Roundtable Session – Table 2 – Strategies and Challenges with Peak Integration in CE

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Abstract:

Peak integration is a critical component of capillary electrophoresis (CE) data analysis and can directly influence the quantitative assessment of product purity, heterogeneity, and product-related species. While many common integration challenges are well recognized, achieving consistent and scientifically justified integration remains difficult as CE methods are applied across different assay formats, products, analysts, instruments, data-analysis platforms, and stages of the analytical method lifecycle. Establishing integration strategies that are sufficiently standardized for reproducibility while retaining appropriate flexibility for complex or atypical electrophoretic profiles remains an important consideration.

Integration challenges and appropriate strategies can differ significantly across CE applications. For example, reduced and non-reduced CE-SDS generate distinctly different electrophoretic profiles, peak complexity, and product-related species, potentially requiring different approaches to peak identification, integration boundaries, shoulders, partially resolved species, and low-level peaks. Other CE applications, including cIEF and icIEF, present additional challenges associated with closely spaced charge variants, baseline definition, and resolution. Differences in data-analysis software, integration algorithms, and parameter settings may further influence integration outcomes and become particularly important when methods are transferred between laboratories, instruments, or software platforms.

Discussion Questions:

1. How should integration strategies differ across CE applications, particularly between reduced and non-reduced CE-SDS, and which integration principles and decision criteria can reasonably be standardized across methods?

2. How do differences in data-analysis software, integration algorithms, and parameter settings affect peak integration and reported results, particularly during method transfer between instruments, laboratories, or software platforms?

3. Should integration consistency be defined by reproducible integration boundaries, comparable reportable results, or both? What level of variability is scientifically acceptable across analysts or platforms?

4. How should organizations establish and document integration rules while maintaining appropriate flexibility for atypical electropherograms, partially resolved species, shoulders, and low-level peaks?

5. How could reference electropherograms or consensus datasets be used for analyst training, method transfer, software benchmarking, and evaluation of automated integration approaches?

Notes:

Question 1.

Discussion points

- Integration strategies may need to be application-specific, particularly because peak profiles and separation characteristics can differ between reduced and non-reduced CE-SDS.
- Shoulder peaks and partially resolved peaks were identified as situations where integration can be particularly challenging and may require application-specific guidance.
- Peak-to-valley ratio was mentioned as a potential decision criterion for determining whether closely spaced/shoulder peaks should be integrated separately.
- Tailing in CE may have different causes and characteristics than in LC, including effects related to absorption and dispersion. Therefore, integration rules should account for the characteristics of CE electropherograms rather than simply applying LC principles.
- Some principles could potentially be standardized, including:
    - Consistent identification of peak boundaries.
    - Defined criteria for handling shoulders and partially resolved peaks.
    - Consistent treatment of low-level peaks.
    - Clear rules for baseline placement.
    - Defined criteria for when manual intervention is appropriate.
- There was also discussion of including integration strategies directly in the analytical method, rather than relying only on analyst judgment.

Summary

The discussion supported having common integration principles and decision criteria, while allowing application-specific rules where electropherogram characteristics differ. The method should clearly define how challenging peak shapes, shoulders, tailing, and poorly resolved species are handled.

Question 2

Discussion points

- Different software platforms and integration algorithms can generate different integration outcomes, even when the underlying electropherogram is the same. This becomes particularly important during method transfer between laboratories or instruments.
- Differences may result from:
    - Integration parameters.
    - Software versions.
    - Algorithm differences.
    - Baseline processing.
    - Manual versus automated integration.
- The same parameters may not necessarily produce the same results across different software versions or generations.
- Software updates can potentially change integration behavior and therefore affect reported results.
- Automated integration was viewed as desirable for consistency, but the group recognized that manual intervention may still be necessary for atypical or difficult electropherograms.
- A key challenge is distinguishing a true method-related difference from a software/integration-related difference.
- A bridging study may be necessary when transferring methods between significantly different software platforms.
- There was also discussion of the need to empower analysts/users to understand and appropriately implement integration within software such as Empower.

Summary

Software and algorithm differences can contribute significantly to integration variability during method transfer. Therefore, integration parameters and software versions should be appropriately controlled and documented, and transfers involving different platforms may require comparability or bridging assessments.

Question 3

Discussion points

- The group discussed the distinction between:
    - Consistency of integration boundaries, and
    - Consistency of the final reported result.

- Two analysts may select slightly different integration boundaries but still obtain essentially equivalent reportable results. Conversely, apparently similar integration boundaries may result in meaningful differences in reported purity/impurity values.
- The question was raised regarding whether experts would necessarily agree on the integration of the same electropherogram. Examples were discussed where the same electropherogram could potentially be interpreted differently by different experts.
- Standardization of system controls was identified as important for reducing variability.
- A few published papers address the challenges and suggest standard practices:
    - Krompholz et al. (2026), **"Improvement of Peak Integration in Capillary Electrophoresis: Reference Data Set No. 1,"** Electrophoresis, 47(4), 276–294. DOI: 10.1002/elps.70077.
    - Blanc, Wätzig & Sänger-van de Griend (2025), **"Peak Integration of Electropherograms in GMP and Research Labs: Navigating Increased Scrutiny Amid Data Integrity Audits and Inspections,"** Electrophoresis, 46(11–12), 653–668. DOI: 10.1002/elps.70002.
- The group discussed using qualification/acceptance criteria and comparisons to a 100% level as potential ways of evaluating integration consistency.
- There was discussion around signal-to-noise expectations when determining quantification limits and the need to establish scientifically justified criteria rather than applying arbitrary limits.
- The overall implication was that integration consistency should likely consider both the integration boundary and the impact on the reportable result.

Summary

Consistency should not necessarily mean that every analyst produces mathematically identical integration boundaries. A scientifically meaningful assessment should consider both boundary reproducibility and comparability of the resulting reportable values, with predefined acceptance criteria for allowable variability.

Question 4

Discussion points

- A major theme was the need for clear integration instructions within the analytical method.
- The method should provide guidance for situations such as:
    - Poorly defined peaks.
    - Shoulder peaks.

    - Partially resolved species.
    - Tailing.
    - Low-level peaks.
    - Peaks approaching or below reporting/quantitation limits.
    - Peaks that are incorporated into a larger main peak.
- Where a peak is not clearly defined, the method should explain how the analyst should determine the integration boundaries rather than leaving the decision entirely to individual judgment.
- The group discussed the need to distinguish between predefined integration rules and justified manual intervention.
- Manual integration should not simply be used to obtain a desired result; it should be supported by documented criteria.
- There was a suggestion to include representative examples/electropherograms in the method, including stressed materials.
- Integration guidance could also be supported through:
    - Training.
    - Development reports.
    - Integration manuals/guidelines.
    - Standardized examples.
- The group discussed whether existing published integration manuals/guidance could be leveraged.
- For atypical profiles, the method should provide enough flexibility to address unusual electropherograms while maintaining a defensible rationale for the integration decision.
- Regulatory scrutiny was specifically mentioned as a consideration: inconsistent or poorly justified integration practices could generate questions from health authorities.

Summary

Organizations should establish predefined, documented integration rules with representative examples, while explicitly defining when and how analyst intervention is permitted. The goal is to minimize subjective decisions without making the method so rigid that it cannot accommodate atypical electropherograms.

Question 5

Discussion points

This topic generated several practical suggestions:

Reference electropherograms

- Establish a reference set of electropherograms representing typical and challenging cases.
- Include examples such as:
    - Typical sample profiles.
    - Stressed materials.
    - Shoulder peaks.
    - Partially resolved peaks.
    - Tailing.
    - Low-level peaks.
    - Difficult baseline situations.
- Include representative E-grams directly in analytical methods or supporting documentation.

Analyst training

- Reference electropherograms could be used to train analysts on:
    - Where integration boundaries should be placed.
    - How to handle shoulders and partially resolved peaks.
    - When manual intervention is justified.
    - How different integration decisions affect reported results.
- This could help reduce analyst-to-analyst variability.

Method transfer

- A common dataset could be analyzed at both the sending and receiving laboratories to assess whether the integration strategy is transferred consistently.
- Reference datasets could help distinguish method differences from software or analyst differences.
- A bridging exercise may be particularly useful when transferring between different software platforms.

Software benchmarking

- The same reference electropherograms could be processed using different software, algorithms, or parameter settings.
- This would allow organizations to evaluate whether different systems produce comparable integration and reportable results.
- Such datasets could also be useful for evaluating the effect of software updates or changes in integration algorithms.

Automated integration

- Consensus/reference datasets could be used to benchmark automated integration.
- Automated results could be compared with an established reference or consensus integration.

- This could help identify where automated integration performs reliably and where manual review/intervention remains necessary.

Summary

A standardized library of reference electropherograms, including challenging as well as ideal profiles, could provide a common resource for analyst training, method transfer, software qualification and benchmarking, and automated integration assessment.