

Welcome to Waters Luncheon Seminar

Your Feedback will be very much appreciated!

Please provide your feedback and you will get a gift set at reception!



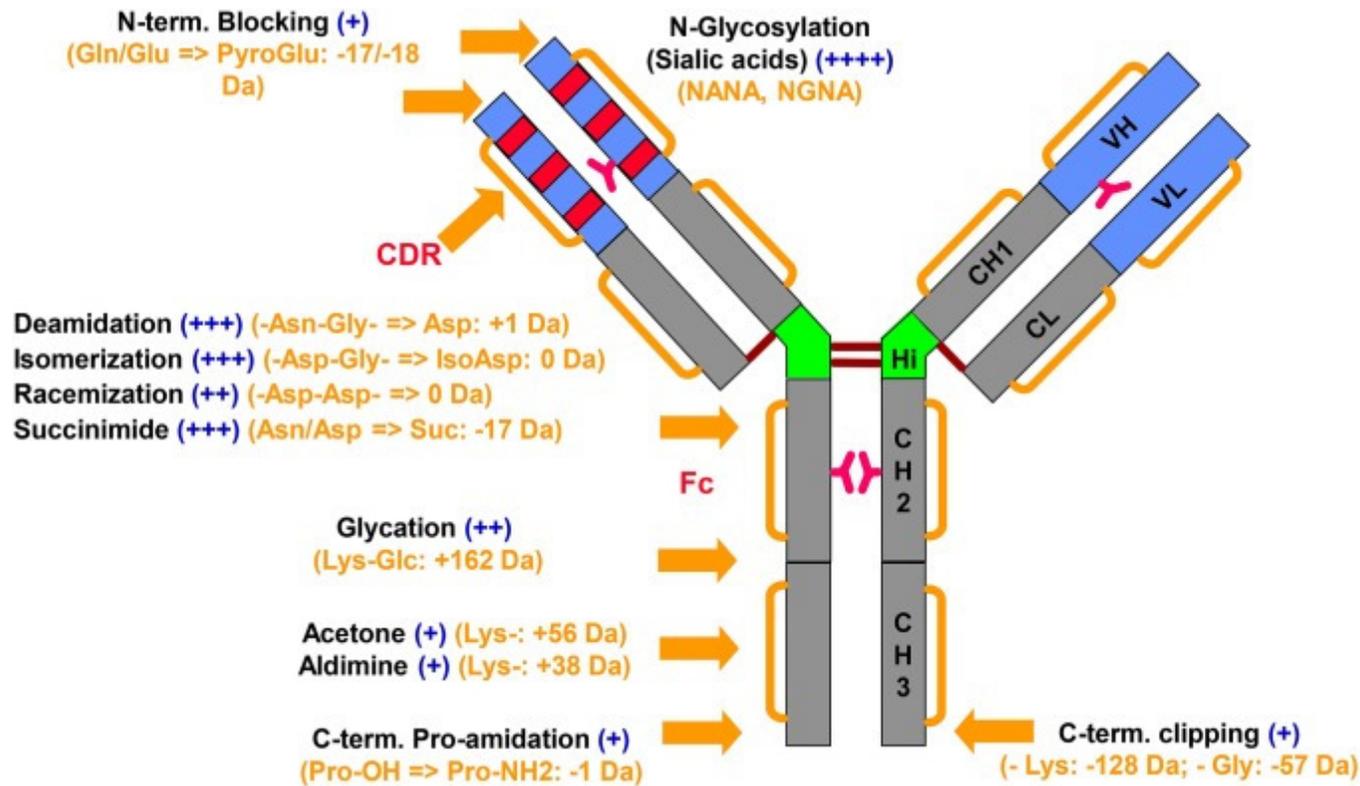
Column-shaped pens

Improving IEX Technology and IEX-MS Applications for Domain Specific Charge Variant Analysis of mAb



ionHance™
CX-MS pH Buffers

mAb Charge Variants

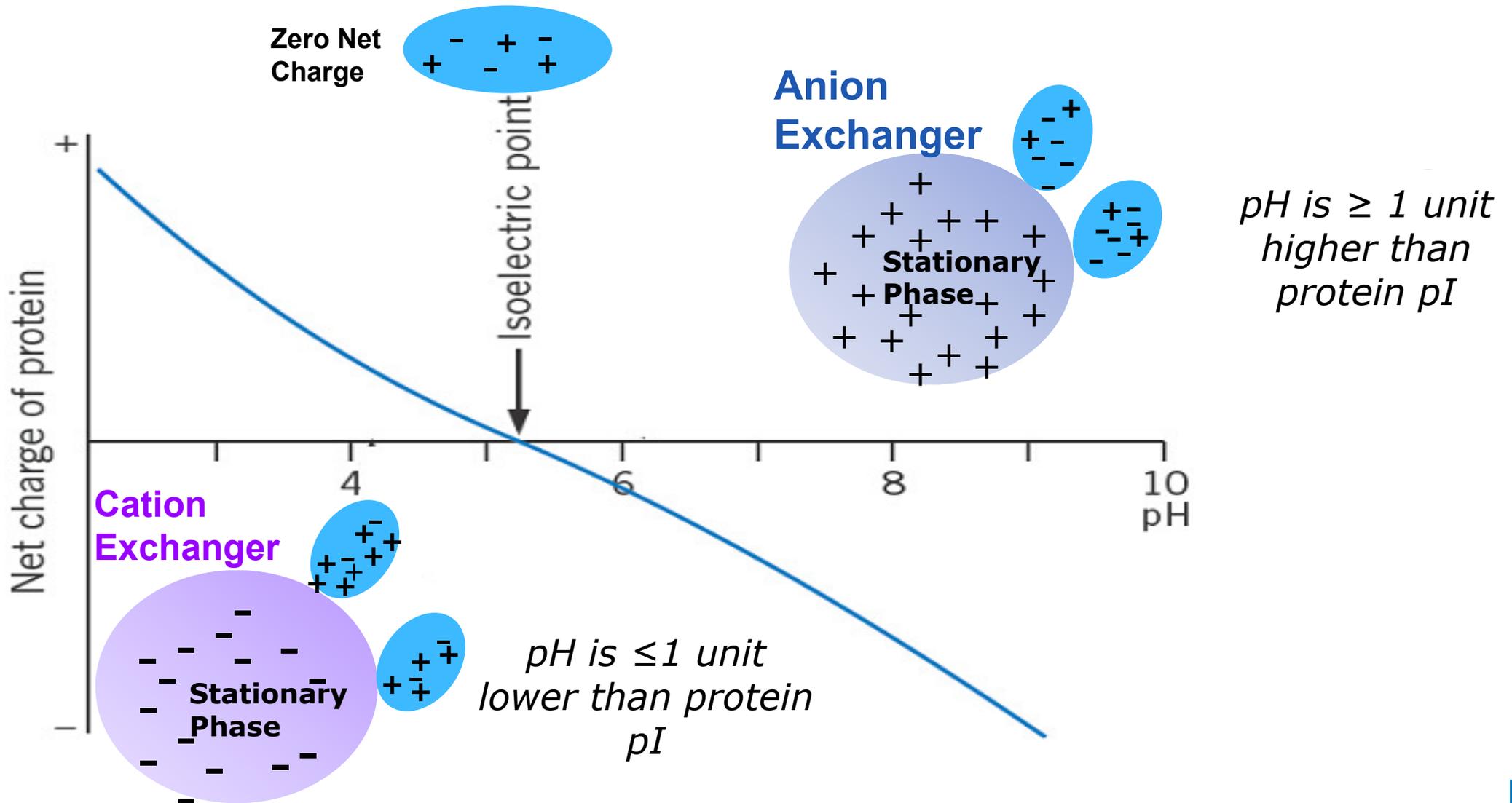


Major chemical degradation pathways	Effect	Species formed
Sialylation	COOH addition	Acidic
Deamidation	COOH formation	Acidic
C-terminal lysine cleavage	Loss of NH2	Acidic
Adduct formation	COOH formation or loss of NH2	Acidic
Succinimide formation	Loss of COOH	Basic
Methionine, cysteine, lysine, histidine, tryptophan oxidation	Conformational change	Basic
Disulfide-mediated	Conformational change	Basic
Asialylation (terminal Galactose)	Loss of COOH	Basic
C-terminal lysine and glycine amidation	NH2 formation or loss of COOH	Basic

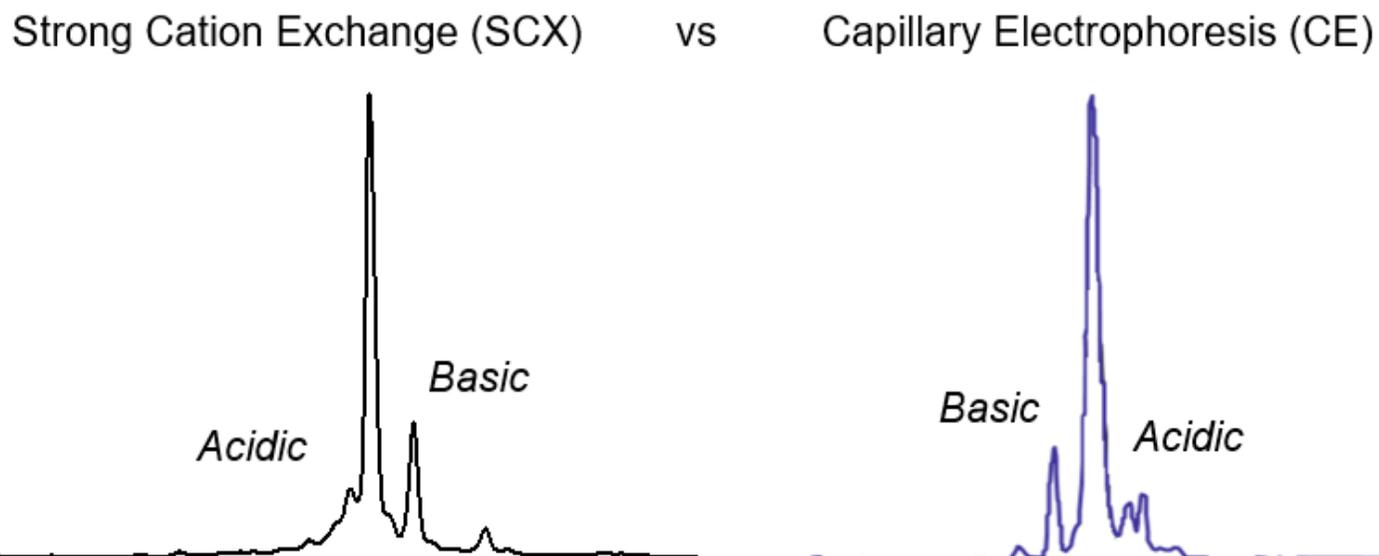
J Chromatogr. A. (2017) 1498:147-154.

mAbs. (2010) 2, 6 :613-624.

Protein Isoelectric Points and IEX



Charge Variant Profiling



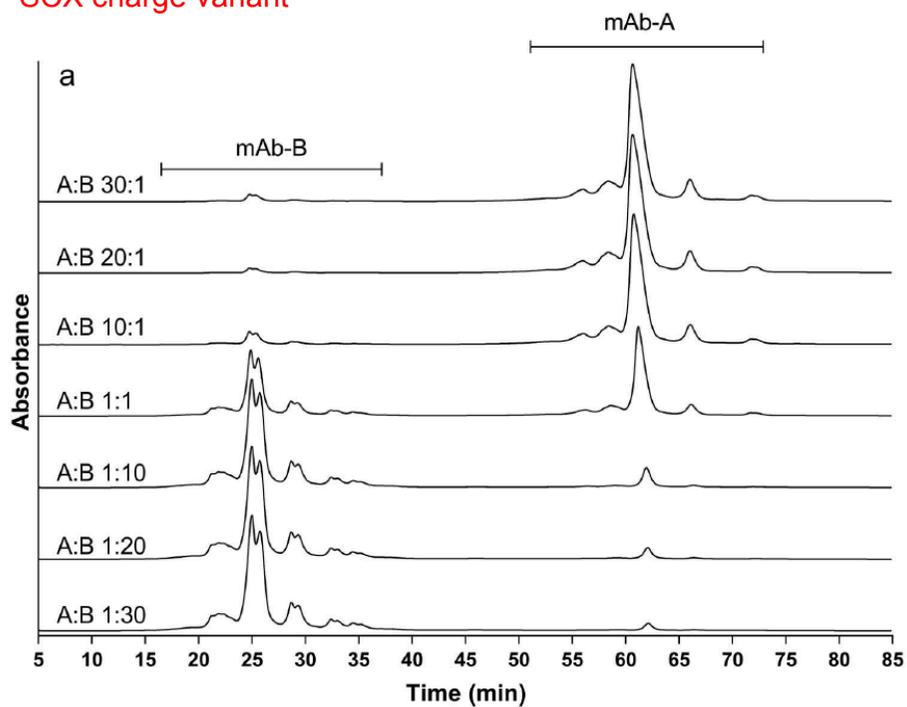
Bio Drugs. 2016, 30, 321-338

- Ion exchange
 - o Reliable, robust separations
 - o Larger sample loads (μg)
 - o Run-run reproducibility

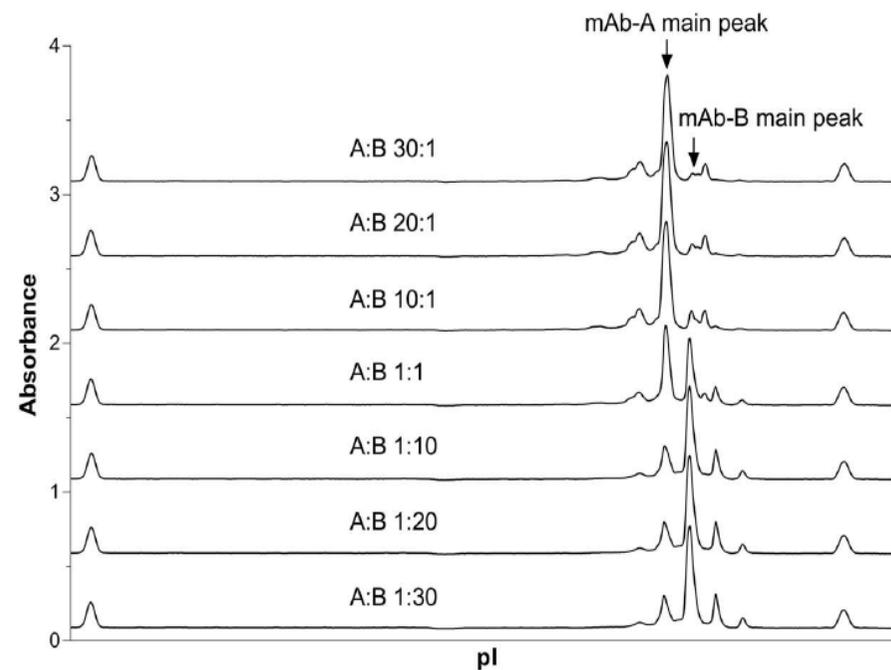
- Capillary electrophoresis
 - o High Resolution, fast separation
 - o Disconnected from 2^o assays
 - o Small sample load (ng)

cIEF and IEX as Orthogonal Methods for mAb Charge Variant Analysis

SCX charge variant

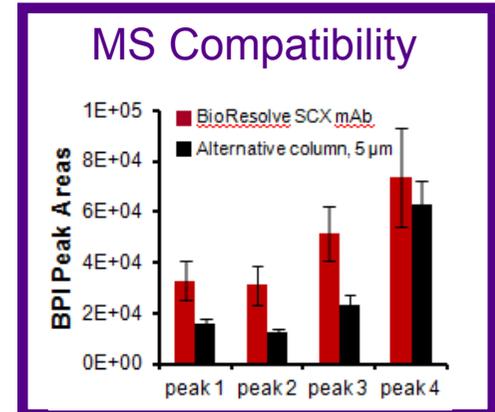
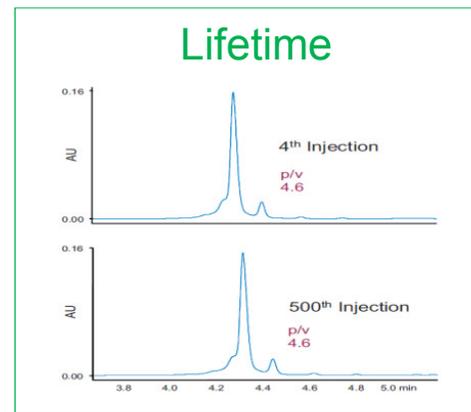
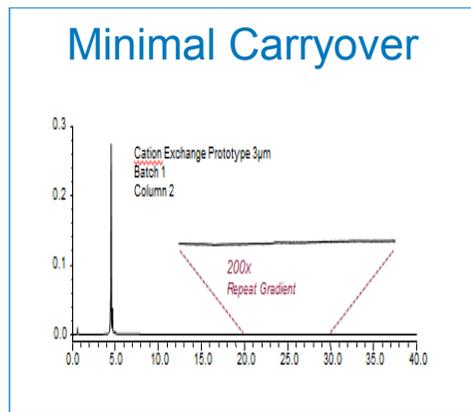
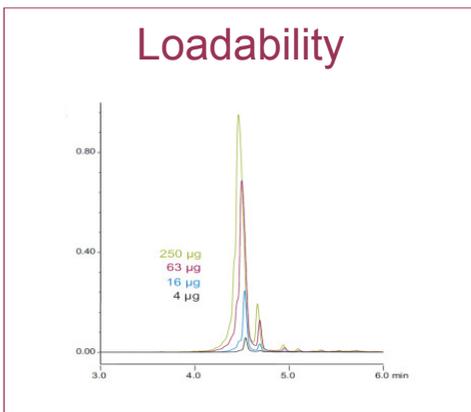
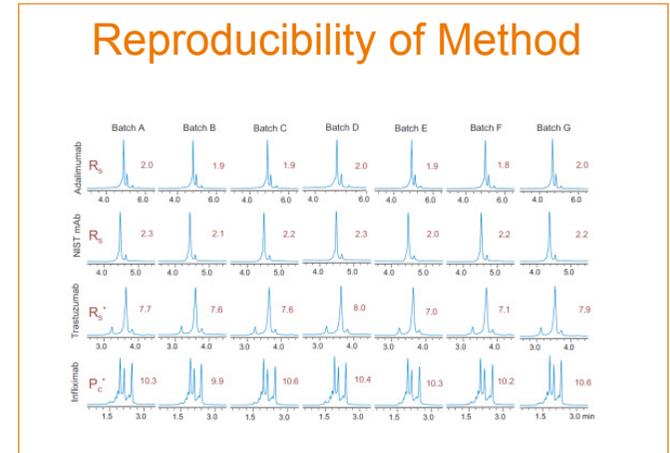
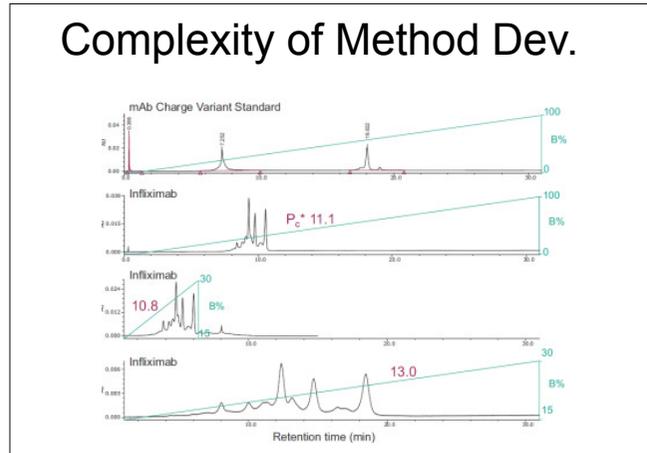
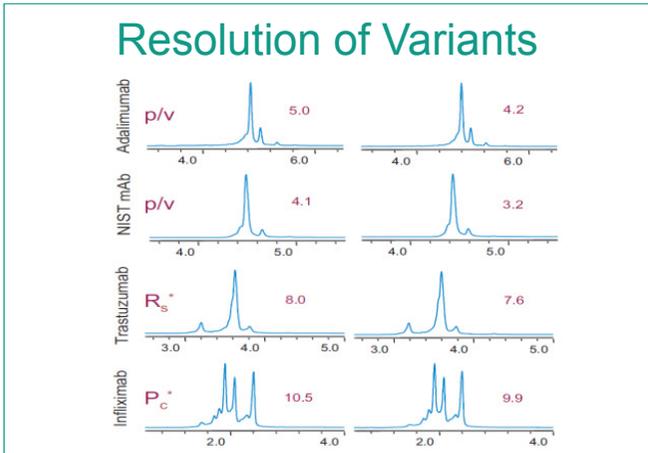


cIEF electropherograms



mAbs. 2019, 11, 3, 489-499

Challenges in IEX for mAb Charge Variant Analysis

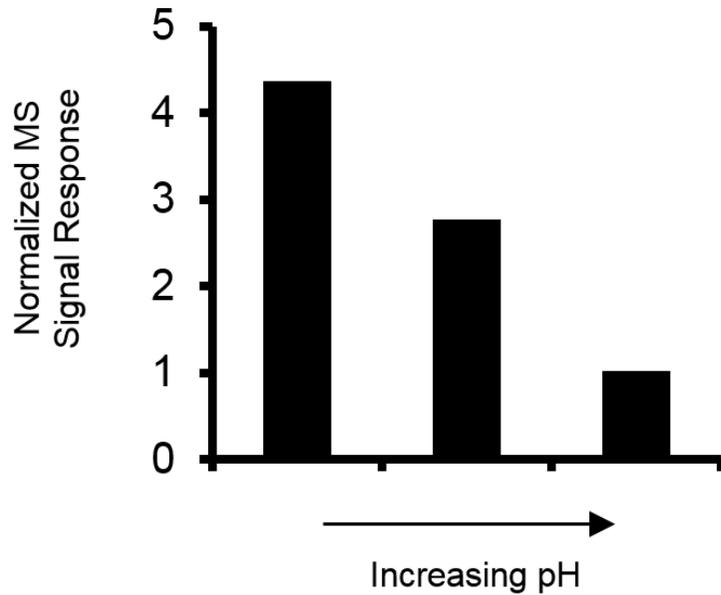


Gradient Options for Online IEX-MS

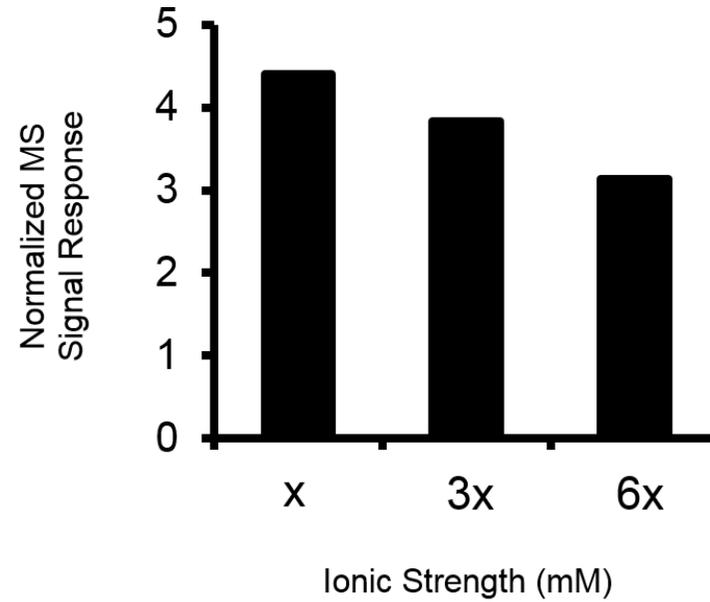
- pH Gradient
 - Ionization efficiency challenges (High pH)

- Salt Gradient
 - MS burden
 - Metal contamination

Fixed Buffer Concentration



Fixed pH



Mobile Phase Options for Online IEX-MS

Mobile Phase	pKa
Ammonium Formate	3.75, 9.25
Ammonium Acetate	4.75, 9.25
Ammonium Bicarbonate	6.40, 9.25

- Volatile mobile phase
 - MS-grade reagents
 - Trace metal certified

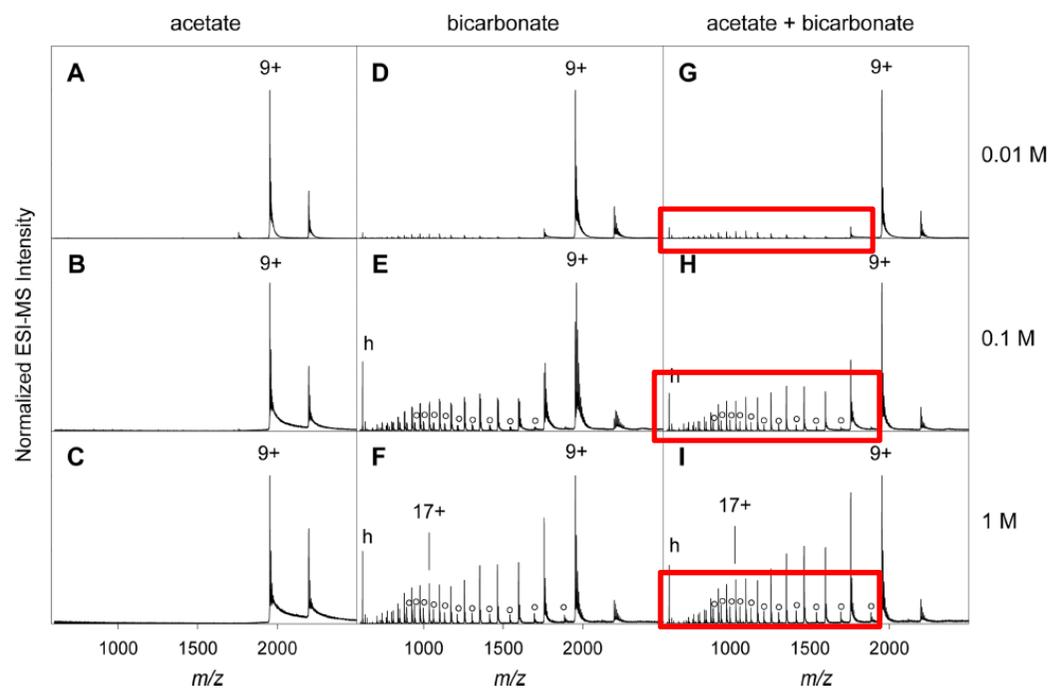
Ammonium Bicarbonate for Online IEX-MS

Effects of Ammonium Bicarbonate on the Electrospray Mass Spectra of Proteins: Evidence for Bubble-Induced Unfolding

Jason B. Hedges, Siavash Vahidi, Xuanfeng Yue, and Lars Konermann*

Department of Chemistry, The University of Western Ontario, London, Ontario, N6A 5B7, Canada

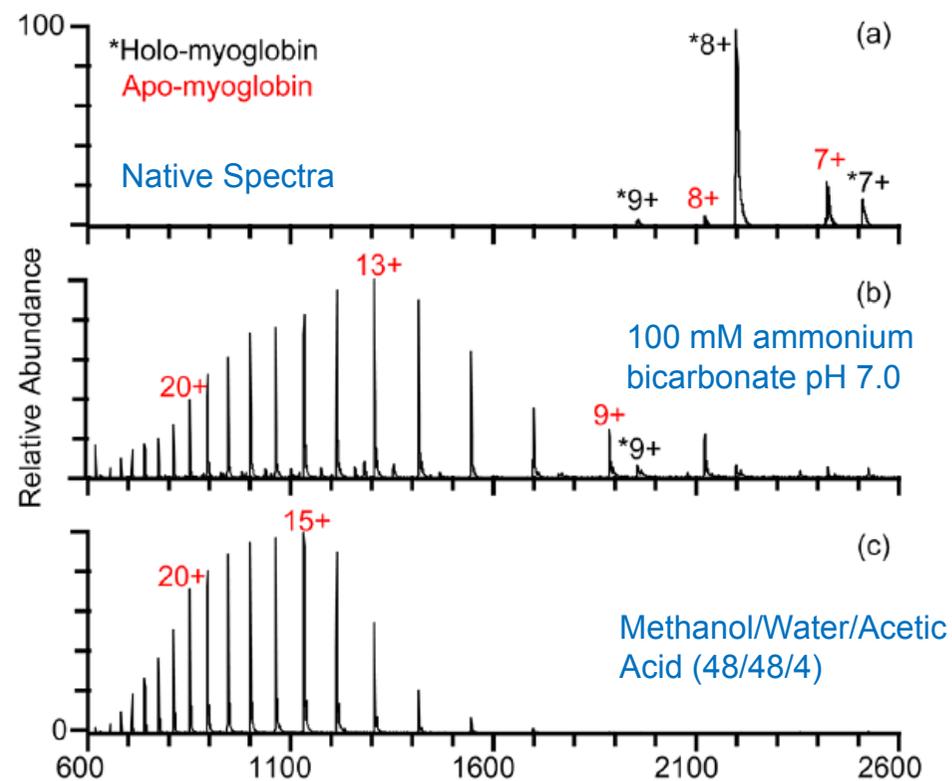
- Supercharging
- Protein Denaturation
- CO₂ adducts
- pH instability



Electrothermal Supercharging in Mass Spectrometry and Tandem Mass Spectrometry of Native Proteins

Catherine A. Cassou, Harry J. Sterling, Anna C. Susa, and Evan R. Williams*

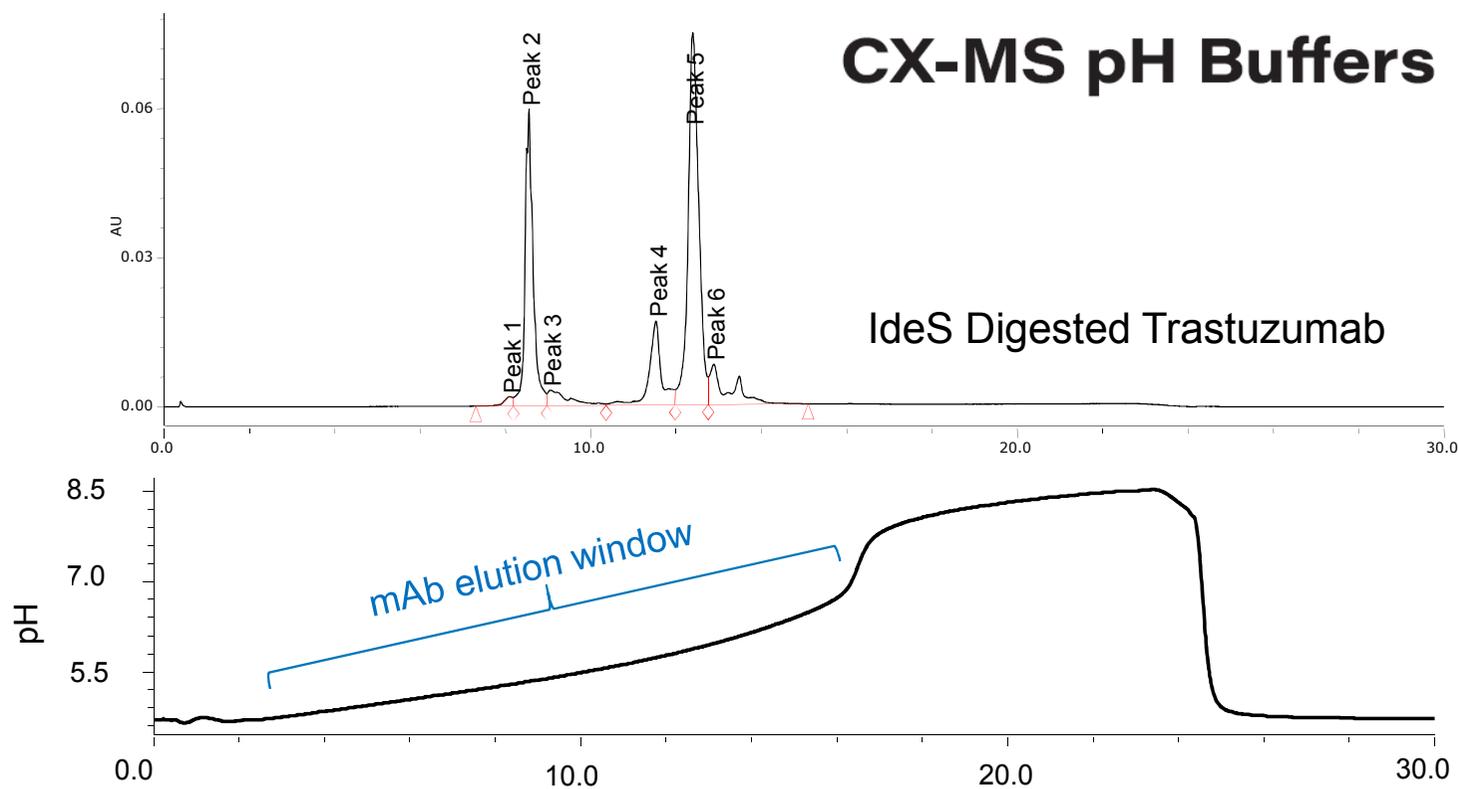
Department of Chemistry, University of California, Berkeley, California 94720-1460, United States



Ammonium Acetate Mobile Phase for Online IEX-MS

IonHance™ CX-MS pH buffer trace
98-2% A linear gradient over 21 minutes

ionHance™
CX-MS pH Buffers

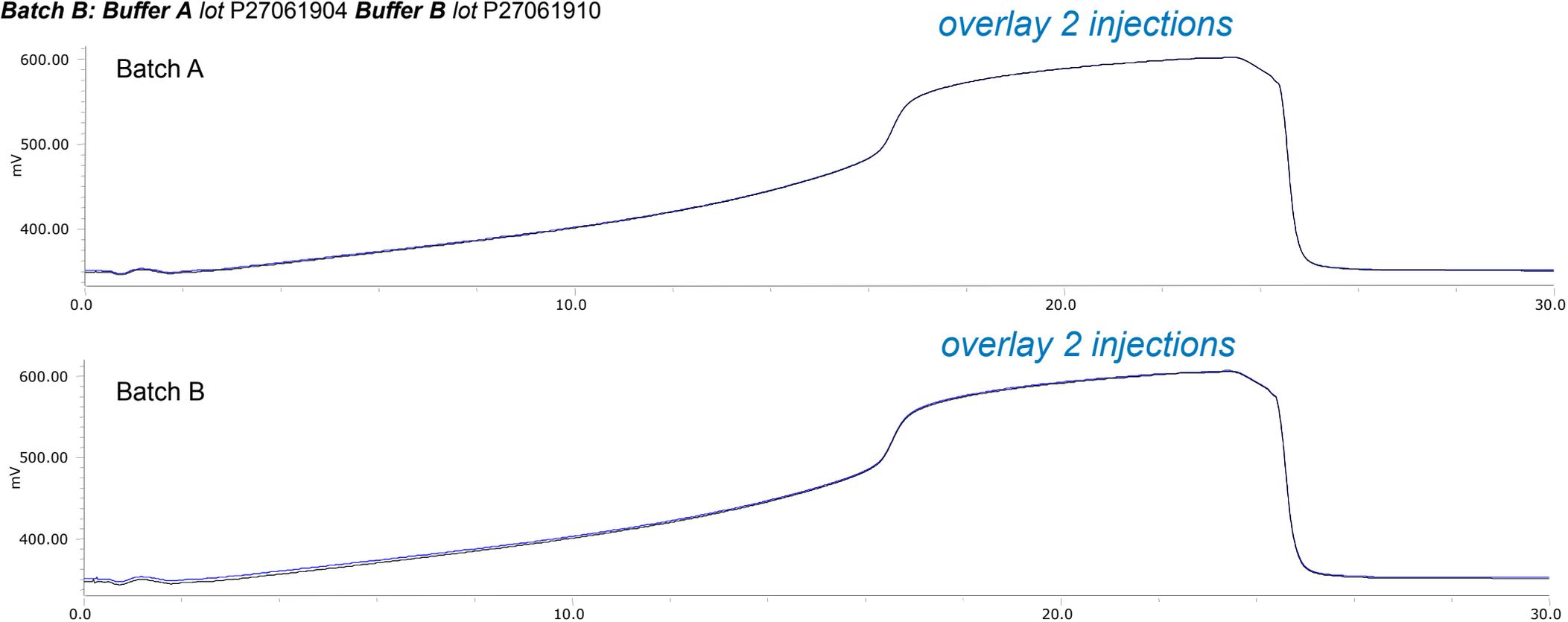


IonHance™ CX-MS pH Buffer Reproducibility

Linear pH Gradients

Batch A: Buffer A lot P27061902 Buffer B lot P27061908

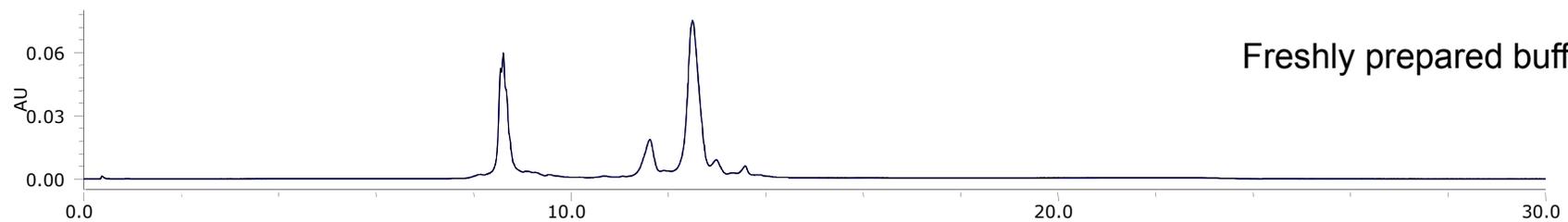
Batch B: Buffer A lot P27061904 Buffer B lot P27061910



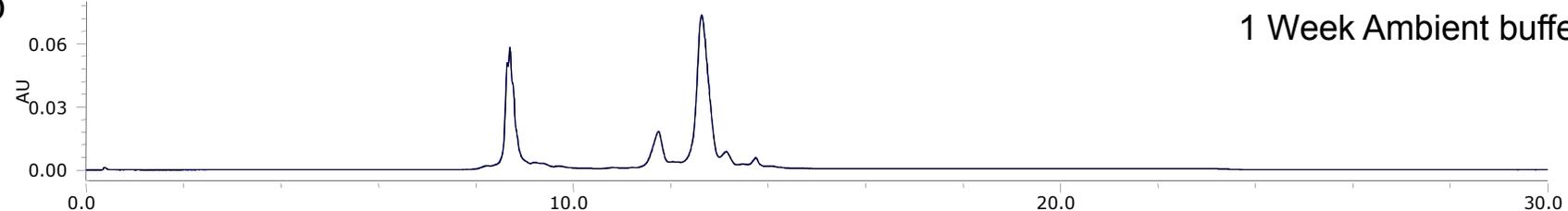
IonHance™ CX-MS pH buffer 1-Week Buffer Stability Tests

overlay 2 injections

IdeS
Digested
Trastuzumab

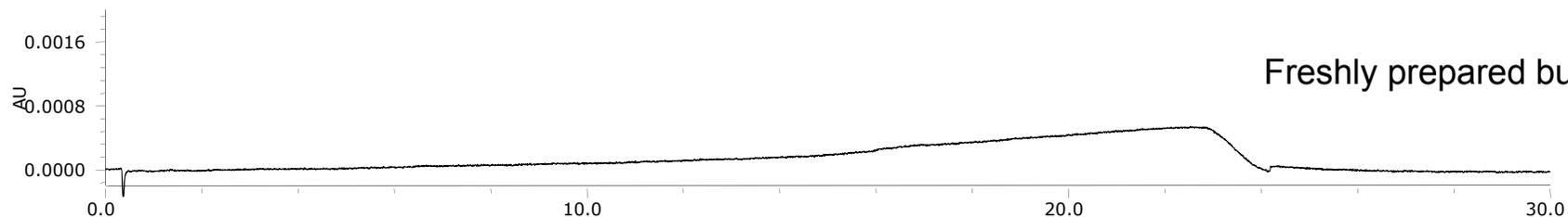


Freshly prepared buffer

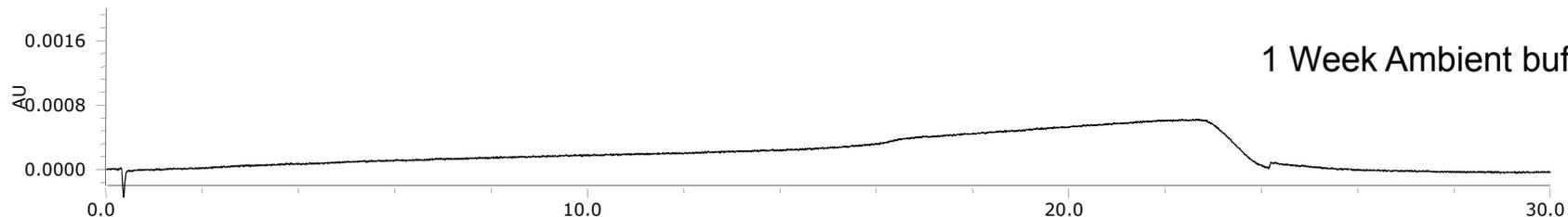


1 Week Ambient buffer

blank



Freshly prepared buffer

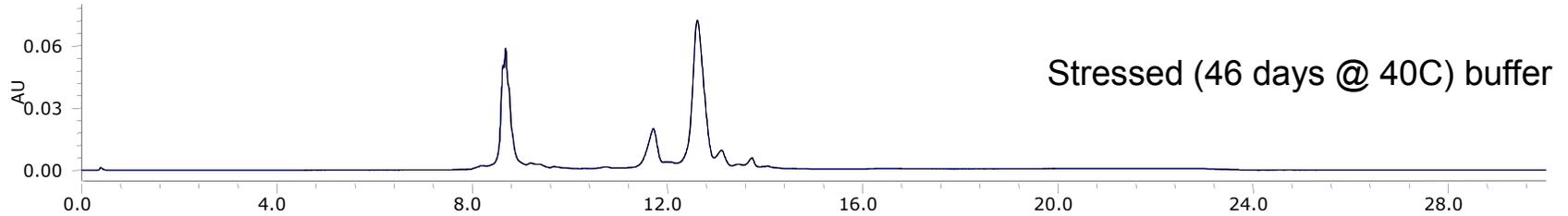
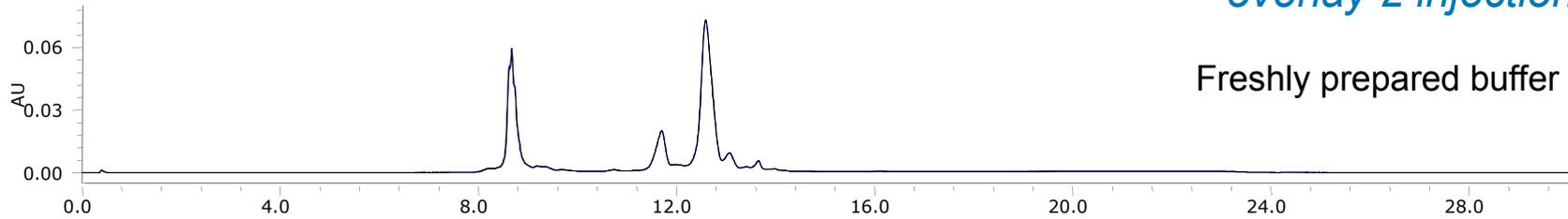


1 Week Ambient buffer

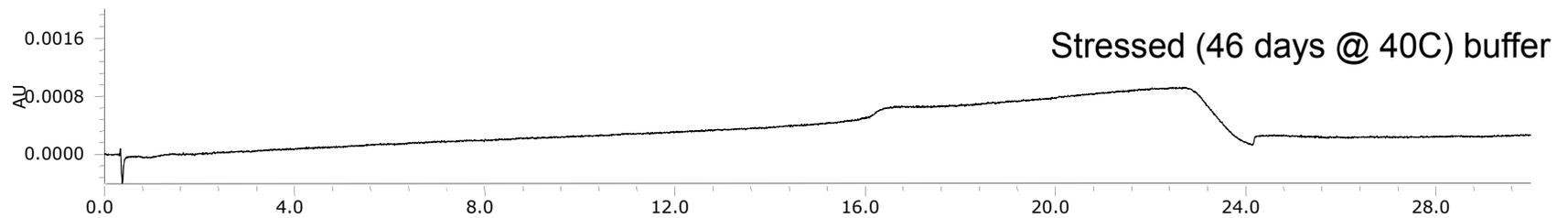
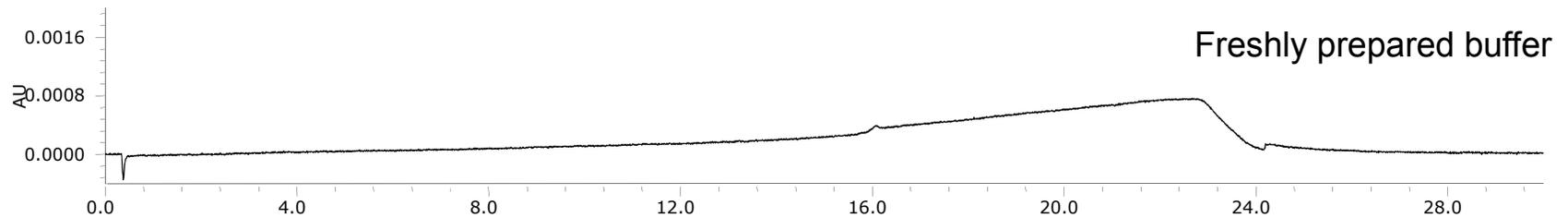
IonHance™ CX-MS pH buffer Long Term Buffer Stability

overlay 2 injections

IdeS
Digested
Trastuzumab



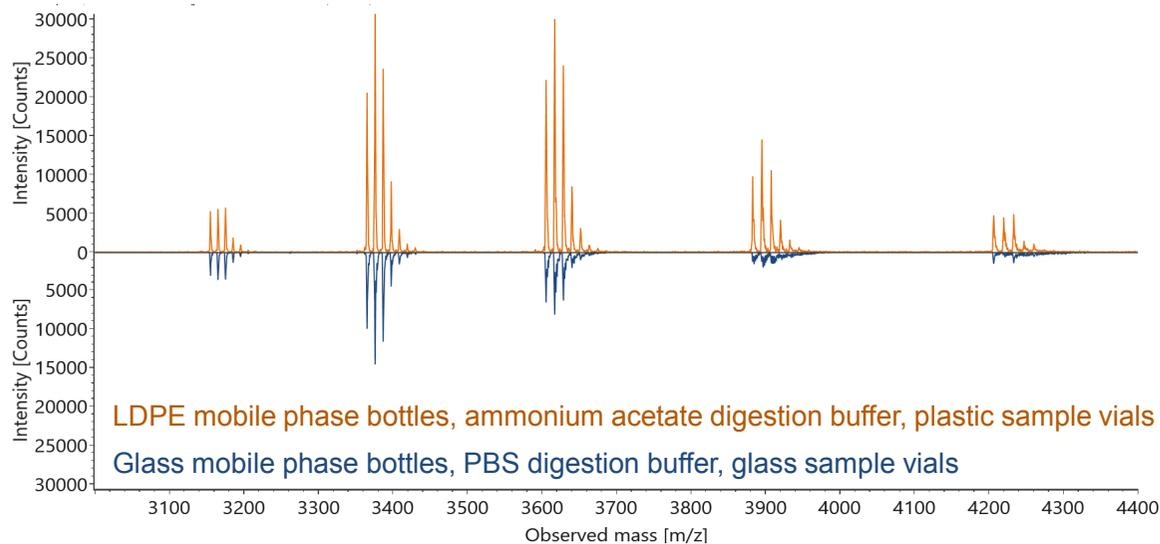
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MS Quality: Eluent Considerations

- Use Plastic
 - CX-MS in trace metal certified LDPE
 - 18.2 MΩ H₂O, not MS-grade H₂O in glass
 - Graduated cylinders, beakers, etc
- Avoid unnecessary contamination
 - Concentrates are trace metal certified
 - Digest without Na, K, Ca

Spectra of NIST mAb (Fc/2)₂ with m/z window 3000-4400

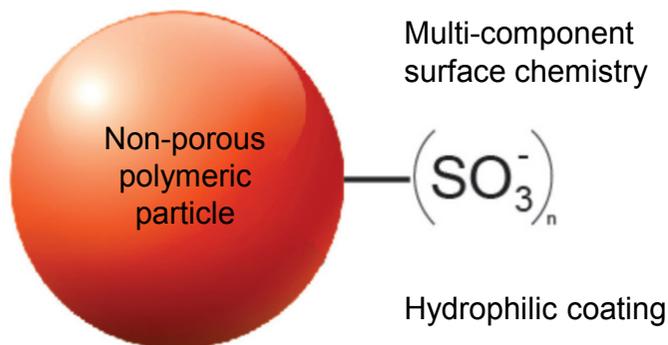


10X Concentrate ICP-MS report

Sample	Replicate	Potassium (K) (ppbw)	Sodium (Na) (ppbw)
IEX MS Buffer B P27061912A	1	23	152
	2	24	160
	3	23	160
	Average	23	157
	Measurement Uncertainty (2σ)	2	9
IEX MS Buffer A P27061904A	1	42	106
	2	49	113
	3	47	110
	Average	46	110
	Measurement Uncertainty (2σ)	6	7

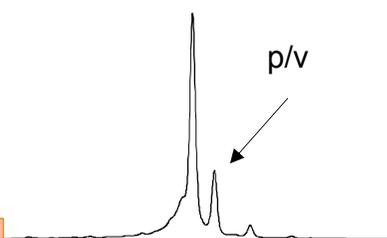
Novel Stationary Phase

New Strong Cation Exchange Phase

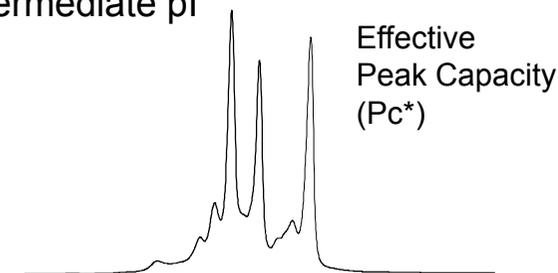


- Novel, specialized polymerization reactions for an optimized hydrophilic surface and sulfonic acid ligand
- **3 μm non-porous particle for optimal diffusion kinetics, high pressure capability and amenability to HPLC, UHPLC and UPLC**
- Developed through purposeful prototyping and comprehensive testing with a wide range of mAbs and separations and both salt and pH gradient chromatography

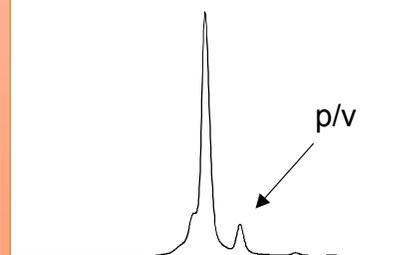
Adalimumab
Basic pI



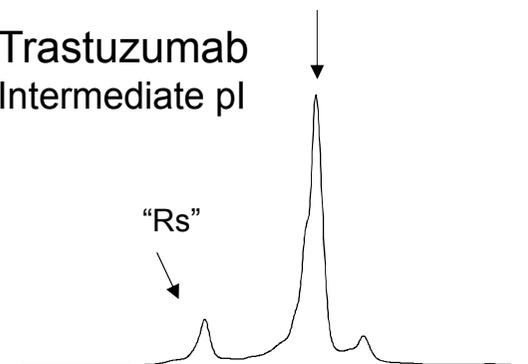
Infliximab
Intermediate pI



NIST mAb
Basic pI



Trastuzumab
Intermediate pI



See: "Designing a New Particle Technology for Robust Charge Variant Analysis of mAbs" Waters Application Note: 720006475EN (January 2019)

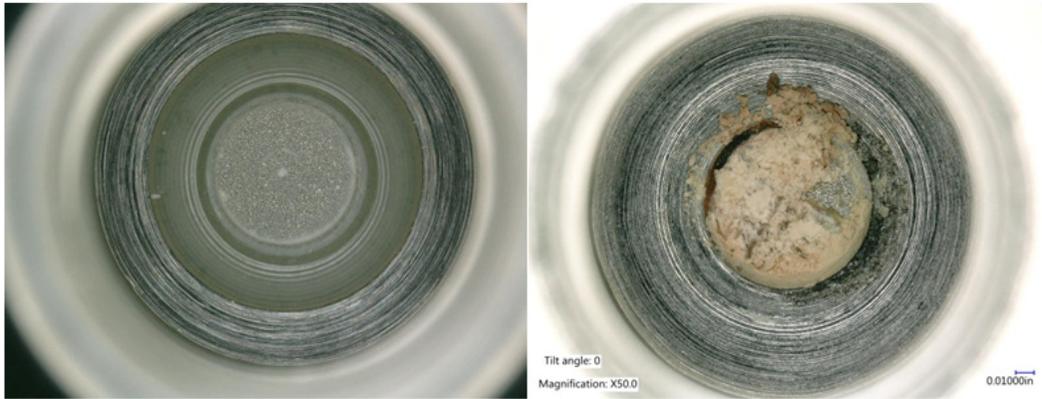
Patent Pending

BioResolve SCX mAb Column Hardware Design

Mitigate corrosion risk, while maintaining UPLC pressure compatibility, column packing performance, and repeatability

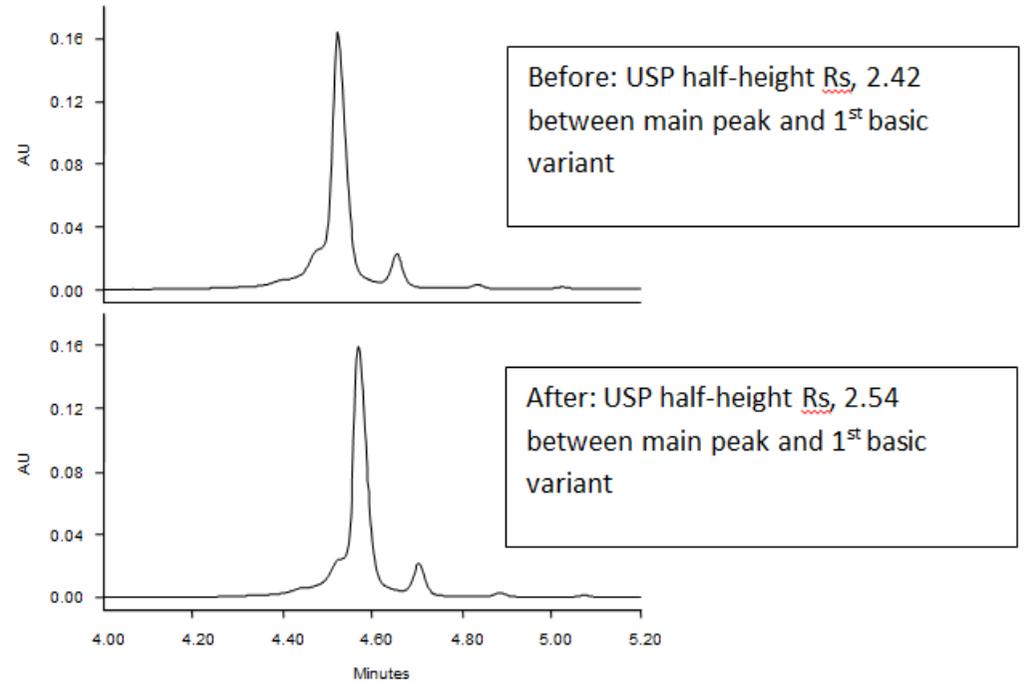
Accelerated corrosion testing of 2.1mm column frits. The stainless steel hardware shows visual rusting in as little as one week, where the BioResolve design has no noticeable corrosion.

Shelf life testing of the improved design has also shown no degradation in separation performance. Columns were evaluated with salt gradients, flushed, and stored for 1 month.



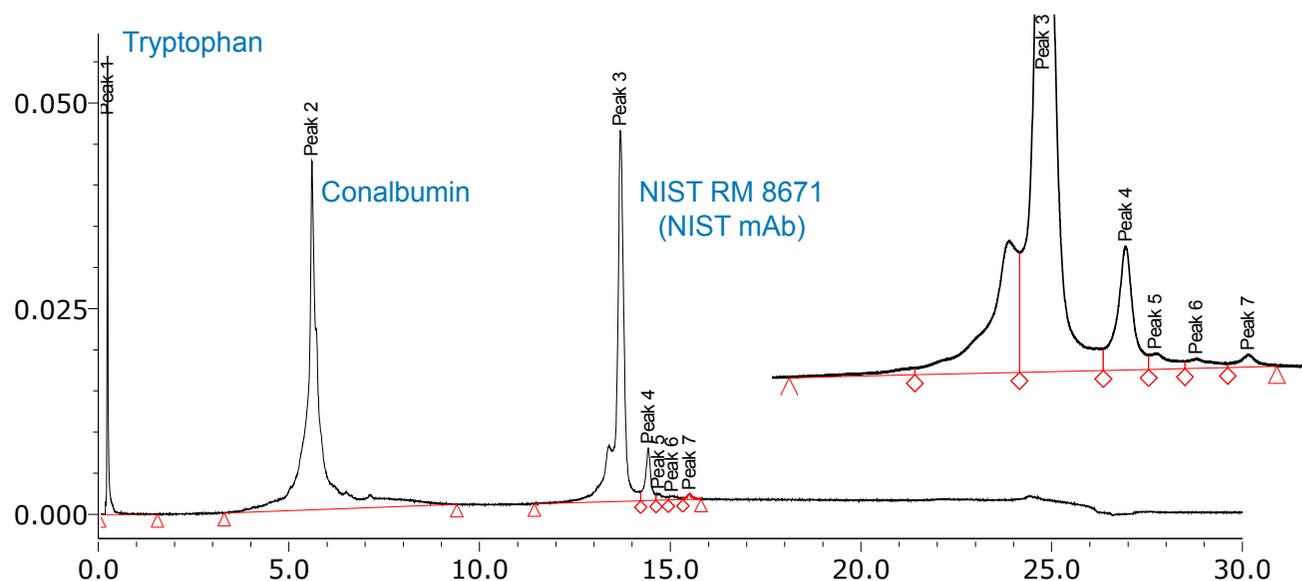
BioResolve SCX mAb
Titanium Hardware

Stainless Steel
Hardware



mAb Charge Variant Standard

A Mixture of Tryptophan, Conalbumin, and NIST mAb



- Retention
- Selectivity
- Resolution
- Recovery

- QC batch testing for a rigorous check on suitability for biopharm applications
- Available for proficiency checks and system suitability

pH Gradient
example

Column dimension 4.6 × 50 mm				
Time	Flow (mL/min)	%A	%B	Curve
Initial	1.44	100	0	Initial
1.0	1.44	100	0	6
23.6	1.44	0	100	6
24.6	1.44	0	100	6
25.6	1.44	100	0	6
30.0	1.44	100	0	6

Lifetime is Important

Recovering from Particulate and Chemical Fouling

■ Problem Statement

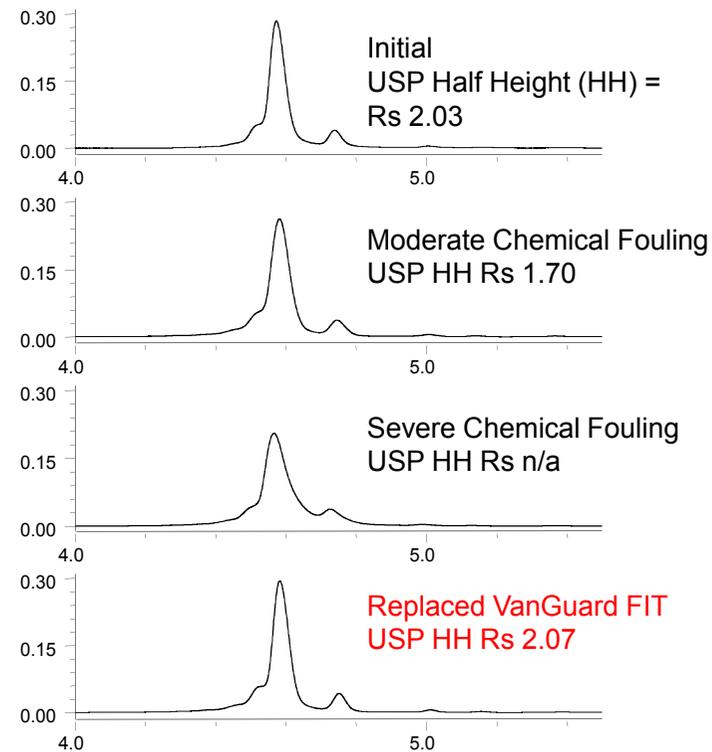
- The injection of column fouling excipients (e.g., polysorbate) and particles (e.g., insoluble sample or microbes) are well known causes of premature failure of columns.
- Traditional guard columns help protect the analytical column. However, they are relatively expensive and introduce compromising levels of additional dispersion

■ Purposefully Designed Solution

- **VanGuard FIT (Fully Integrated Technology)** guard column is a new, simplified guard column design that maximizes column life without degrading biomolecule component resolution.

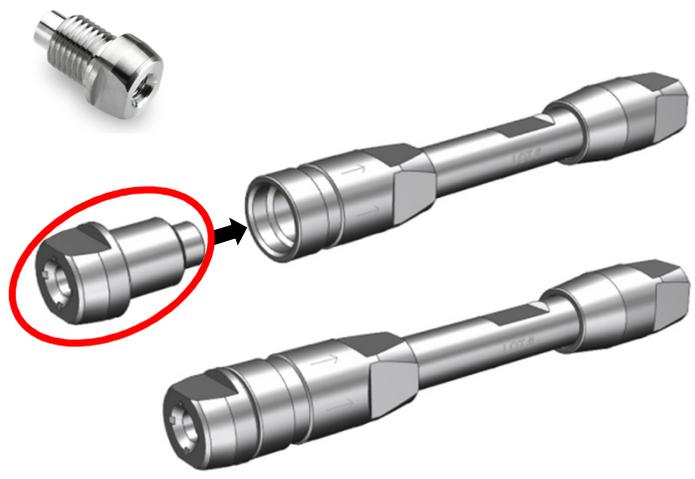
VAN GUARD
FIT

Patent Pending

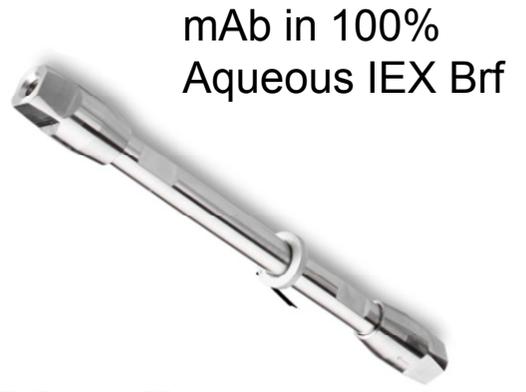
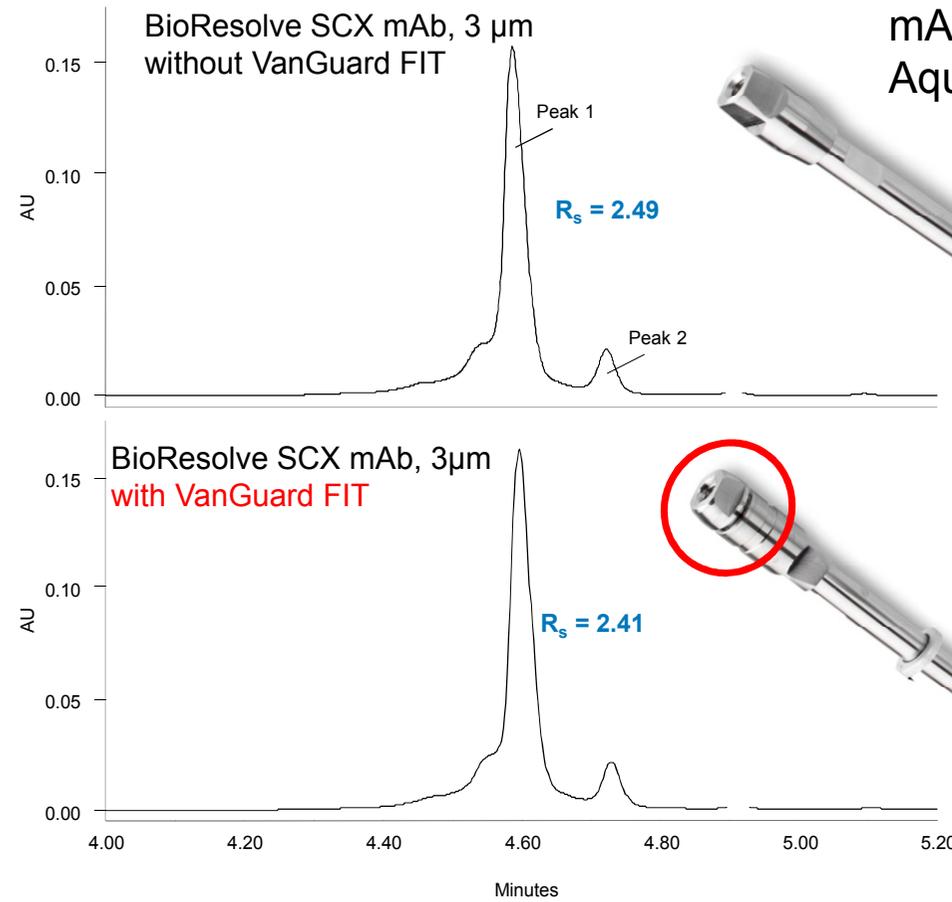


No Compromise Column Protection and Extended Lifetimes with the Use of VanGuard FIT Cartridges

VAN GUARD FIT



BioResolve SCX mAb Column, 4.6 x 50 mm column with integrated VanGuard FIT. Replaceable cartridge screws directly into the column inlet end nut. Optimal installation has never been easier nor more efficient in terms of maintaining analytical columns resolving power.



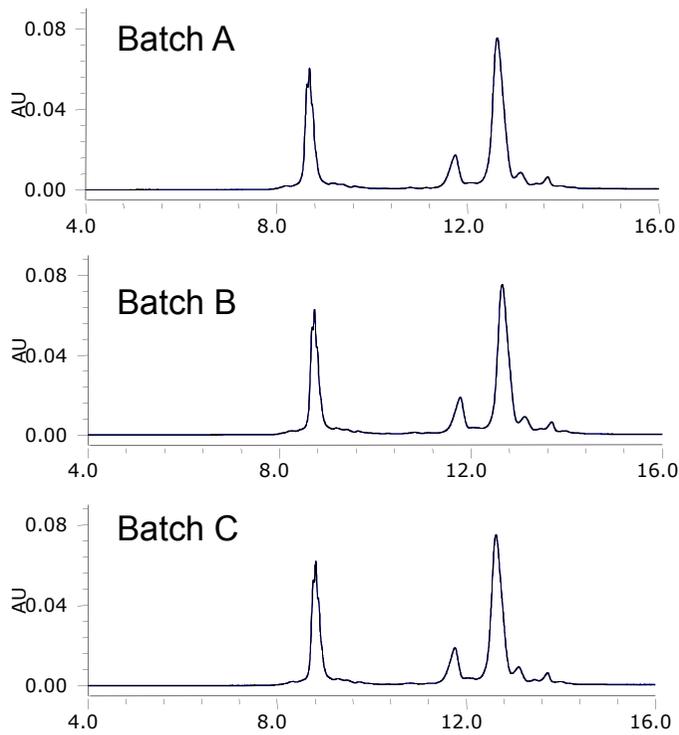
Charge variant profiles¹ for **NISTmAb**. No significant difference in chromatographic performance on BioResolve SCX mAb columns without (top) or with (bottom) VanGuard FIT.

IonHance™ CX-MS pH Buffer Reproducibility

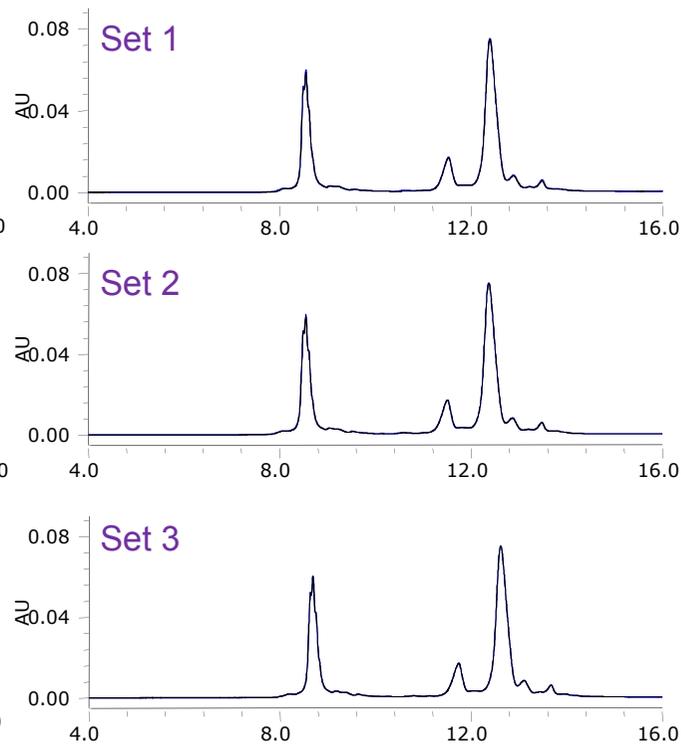
IdeS Digested Trastuzumab

overlay 2 injections

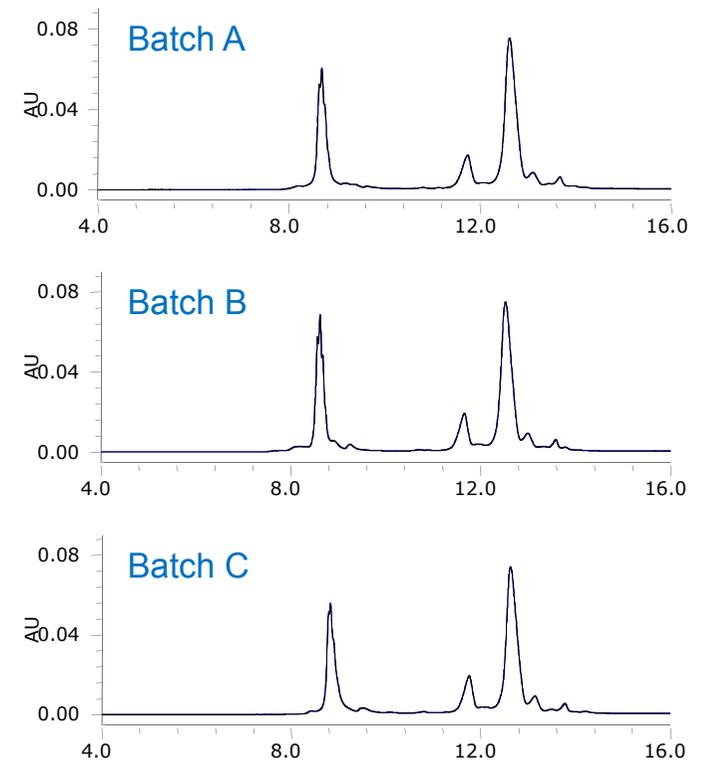
Inter-batch reproducibility



Intra-batch reproducibility (Batch A)



3 Batches of concentrates x 3 Batches of BioResolve SCX mAb columns



BioResolve SCX mAb Portfolio

Developed through Comprehensive Testing and Purposeful Prototyping



- BioResolve SCX mAB Columns
- VanGuard FIT (Fully Integrated Technology)
- mAb Charge Variant Standard
- BioResolve pH Gradient Buffer Concentrates (Optical Only: Non-MS compatible)

ionHance™
CX-MS pH Buffers

Patent Pending

Mass Spectrometry (MS) – an Important Tool for Biopharmaceutical Characterization and Analysis

© American Society for Mass Spectrometry, 2016

ASMS

J. Am. Soc. Mass Spectrom. (2017) 28:786–794
DOI: 10.1007/s13361-016-1531-9

CrossMark

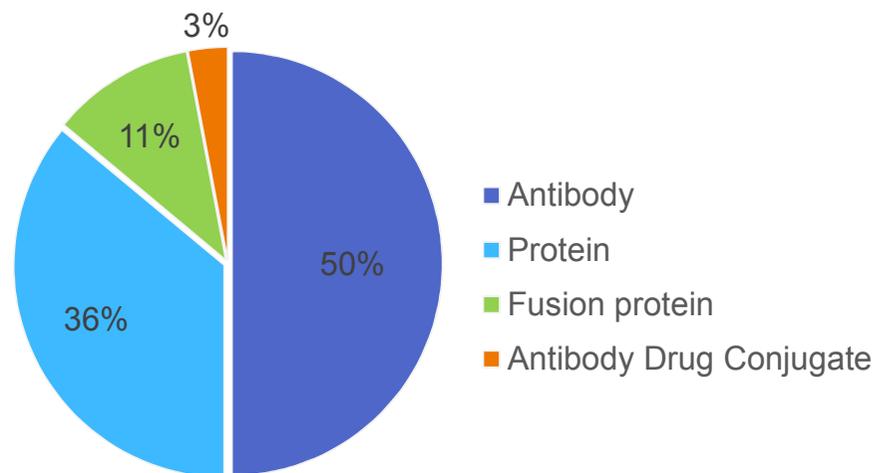
FOCUS: 28th SANIBEL CONFERENCE, CHARACTERIZATION OF PROTEIN THERAPEUTICS BY MS: RESEARCH ARTICLE

A Retrospective Evaluation of the Use of Mass Spectrometry in FDA Biologics License Applications

Sarah Rogstad,¹ Anneliese Faustino,¹ Ashley Ruth,² David Keire,¹ Michael Boyne,² Jun Park³

¹Division of Pharmaceutical Analysis, Office of Testing and Research, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD 20993, USA
²Biotechlogix, Inc., Glenview, IL 60025, USA
³Office of Biotechnology Products, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD 20993, USA

- From 2000-2015 there were 80 approved electronic BLAs
- 79 used MS for protein or impurity characterization



Rogstad et al. A Retrospective Evaluation of the Use of Mass Spectrometry in FDA Biologics License Applications. *J. Am. Soc. Mass Spectrom.* 2017, 28, 786-794

MS in regulatory filings steadily increasing over time

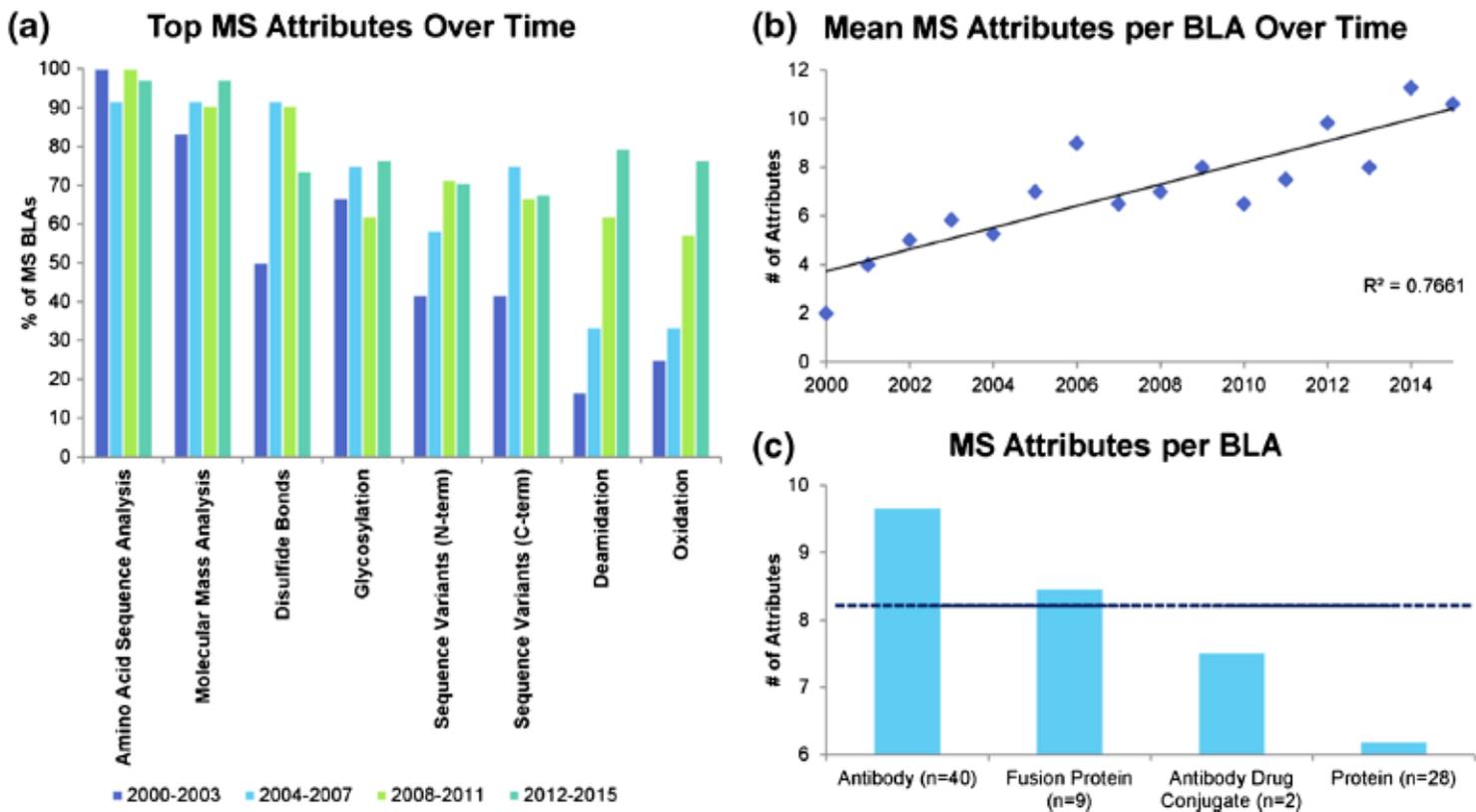


Figure 2. MS attribute analysis. (a) Top eight MS attributes over time. BLAs were binned by year with four-year increments. The percentages of BLAs that examined the top eight MS attributes are shown for each bin. Percentages are based on the total number of electronic BLAs that used MS. (b) The mean number of MS attributes analyzed per BLAs per year. (c) The mean number of MS attributes per BLA is shown based on product type

Rogstad et al. A Retrospective Evaluation of the Use of Mass Spectrometry in FDA Biologics License

Top Unmet Needs for Broader Deployment of LC/MS

Compiled from Hundreds of Surveys and Interviews with Biopharmaceutical Scientists

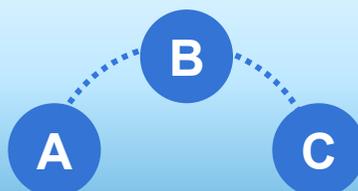
Robust



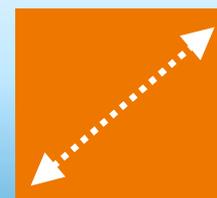
Reproducible



Easy to Learn
& Operate



Small
Footprint



Performance
Informatics



Without compromising the fidelity of the LC/MS data

BioAccord System Integrates Full Waters Portfolio

Separations

ACQUITY I-Class Plus for best reproducibility for demanding biopharma applications

Chemistries

Application-specific installation kits, columns, test standards and LC-MS grade solvent kits

Mass Spectrometry

NEW ACQUITY RDa – the **SmartMS** with small footprint automated setup & self-diagnosis and simple, intuitive user interface



Informatics

UNIFI Informatics support key biopharma applications and is compliance-ready

Service & Support

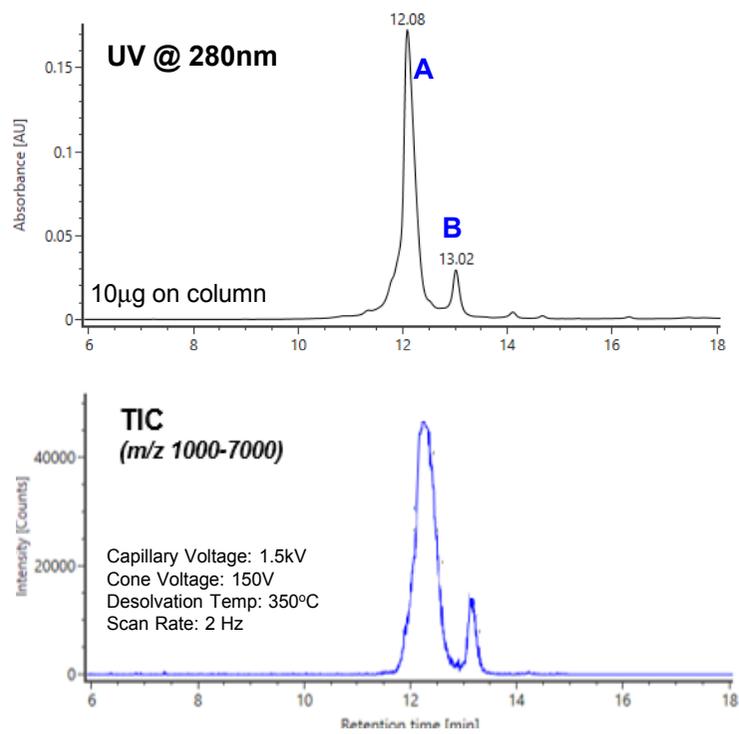
- **NEW** app-specific system install
- **NEW** app-specific customer training
- **NEW** system qualification

Scientific Content

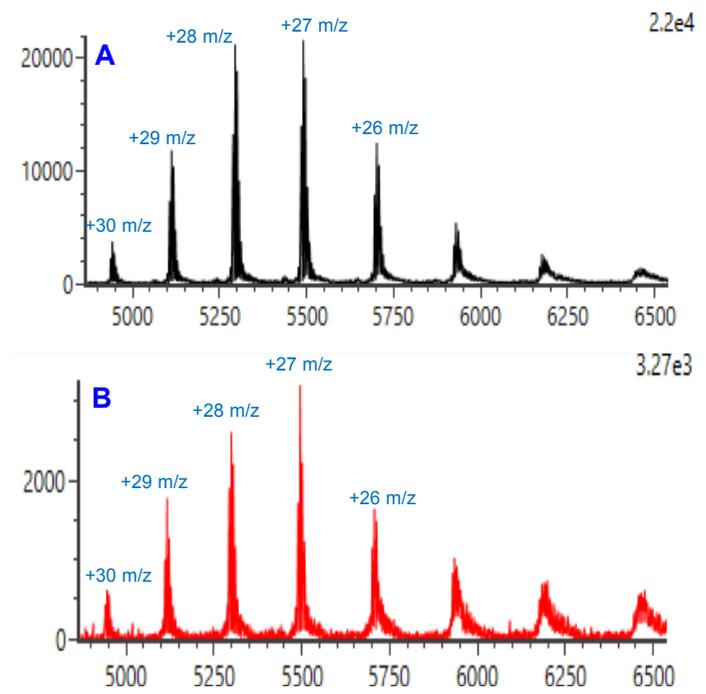
- **NEW** application notes to demonstrate system performance and benefits
- **NEW** example data & method templates to assist new users with getting started



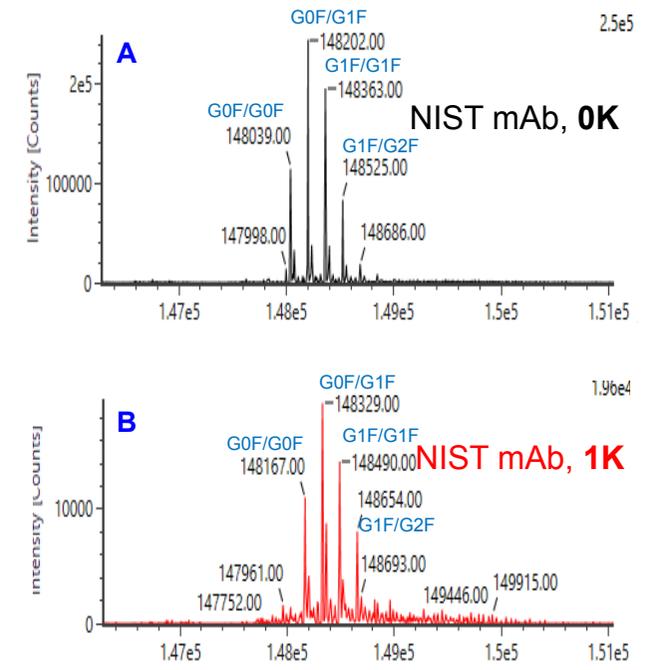
NIST mAb C-terminal Lysine Variant Evaluation on BioAccord System



Combined MS Spectrum of Selected Peaks



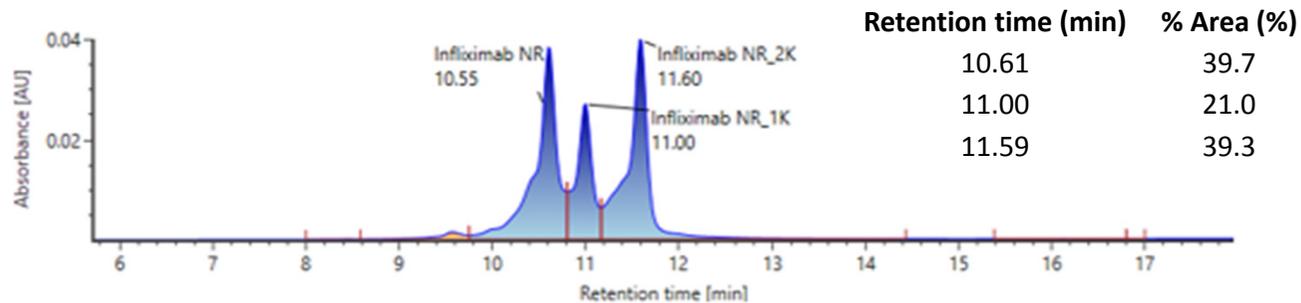
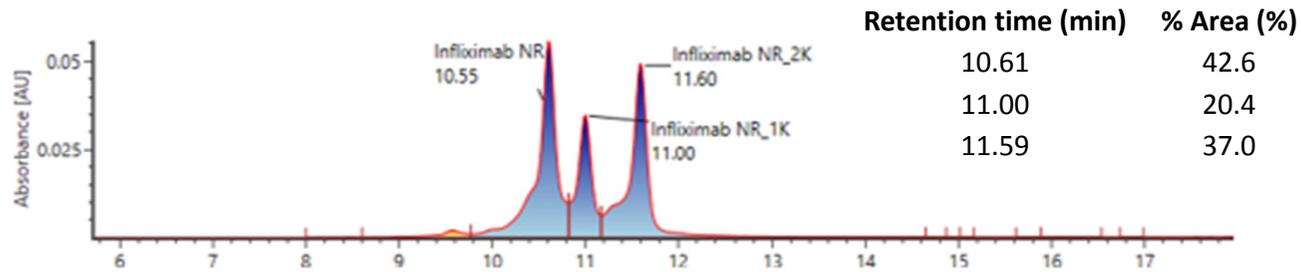
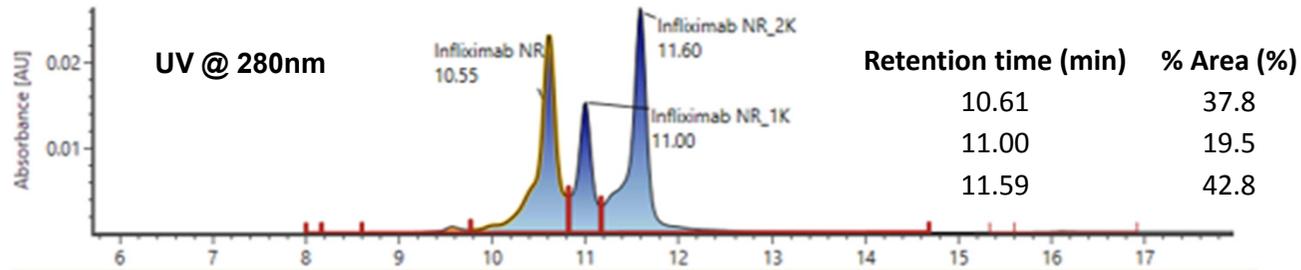
MaxEnt1 Deconvolution



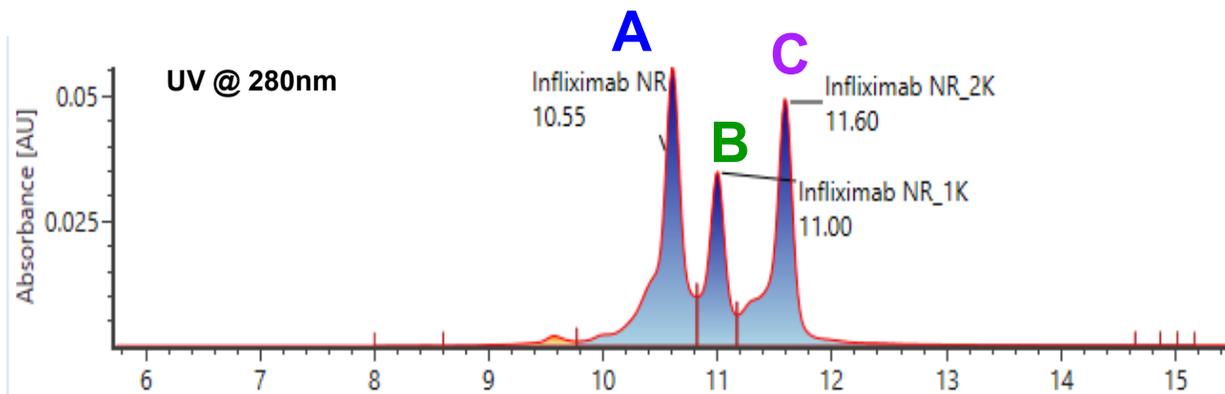
Expected Mass: 148,036.6 Da (G0F/G0F, 2 x pQ1)

Case Study 1: Infliximab Biosimilar Analysis

UV integration for quantitative comparison charge variant profiles

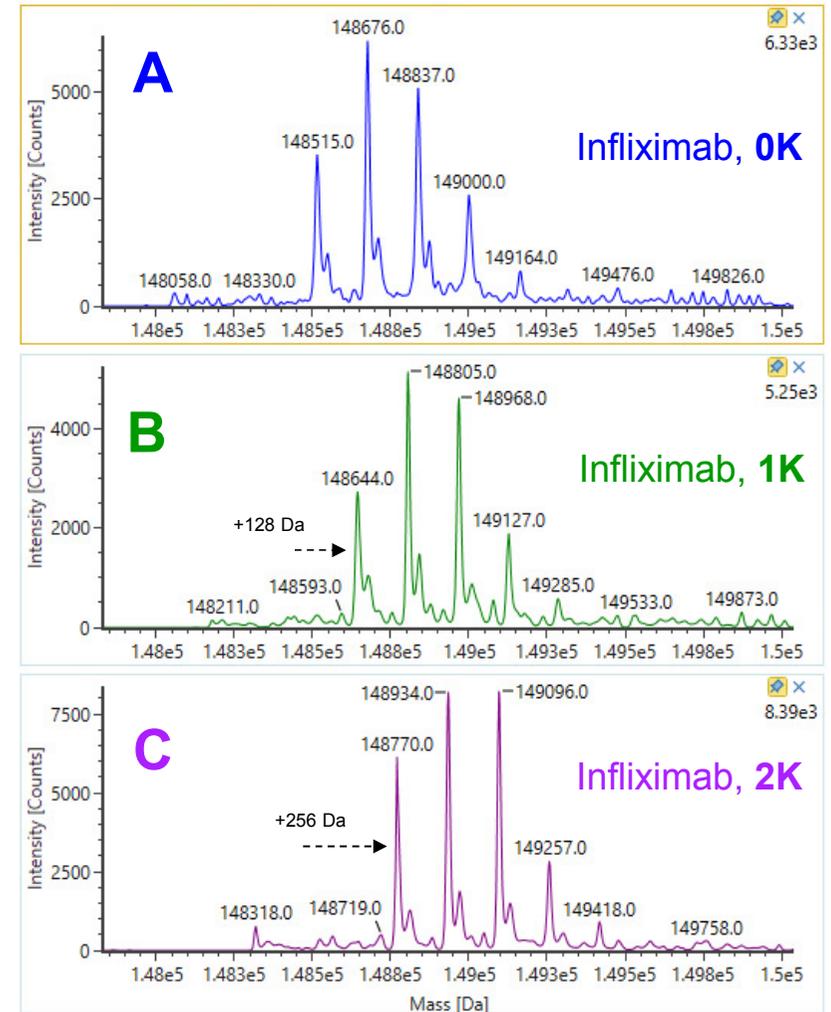


Case Study 1: Infliximab Biosimilar Analysis



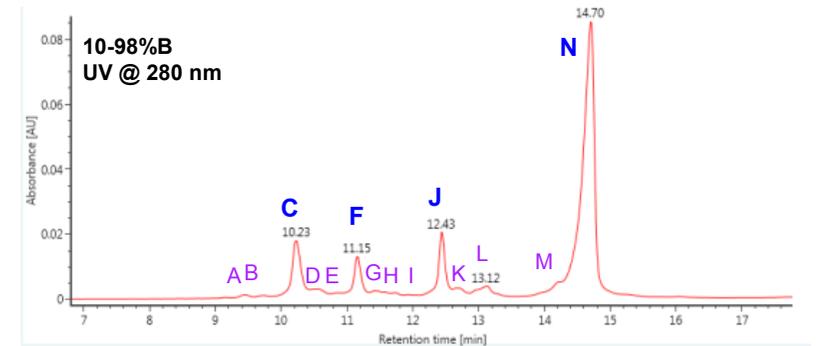
Qualitative online MS analysis to confirm species or investigate differences

MaxEnt1 Deconvolution



IEX-MS Analysis of Infliximab IdeS Subunits

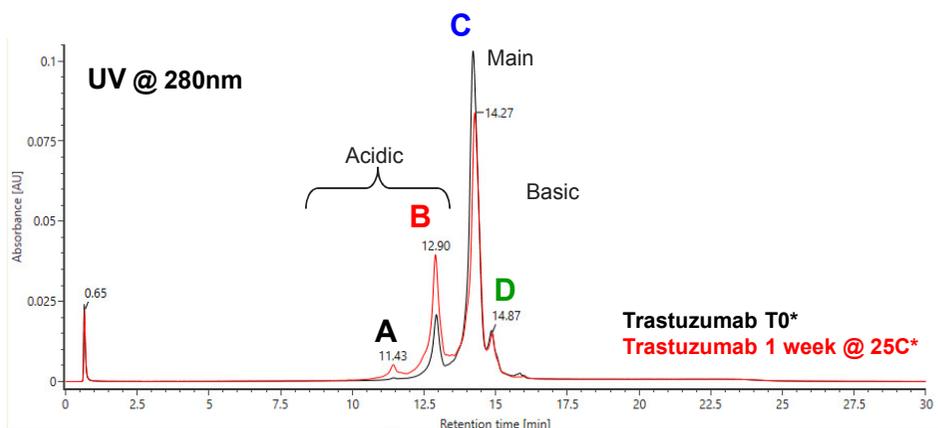
Peak	Lysine Variant	Species Detected
A	0K	Possible G0F-G2F deamidation; G2FS1/ G0-G2
B	0K	Possible G0F-G2F deamidation;  / G0F-G2F
C	0K	Main 0K species G0F-G2F
D	0K 1K	G0F-N/G0F, G0F-N/G1F, G0F-N/G0 (0K) Possible G0F-G2F deamidation (1K)
E	0K	Man5/Man5, Man5/G0F
F	1K	Main 1K species G0F-G2F
G	1K 2K	G0F-N/G0F, G0F-N/G1F, G0F-N/G0 Possible G0F-G2F deamidation (2K)
H	1K 2K	Man5/Man5 G2FS1) / G0-G2
I	2K	 / G0F-G2F
J	2K	Main 2K species G0F-G2F
K	2K	G0F-N/G0F, G0F-N/G1F, G1F-N/G1F, G0F-N/G0
L	2K (Fab) ₂	Man5/Man5, Man5/Man5+HexNAc Possible (Fab) ₂ deamidation
M	(Fab) ₂	Possible (Fab) ₂ deamidation
N	(Fab) ₂	Main (Fab)₂ species



Peaks A-L: Fc-related species, including N-glycoforms containing sialic acid and Man5 N-glycans for each of the 3 C-terminal lysine variants

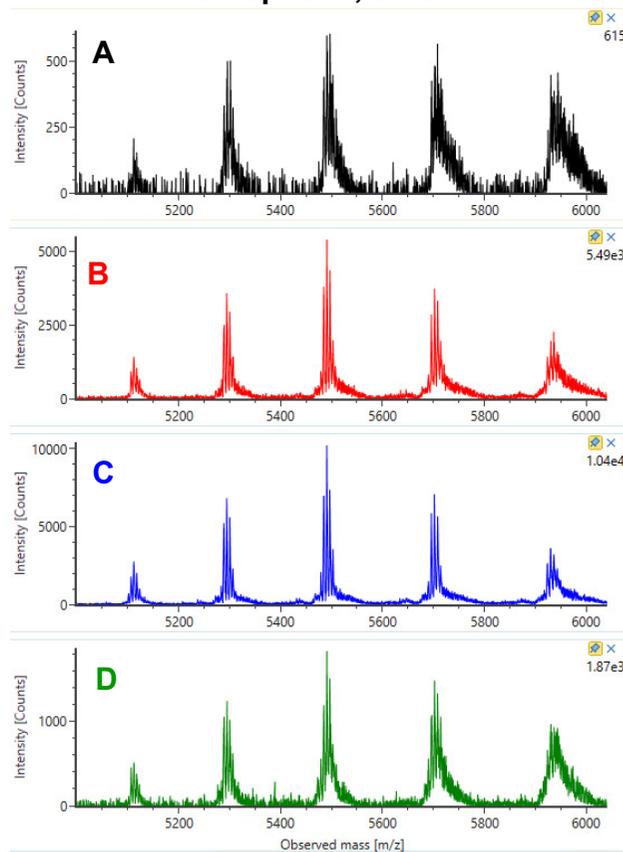
(Fab)₂ acidic variant (likely deamidation) is also detected

Case Study 2: Forced Degradation Study Trastuzumab, pH 8.0 Stress

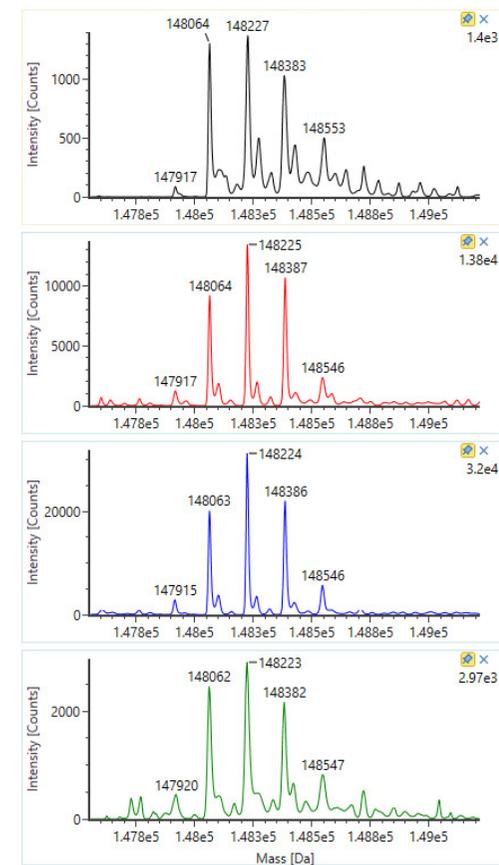


	Acidic	Main	Basic
T0	14.0%	74.7%	11.3%
1wk25C	32.7%	58.3%	9.0%
Δ	+18.7%	-16.4%	-2.3%

Combined Spectra, Smoothed

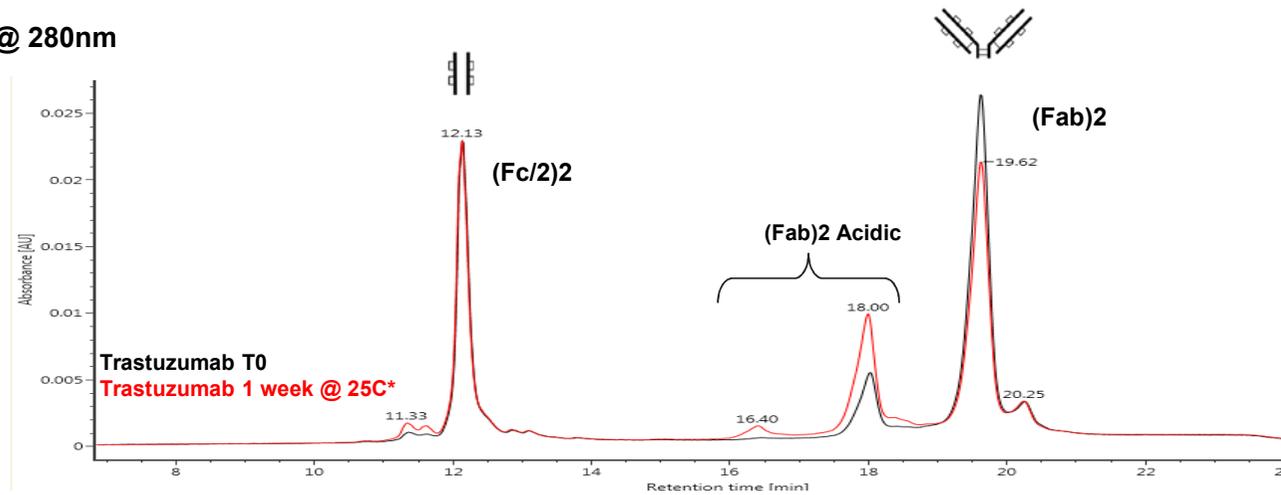


MaxEnt1 deconvolution



Forced Degradation Study Trastuzumab: IdeS Digested Samples

UV @ 280nm



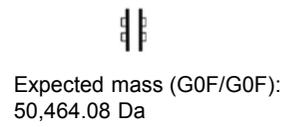
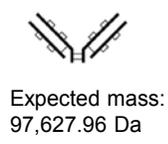
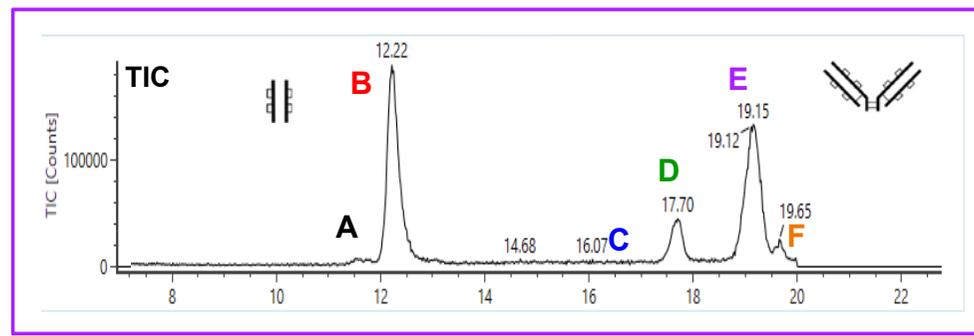
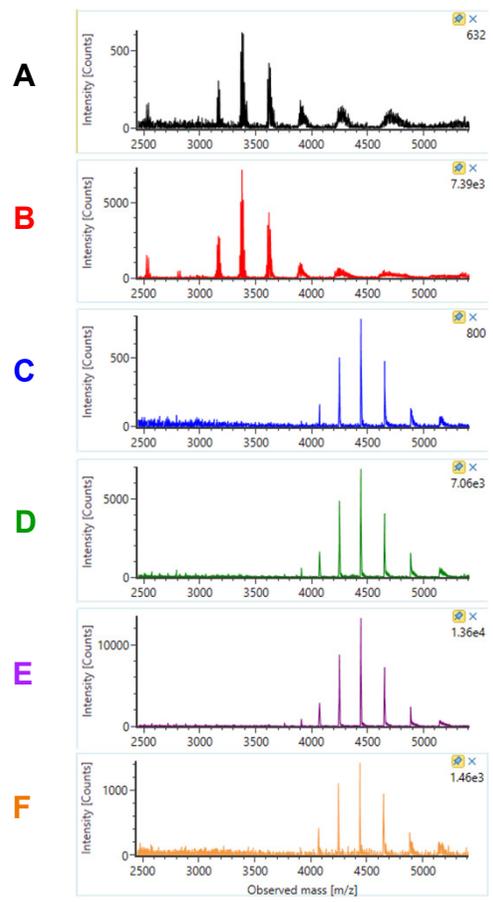
- Further localize the increase in acidic variants to the Fab region
- Good correlation to intact mAb analysis via UV integration
- IdeS digest analysis gives better mass accuracy and greater confidence in assignments

(Fc/2)2	Acidic	Main	Basic
T0	7.1%	84.4%	8.5%
1wk25C	11.6%	81.2%	7.2%
Δ	+4.5%	-3.2%	-1.3%

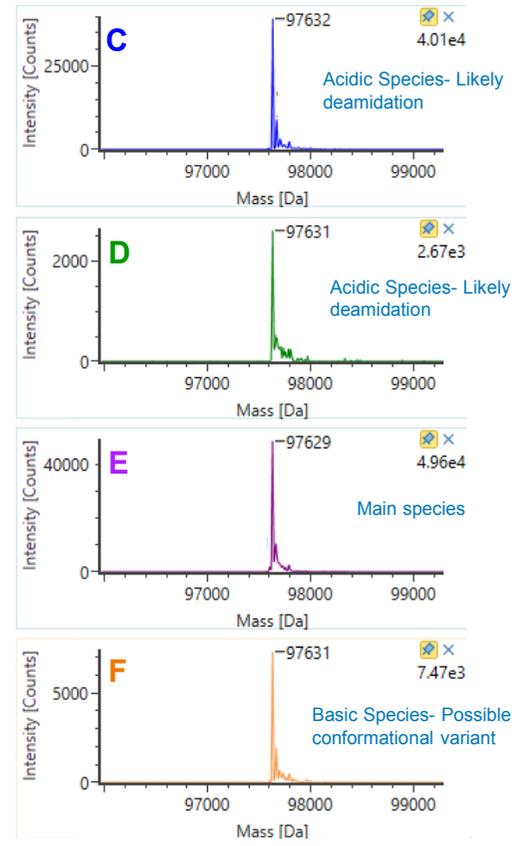
(Fab)2	Acidic	Main	Basic
T0	20.9%	70.3%	8.9%
1wk25C	34.9%	56.4%	8.7%
Δ	+14.1%	-13.8%	-0.2%

Forced Degradation Study Trastuzumab: IdeS Digested Samples

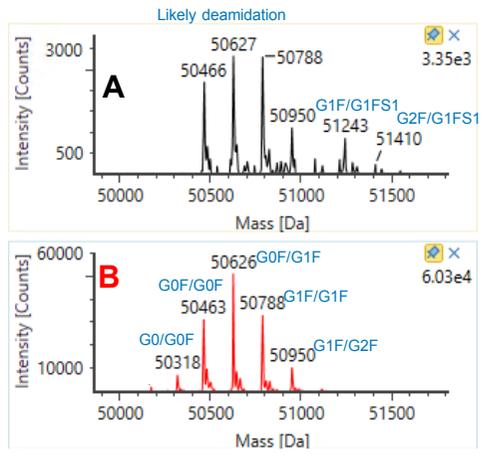
Combined Spectra



MaxEnt1 deconvolution (Fab)2



MaxEnt1 deconvolution (Fc/2)



Summary

- Charge variants are considered critical quality attributes that need to be monitored throughout the life cycle of a drug
- IEX-MS is capable of providing robust, reproducible separations coupled to real time MS data acquisition for peak identification
 - Considerations include buffers, MS settings, reagent and consumable quality
 - IonHance CX-MS buffers provide a flexible platform for easy high quality IEX-MS
 - Applicable to intact and subunit characterization and charge variant monitoring
- Oral Presentation Funai Hall, OR21, 4:50-5:05pm
 - Mechanism of Unusually High Particle Diameter Dependence of Column Backpressure with SCX Polymer Particles, Ed Bouvier
- Please visit PT-2011
 - Improving Online Ion Exchange Chromatography-Mass Spectrometry for Domain Specific Charge Variant Analysis of Monoclonal Antibodies

Your Feedback will be very much appreciated!

Please provide your feedback and you will get a gift set at reception!



Column-shaped pens