Roundtable Session 2 – Table 8 – Peak Integration in CE

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Abstract:

Capillary electrophoresis (CE) often offers superior separation relative to chromatography for macromolecules like monoclonal antibodies (mAbs), a major pharmaceutical class. However, electropherogram baselines pose challenges that traditional chromatography algorithms cannot address, requiring complex integration processes. Integration in GMP laboratories is critically important and has become a focus of data integrity-centric regulatory inspections. The electropherogram integration challenges, the increased use of capillary electrophoresis, data systems developed for chromatograms rather than electropherograms, and the increased regulatory scrutiny call for a resolution.

Let´s find some answers together! Let´s find Good Integration Practices!

Discussion Questions:

- **Integrating Electropherograms – Current Practices:**

What challenging examples do you face? Do you find integrating electropherograms difficult? Is it a time-consuming process for your lab?

- **Integrating Electropherograms – Regulatory Requirements:**

What do the regulatory authorities say about peak integration in general? And, specifically, in CE? What does your QA say? Do you feel "pressure" to only auto-integrate? What is auto-integration? Should manual integration be allowed? Under what circumstances?

- **Integrating Electropherograms – Training:**

Have you had training dedicated to the integration of chromatograms and electropherograms? Do you have a deep Theoretical understanding of the integration functions in your chromatography data system? Or would you consider it an intuitive understanding? Have you received training that helps you understand how electropherograms differ from chromatograms and how that comes into play when integrating electropherograms?

- **Integrating Electropherograms – Documents, Documentation, and Data Integrity:**

Does your company have an overarching policy on the integration of chromatograms and electropherograms? Does your company have general standard operating procedures (SOP) for integrating chromatograms and/or electropherograms? Do your capillary electrophoresis test methods

have enough illustrated examples to make it clear to analysts and reviewers that integration was performed correctly?

What should good documentation of peak integration look like?

Which parameters and examples should be specified in SOPs?

Notes:

1)   General discussion:

   a. Data analysis with the Empower SW is difficult to automate, can be further improved in general

   b. Will (from Protein Simple) once did 4000 integration for a global study comparing Maurice and ICE3. It was determined that if one person analyzes all of the data, the variation caused by different analysts integrating the peaks in different ways can be eliminated. The results were published on Electrophoresis 2-3 years ago.

   c. There is a need to report the results consistently: between labs, between R&D and QC.

2)   Key words:

   a. Accuracy: hard to achieve. What is the true value or a mutually agreed value

   b. Different challenges depending on applications (CE; and also LC!) or molecules. Vendors can help set up standards

3)   Current challenges in peak integration:

   a. Baseline issues:

      i. Complex electropherograms with heavily sialylated mAbs

      ii. Gardasil 9, multivalent HPV vaccine has 20 species to separate. The baseline is often not flat.

      iii. PDL-1 has porcupine peaks, some of them overlap. So, cannot find the tallest peak. Two tricks used: identify the first peak to start; do a lot of peak grouping. Group the low, small peaks; A lot of partial considerations.

      iv. Difficult baseline such as the waviness in CE-SDS, a 45 minute separation.

      v. Applied Bioanalytics has a new gel that runs 15 to 20 minutes. It runs faster to mitigate the wavy baseline issue seen in CE-SDS.

   b. How to calculate S/N ratio?

i. You are measuring millivolts. Take an average baseline from multiple injections of blanks.

ii. You will get different S/N ratios using Chromeleon vs Empower SW. Algorithms used should be given for transparency.

c. How to smooth the baseline? Can do some, but better to do the cut between the valleys. You can do it and explain it to regulators,; as you do it the same way every time.

d. How to group peaks?

e. Training:

i. To ensure it is consistent, need to provide SOP with examples in appendix. (could be 30 pages long, including many illustrations as examples).

ii. Method development report will collect the examples. SOP updates later.

iii. Can also have a default Work Instruction that is generic/universal for the class or platform method.

iv. SOP includes the parameters, and examples to give a visual interpretation of how to adjust them

v. Put a note at the end of the SOP saying "can be adjusted as needed".

vi. Documentation: with a table of parameters for how to use them with Empower.

vii. Many people do peak integration manually while others try to automate.

viii. If we had large enough reference data set, people can agree on how to integrate.

ix. Sometimes, integrating part of the peak helps.

f. Manual integration:

i. If manual integration is needed, it is still possible to do it with good training

ii. Including co-qualification and co-validation in intermediate precision studies for capillaries/instruments/multiple analysts and labs can help justify how peak integration is done. For shoulder problems/over estimating the impurities, use the worst case scenario. Use pool of data to generate RSD% data. Take the raw data and have one person to do the data analysis so you can isolate each factor: variation due to instrument, sample lab, etc. Easy to do with small company.

iii. People hate manual integration because it is time consuming (2-3 hours for 100 E-grams)

iv. SEC and RP-HPLC often have perfect baseline. So, those people don't like to do manual integration. They think it is very subjective.

v. Gradual baseline or sloping baseline with USP mAb1 is difficult to integrate.

4)   Discussions on how to improve peak integration:

a. Hermann has a table of analysis parameters. If you feel additional parameters can be added to Hermann's table, please let him know.

b. Can vendors provide a reference dataset that is complex enough? This dataset can be used to generate standardized data analysis methods.

c. Simple Western SW draws idealized peaks around your peak. Compass SW automatically fit a Gaussian shape about your peak. Autointegration with auto-draw baseline works well. With partial manual integration, you can use the drop down feature to move integration left to right.

d. Would prefer to optimize the parameter settings and do automated peak integration. However, it is difficult to fix theses optimized settings to all samples, especially the unknown peaks.

e. It may be ok to use different models for different ranges of the data: from 1 to 10 minutes: use one model, and from 10 to 20 min, use another model.

f. Send out a set of raw data and have different people analyze them

g. USP is asking Biogen and other companies to run their AAV reference samples with an SOP on PA 800 Plus and Maurice. Therefore, USP will have AAV data sets. These data sets can be useful to study peak integration.

Complex electropherograms could also be obtained from highly sialylated mAbs, or degraded samples.