Roundtable Session 2 – Table 3 - icIEF - Becoming the CE Expert in Your Organization – Best Practices Exchange

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Abstract:

Scope:

Capillary isoelectric focusing has increasingly been used for characterization of charge isoform profiling and stability indication of protein therapeutics. Imaging CIEF (icIEF) is a mode of CIEF that has become commonly applied in both development and QC settings due to its speed and simplicity. As it is a complex technique which is sometimes coupled with fractionation strategies or directly with MS, internal expertise is often developed within and across individual organizations. This roundtable aims to discuss current strategies around choosing a platform and method development. We also aim to highlight gaps that may currently exist with icIEF technology or charge heterogeneity analysis in general. In some cases, this may require the development of new methodologies for existing instrumentation or could require development of completely new instrumentation with enhanced flexibility to allow scientists to work on unique molecules in their portfolio.

Discussion Questions:

1) There is multiple icIEF platforms out there. How do you choose which platform and which parameters?
    a. Some people started on SCIEX (PA 800 from Beckman—cIEF), have to look at parameters and how the instrument performs. Look at Maurice and AES there are a few options out there now.
    b. Preparation of sample and ingredients is critical.
    c. Many people start with platform methods and tweak as needed depending on the molecules.
    d. Transfer from iCE3 to Maurice has been faster by some and slower then others. Contract testing labs may still be on iCE3.
        i. A lot of inertia and work to move to a new platform. When the platform is changed what is new and what is different?
        ii. Software is critical and what software do you want to use is critical. Use of CDS's is very commonplace, but sometimes requiring working with contract lab testers IT to ensure they are collecting and using the software that is desired.
            1. How does different software map to other software's?
    e. Complex molecules often require custom methods. Platform methods do sometimes struggle—what worked for traditional mAbs does not always work for those new modalities.
    f. iCE3 to Maurice Flex is pretty comparable
2) What drove your transition from cIEF to icIEF?
    a. Faster and easier to run then cIEF. Reproducibility was also critical.
    b. Faster is the most critical.
    c. Monitoring with imaging is key

        i. Much easier to troubleshoot and convenient
3) How do you build subject matter expertise on icIEF?
    a. Not always individuals dedicated to charge. Usually methods are handled in full analytical teams focused on separations (CE and LC)
        i. Larger companies have dedicated SMEs
        ii. Smaller companies do engage more with vendors on these topics
    b. Most expertise is held internally and will include heavy method development/method knowledge learning
    c. Reaching out to vendors is always an option though for additional support
    d. Reviewing literature and what has been done before—could provide a good starting point
        i. Journal articles and tech notes/application notes can be good starting points
4) What are some of the gaps in charge heterogeneity analysis that you see?
    a. Debate about cIEF and icIEF versus CEX/IEX
        i. Large comfortability with LC methods
        ii. CE not as widely used
        iii. Everybody has LCs
        iv. CE you are sometimes more reliant on vendors ; versus LC you can make some of the buffers yourself
    b. Baseline resolve of the different variants
    c. Higher throughput icIEF would be good for DOE and method optimization
    d. Collect fractions across the pI range
    e. Higher volumes of fractions collected for additional techniques
    f. Flexibility is critical

Notes:

- Most of the group has done icIEF and has experience with icIEF.
- Difficult sometimes using CDSs and how they map CE to work in it.
- 32 Karat and Compass designed for CE and can be easier to use.
    - It also matters which you learn first—a CDS or 32 Karat/Compass first
    - Data processing and data analysis is much easier in CDS's like Empower.
- Native fluorescence worked very well for low titer samples in particular, AAV
    - What are critical parameters to handle a variety of samples?
    - Little info on low titer samples, so if you find a way that will be critical info to have
- Method development is tough work especially for new modalities. There is not as much historical knowledge and requires a lot of troubleshooting and trying to get something that will work correctly.
- Revvity offers a CZE based method; SCIEX offers a cIEF based method
- AES and Biotechne offer icIEF
- Ideally everything ends up or needs to be planned for being in QC, GMP
- Having experience with analytical development and QC is good as it can be beneficial to knowing what you are handing off and how it will be used
- Constant modality changes and variety make platform methods harder and harder to use
- Workshops and discussions at conferences is critical for idea sharing
- Setting the right acceptance criteria for the assay is key

- Trying different solutions and platforms is critical for innovation
- Sometimes correspondence between CE and CEX works, but if the molecules are complex deep characterization can be necessary such a CE-MS technologies
    - Biotechne- Maurice Flex
    - Advanced Electrophoresis Solutions- CEinfinite
    - SCIEX- CESI; Intabio-MS
    - Agilent- CE-MS
    - CMP