

Strong Cation Exchange Ultra-Performance Liquid Chromatography Coupled to UV and MS Detectors Allows Robust and Reproducible Characterization of mAb and ADC Charge Variants

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SUMMARY

New methods for strong cation exchange (SCX) ultra-performance liquid chromatography (UPLC) have allowed effective resolution and characterization of charge variants throughout the ADC manufacturing process.

Retention time reproducibility after SCX UPLC column equilibration enabled monitoring of important changes in product quality.

SCX UPLC-MS performed with novel MS friendly mobile phases allowed for the confirmation of linker position, number of attachments, and glycoform heterogeneity. The method also provided high mass accuracy MS information for the verification of linker/drug incorporation.

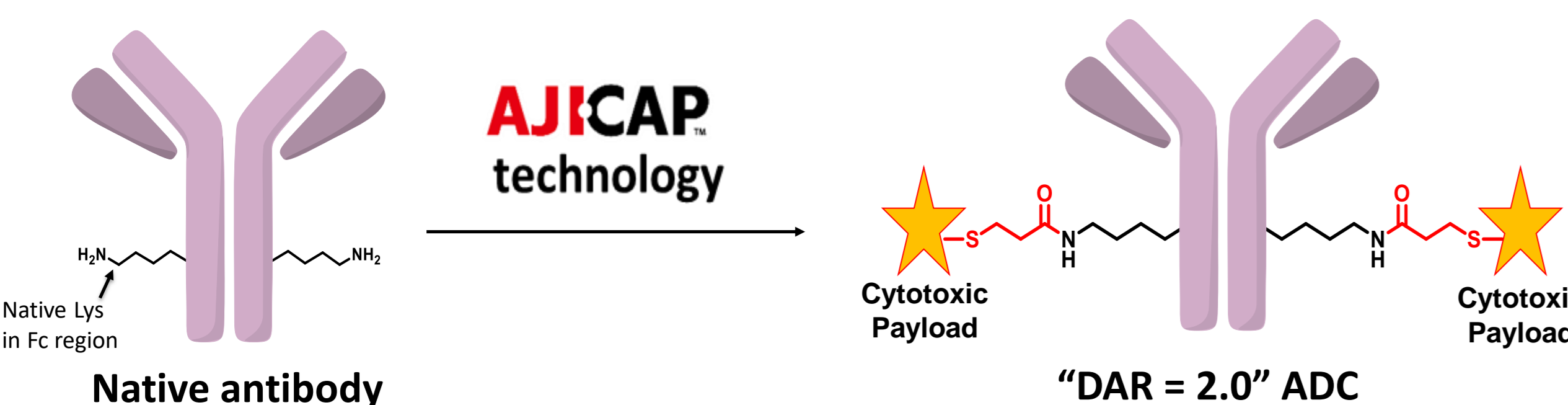
INTRODUCTION

Charge variants have gained considerable attention, especially due to their potential effect on biological activity and stability of mAbs. Modification of isoelectric values in manufacturing arises due to enzymatic or degradation processes, such as deamidation, oxidation, isomerization, and fragmentation.¹

Heterogeneity and the dynamic nature of mAbs demands monitoring by various analytical approaches. Cation exchange chromatography, coupled to UV and mass spectrometry, is quickly becoming the method of choice in characterization of charge variants.²

Typical cation exchange separations require high concentrations of salts, the gradients are based either on varying the concentration of salts or pH; traditional recipes are not compatible with MS detection. Recently, volatile salts have been successfully used for MS based characterization of charge variants.³⁻⁶

In this study, we performed strong cation exchange (SCX) ultra-performance liquid chromatography (UPLC) coupled to UV with a non-volatile salt/pH gradient as well as LC-UV-MS using volatile MS compatible salts. In depth analyses were performed on AJICAP™ mAb and ADC charge variants.



AJICAP™ technology is a novel platform for the site-selective conjugation of antibodies through the use of a class of Fc-affinity compounds to install thiol linkers to well-defined amino acid residue(s).^{7,8}

METHODS

SCX mAb Column Specifications

Strong cation exchange, 3 µm non-porous particle, sulfonic acid functional group ligand, counter ion Na⁺, pH range 3-11, max pressure 10,000 psi (~6.9 kPa), minimum salt concentration 10 mM, maximum organic concentration 50%, column temperature 10 - 60°C

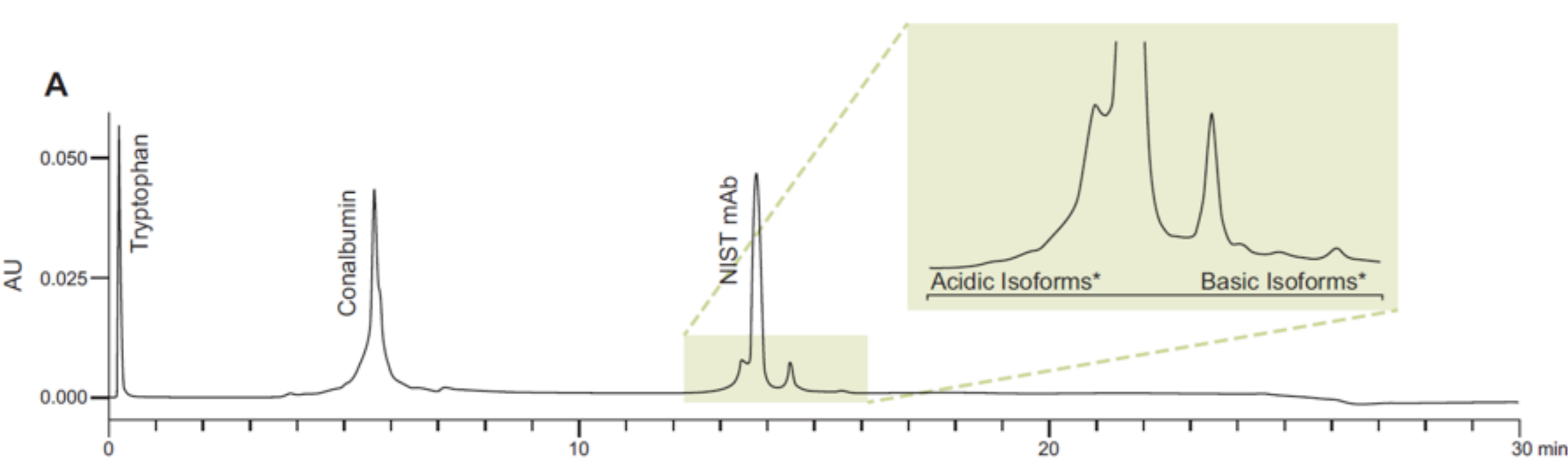


Figure A. Representative SCX chromatogram of NIST mAb Charge Variant Standard obtained using BioResolve SCX pH concentrates. The chromatogram was obtained with an ACQUITY UPLC H-Class Bio instrument and a BioResolve SCX mAb, 3 µm, 4.6 x 50 mm column with a Vanguard FIT Cartridge.⁹

SCX UPLC-UV mAb Mobile Phase Composition – BioResolve SCX

BioResolve CX pH concentrates were used to prepare a mobile phase comprised of four buffer salts, succinic acid, BIS-TRIS propane, triethanolamine and N-cyclohexyl-3-aminopropanesulfonic acid.²

SCX UPLC-UV-MS mAb Mobile Phase Composition

Waters technologies prototype SCX pH buffers A (pH = 5.0) and B (pH = 10.2). This is an ammonium based mobile phase that is optimized to provide salt-mediated pH gradients.⁶

Reduction and IdeS Digestion of Naked (Trastuzumab) mAb and AJICAP™ AJ2 ADC samples

100 µL of original solution of native mAb/ADC (1 mg/mL) was mixed with 1 µL of 1M dithiothreitol (DTT) for reduction of cysteine bonds, incubated for 35 min at 37 °C. 100 µL each reduced sample was diluted with 100 µL of 50 mM Ammonium Bicarbonate and incubated at 37 °C for 30 min with 100 units of FabRICATOR® enzyme (Genovis, A0-FR1-008).

SCX-LC-UV-MS Instrumentation

Analyses were performed on H-Class Plus Acquity UPLC using a BioResolve SCX 2.1x100 mm (for SCX UPLC-UV) and BioResolve 2.1x50 mm (for SCX UPLC-UV-MS) experiments. The BioAccord™ compact TOF MS instrument, used for SCX-UPLC-UV-MS consists of an ACQUITY I-class UPLC with TUV detector and an RDa MS detector.⁶

SCX LC Conditions:

Column: ACQUITY BioResolve SCX (3 µm, 2.1 x 100/50 mm)
Column Temp: 30 °C
Flow Rate: 0.3 mL/min (SCX UPLC-UV); 0.1 mL/min (UPLC-MS)
Mobile Phase: Dual salt/pH gradient (see above)
Grad. (Intact): UV only: Hold at 0% B for 1 min, then 0%-100% B over 22 min (linear), wash at 98%B for 1 min, and re-equilibrate at 0% B for 7 min
UV-MS: Hold at 40% B for 1 min, then 40%-98% B 20 min (linear), wash at 98%B for 1 min, and re-equilibrate at 40% B for 7 min
Grad. (IdeS): UV-MS Hold at 2% B for 1 min, then 2%-98% B over 20 min (linear), wash at 98% B for 1 min, and re-equilibrate at 2% B for 7 min
Total Time: 30 min
Injection: 1-10 µL of 1 mg/mL sample

MS Conditions (RDa detector):

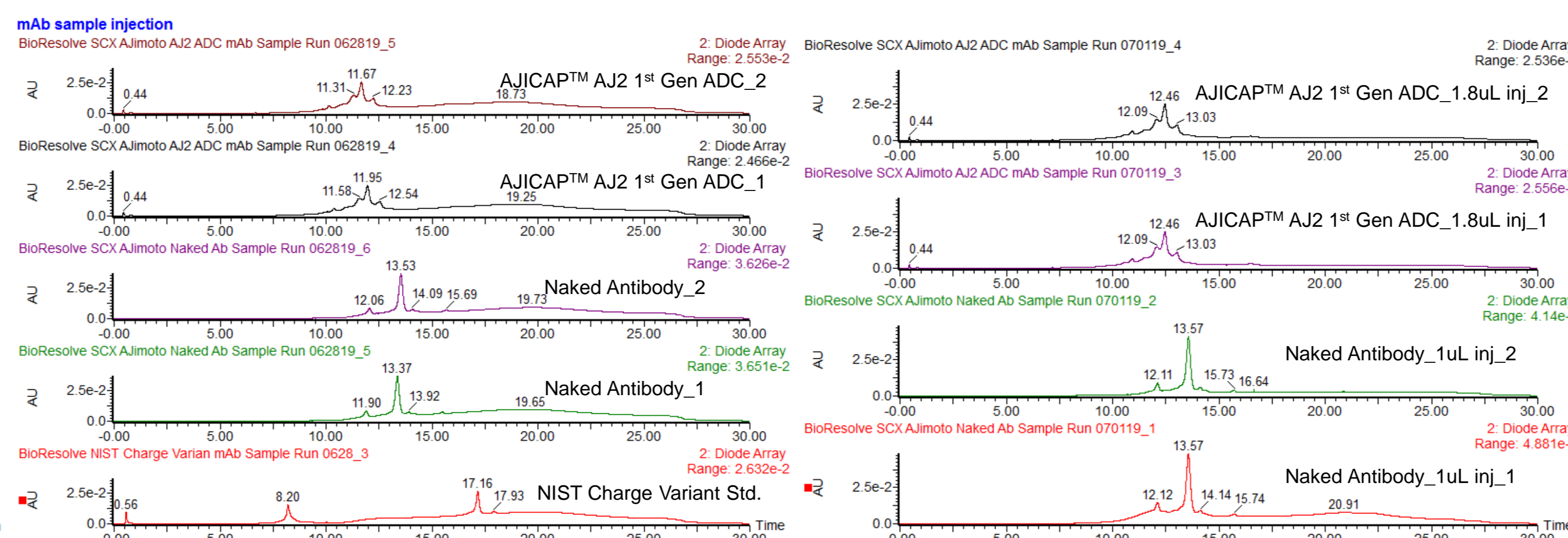
Capillary Voltage: 1.5 kV
Cone Voltage: 150 V
Desolvation Temp: 350 °C



ANALYTICAL RESULTS

SCX UPLC-UV Experiment 1

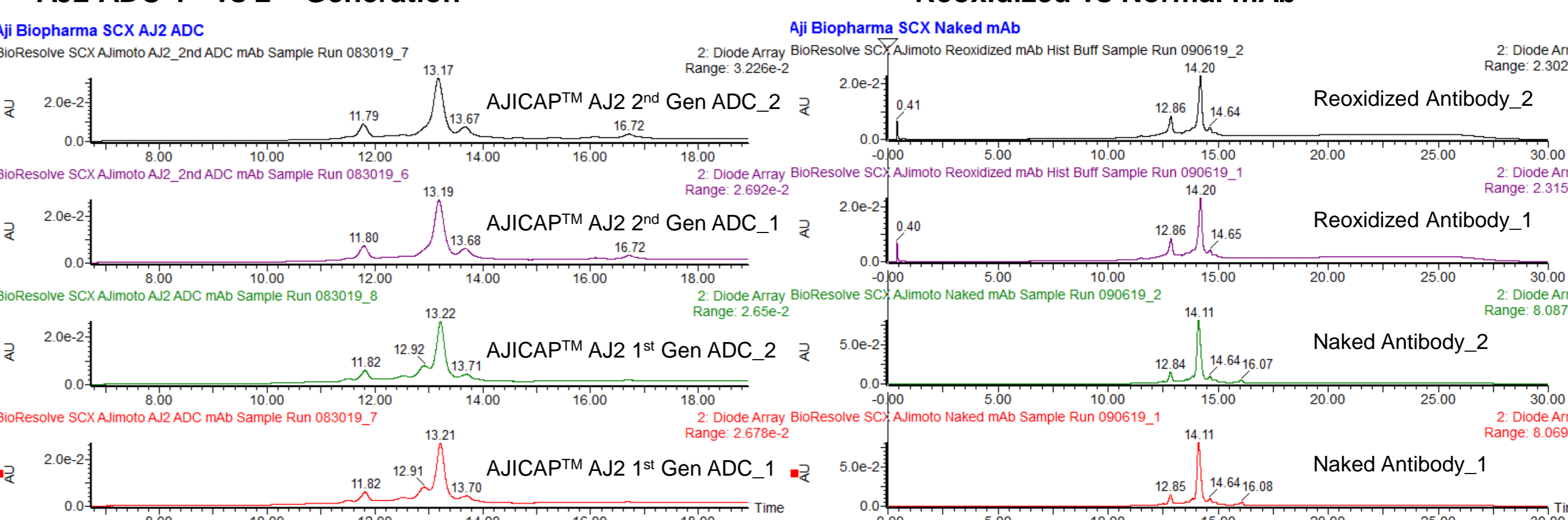
Samples: Waters NIST Charge Variant Standard, Naked (Trastuzumab) Antibody¹⁰, AJICAP™ AJ2 ADC
30 minute SCX Method – Linear Gradient Retention Time Reproducibility Post Equilibration



SCX UPLC-UV Experiment 2

Samples: AJICAP™ AJ2 ADC 1st & 2nd Generation, Reoxidized Naked (Trastuzumab) Antibody

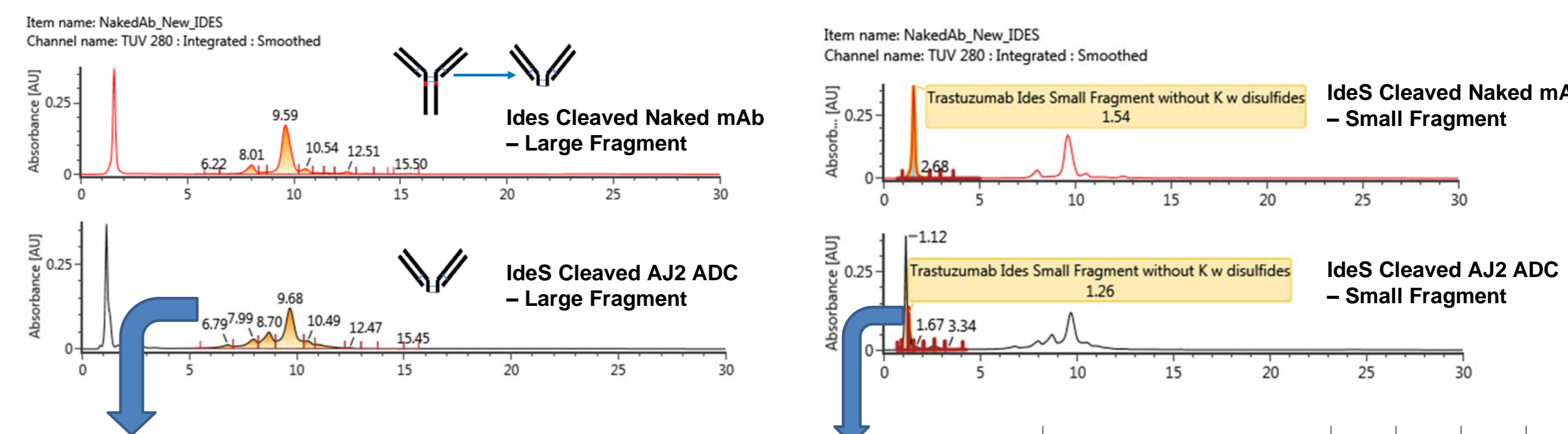
AJ2 ADC 1st vs 2nd Generation



SCX UPLC-UV-MS Experiment

Samples: Naked (Trastuzumab) Antibody, AJICAP™ AJ2 ADC

IdeS Cleaved Naked mAb vs AJICAP™ AJ2 1st Gen ADC IdeS Cleaved Naked vs AJICAP™ AJ2 1st Gen ADC



Protein name	Mods	Response	Observed mass (Da)	Exp. mass (Da)	RT (min)	Protein name	Modifiers	Resp.	Det. mass (Da)	Exp. mass (Da)	RT (min)	
Trastuzumab w disulfides						Trastuzumab IdeS Small Fragment of AJ2 ADC	AJICAP™ DrugPlusLinker (1), Glycosylation G0F N (1)		9681476	26636.75	26637.71	1.25
IdeS Large Frag		45054	97631.92	97627.97	8.1	Trastuzumab IdeS Small Fragment of AJ2 ADC	AJICAP™ DrugPlusLinker (1), Glycosylation G1F N (1)		8514494	26798.90	26799.85	1.25
Trastuzumab w disulfides_1						Trastuzumab IdeS Small Fragment of AJ2 ADC	AJICAP™ DrugPlusLinker (1), Glycosylation G2F N (1)		1481743	26961.83	26961.99	1.25
IdeS Large Frag		7597	97632.90	97627.97	8.7	Trastuzumab IdeS Small Fragment of AJ2 ADC	Glycosylation G0F N (1)		1202377	25232.45	25231.94	1.25
Trastuzumab w disulfides_2												
IdeS Large Frag		292487	97630.79	97627.97	9.7							
Trastuzumab w disulfides_3												
IdeS Large Frag		26119	97632.29	97627.97	10.7							

BioAccord UNIFI software processing confirmed detection of the large IdeS AJICAP™ AJ2 ADC fragment at the retention times of the major resolved peaks. The large fragment does not show any evidence of glycosylation. The additional UPLC-UV peak at 8.7 min retention time has the same MS profile as the other resolved peaks.

BioAccord UNIFI software processing confirmed detection of the small IdeS fragment of the AJICAP™ AJ2 ADC antibody at the 1.25 min UPLC-MS retention time. Notice the downshift of retention time due to the presence of the linker-drug. As expected, various glycoforms of the small fragment were detected, and linker-drug modifications were observed.

CONCLUSIONS

- ✓ Acquity UPLC with a BioResolve SCX column affords effective charge variant resolution, retention time reproducibility, and highly sensitive UV detection, thus allowing for small changes in product quality to be monitored.
- ✓ Novel MS friendly SCX mobile phase enabled in depth characterization of mAb and ADC molecules on the benchtop BioAccord TOF MS system with UNIFI software.

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