Table 1: Advantages and Challenges for Implementing CE-MS and Other New Technologies

Session 1:	Session 2:
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Scope:

Capillary Electrophoresis-Mass Spectrometry (CE-MS) has gaining more importunate over the last two decades for biopharmaceutical industry with the improvement of the CE-MS interface and MS instrumentation including traditional CE-MS and chip-based CE-MS. CE-MS as an orthogonal technique of LC-MS not only provide complementary information to LC-MS but also have its unique applications especially for charged and polar molecules. For example, CE-MS can provide intact and bottom up protein characterization, charge variant identification, glycoprotein profiling, protein-ligand complex, protein integrity in vitro and in vivo, as well as in biomarker characterization. This table will focus on the "Advantages and challenges for implementing CE-MS (including chip-MS) and other new technologies such as affinity CE in development of biopharmaceuticals" discussions and explore the future directions.

Questions for Discussion:

1. What types of CE-MS methods do you think are promising to add value to cQA monitoring during process development, compared to the existing and commonly used method panel? (like e.g. CE-MS-based glycan profiling, CE-MS-based peptide mapping, or CE-MS-based top-down / middle-down approaches)
2. What are barriers for implementing CE-MS (including chip-MS) in development of biopharmaceuticals? What's the advantages and challenges for implementing CE-MS?
3. What are the existing techniques and how does CE-MS to help with definitively identify all the peaks in CE separations?
4. Which new applications such as affinity CE could be of high interest in the development of biopharmaceuticals?

Discussion Notes:

Session 1:

Ten scientists participated the discussion:

1) From vendors' points of view:
    a. What do the customers want/need?
    b. Where is this space going?
    c. Need engineers' input?
2) From industry's point of view:
    a. CE-MS method needs to be or have the following criteria:
        i. Robust
        ii. High-resolution
        iii. Sensitive – even at early stage
        iv. Can be utilized to different applications
        v. Instrumentation must be suitable for varied purpose
3) Sample criteria for MS
    a. Purity – baseline resolved
        i. Integrate with dropped line
        ii. Up to neighboring peaks
        iii. More separation is better

1. "Profile is nice"

b. Quantity
c. Sample volume is different from LC
d. BGE – must be compatible with MS
    i. Need to vaporize
    ii. Analytes – essential to ionize properly

4) MS requests from Industry
    a. Provide another detector, either LIF or UV before MS, before electrospray
    b. Sample inline – better for fraction collection
    c. Window in chip?
    d. Smaller volumes
    e. Prep scale
        i. Require bigger capillary
        ii. IEF – concentrate the molecule
5) Gaps
    a. Fraction collection issues
    b. Label handling in MS
    c. Online collection
        i. No protein change is better
    d. Other assays – demand bigger capillaries
6) Fraction collection issues
    a. Labor intensive; time consuming
    b. "Lose" samples
    c. Need desalting/buffer exchange
    d. LC sample gets pumped through UV flow cell to MS spray
7) Solution to sensitivity issues
    a. Add DMSO
    b. ZipChip's new protocol
        i. Less load at 0.25 mg/mL
    c. 0.1% LOD vs LOQ
        i. iCE vs ZipChip
8) Proteomics – lower concentration
    a. Problematic for peak capacity
        i. Digest proteins into thousand peptides
    b. Lumos MS – not an issue for peak capacity
9) Possible solutions to other issues
    a. Need a shorter window
    b. Change capillary chemistry, before MS
    c. "Hybrid" type
        i. ZipChip – working on EOF
        ii. Fractionate through ZipChip
            1. Compare manual vs. ZipChip
    d. Automate FFE
        i. Collect different charge variants
    e. Collect and reinject cIEF then to MS
        i. Need online and real time
        ii. Other assays besides MS
            1. Binding assay
    f. Use pre-mix to avoid analyst error
    g. Improve reproducibility by injector
        i. Fixed loop for large volume

10) CE-MS vs LC
    a. Good resolution
    b. Good sensitivity
    c. Biotransformation
        i. Collect plasma/serum
        ii. Protein integrity
            1. Antibody – good
            2. ADCC/Fusion protein – at half-life, concentration decrease
    d. Interaction with HPLC columns
        i. Need to optimize the methods
        ii. Aggregates – change sample prep
    e. Ratio of use in PKDM
        i. 85% LC
        ii. 15% MS
    f. Cleaner MS data
        i. Nanoflow
        ii. LC – microflow
    g. CE-MS throughput – not as good as LC
    h. Intact mass with Agilent for mAb
    i. % of impurity – only for characterization, not for quantitation
        i. Relative quantitation
    j. Advantages of LC vs CE MS sample prep
        i. Same
        ii. Training within a week
            1. Takes years for data analysis to be proficient
            2. Routine running vs troubleshooting
        iii. CE-MS- small peptides, 1 day done
        iv. Cleaning of CE-MS is less than LC-MS
            1. Maybe 2X per year
        v. LC – only concern is column
        vi. CE – lots of moving parts
        vii. LC – lots of flexibility with columns packing
    k. Issues/Concerns
        i. Analysts get trained on LC MS, not CE-MS
            1. CE-MS is deemed easier though
        ii. HPLC – older technology than CE
        iii. LC buffers are not CE-MS friendly
        iv. Room for improvement
            1. History: CE-SDS (peaks) vs SDS-PAGE (bands)
            2. Relative ratio vs relative migration time
            3. CE – no sample carryover
            4. Retention time shift – day to day
        v. CE-MS in USP – not yet
            1. Depends on money, resources. allocation

<u>Session 2:</u>

Nine scientists participated the discussion. Key points were captured as following:

1. Chip based CE-MS is useful for cQA monitoring:

    a. User-friendly hyphenation helps traditional LC user to get comfortable with CE
    b. Short analysis time (e.g. 3min run)
    c. Fit for purpose analysis meets the needs (e.g. cell line monitoring with portable CE-MS analyzer)
2. Barriers for CE-MS:
    a. Need user-friendly walk-up system for process control
    b. High end MS instrumentation requires training and expertise
    c. Fewer CE-MS specialist compared to LC-MS. Lack of CE-MS training even in academia.
    d. Need more communication within organizations to promote technologies. Bridging CE separation with MS (suggestions: to have CASSS CE meeting together with MS meeting?)
3. Solutions:
    a. Enable automation and robotic system
    b. Need pioneers to take the lead and try out (i.e., raise the bar and set standard to push forward the technology)
    c. Mindset changes (open to new technologies and new ways to do things) to overcome hurdles.
    d. Highly trained scientist for high quality data
    e. CE-MS training course or workshop will be very useful.
    f. Vendors' effort to make MS instrument more user friendly and less requirement for training. Make MS more accessible.
    g. Data science (e.g. AI) to help.
4. General:
    a. All tools are needed depending on the analytical task. CE-MS won't replace LC-MS, but is a powerful complementary tool (example of peptide mapping: LC-MS requires 2 enzyme digestion, CE-MS only need one)
    b. CE-MS to help MS ID of established standard separation, such as CE-SDS, cIEF, IEX, is much needed