Table 7: Peak Characterization of CE-based Size and Charge Heterogeneity Methods?

Session 1:
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Scribe: Handy Yowanto, *SCIEX*

Session 2:
Facilitator: Merry Christie, *CDER, FDA*
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Scope:
The development, manufacturing and quality control of protein therapeutics requires state-of-the-art analytical technologies to elucidate product purity, structure, and chemical stability. Capillary electrophoresis (CE) plays an important role in the analysis of proteins by providing a complementary methodology in the characterization of size and charge attributes. While CE provides exquisite efficiencies and high resolution of protein variants, the technology suffers from direct peak characterization due to the challenges associated with mass requirements, fractionation, buffer/gel complexity and direct coupling to mass spectrometers. This roundtable aims to discuss the technical aspects, strategies and solutions in using currently available tools to (in)directly identify size and charge variants, or others, separated by various CE methods.

Questions for Discussion:
1. How do you perform integration of CE-based size and charge heterogeneity methods? Do you allow
1. for manual integration? How do you control integration to ensure consistency in the data analysis?
2. What methods do you use to identify the species present in individual peaks?
3. What are challenges you face when identifying the species present in each peak and how do you
4. overcome these challenges?
5. What is your approach to assess if the identified species impact product quality or safety?
6. When during product development do you conduct peak characterization?

Discussion Notes:
Session 1:
1. How often are you asked to perform full peak characterization of your products?
    - Most participants at the table indicated twice a year, with the majority working on monoclonal antibody products.
    - Depend on the molecules, usually 3 to 4 times a year. Needs may be greater for peak characterization of new molecules such as fusion, bispecific, etc.
    - There is a need to use orthogonal methods and collect fractions for analysis.
    - Full characterization is done in later stages of development.

2. What point in the product development phases do you apply in-depth identification of product variants?
    - Some have a need for peak characterization at Phase 1 pre-tox human study where methods need to be developed.
    - For most participants, a deeper characterization of minor forms is typically done between phase 3 and BLA, typically with pivotal material
    - Cycle 2 pre-clinical trial development in some cases cycle 1 post R&D
    - Leverage tox studies: during tox campaign, safety profile from animal study are typically established and can be leveraged if good comparability is observed with tox and clinical materials. The need to do deep characterization in early phase should be rare.

3. What experiences do you have in identifying product variants separated by CE? What commercially available technologies or tools have you used that have helped you to achieve CE peak identification?
    - SEC fractions can be collected and analyze for each peak; one example using this approach was to determine the root cause of protein cleavage due to trace metal from the fermentation tank.
    - Some have manually collected peaks or regions from ion exchange (IEC) columns and then analyzed by iCIEF, some have used CEInfinite to collect charge isoforms, some have used Agilent OFFGEL (pI strips) to enrich variants; 908Device's Zipchip has been successfully used by participants for analyzing samples via CZE-MS; Intobio's Blaze is another tool (CIEF-MS) that folks will explore when the system becomes commercially available.
    - Charge or size variants need to be analyzed, particularly the acidic region which tend to accumulate more pCQAs than the basic region. Deamination, glycation, etc are characterized by MS. These new peaks are observed in typically 20 – 30% of the products.
    - Perform enzyme digestion to indirectly ID minor forms (e.g., hinge fragments).
    - HPLC, MS, CE-SDS are used collectively to characterize glycans as well as reduced & non-reduced size variants of proteins.
    - Rotofor or OFFGEL are other tools used for enrichment. Gradient strips for enrichment is time consuming and this is sufficiently pure to analyze new peak. Free thiol reacts with product and shift the product peaks during analysis. In this example, process development team was able to modify the condition upstream.
    - CE SDS peak patterns are different for bispecific. The 2 different heavy chains and 2 different light chains that makes this different from conventional monoclonal antibody.
    - CE-SDS under reducing conditions result in the kappa and lambda chain migrating at different rate (migration shift) despite having similar molecular weights.
    - CESI using uncoated capillary for peptide Mabs work well. Niche applications and in glycan work it is easy to connect LC into MS, in additions, the 908 devices work well too. The Zip Chip works well when following their protocol.

4. What has been most successful to you? Most importantly, where/when did you fail?
    - This question was not reviewed by the table.

5. What strategies have you taken that you can share today that will help the CE community to perform such studies with minimal burdens and costs?
    - Level of characterization should depend on how active your minor variants are; for example, enriched basic (e.g., 90-100%) and acidic variants (e.g., 60-80%) typically remain active for various antibody products; HMW forms can be hyperactive (e.g., 200%)
    - Is there a need to do individual peak characterization or is "regional" assessment good enough for minor variants that have reasonable activity?
    - Perform a risk assessment with appropriate SMEs to determine a strategy for characterization, resources with depend on the level of risk