools like WaterConnect, PMI, and Genedata Expressionist, with a consensus on implementing analysis rules to accelerate workflows.

Finally, we touched on advanced deconvolution techniques, such as 2D deconvolution (RT as a function of mass) and progressive (sliding window) deconvolution. Vendors need solutions that are fast, reliable, compliance-ready, and cost-effective.