Table 2: New CE Modalities- How to Analyze Complex Biomolecules

Facilitator: Andrei Hutanu, *F. Hoffmann-La Roche Ltd, Basel, Switzerland*

Scribe: Eleanor Le, *Amgen Inc., Thousand Oaks, CA USA*

Scope:

Rapid advancements in science and technology create new therapeutics for disease mechanisms which were previously considered difficult, if not impossible, to target. This ever-growing diversity of modalities significantly affects patient wellbeing. However, these new modalities often bring major challenges for analytical quality control. Capillary Electrophoresis has proven to be a valuable technique in the analytical toolbox for characterization of various novel pharmaceuticals, including, bsmAbs, Co-Formulations, ADCs and AAVs. The scope of this roundtable is the discussion of current challenges, approaches and chances offered by CE for new modalities.

Questions for Discussion:

1. Which new modalities are currently investigated/of most interest?
2. Which of these seem most challenging?
3. What benefits can CE offer?
4. What are the disadvantages of using CE vs. LC methods for these new modalities?
5. Should CE be avoided for any new modalities?
6. Which sub mode of CE has the brightest future regarding different new modalities?
7. What are the CE technologies available for running these new modalities, considering there are no historical data?
8. What caveats will be considered for CE in running these modalities?
9. Are there any CE standardized or platform methods available for consideration to ensure data quality?
10. What is the justification of using CEX-LC method vs. cIEF or CZE methods for charge-based assays?
11. Besides SDS-PAGE and SEC-LC methods, what are the orthogonal methods that can be used to correlate the CE data?
12. What are the different statistical tools available to use for DOE in CE method development?

Discussion Notes:

1) Which new modalities –
    a. fusion proteins;
    b. AAVs, viruses behave different completely;
    c. half-life extended molecules;

    d. PEG molecules – developed NR CE but causes dePEG; caused it to be unstable – lowering temp at 50C and added antioxidant;
    e. fusion protein – use iCE and Wes from ProteinSimple;
    f. bi-specific;
    g. DNA; use Sciex kit but didn't get good response for dye for signal;
    h. AAV for new product;
    i. 3 kDA proteins; 12 KDa proteins – 75 mins separation; bigger than internal standard; but may interfere with system peaks; still use Sciex gel from PA800; change voltage? Other parameters?
    j. 5 AAVs – good for CEs; formulations issue with quick buffer exchange;
2) Which are more challenging –
    a. co-formulations of 2 Abs that are the same mixture;
    b. challenging from charge separation;
    c. compare LC and CE; empty has lots of peaks still with profile maybe due to isomers;
    d. use AEX; can't see the partial ones, use HPLC method to optimize;
    e. AAVs difficult with cIEF, different pIs of virus; CZE – virus always uncharged; basic regions variable; virus starts to unfold in 3M urea causes partially charged; try non-reduced method?;
    f. intact virus – pH 3-9 migration; limited material and most of the time not pure from vendors;
    g. cIEF – bump up urea and time sensitive;
    h. Maurice – onboard mixing capability might help on some situations;
3) Benefits –
    a. CE-SDS method robust;
    b. good solutions for stressed samples;
    c. standard for technique separation;
    d. iCE – monitor degradation;
    e. use ratio for CE-SDS and iCE for peaks changing;
    f. late stage -SEC as release for empty vs full, AUC, charge detection MS – for stability and release;
    g. lots of AAV materials needed for 100%, 60%, etc. for empty vs full, partial;
    h. FIO – iCE data
4) Disadvantage of CE:
    a. simple characterization;
    b. peaks IDs difficult; intact mass – PTMS:
    c. need binding assay;
    d. need LC assays to confirm;
    e. cIEF is preferred for pI;
    f. buffer exchange and fraction collection;
    g. need orthogonal assays;
    h. co-formulated drug products for cIEF; pIs overlap – argument for chromatography;
    i. CE-SDS standard for fragmentation;
5) CE avoided for new modalities –
    a. incomplete denaturation;

    b. binding with SDS;
    c. CE is always worth the look in a positive way; always worth trying;
    d. fusion protein – 30-40 peaks? Need to use enzyme to simplify profiles;
    e. 17-18 positions; need to tag and purify – but is this a new molecule?
    f. Need a different method for release;
    g. good for characterization;
6) Sub-mode of CE:
    a. difficult question;
    b. charge hetero assays will increase from several pipelines;
    c. CZE vs. iCE; CE-SDS will keep its role;
    d. more CE-MS;
7) CE new technologies –
    a. PA800 first, platform methods first;
    b. some companies develop their own buffers;
    c. HHS buffer vs SDS buffer for hydrophobic samples;
    d. go with manufacturer's recommendation, then troubleshoot;
    e. go off prior knowledge;