Table 1: New Mass Spec Methods to Tackle New Biotherapeutic Challenges

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Key Words: New Modalities, Accurate Mass, Biotherapeutics

Table Scope:

Newness: many 'new' things in mass spectrometry have been around a long time, are hailed as revolutionary, but do not get adopted widely. We will examine some examples to determine what are the major barriers and uncover better pathways.

Techniques: to enhance mass spectrometry we can go beyond just 'sample prep' - there are very evident techniques such as labelling, mass tags, and gas phase chemistries that appear easy to implement. So why aren't they used more widely? We use acid modifiers in solvents, so what's so difficult?

Hyphenation: we can all recognize that mass spectrometry would not be where it is today without hyphenation to other techniques like liquid chromatography, so why not other separation techniques that should be easy for any user? Are the barriers real or imagined?

What's the best mass spectrometry joke you know? Tell one, and if it's voted a winner, there will be a prize!

- Do we need new mass spectrometers at all – aren't the current ones just fine?
- What should be hyphenated to mass specs – do we lack imagination, or is it laziness?
- Why do we use so few of the techniques that mass spec uses – are we so pressed for time that we can't expand our horizons and invest to save effort?
- A mass spec is not just for Christmas – what keeps them running into old age , and how much do we need the service engineers when modalities change?

Discussion Notes:

Attendee Backgrounds:

- Experienced scientists from pharmaceutical industry
- Mass spec vendors
- Development companies

1. How do people define new mass spec methods?
   The group thinks not only new mass spectrometry technologies are considered; new applications of existing technologies are also part of the new methods. Some technologies do not get adopted until they have an appropriate application (iCIEF-MS for example). Some 'new' technologies are not adopted until they are robust.

2. CDMS usage:
   a) CDMS could be achieved by using Thermo UHMR or sending samples to Megadalton Solutions.

b) The CDMS technology utilized by Megadalton Solutions has not been commercialized yet which might also limit the broad usage.

3. CRO offers service with new technology:
   a) Megadalton Solutions provides CDMS services; Immuto Scientific provides reactive radical footprinting services.
   b) It's often more cost-effective to have the CROs which are equipped with the new technology to run samples and provide reports.

4. Factors that make a new technology more applicable to industry:
   a) High throughput
   b) Robust
   c) Small footprint, benchtop
   d) Easy to maintain
   e) Automated data analysis.

5. Covalent labeling:
   a) FPOP in some sites is restricted based on laser usage and safety guidance.
   b) It's more effective to have CRO to run the samples: expertise; time; number of projects in a year.
   c) Cases until now have been rare when covalent labeling but not HDX is needed in pharmaceutical industries. May be changing with recent developments and proof of capabilities。

6. Barriers for a new technology to get into industry:
   a) It's hard to come to a decision to acquire new technology because new technology does not always help make more money. "Return on Investment" for adoption of new technology is a difficult argument versus tried-and-trusted techniques.
   b) New technologies might require a lot of fine tuning to make it work and be applicable. It might work after tuning but may not be dramatically better than traditional methods. Scientists typically have no time to try new methods and test them out. Companies typically do not have dedicated groups to evaluate new technology. Postdocs might be hired to assess a specific technology.
   c) The appropriate project is often needed to justify if necessary to develop the new methods – unlikely to simply try new technology without a specific goal in pharma.
   d) In QC labs, not everyone has the expertise in MS. Special training is needed in instrument operation and data interpretation. Having standard protocols could be helpful. An internal MS training course could be helpful. Relationship with mass spec vendors is important here.

7. Hyphenation:
   a) CE-MS: useful to resolve charge variants.
   b) But instead of coupling CE to MS, offline fraction (cIEF) seems to provide reliable results.

8. Vendors and training:
   a) The location and size of the company affects how much the company relies on vendors.
   b) Instrument purchasing and training are often on different budgets (Training is expensive).

c) Application scientists from vendors often need to learn new technologies alongside the users, during training.
d) It's hard to get technical contact by connecting the sales. Finding the correct contact person from vendors is critical.

9. How often to clean the source for techniques like SEC-MS:
   Some scientists prefer preventative maintenance so that venting the instrument and cleaning the source are done each month. (therefore, time constraints for new techniques)
   Others state that ammonium acetate itself is not dirty; but other salts etc. coming from the samples are making the instrument dirty.

10. When does a revolution in LC or column become relevant:
    a) It's often motivated by new modalities.
    b) When the need comes, seen in publications or used by other companies.
    c) If there are research-based projects.
    d) Some people tend to figure out first, but others prefer to wait for other companies to try it out. Adoption curve is not always rapid.

11. Which is preferred: nano flow or regular flow LC:
    a) High flow is reliable and needed for product release and GMP purposes.
    b) Routine analysis: nano flow is fine (non-GMP); but prefer high flow.
    c) uPAC column work well.

12. Instrument lifetime:
    a) Some people refresh every 2-5 years; others refresh every 8 years.
    b) Justification of a new instrument also takes years.
    c) Instruments are replaced more often when high sensitivity is needed, or the sample amount is low.
    d) Looking at small molecules might not need replacing instruments very often.

13. Discovery mode or MRM mode? More information may not be necessary.

14. Does MALDI-MS have a place in future: MALDI-MS are used when LC-MS fails (heavily glycosylated) for example spike protein.

15. DESI-MS are used for high throughput intact mass analysis.

16. New challenges in pharmaceutical industry that might need new mass spec technologies:
    a) Characterizing new modalities: ADC; multispecific antibodies; AAV; fusion protein; more and more amino acid and glycosylation
    b) Heterogeneity caused by glycan envelopes on large antibodies: deglycosylation is also challenging.
    c) Hard to separate mRNA variant.
    d) One attendee pointed out that ETCR (electron transfer charge reduction) might be a tool to reduce heterogeneity, but the tool is not widespread.

17. Is 2D LC useful or needed?
    Prefer offline, then bring the sample from the first LC into autosampler for the normal HPLC analysis. E.g. high pH reverse phase LC can be achieved offline (can be automated with robot).

18. Protease?
    a) High specificity is preferred. GluC might not be specific.
    b) Other digestion methods: pressure cycling-accelerated digestion; microwave-assisted digestion with acetic acid or formic acid (cleavage at D and E).

Summary:

- Adoption of new technologies may be constrained by a number of factors, including ROI, expertise, and workload, irrespective of modalities.
- New modalities may well drive adoption of new techniques and tools because of complexity
- New modalities also drive new instrumentation as capital expense, balanced with outsourcing to CROs.
- New mass spec capabilities may not be adopted until they have an appropriate application.