Table 6: Dips, Peaks, and pI Shifts: Mitigating Pharmalyte Lot Variability Impacts on cIEF Profiles

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Scope:

Capillary Isoelectric Focusing (cIEF) is an effective method for determining charge variants of a product and determining their relative change in stability samples. As such, cIEF, is an important product characterization technique in order to monitor product consistency and monitor for variations. Pharmalytes are required to generate a pH gradient for which the charge variants migrate to the location where their pI equals the pH, as such, variations in the ampholyte lots can impact the resulting electropherograms produced. This roundtable aims to discuss the challenges presented to the cIEF assay due to variability of pharmalyte lots.

Questions for Discussion:

1. What challenges are presented due to variability in pharmalyte lots?
2. What method conditions/controls can be put in place to mitigate impact due to pharmalyte variability?
3. How are pharmalyte lots assessed prior to allowing use in testing?
    a. Qualification of pharmalyte lots and/or lot characterization?
    b. How is an unacceptable pharmalyte lot identified?

Discussion Notes:

- Dips
    - Dips are observed due to pharmalyte lots and sample excipients
    - 3-10 pharmalytes
        - Noticing dips in the baseline in the acidic region, which can interfere with integration
        - Addition of 8-10.5 pharmalytes still has the observed dip
    - A dip is most commonly observed around 6.8 (from 6.2-7.3)
        - Observation with a specific molecule that focused in-between the histidine and pharmalyte dips
            - Trending of data over time for this molecule observed high variability resulting in difficulties during validation

        - To mitigate differences additional guidance was provided to analysts on how to integrate specifically taking these dip features into account for this program
            - Product specific operating procedure
            - Multiple figures for guidance on integration
            - Multiple examples for the molecule at different stages eg. FDS, Stressed
- Dip will vary lot to lot
    - Does a specific lot for a program need to be labeled as a critical reagent and designated on a per program basis
        - May need to be assessed depending on impact to quantitation of variants
- Dip impact to quantitation
    - Molecule dependent
    - Good practice to compare lots with and without the dip
        - Has been observed for some molecules that there is no impact to quantitation
        - Include multiple images for what is acceptable for the molecule, with and without the dip present
    - When trending long term: estimates for %RSD
        - MP: 2-3%
            - One example of dip impact caused this to rise to 5-7% over time, which is an issue to be further evaluated
        - Acidic: 5%
        - Basic: 7-20%
- Lot Selection
    - Can establish a qualification process on critical programs where lot impact wants to be minimized
    - More directed towards commercial programs
- Consider alternate vendors if having issues with Pharmalytes
    - Servalytes
        - Some have had good experience with these, good resolving power

        - For the higher end of the range 9-11 it is recommended to aliquot these, as going from 2-8 to RT repeatedly can cause them to crash out
    - AESalytes
- Dip Mitigation
    - Is unknown what exactly is causing the dip
    - Can mix in other ranges of pharmalytes
        - 5-10, or 8-10.5
    - Dropping the concentration of pharmalyte
        - Lowest recommendation would be 2%, any lower wont establish a proper gradient
        - Baseline noise is decreased and flatter
        - Minimizes impact of the dip
- Native Fluorescence on Maurice
    - Histidine interference will be removed
    - If there is a dip due to the pharmalytes in the UV, this will still be present in the NF, something is creating a gap in the gradient
- Is there a range of pharmalytes where peaks are more frequently observed?
    - 3-10 acidic range
    - Mixed pharmalytes 5-8 and 8-10.5
        - Something in the 8-10.5 will create a gap in the 5-8

- pI Shifts
    - pI Shift can occur between multiple runs but is consistent within run
    - Attributed to pharmalyte lots
    - How is this controlled and how does it impact projects?
        - Mixing of pharmalytes is common
            - By using a different lot this can result in a pI shift of up to 0.3 units
            - This can raise concerns around method qualification
                - Concern was mitigated as it is explained to be caused by lot to lot differences

- Shifts in pI does not impact the quantitation
- For one acidic molecule it was observed to have shifting pI from 5.9-6.7
- Need to keep in mind: What is the purpose of the method?
    - Intended purpose is charge heterogeneity
    - Is pI relevant?
        - Maybe for ID
        - This is typically only calibrated over the 2 pI markers
    - Instead of pI, it may be advantageous to move to relative pixel position

Eliminates concern and mitigates any communication breakdown due to observing varying values for pI

Useful metric for troubleshooting

Some movement for use in IND and publications, has not been questioned yet

- Variability in pharmalyte lots is expected due to the manufacturing of lots itself containing a of variables
- Air spikes
    - Centrifugation prior to transferring samples has helped mitigate
        - Onboard mixing has caused increased spikes
            - Methylcellulose is very viscous
    - Cartridge age, as it sees more wear and tear, more artifacts may present themselves
- Excipients that may cause Dips
    - 7.5-7.6 due to histidine
    - Pluronic is bad
        - Requires running samples prior to DS
    - Anti-oxidizers
    - Triton x10/100
        - Causes interference with profiles