Roundtable Session 2 – Table 9 – Becoming the CE Expert in Your Organization – Best Practices Exchange

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Abstract:

Imaged capillary isoelectric focusing (iCIEF) is an industry standard technique for determining the relative distribution and calculated isoelectric point (pI) of charge variant species in monoclonal antibodies. The high resolution, reproducibility and minimal method development requirements of this technique have resulted in its extensive use for product development and release testing. In this round table session, we will discuss best practices surrounding method optimization, system suitability, troubleshooting, and the evolution of iCIEF methods to support newer modalities of biologics.

Discussion Questions:

1. What factors contribute to run-to-run variability, and how can we address them?

2. How can we improve resolution of poorly resolved peaks while minimizing method development time?

3. What system suitability criteria are most critical for iCIEF release testing, and how can we strike a balance between regulatory compliance and operational practicality?

4. How might iCIEF methods need to evolve to support newer modalities such as AAVs, fusion proteins, and other non-mAb biologics?

5. What are the key advantages and limitations of different iCIEF platforms (e.g., Maurice, iCE3, SCIEX systems) and how should these factors influence instrument selection for development vs. release testing?

Notes:

1. What factors contribute to run-to-run variability, and how can we address them?

(1) Ampholyte Reagent Consistency:
Pharmalyte, the most widely used ampholyte for isoelectric focusing, is facing significant lot-to-lot consistency issues. This known variability is a major concern for reliable and reproducible results. To better manage this problem, it would be helpful if vendors could provide detailed lot information or evaluation data to users.

(2) Supply Chain and Alternatives:
Earlier this year, a supply chain shortage of Pharmalyte highlighted the urgent need for reliable alternatives. Evaluating backup ampholytes has become crucial. Several viable options are now on the market, including AESlyte®, SERVALYT™, BioLyte® and Sinulyte™.

(3) Ampholyte Range and Robustness:

Using a wider range of ampholytes, such as the 3-10 range, can lead to less robust results depending on the lot-to-lot difference. A more reliable approach is to mix Pharmalytes with a narrower pH range.

2. How can we improve resolution of poorly resolved peaks while minimizing method development time?

(1) Using Narrower-Range Ampholytes:
There are some narrower-range ampholytes available, such as AESlyte. By mixing different ratios of narrower-range ampholytes, peak resolution can be improved. But longer focusing time will be needed.
(2) For icief-fractionation method development: method can be developed using the analytical cartridge, and the fractionation process still requires further optimization when changed to prep cartridge.

3. What system suitability criteria are most critical for iCIEF release testing, and how can we strike a balance between regulatory compliance and operational practicality?

A robust system suitability test should utilize a stable, standard sample with a fixed profile. The criteria for this standard are:

(1) Peak Profile: The percentage of acidic, main, and basic peaks must remain consistent across all runs.
(2) Peak Resolution: The resolution between these peaks should be limited to a small, acceptable range.
(3) pI and pI Difference: The isoelectric point (pI) values of the peaks, as well as the pI difference between them, should remain within a defined range.

Besides, to minimize lot-to-lot differences from commercially purchased standards, some companies opt to develop and use their own internal standard material. It's essential to use proper pI markers as part of the platform method. If any issues arise, these markers will show a deviation, alerting the user to a problem with the run. If needed, additional pI markers can be included.

4. How might iCIEF methods need to evolve to support newer modalities such as AAVs, fusion proteins, and other non-mAb biologics?

AAV: reduced icIEF methods to get clean baselines and monitor the deamidation. The native method is still under development. Adding some BioLyte with pharmalyte can help to achieve better resolution.

For some of ADC samples, due to stability, the samples will need to be prepared on ice and kept in autosampler at 4 C. Depending on molecules, urea is not a stabilizer for ADC, in such cases, formamide may be a more suitable alternative.

SERVALYT works better for fusion proteins.

5. What are the key advantages and limitations of different iCIEF platforms (e.g., Maurice, iCE3, SCIEX systems) and how should these factors influence instrument selection for development vs. release testing?

(not having enough time to go through this question but discussed about Maurice).

The Maurice system has limited temperature control. To mitigate condensation issues in the autosampler, a well-plate cover with pre-slits can be used when the sample is stored at 4 °C.

It is a good practice to run a self-test on a new cartridge before use to ensure it meets specifications. A coating issue with the cartridge is often indicated by problems with the basic peaks, which are typically the first to show issues.