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:

- Application/methods:
    - CE-MS can handle samples with little amounts: such as proteins purified from sera, tissues, or cells. Eg. using Agilent 7100 CE-MS system through CMP EMASS-II ion source to analyze samples at intact, reduced, and subunit levels (Anal. Chem. 2021, 93, 13, 5562–5569)
    - Online iCIEF-MS methods can identify proteoforms at intact level
    - Charge variant profile changes can be dramatic for forced degradation samples, peak identification with CE-MS is helpful to set specifications
- Comparison to other technologies
    - CE consumes smaller volumes of samples comparing to LC, high sensitivity can be achieved when coupling with nanospray ionization
    - CE can separate some small peptides that have low column binding with LC
    - LC and CE have different selectivities, the two techniques are complementary methods
- Hardware:
    - CE-MS technology has become more plug-and-play
    - nanoCEasy Interface: improved interface robustness (Anal. Chem. 2021, 93, 44, 14593–14598), the MS data quality is good in spite the use of ampholytes
    - 908 Devices: good resolution; recently improved surface coating; expensive to use (chips and reagents, shelf-life is short for reagents after opening); need MS instrument with high mass range; sample delivery with autosampler takes 10 minutes; need to decrease surface interactions with high hydrophobicity molecules
    - Is there a way to desalt CIEF samples when using urea, similar to divert valves in LC?
    - For peak characterization, HIC can be a good option because of good separation resolution
- Future direction
    - Sample preparation: plate-based sample preparation with robots
    - Instrument hardware:

        - more CIEF-MS instruments are going to be available (offline fractionation and online MS)
        - multichannel CE to interface with MS would improve method throughput
    - Data analysis:
        - batch process (it is time-consuming to deconvolute manually)
        - more reliable deconvolution algorithms for intact mass spectra, especially for early phase molecules
        - would be helpful to label PTMs on electropherograms automatically
        - In silico tools to correlate the results from peptide and intact protein levels