Table 9: DoE and QbD

Facilitator: Kathir Muthusamy, *Regeneron Pharmaceuticals, Inc., Tarrytown, NY, United States*

Scribe: Toby Reichenberg, *Genentech, a Member of the Roche Group, South San Francisco, CA, United States*

Scope:

Capillary electrophoresis (CE) methods have become integral to analytical quality control throughout the biopharmaceutical industry. Automated instrumentation, powerful separation efficiency, low sample requirements, and fast analysis times are characteristics that have established CE as an essential tool for product characterization and routine quality determination. The flexibility of CE to interrogate an extensive array of product quality attributes, including size, mass, charge, carbohydrate content, identity and even affinity, drives the increasing number of applications. As CE methods have become mainstream, the industry has transitioned to Quality by Design (QbD) for process development. QbD is a risk-based approach, typically involving Design of Experiments (DoE), to achieve a consistent, well understood, high quality product. The analogous concept for method development, Analytical Quality by Design (AQbD), is intended to confer similar benefits to analytical methods by the same means.

Questions for Discussion:

The goal of this roundtable discussion is to connect CE users of diverse backgrounds to share commonly observed issues and lessons learned in context to Analytical Quality by Design and DoE.

Discussion Notes:

Attendance by 5 participants

- Experience in biopharmaceutical analysis using CE
- Experience in CE technical service, engineering, sales
- Good understanding of DOE and QbD conept
- Interest in practical implemention of QbD and DOE
- Introduction by Facilitator
- Examples of CE applications
- CGE, CZE, CIEF, oligo fingerprinting
- Most recently applied to ADC’s, bispecifics and even AAV’s

- Roundtable is about how CE fits into a QbD structure the what is the role of DOE
- Consensus of timing for assessing method performance
- Use platform method initially with minimal performance assessment
- DOE and targeted performance assessment between early and late stage of product development
- Sometimes may not be considered until late stage
- Survey of CE methodologies and applications used to guide bioprocess
- development and to ensure product quality
- CESDS (CGE), CIEF by far most commonly used
- icIEF with and without mobilization (without preferred, with is usually legacy)
- CESDS-MS is gaining interest
- A few CZE applications
- CESDS, reduced and non-reduced was most commonly used as a platform method, having well understood, standard sample preparation and analysis parameters.
- Method Development OFAT (One Factor at a Time) or MFAT (Multiple Factors at a Time)
- OFAT is still most commonly used
- To evaluate the main effects of many factors without confounding by interactive effects, a fairly recently devised approach of definitive screening (JMP, Minitab, etc.) was described.
- This followed an earlier observation by an attendee that most of the time, main effects, rather than interactions are the most important focus for method development.
- Definitive screening was described as a much more efficient approach (less resource intense) than OFAT to assess multiple main effects.
- Prior to definitive screening, most typical screening designs (highly saturated fractional factorial, Plackett-Burman) allowed varying degrees of confounding with interactive effects.
- Companies often transfer methods to different sites
- Typically part of method qualification/validation and performed according to protocol with predefined acceptance criteria
- Example given was intermediate precision with acceptance based on relative standard deviation of combined data meeting criterion.
- Intermediate precision should include lab to lab, analyst to analyst, instrument to instrument, basically all the factors that can contribute to variability between CE analyses.
- Rely on method development knowledge for the for troubleshooting in the event of transfer problems.
- MFAT DOE during method development was considered especially useful for this purpose.
- Troubleshooting

- From the vendor side a robust investigation service is necessary for troubleshooting using dedicated personnel following a systematic, dedicated process.
- Some vendors use DOE to investigate problem materials (usually reagents)
- CIEF user proactively assesses complex, variable reagents, i.e. ampholytes using predefined screening designs.

- Different instrument platforms offer capabilities best suited to product quality attribute of interest, supporting process development, and QC lot release testing .
  - PA800 for CESDS (CGE) because it's the standard for CESDS lot release and CZE for flexibility.
  - ICE for benefits from lack of mobilization, Maurice same benefits as ICE plus Empower integration (served data acquisition and analysis)
  - Caliber for high throughput (process development support)
  - Some interest in Caliper multi-channel QC use
  - In general, CE analyzer design is purpose optimized, some more flexible than others, but no one size fits all.
  - Flexible analyzer configurations (different modes of detection, separation, etc.) offer power to further refine and tune platform methods to very specific applications for one-off product attributes (IgG kappa vs. lamba light chains)
- DoE relevance to method development and Validation
  - Development of a platform method for multiple IgG products was based on DOE because knowledge of effects gained in early development would inform method robustness (a QbD concept)
  - Late phase, a streamlined multifactor DoE can be performed during method validation to demonstrate robustness.
  - To accelerate time to validation of a CZE method, the example of an MFAT, half factorial DOE 2 (2-way interactions without confounding) was performed after some method development and modeled. The modeled results were successfully applied to define test procedure method parameters that were verified in pre-method qualification accuracy, linearity, precison and specificity. Robustness was already shown by the DOE.
- Predictive modeling of method performance
  - Questions about of the utility of a model to predict method performance were addressed
- Modeling based on a prospective DOE can be used to predict method performance based on the hypothetical level of a DOE effect factor (temperature, protein conc., pH, etc.)
- Optimum factor levels can be estimated by inputting performance goals (maximize resolution, minimize undesirable peak area, etc.) into a model based on linear regression of the effect factors of the DOE.

- Known or estimated method variability at hypothetical factor levels can be input into the model to yield a predicted distribution of results around the expected mean at the selected factor levels.
- When performance requirements are defined, method variability is known or can be estimated, and a linear regression model based on actual data from different factor levels is available, the failure rate of the prospective method can be predicted.
- Consideration of ICH design space concept for CE methods
  - Modeled MFAT results can enable prediction of method performance at different critical factor levels.
- Allows selection factor levels within a method to accommodate analysis of different molecular entities
- Allows flexibility in factor levels that can accommodate different vendor sourced reagents that may be different ionic strength, pH, etc. because the model predicted acceptable results using reagents with those characteristics.
- Automation of varying DOE factor levels to facilitate experimentation
  - Currently must create separate instrument programs for each set of instrument parameters in a DOE
  - Software to automate this is available for chromatography but not CE
  - There is interest in software to alleviate the burden of instrument method programming for QbD/DOE
- Emphasis has been on instrument parameters, what about sample preparation?
  - AAV analysis by CE is sample prep intensive and to develop kits a DOE approach would improve kit quality and the knowledge base to troubleshoot for customers
  - Also applicable to most various test kits supplied by vendors to industry.