Roundtable Session 1 – Table 1 – Strategies and Challenges with Peak Integration in CE

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Abstract:

Peak integration in capillary electrophoresis (CE) is a critical step for accurate quantitation and characterization of biomolecules, yet it presents a range of technical and analytical challenges in both R&D and GMP environments. This roundtable will explore and discuss strategies employed to achieve reliable peak integration, including the selection of optimal integration parameters, management of baseline drift and noise, and approaches for resolving overlapping or poorly defined peaks. By sharing experiences and solutions, participants will gain insights into improving data quality and reproducibility in CE assays.

Questions

1. What are the biggest challenges you've experienced with peak integration? How do you resolve them?
    a. Software, data acquisition, peak integration protocol
    b. Test procedure instructions, Training, Method Transfer
    c. Data quality, noisy baselines, Matrix interference, peak migration variance
    d. Inconsistent resolution, Impact of peak shape, Tailing peaks, matrix effects
    e. Low abundance peaks, signal to noise

2. How do baseline drift and noise affect peak integration, and what strategies can be used to mitigate these issues?
    a. Baseline correction, smoothing algorithms
    b. Manual vs automated integration

3. Is there an opportunity to provide new innovative solutions such as leveraging AI-driven algorithms or automated integration strategies?

4. How do molecule type and assay format impact peak integration?

a. RNA, DNA, fusion proteins, mAb, ADC, ...
b. CE-SDS, CGE, CZE, iCIEF, CIEF, CZE, CE-MS, ...

5. What approaches are most effective for integrating closely spaced or overlapping peaks?

6. How do you ensure consistency in peak integration when transferring methods between labs or instruments? What are the most critical peak integration instructions to document when training and transferring methods?

Notes:

Overall summary:

Peak integration in CE faces challenges with inconsistent parameters, shifting peaks, and baseline noise. Analysts struggle to distinguish real peaks from artifacts, especially in complex profiles like CGE or iCIEF. Strategies include using overlays of blanks, defining consistent integration bounds, and leveraging software tools like Empower for custom LOD settings. While automation works in most cases, manual review is often needed for edge cases. There's a strong need for standardized methods, reference datasets, and formal training to ensure consistency across labs and analysts.

1. Biggest Challenges & Resolutions

a. Software, Data Acquisition, Peak Integration Protocol

- Empower software allows custom fields for peak bounds and LOD.
- Integration parameters not consistently applied across runs.
- Automated boundary setting works ~90% of the time; manual adjustments needed for edge cases.

b. Test Procedure Instructions, Training, Method Transfer

- No formal training programs exist for peak integration.
- Method transfer issues: inconsistent interpretation across labs.
- Suggested solution: reference datasets, standard work, pass/fail criteria for training.

c. Data Quality, Noisy Baselines, Matrix Interference, Peak Migration Variance

- Baseline drift and noise complicate peak identification.
- Peak migration between runs affects % area and integration accuracy.
- Matrix effects in CGE/iCIEF cause real vs. artifact peak confusion.

d. Inconsistent Resolution, Peak Shape, Tailing Peaks, Matrix Effects

- iCIEF/CZE resolution issues: overlapping peaks in tailing/shoulder.
- Tailing and shoulder peaks challenge automated integration.
- Baseline curvature complicates valley-to-valley integration.

e. Low Abundance Peaks, Signal-to-Noise

- LOD strategies vary: static vs. dynamic depending on normalization. With static seems to be the preferred approach
- Signal-to-noise ratio used to validate low abundance peaks.
- Peak area filtering used to exclude noise but may be subjective.

2. Baseline Drift & Noise Impact + Mitigation Strategies

a. Baseline Correction, Smoothing Algorithms

- Generic baseline drawing works for ~70–80% of cases.
- Overlay blanks to identify shifts and define consistent bounds.

b. Manual vs Automated Integration

- Manual integration often required for stressed samples or complex profiles.
- Automated methods work well for most cases but struggle with shoulders and peak shape variability.

3. Opportunities for Innovation

- Interest in modeling baseline as an extension of blank sample.
- Potential for instrument-dependent modeling.

- No direct mention of AI, but challenges suggest room for AI-driven integration and adaptive algorithms.

4. Molecule Type & Assay Format Impact

a. Molecule Types

- Mention of LNP with no RNA as a blank.
- mRNA discussed in context of resolution standards and assay purpose.

b. Assay Formats

- CGE, iCIEF, CZE discussed extensively.
- Maurice CIEF uses baseline-to-baseline with vertical drop splitting.
- LabChip mentioned with moving peaks/shifting peak make overlay difficult

5. Approaches for Closely Spaced or Overlapping Peaks

- Overlay sequences to identify consistent patterns.
- Use pI markers to define start/stop bounds.
- Valley-to-valley and baseline-to-baseline methods discussed.
- Manual integration and example profiles used for shoulder peaks.

6. Ensuring Consistency Across Labs/Instruments

- Reference datasets and standard work suggested.
- New analysts should integrate sample data and compare results.
- Define pass/fail criteria and set parameters within methods.
- Integration events must align with method-defined parameters.