Roundtable Session 2 – Table 3 – CE/MS: Currents Trends and Applications in Biopharmaceutical Development

Facilitator: Tim Blanc, *Eli Lilly and Company*

Scribe: Caitlin Kerr, *Bristol Myers Squibb*

Abstract:

Capillary Electrophoresis-Mass Spectrometry (CE/MS) continues to gain momentum as a transformative analytical tool in biopharmaceutical development. By uniting the high-resolution separation power of capillary electrophoresis (CE) with the structural specificity and sensitivity of mass spectrometry (MS), CE/MS is uniquely positioned to address evolving analytical needs in the biopharmaceutical industry.

This roundtable will focus on current trends and applications of CE/MS in biopharmaceutical development, highlighting recent advancements in instrumentation, workflows, and data analysis strategies. Discussion will explore how CE/MS is being applied to cutting-edge modalities such as cell and gene therapies, bispecifics, and other complex biologics, while also addressing its role in critical areas including charge variant analysis, glycosylation profiling, and impurity characterization.

Participants will share insights on how CE/MS is advancing from a specialized technique to a more broadly adopted tool, identify opportunities for innovation, and discuss practical challenges related to data interpretation, method transfer, and regulatory acceptance. Join us to exchange experiences and perspectives on how CE/MS is shaping the future of biopharmaceutical development.

Discussion Questions:

1. What are the most significant emerging trends in CE/MS technology and workflows that are influencing biopharmaceutical development?

2. How is CE/MS being applied to new therapeutic modalities such as cell and gene therapies, bispecific antibodies, or fusion proteins?

3. What recent innovations in CE/MS instrumentation or interfaces have improved sensitivity, robustness, or ease of use?

Applications

1. In which analytical applications has CE/MS shown the most value in the past year (e.g., charge heterogeneity, glycosylation, peptide mapping)?

2. How is CE/MS helping to address evolving analytical challenges associated with increasingly complex biopharmaceuticals?

3. What are some case examples where CE/MS provided insights that were not achievable with traditional orthogonal methods?

Data and Integration

1. What strategies are being developed for effective data analysis, processing, and integration of CE/MS into broader analytical platforms?

2. How do we ensure comparability and reproducibility of CE/MS data across labs and platforms?

3. What role can automation, machine learning, or AI play in advancing CE/MS data handling and interpretation?

Future Outlook and Adoption

1. How close is CE/MS to broader adoption in development pipelines, QC, or regulatory submissions?

2. What are the current barriers to more widespread use of CE/MS in biopharma, and how might these be overcome?

3. Looking ahead, where do you see CE/MS providing the greatest impact in the next 3–5 years?

Notes:

Opportunities for Innovation

- There is interest in advancing cIEF-MS connections, particularly in overcoming challenges such as ampholyte suppression and contamination in mass spectrometry.
- Attendees expressed a desire to learn more about CE-MS, with their companies interested in incorporating it when beneficial and necessary.
- A key goal is to identify artifacts observed in CE analysis.
- While some groups perform CE-SDS more frequently than CE-MS, there is growing interest in expanding CE-MS capabilities.

Technical Developments & Trends

- AESlytes vs Pharmalytes: AESlytes are being explored as an alternative to Pharmalytes to improve MS signal.
- A longer capillary column has been manufactured for short-column AESlytes to enhance resolution prior to MS.
- Current CE-MS Trends:
    - Two needs in iCIEF-MS coupling: peak purity and peak identification. Among these, peak ID is considered most critical.
    - Historical evolution of CE-MS interfaces:

        - Beckman CE-MS (first interface)
        - Agilent CE-MS
        - Sciex CZE-MS solution
        - CMP Scientific interface (developed by Dovichi)
        - Intabio microchip iCIEF-MS (most recent)
    - These developments have improved automation and industry viability, and CE-MS usage is expected to grow.

Applications & Use Cases

- Peptide Mapping:
    - Important for glycosylation analysis.
    - CE-MS shows high potential in separating glycosylated forms, which can be challenging with other methods.
- CE-SDS:
    - Still not compatible with MS; no commercial options currently available.
- CZE-MS and iCIEF-MS:
    - Commercial solutions exist that have limited/addressed buffer compatibility issues.
- Charge Heterogeneity:
    - CE-MS is valuable for analyzing charge variants; CMP, AES, and Sciex offer relevant applications.
- Glycan and Oligosaccharide Analysis:
    - Interest in these applications has grown significantly.
- Proteomics Outside Biopharma:
    - CZE is increasingly used for protein ID in single-cell analysis, offering sensitivity gains at low flow rates and high-resolution separation.
- Complex Modalities:
    - Native mode analysis of ADCs and bispecifics often requires CE-MS over LC-MS or standalone CE.
    - Antibody-RNA conjugates (AOCs) are also gaining popularity.
- CE-MS has provided insights where other methods fall short, such as DAR ratio changes and their characterization.

Methodology & Data Integrity

- **MS in QC**:
    - Typically used for peptide mapping (PMAP), but PMAP leads to loss of proteoform information due to digestion.
    - Intact MS provides complementary information to PMAP.
- **Barriers to CE-MS Adoption**:
    - Robustness and reproducibility across labs and platforms remain major challenges.
    - CE-MS transfer is significantly more difficult than CE transfer due to variables like sprayer voltages, distances, and buffer complexities.
    - Integration with systems like Empower is complicated by these factors; currently, this remains a manual process.
- **AI and Machine Learning**:
    - Expert intelligence is exploring AI/ML to assist with integration and method development.
    - While AI may help with repetitive tasks (e.g., data verification), human oversight is still essential, especially in biopharma where risk tolerance is low.

Future Outlook

- The future of CE-MS lies not in broadening its scope but in improving robustness.
- Currently, CE and CE-MS are primarily used in early-phase method development, not in QC.
- Training QC labs for CE-MS is a significant barrier due to the complexity of the technique.
- Simplifying CE-MS and improving its reliability will be key to broader adoption.