

Accelerating Charge Variant Characterization: Deployment of Supersonic cIEF for Rapid IEX Fraction Evaluation

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2026

Contents

- Regulatory and biological importance of charge variants.
- Technological Overview: Supersonic vs. Traditional cIEF.
- Case Studies: Successes and Challenges.
- Technical Outlook: Reproducibility, sensitivity, and further optimization.
- Q&A



01 Charge variant characterization intro –What, why, how

Monitoring Product Variants: A Regulatory Mandate

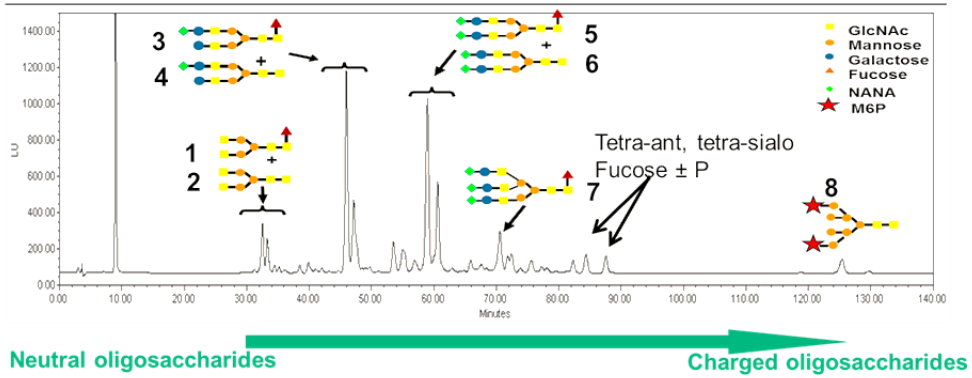
Heterogeneity is Inherent: Proteins are not a single species; they are a population of variants (Size, Charge, Glycans).

Regulatory Requirement: Health authorities (FDA/EMA) require characterization to ensure safety and efficacy.

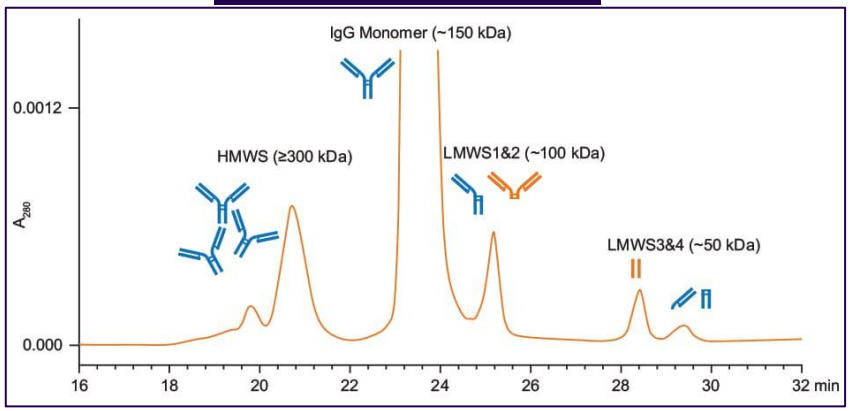
Impact:

- Potency and PK/PD.
- Batch-to-batch consistency.
- Structure-Function relationship.

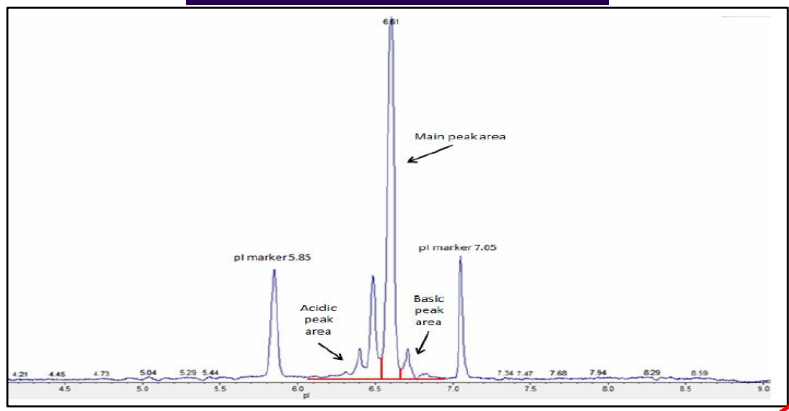
Glycoforms



Size variants



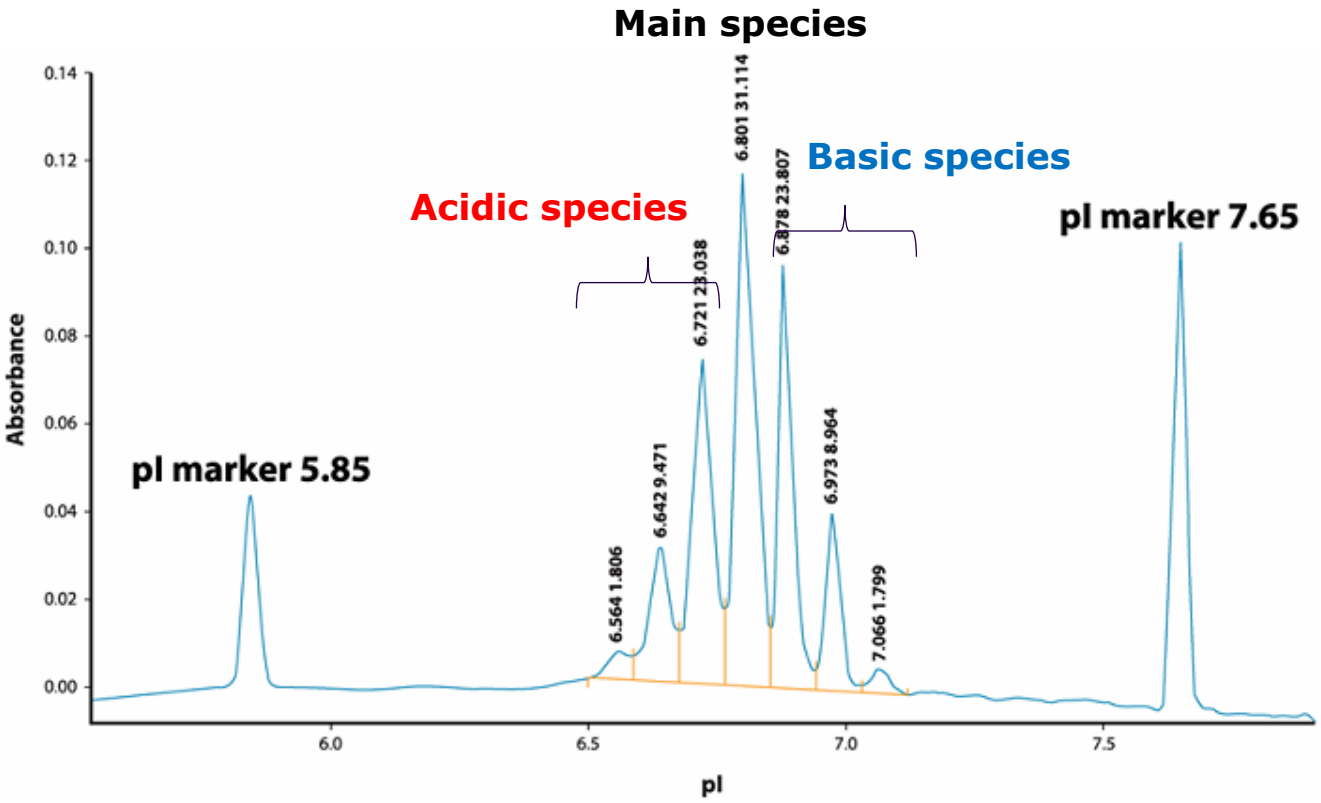
Charge variants



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Objective: Isolate and characterize individual charge variants

Acidic species	Basic species
Sialic acid	C-terminal Lys
Deamidation	N-terminal PyroGlu
Trisulfide bonds	Isomerization of Asp
Cysteinylation	Succinimide
Glycation	Amidation



Questions to Answer:

- What specific post-translational modifications (PTMs) are involved?
- How do these modifications impact product potency?

Opportunities for Charge Variant Characterization



- Early CQA assessment.
- Informing Process Development (PD) activities.
- Proactively addressing potential regulatory queries.

- Full product/impurity understanding.
- Confirming CQAs
- Link to potency (cell-based assays).

02 The Reality in the Lab: Navigating the charge variant Characterization Bottleneck

Why AKTA-based IEX Remains the Gold Standard

- **Quantity Requirements:** Comprehensive structural and functional assays require significant sample amounts (milligram scale).

- **Downstream Testing:** PTM identification (Mass Spec) and potency (cell-based assays).

- **Technical Constraint:** cIEF fractionation cannot yet provide the material needed for a full characterization suite.

COLUMN CHROMATOGRAPHY

AKTA

'gold standard'

Preparative tool



CAPILLARY ELECTROPHORESIS

MAURICEFLEX



MAURICE

'gold standard'

analytical tool

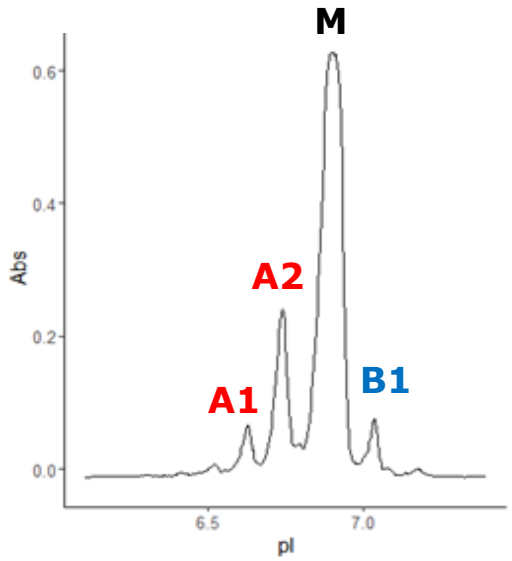


Caveat: isolation ***quantity*** is limited

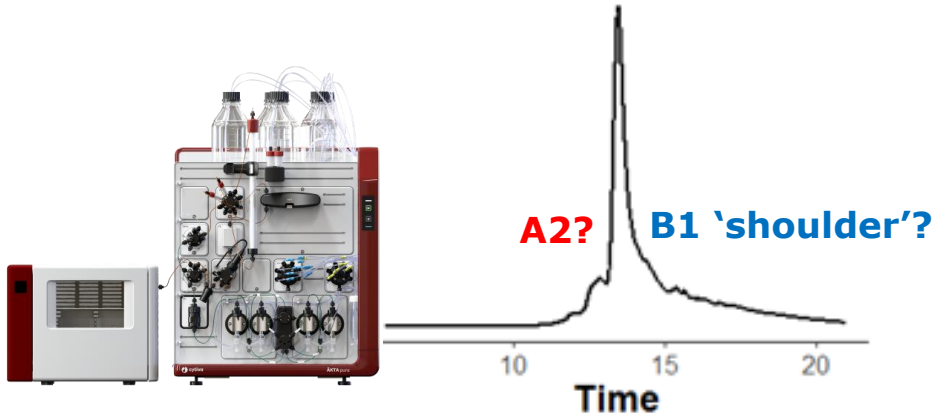
The Disconnect: Analytical cIEF vs. Preparative Chromatography

cIEF profile:





Chromatography (IEX) profiles:



- High Resolution
- Analytical Scale
- Calibrated pI results

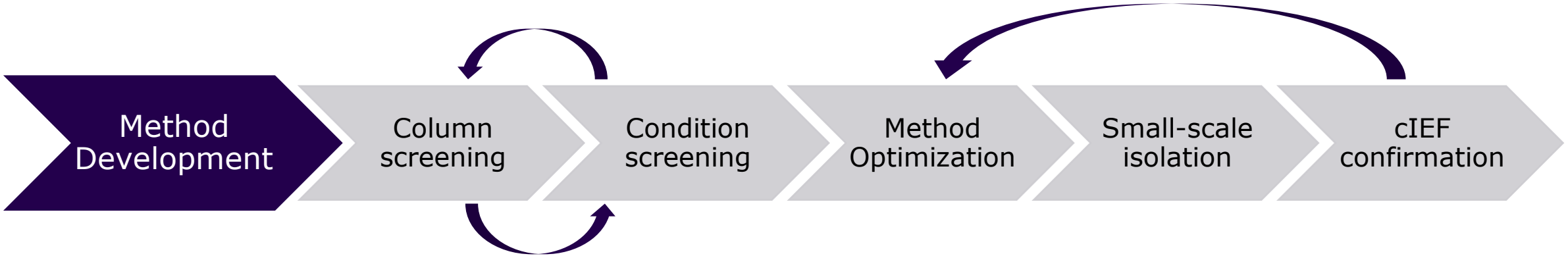
- Lower Resolution
- Preparative Scale
- Physical Fractions

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Electropherograms from Steve D'Eri

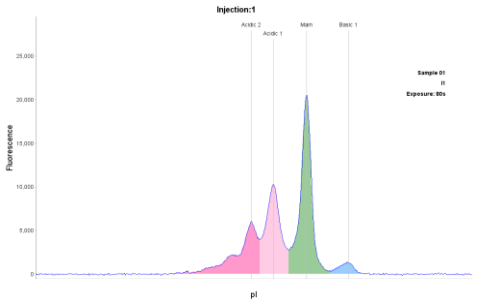
Method Development: A Sea of Parameters

Parameters	Variable 1	Variable 2	Variable 3	Variable 4
Column type	Strong AEX	Weak AEX	Strong CEX	Weak CEX
Column chemistry	Resin 1	Resin 2	Resin 3	Resin 4
Bead size	Small (↑resolution↑pressure)		Large (↓resolution↓pressure)	
Elution mode	pH gradient		Salt gradient	
Mobile phase composition	pH	Molarity	Buffer/ion species	
Gradient type	Linear	Step	Complex	
Loading	µg – mg	Dilution	Buffer exchange	

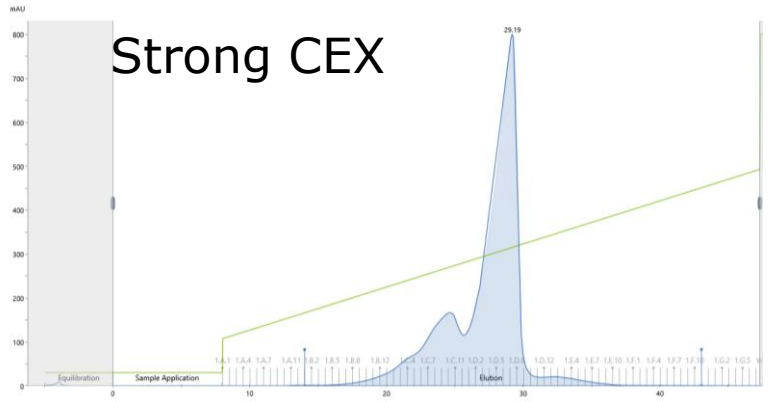


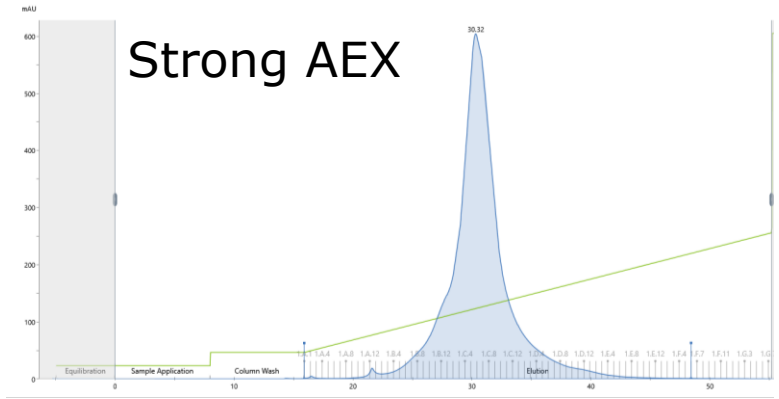
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Example: Column screening



Target Profile

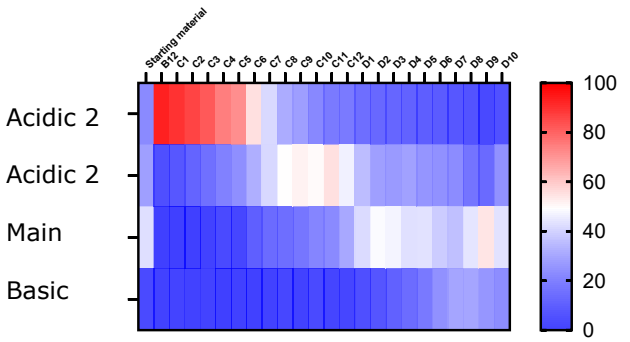


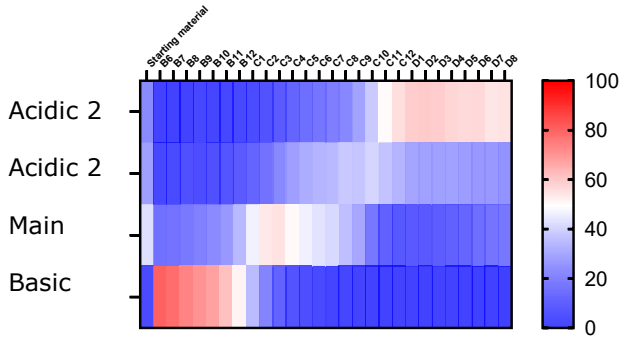


Resin Comparison



Buffer exchange to remove salt





Results:

CEX: Good for A1&2; poor for Basic

AEX: Good for B&A2; poor for A1

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The Pain Point: Analysis Paralysis

Sample Load: 30+ samples per IEX condition.

Batch Run: 3 conditions = 96-well plate.

Maurice Run Time: >24 hours per plate.

Bottleneck: We generate fractions faster than we can analyze them.



Standard cIEF Methodology

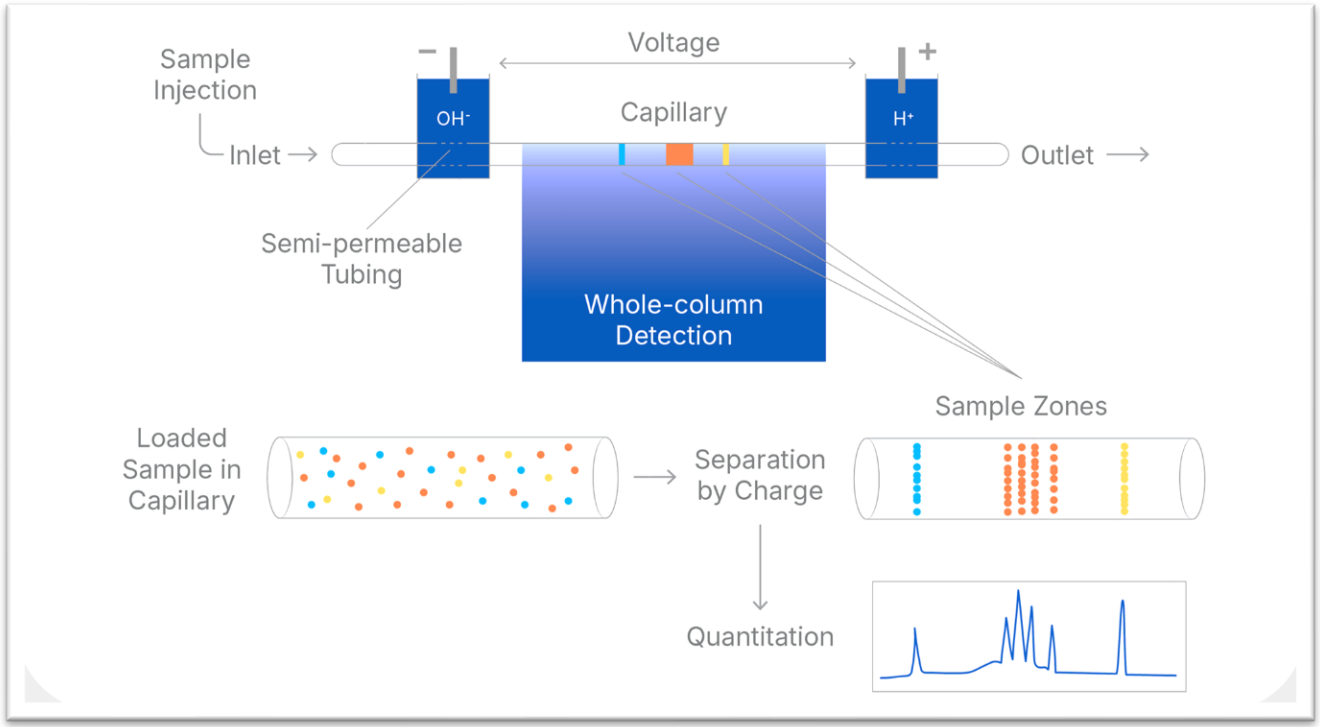
Master Mix	Purpose
Water	
8M urea	Denatures proteins, maintains solubility
1% Methylcellulose	Improve protein separation, act as capillary coating
3-10 Pharmalyte	Establishes pH gradient range
8-10.5 Pharmalyte	
Low pI marker	Creates standardization for sample measurements
High pI marker	
Sample	

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Two-Stage Separation (8 min run)



- **Stage 1 (Pre-focusing):** 1500V for 1 min.
  - Establishes pH gradient gently.
  - Prevents "Joule Heating" .
- **Stage 2 (Focusing):** 3000V for 7 min
  - Sharpens zones into high-resolution peaks.
  - Overcomes diffusion



03 Beyond Traditional Constraints: Accelerating toward SupersonicIEF.

The Supersonic iCIEF Concept

Electrophoresis

WILEY-VCH

ELECTROPHORESIS

RESEARCH ARTICLE OPEN ACCESS

Development of the SupersonicIEF Method for High-Throughput Charge Variant Analysis

Will McElroy | Christopher D. Heger

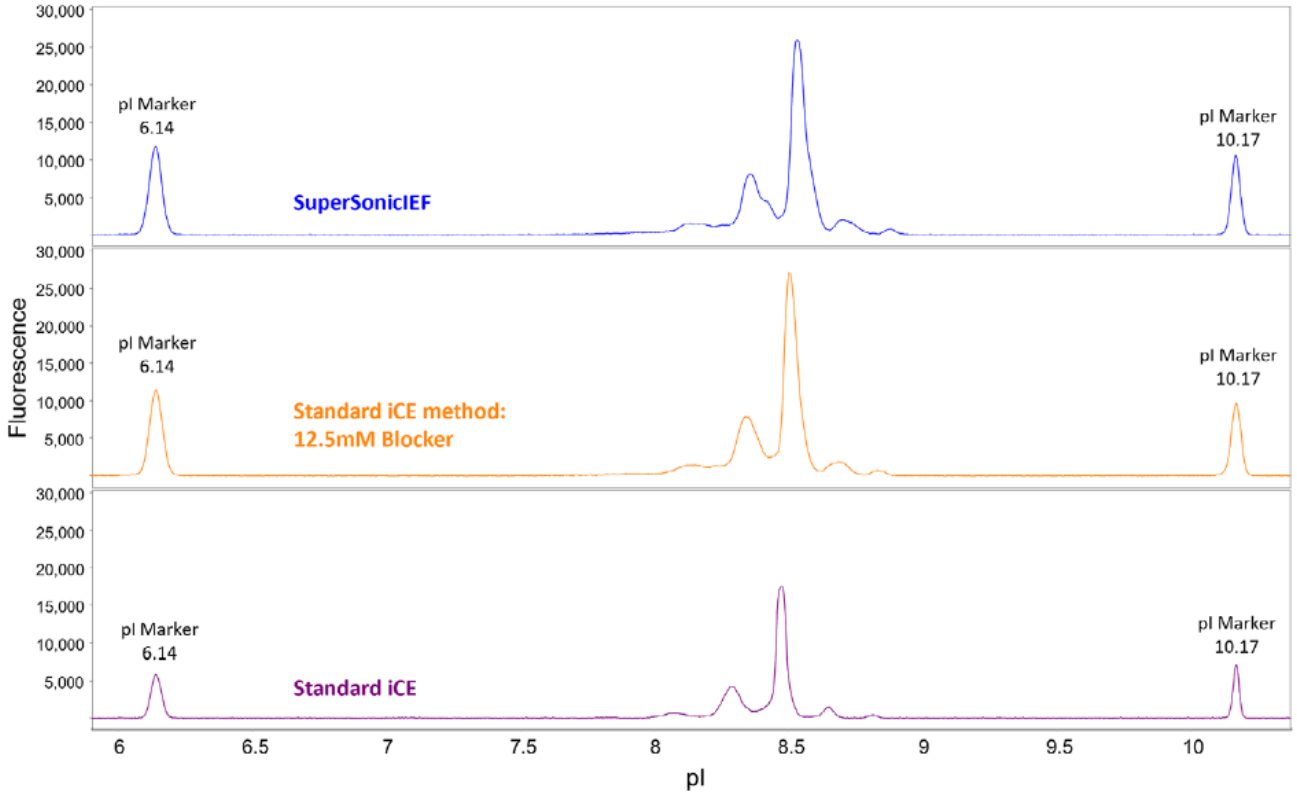
Applications Science, ProteinSimple, a Bio-Techne Brand, San Jose, California, USA

Correspondence: Will McElroy (will.mcelroy@bio-techne.com)

Received: 7 June 2024 | **Revised:** 6 September 2024 | **Accepted:** 16 September 2024

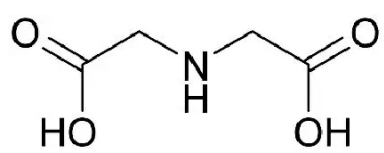
Keywords: charge heterogeneity | fusion proteins | imaged capillary isoelectric focusing | monoclonal antibodies | platform method

	Platform Method		SupersonicIEF method	
	Time	Voltage	Time	Voltage
Focusing Step 1	1 min	1500V	0.3min	1500V
Focusing Step 2	7-10 min	3000V	0.2min	2500V
Focusing Step 3	N/A	N/A	0.2 min	3000V
Focusing Step 4	N/A	N/A	3.5 min	4300V

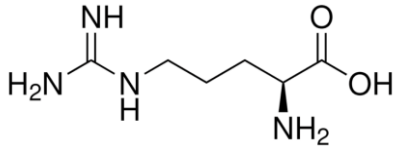


Comparison of Belimumab analyzed

Blockers: Iminodiacetic Acid (IDA)



L-Arginine



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What Do Blockers (Spacers) Do?

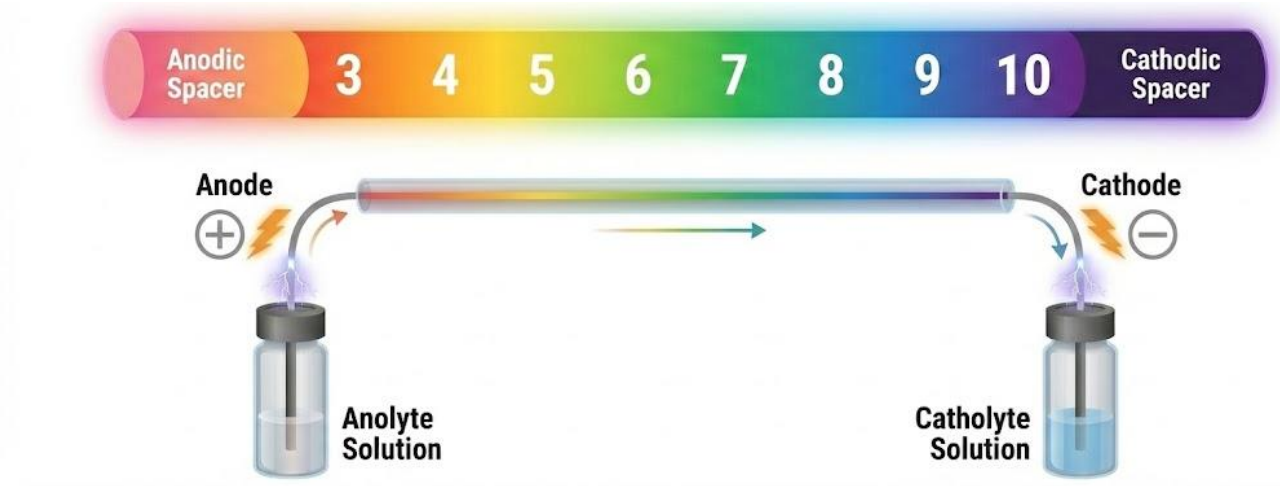
Problem: Proteins can focus at capillary ends (and be out of detector view).

Action:

IDA: Blocks the Anode end segment.
L-Arginine: Blocks the Cathode end segment.

Effect: Squeezes the pH gradient to keep focused bands within the 280nm detector window

IDA L-Argine



Platform Adaptation with blockers and high voltage

	Platform Master Mix	SupersonicIEF Master Mix
Methyl Cellulose	=	=
Urea	=	=
Pharmalytes	=	=
pI Markers	=	=
IDA	N/A	12.5mM
L-Arginine	N/A	12.5mM
Sample	=	=
Water	↑	↓

Modified Recipe

	Platform Method		SupersonicIEF method	
	Time	Voltage	Time	Voltage
Focusing Step 1	1 min	1500V	0.3 min	1500V
Focusing Step 2	7 min	3000V	0.2 min	2500V
Focusing Step 3	N/A	N/A	0.2 min	3000V
Focusing Step 4	N/A	N/A	3.5 min	4300V
Total Time (per injection)	8 min		4.2 min	

Standard voltage

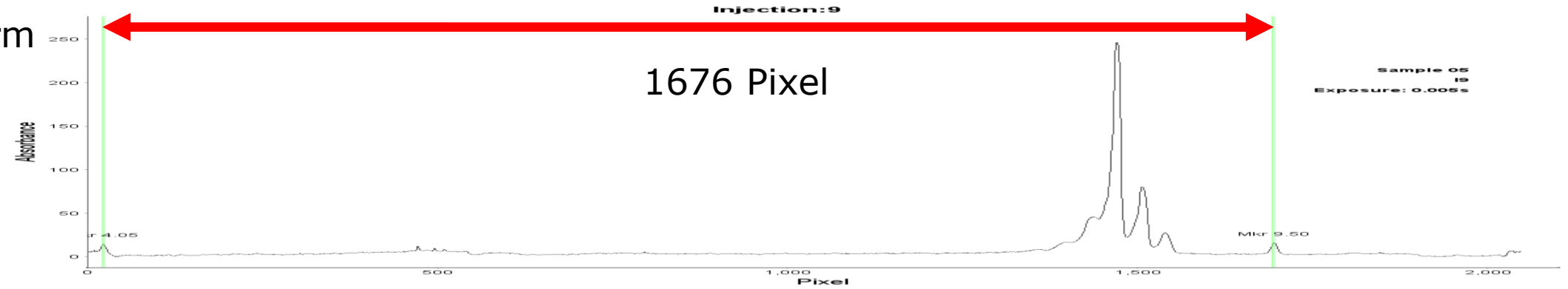
High voltage

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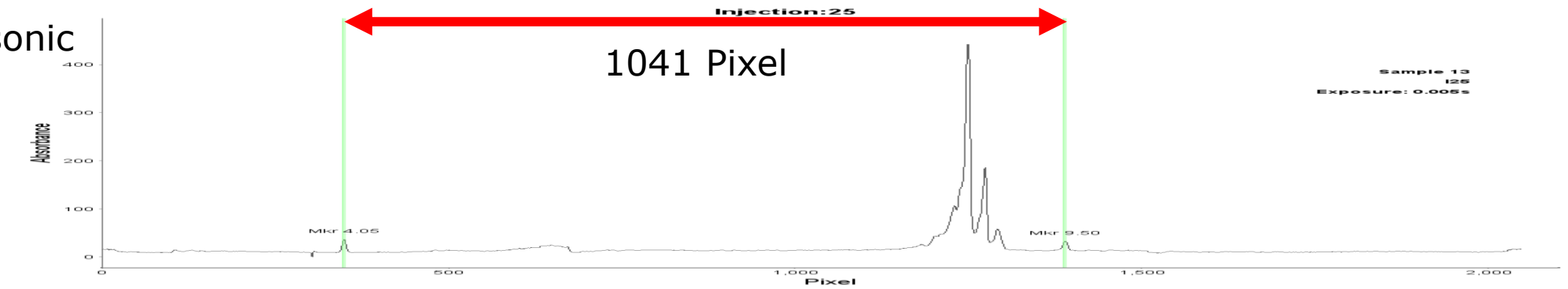
Note: using existing Maurice instrument

Case 1: mAB A (IgG1)– Raw Pixels

Platform



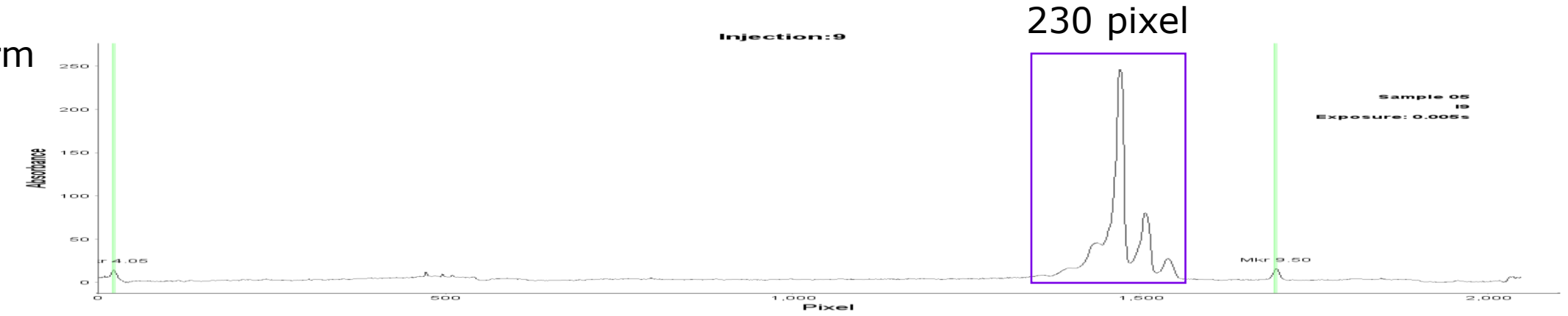
Supersonic



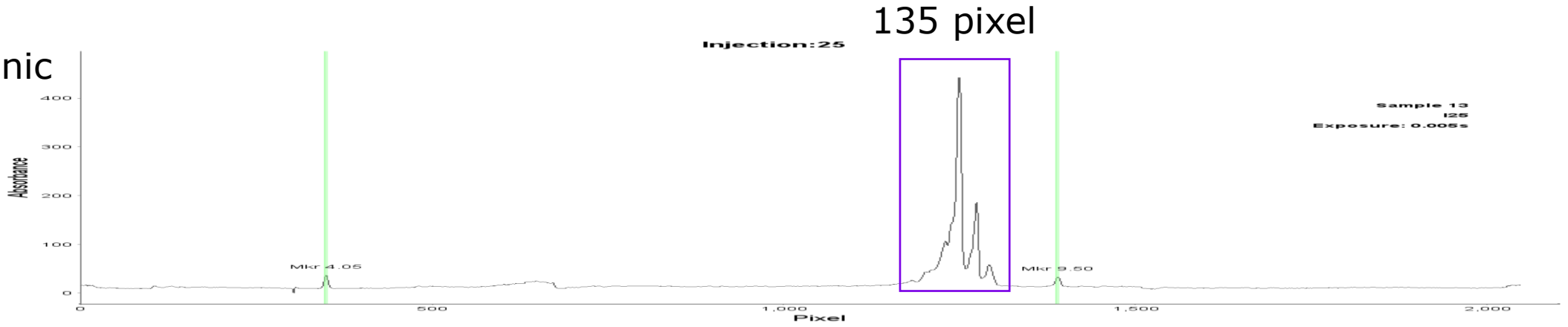
Result: Visual evidence of blocker-induced gradient compression

Case 1: mAB A – Peak width

Platform

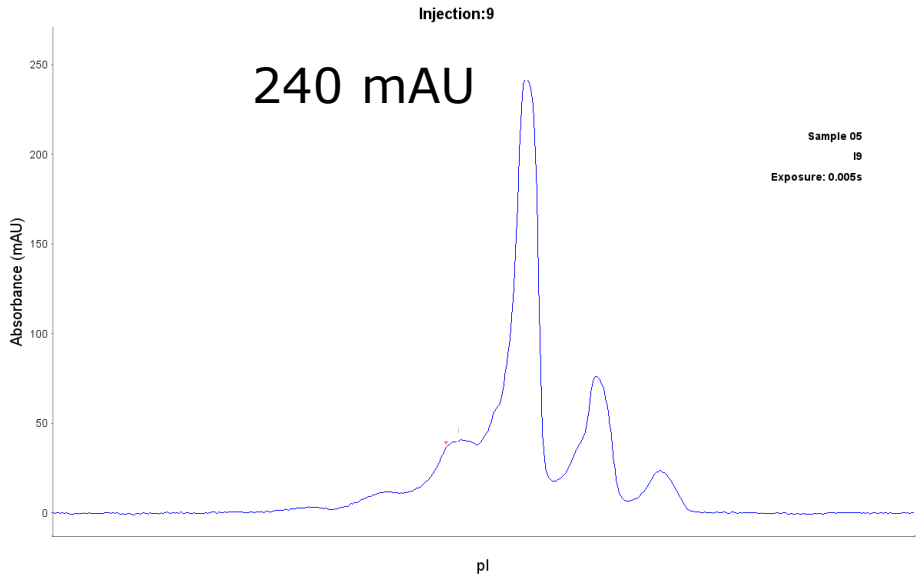


Supersonic

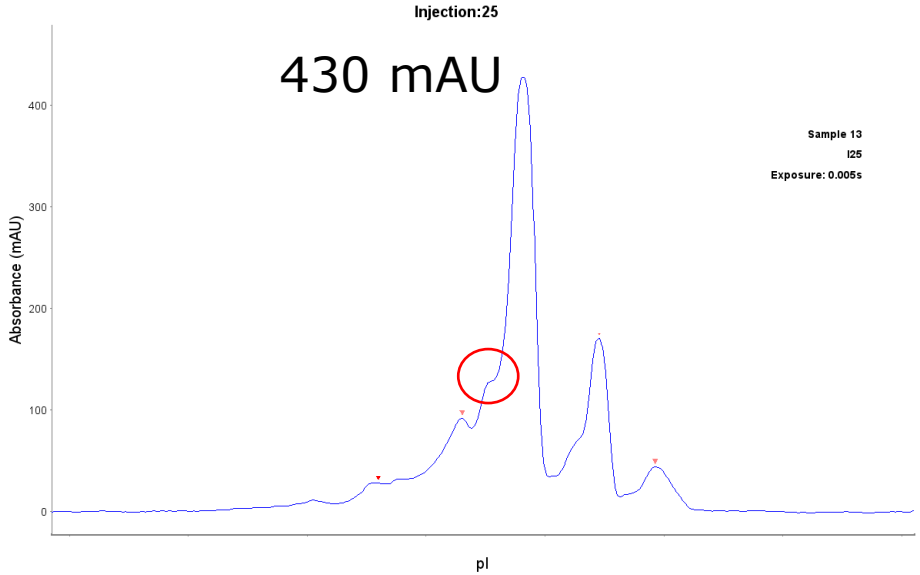


Observation: Significant narrowing of isoforms

Case 1: mAB A – pI Calibrated Profile



Platform

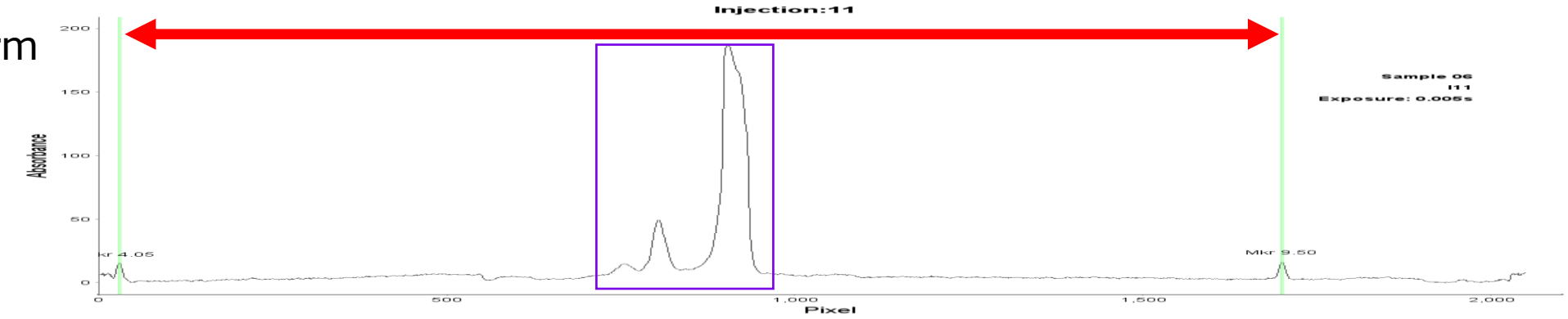


Supersonic

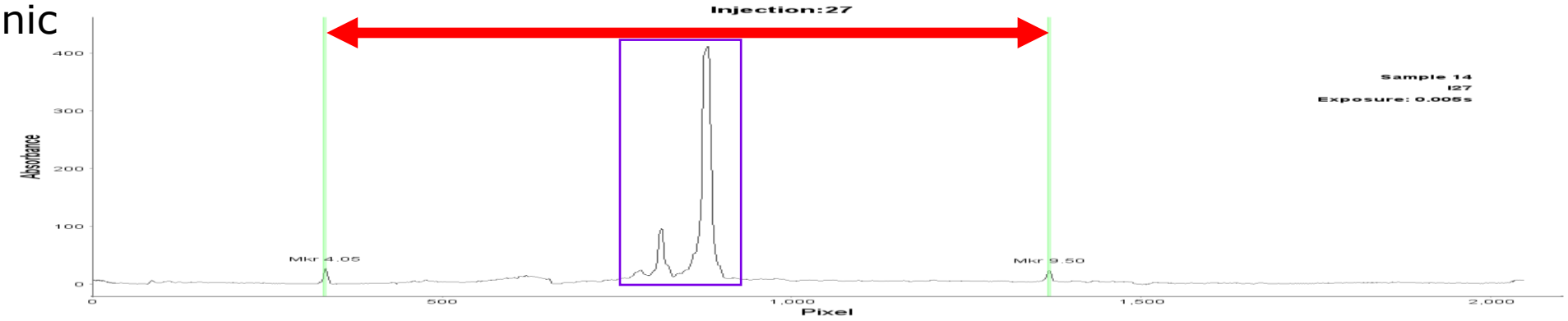
	Platform method	SupersonicIEF
Main pI	8.5-9.0	=
Acidic peak area percentage (%)	18.8	19.9
Main peak area percentage (%)	55.8	54.7
Basic Peak area percentage (%)	25.4	25.3

Case 2: mAB B (IgG1) – Raw Data

Platform

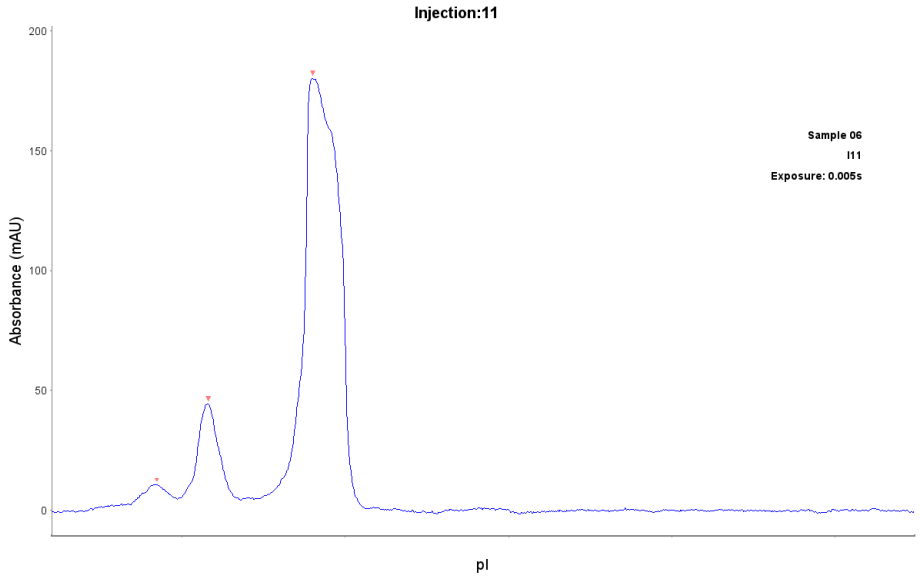


Supersonic

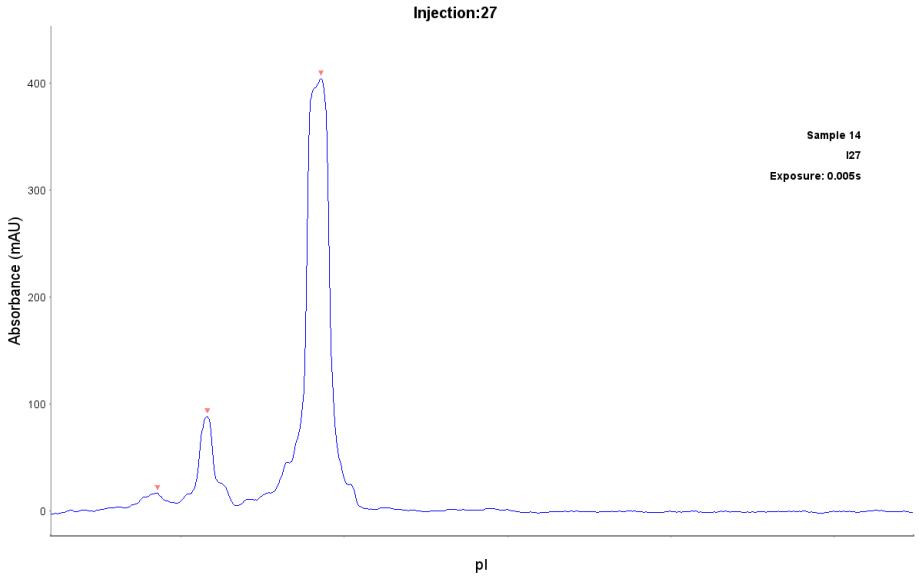


- Confirmed compression of marker-to-marker distance.
- Confirmed decrease in protein peak width.

Case 2: mAB B – Calibrated Results



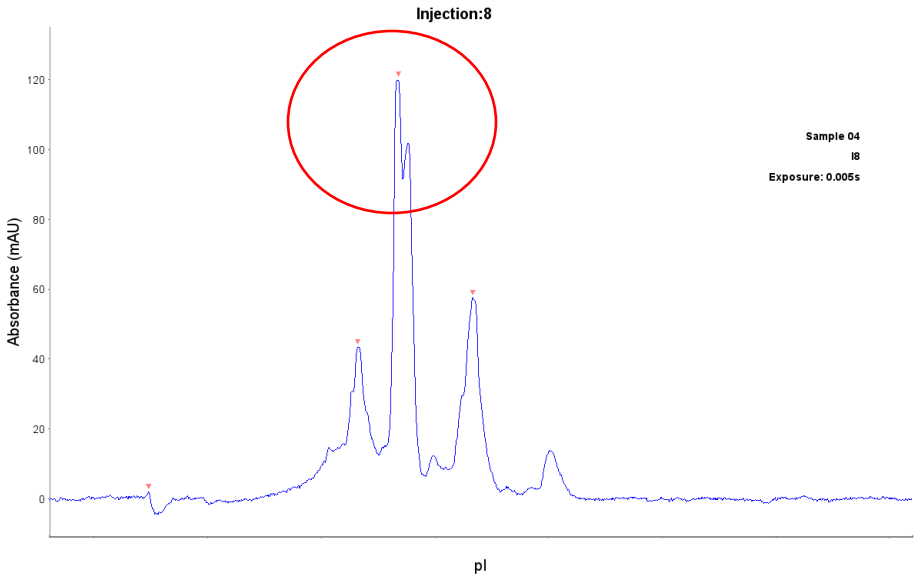
Platform



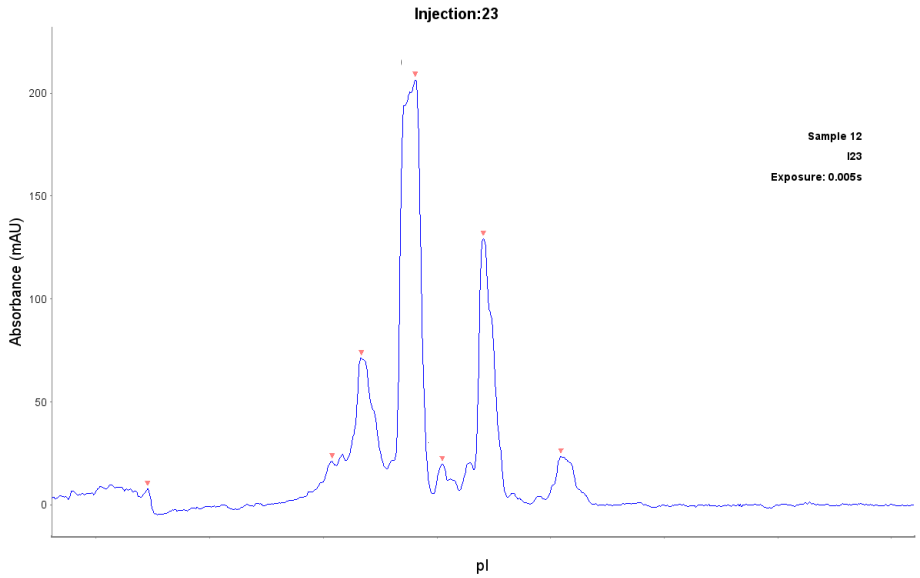
Supersonic

	Platform method	SupersonicIEF
Main pI	6.5-7.0	=
Acidic peak area percentage(%)	18.1	18.7
Main peak area percentage(%)	81.6	81.0

Case 3: mAB C (IgG4)



Platform



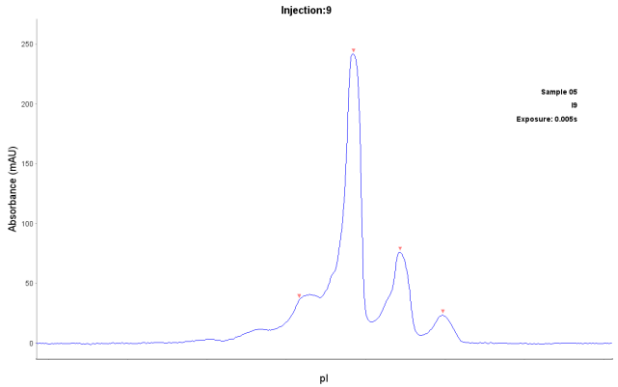
Supersonic

	Platform method	SupersonicIEF
Main pI	6.5-7.0	=
Acidic peak area percentage(%)	24.4	23.9
Main peak area percentage(%)	45.0	44.3
Basic Peak area percentage(%)	30.5	31.9

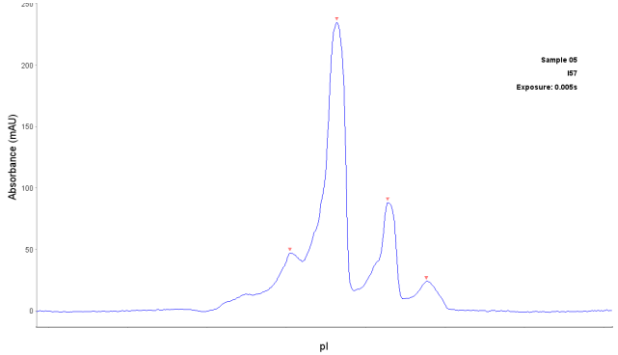
Do We Really Need the Blockers?

mAB A

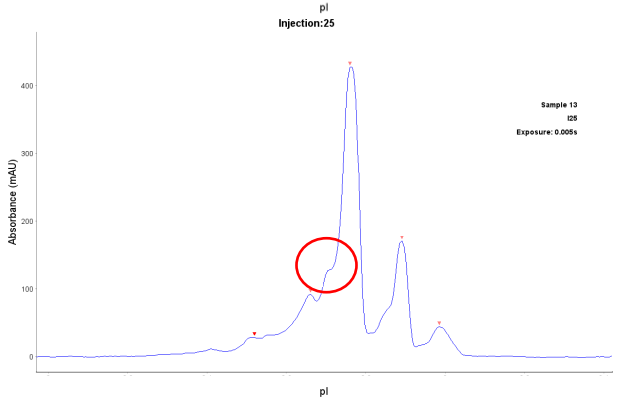
Platform method



Supersonic Voltage + Platform Master Mix (No Blocker).



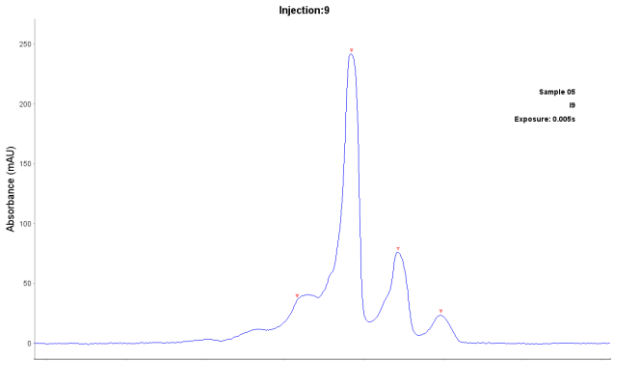
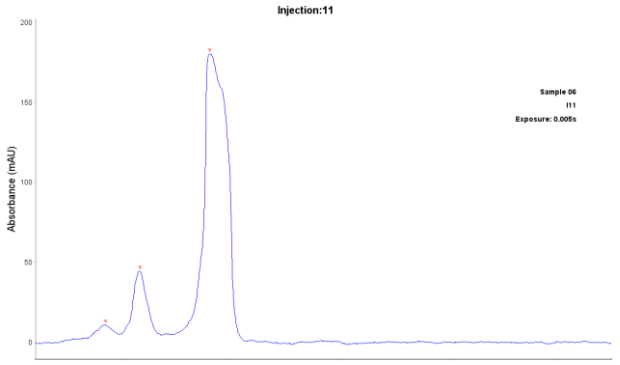
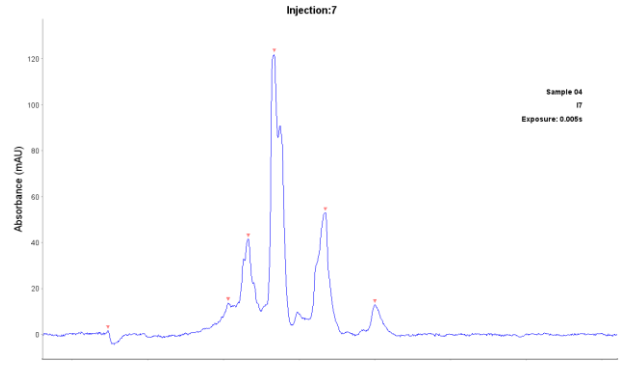
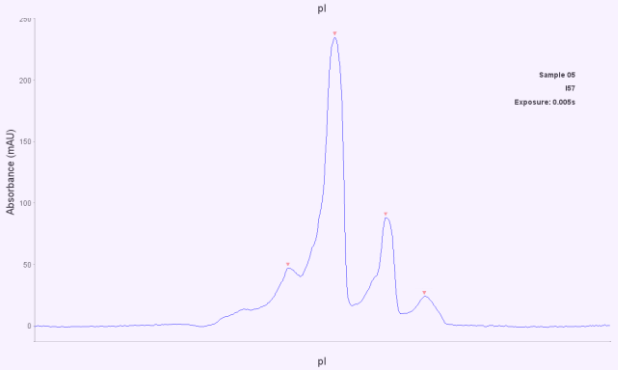
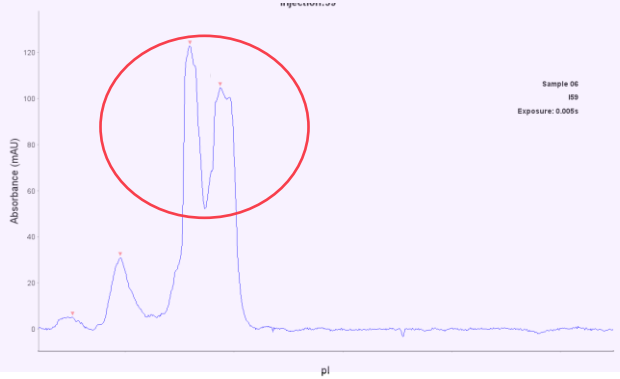
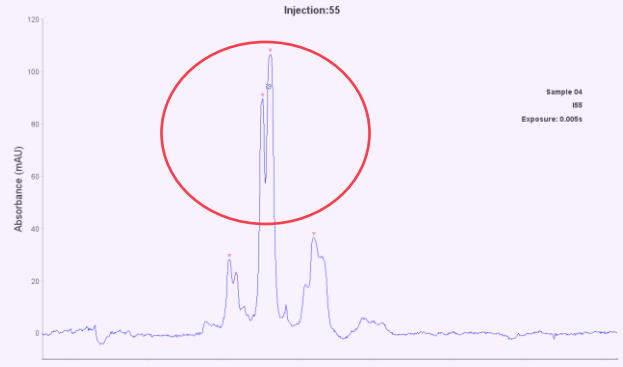
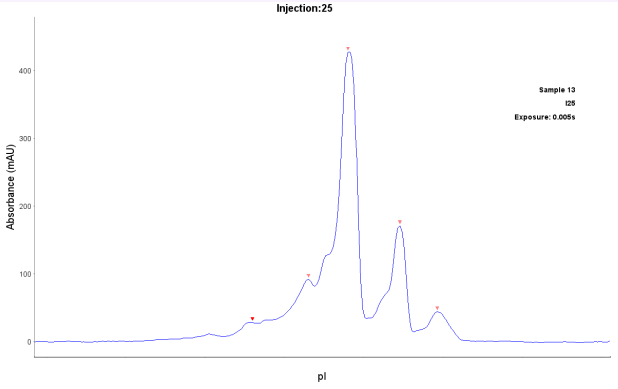
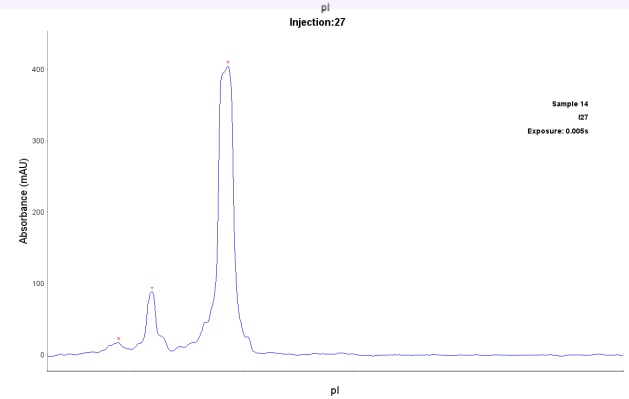
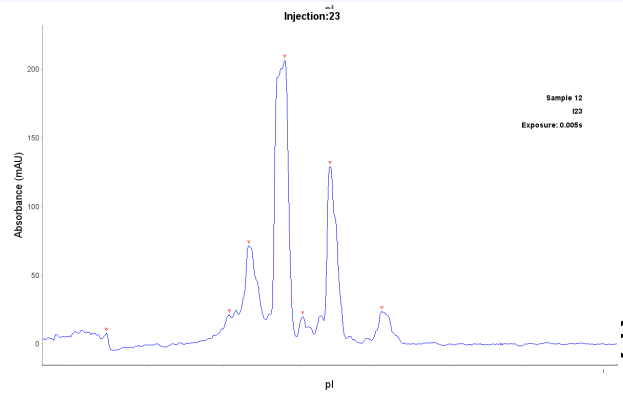
SupersonicIEF (blocker & voltage)



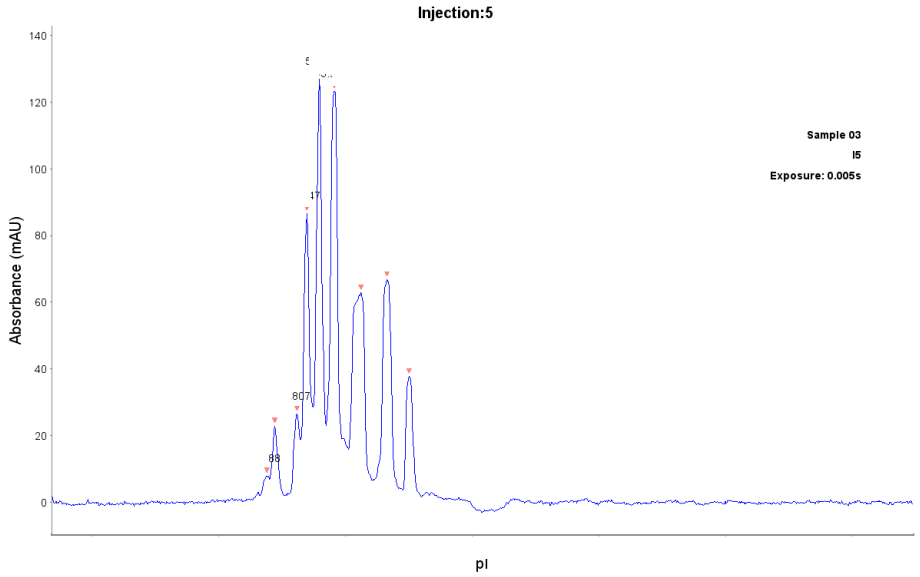
'No' in the case of mAB A, however…

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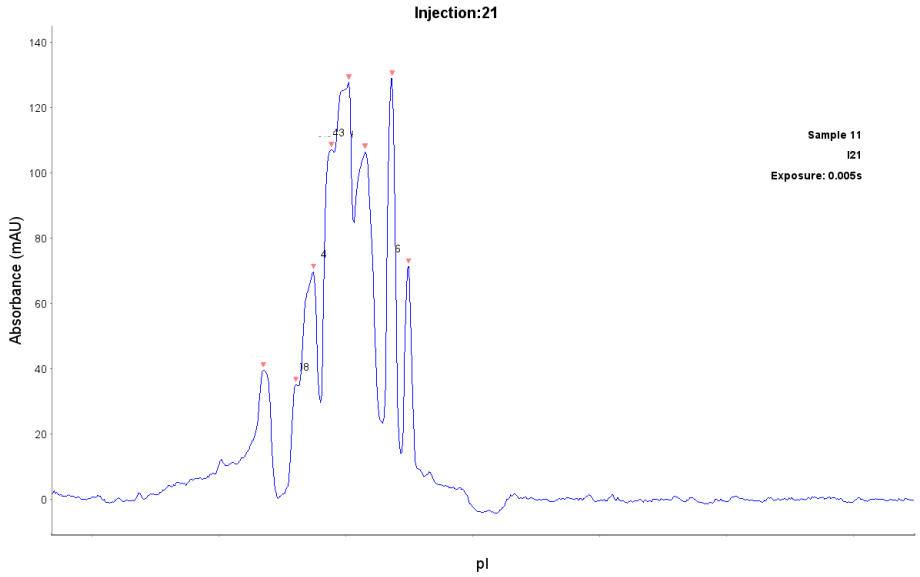
The Blockers are required in some cases

	mAB A	mAB B	mAB C
Platform method			
Supersonic Voltage + Platform Master Mix (No Blocker).			
SupersonicIEF (blocker & voltage)			

Limitations: mAB D (Bi-specific)



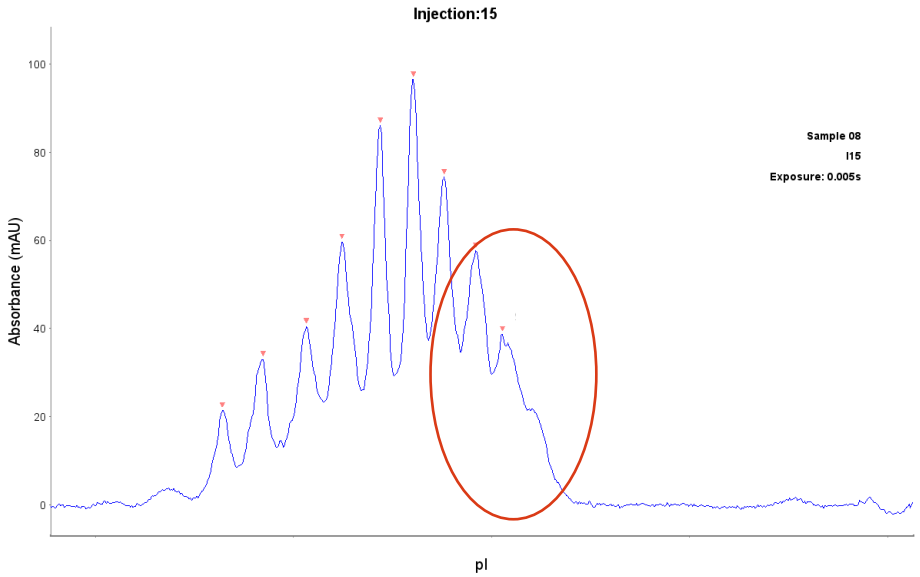
Platform



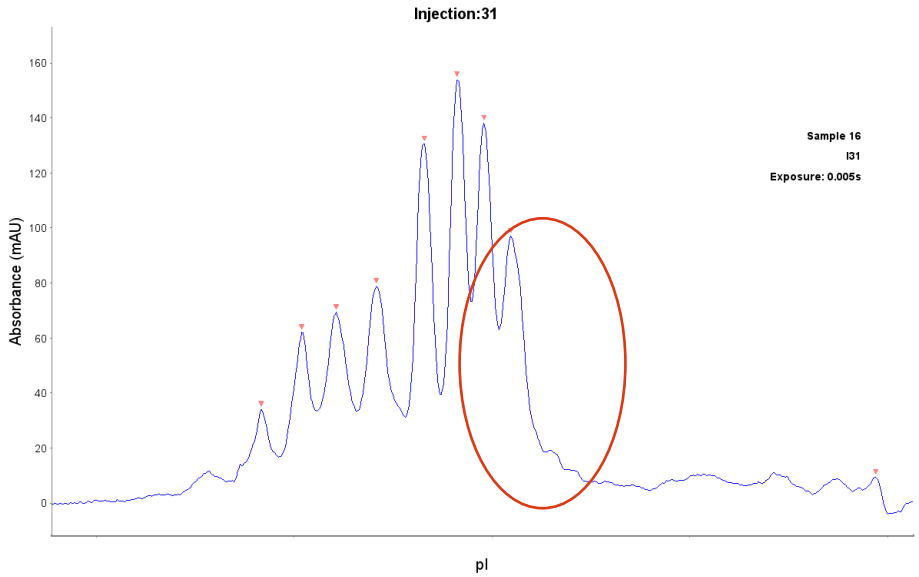
Supersonic

- **Challenge:** Fab N-glycosylation sites in addition to Fc sites.
- **Result:** "Hedgehog" profile (8 peaks in platform) compressed into **6 peaks** in Supersonic.
- **Verdict:** Over-compression lost critical resolution

Limitations: Enzyme



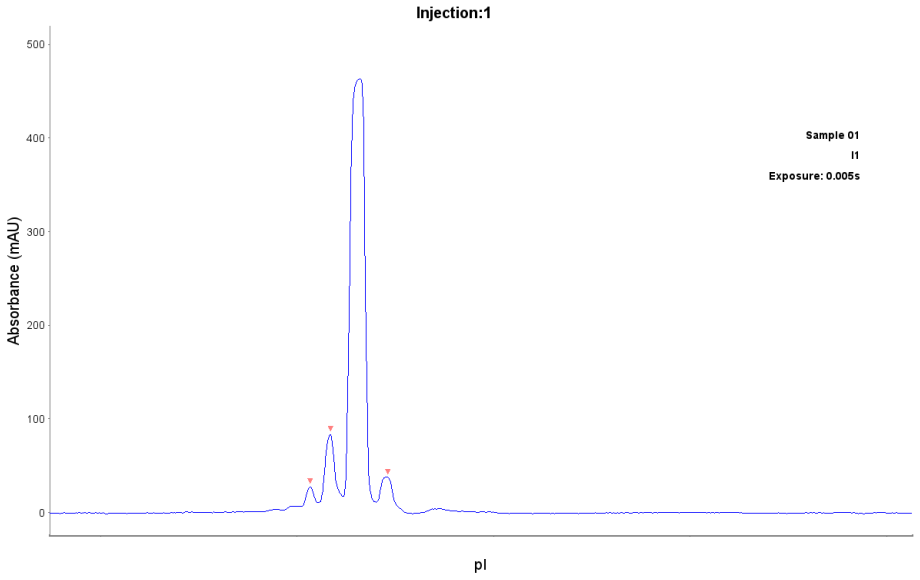
Platform



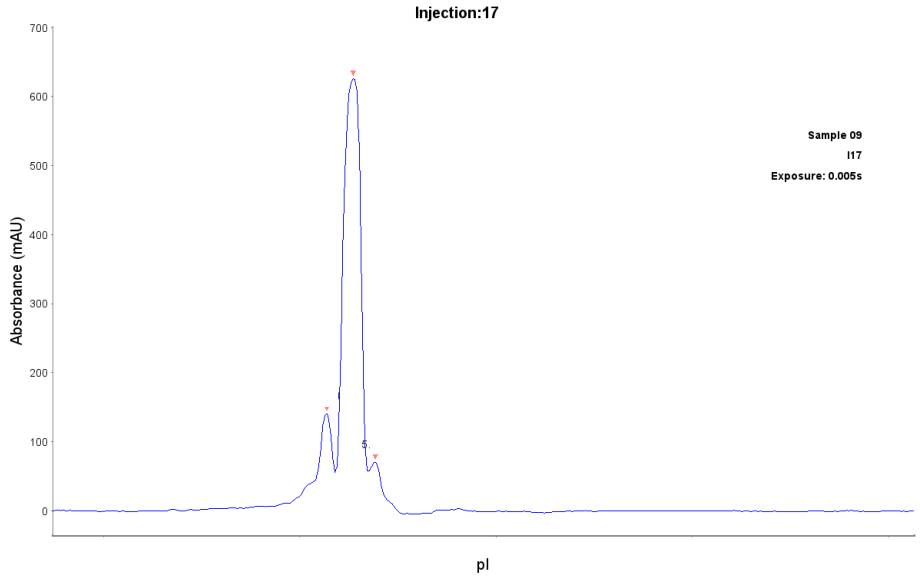
Supersonic

- **Challenge:** Multiple N-glycosylation sites.
- **Result:** Platform (10 peaks) vs. Supersonic (**8 peaks**).

Limitations: mAB E (IgG1)



Platform



Supersonic

- **Result:** Poor separation in Supersonic.
- **Observation:** Charge variants became shoulders of the main peak rather than distinct peaks.

Identifying the Pattern: The pI Correlation

Molecule	Type	Main pI	Supersonic Result
mAB A	IgG 1	8.5-9.0	Excellent
mAB B	IgG 1	6.5-7.0	Excellent
mAB C	IgG 4	6.5-7.0	Excellent
mAB D	Bi-spec (multiple N-glycan site)	5.5-7.0	Poor
mAB E	IgG 1	5.5-7.0	Poor
Enzyme	Enzyme(multiple N-glycan site)	5.0-5.5	Poor

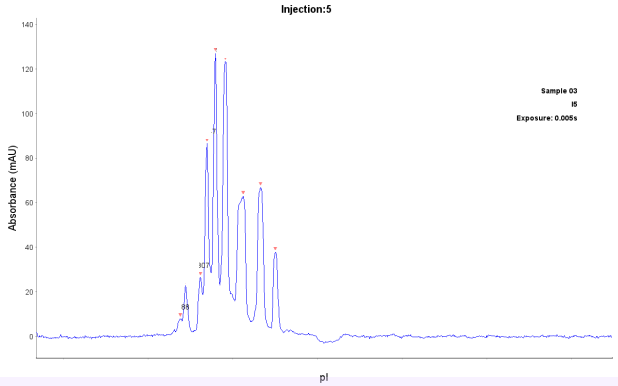
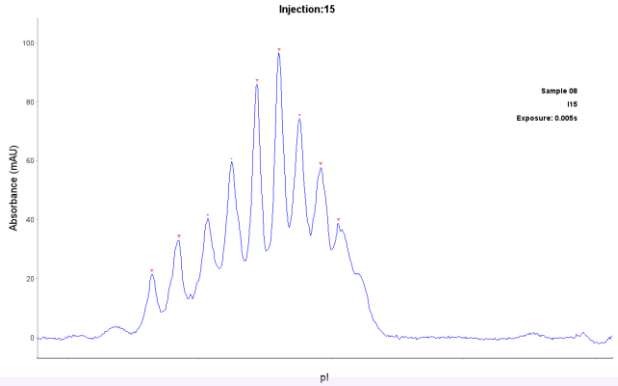
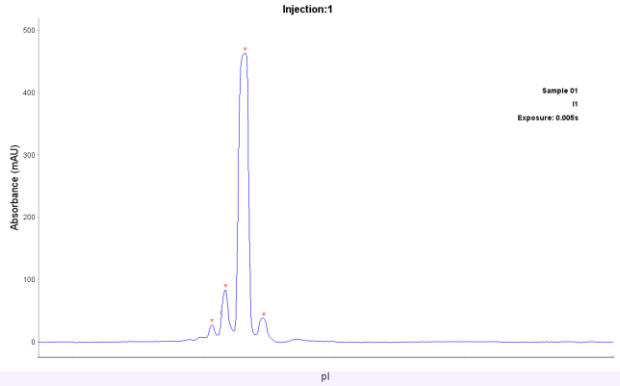
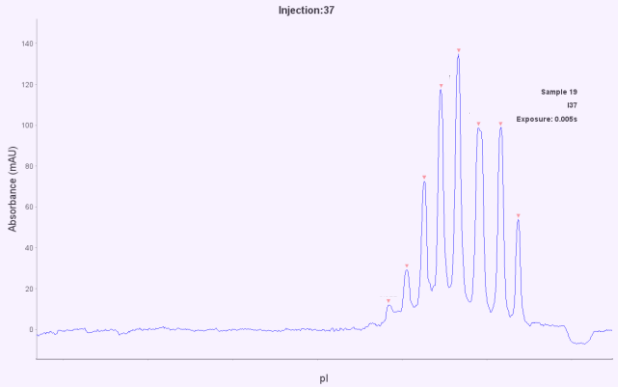
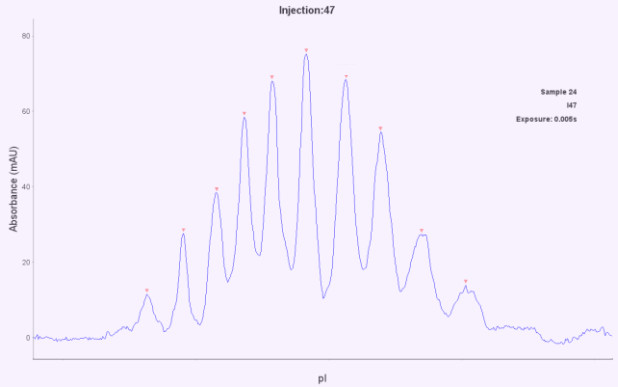
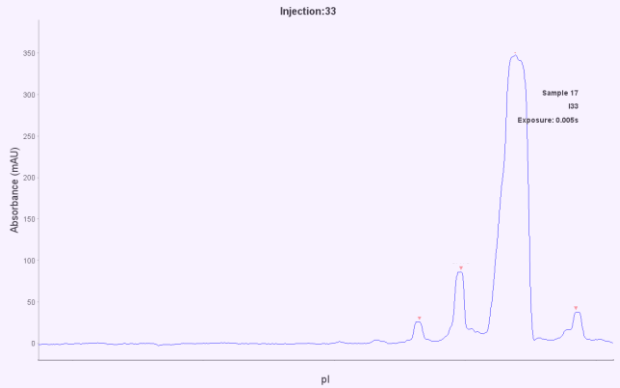
Tuning the Platform for Low pI

Master Mix	Purpose
Water	
8M urea	Denatures proteins, maintains solubility
1% Methylcellulose	Improve protein separation, act as capillary coating
3-10 pharmalyte	Establishes pH gradient range
8-10.5 pharmalyte	
Low pI marker	Creates standardization for sample measurements
High pI marker	
Sample	

- **Problem:** Mismatch for low pI molecules.
- **The Fix:** Replace 8-10.5 Pharmalyte with **5-8 Narrow Pharmalyte**.

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Success with Tuned Supersonic

	mAB D	Enzyme	mAB E
Platform method			
Supersonic Replace 8-10.5 Pharmalyte with 5-8 Narrow Pharmalyte			

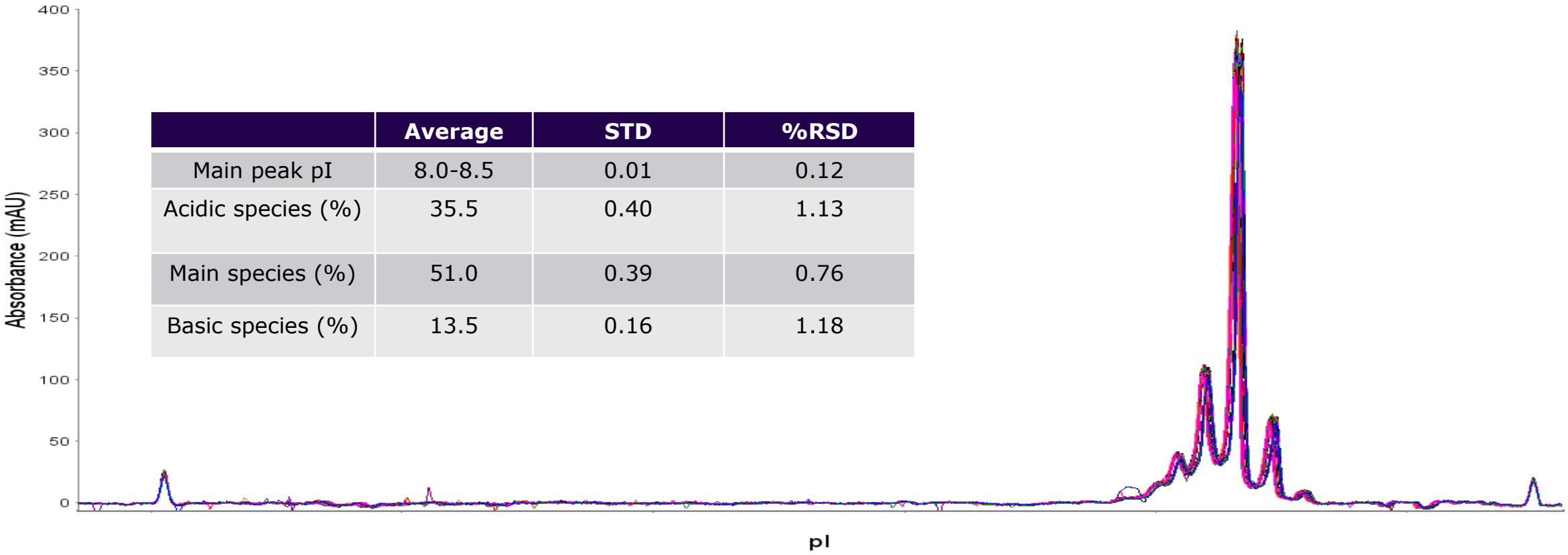
Result: Restored 8 peaks for mAB D and 10 peaks for Enzyme.

Benefit: Improved peak resolution over the original platform method

Method robustness

'scientifically sound' method to support characterization work

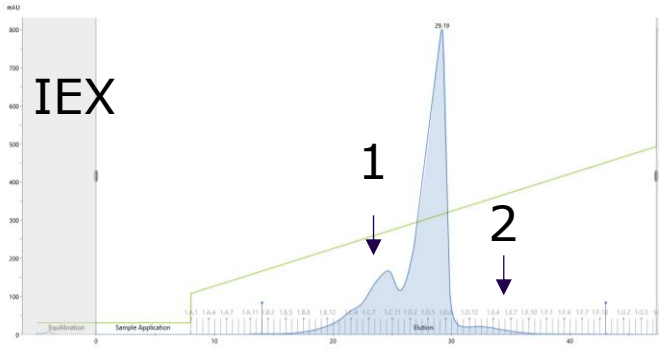
	Average	STD	%RSD
Main peak pI	8.0-8.5	0.01	0.12
Acidic species (%)	35.5	0.40	1.13
Main species (%)	51.0	0.39	0.76
Basic species (%)	13.5	0.16	1.18



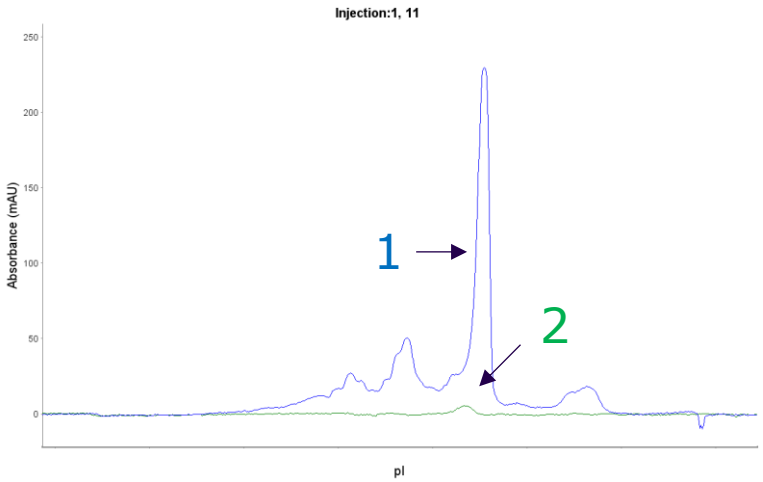
Test Conditions: Multiple wells, cartridges, and operators

Profile compression has the unexpected benefit of better Sensitivity and LOQ

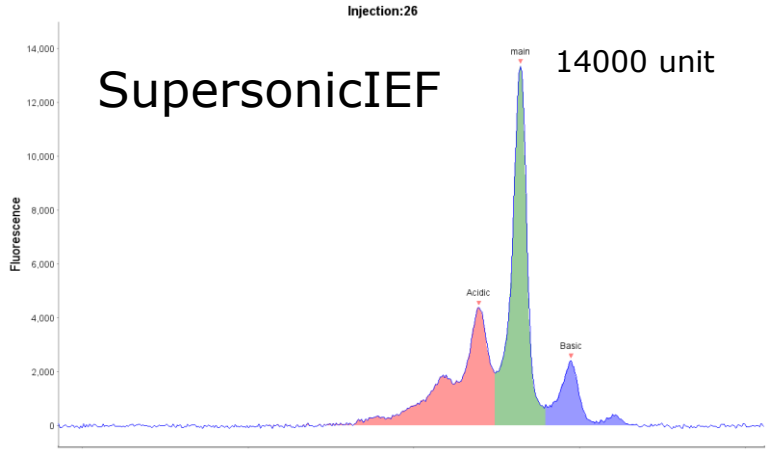
Problem: IEX fractions can be too diluted (below UV LOQ).

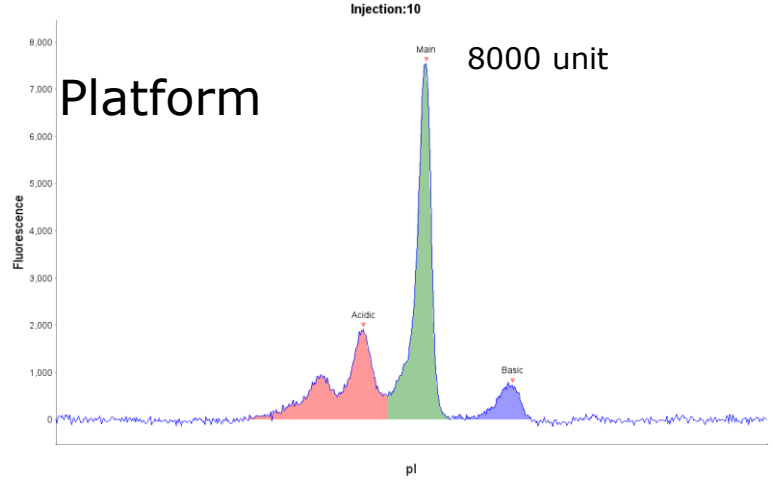


UV mode



Fluorescence mode



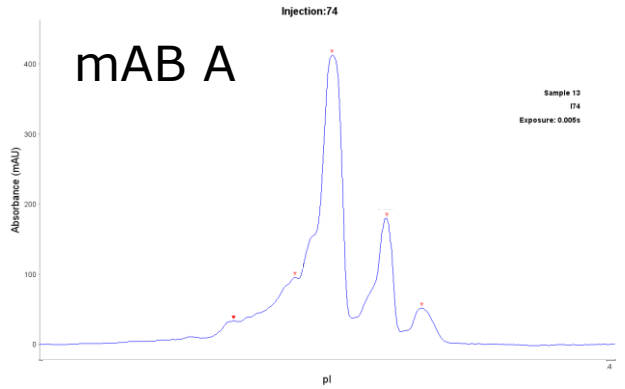


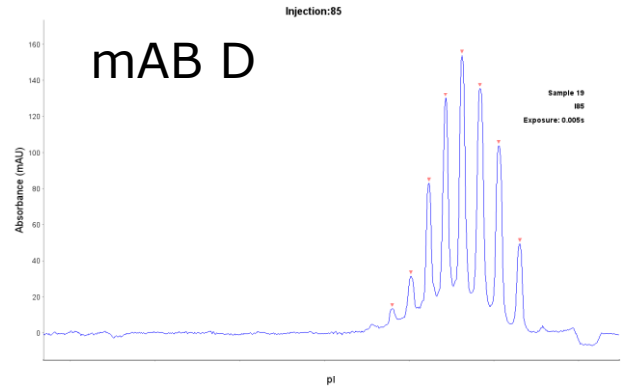
- **Solution:** Maurice Native Fluorescence (Ex 280nm / Em 350nm).
- **Case Study (0.025 mg/mL):** Supersonic detected two basic peaks where the platform method only detected one.
- **Outcome:** Higher peak intensity improves the LOQ.

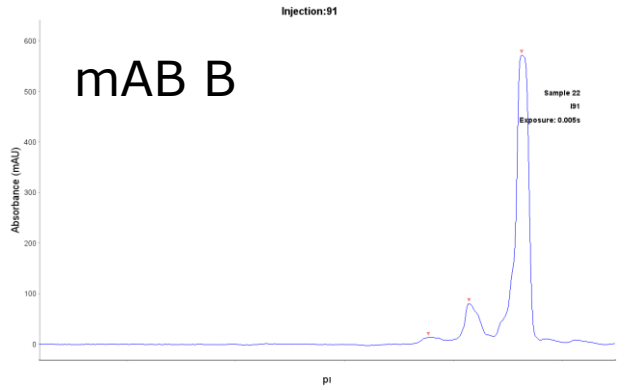
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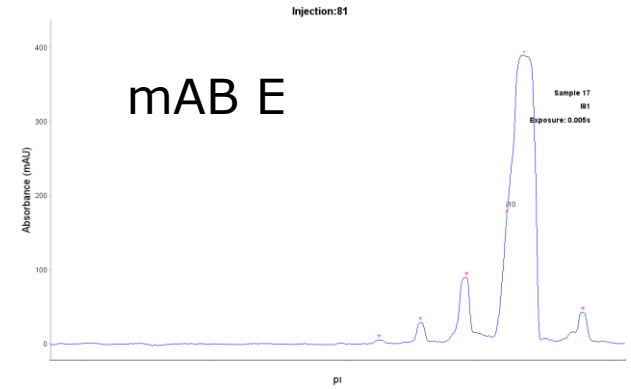
Pushing the Limits: 3.2 Minute Run

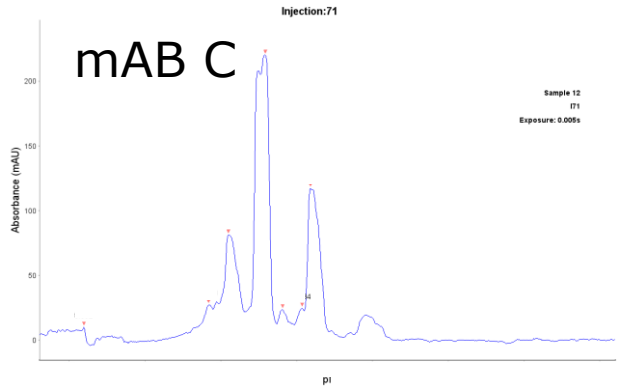
	Platform Method		SupersonicIEF method		HypersonicIEF method?	
	Time	Voltage	Time	Voltage	Time	Voltage
Focusing Tim 1	1 min	1500V	0.3min	1500V	0.3min	1500V
Focusing Tim 1	7 min	3000V	0.3 min	2000V	0.3 min	2000V
Focusing Tim 2	N/A	N/A	0.2 min	3000V	0.2 min	3000V
Focusing Tim 4	N/A	N/A	3.5 min	4300V	2.5	4300V
Focusing Time (one injection)	8 min		4.2 min		3.2 min	
96- well plate time	~21 hours		~12 hour		10 hour	

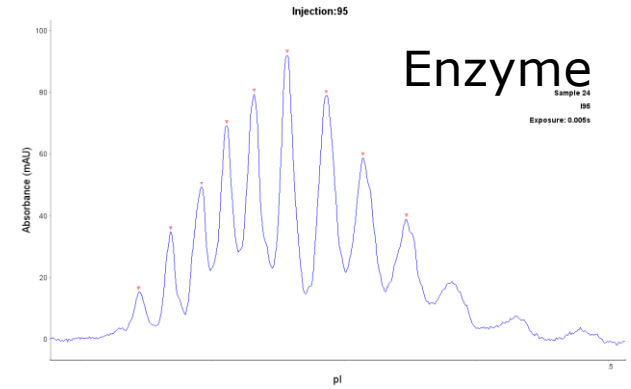












Conclusion

Efficiency Gains: A 50% reduction in runtime is achieved by Supersonic cIEF, utilizing zero new reagents or instrumentation.

Analytical Robustness: Excellent reproducibility and an improved LOQ for dilute fractions are demonstrated by the Supersonic method.

Broad Applicability: Supersonic cIEF is successfully applied across diverse modalities, facilitated by targeted Pharmalyte tuning.

Thank You

Acknowledgements

Sanofi BioAnalytics Characterization
Anny Ali
Bertie Chi
Heather Hughes

Bio-techne
Anna Amato
Baburaj Kunnummal
Will McElroy
Ed Chase