Roundtable Session – Table 9 – CE Troubles: Root Causes and Potential Solutions

Facilitator: Ben Clarke, *USP*

Scribe: Chloe Lipovsky, *Pfizer, Inc.*

Abstract:

CE methods are versatile and performant workhorses of the biopharmaceutical industry. However, their failure modes can be difficult to troubleshoot or assign to a well-supported root cause. Common performance issues often have several potential causes. Issues may also be transient and appear only in a sample (e.g., bubbles, sample preparation irregularity), a run (e.g., temperature, current instability) or a lot of capillary, cartridge or reagent. To ensure that a CE method is robust in a regulated environment, methods should be designed not only to minimize failure but to support root cause identification. This roundtable will discuss CE problems and approaches for investigating and avoiding them.

Discussion Questions:

1. What is a challenging CE problem that you had to troubleshoot and how did you resolve it?
2. What controls and procedures do you routinely use to support troubleshooting and root cause investigations?
3. What strategies do you use when communicating with QA, management and external partners about CE problems and root causes?
4. What troubleshooting challenges are presented by new modalities (e.g., AAV, conjugates, mRNA-LNP)?
5. What parameters do you optimize for CE that improve overall robustness for the method? (i.e lower pH, alternative alkylants, developing own buffer to minimize post translational sieving)

Notes:

1. What is a challenging CE problem that you had to troubleshoot and how did you resolve it?

- Ghost peak story (reference talk: “Differentiating Product-Related Species from Preparation-Induced Artifacts in Reduced CE-SDS – Root Cause Analysis Investigation”, Thilina Jayarathne, Bristol-Myers Squibb Company)

- Under non-reduced conditions – method induced fragmentation (increase of L chain) based on pH of sample buffer.
    - Kappa vs lambda L chain differences
        - Low pH vs high pH tris buffer (only for lambda L chain not kappa L chain)
    - Low tris vs low phosphate (low phosphate outperforms low tris – not much buffer capacity for tris)
- Orthogonal method troubles/method bridging
    - Problem: Lots of peak tailing and loss of resolution on Maurice, higher than on PA800+
        - Might have been evaporation – liquid level had dropped substantially
        - Weather dependent condensation (look for seasonal trends)
- General tip for troubleshooting: make sure you are looking at both electropherograms vs quantitative data. Problems often show up in electropherograms before quantitative data would flag as issue.

2. What controls and procedures do you routinely use to support troubleshooting and root cause investigations?

- Running internal reference standards and SOP system suitability
    - When troubleshooting, typical first steps – how did reference standards/system suitability injections look? Also, go to system and have a look to see if anything looks wrong
- General tip: keep up with maintenance – service engineers for PM and routine maintenance
- System suit to control assay – was there a change in instrument or manufacturer recently?
    - Coolant in Sciex has changed
- Problem: CZE method for multiple programs, different resolution with new vs old Sciex coolant. Sciex flushed old coolant and will replace new coolant – can't go back to old coolant. Low resolution at first, sometimes it's high resolution, then back to low resolution – previous coolant in QC, can't release without. Currently in development doing comparability to new and old coolant.
    - If the issue is sporadic, could be arching issue
        - Observations: new coolant is more drippy than old one, have to be more mindful with interface block
        - Sciex has introduced new cleaning procedure – cleaning red seal rings
    - For troubleshooting – increase and decrease capillary temperature (even if you can't change method, could help with understanding issue
- Problem: Self-replicating RNA. Ladder – nothing goes high enough with MW. Currently, there is no good standard in the industry. Typical control includes construct and backbone specific control with internal standard

3. What strategies do you use when communicating with QA, management and external partners about CE problems and root causes?

- Ex. Find root cause → open quality event → correct in testing lab → update method → potentially file an IND amendment
    - Perform impact assessments
- Emphasizing if there is impact to data or not when communicating
- General watchout: sometimes people try to apply chromatography to CE principles, but technologies are different
    - Ex. Migration time shift in CGE is okay and not a cause for concern

4. What troubleshooting challenges are presented by new modalities (e.g., AAV, conjugates, mRNA-LNP)?

- Self-amplifying mRNA (saRNA)– hard to find control, no sufficient ladder
- For some new modalities, there has been success with making own gels (proprietary blend) for better resolution
    - Sciex kit has poor resolution for some new modalities
- Problem/solution to share: due to the formulation buffer the saRNA was in, it was adhering to the plastic. Can't concentrate or buffer exchange. Thought the issue was related to the concentration of the sample but actually was sample prep conditions. Isolating sample prep conditions from everything else
- Important to note that some of the more sticky AAVs, can lose 20-30% in pipette tip
    - Specify minimum volume in method
- Info pass: Biophase becoming more common – issues with cartridges clogging – Sciex recommends reconditioning.
    - Sometimes cartridges are bad out of the box, even when using Sciex kit. Not all capillaries are bad, sometimes 3/8, etc.
    - Suggestion: do more conditioning rounds to unblock clogs. Try high pressure injection
    - Capillary storage – normal shutdown: gel, water, air
- cIEF – issues with long-term storage, even though short-term storage is working fine
    - For long-term storage: triple rinse catholyte and anolyte ports and then leave in water for a while before removing water. Important to make sure membranes are clean. Make sure to remove final water though and store tanks in air

5. What parameters do you optimize for CE that improve overall robustness for the method? (i.e lower pH, alternative alkylants, developing own buffer to minimize post translational sieving)

- PA800+ – always store capillary in water, not air
- Ghost peaks talk example: looking at method parameters earlier stage – ghost peaks in product

    - Tips: start looking at trending sooner for method lifecycle data
- Method parameters – buffer capacity is often something missed (i.e. tris does not have great buffer capacity)
    - When developing buffer in-house, calculate buffering capacity. Also keep in mind there is a chance your pH will change during sample prep
- DOE tips with buffering capacity: if your lower DOE conditions are below buffering capacity, then you will be looking at something else. Make sure your DOE upper and lower limits make sense with buffering capacity
- You can get so much info out of good DOE. Start with wide DOE to narrow in and then robustness DOE with narrower range of instrument and method parameters
- Using AI for solving CE problems – having AI check internal and external resources to see if someone has run into same problem

Last thoughts:

- Info pass: problem in 2 sites – PA800+s – recent issues with PA800s installed in last couple years – coolant system was broken from the start, quality has decreased?
    - Coolant leak due to garage – it wouldn't open, it kept hitting the bar – happened multiple times