Table 8: CE/MS: Method Development, Application and Implementation in Biopharmaceutical Development

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Scope:

Capillary electrophoresis (CE) is widely used in biopharmaceutical development as a tool for both characterization and quantitation. While CE has many possible uses it is most notably employed to understand the size and charge variants of therapeutic proteins. Unfortunately, the direct identification of CE peaks and the in-depth characterization of therapeutic protein variants is often not possible due to the challenges of fractionating CE peaks and/or interfacing CE assays directly with mass spectrometry (MS). The last decade has seen significant advancements in CE technologies that directly hyphenate with MS leading to the availability of commercialized CZE-MS and cIEF-MS platforms. This roundtable will discuss the advancements in CE-MS technologies and areas for potential growth and improvement. Additionally, we will aim to discuss the current applications for CE-MS within the biopharmaceutical development space and identify future opportunities for this exciting technology.

Questions for Discussion:

1. Application/method: What's your application using CE-MS, for example, CE-MS for process development testing, protein characterization, peptide, and metabolite analysis? Is it a routine practice? Is there a generic method? Do you only use CE-MS for a specific application?
2. Comparison: Why do you choose or are interested in CE-MS for your application? Have you tried LC-MS or other techniques? How's the comparison between the methods?
3. Hardware: Which commercialized or self-build platform do you use? Can you introduce and comment on the platform? What are the pros and cons? Do you have tips and tricks to share? How to improve the robustness of the technologies?
4. Future direction: What's the most exciting CE-MS progress for you in recent years? What do you see it's the biggest opportunity for CE-MS? Where could this tech be most impactful? Would you used CE-MS in a regulatory filing. Finally, what's the future direction of CE-MS in the pharmaceutical industry?

Discussion Notes:

1. **Application/method: What's your application using CE-MS, for example, CE-MS for process development testing, protein characterization, peptide, and metabolite analysis? Is it a routine practice? Is there a generic method? Do you only use CE-MS for a specific application?**

    - UV trace and BPE in coordination with CE-MS.

2. **Comparison: Why do you choose or are interested in CE-MS for your application? Have you tried LC-MS or other techniques? How's the comparison between the methods?**

    - CE-MS can be orthogonal to LC-MS
    - Charge heterogeneity
    - Ambiguous assignments/shoulders in MS characterization. Typical separation methods lack resolution, and Peptide mapping is insufficient. Application requires digest. Does it need to be completely online – fractionation is good. Developing online capability for digestion or modification.
    - In capillary digest for peptide level information. Almost 100% coverage. Requires pepsin and depends on the individual protein sequence. Data is difficult to parse.
    - 5 or more micrograms is common concentration for samples. 5mg/mL – can be as low as 1 microgram/ 0.05 mg/mL
    - Information from peptide level is very limited.
    - Drug product development from early to late stage. Many different conditions. Stability studies. CE separation give less complicated spectra that are easier to analyze.
    - Capillary electrophoresis is less robust than LC-MS and can lead to scientists abandoning the system.
    - Base CE-MS is very hands on and difficult. ZipChip makes things much easier.
    - Characterization of charge variants.
    - Better fragmentation technologies to keep up with CE migration times.
    - Middle up approach. Localize modifications and clippings. Allows isotope resolution. Routine application & characterization (prefer offline fractionation for characterization) – non-denaturing conditions are required
    - Vendors are looking for opportunities to meet scientists needs – how can we facilitate this? Alpha and Beta testers are required and vendors have to ask what is needed. What are the new modalities? What molecules are people working on? What molecules work well with this system?
    - More collaboration will lead to CE-MS becoming more robust.

- MAM (multi-attribute method) is a buzzword – replacing in use assays with MS is very difficult – do CE-MS in parallel with existing assays to prove worth.
- MS PTM analysis = you find what you're looking for, but potentially lose information – multiple MS assays are required to verify certain things.
- Potency assays. Who does native fractionation? Need to prove that it's native. Will it interfere with the binding assay?
- Affinity CE - binding on the capillary – mobility change with or without the antigen.
- Christopher Duecey?
- Charge heterogeneity
- Peptide mapping with CE-MS is possible and is done Wendy Lan – but LC is already very well established – but LC can fail to bind certain kinds of peptides – when is it worthwhile to CE-MS?
- CE-MS can be a last resort to solve problems when other assays fail.
- What instrumentation would you use for routine assays – chip-based CE is the way here.
- Any time you go below normal analytical flows robustness becomes an issue – even with UPLC

3. **Hardware: Which commercialized or self-build platform do you use? Can you introduce and comment on the platform? What are the pros and cons? Do you have tips and tricks to share? How to improve the robustness of the technologies?**

    - Agilent 7100 – EMASSII interface – Agilent TOF & QTOF

4. **Future direction: What's the most exciting CE-MS progress for you in recent years? What do you see it's the biggest opportunity for CE-MS? Where could this tech be most impactful? Would you use CE-MS in a regulatory filing. Finally, what's the future direction of CE-MS in the pharmaceutical industry?**

    - 2D work seems very promising – good for very complex samples
    - CE-SDS in mass spec
    - Clean MS
    - Fragmentation methods
    - Examination of complex mixtures of small molecule drugs – heterogeneous small molecules – carbohydrates (heparins)
    - AAB (AEB? AAV?) – living virus - roundtable tomorrow – high priority for – just like pokemon
    - Can intact mass data help direct your bottom-up work? – observe things that you weren't expecting – if CE-MS has an easy enough work flow this could be a good workflow. – Glycation is observed in intact & peptide mapping can localize

- Amgen is trying to implement CE-MS in parallel with other assays – pushing it hard
- For investment - CE must survive for decades and outlast other new systems that appear – relative quantification – very expensive method (can you do it with other easier assays) – have to transfer samples