Roundtable Session 2 – Table 9 – CE-MS in Regulatory Filings

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Abstract:

Capillary electrophoresis has been coupled to mass spectrometry for decades now and numerous publications exists showing the applicability and benefit of CE-MS for the characterization of (bio)pharmaceuticals. However, in the past CE-MS was considered a rather unrobust technology that is difficult to use in routine practice. This seems to be changing in recent years and more and more commercial systems are becoming available: These facilitate the coupling of CE to MS for multiple CE assay formats and make the technology more applicable to routine use. The increased use of CE-MS in routine should also lead to these assays and data being used in regulatory filings. This roundtable focuses on the use of CE-MS assays and data in regulatory filing and is intended to exchange experiences made for this topic.

Discussion Questions:

Q1: What types of CE-MS assays are you using in your lab / you company?

Q2: At what stages during development are you using CE-MS assays and for what purpose?

Q3: Do you use CE-MS assays or data in regulatory filings?

Q4: If yes, are you using the assays or data for characterization purpose, or under GxP?

Q5: In case of use for characterization purpose, what do you do with regards to method qualification to prove the assays or data are scientifically sound?

Q6: Are there any examples for the use of CE-MS assays under GxP?

Q7: Can you share your experience with regulatory agencies with regards to CE-MS assays or data?

Notes:

What brings you to this table today? What do you want to know about CE-MS in regulatory filings?

- To see the future of this method
- To see what other people are using CE-MS systems for
- To see what people who use this tool want from the technology
- To look at the different modalities and how the technology is implemented
- To gather information on the regulatory attitude and how to integrate CE-MS into the analytical workflow
- To know regulatory expectations for CIEF fractionation for MS
    - Note: No agency employee was at the table so this expectation could not be met
- To see what other people's experience with their country's health authorities is

Q1: What types of CE-MS assays are you using in your lab/company?

- ZipChip (908 Devices) for intact mass for filing; an advantage of CE-MS is for intact so can see free thiol and succinimide
- CMP Scientific instrument for reduced peptide mapping--it covers short peptides not seen on LC-MS
    - LC rather than CE is still needed for routine testing so the assay can be transferred to GxP
- IntaBio (Sciex) because it gives a separation like that seen on CIEF as opposed to no photometric readout as with the ZipChip
- CEInfinite (Advanced Electrophoresis Systems), but it has been challenging to connect it to the mass spectrometer because a very good mass spectrometrist is needed to get the right connection
    - it is more challenging when the operator doesn't have an MS background, but it is getting easier
    - that is an advantage of the CIEF junction sprayers--the chip can make the junction reliably and zero blind section volume
- It's helpful if the intact CE-MS analyst has peptide mapping experience so they have in mind the applicable mass differences
    - one of the challenges with intact CE-MS are with the clips--they have higher ion mobility
    - another challenge of intact CE-MS vs. peptide mapping CE-MS is that CIEF peaks can easily bleed into one another
        - continuous MS monitoring is needed to be able to see the mass changes as the separations occur

Is anyone doing subunit analysis?

- It can help to pinpoint changes vs. intact analysis, but the challenge is getting digests to work properly
- Subunit analysis is seeing strength with ADCs; MS sees differences at intact level also because of ADC linkage structural heterogeneity; subunit analysis simplifies the process
- It would be nice to have LC's loading capacity so a large volume of sample could be put on and do EAD

Is anyone doing affinity CE-MS?

- It could be useful with force degraded samples to test target binding
- A system that can do both free zone CZE and CIEF would be needed
    - this would also be useful for LNPs

Are there other applications using CE-MS? Is anyone using it for DNA?

- There are small molecule applications but we had no participants with experience in this area
- Using it for siRNA in positive ion mode (although DNA MS normally uses negative ion mode)

Q3: We are usually using CE-MS for characterization. Do you use CE-MS assay or data in regulatory filings?

- four attendees out of approximately ten answered ‘yes’
- CE-MS characterization data has been collected, but whether to use it or not is up to more senior management
    - not interested in non-CQA changes when considering whether to include
- Including CE-MS data might make filings easier because providing both LC-MS peptide mapping and intact CE-MS to health authority could be complementary
- When looking at free thiol, 2 Da mass differences can be tracked across peaks, but a photometric profile is needed for a cross-reference
- CE-MS can be an assay with great resolution, but it is necessary to prove what is being seen is not an artifact

Does the health authority require exact size?

- It depends on the control strategy
- A combination of MS and charge heterogeneity can be used

How useful would CGE-MS be?

- To make CGE-MS viable with industry the goal would be to create a new cartridge rather than a whole new instrument
- CGE usually gives good separation with a great size range, but that range is harder for mass spectrometry
    - Is it better to have apparent mass or intrinsic mass?
- It is usually acceptable to fractionate the material, but characterization scientists would really like to *know* that the peaks seen in CGE are the same as the material analyzed by MS
- Indirect characterization can usually be used when questions are received from the health authorities, but must be prepared for pushback
    - it is usually adequate to just provide an orthogonal assay, but the results must be consistent
- CIEF-MS is more powerful because it can provide charge, glcyan, and deamidation

Q6: Are there any examples for the use of CE-MS assays under GxP? Can you foresee a time when we would have CE-MS in GxP?

- CE-MS is really powerful in DOE for process conditions, but that's still characterization
- Johnson & Johnson is known to be filing a peptide mapping MAM assay to replace their charge heterogeneity assay
    - they will be tracking only a few selected modification events by SIM and disregarding other possible changes
    - there is a risk of missing important information when relying solely on MAM
    - an omission of charge heterogeneity would be surprising considering its power to monitor process consistency
- For implementation of CE-MS in GxP something like the BioAccord but for CE--an integration of instrumentation and total software automation--might be needed