MERCK

Small Peak – BIG Problem!

Distinguishing between protein degradation and contamination peaks in capillary gel electrophoresis (CGE-SDS)



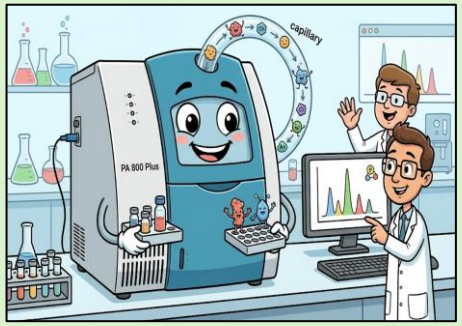
Presenter: Jeremy Osko

Large Molecule Analytical; Merck & Co., Inc., Rahway, NJ, USA

Proprietary

Presentation Agenda

CGE-SDS Overview



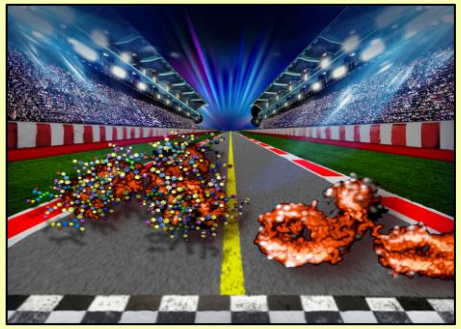
- ❑ Hallmark Technique
- ❑ Fundamentals of CGE-SDS
- ❑ Instrumentation Options

Industry Challenges



- ❑ Standard Operating Procedures (SOPs)
- ❑ Integrated Control Strategy
- ❑ Elusive Challenges

Case Study #1: Characterization of Protein-Related CGE-SDS Peaks



- ❑ Identification of New Peak
- ❑ Ruling Out Protein Degradation
- ❑ Enzymatic Digestion

Case Study #2: Exploring Contaminant Related CGE-SDS Peaks



- ❑ Uncharacterized New Peaks
- ❑ Contamination Investigation
- ❑ Implementation of Environmental Controls

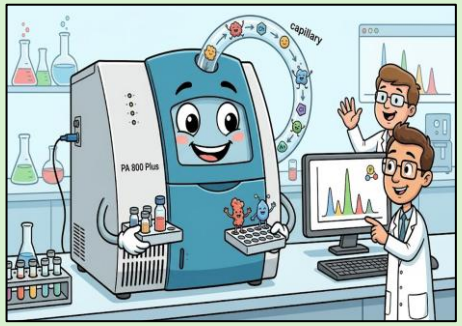
Limitations & Future Directions



- ❑ Mass Spectrometry Availability
- ❑ Sample Robustness
- ❑ Alignment Of Best Practices

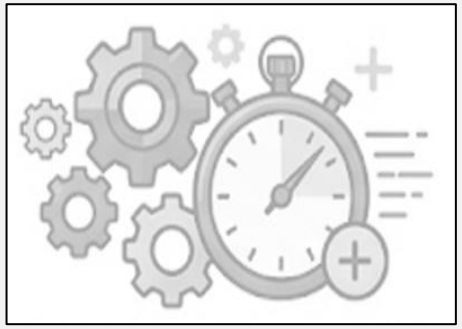
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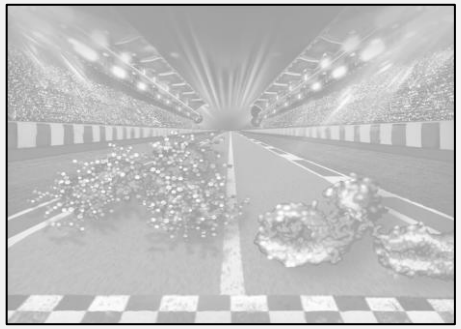
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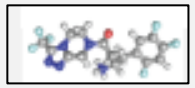


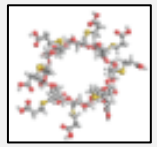
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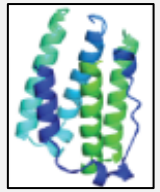
Proprietary

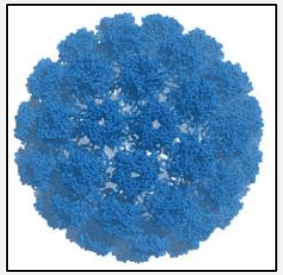
Critical Quality Attribute Fragmentation Is Monitored By CGE-SDS

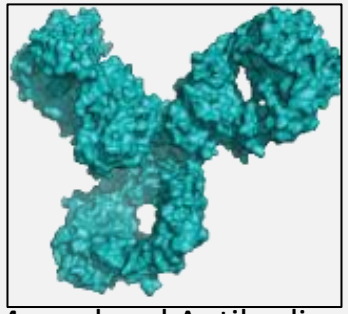
CGE-SDS Technique Must Remain Capable Of Quantifying Fragmentation Across Increasingly Divergent Pipeline

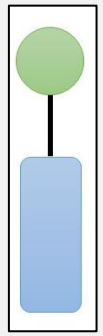

Small Molecules

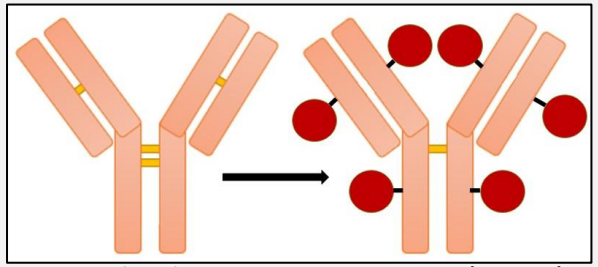

Peptides


Proteins


Viruses


Monoclonal Antibodies


Bioconjugates


Antibody Drug Conjugates (ADCs)



PA 800 Plus



IgG Purity Kit [Range: 10-225 kDa]

Table A-3 Sample Temperature Control Specifications

Range	20 °C below the ambient temperature to 60 °C (140 °F) Minimum value: 4 °C (39 °F)
Stability	±1 °C at 25 °C (77 °F) ±3 °C at 4 °C (39 °F) and 60 °C (140 °F)
Accuracy	±2 °C within a range of ±15 °C from the ambient temperature ±3 °C outside a range of ±15 °C from the ambient temperature

Table A-4 Capillary Temperature Control Specifications

Range	10 °C below the ambient temperature to 60 °C (140 °F) Minimum setting: 15 °C (59 °F)
Stability	±1 °C at 25 °C (77 °F)
Accuracy	±1 °C within a range of ±1 °C from the ambient temperature ±2 °C outside a range of ±5 °C from the ambient temperature

Table A-8 PDA Detector Specifications (continued)

Scan collection frequency	0.5 Hz to 32 Hz
UV source	30 W pre-aligned deuterium lamp
UV source lifetime	1,000 hours
Wavelength accuracy	2 nm
Wavelength range	190 nm to 600 nm

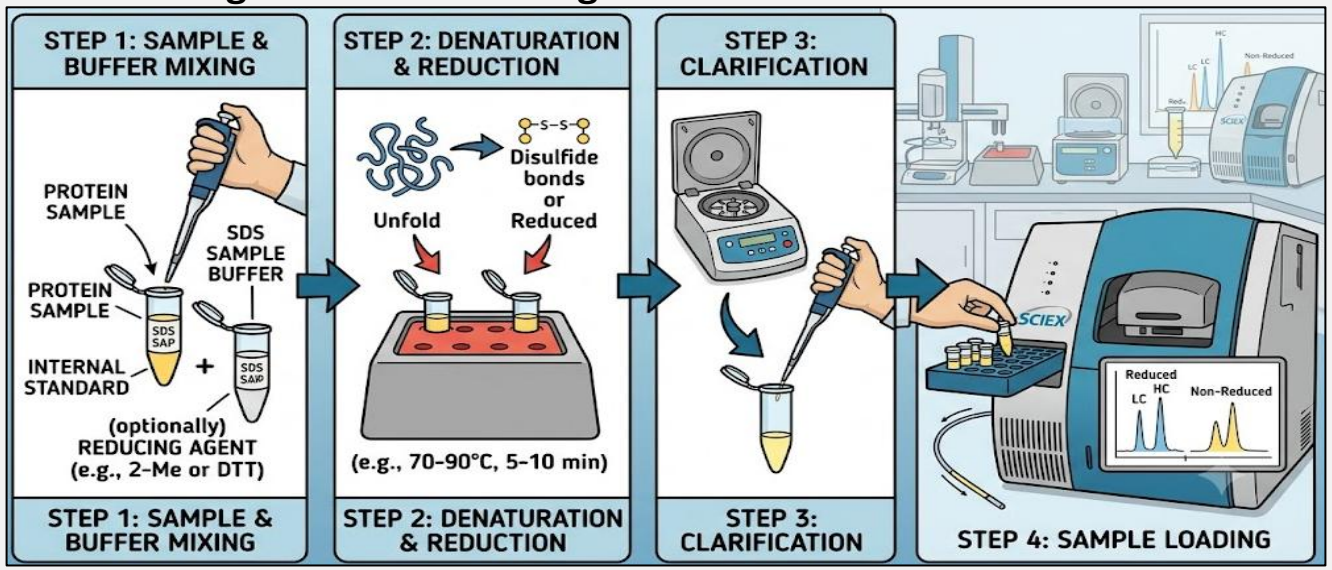
https://sciex.com/content/dam/SCIEX/pdf/customer-docs/user-guide/pa-800-plus-system-overview-guide.pdf

Proprietary

Protein Separation Highly Dependent On Sample Preparation

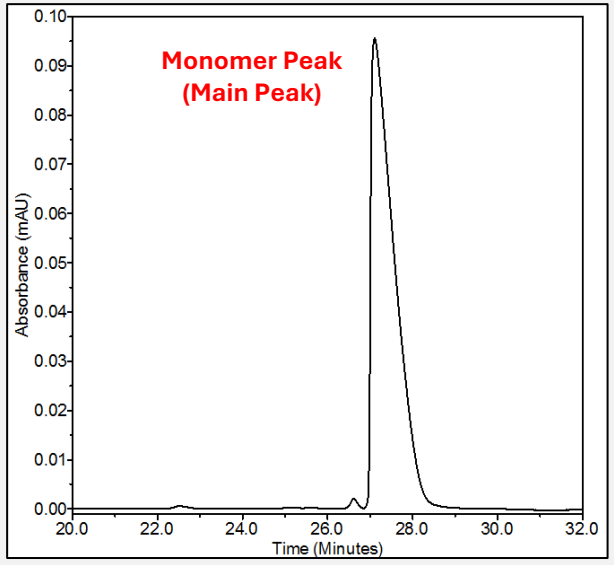
Protein Susceptibility To Efficient SDS Binding Drives Relative Migration Times

Alkylation Reagents
NEM vs IAM
&
SDS Buffer



Reducing Reagents
BME vs DTT vs TCEP
&
Sample Reducing Buffer

NR CGE-SDS Example

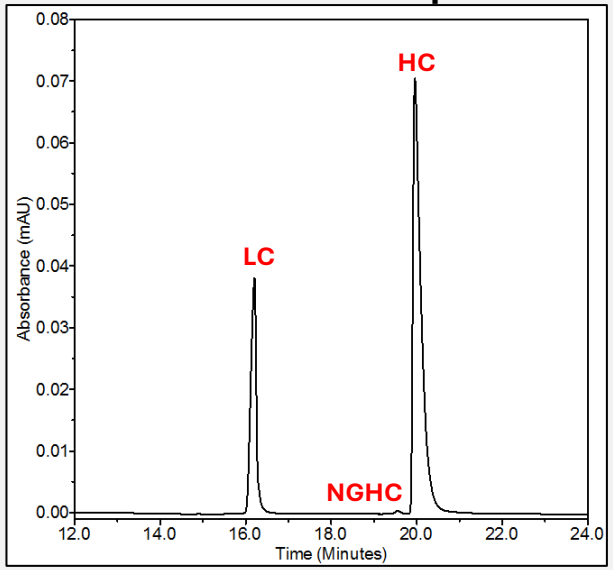








R CGE-SDS Example



Sample preparation image generated through AI

CGE-SDS Application & Challenges Dependent On Instrument Selection

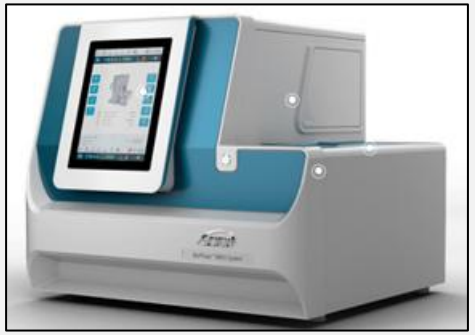
Large diversity of analytical instrumentation available for CGE-SDS workflows, each with their own proprietary reagents and detection mechanisms



PA 800 Plus













https://sciex.com/products/capillary-electrophoresis/pa-800-plus-pharmaceutical-analysis-system

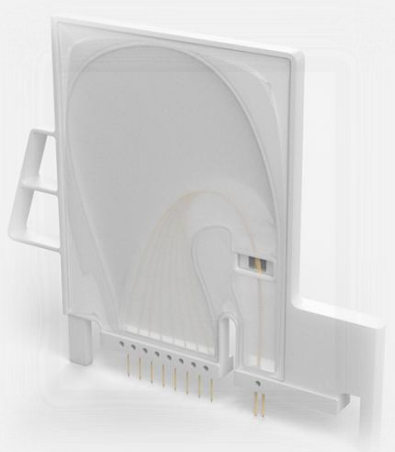
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BIOPHASE 8800













https://sciex.com/products/capillary-electrophoresis/biophase-8800

CGE-SDS Application & Challenges Dependent On Instrument Selection

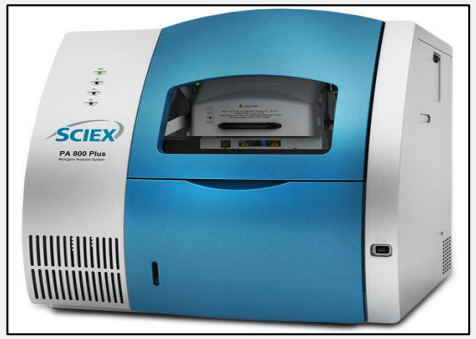
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Maurice









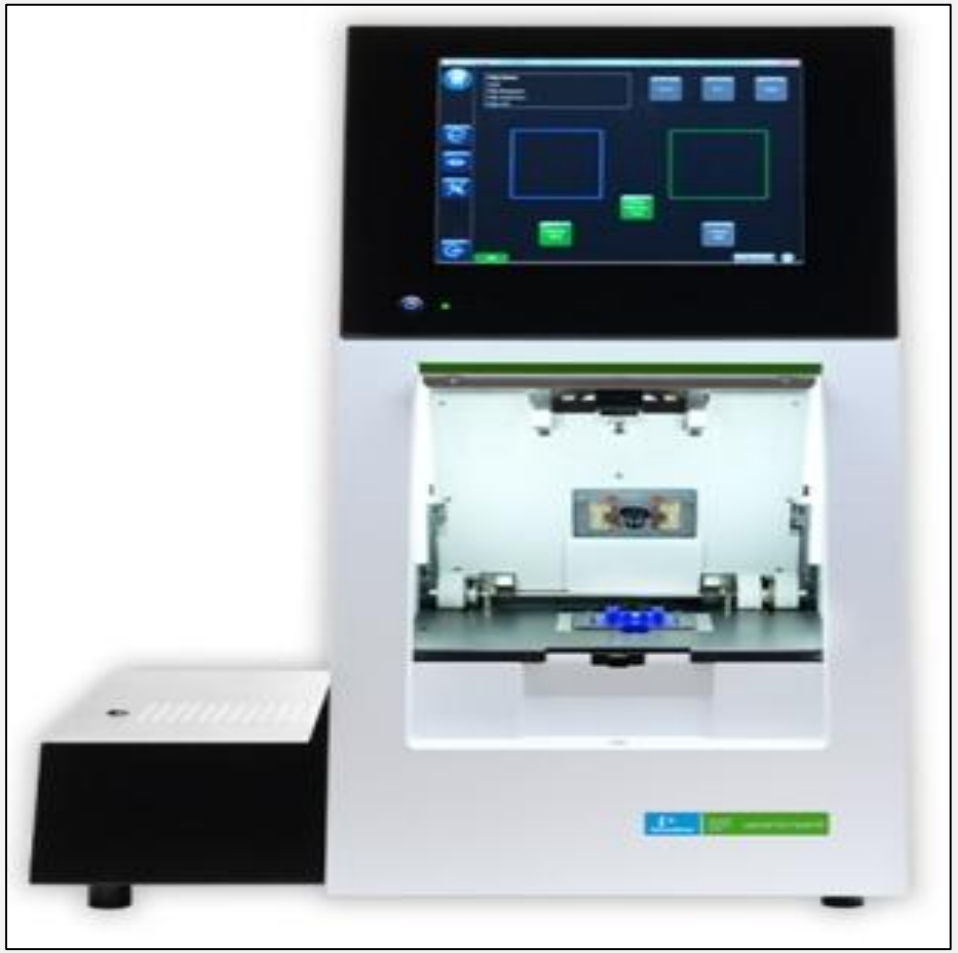




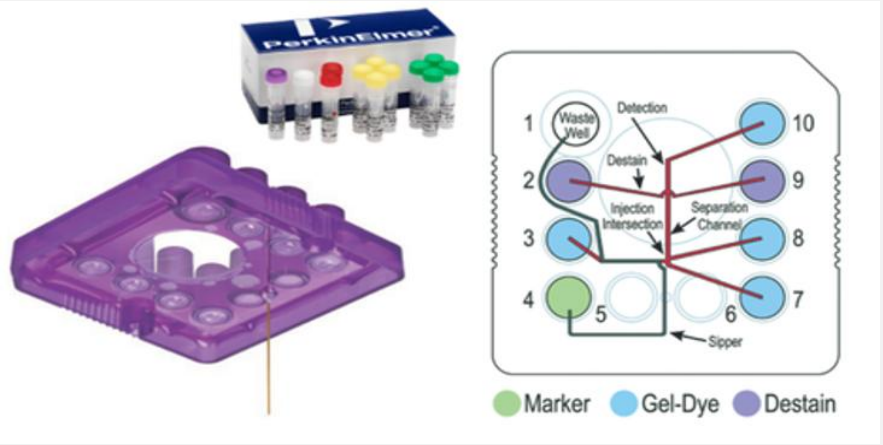
https://www.rndsystems.com/instruments/capillary-electrophoresis-instruments/models/maurice

CGE-SDS Application & Challenges Dependent On Instrument Selection

Large diversity of analytical instrumentation available for CGE-SDS workflows, each with their own proprietary reagents and detection mechanisms



LabChip









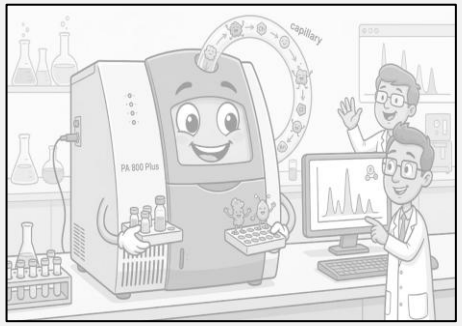


https://www.revvity.com/product/labchip-gx-touch-24-cls138162

Proprietary

Presentation Agenda

CGE-SDS Overview



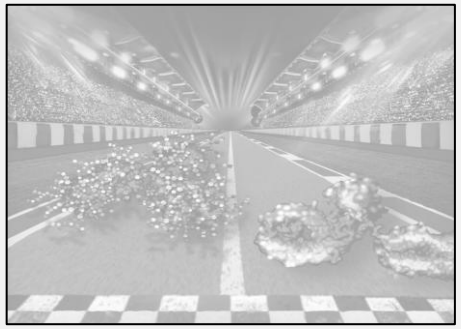
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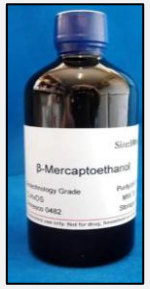


- Mass Spectrometry Availability
- Sample Robustness
- Alignment Of Best Practices

Standard Operating Procedures & Best Practices Integrated Into Control Strategies

USP General Chapter 126 Utilizes Sciex IgG Heterogeneity Assay Kit & ICH Q2 (R2) Informs On Validation Of Analytical Procedures

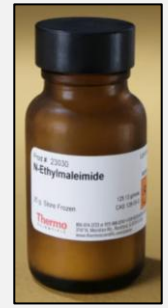
BME SDS





Use personal protective equipment as required. Ensure adequate ventilation. Keep people away from and upwind of spill/leak. Evacuate personnel to safe areas. Remove all sources of ignition. Take precautionary measures against static discharges.

NEM SDS





Use personal protective equipment as required. Evacuate personnel to safe areas. Ensure adequate ventilation. Keep people away from and upwind of spill/leak. Avoid dust formation. Remove all sources of ignition. Take precautionary measures against static discharges.

Best Practices & Company Specific SOPs May Open The Door For Ambiguity

Examples:

- ❑ Sample Concentration: “Dilute samples to a concentration of 10 mg/mL”
  - ❑ What if samples are < 10 mg/mL?
  - ❑ Dilute in water, formulation buffer, Sample reducing buffer, Low pH sample reducing buffer, or SDS?
- ❑ Sample Mixing: “Thoroughly mix samples” or “Briefly vortex samples”
  - ❑ Is there a time period that is considered “thorough”?
  - ❑ Is there a speed setting on the vortexer?
  - ❑ What if sample is inverted or using electronic pipets (volume mixing ratio, speed of mix, # of cycles)?
- ❑ Solution Stability: “Solution stable in CE-SDS matrix for up to 48 hours”
  - ❑ Has solution stability been verified for your specific asset?



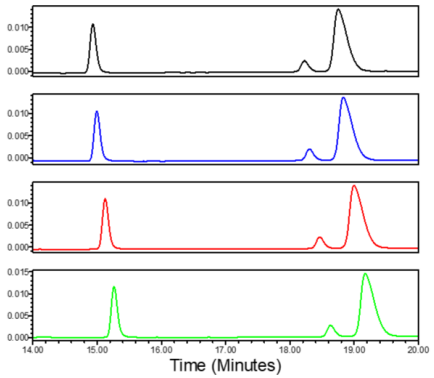
https://database.ich.org/sites/default/files/ICH_Q2%28R2%29_Guideline_2023_1130.pdf
https://www.usp.org/events-training/course/usp-standards-support-characterization-mabs-applications-and-general-chapter

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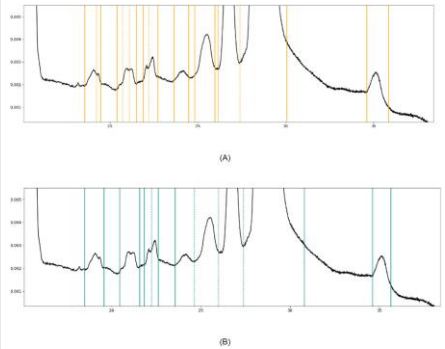
Avoiding The Unavoidable – Method Challenges Still Persist In Regulatory Filings

CGE-SDS Challenges Continue To Be Present Despite SOPS, Best Practices, And Larger Collaborative Efforts

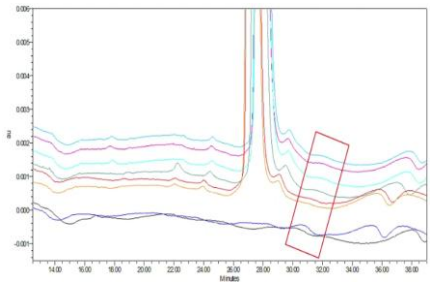
Baseline Drift



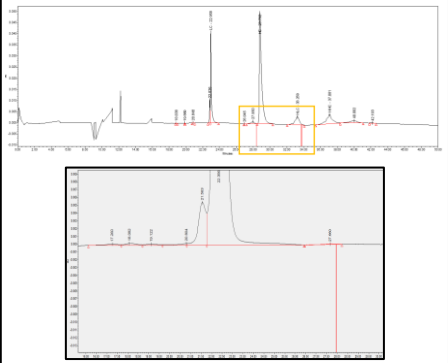
Integration Practices



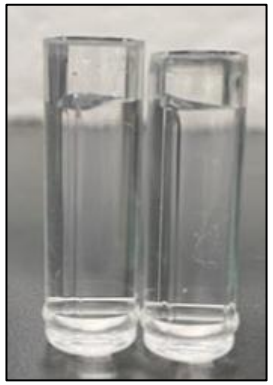
Baseline Hump Elevation



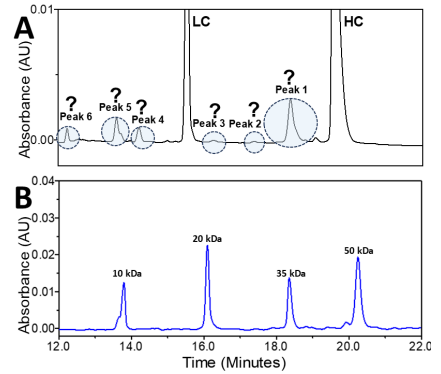
Empower Integration I17 Error



Manufacturing Defects



Additional Peaks

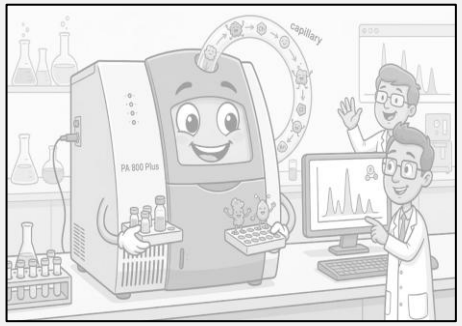


https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/elps.70077

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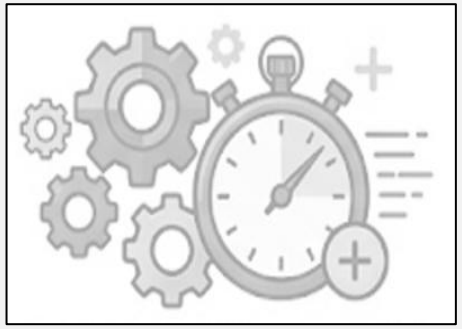
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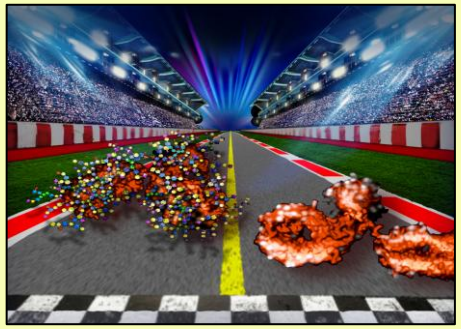
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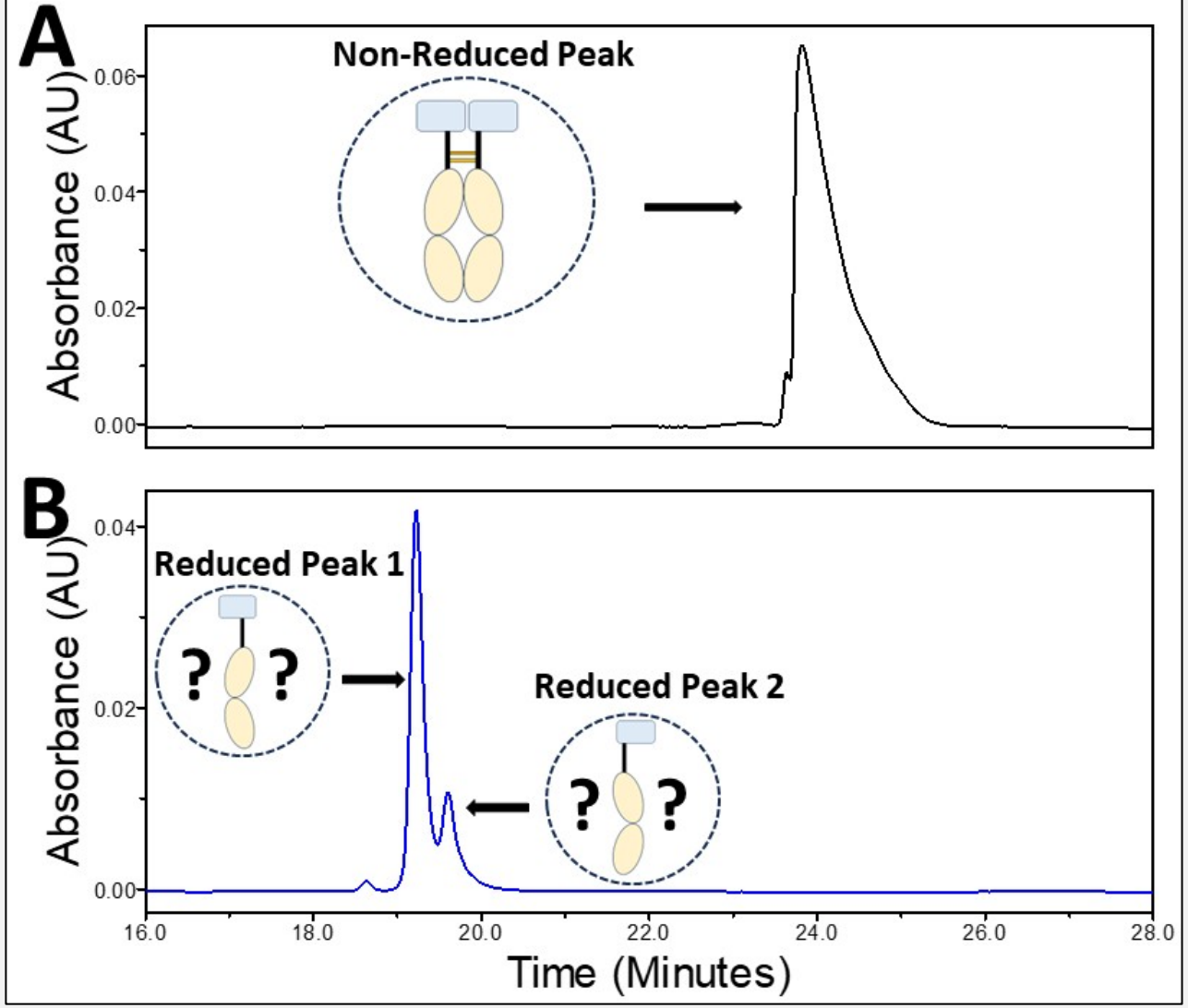


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- Sample Robustness
- Alignment Of Best Practices

Proprietary

Case Study #1: Characterizing Protein Peaks In CGE-SDS Under Reducing Conditions

Expecting 1 Peak, But Getting 2 – Ruling Out The Obvious



Make No Assumptions

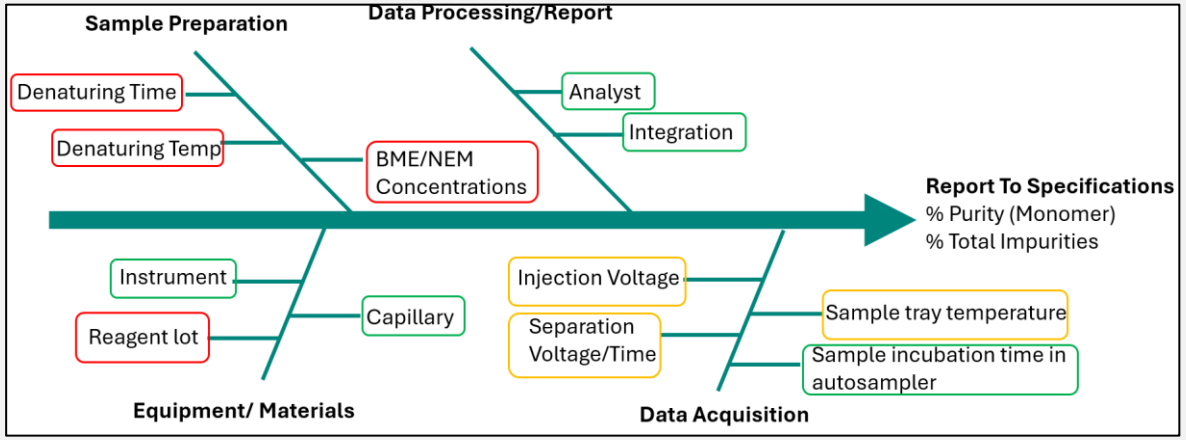








Review Fishbone Method Parameter Diagram



Investigate Typical Suspects

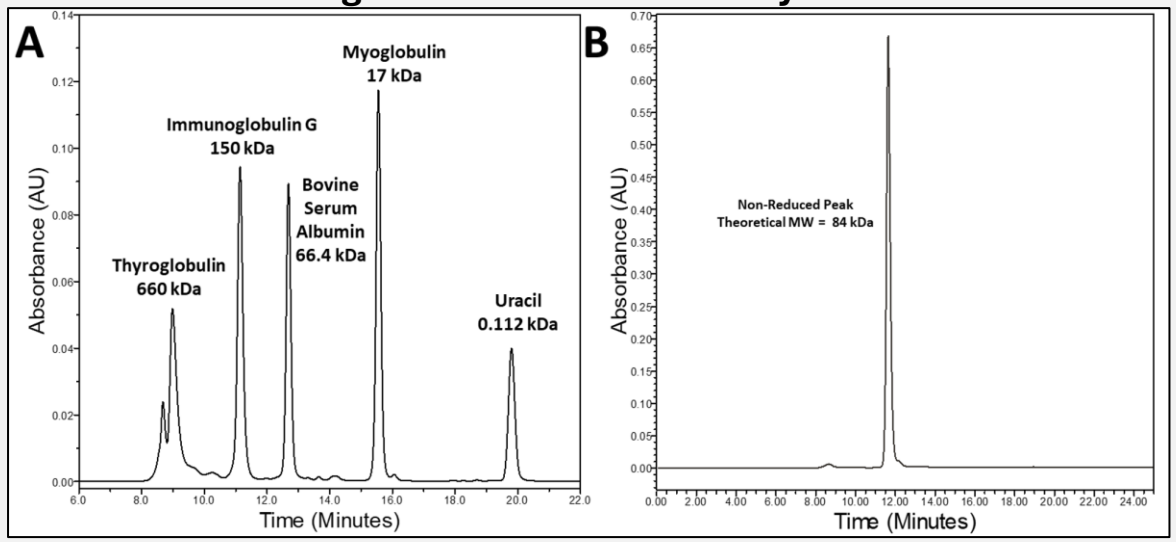
- ❑ NOT Result Of Robustness Concerns
- ❑ NOT Result Of Forced Degradation Pathways
- ❑ Impurities NOT Present On Orthogonal Analytical Methods
- ❑ MS Capabilities Unavailable During Time Of Observation

https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/elps.70006

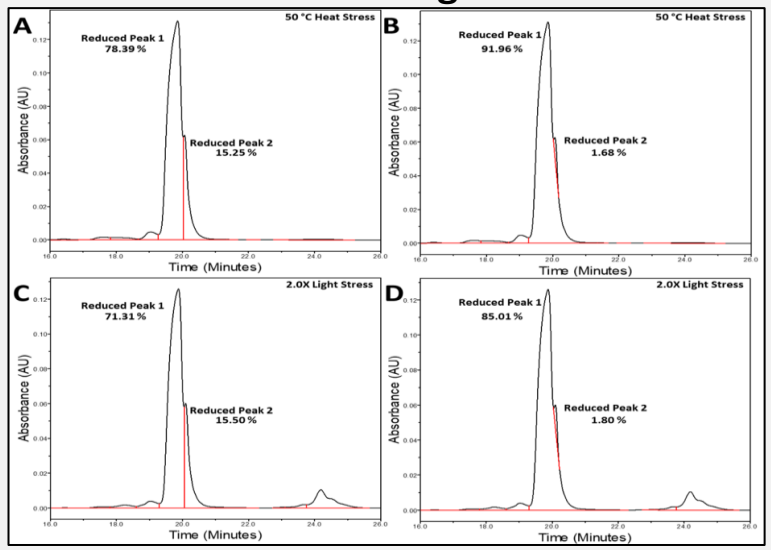
Proprietary

Case Study #1: Characterizing Protein Peaks In CGE-SDS Under Reducing Conditions

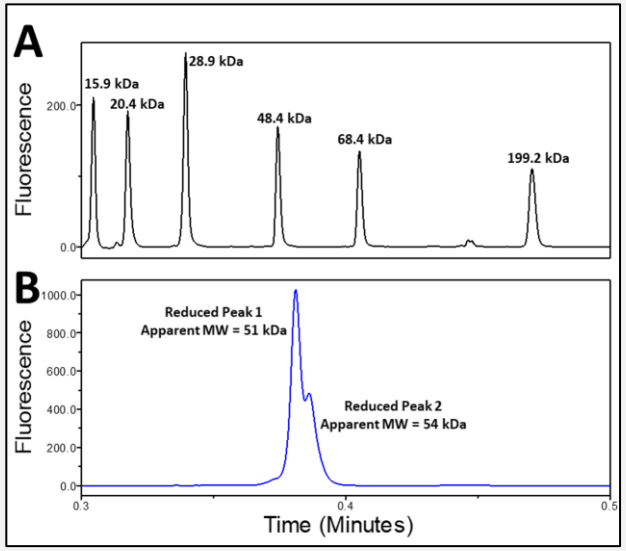
One Single Peak In UP-SEC Purity Assessment



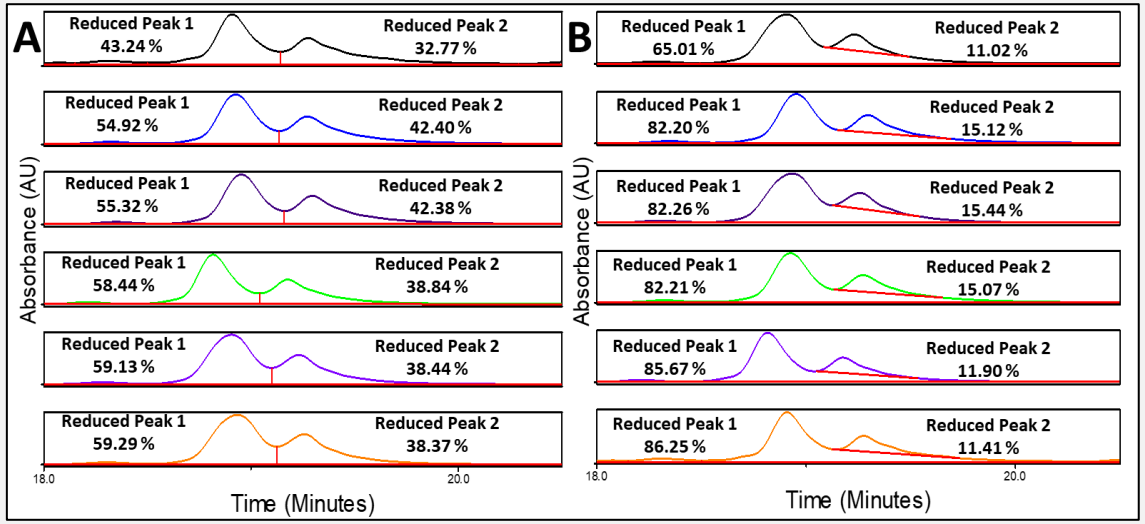
Peaks Present In Forced Degradation Profiles



Reproducible on R CGE-SDS LabChip Experiments



Peaks Present Throughout Purification Scheme

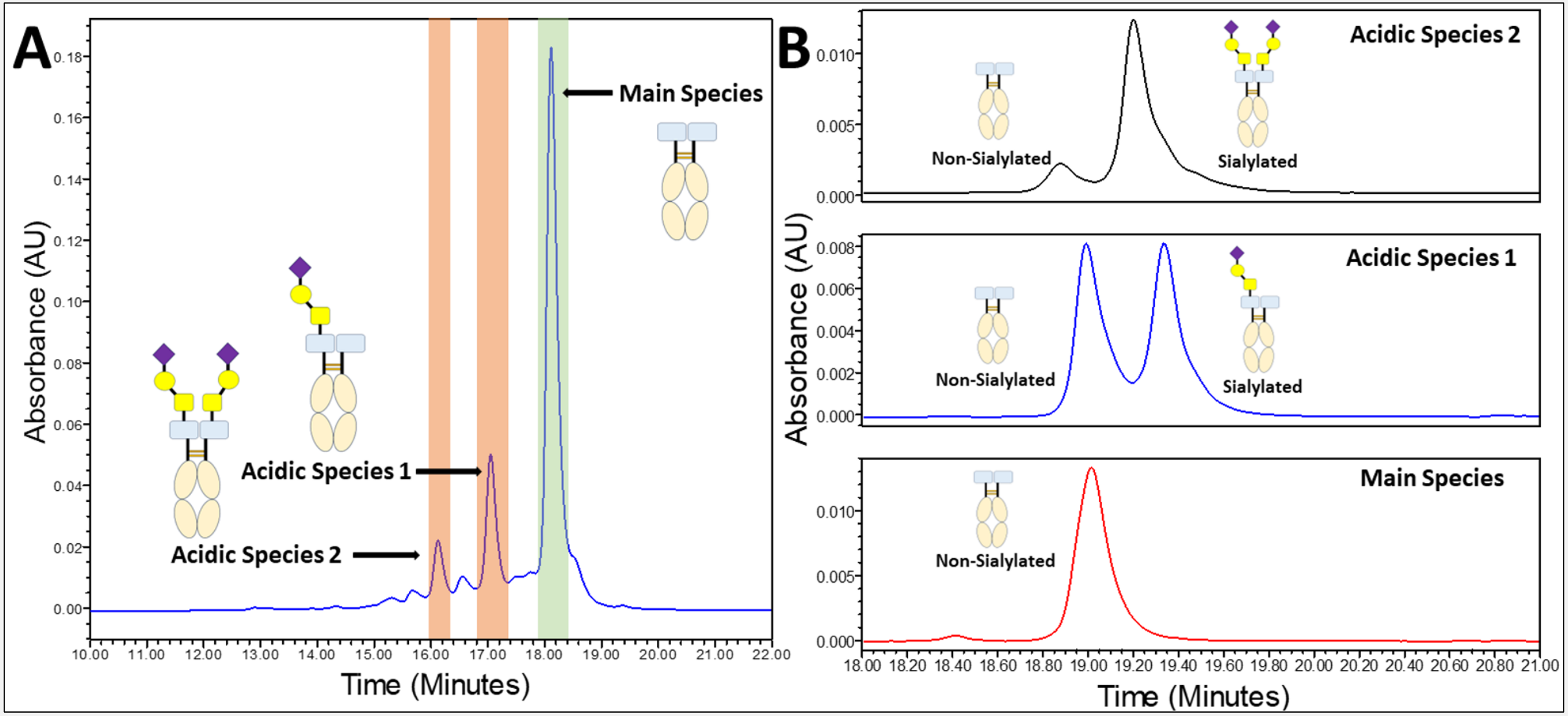


https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/elps.70006

Proprietary

Case Study #1: Characterizing Protein Peaks In CGE-SDS Under Reducing Conditions

Isolated Characterization Of iCIEF Charged Variants Indicated O-Linked Sialylated As Potential Root Cause

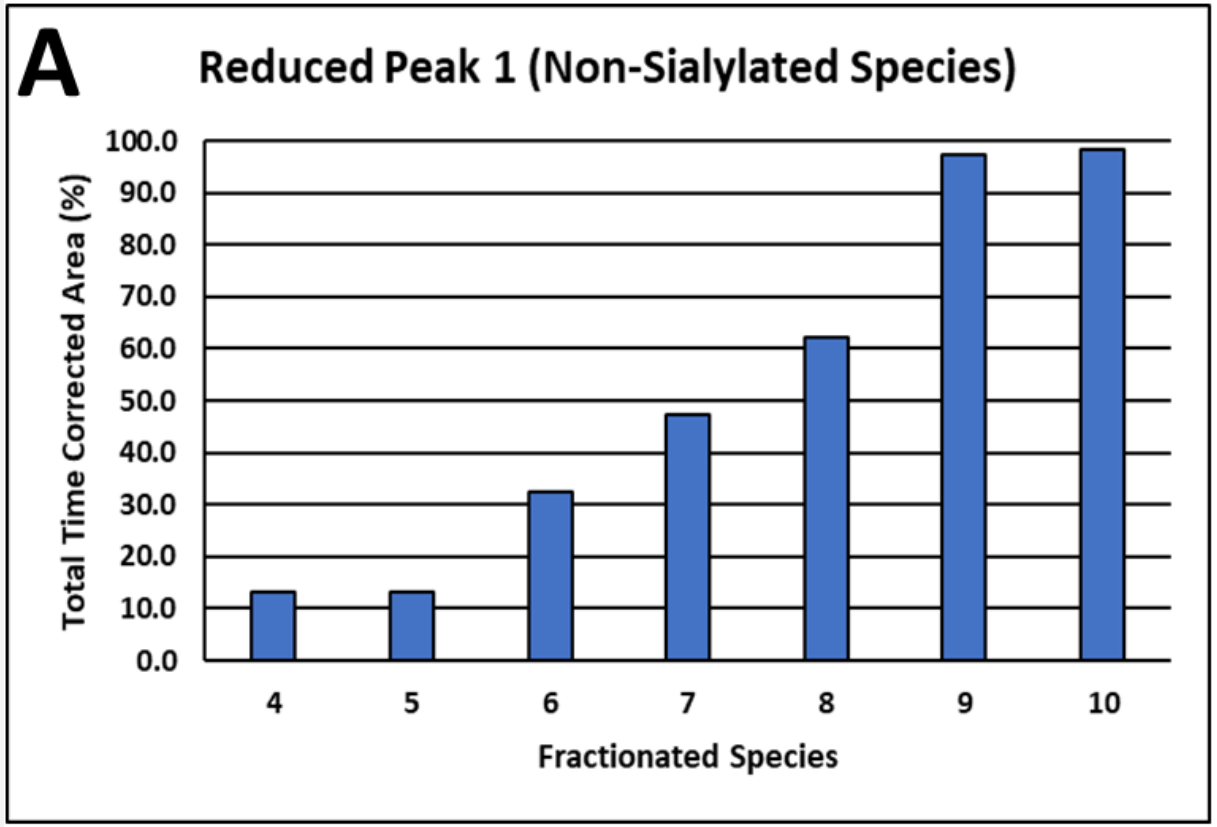


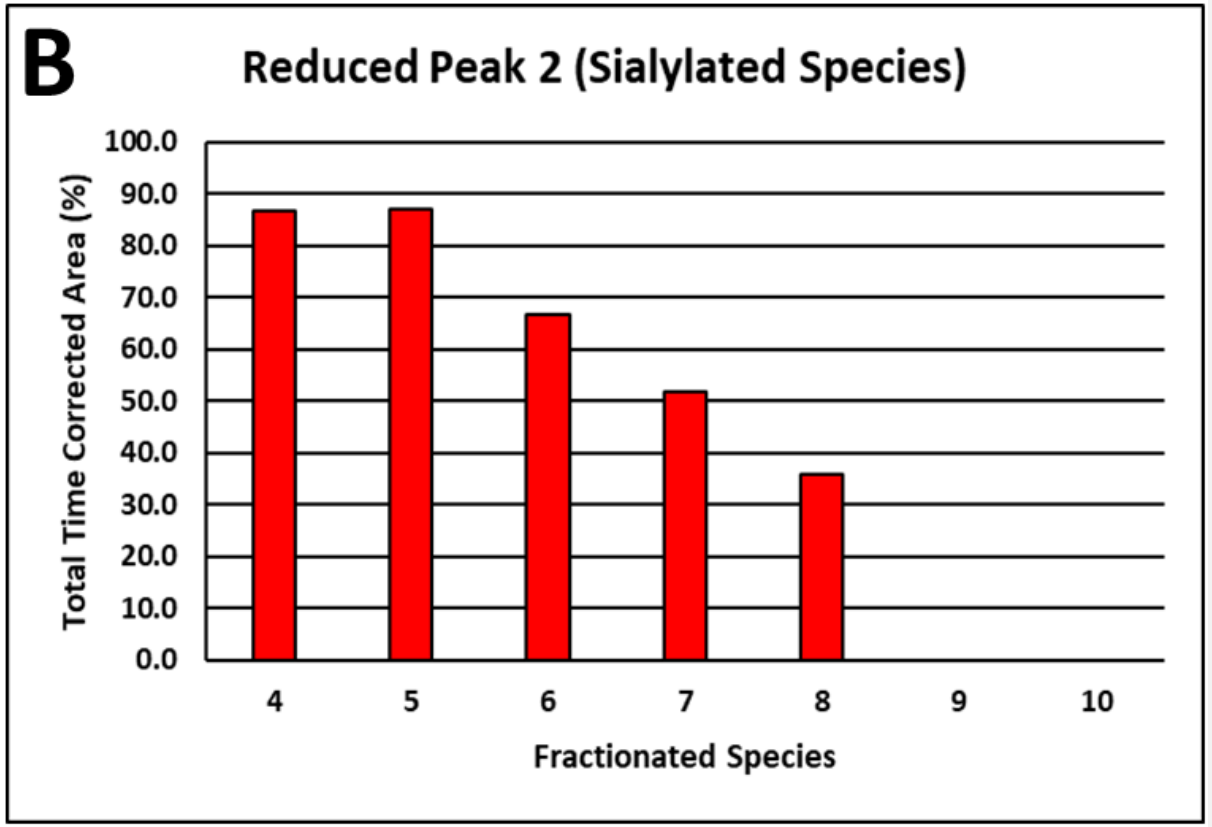
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Case Study #1: Characterizing Protein Peaks In CGE-SDS Under Reducing Conditions

Re-Injection Of iCIEF Sialylated Charge Variant Fragments Into CGE-SDS Directly Correlated With Reduced Peaks 1 & 2



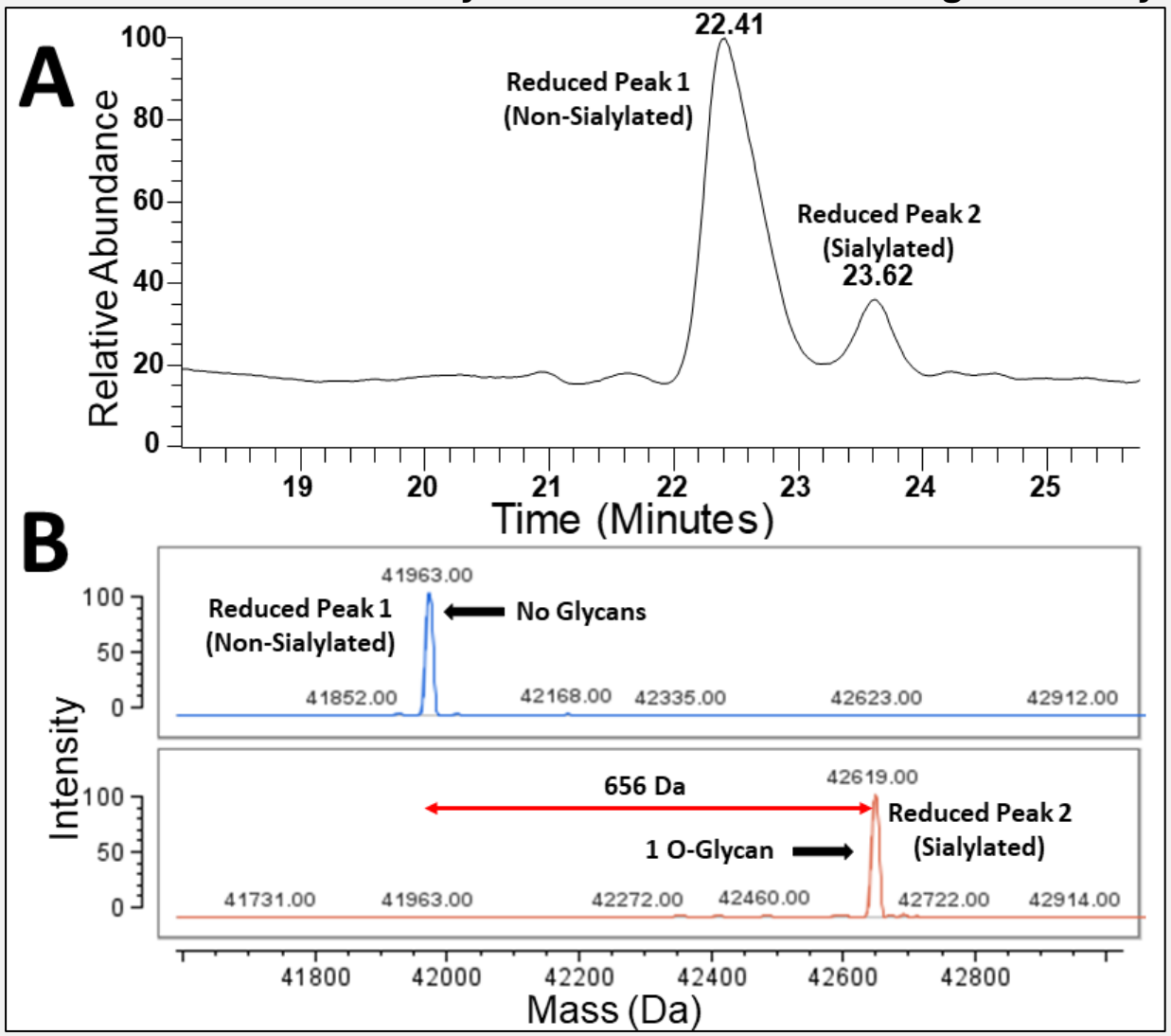


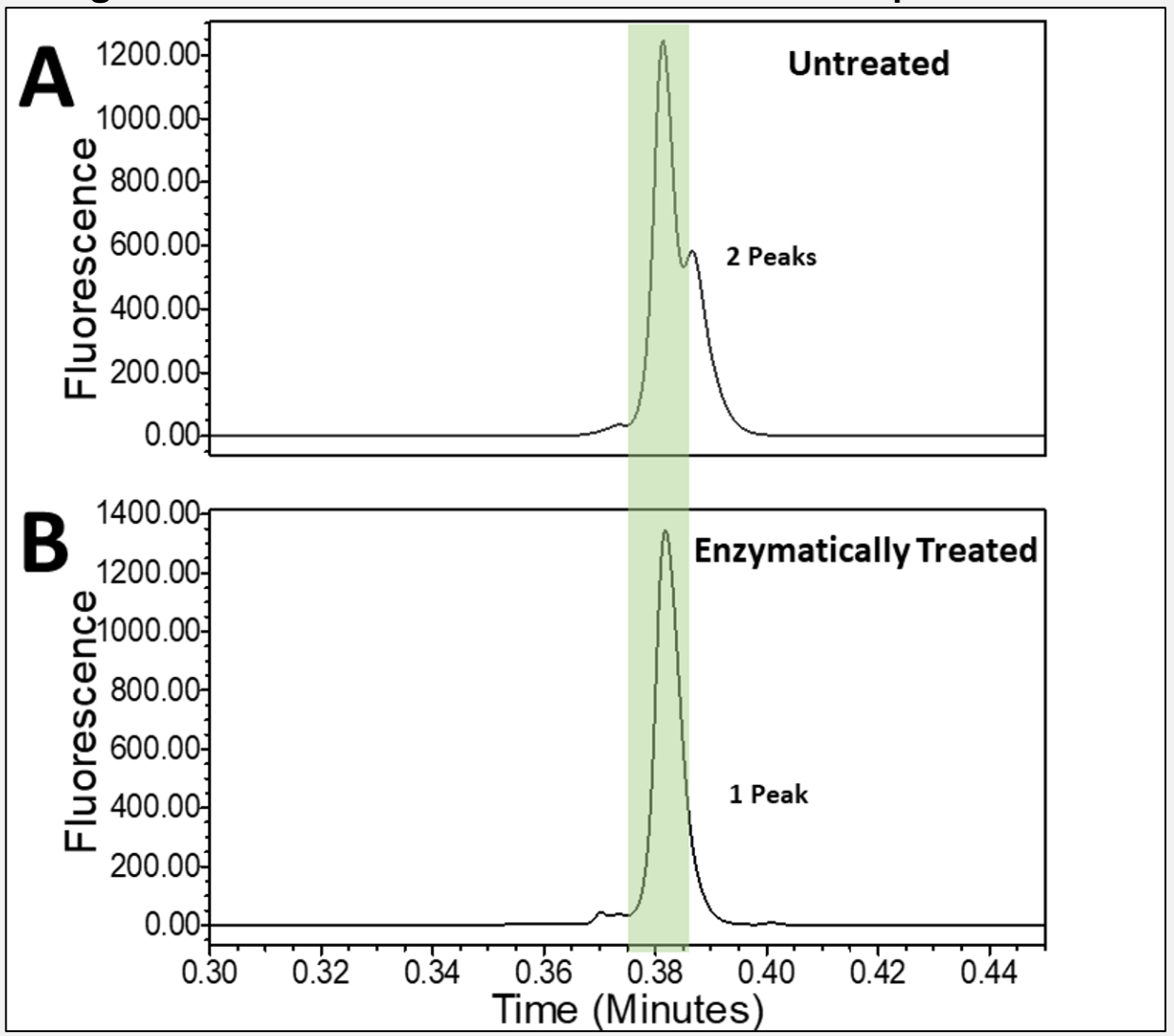
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Case Study #1: Characterizing Protein Peaks In CGE-SDS Under Reducing Conditions

HILIC-MS Was Eventually Able To Confirm The Findings With Enzymatic Digestion As A Potential Resolution For Future Implementation





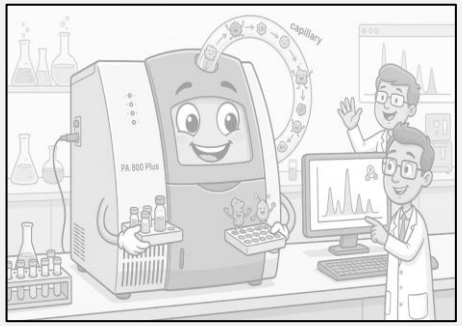
One Single PTM Is Enough To Drastically Change Electropherogram & Impact Purity Assessment

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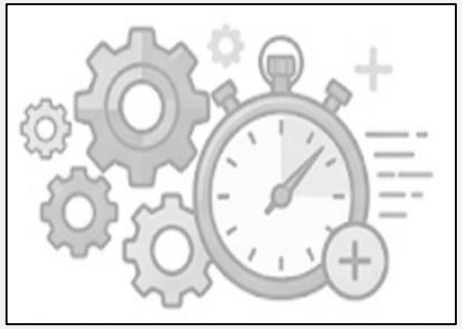
Presentation Agenda

CGE-SDS Overview



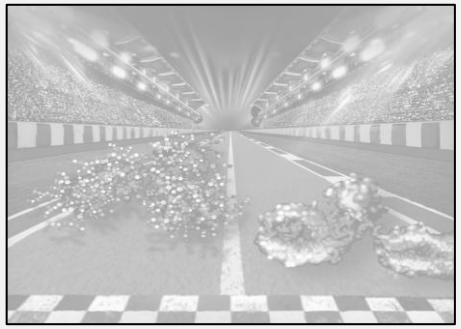
- Hallmark Technique
- Fundamentals of CGE-SDS
- Instrumentation Options

Industry Challenges



- Standard Operating Procedures (SOPs)
- Integrated Control Strategy
- Elusive Challenges

Case Study #1: Characterization of Protein-Related CGE-SDS Peaks



- Identification of New Peak
- Ruling Out Protein Degradation
- Enzymatic Digestion

Case Study #2: Exploring Contaminant Related CGE-SDS Peaks



- Uncharacterized New Peaks
- Contamination Investigation
- Implementation of Environmental Controls

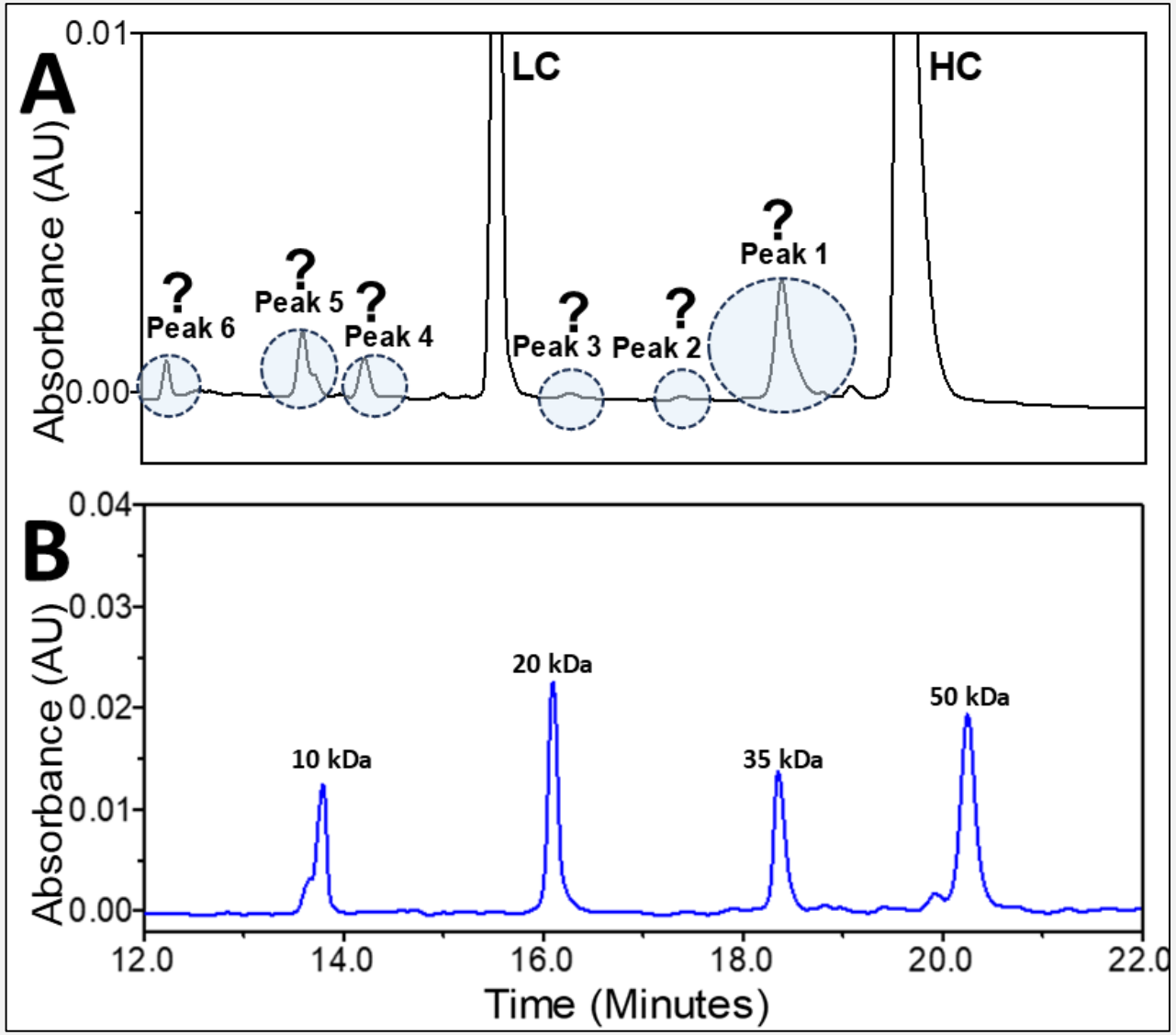
Limitations & Future Directions



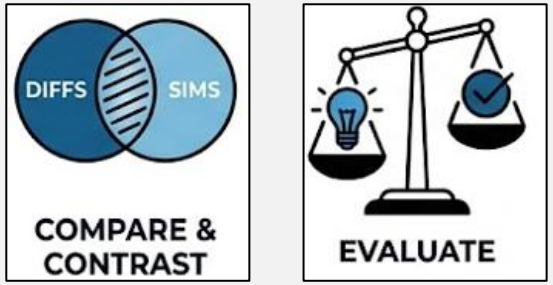
- Mass Spectrometry Availability
- Sample Robustness
- Alignment Of Best Practices

Case Study #2: Exploring Contamination Peaks In CGE-SDS Under Reducing Conditions

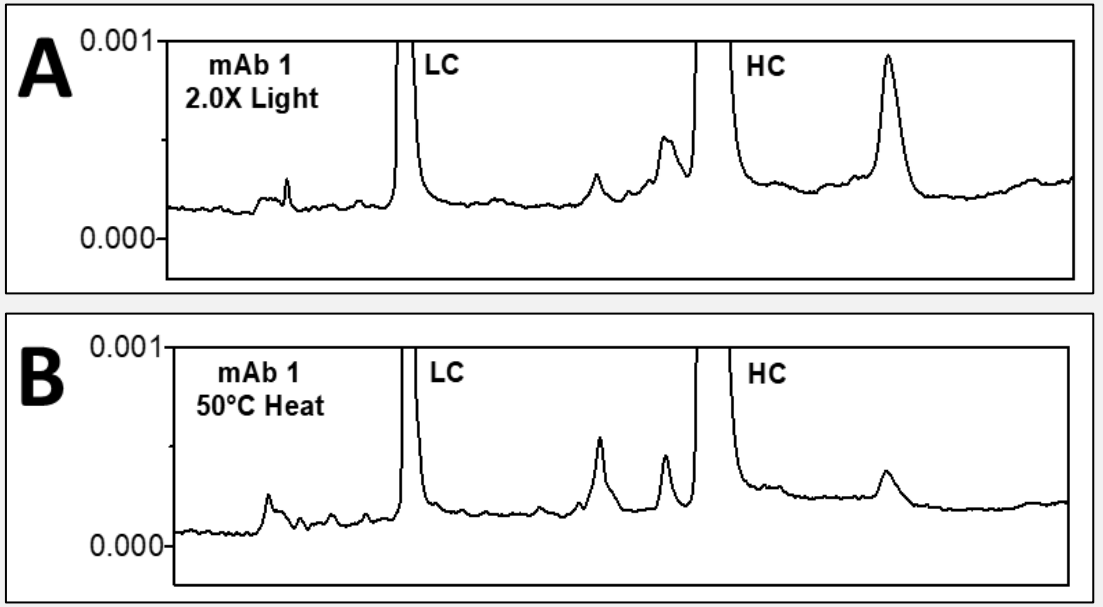
Expecting 2 Peaks, Getting 6 More! – Enough To Make Or Break A Specification



Repeating Assessments From Case Study #1



No Correlation In Light Or Heat Stressed Samples



Investigate Data Trending

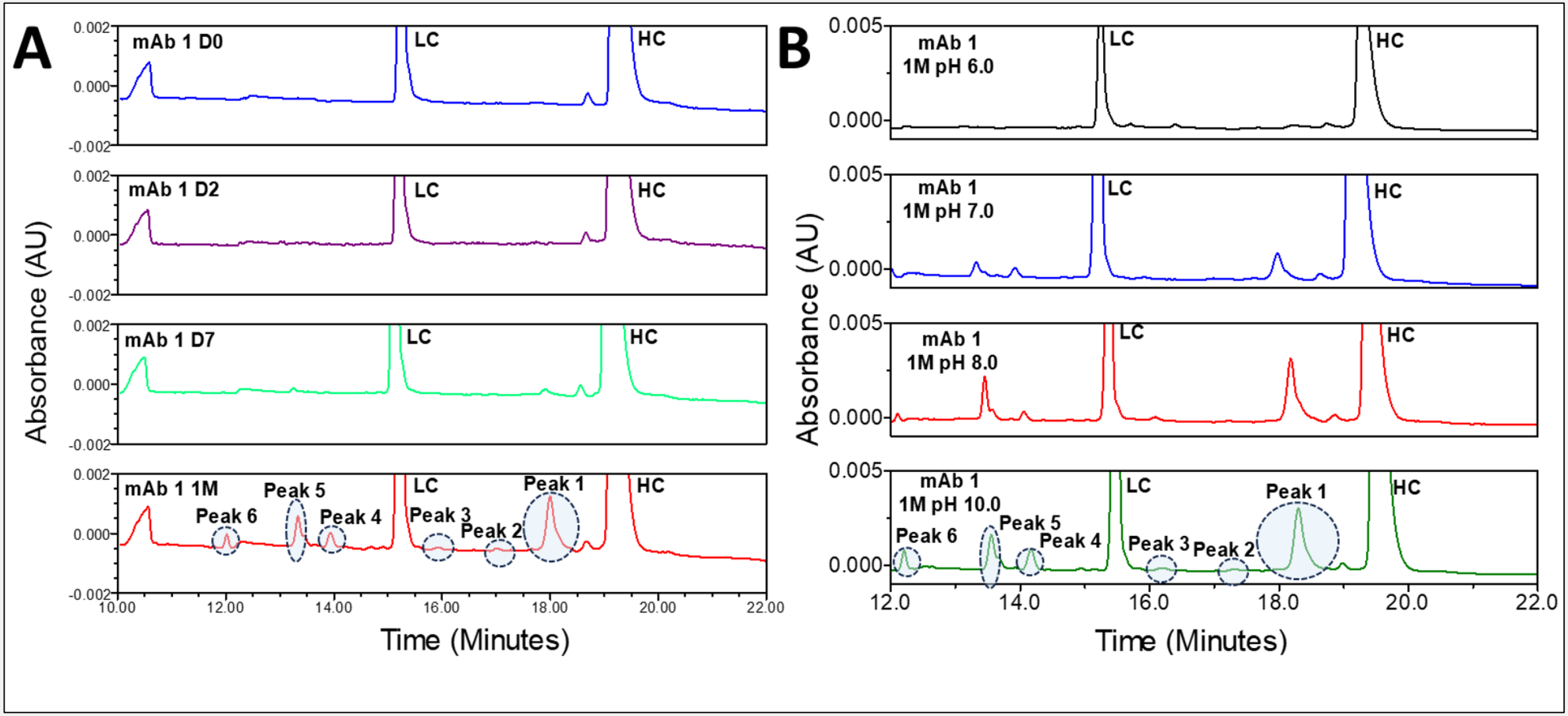
- ❑ Low Occurrence Rate: ~1 in 200 injections
- ❑ Higher frequency in longer datasets and at end of datasets

Unexpected Peaks In Reduced Capillary Gel Electrophoresis Associated With Hair And Dust Contamination. Manuscript Submitted And In Review.

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Case Study #2: Exploring Contamination Peaks In CGE-SDS Under Reducing Conditions

Time Dependence & pH Dependence On Peak Formation! – BUT Not Within Assay Testing Window Of 48 Hours…

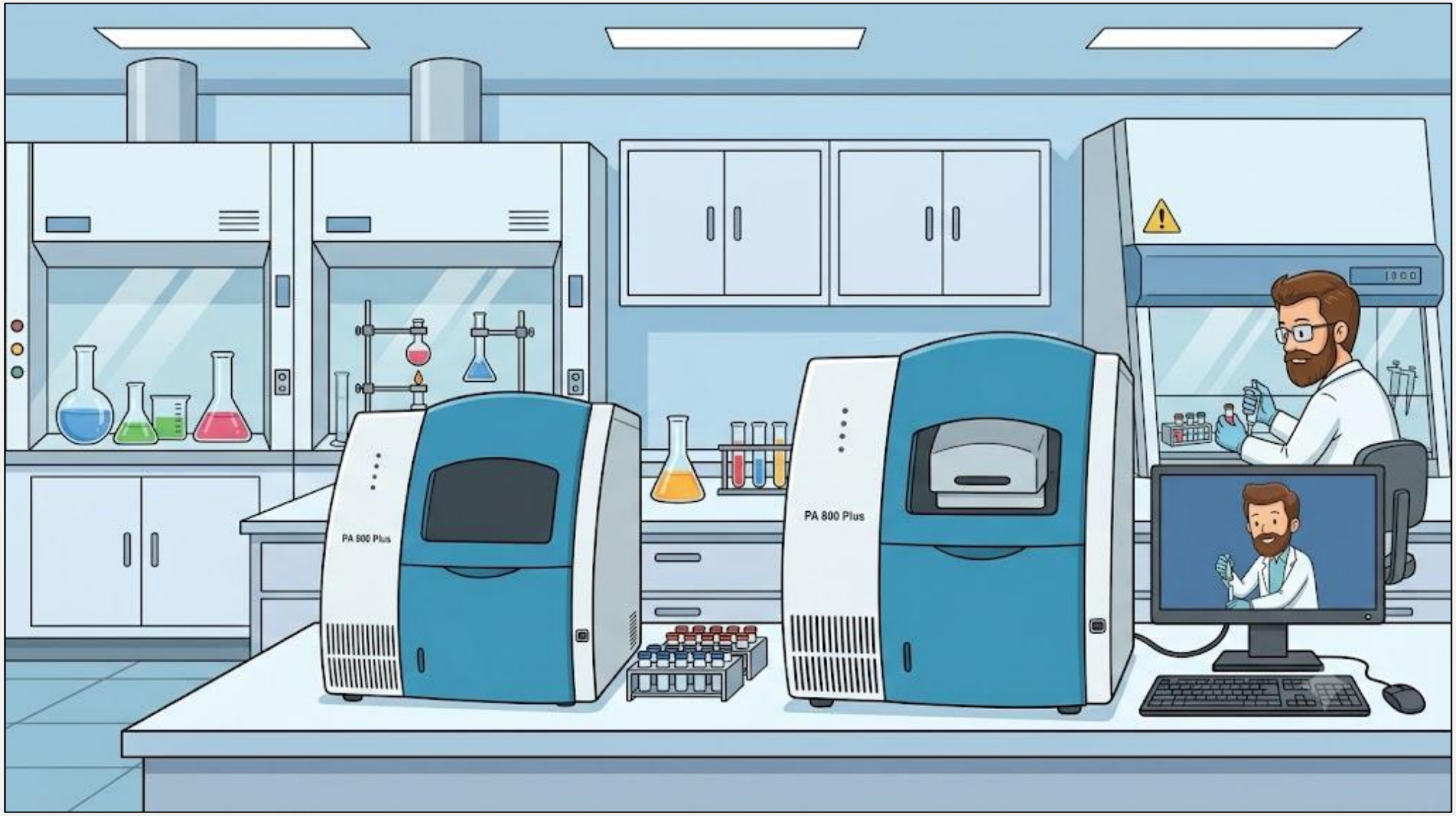


SDS MW Sample Buffer: 100 mM Tris-HCl @ pH 9.0 with 1% SDS. Low pH Sample Buffer: 100 mM Tris-HCl @ pH 6.8 with 1% SDS.

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Case Study #2: Exploring Contamination Peaks In CGE-SDS Under Reducing Conditions

Activity – Let's Review A Traditional Lab Setup



Laboratory image generated through AI

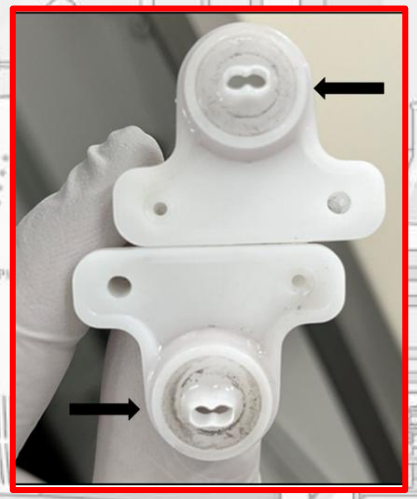
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Case Study #2: Exploring Contamination Peaks In CGE-SDS Under Reducing Conditions

Contaminants Could Be Everywhere! Especially In Area's Not Explicitly Mentioned In Analytical Method Or SOPs



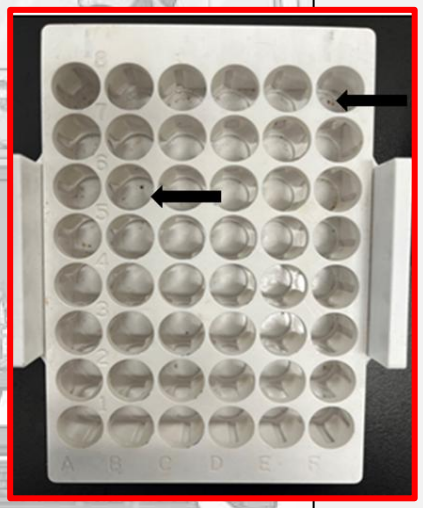
Exposed Vial Caps



O-Ring Dust



System Interior Cleanliness



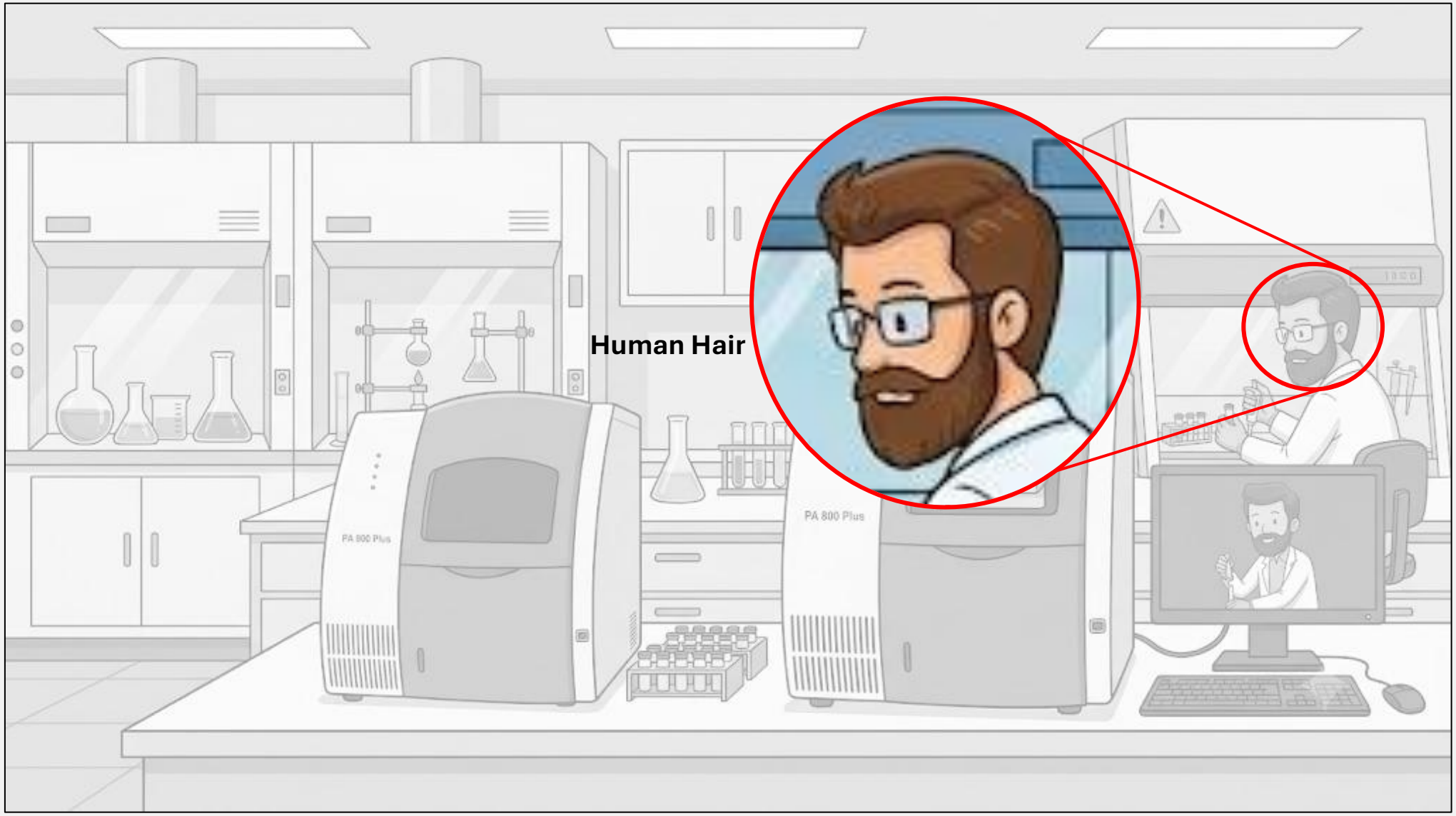
Tray Debris

Laboratory image generated through AI

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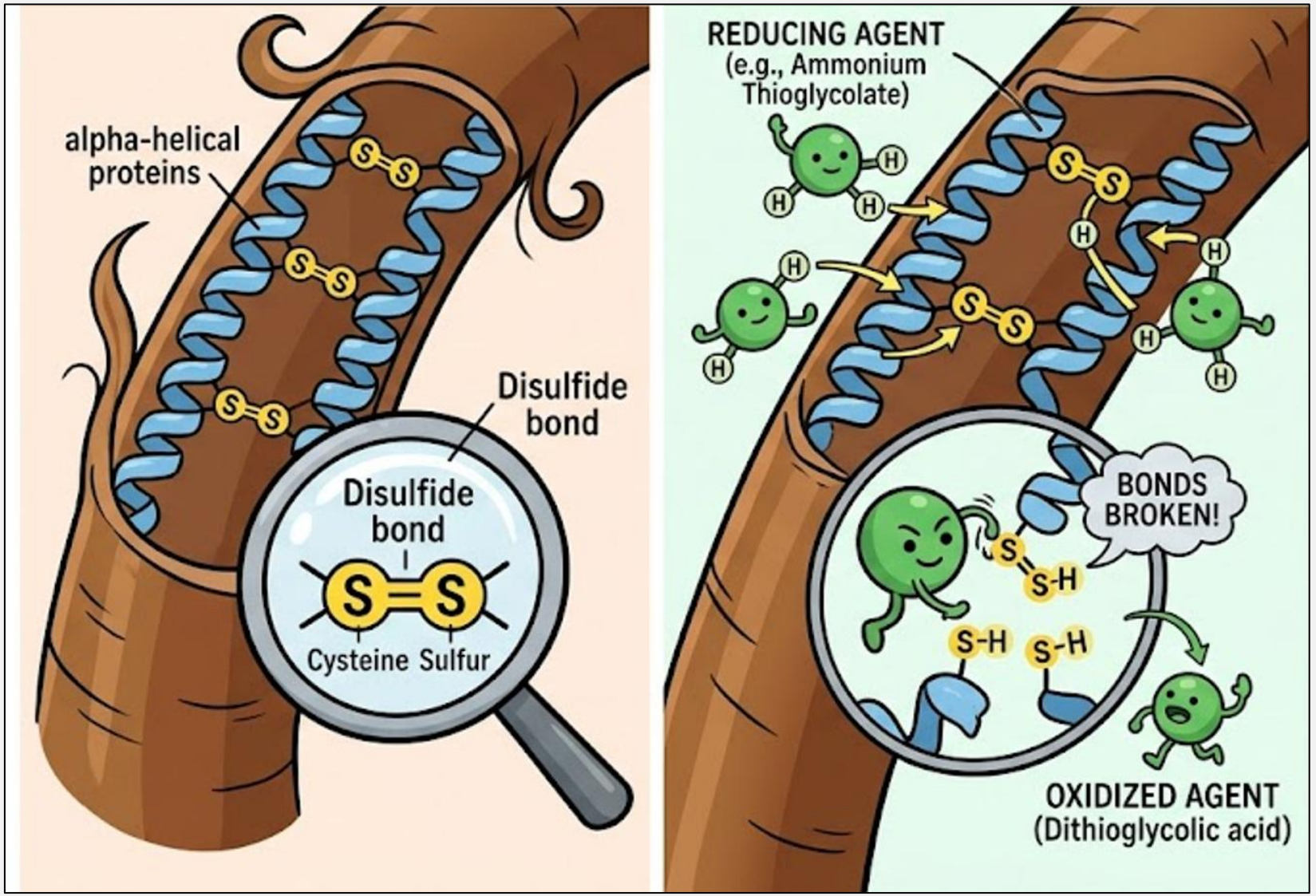


Laboratory image generated through AI

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Case Study #2: Exploring Contamination Peaks In CGE-SDS Under Reducing Conditions

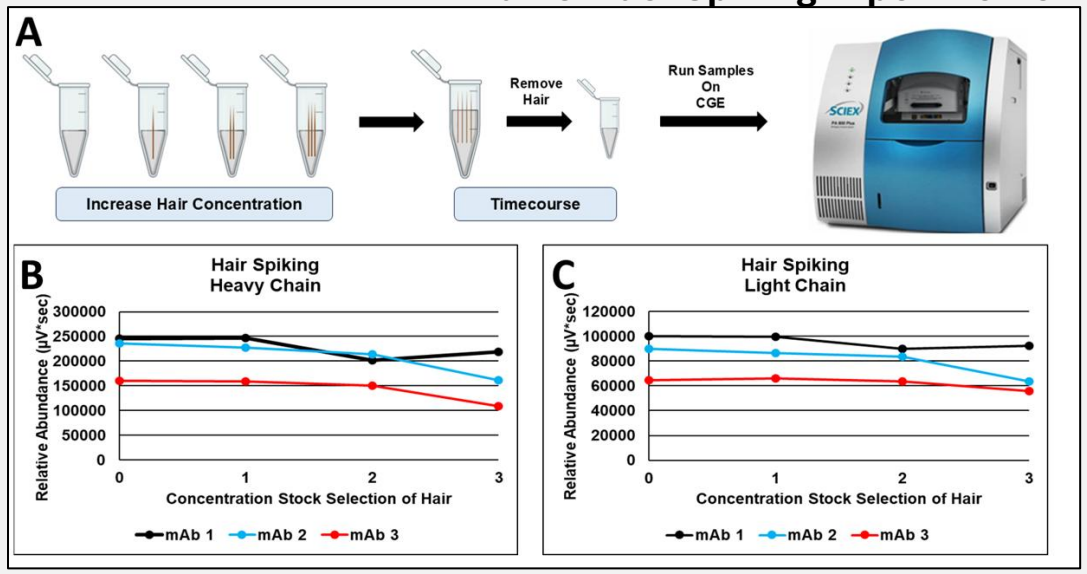
Hair Contains Keratin Which Can Reduce To MWs Similar to mAbs

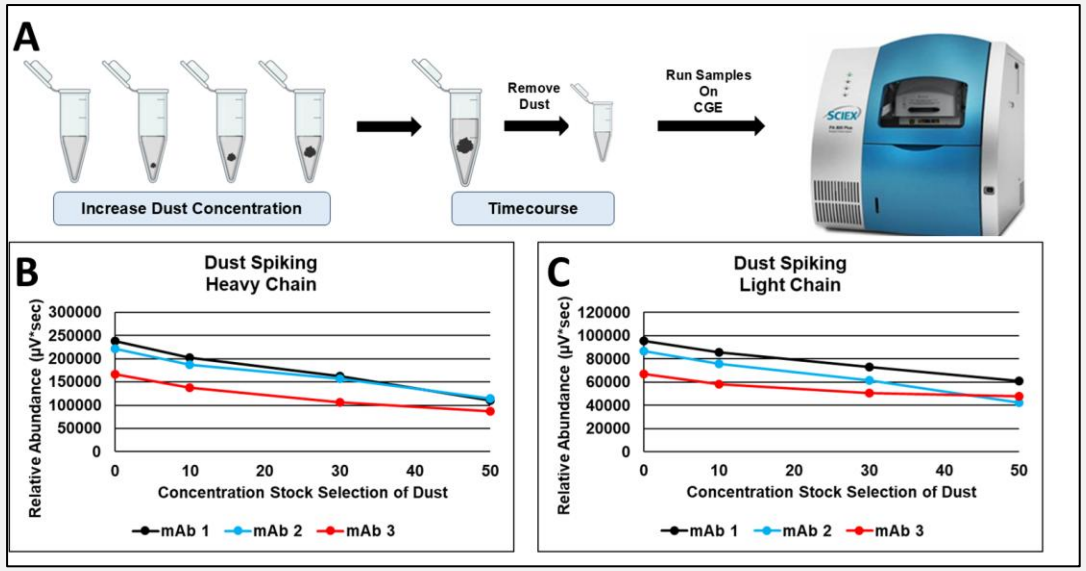


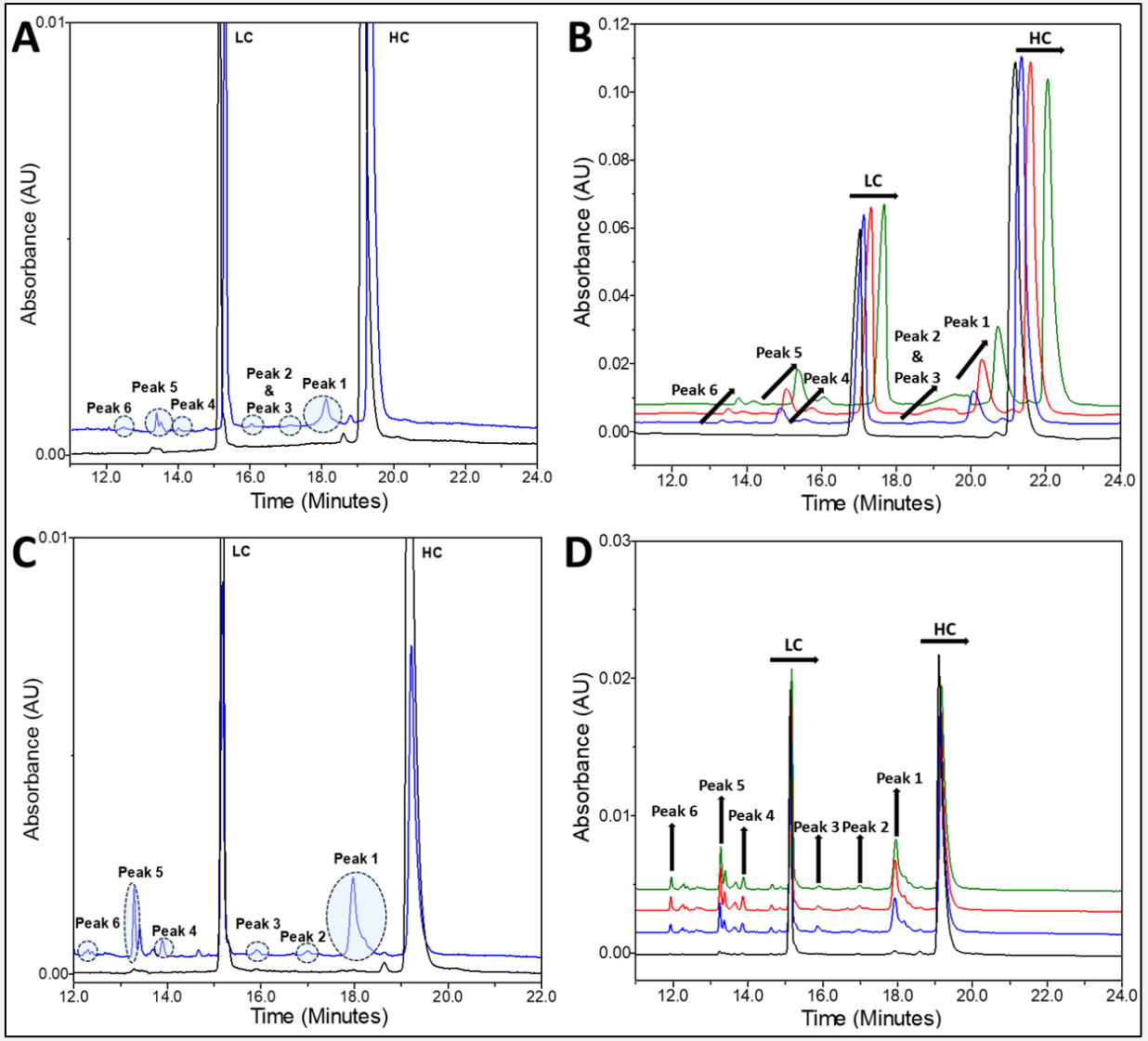
Hair reduction image generated through AI

Case Study #2: Exploring Contamination Peaks In CGE-SDS Under Reducing Conditions

Hair & Dust Spiking Experiments Positively Correlated With Increased In Peaks 1-6





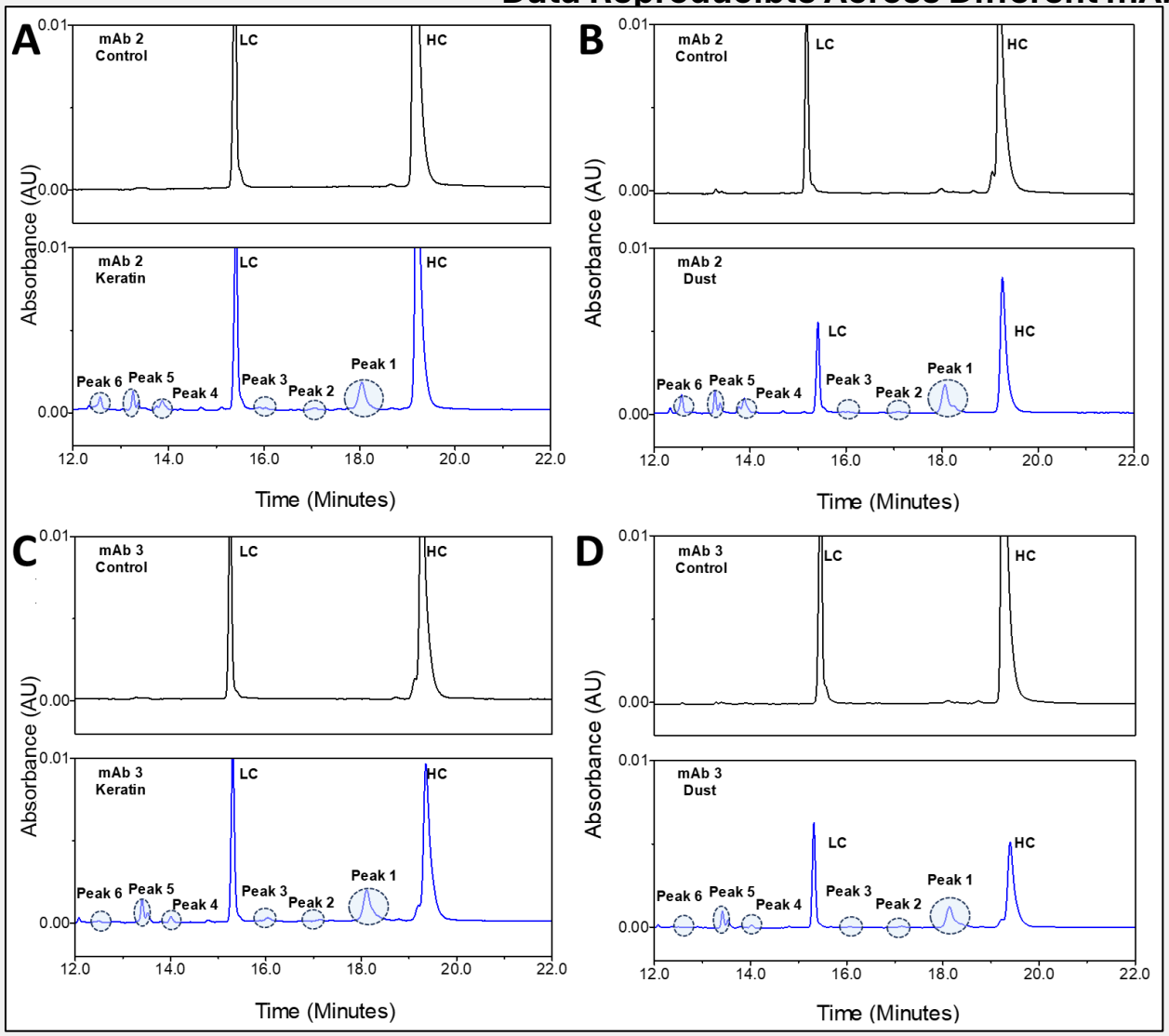


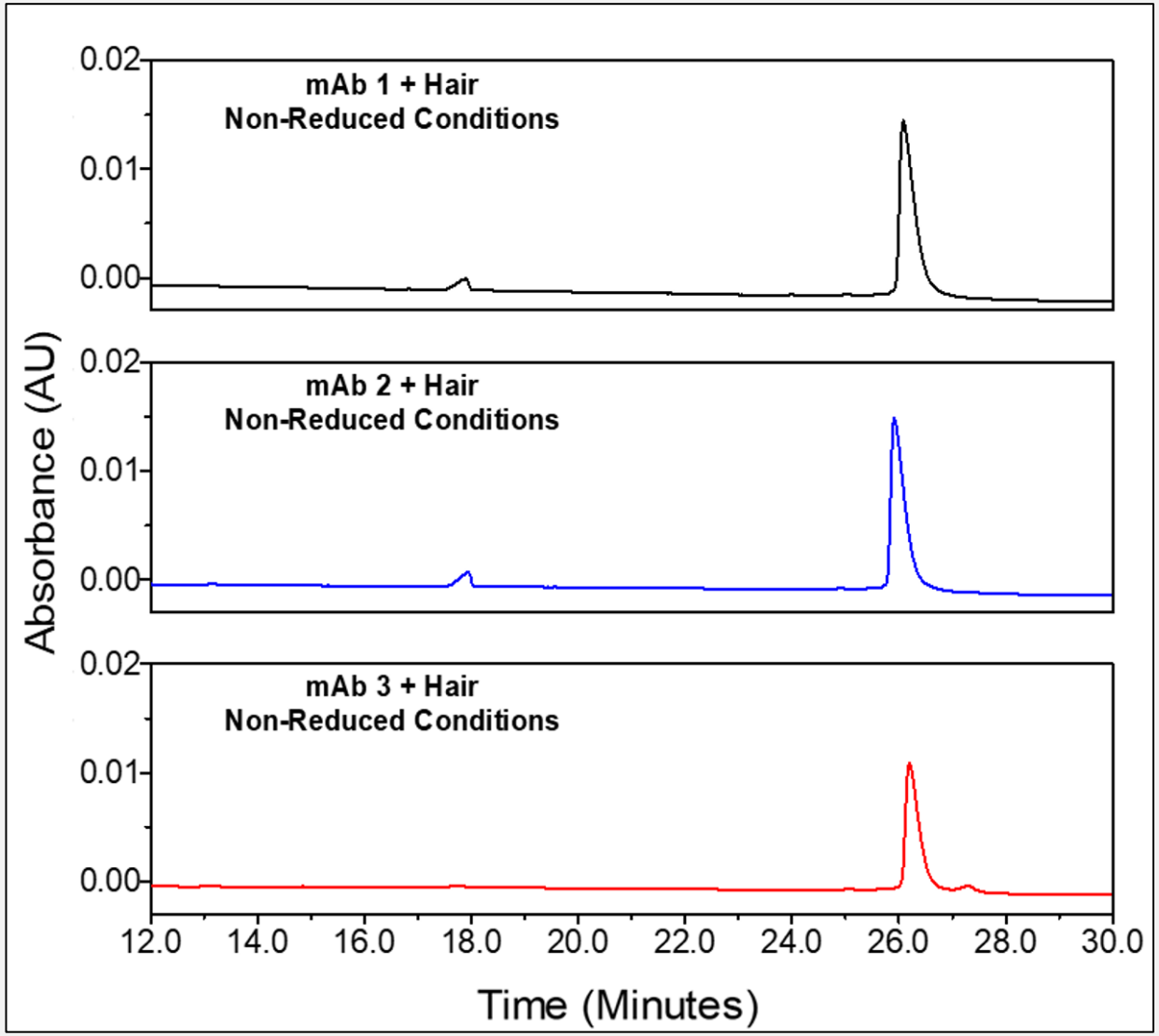
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Case Study #2: Exploring Contamination Peaks In CGE-SDS Under Reducing Conditions

Data Reproducible Across Different mAbs & Absent From NR CGE-SDS Controls



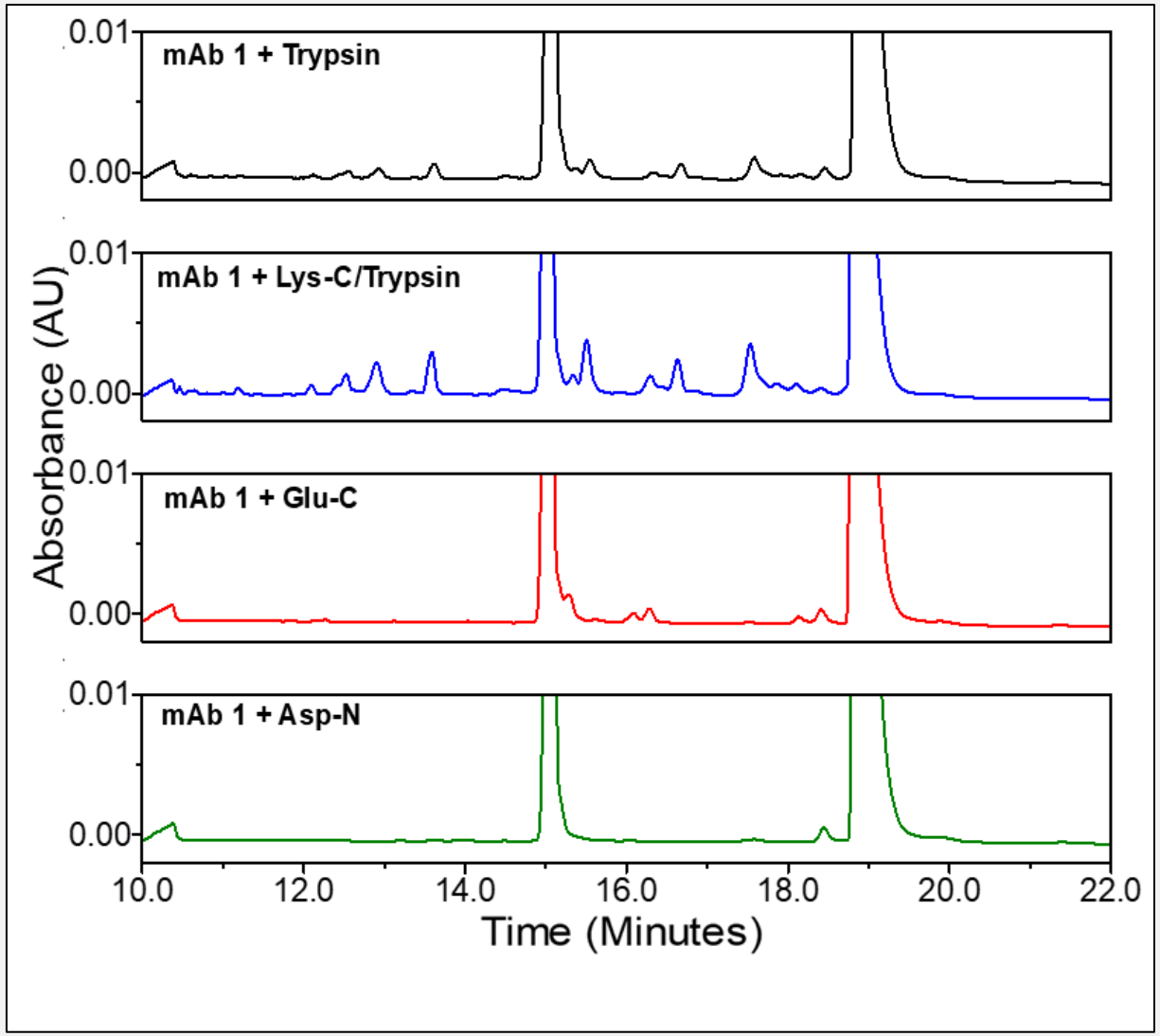


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Case Study #2: Exploring Contamination Peaks In CGE-SDS Under Reducing Conditions

No Trend Observed In Proteases Tested To Date



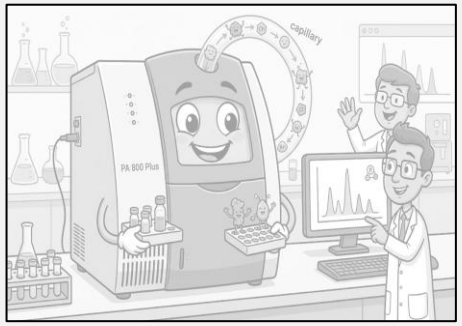
Contamination May Unintentionally Be Introduced During The Sample Preparation That Can Impact Final Results

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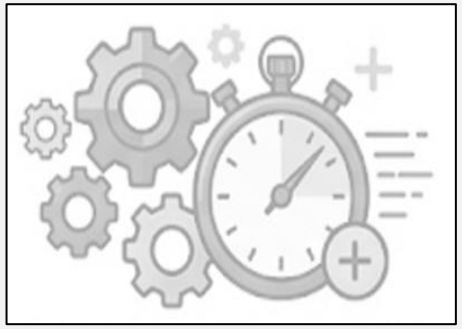
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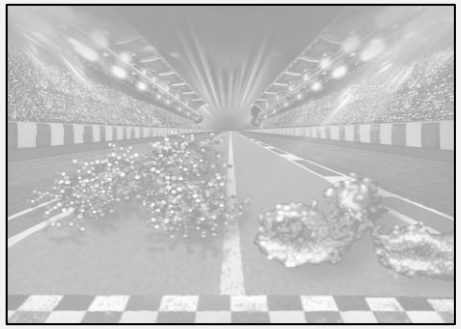
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- Contamination Investigation
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Limitations & Future Directions



- Mass Spectrometry Availability
- Sample Robustness
- Alignment Of Best Practices

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Current Limitations & Future Directions

Complex CGE-SDS Profiles	Increasingly diverse modalities may produce complex electropherograms in CGE-SDS workflows that require further understanding of SDS binding and mechanistic interactions
Industry Alignment	As more data is generated across different analytical laboratories, new artifacts may be identified that require larger strategy alignment on root cause and potential resolutions
Mass Spectrometry Readiness	The characterization of unique CGE-SDS peaks may require establishment of mass spectrometry instrumentation, method development, buffer compatibility assessment, and overall testing that may not be available during time of observations
Protease Assessments	While the exact proteases tested did not correlate to Peaks 1-6, different hair samples may contain different proteases or levels of proteases. Additional proteases not yet identified may play a role in peak formation. Future MS experiments in preparation.
Conclusion	As analytical control strategies are implemented, characterization of new peaks in CGE-SDS is critical. Future work will continue to probe and monitor these observations.

Acknowledgements

Large Molecule Analytical

Biologics Analytical Research & Development (B-AR&D)
- Thomas Niedringhaus
- Mark Haverick
- Joop Waterval
- Lauren Favell
- Andrew Semple
- Karen Bern
- Xiaoyu Yang

Cell-Based Sciences (CBS)
- Julie McIntosh

Pharmaceutical Analysis & Digital Technologies (PADT)

Analytical Enabling Capabilities – Biologics Mass Spectrometry (AEC- MS)
- Zhengqi Zhang
- Shannon Rivera

Historical CASSS CE-Pharm Presentations

Larger CE Community

All Of You For Attending!

MERCK

THANK YOU!!!

ANY QUESTIONS?

