Roundtable Session – Table 5 - Best Practices of Extended Characterization Mass Spec Methods

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Abstract:

Modern therapeutics are increasingly complex and rely on a growing suite of extended characterization workflows to develop a complete picture of structure-function relationships. Mass spectrometry (MS) has become the gold standard for elucidating molecular detail, but it really shines when paired with other orthogonal assays that confirm and complement the information afforded by MS. In this roundtable, we'll discuss emerging techniques and ongoing challenges associated with end-to-end characterization strategies from early development through product commercialization.

In particular, this roundtable will discuss common MS workflows (intact, subunit, native, and peptide mapping) and ideate on how each can help answer different questions. We will address the interplay of MS, biophysical, and purity methods and explore how complementary techniques like CE-SDS, SEC, MALS, and CEX help verify and strengthen the story your MS data is telling. This roundtable will examine how charge- and size- variant characterization and forced degradation studies contribute to a comprehensive extended characterization package.

Discussion Questions:

1) How do you balance the depth of characterization with development timelines? How does your product team decide the molecule has been sufficiently characterized?
2) What are the current bottlenecks in extended characterization, and are there any ways to ameliorate these bottlenecks?
3) How do you prioritize characterization assays for samples where material may be limited (ie trace charge-variant fractions)?
4) What are the main challenges faced when implementing new technology or workflows to enable extended characterization?
5) In your experience, what are the new trends for characterization tools? Are there any new technologies that you expect to become standardized in the near future?

Notes:

Question 1)

In the Early Phase of development, the primary focus is on speed and standardization. We utilize more platform methods, high-throughput assays that provide essential data on quality, stability, and function. This approach ensures we generate decision-relevant data quickly to keep the project on schedule, effectively balancing time constraints with the need for initial characterization.

The characterization strategy is different for bispecifics. Given their inherent complexity regarding correct assembly and stability, these molecules often require a more bespoke and comprehensive set of analyses from the outset, even in early phases, to proactively manage higher development risks.

A trigger for a deeper investigation is if the potency assay looks good but the pepmap shows unexpected signals (e.g., concerning cross-reactivity or epitope shifts). This becomes a mandatory point for investigation. These discrepancies must be immediately addressed with in-depth analysis to prevent costly failures later on.

If time and material are available, an in-depth analysis may be conducted already early, even if not strictly required for the immediate go/no-go decision. This is done to build a richer understanding of the molecule's behavior or if there is personal interest from the scientific team. While not mandatory for "sufficient" characterization, this proactive approach helps mitigate potential risks down the line.

Question 2)

The current bottlenecks in extended characterization primarily revolve around material quantity, method specificity, and the time required for peak isolation and confirmation.

A major bottleneck is the full structural characterization of low-level unknown peaks observed in electrophoretic methods like CE-SDS.

- Bottleneck: Isolating sufficient material for subsequent in-depth analysis (e.g., Mass Spectrometry) when the variant is present at a very low percentage.
- Amelioration: The process requires fraction collection. A common strategy is the option: fraction collection with RP chromatography, followed by confirmation: reinject the peak on CE SDS. While effective, this process is inherently time-consuming and material-intensive.

Nano-Scale Techniques:

- Bottleneck: Standard methods, such as those that require higher material input, are used because usually we have enough material. This makes the integration of highly sensitive but lower-throughput Nano applications challenging for routine work.
- Amelioration: Reserve Nano for trace variant characterization, using it only for minor fractions or as backup. Integrating microflow or nanoflow systems into routine analysis can improve sensitivity and reduce sample consumption over time, but requires significant upfront investment and method development.

Question 3)

When material is scarce, the priority shifts to techniques that offer maximum information from minimal input, ensuring that the functional consequence and structural identity of the variant are understood. The priority order is typically:

1. Bioassay: The Bioassay would have priority over other methods. The primary concern is whether the structural change in the variant impacts its biological activity or potency. If the variant shows a significant shift in function, it necessitates deeper investigation regardless of its low abundance.

2. Intact Mass Analysis: Provides the overall molecular weight of the variant, which can often immediately suggest the type of modification (e.g., glycosylation, deamidation, or oxidation).

3. Peptide Mapping: This provides site-specific localization of the modification. Since it requires digestion and often more material, it is highly critical but challenging to perform on trace amounts.

Fraction Collection Challenge: CE-IEF peak collection is still a challenge if you want to get enough material for pepmap from each peak. Standard preparative techniques often fail to yield the necessary microgram quantities.

To address this, nano applications would apply. High-sensitivity mass spectrometry coupled with nanoflow liquid chromatography can drastically reduce the required material, making it possible to run peptide mapping or even intact mass analysis on the minute amounts recovered from trace variant fraction collection.

Question 4)

The primary hurdle is providing clear evidence that the new technology offers a substantial improvement over existing methods.

It's essential to prove that it has a benefit before making a full investment. This often involves preliminary testing, such as conducting test measurements at the vendor or collaborating with a key user to establish feasibility and utility.

The new technology must integrate with, or significantly enhance, established techniques. Mass Spectrometry (MS) is usually the anchor technique for biopharma characterization. New workflows must either feed into or be complementary to the MS platform to be accepted and utilized.

A system like Maurices Flex capillary electrophoresis platform is often resistant to immediate replacement unless the new option clearly solves a major bottleneck.

New technology adoption is often limited by human and time resources, not just cost. You need time, capacities, and interest for new technologies. Scientists are typically fully engaged with ongoing pipeline projects, making it difficult to allocate the significant time required for method development, optimization, and system validation.

And successful implementation requires someone who is dedicated to learning the system, writing the SOPs, troubleshooting, and training others. Without this dedicated ownership, new instruments often sit idle after the initial novelty wears off.

Question 5)

Hardware trends:

Advanced Mass Spectrometry for Mega-Dalton Molecules to handle increasingly complex modalities like AAVs , mRNA, and large protein complexes.

Charge Detection Mass Spectrometry (CDMS): CDMS will be a success because no other instrument delivers this information. It is becoming an essential tool because it can directly measure the mass of mega-dalton ions and highly heterogeneous mixtures without relying on charge state deconvolution, providing an alternative to traditional MS.

Direct Mass Measurement: Techniques that allow for direct mass measurement on increasingly large molecules will be of significant interest, driving a shift toward top-down and middle-down analysis methods for rapid integrity checks.

Mass Photometry (MP): This is a very fast and promising technology. MP is quickly gaining traction in AAV and protein interaction labs.

Software trends:

The next level will be the implementation of AI, machine learning and fast automated data evaluation and reporting.

There is a clear need for full automation from the sample to the final report within a few hours to accelerate development timelines.

Release testing is the best training for machine learning, as it provides large sets of standardized, validated data. However, training these models is difficult for new modalities where historical data is sparse.

Vendors are working on Chat GPT style solutions for labs for tasks like trouble shooting and data analysis.

Real time analytics is of interest. It is crucial for modern manufacturing control. The challenge lies in data handling, data transfer will be a bottleneck Therefore, a system for on the fly detection and analysis where data is analyzed immediately at the source will be key to managing high-speed data flow and enabling fully automated, self-correcting systems.

The major implementation challenge will be ensuring regulatory compliance and confidence in controlling fully automated systems, requiring robust validation of AI/ML models.