Characterization of Complex cIEF Electropherograms from mAb and Antibody-Drug Conjugate (ADC) Using a Novel icIEF-UV/MS System

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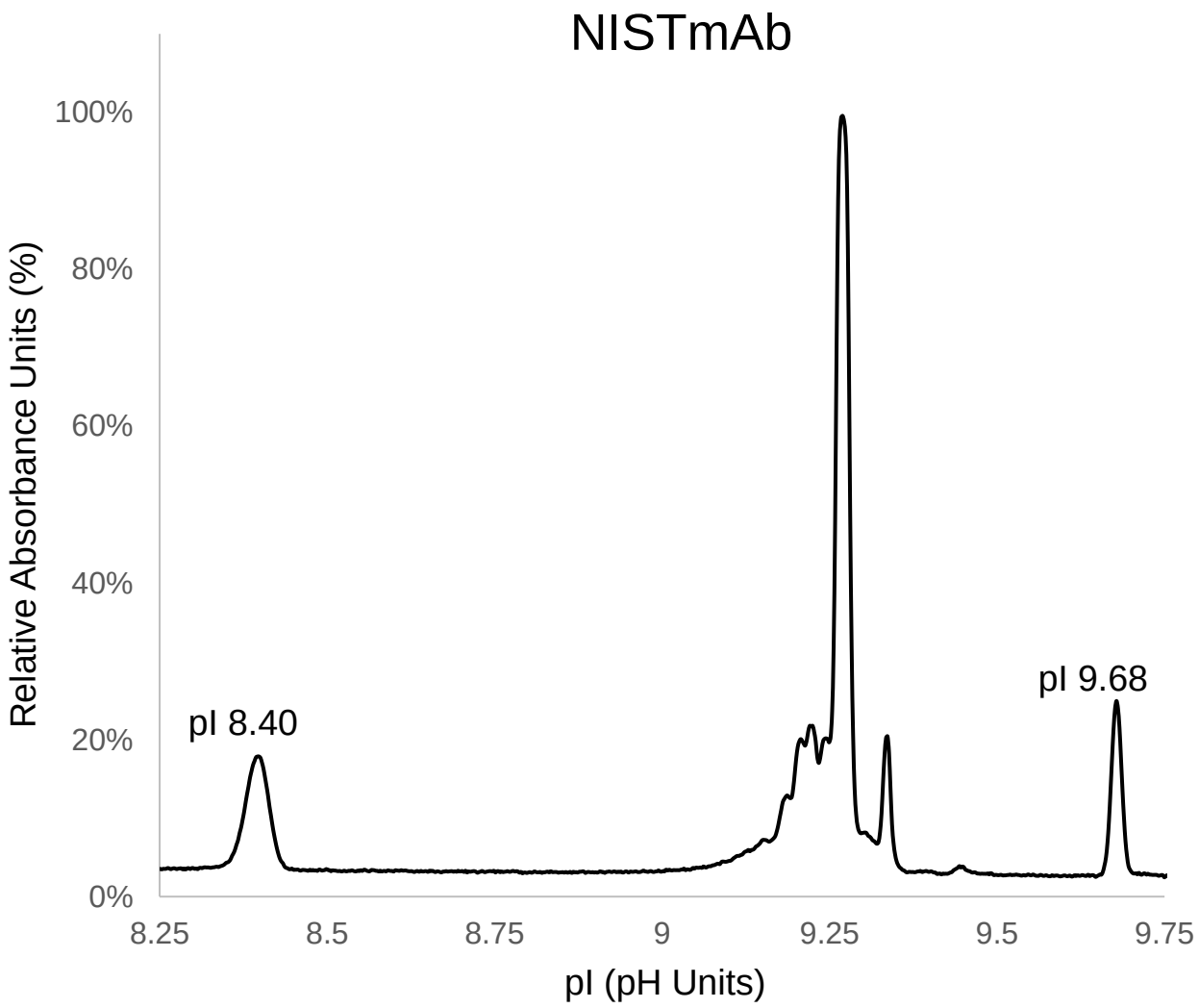
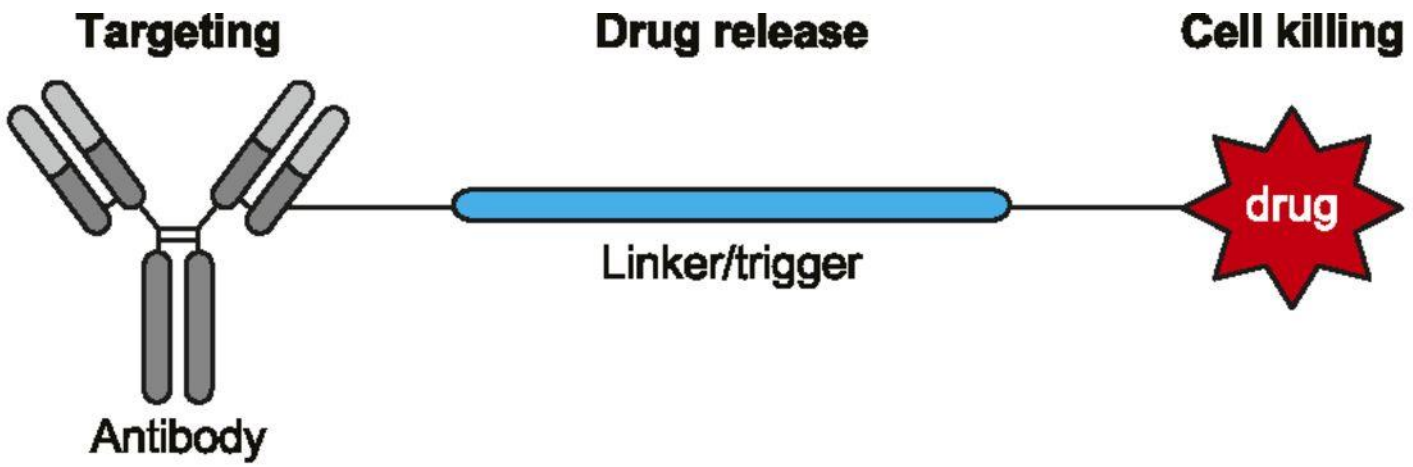
Charge Heterogeneity Analysis Plays a Critical Role in Biopharmaceutical Development

- Charge heterogeneity is present in most biopharmaceutical protein products
  - The pKas of the amino acids and post translation modifications impart charge
  - In some instances, critical quality attributes are monitored by these methods (deamidations, oxidation, isomerization, glycation…)
- Charge heterogeneity profiles reflect process consistency, and constantly involves in the in-process and release testing
- Isolation and identification of charge variants is an important part of product characterization and manufacturing control strategy development

icIEF Can Tell the Charge Heterogeneity



Antibody Drug Conjugate General Introduction

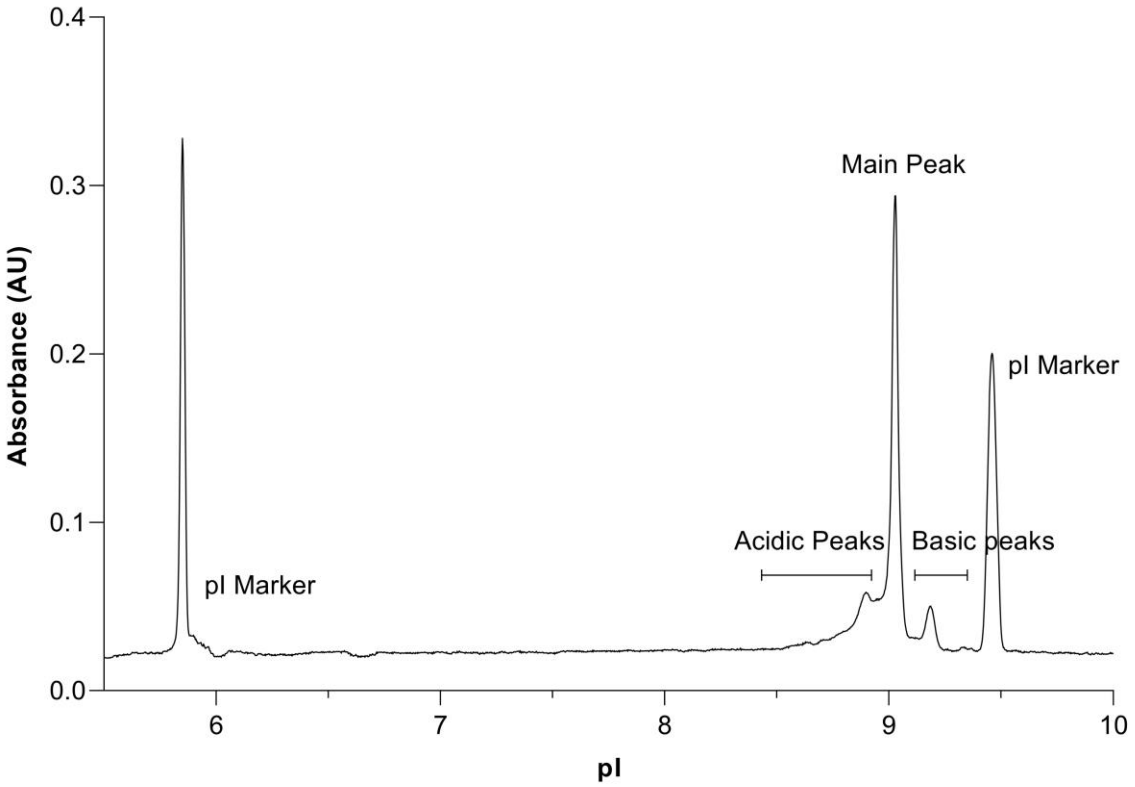


Targeted antigen	Release mechanism	MOA
Tumor Specific	Cleaved by reduction	Microtubule disruption DM1, DM4, MMAE, MMAF
Minimal Normal Expression	Cleaved by low pH	DNA damage calicheamicin, duocarmicin, SN-38, D6.5, PDB dimers
Internalizing	Cleaved by proteases	Transcriptional Inhibitor amanitin
Prevalent in cancers	Non-cleavable	
Abundant in cancers		

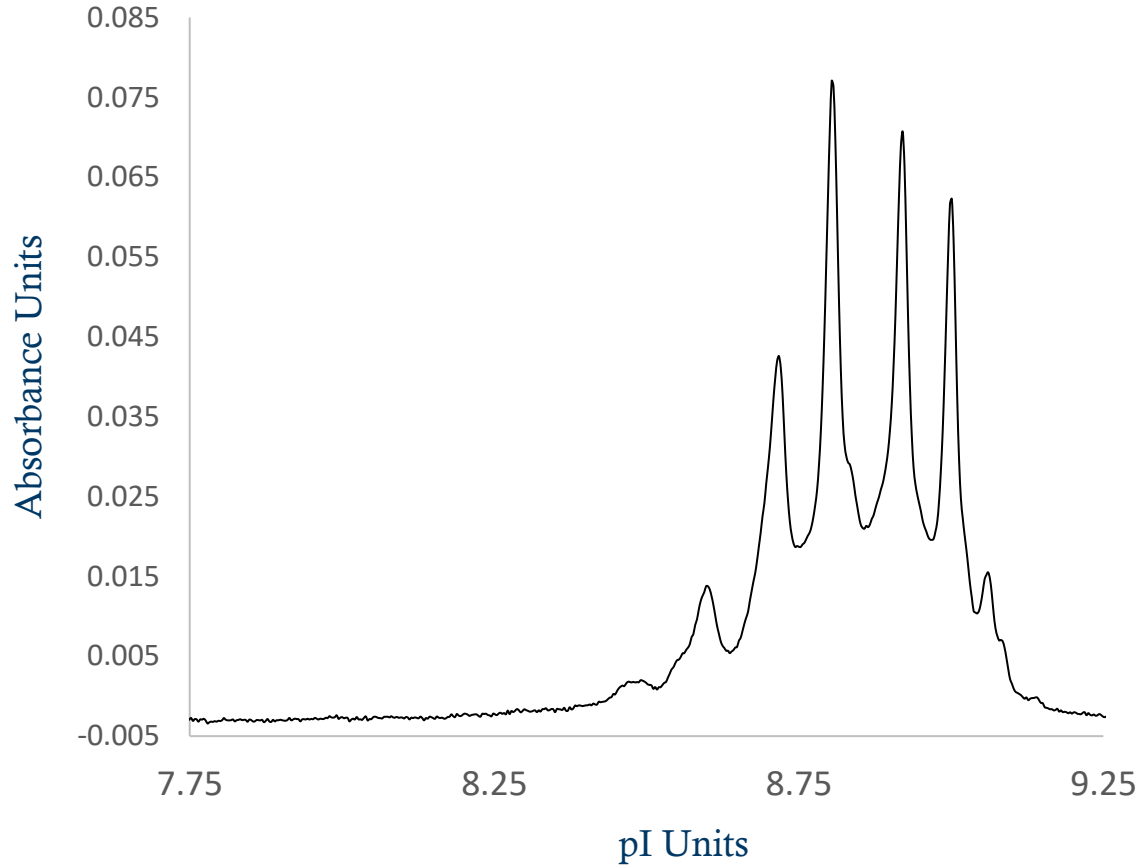
Paul Polakis Pharmacol Rev 2016;68:3-19

The Unexpected: ADC Profile post Conjugation

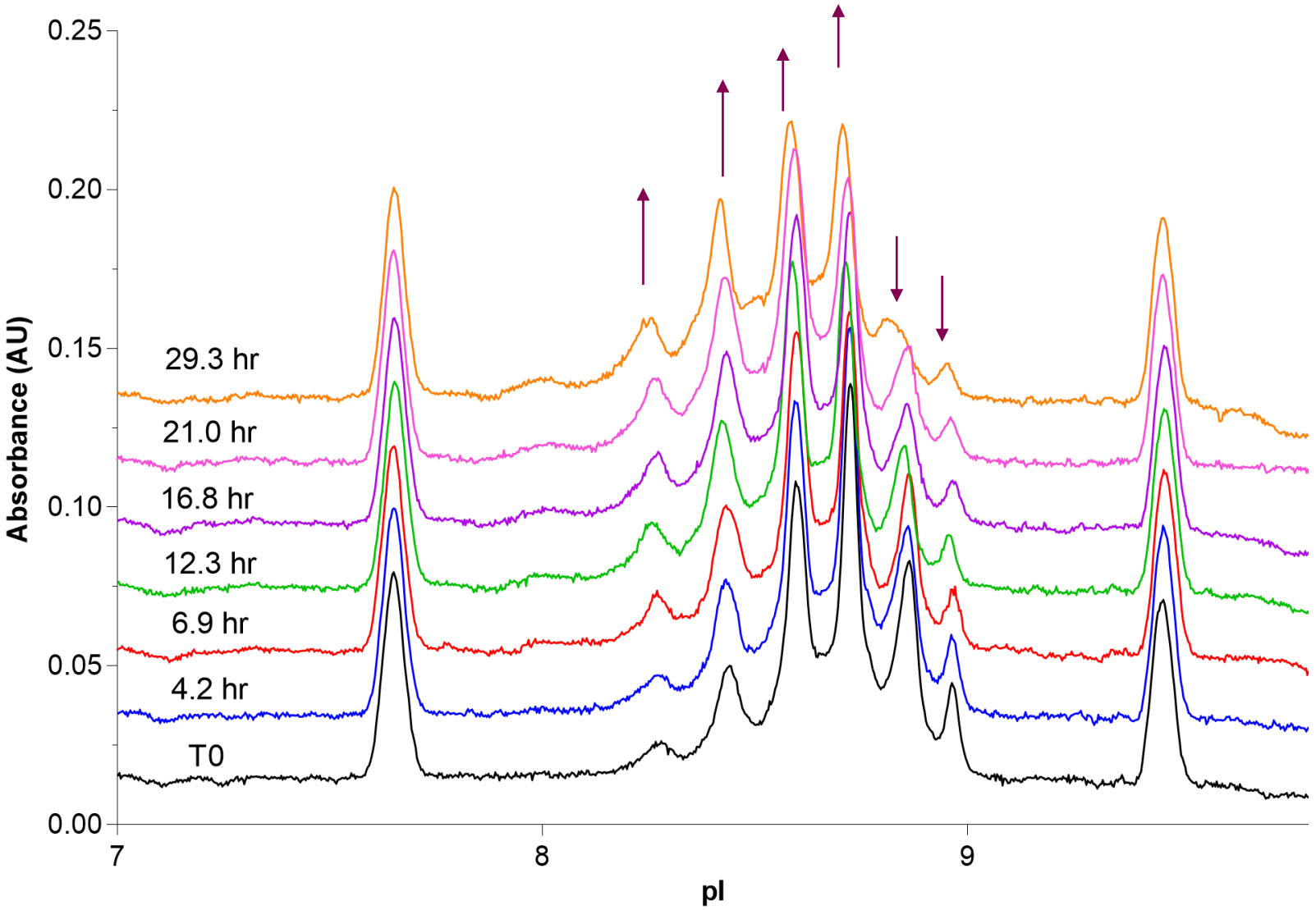
Int-01 icIEF Profile



ADC-1 icIEF Profile

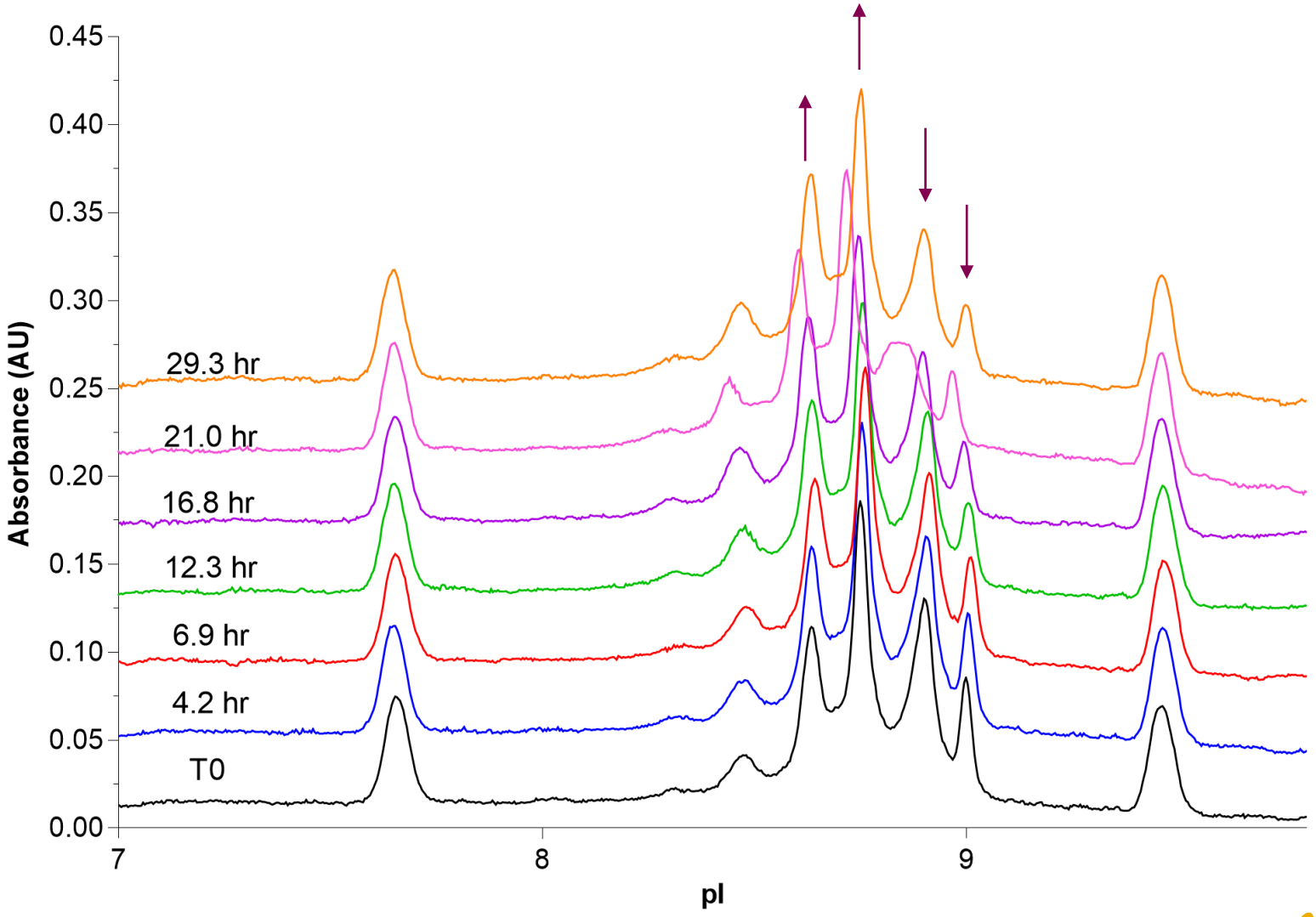


The Unexpected: Autosampler Stability for ADC-1

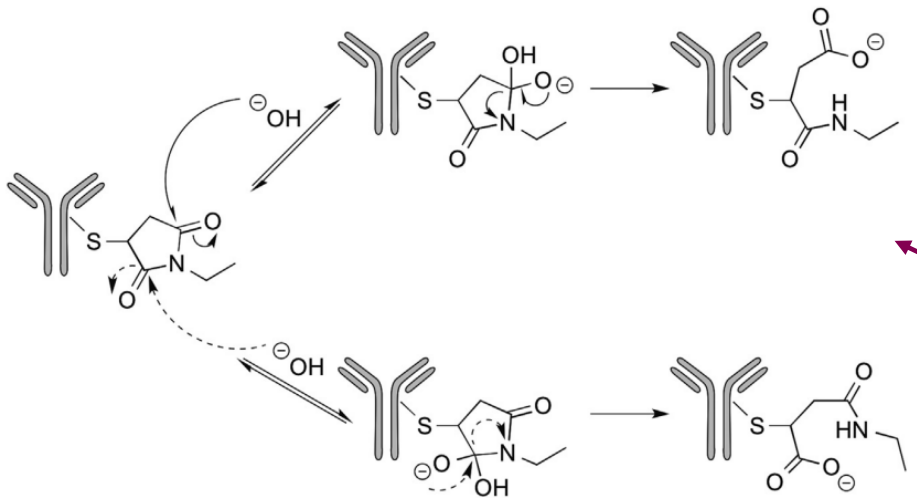


cIEF Method Optimization Could Improve Some Autosampler Stability, But these ADC Issues Still Remains

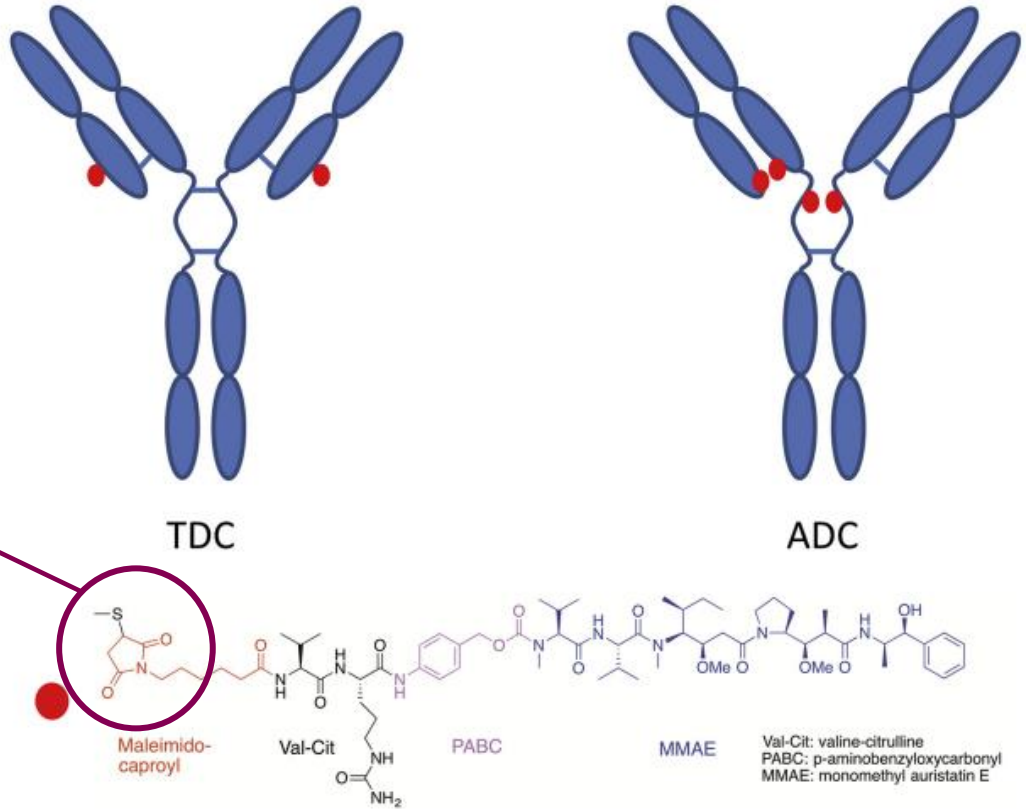
- Increasing the pharmalyte concentration and decreasing the mixture pH slowed the loss in basic peaks and growth in the acidic peaks
- Are the issues related to payload?



Could It Be the Payload?

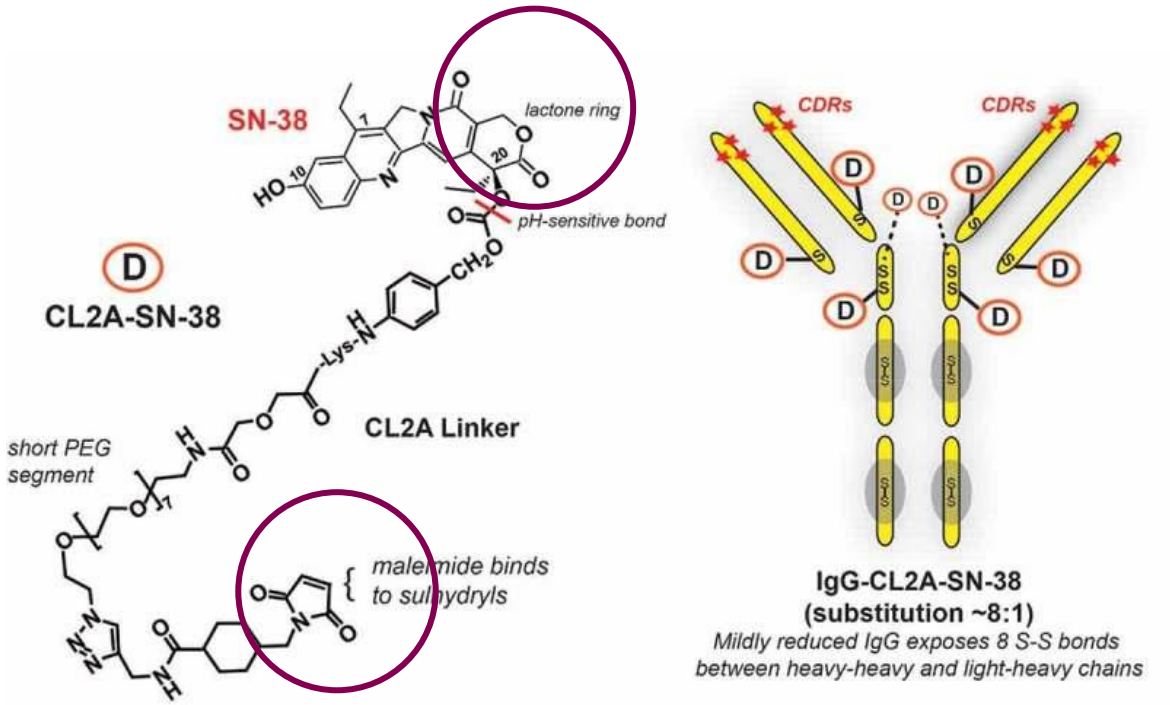


K. Zheng, *et al.* ,Journal of Pharmaceutical Sciences 108 (2019) 133-141

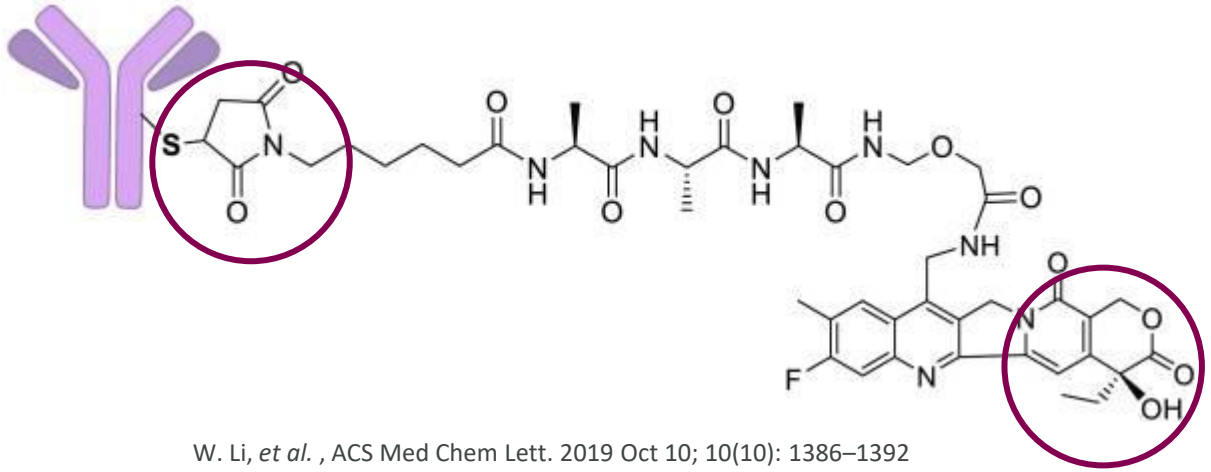


K. Zheng, *et al.* ,Journal of Pharmaceutical Sciences 108 (2019) 133-141

Charge Change in Cysteine Conjugated Payloads?

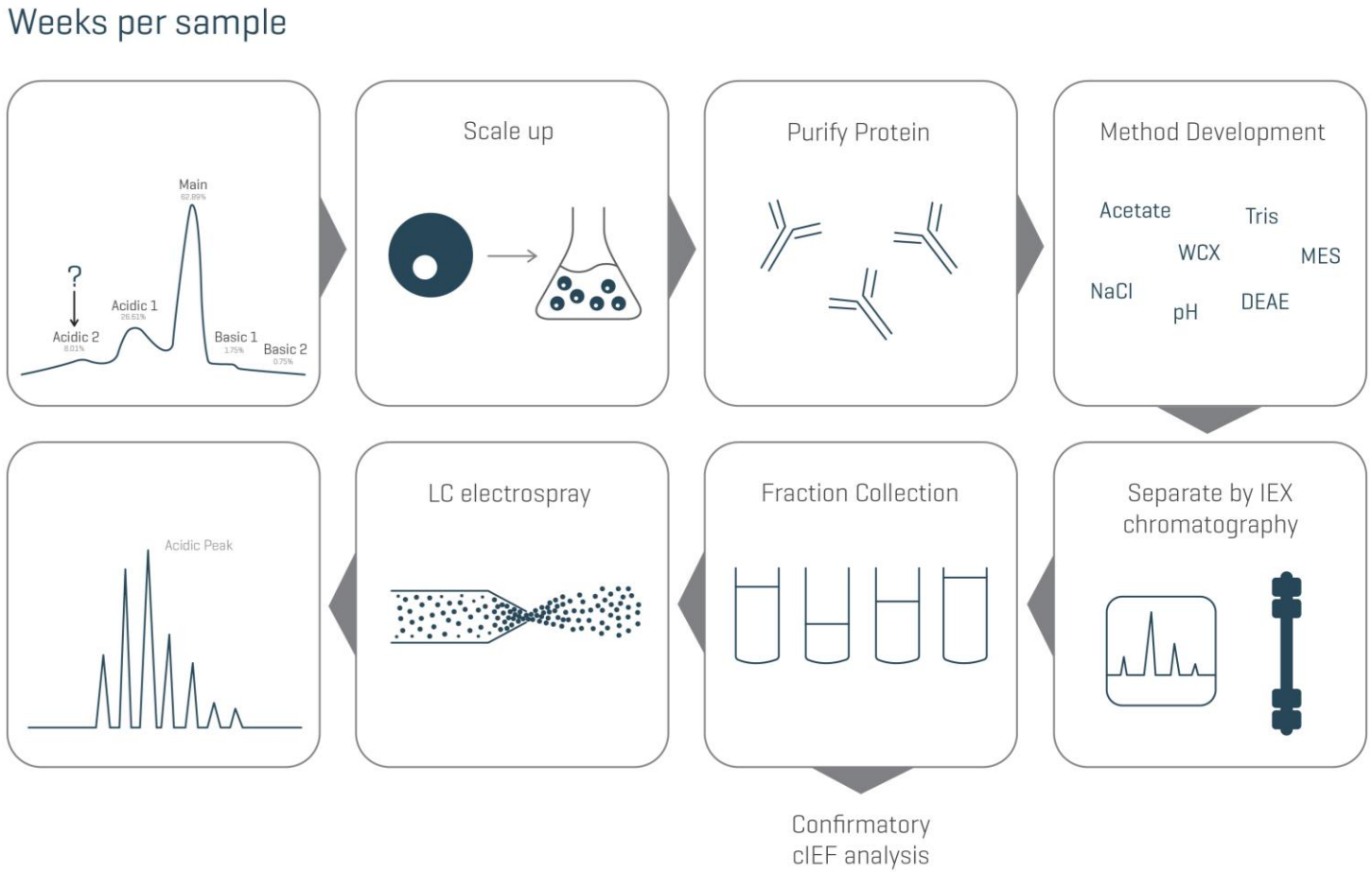


D. Goldenberg, R. Sharkey, MAbs. 2019 Aug-Sep; 11(6): 987–995.



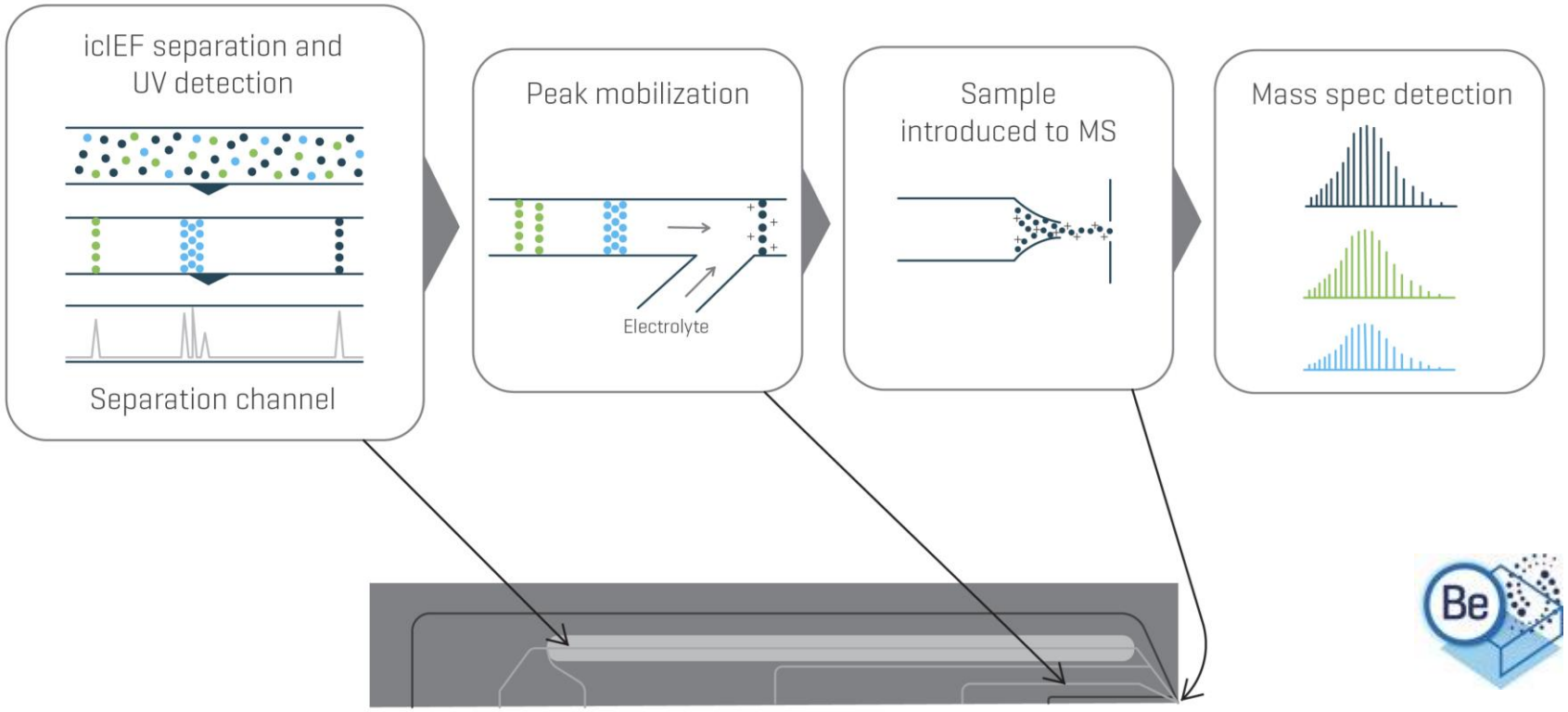
W. Li, *et al.* , ACS Med Chem Lett. 2019 Oct 10; 10(10): 1386–1392

What Can We Do When icIEF Does Not Connect to MS?



- Sample abundance
- Sample stability
- Turn around time for the characterization

SCIEX's IntaBio ZT Microfluidic Chip icIEF-UV/MS



- icIEF separation
  Real-time UV absorbance imaging
- ~30-min sample analysis
- High-sensitivity and high-resolution MS data from ZenoTOF 7600 system
- Biologics Explorer software for data analysis

icIEF-UV Analysis

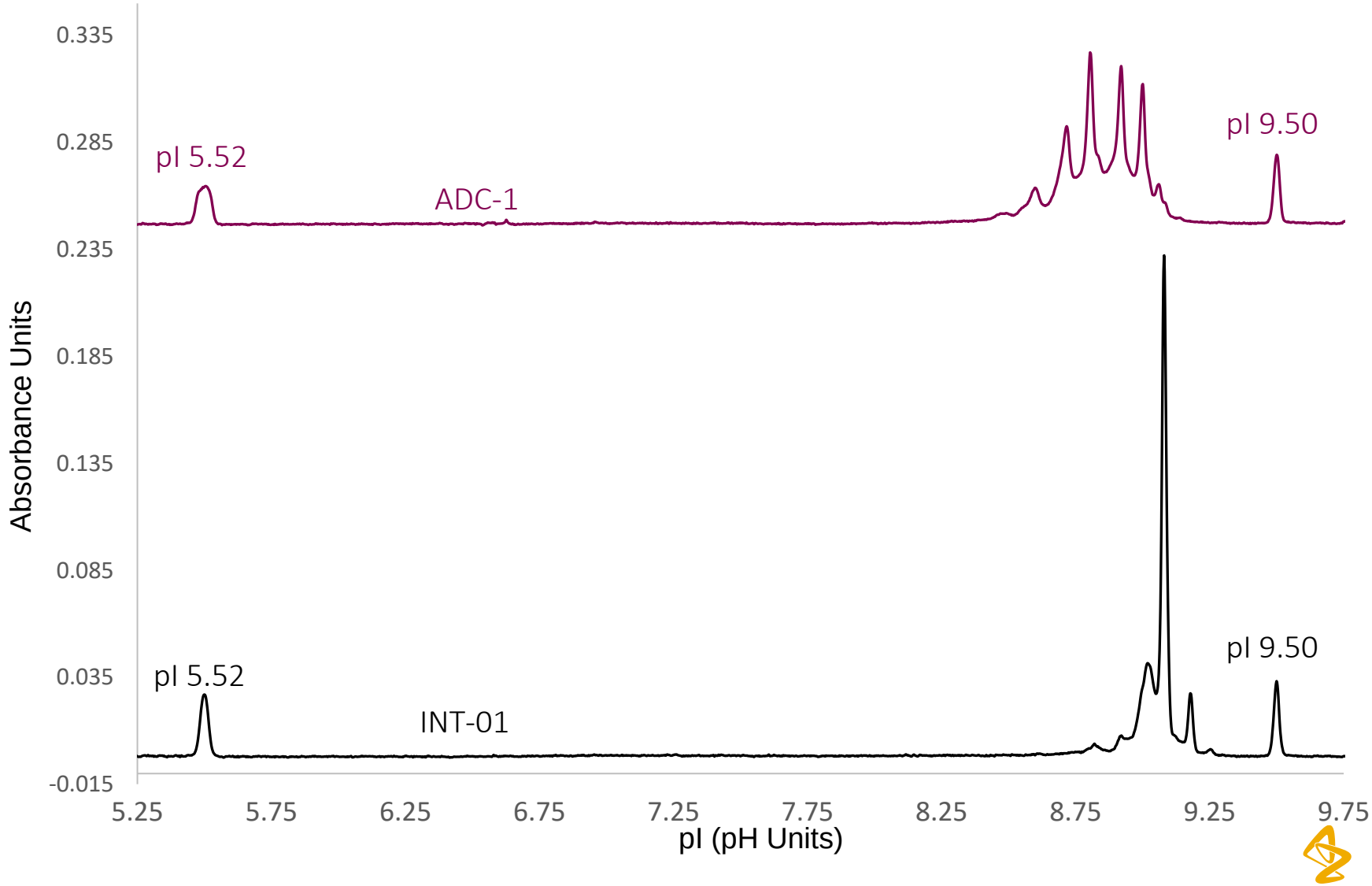
Peak Characterization and Quantitation

INT-01 and ACD-1 icIEF-UV Methods

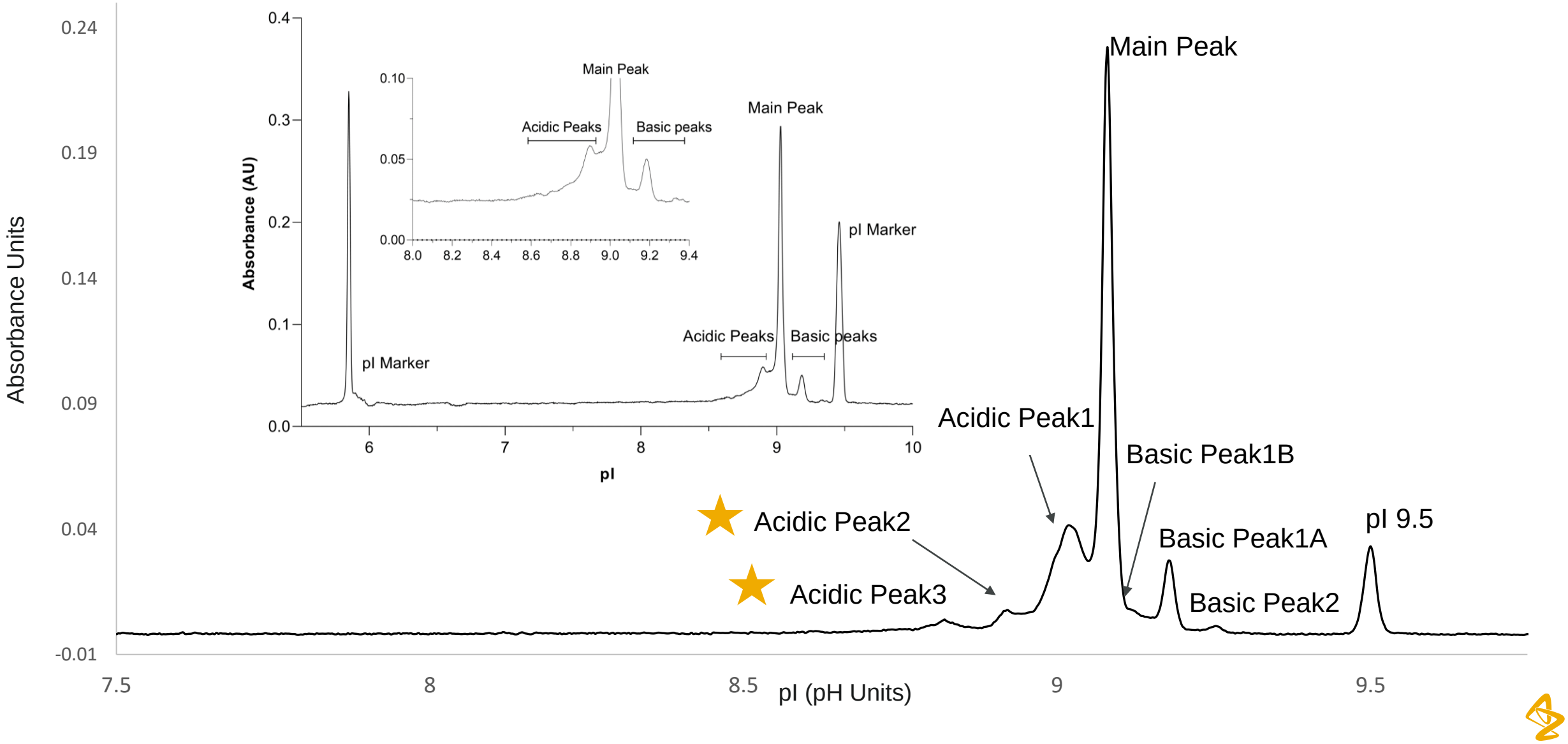
Platform Method

- 3% Pharmalyte 8 to 10.5
- 3% Pharmalyte 5 to 8
- 15 mM Arginine
- 400-1000 µg/mL Protein
- pI estimated with pI 5.52 and 9.50 peptide markers

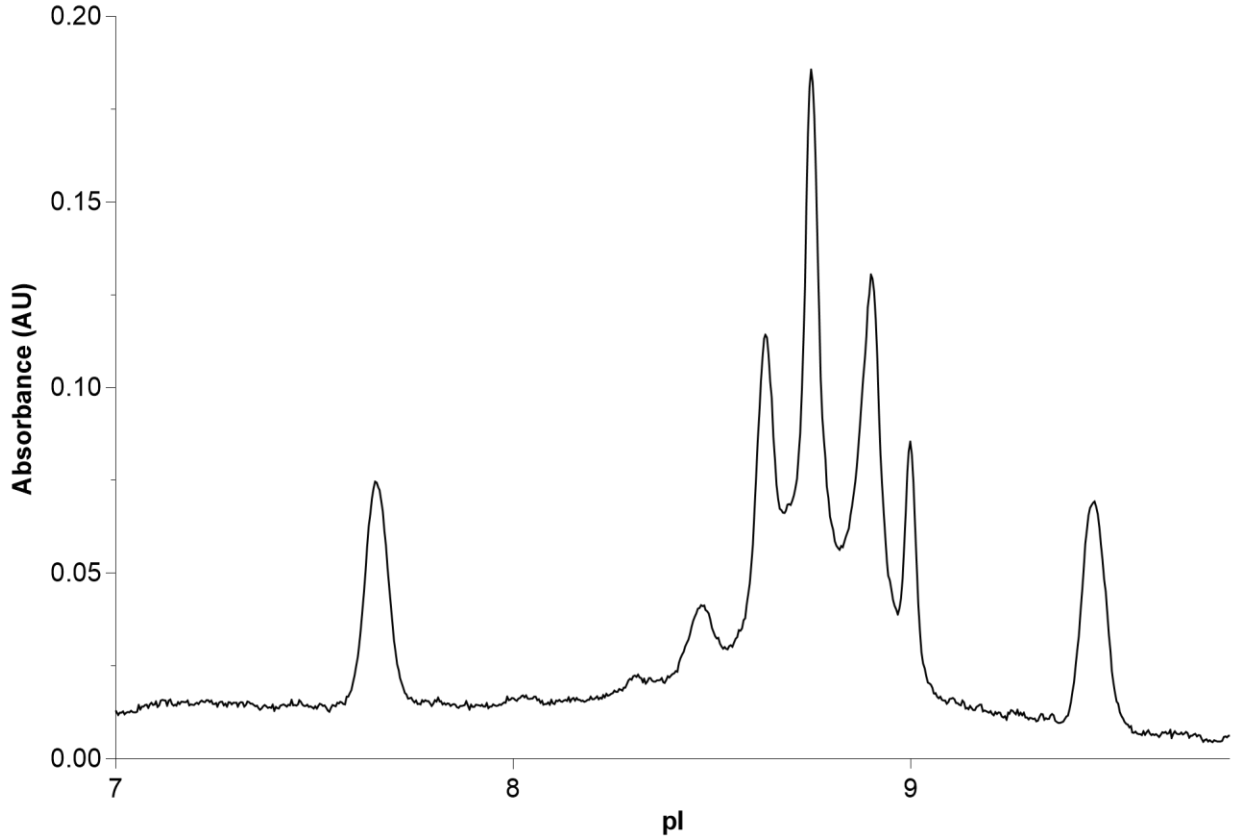
- Focusing time 6.5 Min
  - 1500 V 1 Min
  - 3000V 1 Min
  - 4500V 4.5 Min
- Mobilization time 10 Min
  - Mobilization 3000V
  - ESI Tip 5500V

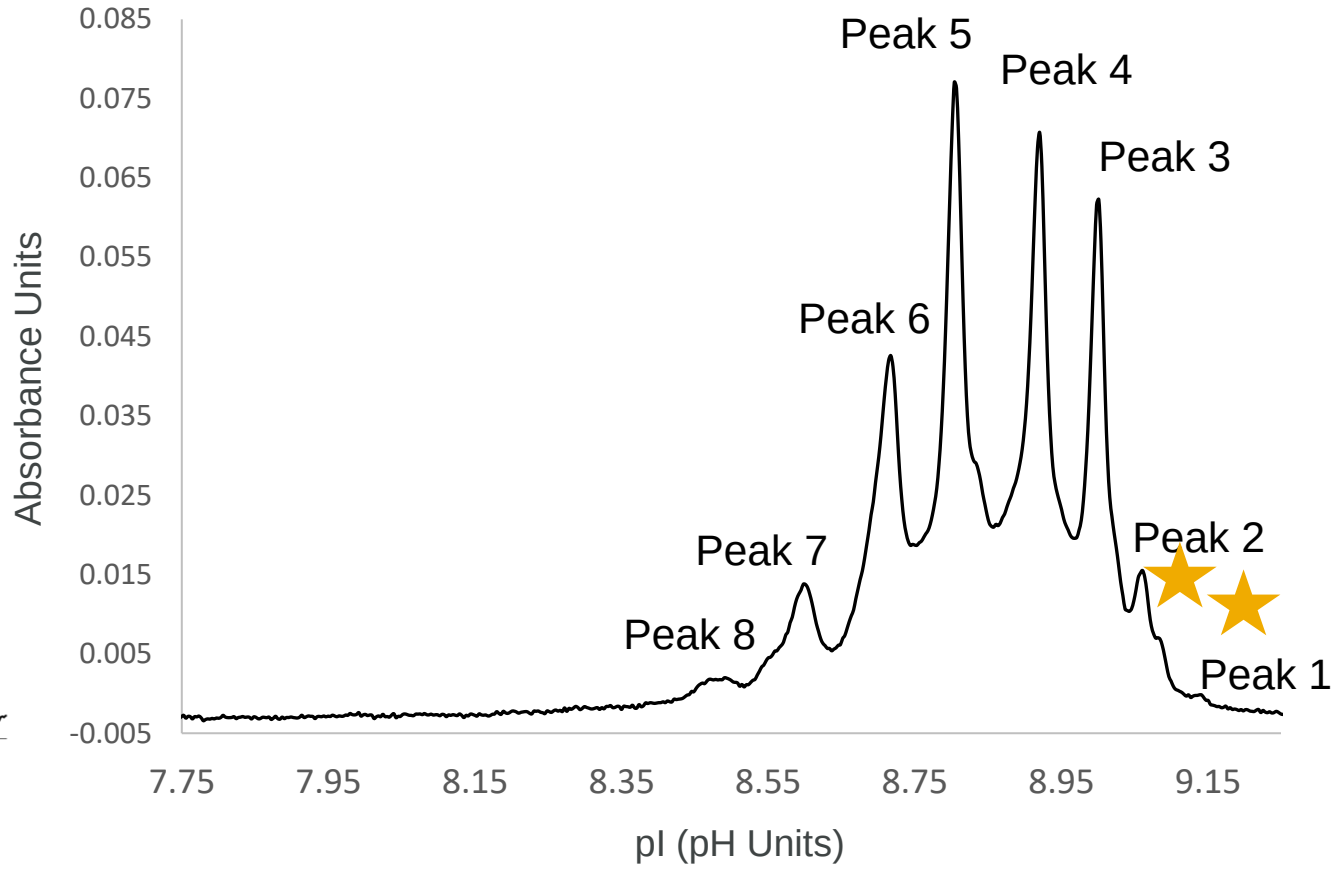


INT-01 IntaBio ZT icIEF-UV Profile is Similar to ProteinSimple iCE3



The ADC-1 icIEF-UV Charge Profile Shows Similarity to iCE3

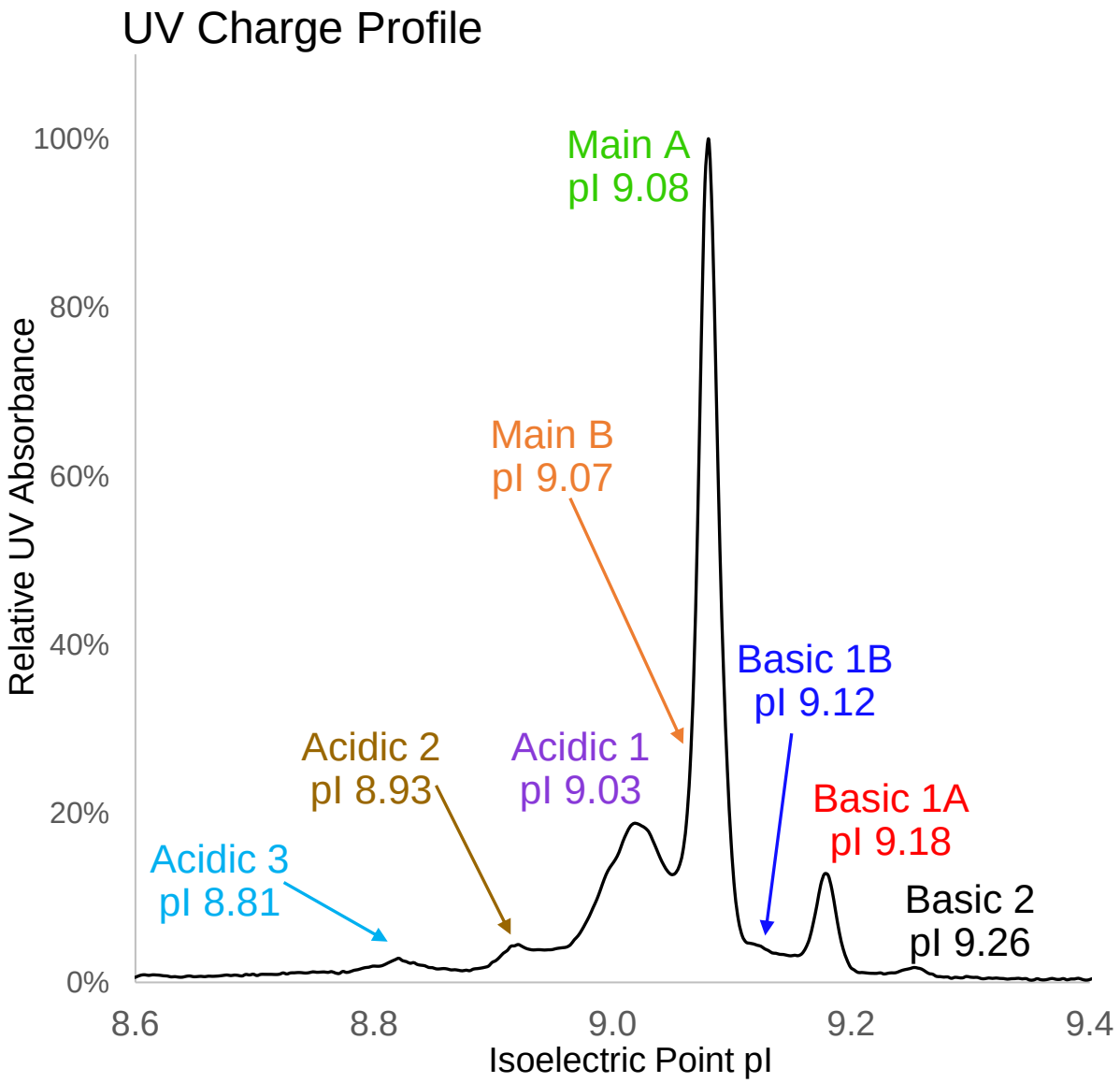


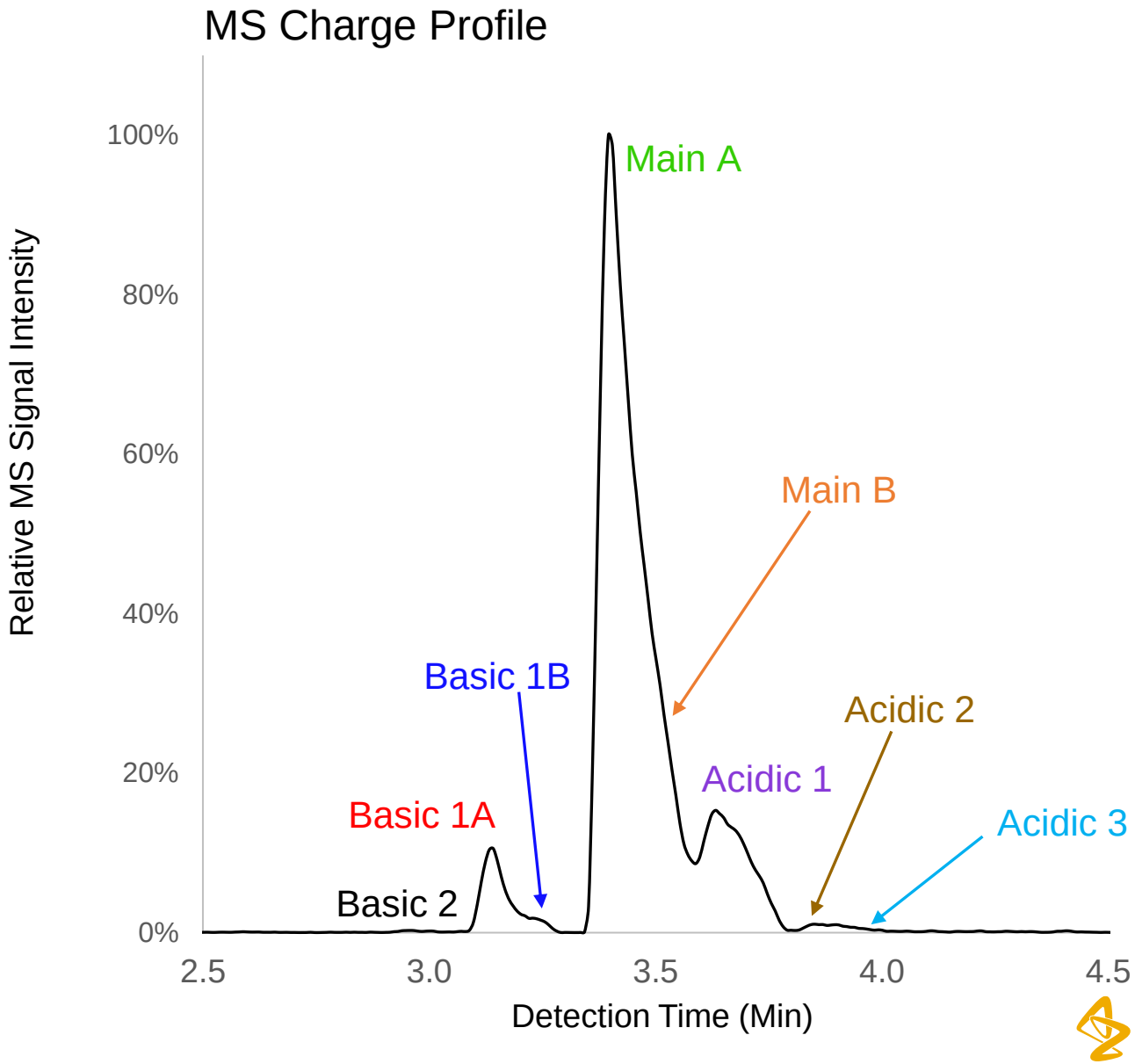


icIEF-MS Analysis

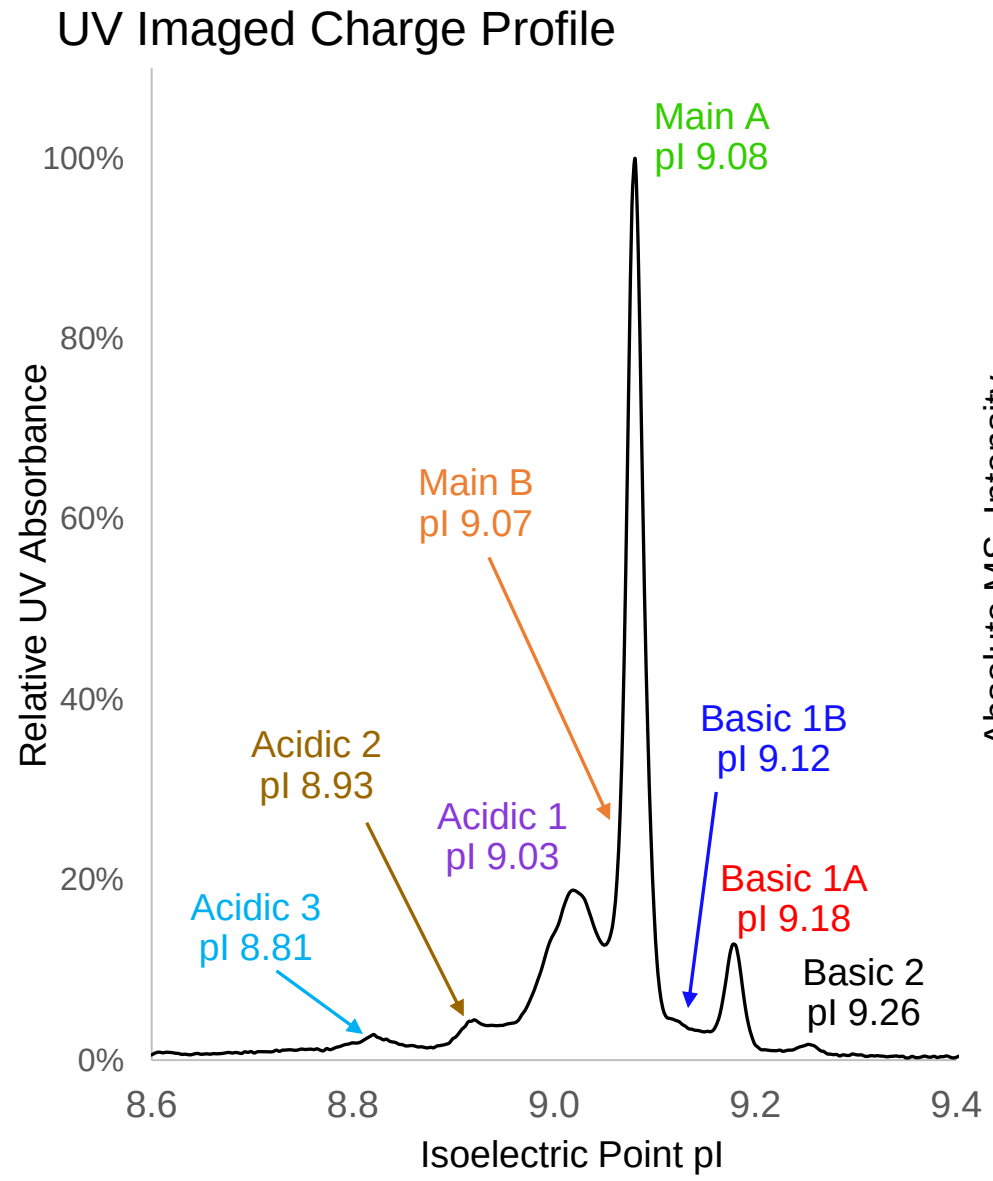
Peak identification

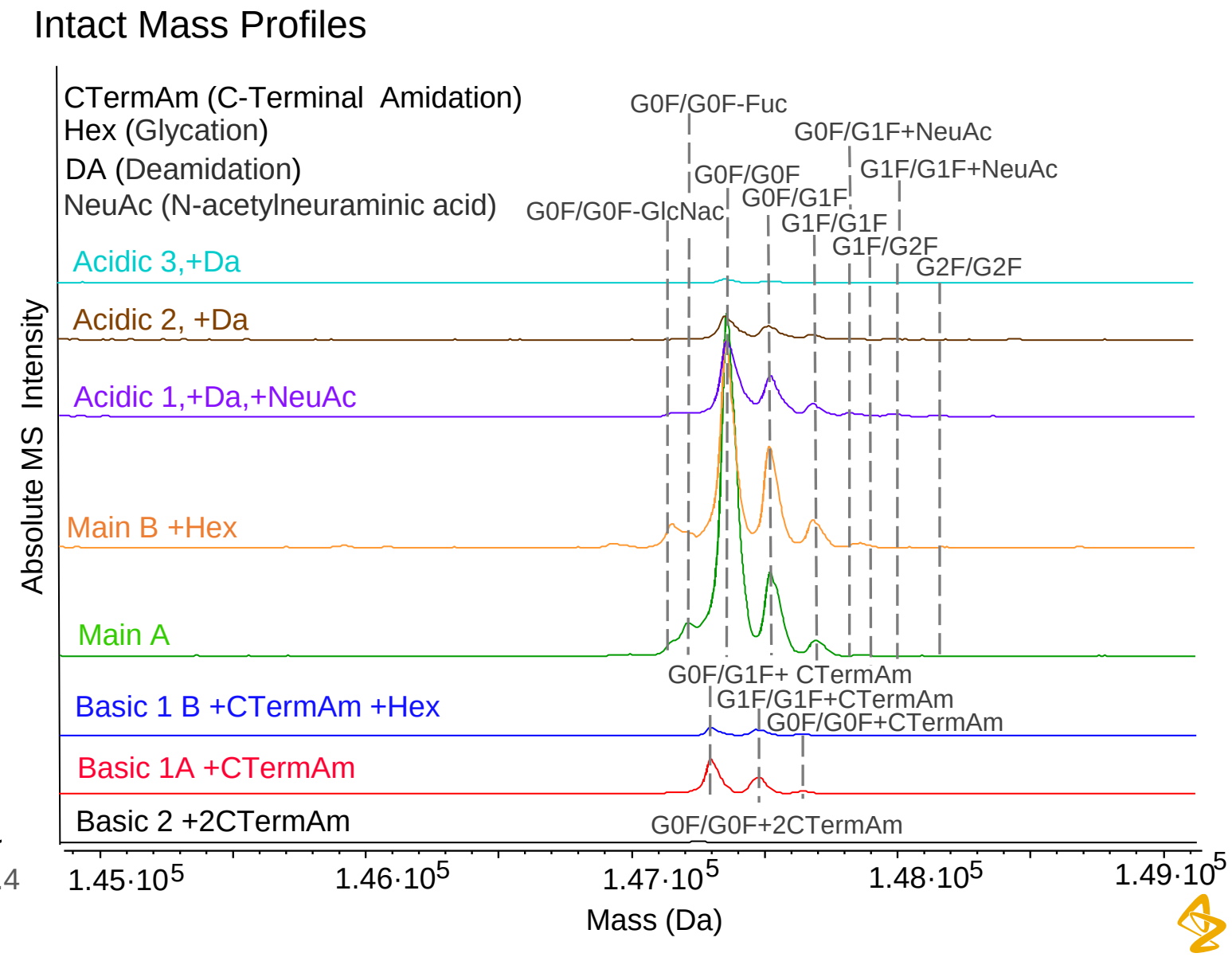
INT-01 UV Charge Profile Shows Good Comparability with the icIEF-MS



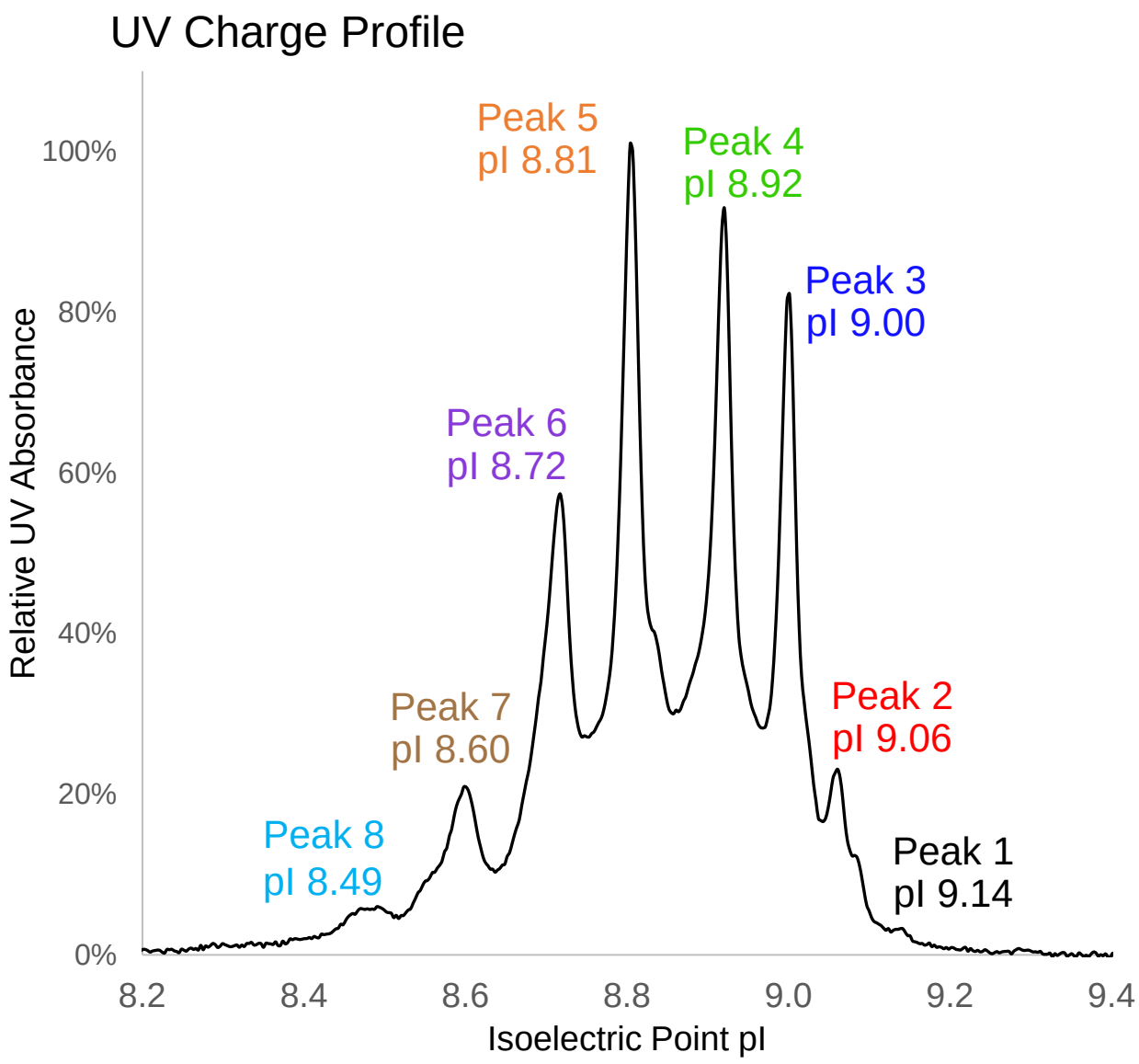


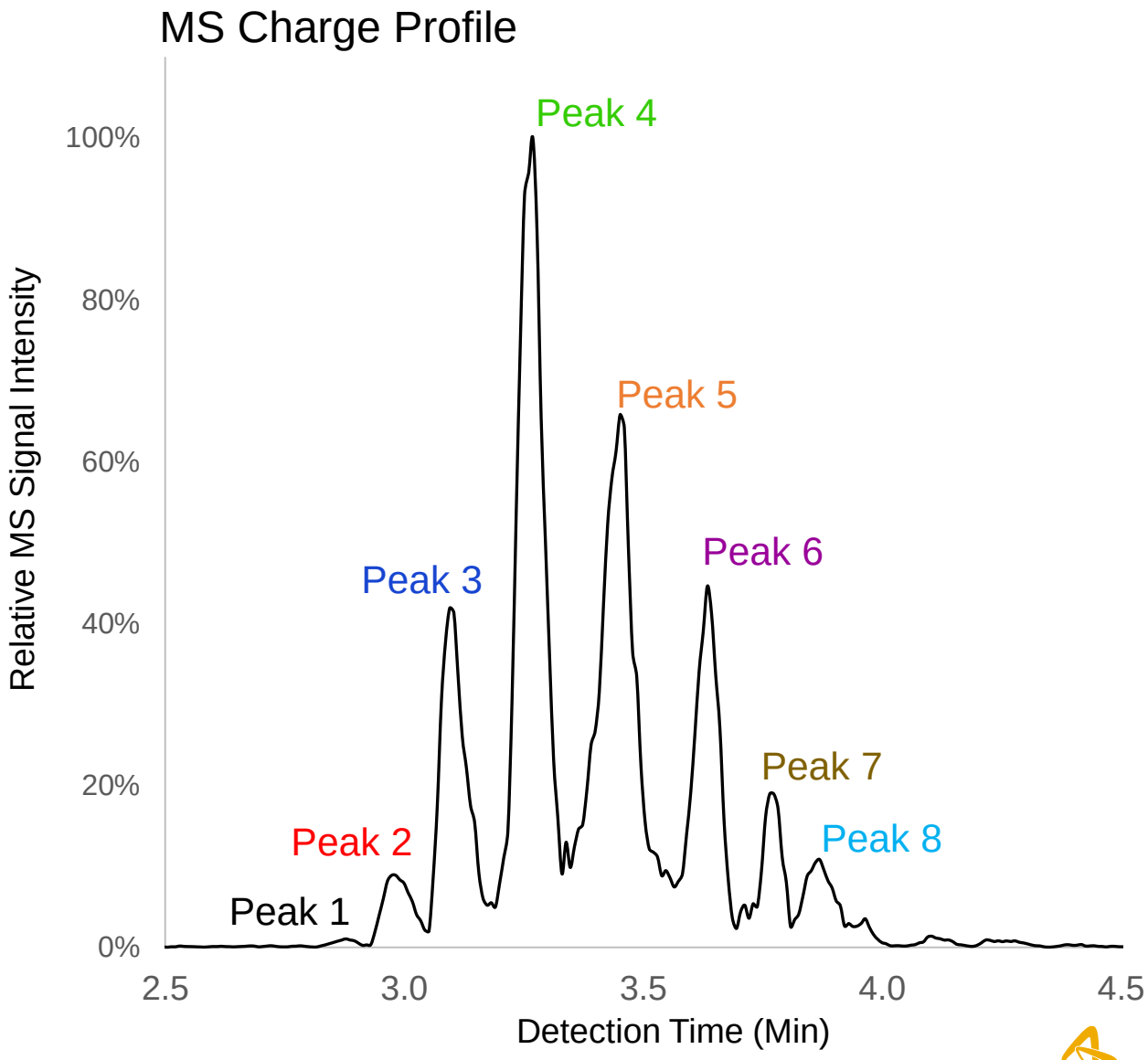
INT-01 icIEF-UV/MS Charge/Mass Isoform Identification with Typical mAb Quality Attributes



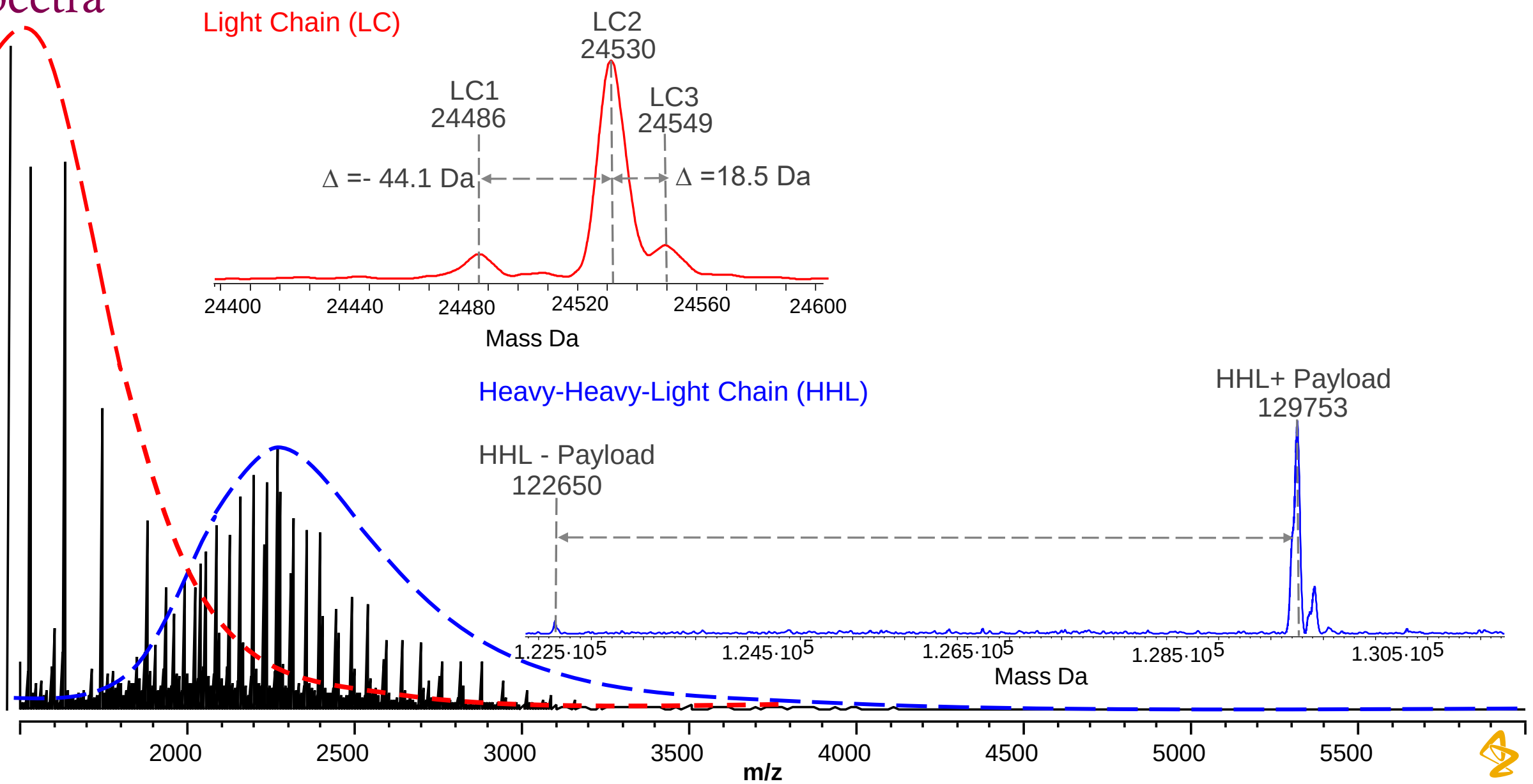


ADC-1 UV Chrage Profile Shows Good Comparability to the icIEF-MS

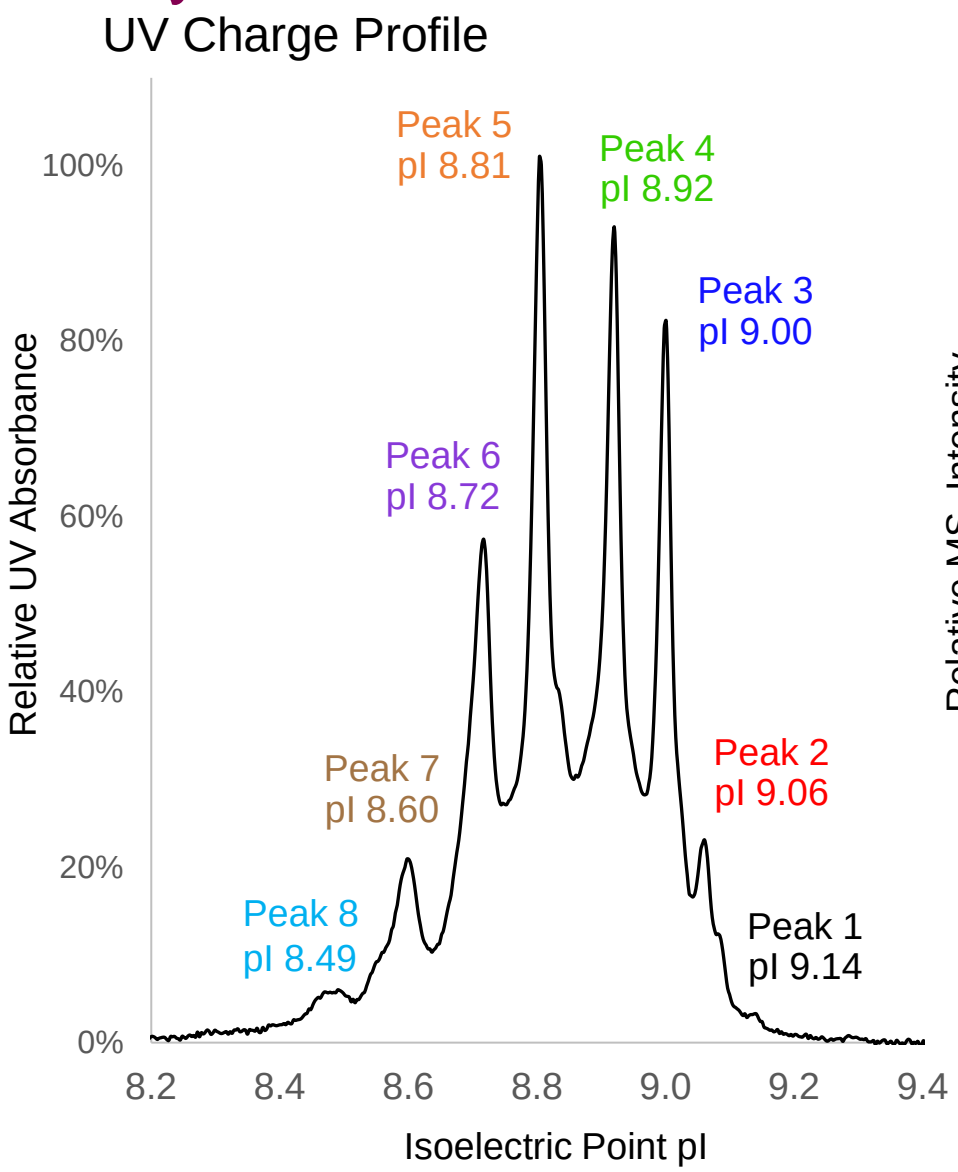


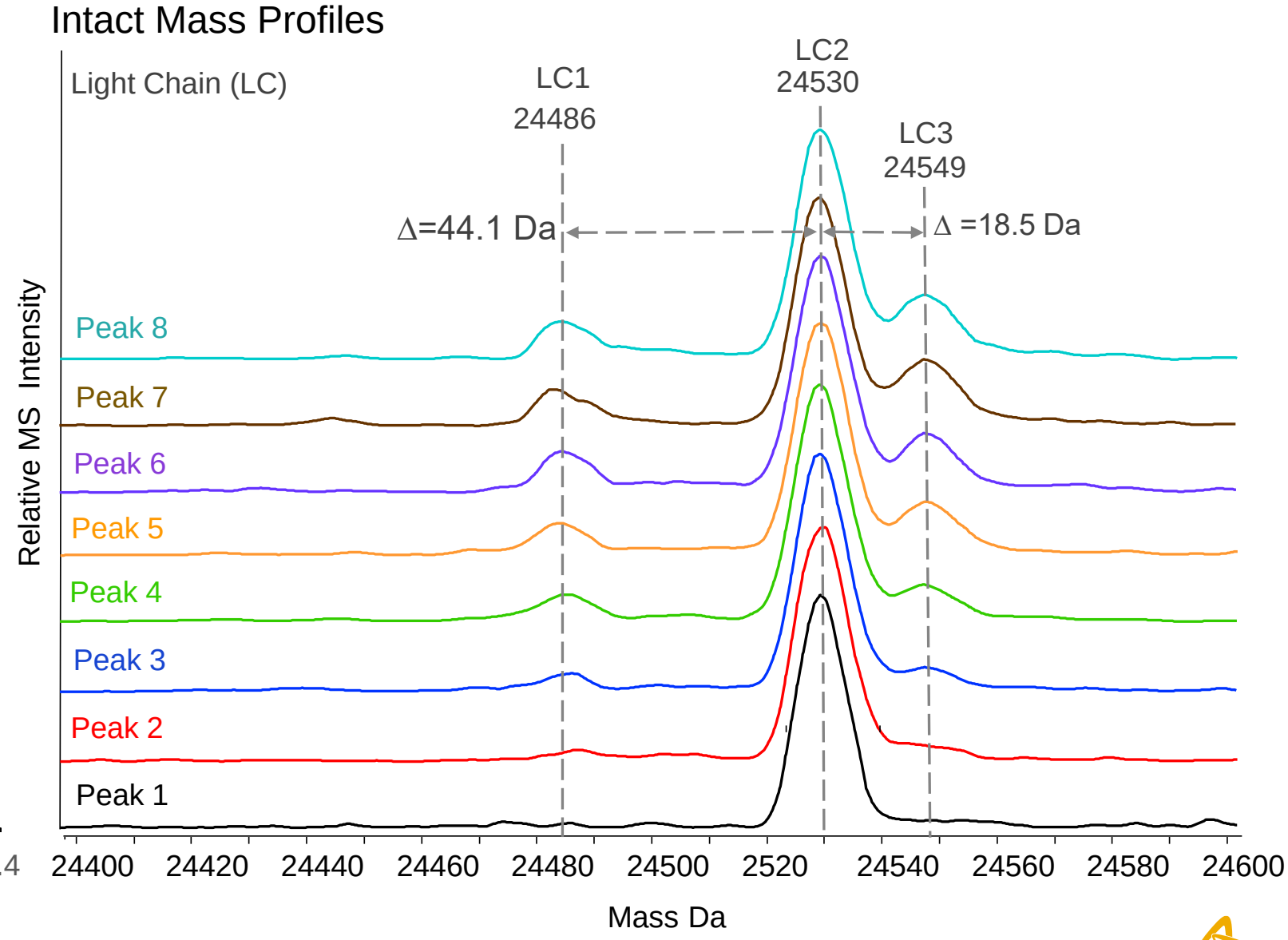


Two Charge Envelopes in the Deconvolution of the ADC-1 Peak Mass Spectra

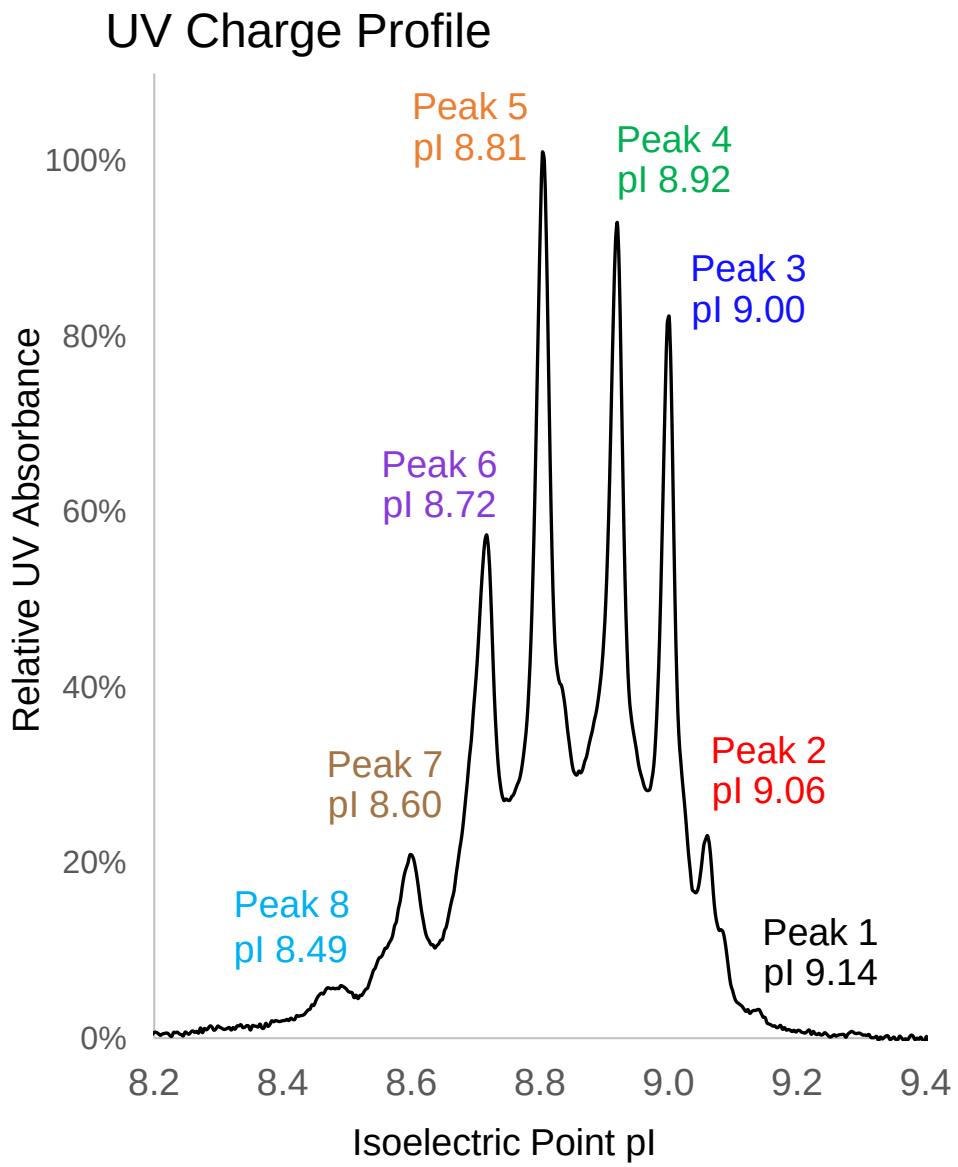


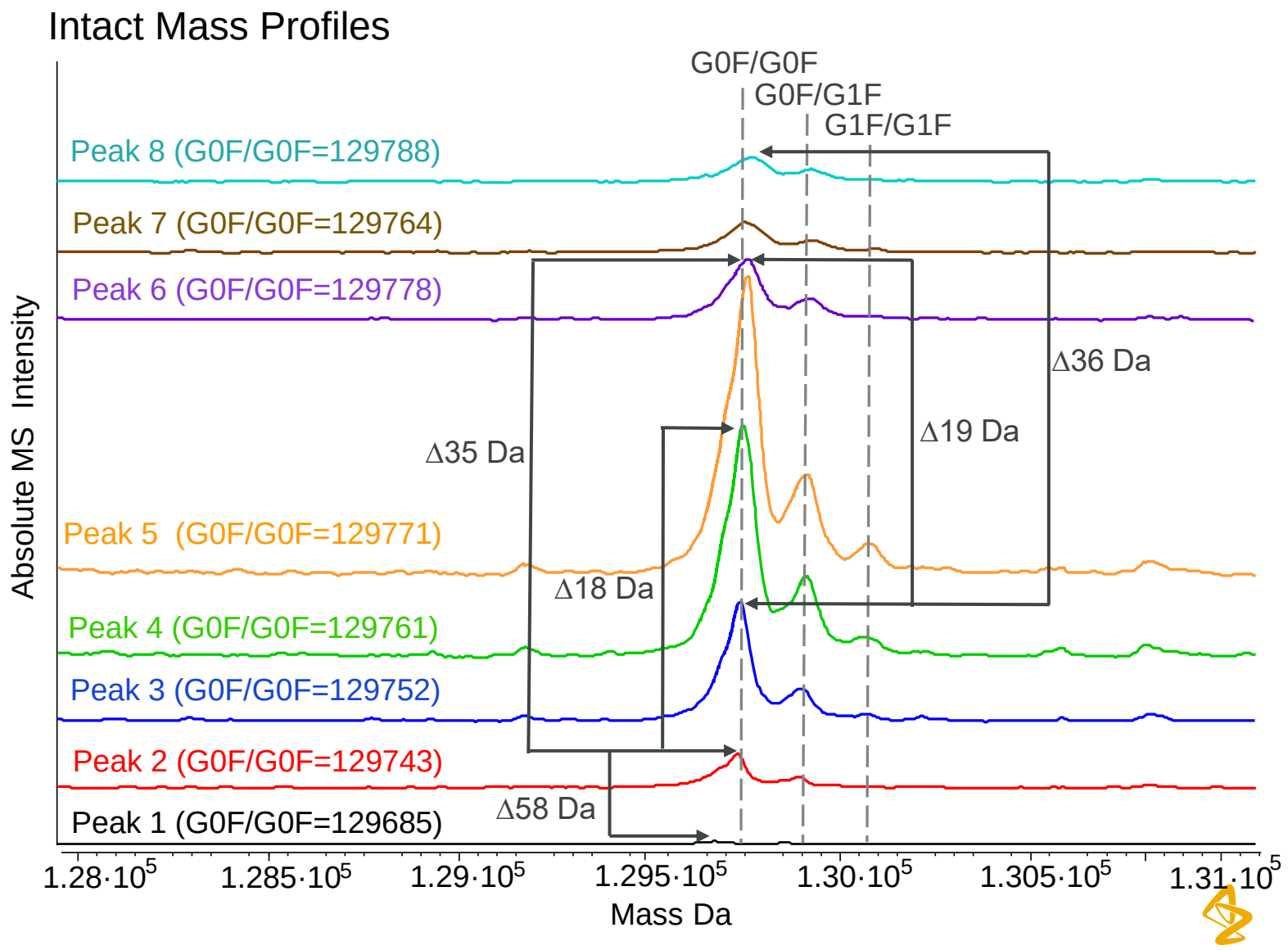
ADC-1 Light Chain Charge/Mass Isoforms Show Shifts Related to Payload Modifications





ADC-1 Heavy-Heavy-Light Charge/Mass Isoforms Show Shifts Related to Payload Modifications



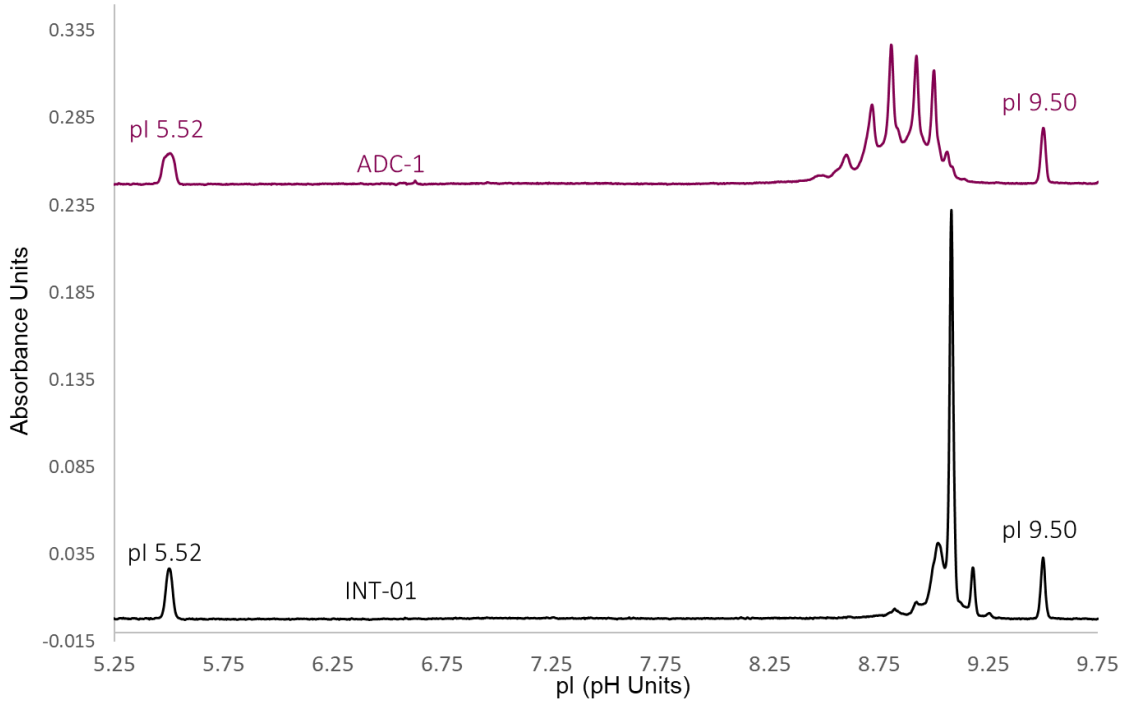


icIEF-UV/MS Peak Characterization Discussion and Summary

- Both INT-01 and ADC-1 were separated under IntaBio ZT platform icIEF-UV/MS conditions. Conjugation of INT-01 into ADC-1 resulted in both a reduction in pI and increase in charge heterogeneity
- Deconvoluted icIEF-MS of INT-01 shows that charge heterogeneity was the result of C-term amidation, deamidation and glycation
- The acidic charge variants in ADC-1 had light chain (LC) and a heavy-heavy-light chain (HHL) with mass isoforms shift by approximately 18 and or 36 Da
  - The shift in pI and molecular weight is consistent with carboxylic acid formation from succinimide ring and lactone ring hydrolysis
  - The payload modifications are fast process, where conventional offline fractionation LC-MS may not identify
- There is an inverse relationship between pI of the ADC and relative abundance of the +18 Da isoform indicating that the noncovalent interactions between LC and HHL was preserved during the icIEF separation

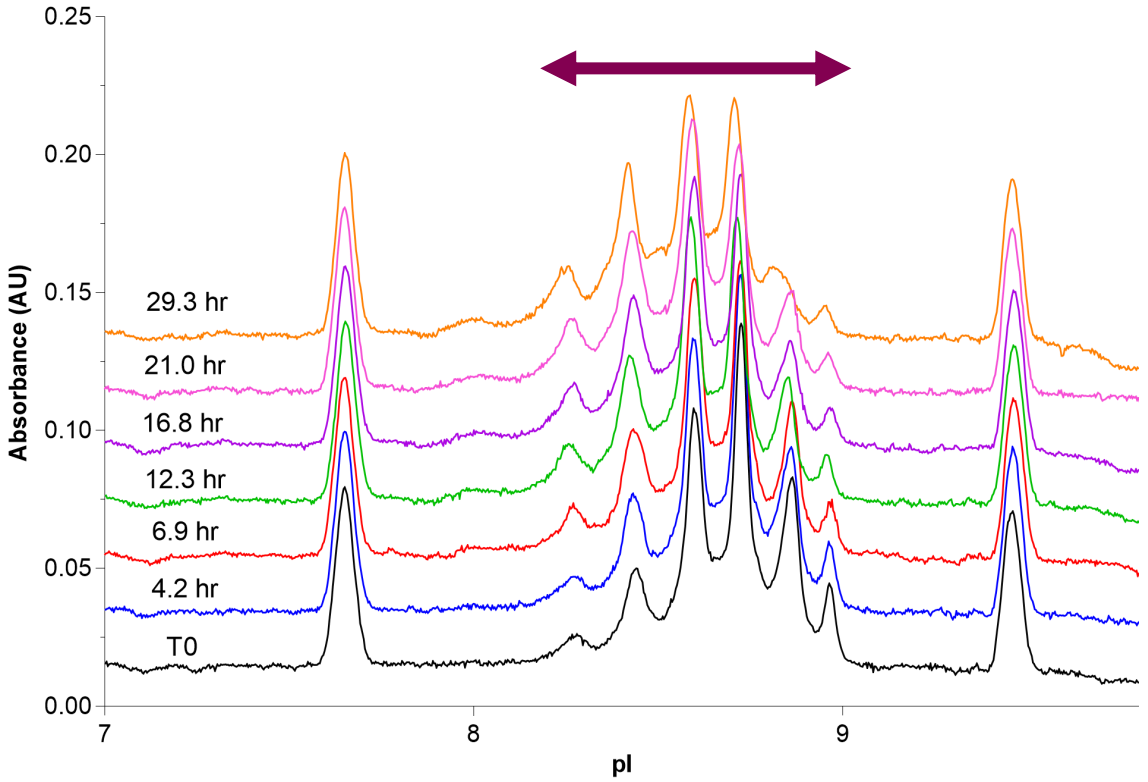
Challenges with icIEF as ADC-1 Release Method due to Payload Instability

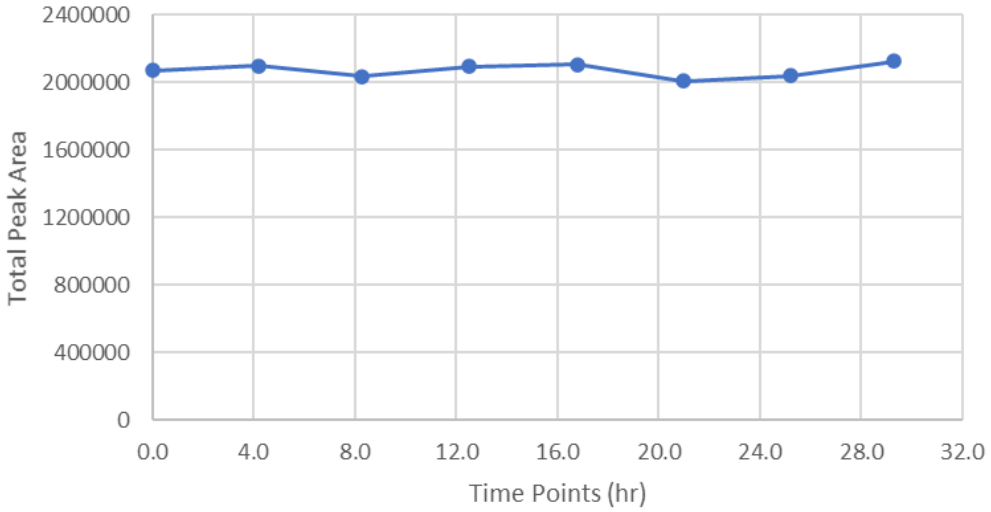
- The modifications on the payloads are not necessarily critical quality attributes
  - This means a traditional cIEF specification may not be appropriate for ADC-1
- In lieu of icIEF analysis, AZ has been collecting peptide mapping data for ADC-1 GMP stability and release samples
  - The characterization data package collected on SCIEX's Intabio ZT system provides the opportunity to explore a new specification strategy



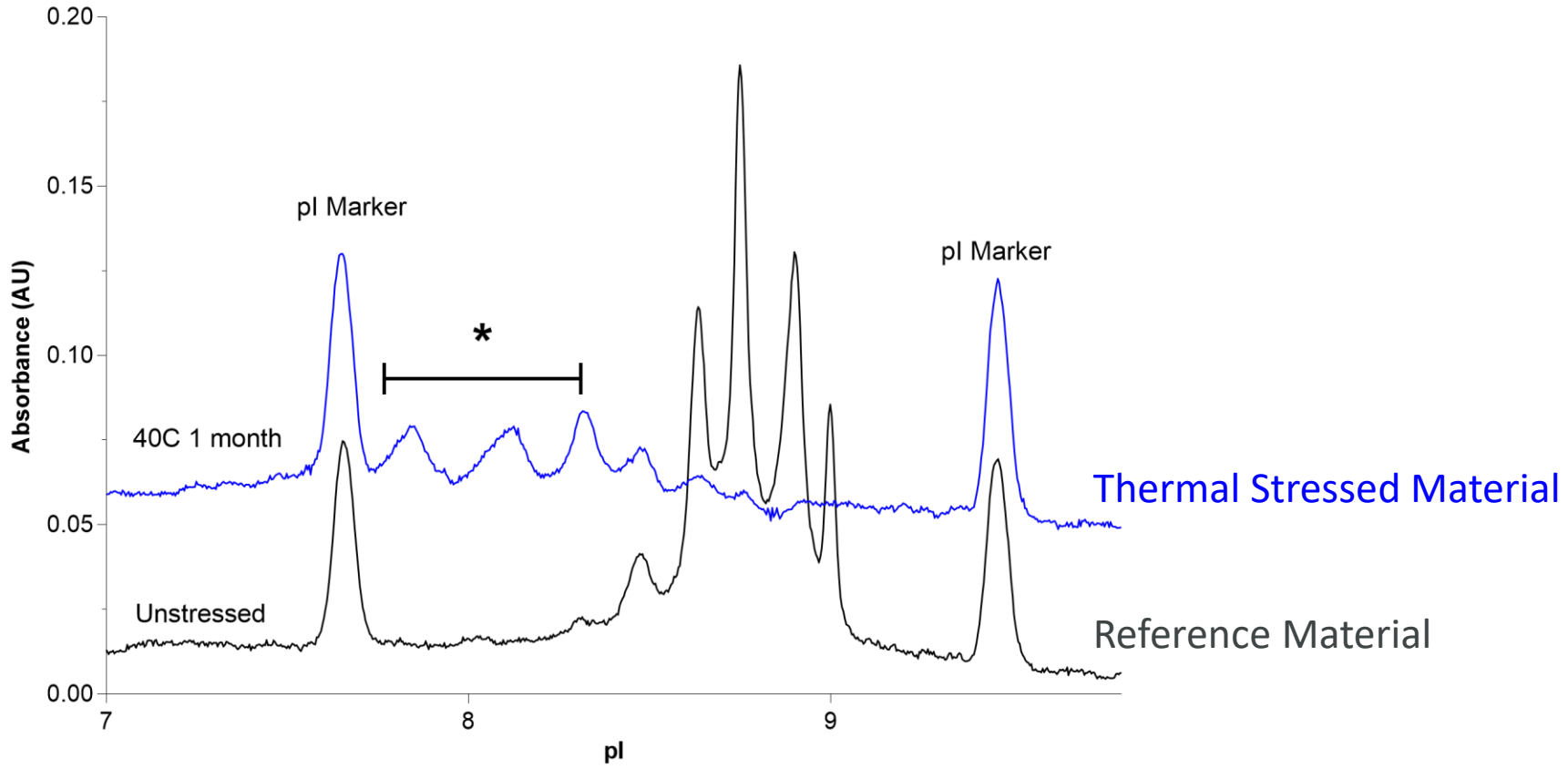
Thinking Outside The Box for cIEF Method Specifications

- Individual peaks shift intensities over time, but let's widen the focus…





If Not Individual Peaks, Then What Do We Set The Specification On?



* How about we base the specification on the total area around the region of these stressed peaks and not individual peak groups?

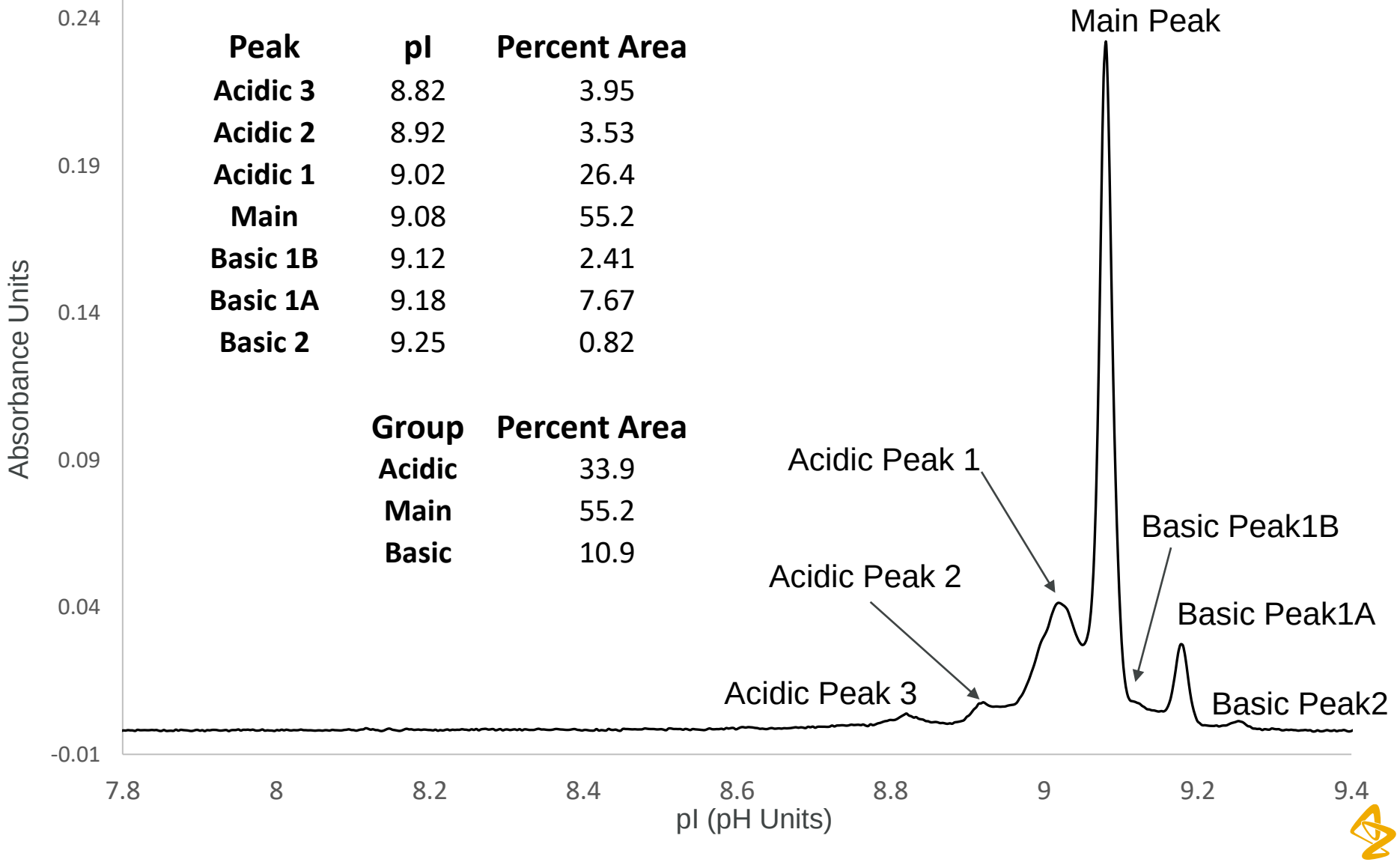
Back-up slides

INT-01 Quantitative Analysis Shows Good Comparability to Quantitative Results from the iCE3

- The isoelectric points (pI) for charge variants range between 8.82 and 9.25 pH units
- Percent areas for charge variants range between 0.82 and 55.2%
- AZ's data: 22.8% Acidic, 68.6% Main, 8.6% Basic

Peak	pI	Percent Area
Acidic 3	8.82	3.95
Acidic 2	8.92	3.53
Acidic 1	9.02	26.4
Main	9.08	55.2
Basic 1B	9.12	2.41
Basic 1A	9.18	7.67
Basic 2	9.25	0.82

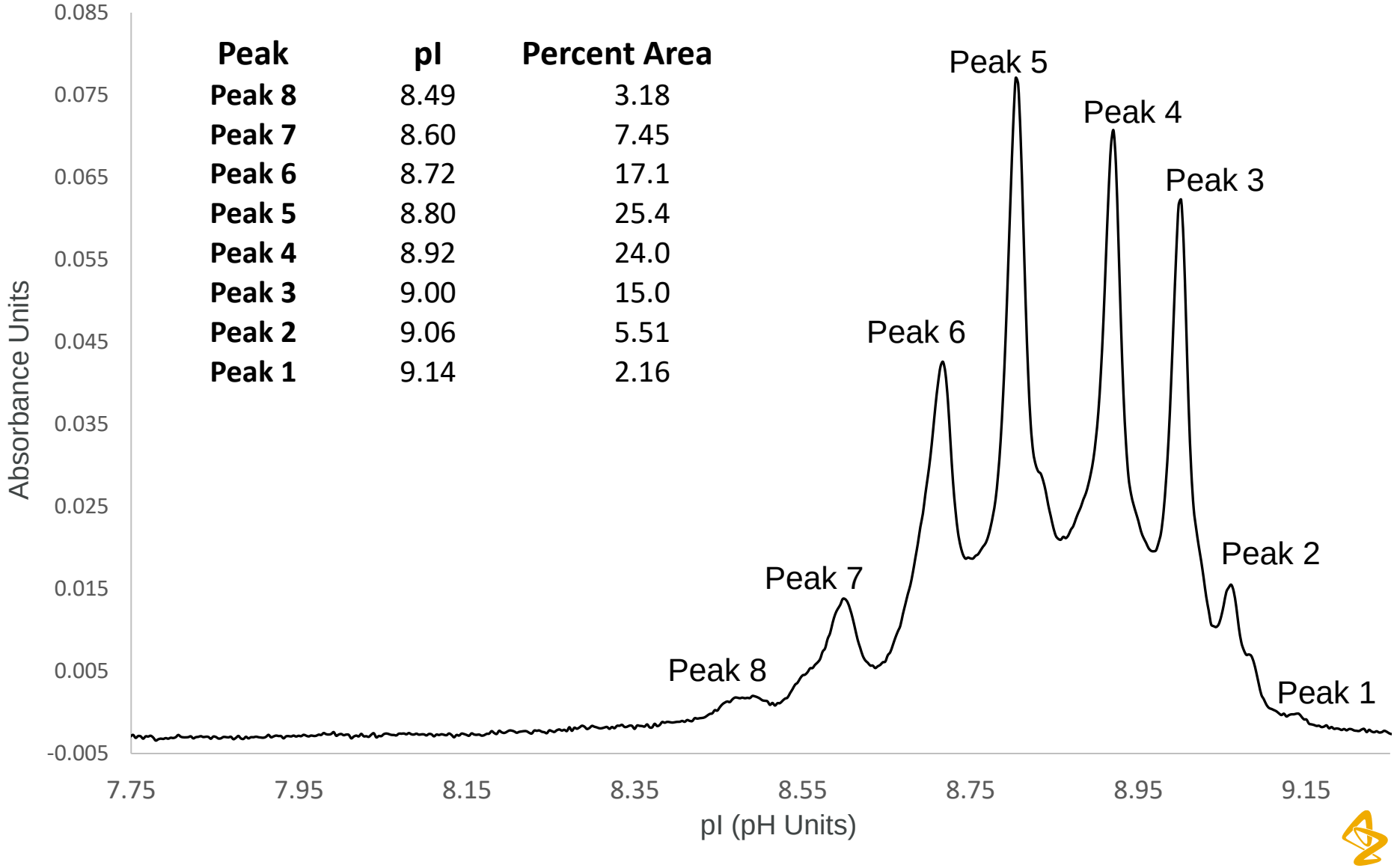
Group	Percent Area
Acidic	33.9
Main	55.2
Basic	10.9



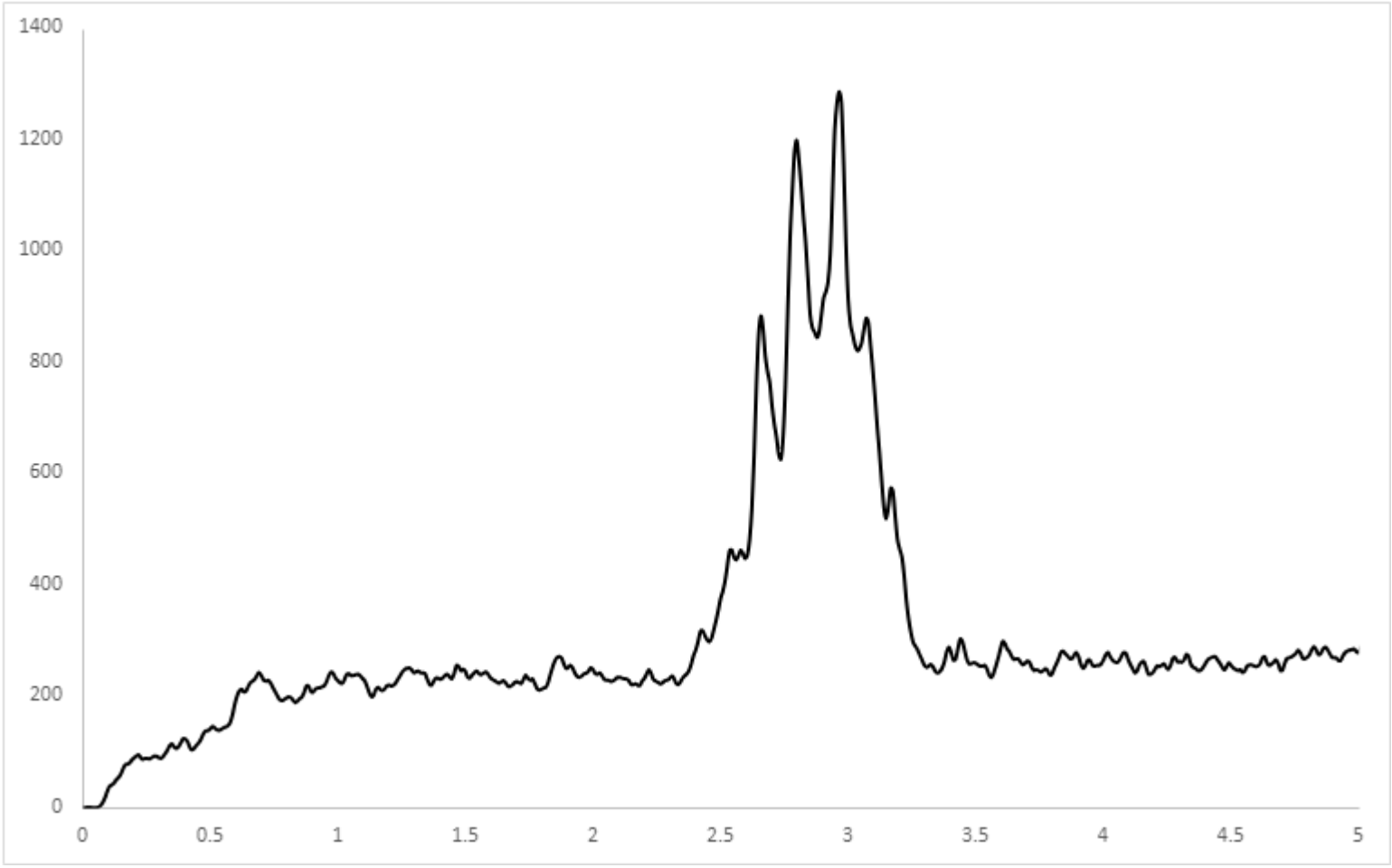
ADC-1 Quantitative Analysis Shows Good Comparability with Quantitative Results on the iCE3

- Conjugation results in shifts the distribution of charge variants to lower pI and an increase in heterogeneity
- The isoelectric points (pI) for charge variants range between 8.49 and 9.14 pH units
- Percent areas for charge variants range between 2.16 and 25.39 %

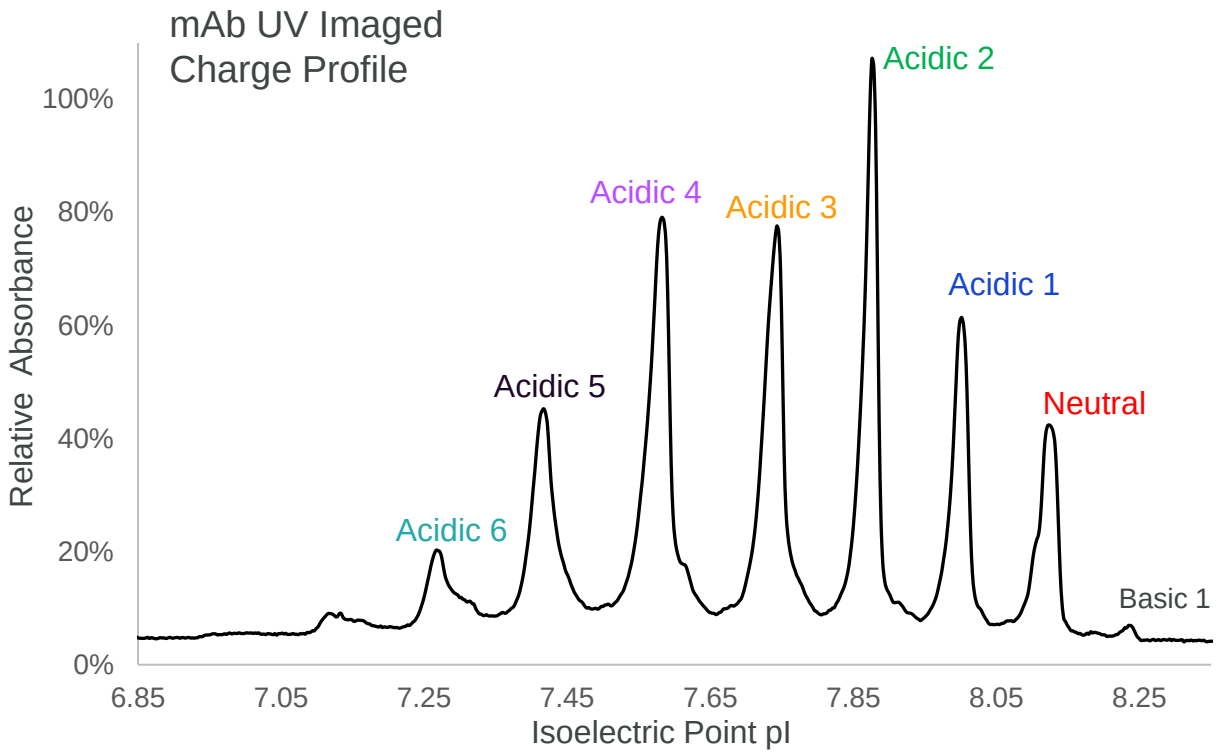
Peak	pI	Percent Area
Peak 8	8.49	3.18
Peak 7	8.60	7.45
Peak 6	8.72	17.1
Peak 5	8.80	25.4
Peak 4	8.92	24.0
Peak 3	9.00	15.0
Peak 2	9.06	5.51
Peak 1	9.14	2.16

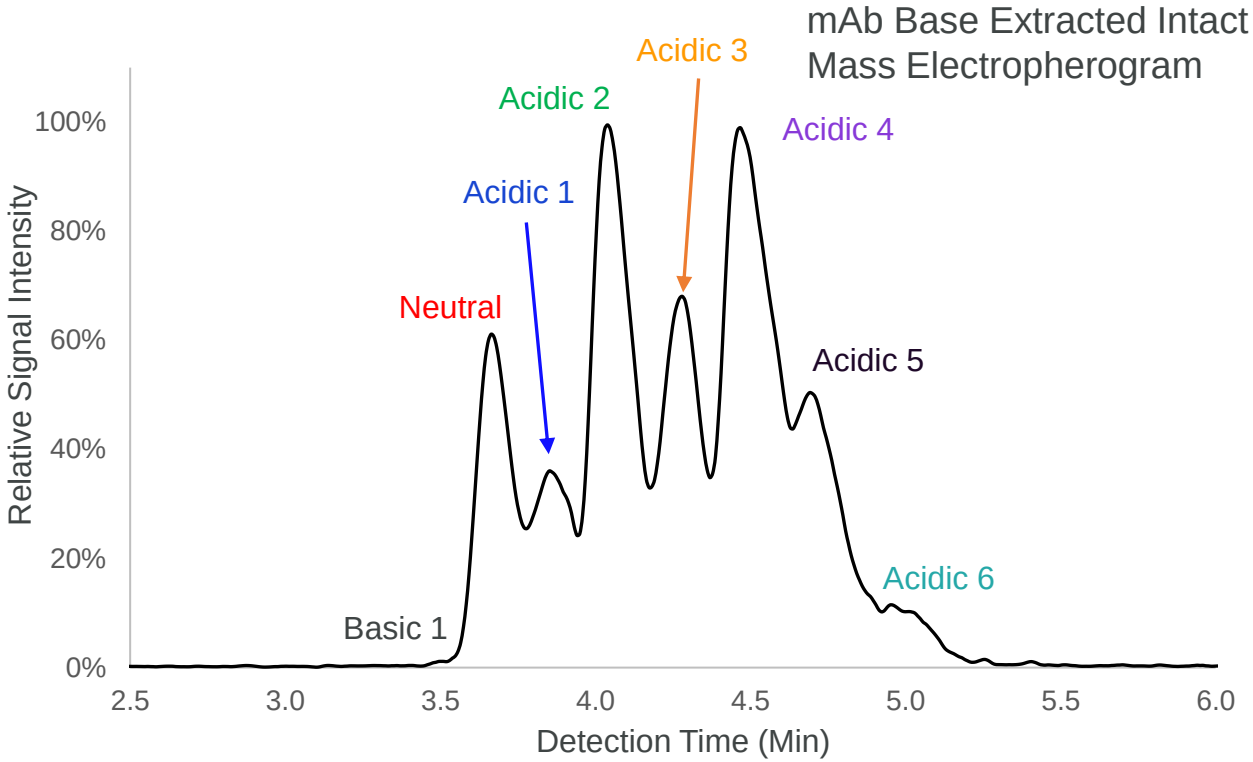


ADC-1 raw BPE profile



mAb-1 highly glycosylated mAb, Fab N-glycan





SCIEX Biologics Explorer

mAb Charge/Mass Isoforms

ProAm (Proline Amidation)

NLG (N Linked Glycan)

NeuAc (N-acetylneuraminic acid)