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

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Abstract:

In recent years, the biopharmaceutical industry has undergone significant shifts. Monoclonal antibodies (mAbs) continue to dominate pipelines, but more complex molecules like antibody-drug conjugates (ADCs) are gaining prominence. Additionally, cell and gene therapies are rapidly growing.

Gene therapies, which utilize large viral vectors (such as adeno-associated viruses or AAVs), are becoming increasingly popular. As biopharmaceutical complexity rises, innovative approaches are essential for effective characterization.

As quickly as the therapeutic modality landscape evolves, the availability of new mass spectrometry (MS) based methods is also evolving. Techniques such as electron-activated dissociation (EAD), charge detection mass spectrometry (CDMS), and fast photochemical oxidation of proteins (FPOP-MS) are being employed is being tackle challenges in some of the more complex molecules that are becoming increasingly common, in addition to the ever-increasing array of technology improvements provided by the instrument manufacturers.

Discussion Questions:

- What are the main challenges of current Mass Spec methods when analyzing new modalities (ex: AAV,…) and are they more related to sample preparation, separation or data acquisition?
- What role does data and analytics have to play alongside advances in hardware development?
- Have some technologies not lived up to their promises? Or are these simply not as valuable or required by regulatory agencies? E.g., Ion Mobility
- How to best facilitate the adoption of a ‘new’ method as ‘standard’?
- Do we need more MS performance? Or could automation and/or software provide the answers?

Notes

- *What are the main challenges of current Mass Spec methods when analyzing new modalities (ex: AAV,…) and are they more related to sample preparation, separation or data acquisition?*
    - First limitations and challenges what analytical scientists deal with when a new modality comes into play- sample preparation
        - MS friendly solvent, without losing structural info and getting it into MS to get answers.
        - Lot of customers struggle with this as a first step.
    - Data processing is another challenge - instruments are well established. But what does the data mean.
- ***Most urgent need in terms of new modalities right now? Most attention needed for which modality?***
    - Automation is needed in the future - Lowers the need for expertise -
- Gene Therapy as new modality - sample availability is a challenge and the processing.
    - *What is the solution?*
    - To use smaller quantities - but there is no real answer.
    - Customers may not have any ideas at all about how to get the analytical results they need to answer questions
    - Vendors have some developed methods but not validated methods and get challenged a lot - daily operation cannot support that much sample consumption.
    - Vendors depend on collaborators - they cannot ask for much.
    - Lucky with couple of modalities - some are now available to purchase so vendors can start with something to have an idea on the applicable methods to share when they work with customers.
    - Nothing historical available for new modalities - so no straightforward transferability.
    - On Customer side – they must have early engagement with research teams to provide more materials
    - CRO perspective for AAV analysis - trying to buy the material to develop the methods. Pep map is difficult on AAVs - Not enough material seems to be a key challenge here too.
    - Commercially available standards are small amounts and very expensive. Vendors need collaborators to support customers with their needs.
    - Need to use alternative methods not MS.
    - If program progresses - later stage has more samples

- *Have some technologies not lived up to their promises? Or are these simply not as valuable or required by regulatory agencies? E.g., Ion Mobility*

- ***What is one of the most promising new technologies which has been released recently?***
    - Charge Detection - it is clearly promising - high mass transmission
    - MS with e-MSion to allow all fragmentation (ExD cell).

- Customers want to use something they are familiar with - so Orbitraps are often favoured in combination for new technologies.
- Charge measurement accuracy is a question.
- Wyatt - SEC-MALS has a lot of promise Putting an LC in front of CDMS - requires a lot of work and software update.
    - Requires more material, and more concentrated samples - but has potential, promise and reliable results.
- Users want to see intact - as they want to see the whole molecule - and what is happening.
- Lipid nanoparticles - how to prevent the particle from sheering, or degrading in the MS process, in the source and during ionization.
- CDMS from Megadalton works in negative mode too
    - Nucleic acids - negative mode works better.

- ***How do you validate the method if you don't know what to expect?***
    - Reference Standards and standard organizations are needed.
    - Vendors need to and can work with standard bodies - like USP - who is developing AAV standards.
    - Vendors need to validate their own instruments.
    - The amount of ions needed - how it is decided what is needed, what is enough - how can that be validated for a regulatory environment? These are specific questions.
    - System suitability tests need to be developed - but what is the reference? How can you assure your system is suitable - need collaborations with industry and academia to work through these questions.
    - New modalities are very project specific.

- ***Ion mobility as another newer technique Where is it applied most in BP industry and which modality benefits most?***
    - Collision induced unfolding (CIU) studies - suitable for proteins more - there is a place for that. It seems to be repeatable - would not call it robust.
        - Vion was purchased by one attendee, but they never got to use it - no real customer needs were identified.
    - FDA is looking into methods with IM with collaborators
    - Looking at HOS information usability - which must be looked at for regulatory filings.
    - CIU - is like quick and dirty analysis,
        - intact level - so can complement or help instead of lengthy HDX - which is a long method and challenging.
    - Reproducibility is achievable but only on one modality.
    - Limitation - it is also a gas phase structure - so more like a fingerprint information.
    - Lot of modalities are protein-based and have some general behaviour which can be analysed with IM techniques.
    - Biosimilars must have the same primary structure - so comparing CIU can help with extra level of comparison.
    - Attendees view on whether the FDA gives any advice on how to address these needs - no specific advice.

- Consensus - There is a need for higher order structure studies and that will utilise new methods if available.

- *How to best facilitate the adoption of a 'new' method as 'standard'?*

- Around this topic MS for HCP analysis has been discussed - Discrepancies between ELISA and MS
- They will never be the same - absolute quantitation is not easy.
- It is important for the drug in development How to approve the safety - not on a limit of specific HCPs, it has to be a reproducible profile and safe.
- Need to have proved HCP present is no risk at the level it is present.
- The bigger emphasis is on risk assessment per drug product - it is very difficult but has to be done. There is guidance but no strict expectations on exact levels - It is up to the developer of the biopharmaceuticals.
- Methods need to be robust is the criteria.
- With new technologies, if they are more sensitive - new HCPs may be detected and identified - but still safe is important – based on available information and studies.
- Regulators will never characteristically say this method is better.
- Methods need to be reliable – primary Regulator expectation and guidance
- But for now, ELISA is the gold standard.
- ***Could MS be the predominant method*?**
  - No, will likely never replace ELISA, as that is simpler, robust and reliable.
  - Most companies submit that data too - but not necessarily used routinely for QC. Not fit for purpose for release. It's great for process development, and to support studies which HCP gives that immunogenetic response - good to monitor them. More direct approach on HCP ID.
  - MS is great for ID.
  - Lot of companies do not have methods early on - but they do it later.
  - AAV example - depending on the supplier of AAV – starting material can be very different - more complex.
  - MS is the only method which can help with it early.
  - AUC takes overnight, most manufacturing methods take 24 hours.
  - Customers want as many techniques as possible - so they can go to the FDA with the most knowledge.