CE Pharm 2022

Charge Variant Characterization of Therapeutic Proteins using Preparative iCIEF and Mass Spectrometry

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Bristol-Myers Squibb, Biologics Development

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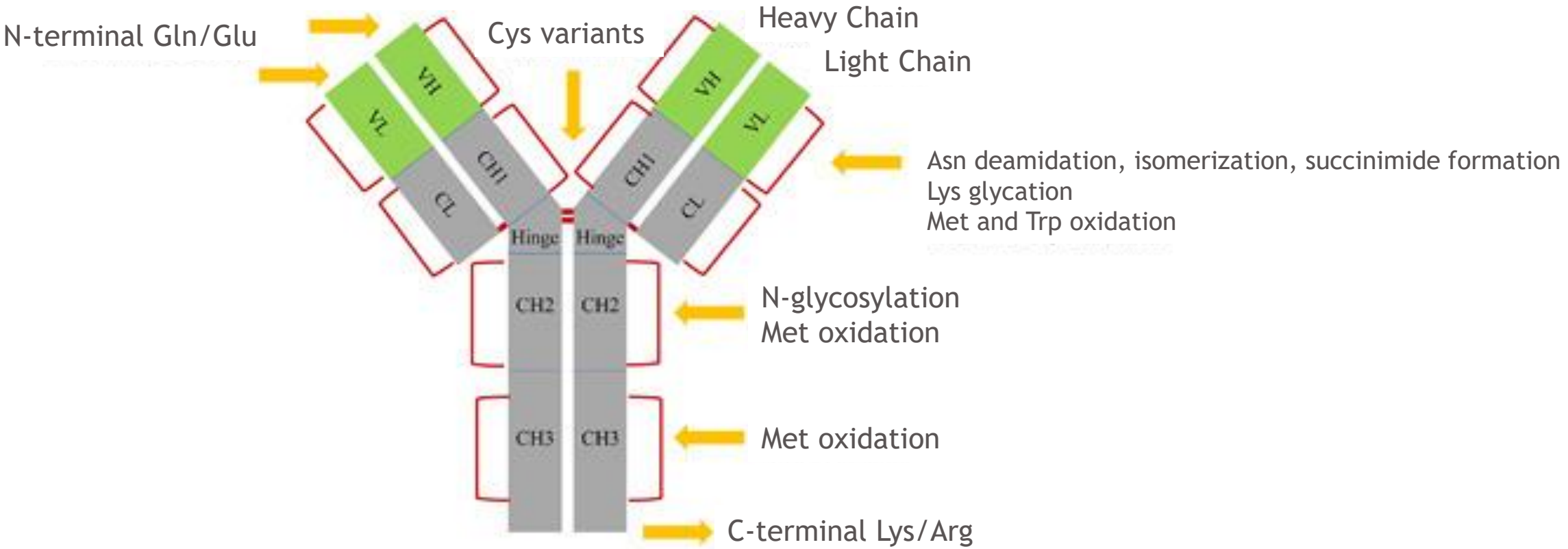
Post-translational Modifications and Charge Variants



Chung S, Tian J, Tan Z, Chen J, Lee J, Borys M, Li ZJ. Biotechnol Bioeng. 2018 Jul;115(7):1646-1665.

Impacts of Charge Variants

Modifications	Affected amino acids	Impact on clinical efficacy/pharmacokinetics
Acidic species		
Deamidation	Asn, Gln	14-fold reduced antigen binding (Huang et al., 2005) (Asn55 located in the CDR2 region of the heavy chain of IgG1)
Oxidation[a]	Cys, Met, Trp, His, Tyr	Reduced binding with Protein A and FcRN (Bertolotti-Ciarlet et al., 2009; Gaza-Bulseco, Faldu, Hurkmans, Chumsae, & Liu, 2008; Pan et al., 2009)
		Reduced half-life (Gaza-Bulseco et al., 2008)
		Loss of target binding and activity (Hensel et al., 2011)
Glycation	Lys	No significant impact on half-life or potency (Alt et al., 2016; Khawli et al., 2010; Miller et al., 2011; Quan et al., 2008)
		May illicit response with AGE pathways (Ott et al., 2014)
Basic species		
Isomerization	Asp	Complete inactivation (Rehder et al., 2008) (Aspartate 92 located in the antigen-binding region of the light chain of IgG2)
Succinimide	Asn, Asp	May illicit immune response (Chen et al., 1996)
C-terminal Lys/Arg	Lys, Arg	No significant impact on binding, PK, or half-life (Alt et al., 2016; Antes et al., 2007; Khawli et al., 2010; Lyubarskaya et al., 2006)
C-terminal amidation	Gly	No known impact in MAbs
N-terminal pyroGlu	Gln, Glu	Potency not significantly impacted (Manning et al., 2010)

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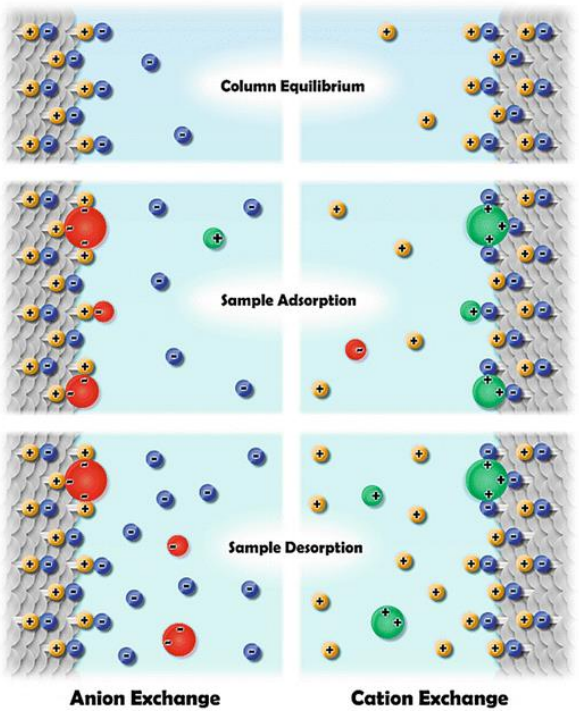
Outline

- Charge Variant Analysis
- Mass Spectrometry Characterization of Charge Variants
- Case Study 1: mAb1
- Case Study 2: mAb2
- Summary

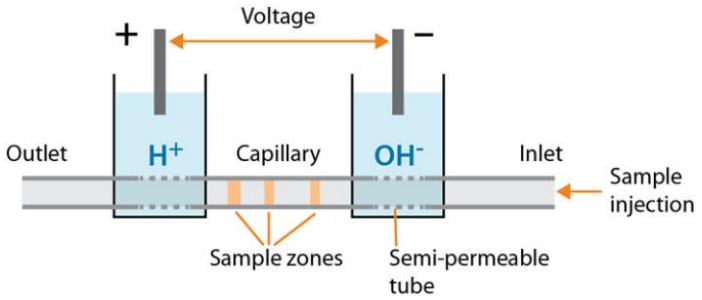
Techniques for Charge Variant Analysis

Ion exchange chromatography (IEX)

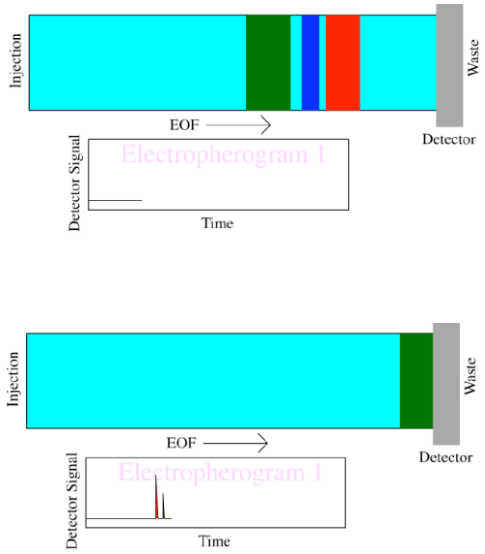


Capillary Electrophoresis (CE)

Capillary Isoelectric Focusing (CIEF)



Capillary Zone Electrophoresis (CZE)

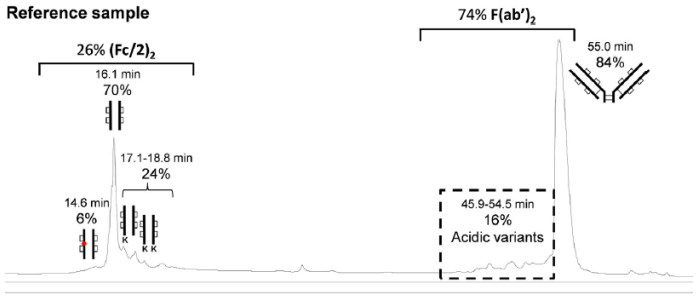


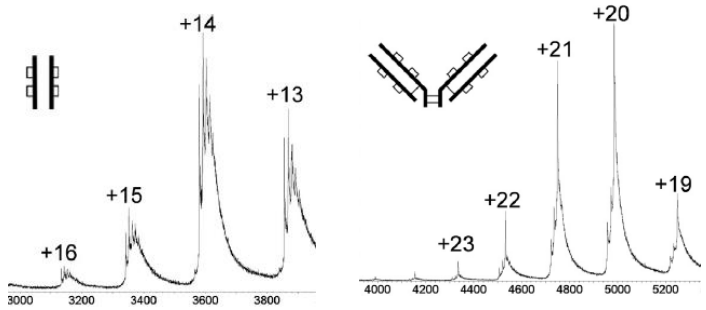
1. Cummins P.M., Rochfort K.D., O'Connor B.F. (2017) Ion-Exchange Chromatography: Basic Principles and Application. In: Walls D., Loughran S. (eds) Protein Chromatography. Methods in Molecular Biology, vol 1485. Humana Press, New York, NY
2. https://www.proteinsimple.com/ice3.html
3. https://www.shsu.edu/~chm_tgc/primers/pdf/CEs.pdf

IEX-MS

Dual Salt/pH Gradient

Mobile Phase A	50 mM ammonium formate (pH 3.9)
Mobile Phase B	500 mM ammonium acetate (pH 7.4)

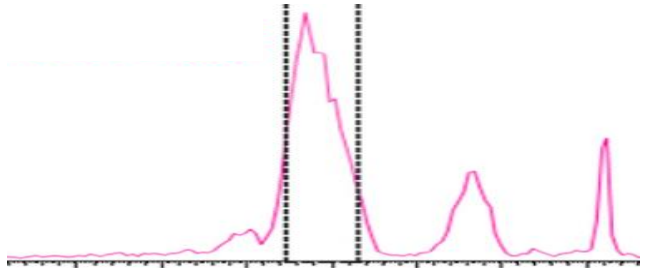


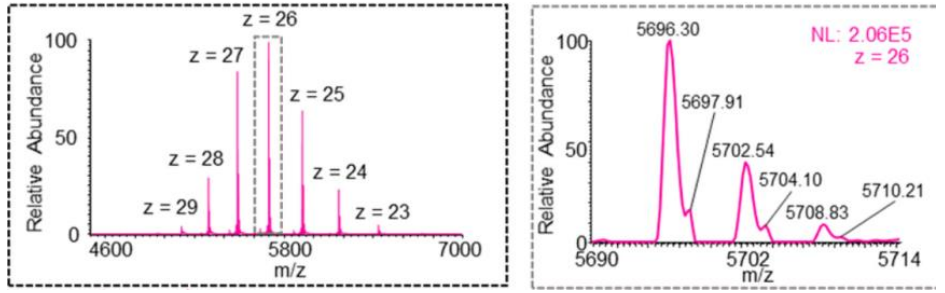


J Chromatogr B Analyt Technol Biomed Life Sci. 2017;1048:130-139

pH Gradient

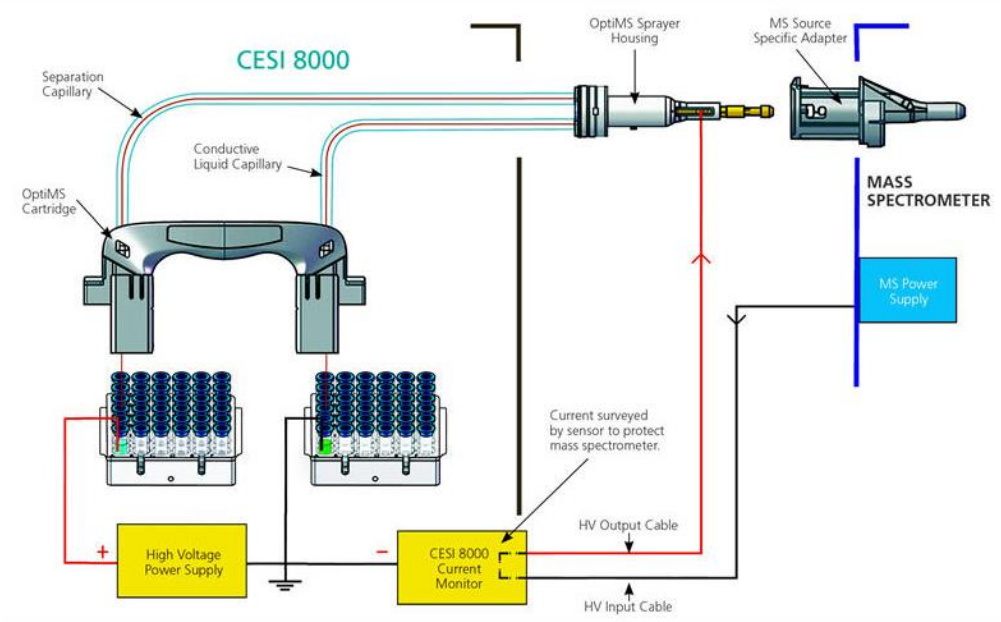
Mobile Phase A	25 mM ammonium bicarbonate and 30 mM acetic acid (pH 5.3)
Mobile Phase B	10 mM ammonium hydroxide in 2 mM acetic acid (pH 10.18)

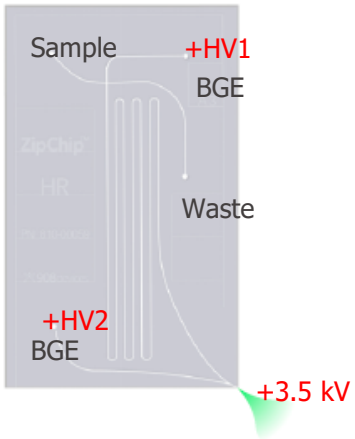


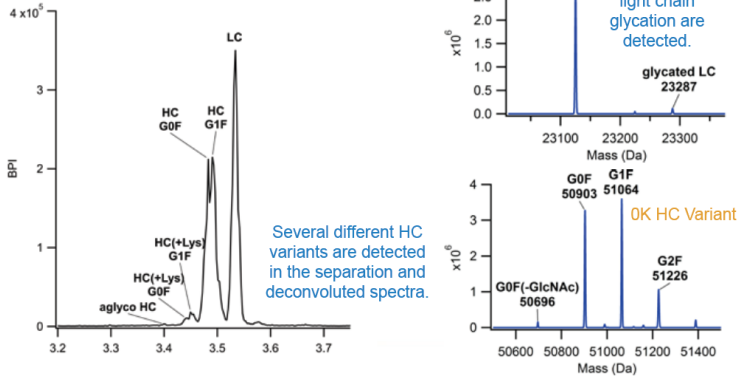


Anal Chem. 2018; 90 (7): 4669-4676

CZE-MS



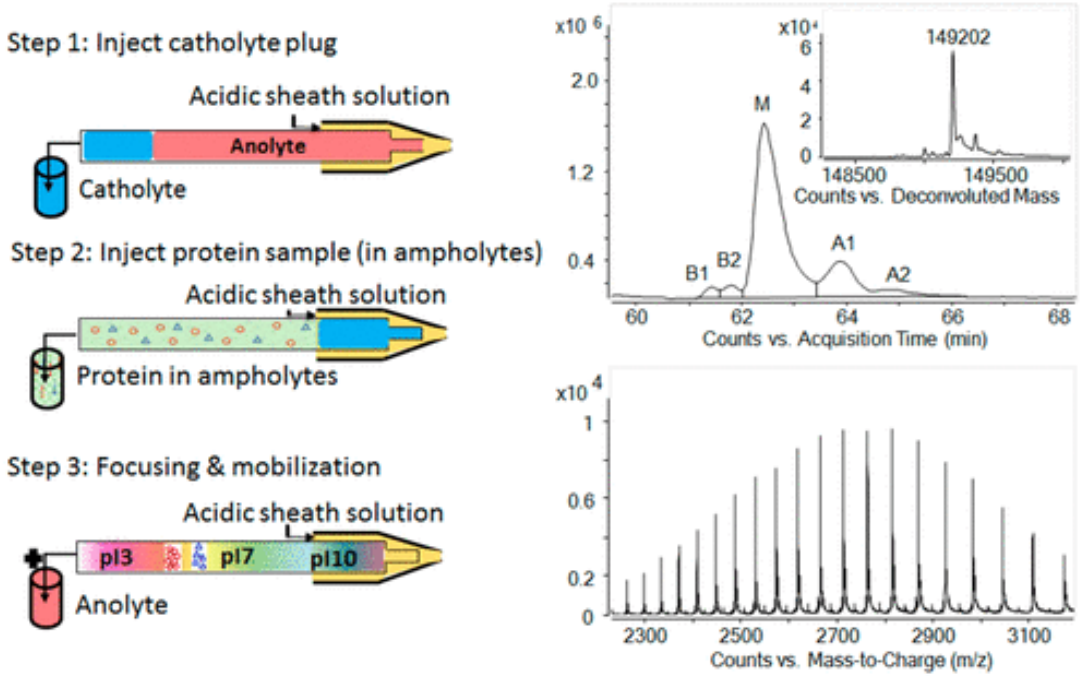


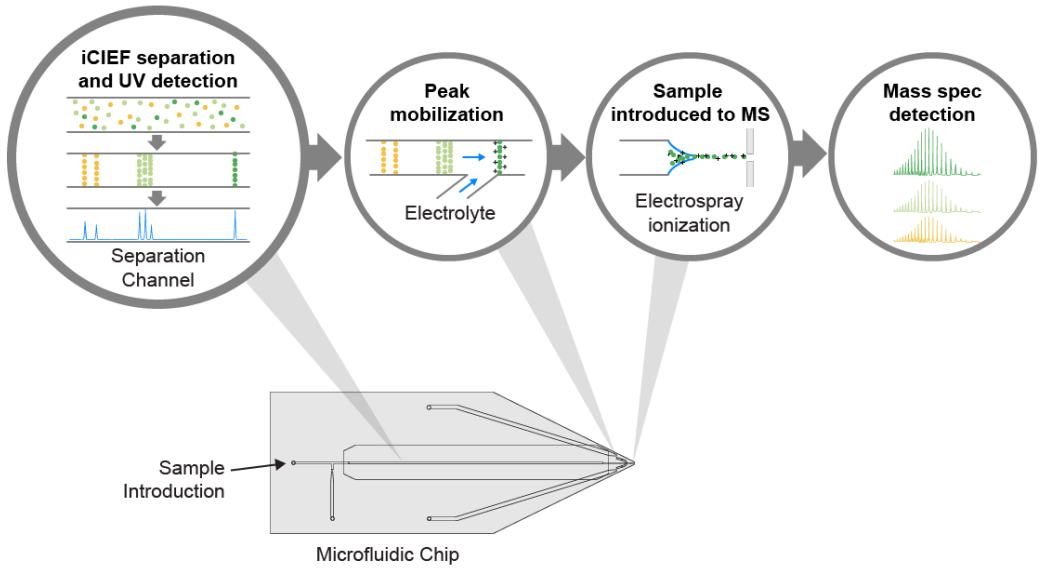


https://sciex.com/ce-features-and-benefits/ultra-low-flow-cesi-ms-technology

https://908devices.com/wp-content/uploads/2017/11/LCGC1017-908dev-9-27-ES-FINAL-web.pdf

CIEF-MS

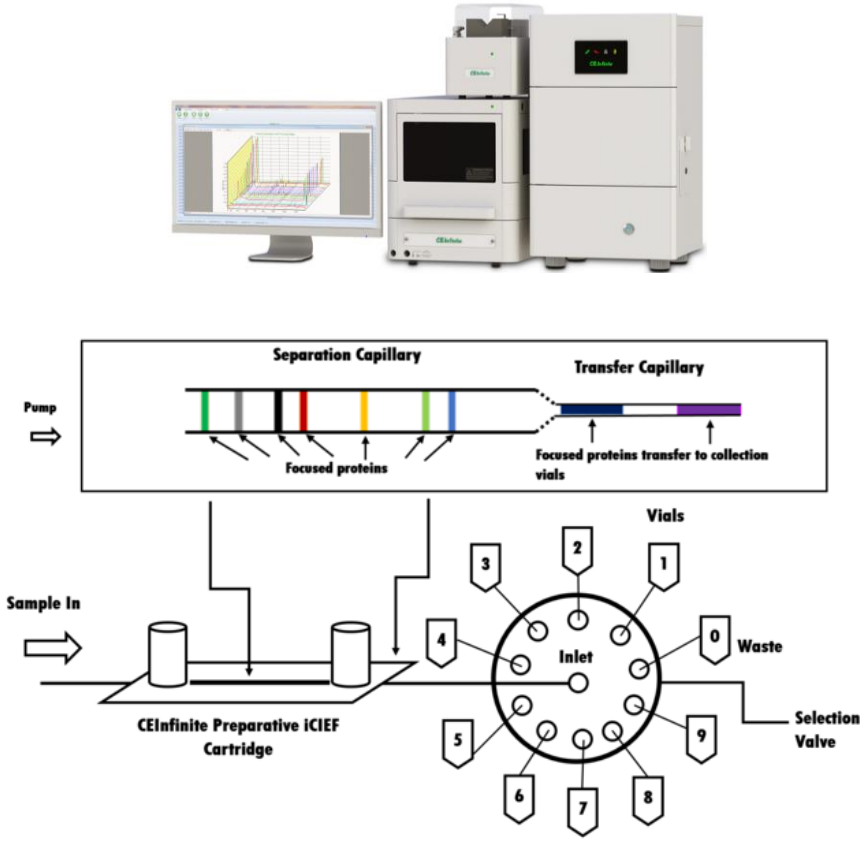


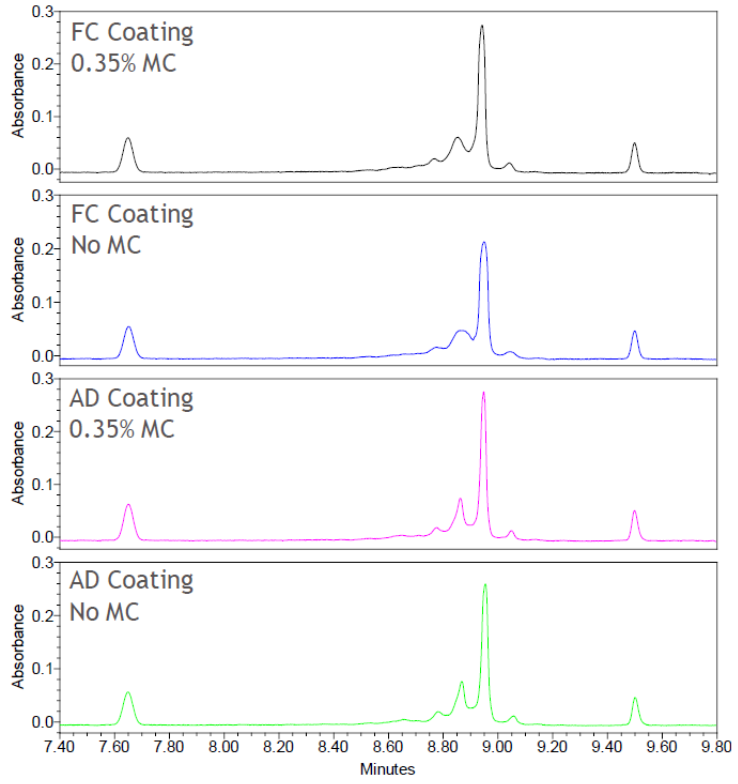


Anal. Chem.2018, 90 (3), 2246-2254

https://sciex.com/technology/icief-ms-technology/

Preparative iCIEF system and Workflow for MS Analysis



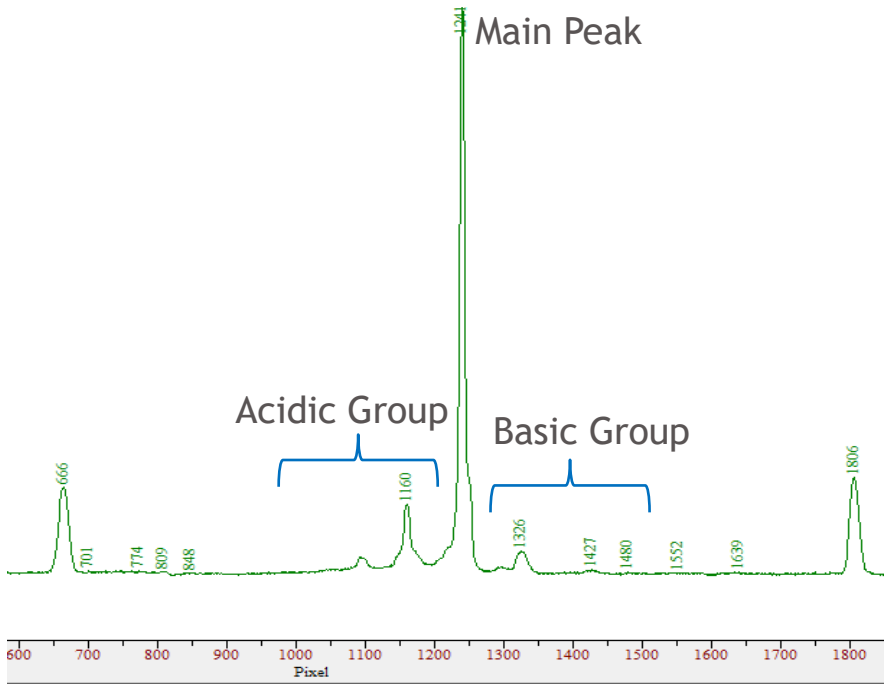


- *On a cartridge with fluorocarbon (FC) coating, absence of methyl cellulose resulted in loss of resolution*
- *On a cartridge with acrylamide derivative (AD) coating, the same peak profile was observed with or without methyl cellulose*

Case Study 1: mAb1
Background

- During process development for mAb1, a different cell line was introduced. Comparing to Process A, which showed mean % acidic group of 33.9%, lower mean % acidic group was identified in Process B (25.5%).
- Using peptide mapping, lower deamidation and oxidation levels, similar sialylated glycans levels, slightly higher glycation levels were observed for Process B vs Process A.
- Objective of this study:
  - To show comparability between Process A and Process B material in terms of the acidic species.

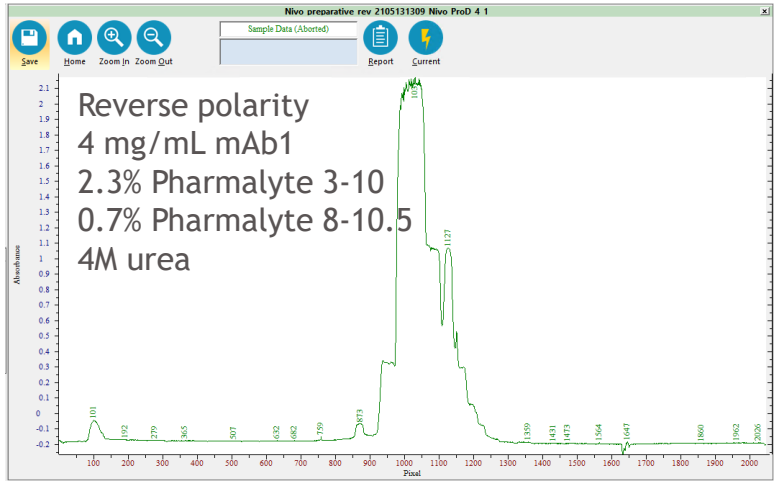
Case Study 1: mAb1
Analytical iCIEF

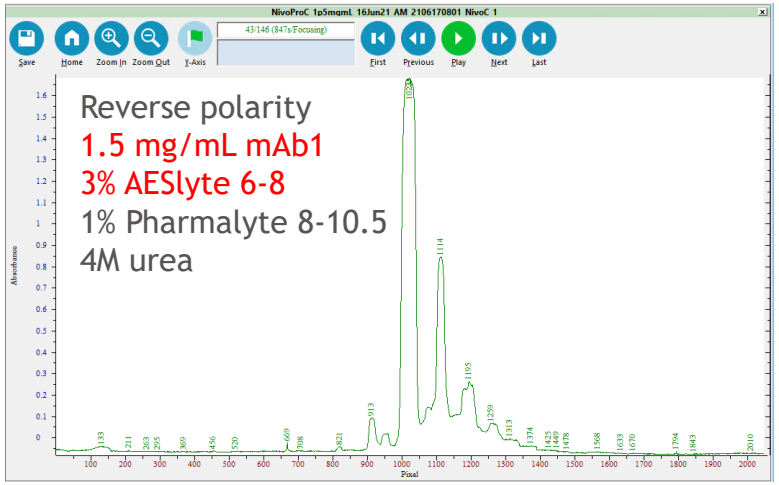


	Method parameters
cartridge	fluorocarbon coating cartridge (100 µm ID)
Methyl cellulose	0.32%
Ampholytes	2.3% Pharmalyte 3-10 0.7% Pharmalyte 8-10.5
Additives	0.95 M Urea
pI Marker	6.14 and 9.46
Protein conc.	0.25 mg/mL
Focusing	8 min

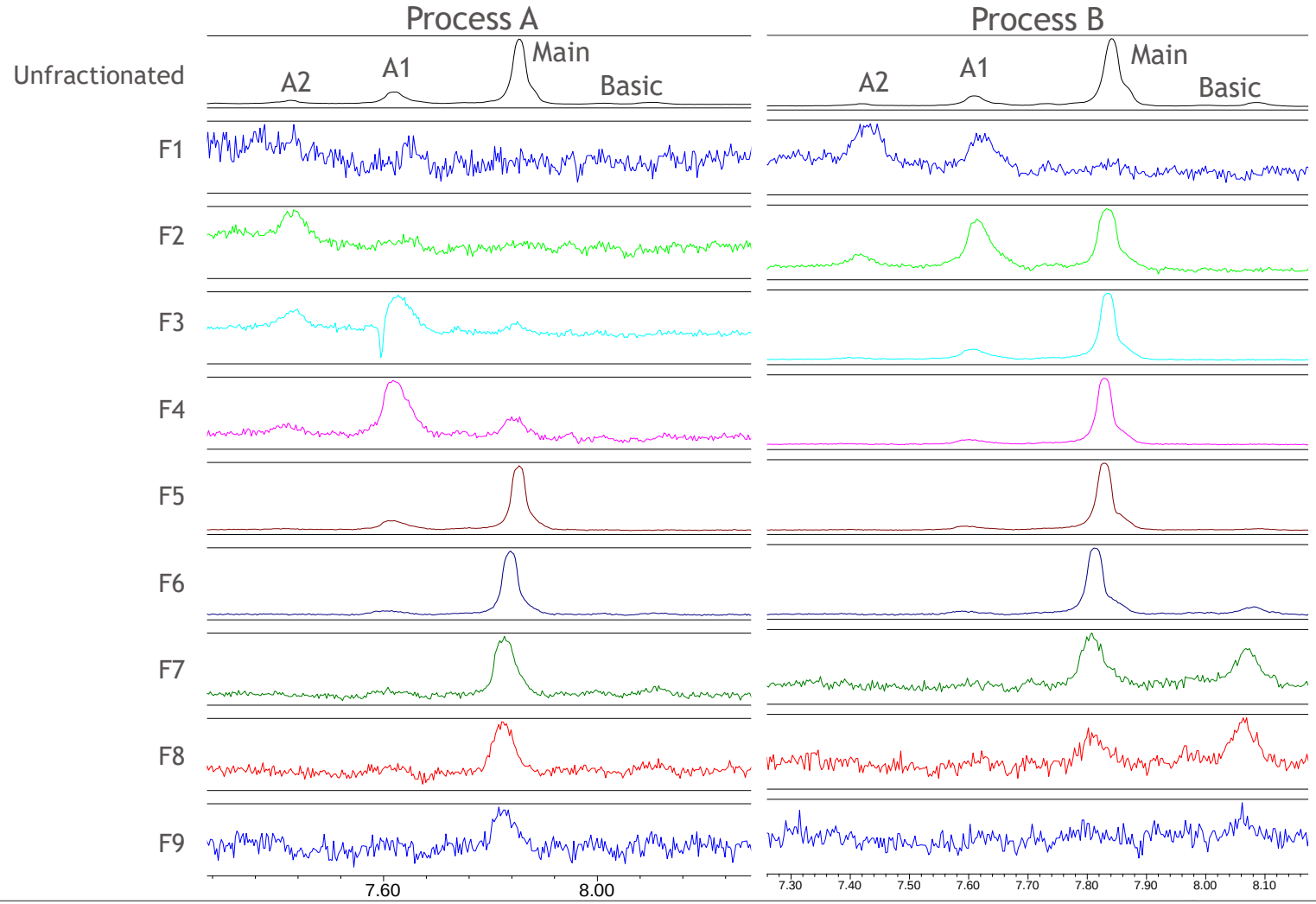
Case Study 1: mAb1

Method Development on Preparative iCIEF





Peak identification of collected fractions using analytical iCIEF



Case Study 1: mAb1

Analysis of Reduced and Deglycosylated Samples using LC-MS

Process A

Process A	Peak ID	HC C-term Lys	Total Glycation
unfractionated control	N/A	1.6	5.3
Fraction 1	N/A	ND	8.2
Fraction 2	A2	ND	8.7
Fraction 3	A2 & A1 (major)	ND	8.7
Fraction 4	A1 (major) & Main	ND	8.0
Fraction 5	Main	ND	4.7
Fraction 6	Main	1.2	4.3
Fraction 7	Main (major) & Basic	5.0	4.3
Fraction 8	Main (major) & Basic	8.4	3.8
Fraction 9	Main (major) & Basic	9.4	3.8

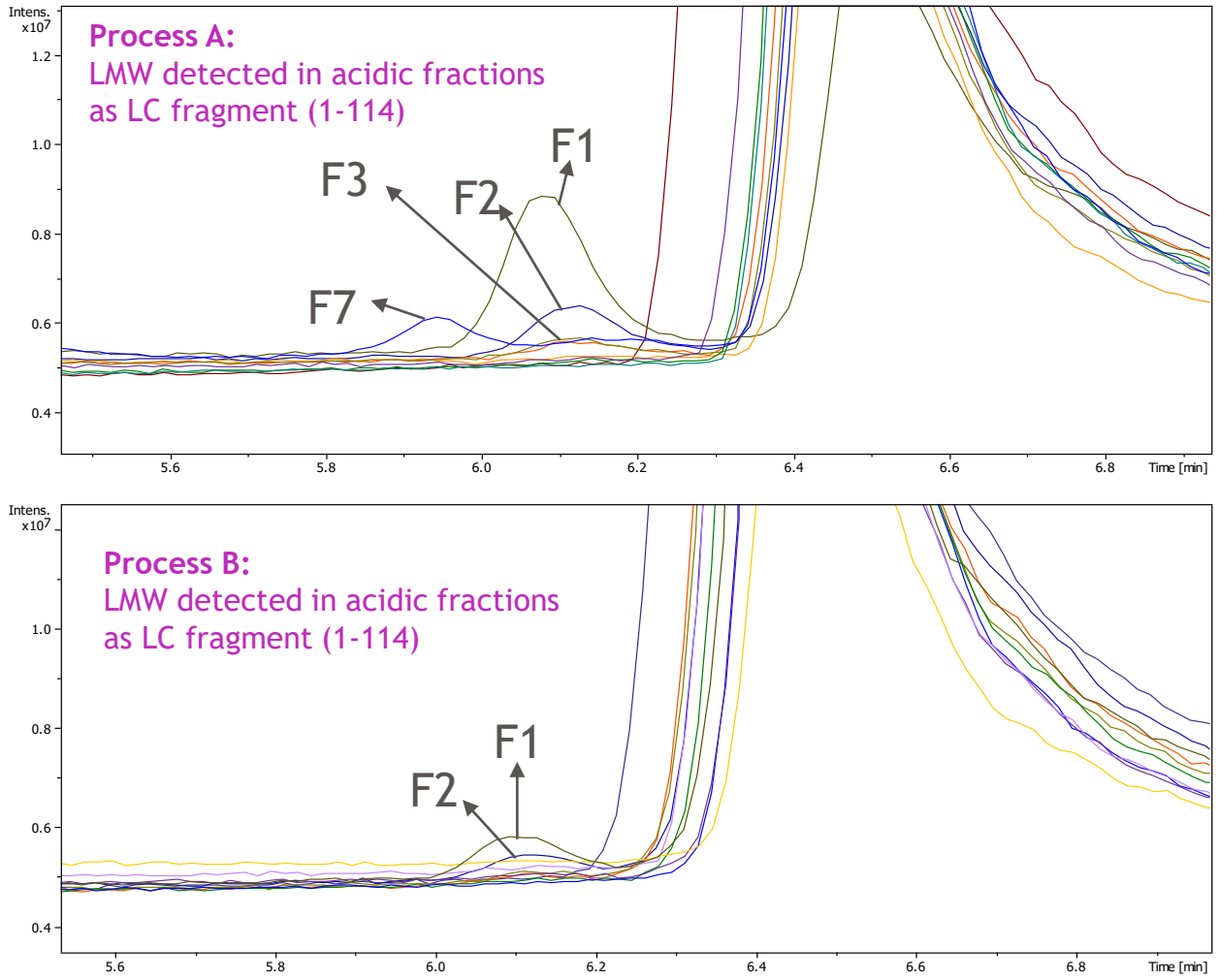
Process B

Process B	Peak ID	HC C-term Lys	Total Glycation
unfractionated control	N/A	2.9	6.6
Fraction 1	A2 & A1	ND	10.7
Fraction 2	A1 & Main	ND	8.5
Fraction 3	Main	ND	6.4
Fraction 4	Main	ND	6.3
Fraction 5	Main	1.1	6.1
Fraction 6	Main (major) & Basic	5.2	6.1
Fraction 7	Main & Basic	21.0	5.7
Fraction 8	Main & Basic	32.7	5.7
Fraction 9	Basic	36.4	5.9

- *C-terminal lysine was enriched in the basic fractions*
- *Glycated species were enriched in the acidic fractions*

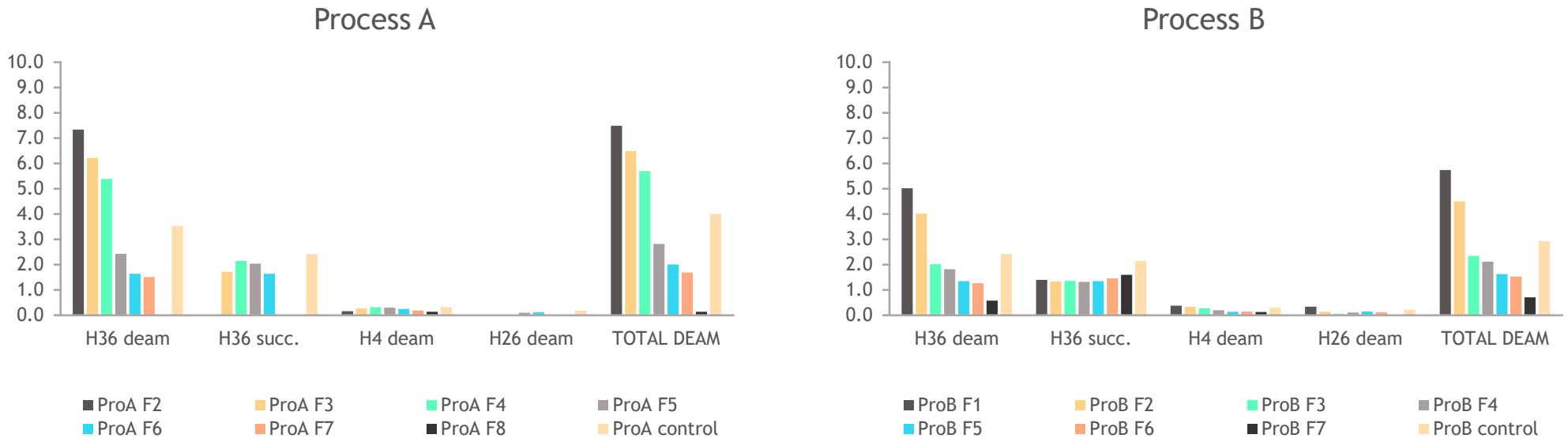
Case Study 1: mAb1

Analysis of Reduced and Deglycosylated Samples using LC-MS



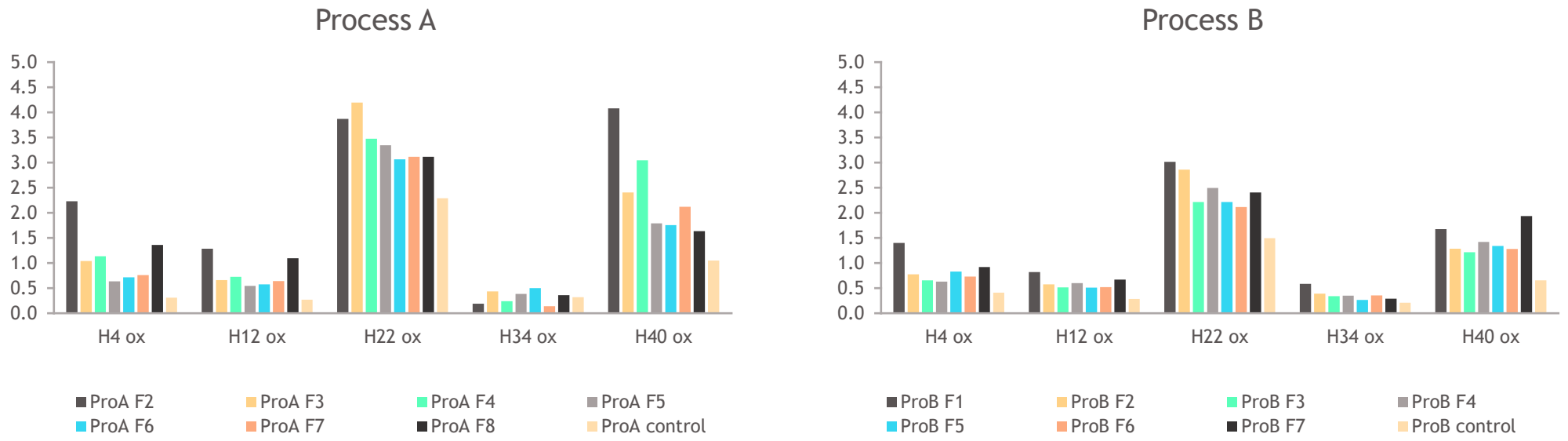
- *LC fragments were observed in acidic fractions*

Case Study 1: mAb1
Peptide Mapping - Deamidation



- *The same deamidated species were observed for Process A and Process B*
- *Deamidation was enriched in the acidic species in iCIEF*
- *Slightly higher levels of H36 deamidation were observed in Process A comparing to Process B*

Case Study 1: mAb1
Peptide Mapping - Met Oxidation

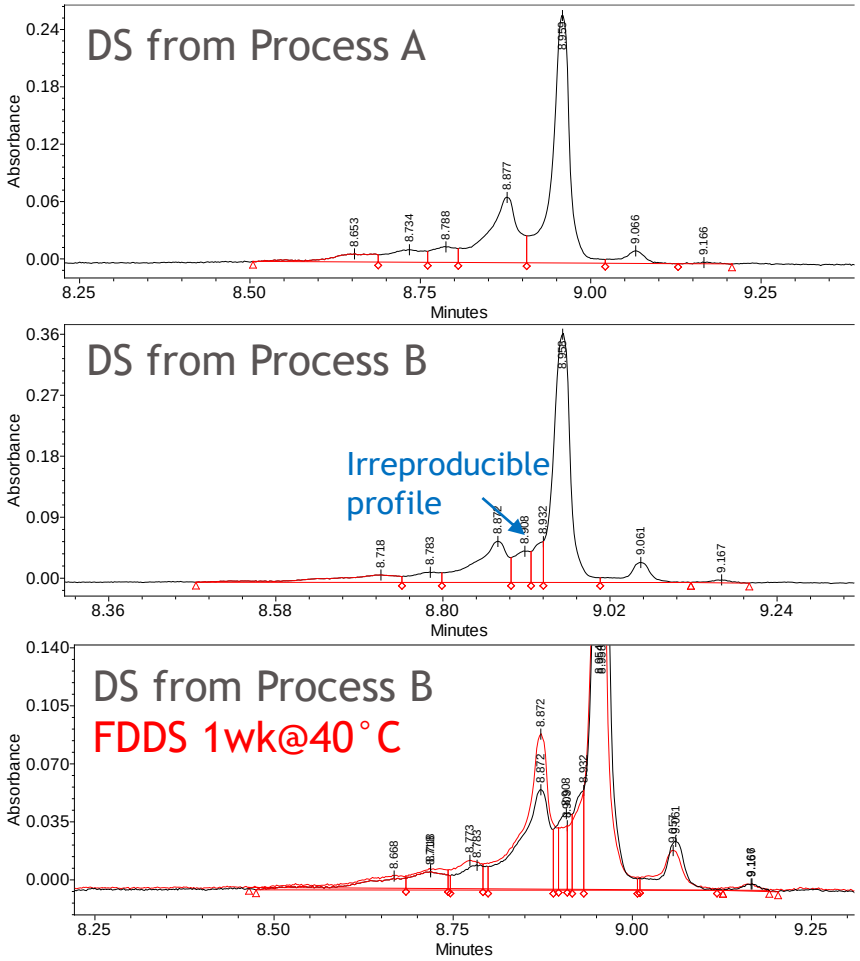


- *The same oxidized species were observed for Process A and Process B*
- *No obvious charge variant separation with iCIEF was observed for oxidation*
- *Slightly higher levels of H22 and H40 oxidation were observed in Process A comparing to Process B*

Case Study 2: mAb2
Background

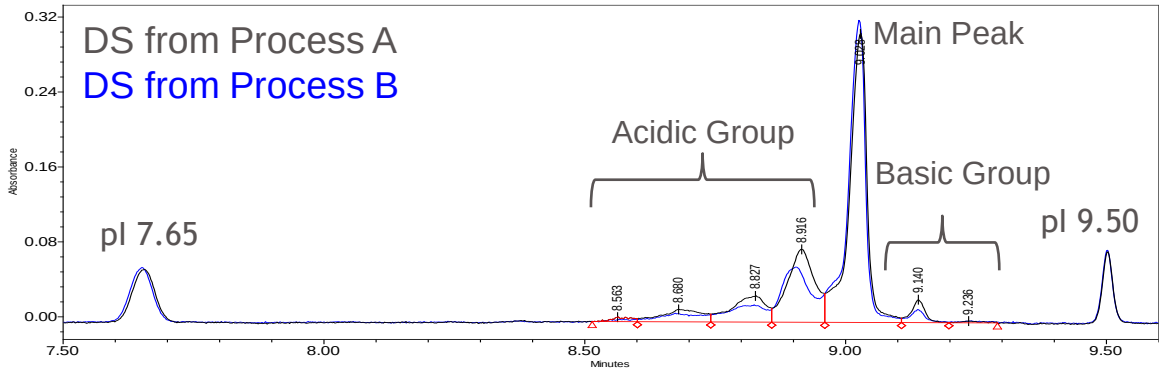
- During process development for mAb2, a different cell line and process was introduced. Comparing to Process A, irreproducible peak profile was observed using a platform iCIEF method.
- Objective of this study:
  - Method development for release and stability
  - To show comparability between Process A and Process B material

0.35% Methyl Cellulose
1% Pharmalyte 5-8, 3% Pharmalyte 8-10.5
3 M Urea
0.25 mg/mL of mAb2



Case Study 2: mAb2

Analytical iCIEF Method Development for mAb2 for Release and Stability

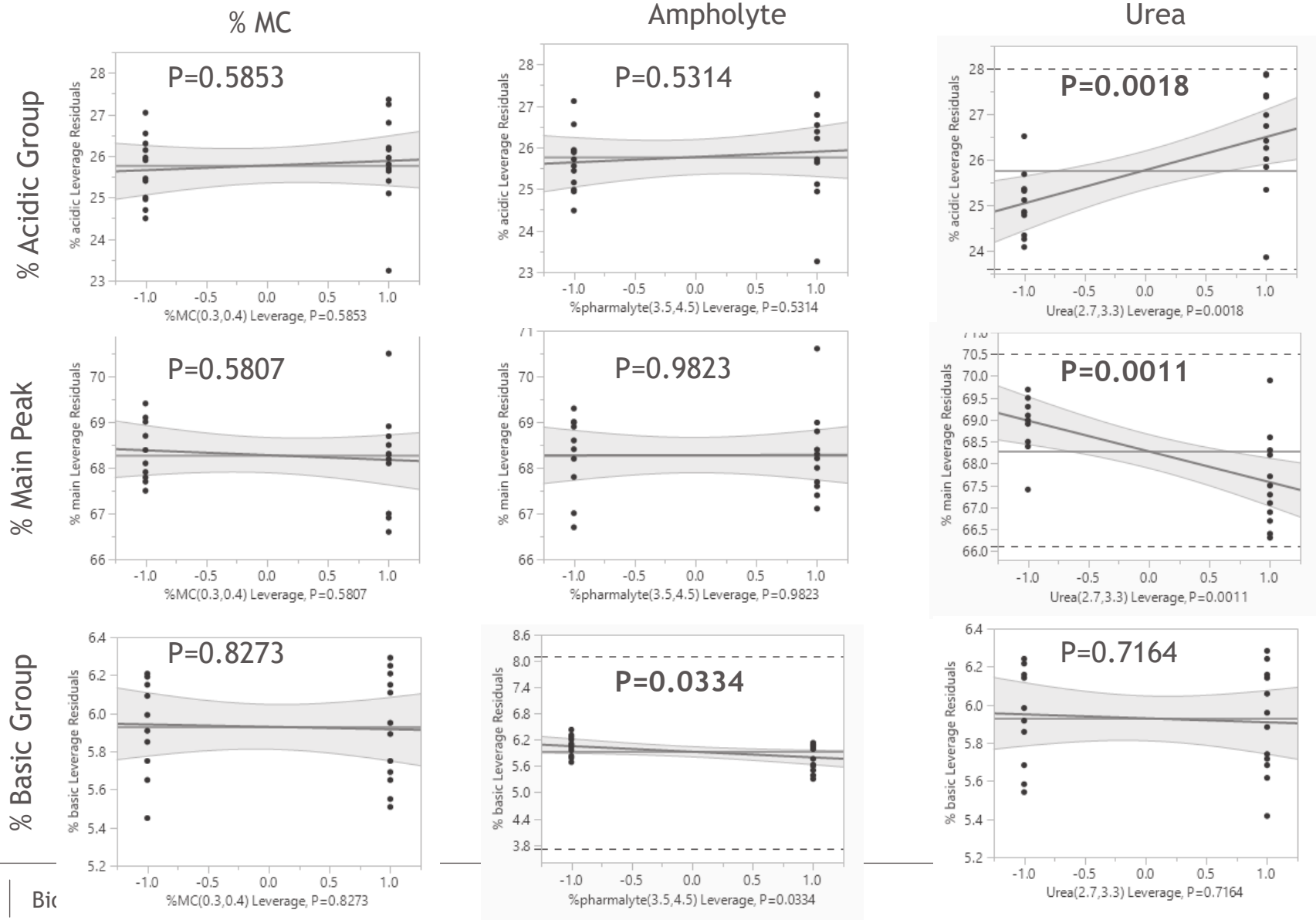


	Platform method	New Method for Process B
Methyl Cellulose	0.35%	0.35%
Ampholytes	1% 5-8 Pharmalyte, 3% 8-10.5 Pharmalyte	2% 6-9 Servalyt, 2% 8-10.5 Pharmalyte
Additives	3M Urea	3M Urea, 5mM Arg
Focusing time	10 minutes	10 minutes
Low pI marker	7.65 (0.5%)	7.65 (0.5%)
High pI marker	9.77 (0.5%)	9.50 (0.5%)

Case Study 2: mAb2

Method Robustness

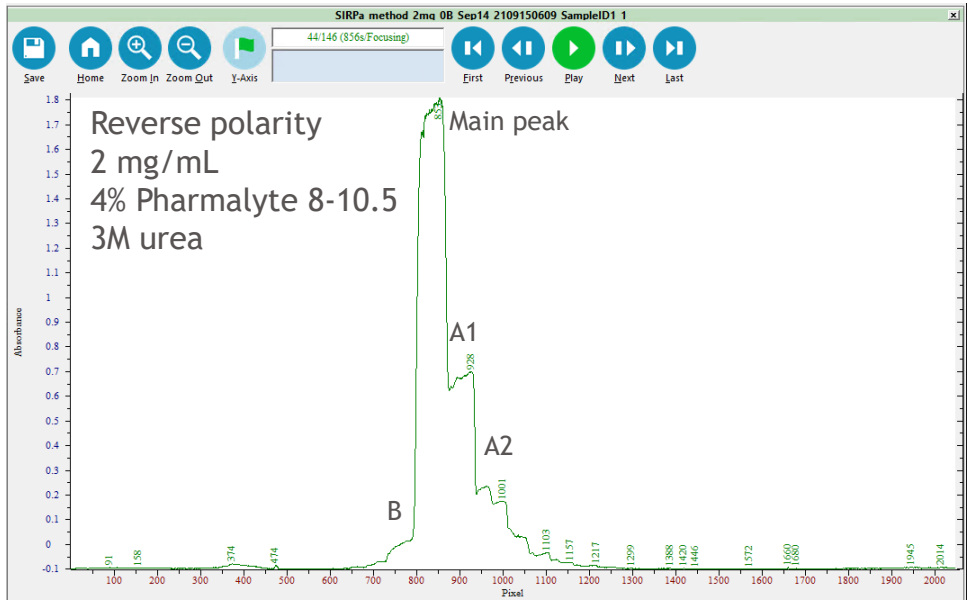
DoE: MC (0.3-0.4%), Ampholyte (3.5-4.5%), Urea (2.7-3.3 M)



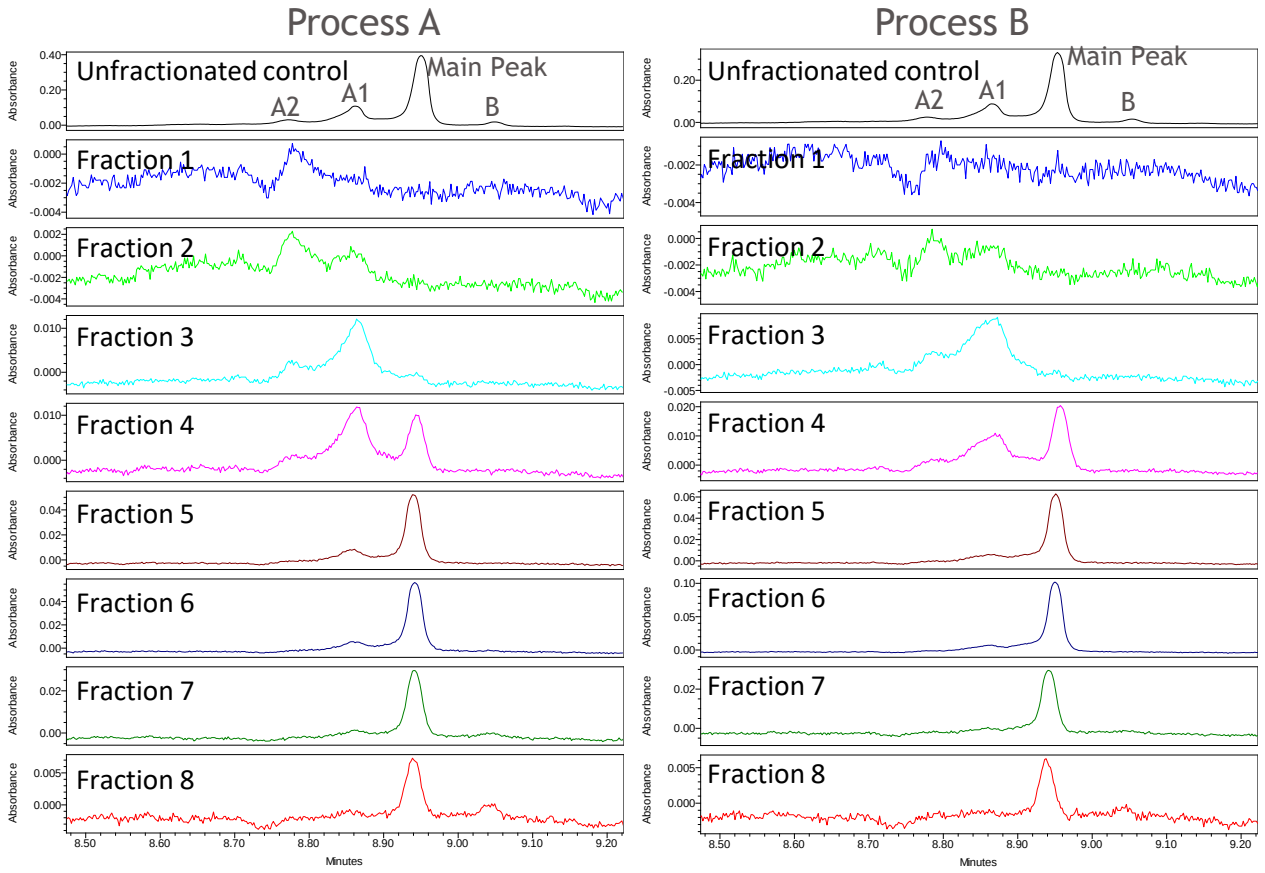
Case Study 2: mAb2

Preparative Fraction Collection for Acidic Peaks Characterization

Electropherogram after focusing on CEinfinite Preparative iCIEF system



Peak identification of collected fractions using analytical iCIEF system



Case Study 2: mAb2
Intact MS Analysis

Relative Intensity (%)	Fraction 1 A2		Fraction 2 A2 (major) +A1		Fraction 3 A2 + A1 (major)		Fraction 4 A1 (major)+main		Fraction 5 A1+Main (major)		Fraction 6 A1+Main (major)		Fraction 7 A1+Main (major)+B		Fraction 8 A1+Main (major)+B	
	Process A	Process B	Process A	Process B	Process A	Process B	Process A	Process B	Process A	Process B	Process A	Process B	Process A	Process B	Process A	Process B
Intact mAb2+ G0F/G0F-GlcNAc-Hex	ND	ND	ND	ND	ND	ND	ND	ND	1.4	1.1	1.3	1.2	1.5	1.1	1.6	1.2
Intact mAb2+ G0F/G0F-GlcNAc	ND	ND	ND	2	4.6	2.3	5.3	3.3	5.3	3.6	5.9	3.9	6.2	4	6.2	4.2
Intact mAb2+ G0F/G0F-Fuc	ND	ND	4.9	2.5	4.4	2.2	3.8	4.2	3.5	5.8	3.3	5.9	2.9	6.3	2.4	5.6
Intact mAb2+ G0F/G0F	24.8	13.2	29.4	14.5	37.5	20	48.2	35.8	58	45.2	58.5	47.6	59.7	49.8	59.3	50.2
Intact mAb2+ G0F/G1F	25.1	22.2	25.2	29.7	27.8	36.5	23.5	31.8	20.7	28.2	20.4	27.8	19.1	27.2	19.4	26.7
Intact mAb2+ G1F/G1F	23.9	24.3	19.3	25.4	14	23	11.6	15.8	8.4	11.1	8.1	9.8	7.8	9.2	8	8.7
Intact mAb2+ G1F/G2F	16	18.1	12.9	13.7	7.3	9.8	5.1	5.6	2.7	3.5	2.6	2.7	2.8	2.4	3.1	2.4
Intact mAb2+ G2F/G2F	10.2	13.5	8.3	7.6	4.4	4.1	2.4	2.2	ND	1.5	ND	1.1	ND	ND	ND	1
Intact mAb2+ G2F/G2F+Hex	ND	8.7	ND	4.7	ND	2.1	ND	1.3	ND	ND	ND	ND	ND	ND	ND	ND

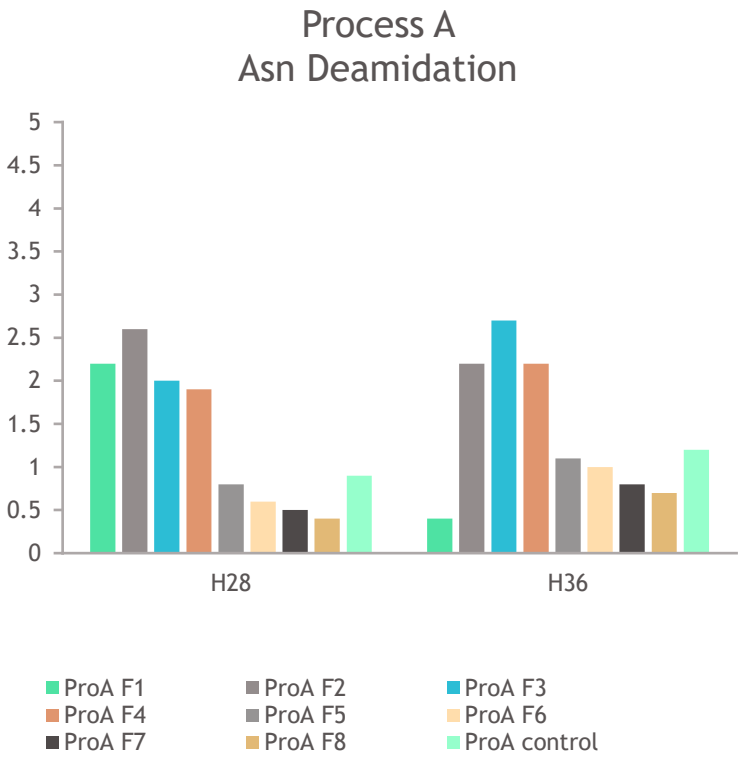
- *Similar proteoforms were identified in the fractions from Process A and Process B*

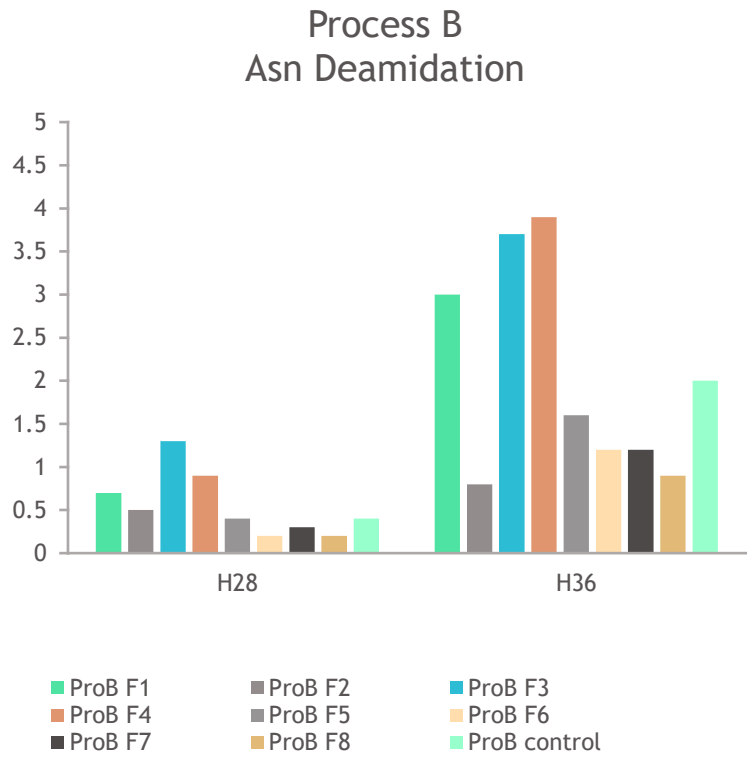
Case Study 2: mAb2
MS Analysis of Deglycosylated Fractions

Relative Intensity (%)	Fraction 1 A2		Fraction 2 A2 (major) +A1		Fraction 3 A2 + A1 (major)		Fraction 4 A1 (major)+main		Fraction 5 A1+Main (major)		Fraction 6 A1+Main (major)		Fraction 7 A1+Main (major)+B		Fraction 8 A1+Main (major)+B	
	Process A	Process B	Process A	Process B	Process A	Process B	Process A	Process B	Process A	Process B	Process A	Process B	Process A	Process B	Process A	Process B
Intact mAb2	50.3	40.5	50.9	30.2	70.2	36	79.1	61.9	87.8	76.7	89.4	79	91.8	83.9	94.5	89.4
intact mAb2+ Hex	17.9	24.9	22.2	39.4	19.3	46.1	12.8	26.6	4.2	13.3	3.1	10.3	2.5	8.7	1.8	6.5
intact mAb2+ 2Hex	20.7	19.5	20.9	18.8	8	11.9	5.9	7.7	6.1	7	5.8	7.6	4.6	5.5	3.7	4.1
intact mAb2+ 3Hex	11.1	11	6	8	2.5	4.8	1.5	2.5	0.6	1.5	0.5	1.4	0.5	0.9	ND	ND
intact mAb2+ 4Hex	ND	4	ND	3.6	ND	1.1	0.8	1.3	1.3	1.5	1.1	1.7	0.6	1.1	ND	ND

- *Glycation was enriched in acidic peaks in iCIEF*
- *Higher levels of glycation were identified in Process B comparing to Process A*

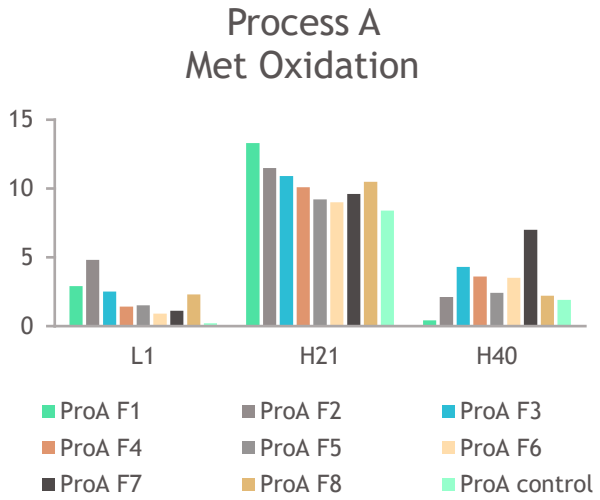
Case Study 2: mAb2
Peptide Mapping – Asn Deamidation

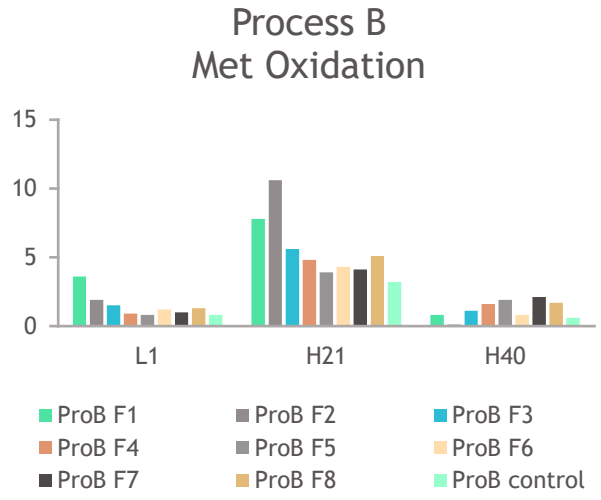


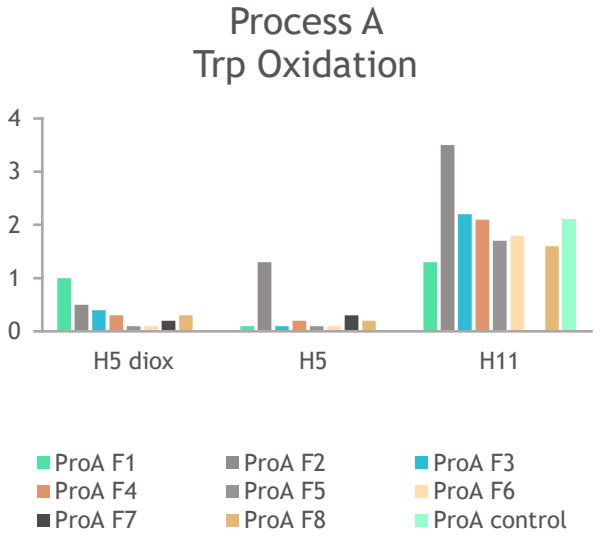


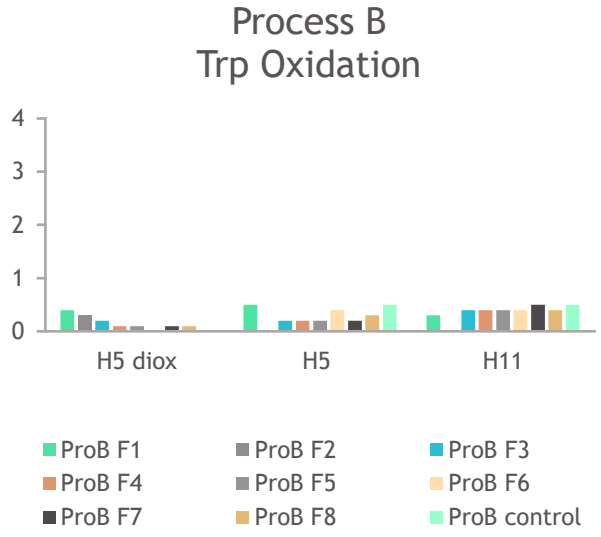
- *Similar deamidations were identified in the fractions from Process A and Process B*
- *Asn deamidation was enriched in acidic peaks in iCIEF*

Case Study 2: mAb2
Peptide Mapping – Oxidation



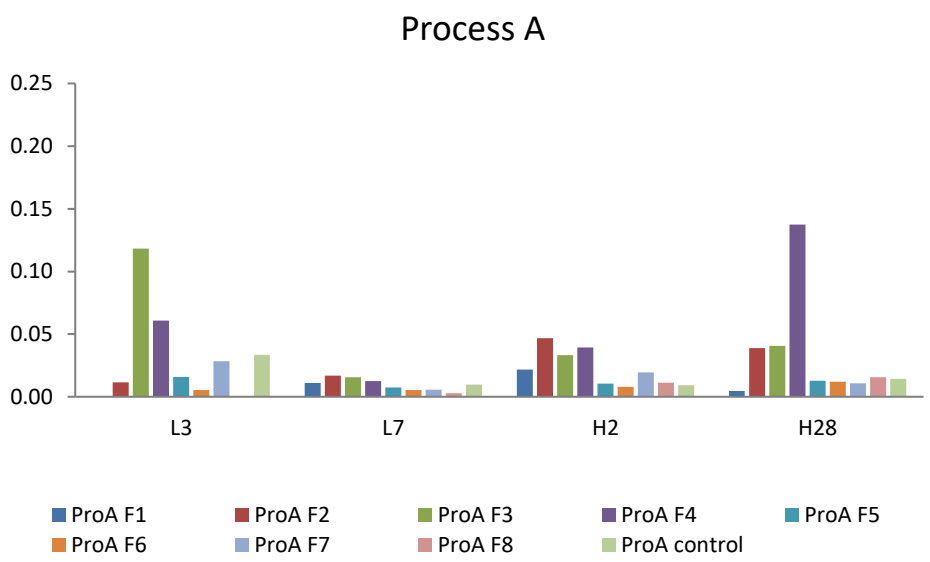


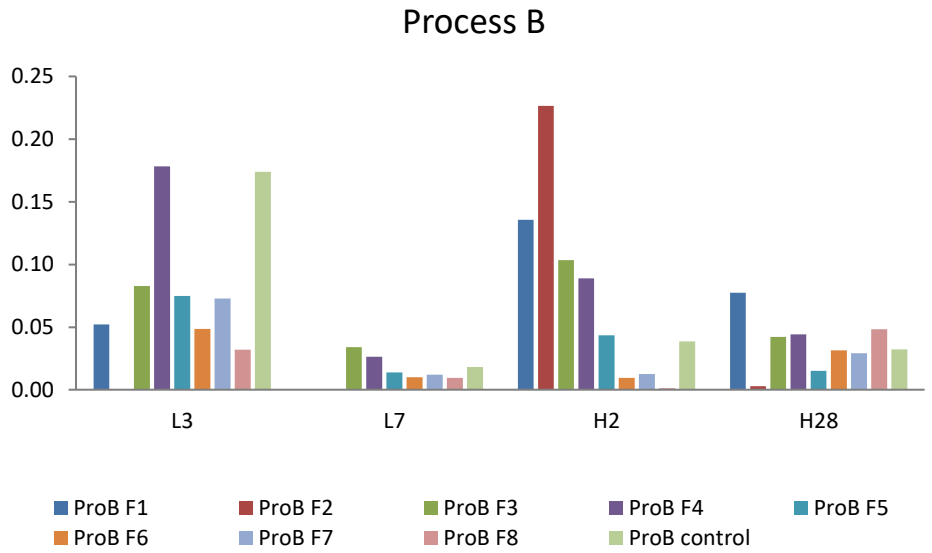




- *Similar oxidations were identified in the fractions from Process A and Process B*
- *Slightly higher levels of H21 and H40 Met oxidations and H11 Trp oxidation were identified in Process A*

Case Study 2: mAb2
Peptide Mapping - Glycation





- *Similar glycations were identified in the fractions from Process A and Process B*
- *Glycation was enriched in acidic peaks in iCIEF*
- *Slightly higher levels of L3 and H2 glycations were identified in Process B*

Summary

- Two iCIEF methods were developed for preparative separation and fraction collection of charge variants from mAb1 and mAb2.
- MS analysis of charge variant fractions from iCIEF showed comparability between Processes for mAb1 and mAb2.
  - Case 1:
    - The same deamidated and oxidated species were observed for Process A and Process B of mAb1.
    - Deamidation was enriched in the acidic species in iCIEF. Slightly higher levels of H36 deamidation were observed in Process A comparing to Process B.
    - No obvious charge variant separation with iCIEF was observed for oxidation. Slightly higher levels of H22 and H40 oxidation were observed in Process A comparing to Process B.
  - Case 2:
    - For mAb2, irreproducible acidic shoulders of the main peak were observed from Process B DS using a platform method, which could impact method reproducibility.
    - An iCIEF method has been developed for charge variant analysis of mAb2 Process B samples with improved resolution between the acidic and main peaks. The method has been demonstrated robust and method qualification has been completed.
    - Assay characterization was performed to elucidate the difference in charge variants from Process A and Process B using CEInfinite iCIEF system and mass spectrometry analysis. In the acidic peaks of iCIEF, higher levels of glycation were identified from Process B, which could contribute to the irreproducible acidic shoulders of the main peak. Overall, MS analysis of charge variant fractions from iCIEF showed comparability between Process A and Process B.

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