

A photograph of a female doctor in a white lab coat with a stethoscope, wearing glasses, and a female patient. They are both smiling and looking at a tablet held by the doctor. The background is a blurred clinical setting.

Together, we turn
treatment into

time

Expanding Mass Spectrometry for Challenging Biotherapeutic Characterization

Dominick Yeo (Field Scientist)

September 2026

Catalent
Pharma Services™

Dominick Yeo

Field Scientist, Biologics Analytical Services

- 8+ years working for Catalent SMARTag® Team founded by Nobel Laureate, Dr. Carolyn Bertozzi as the Lead Mass Spectrometry Scientist
- Key contributor to ADC discovery decisions
- Key contributor as a bioconjugation & purification SME
- Contributor to 4 publications related to ADCs
- Inventor on 6 ADC-related patents (4 patent pending)

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Agenda

- 1 Catalent Biologics Analytical Services Overview
- 2 Adding Mass Spec to Size Exclusion Chromatography (SEC)
- 3 Host Cell Protein Release Testing
- 4 Adding Mass Spec to Hydrophobic Interaction Chromatography (HIC)

Pharma & Consumer Health (PCH)



Oral Rx
(Zydis®, Softgel,
Oral Solids)

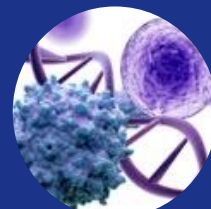


Consumer Health

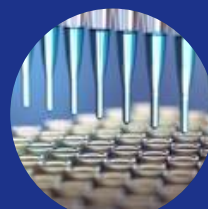
Biologics (BIO)



Core Bio



Cell & Gene Therapy



Biologics Analytical Services

Clinical & Specialty Services (CSS)



Clinical Supply



Inhalation



Clinical Packaging



Micronization

Guided by Patient First & Committed to Unparalleled Customer Service

Biologics Analytical Center of Excellence

30+ Years of Experience

- 300+ Programs Supported
- 30 Audits per year
- Zero 483-form

High-Client Satisfaction

- 90%+ returning clients
- 9+ out of 10 would recommend
- Dedicated Project Manager & Technical lead
- > 98% on-time delivery

800+ assays and techniques

- 180+ scientists (<10% turnover)
- 47,000+ ft² Facility
- 31,000+ ft³ stability storage





Mexico Ministry of Health



Drug Enforcement Administration



Republic of Turkey Ministry of Health



Pharmaceuticals and Medical
Devices Agency (Japan)



Food and Drug Administration



Medicines and Healthcare products
Regulatory Agency (UK)



Jordan Food and Drug Administration



Brazilian Health Regulatory Agency
(Anvisa)



Medicines and Healthcare products
Regulatory Agency



Ministry of Health
Canada



Ministry of Healthcare of
the Republic of
Kazakhstan



Ministry of Health of the Russian
Federation



With the help of our experienced
**scientific team and range of
capabilities, our goal is to serve as
your strategic partner to support
all your biologics analytical
development and testing needs
under a rigorous cGMP
environment.**

180+ Scientists | 30+ Years Experience | 11+ Regulatory Agencies

Contract Biologics Analytical

7

From traditional to emerging modalities, and from discovery to commercial



MABS & BISPECIFIC
ANTIBODIES



PEGYLATED
PEPTIDES



BIOCONJUGATES &
ADCs



RECOMBINANT
FUSION PROTEINS

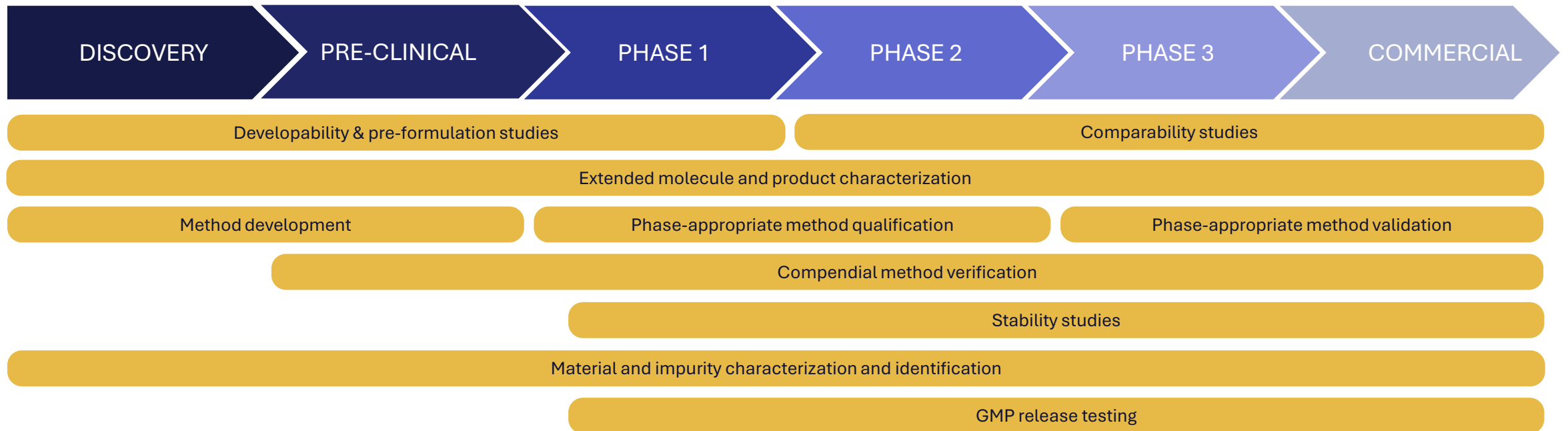


CELL & GENE
THERAPIES



OLIGONUCLEOTIDES,
NUCLEIC ACIDS

Supports All Modalities



Catalent®

Biologics Analytical Capabilities at a Glance

8

Accelerate your biologics' path to patients

Bioassay/Cell-Based Potency

- ▶ Binding potency: ELISA, AlphaLISA, surface plasmon resonance, Octet, etc.
- ▶ Relative potency: infectivity, cytotoxicity, mRNA transcription, neutralization, etc.
- ▶ Cell banking/storage

Method Establishment

- ▶ Method development, evaluation, and optimization
- ▶ Method transfer, qualification, phase-appropriate validation, method validation remediation

Mass Spectrometry

- ▶ State-of-the-art mass spectrometry core with high-resolution LC/MS; GC/MS and ICP/MS; Protein metrics software for data processing
- ▶ Size & charge variant characterization including fraction collection for impurity ID

Extended Characterization

- ▶ Secondary & higher order structural characterization using orthogonal approach
- ▶ Size & charge variant characterization
- ▶ Forced degradation study; comparability study

Release & Stability Study & Storage

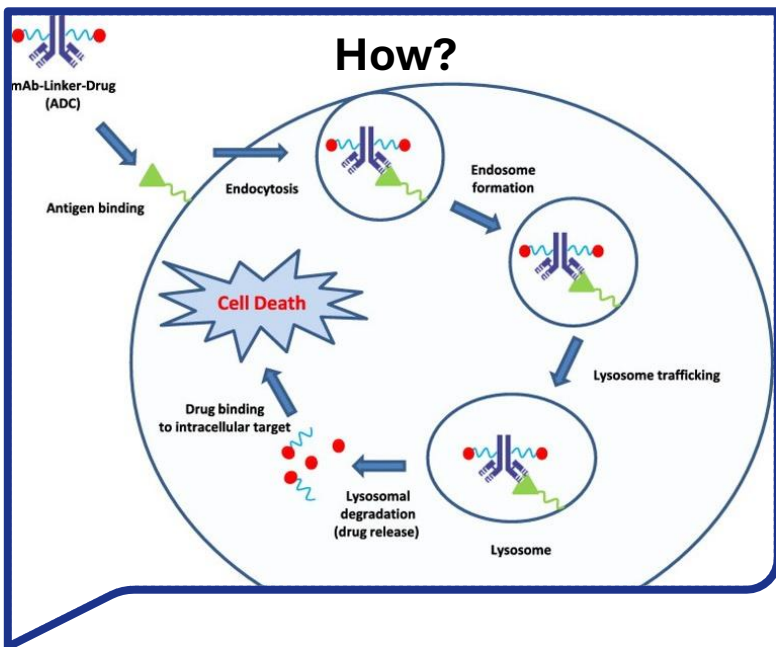
- ▶ Release testing; specialized expertise in temperature excursion, photostability
- ▶ -80 °C to 60 °C, RH 25% to 90%, and customizable chamber conditions
- ▶ In accordance with ICH/WHO/ASEAN guidelines

Catalent®

The Three Pillars of Analytics

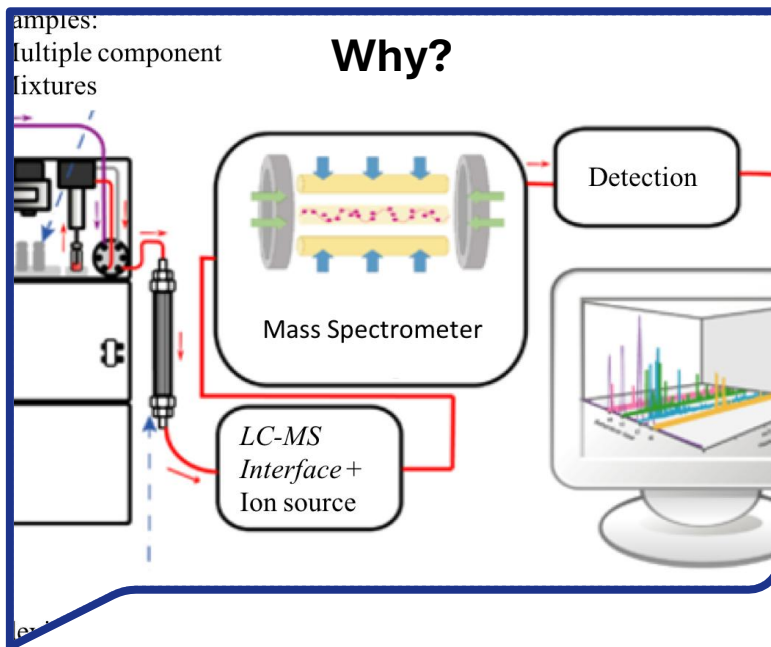
The How, What, and Why

9



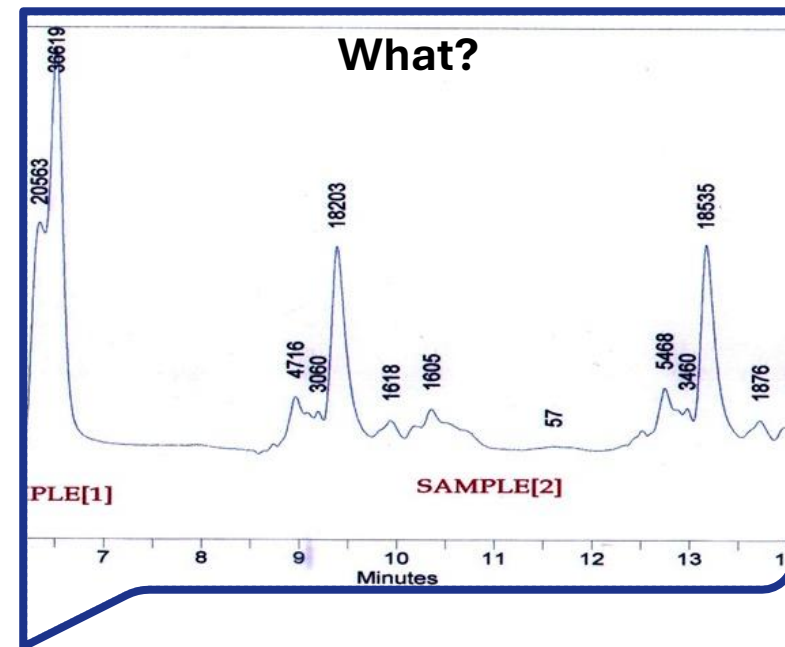
How does this therapeutic work?
Does it work as intended?

- Cell based assays
- Binding kinetics assays
- Etc.



Why are we seeing X?
Why does this work/not work?

- Mass Spectrometry



What is present in this therapeutic?

What attributes are we tracking?

- HPLC
- icIEF
- Etc.

The Nature of the Molecule

- Some molecules cannot withstand conventional analytical conditions
- Structural complexity may prevent direct measurement

Sample Matrix

- The surrounding sample components can obscure the analyte of interest
- Interferences may limit sensitivity or specificity

Mobile Phase

- Some separation methods rely on mobile phases that are incompatible with mass spectrometry

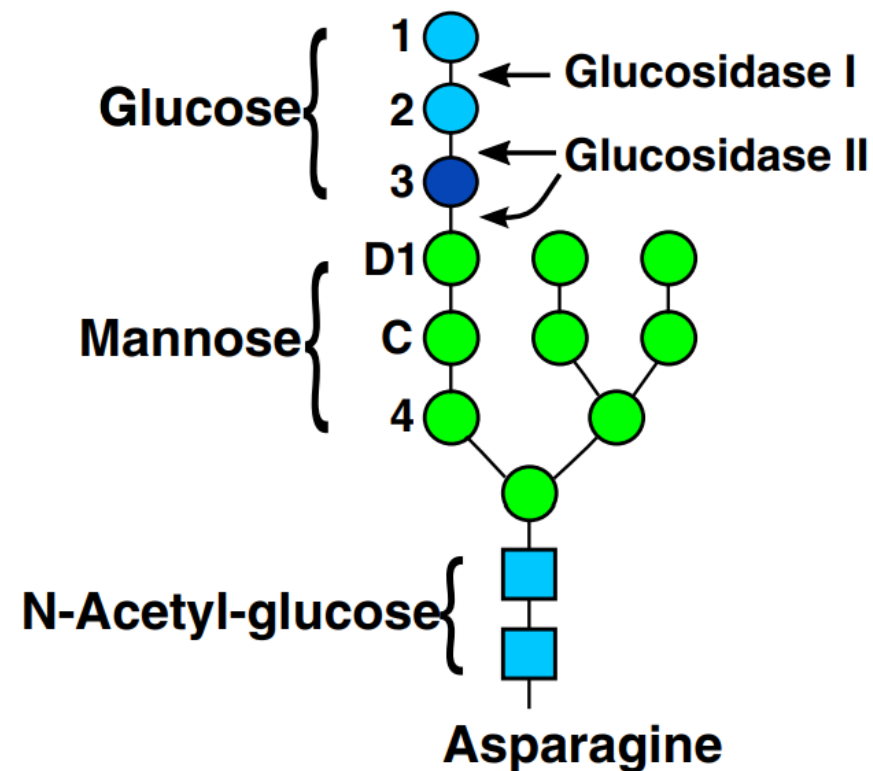
Native SEC-MS for Glycoprofile Determination of Cysteine-Conjugated ADCs



Glycprofile/Glycoform Analysis

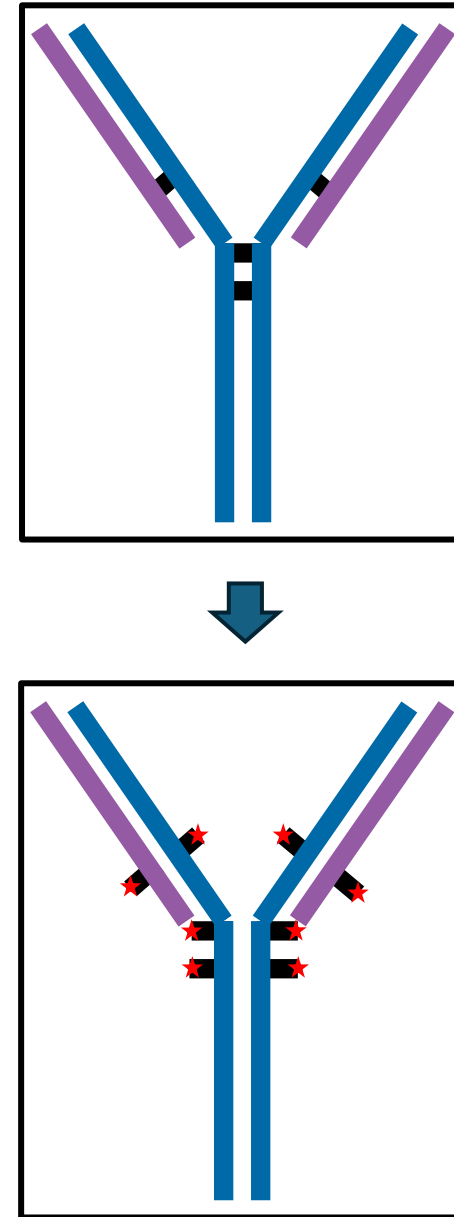
12

- Glycosylation is often a critical quality attribute for both monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs), impacting product safety, efficacy, and consistency.
- Certain glycoforms can alter serum half-life and clearance rates. High-mannose species, for example, may result in faster in vivo clearance.
- Unusual or non-human glycans can increase the potential for unwanted immune responses, making glycan characterization essential during development and comparability assessments.
- Glycosylation profiles are sensitive to cell line selection, culture conditions, media composition, and process changes. Monitoring glycoforms helps ensure batch-to-batch consistency.



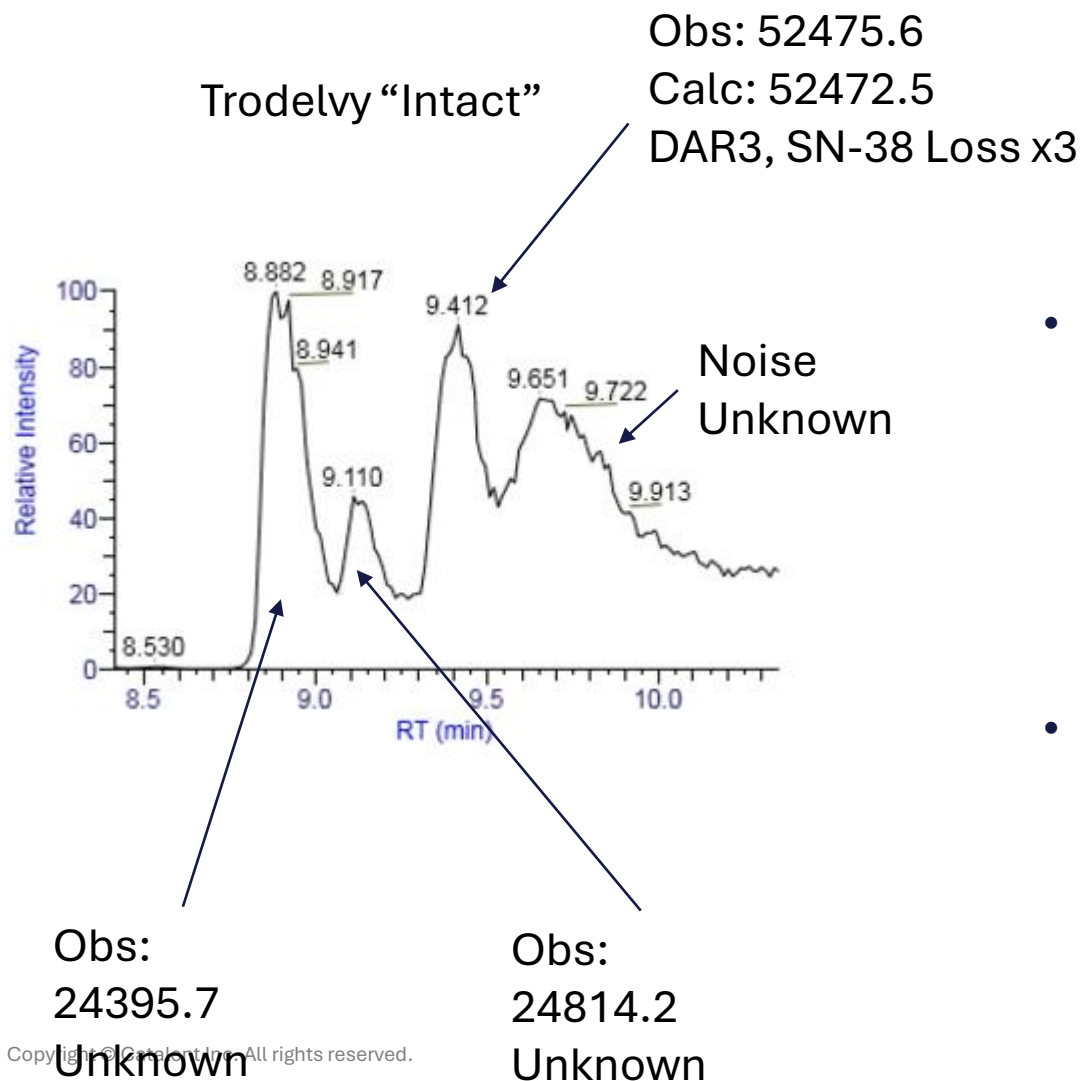
The “Cysteine Problem”

- The creation of a classical DAR8, cysteine-conjugated ADC results in the complete reduction of the disulfide bonds holding the antibody together.
- Any RP-HPLC-MS method will result in the denaturing of the ADC and separation of HC and LC, making intact mass determination impossible.
- Typical SEC solvents, which maintain the integrity of the antibody, have non-volatile salts which are harmful to mass spectrometers.
- Therefore, determining the glycoprofile of the intact ADC is difficult to do.
- In the past, we used the glycoprofile of the mAb and assumed it transferred to the ADC.
- However, conjugation conditions, purification methods, and effects on solubility by the linker-payload have the potential to shift this profile

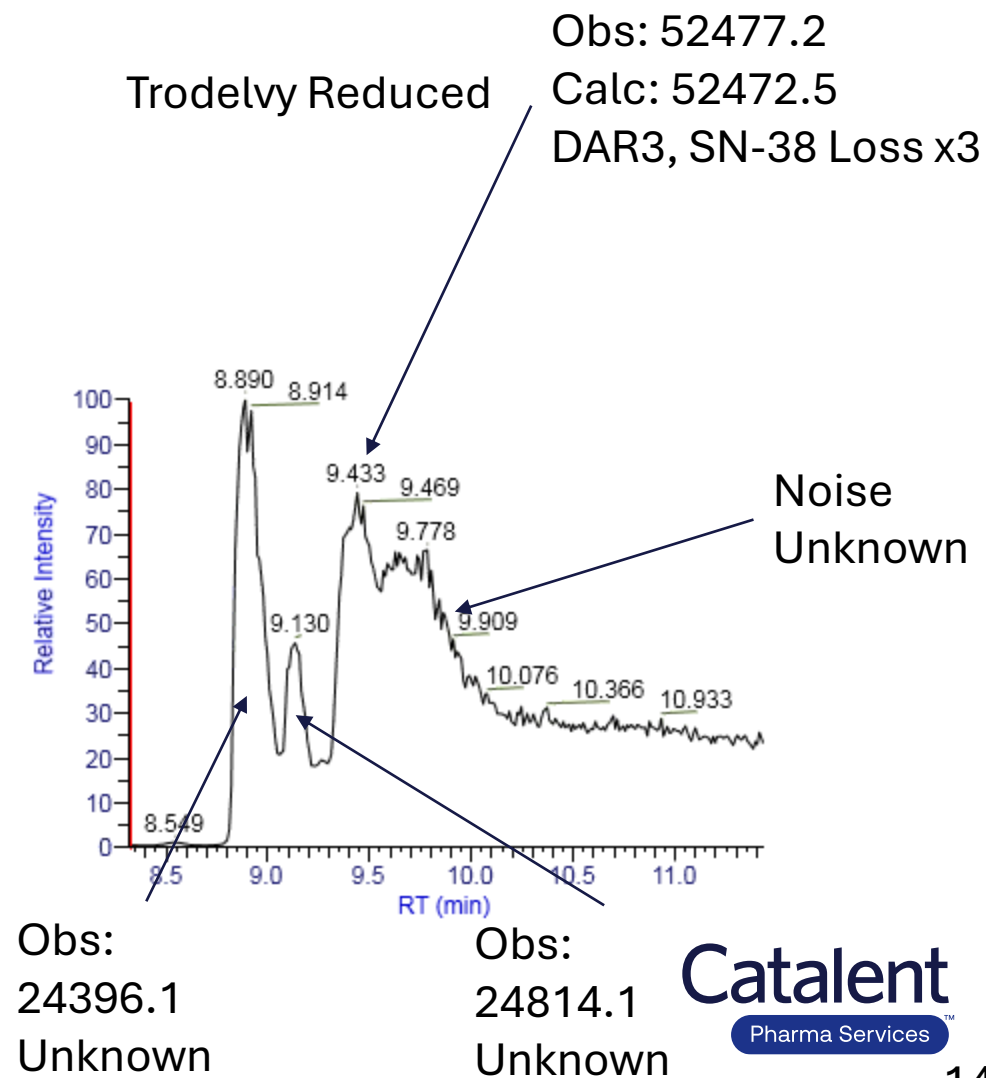


Intact and Reduced Trodelvy Have the Same RP-HPLC-MS

14

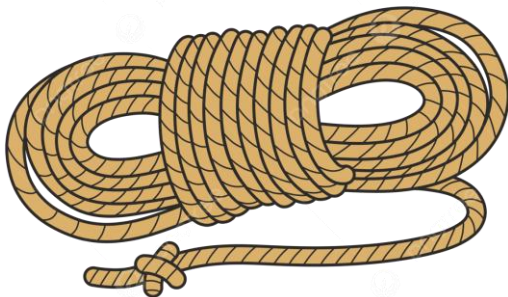


- Even without adding a reducing agent like DTT, Trodelvy naturally split into HC and LC forms
- Without Native MS, intact MW measurement of these ADCs is impossible

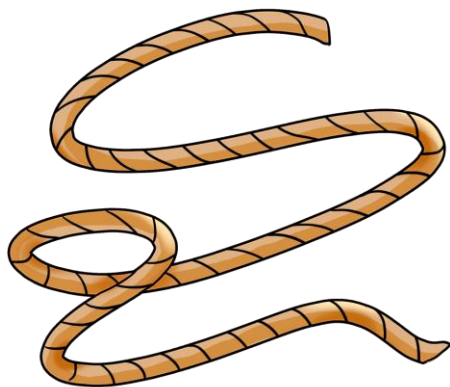


What is Native MS?

Native



Denatured



- Native mass spectrometry (Native MS) enables the study of intact protein, non-covalent protein-protein, and protein-ligand complexes in their biological state.
- For antibodies, heavy and light chains are bound together by covalent disulfide bonds.
- In cysteine-conjugated ADCs, these disulfide bonds are broken to enable conjugation. As a result, the heavy and light chains are held together non-covalently.
- Denaturing solvents (e.g. organics, acids, chaotropes) disrupt these noncovalent bonds

SEC-MS Method Development Objectives

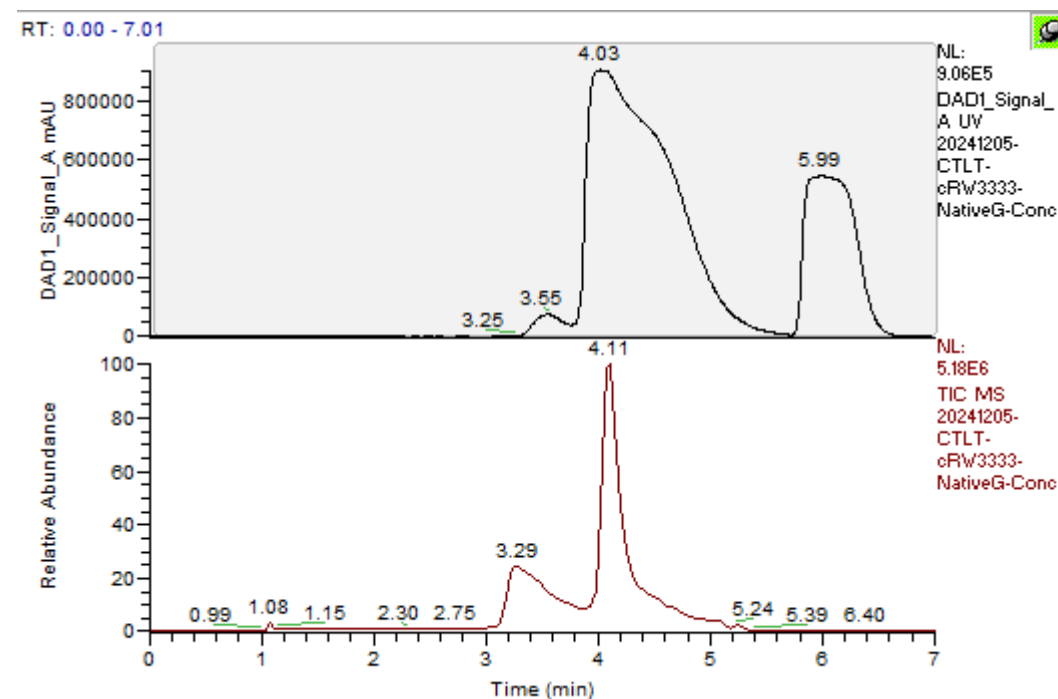
16

- Develop an SEC method that can provide translatable data between an HPLC-SEC method and LC-MS.
 - Typical SEC methods are very salty
- Create an MS method, including solvents and MS conditions, that allows for native analysis of antibodies and ADCs on the QExactive
 - Most Native MS methods require a BioPharma-Capable MS with an upper range of 8000 m/z or more. QExactive only supports up to 6000 m/z. This requires the buffer to be slightly acidic to increase the charge state of an antibody to 25 or more without being unfriendly to the antibody.
 - MS also requires non-volatile buffer, so our current sodium-containing buffer will not be compatible.

Intact MW SEC-MS Method Parameters

17

- Buffer: 50 mM Ammonium Acetate, pH 4.7 (half-equivalence point, pH = pKa)
 - pH only affects charge state in MS, does not affect retention time
- Column: Phenomenex Biozen 1.8 μm dSEC-2, 200 Å, 150 x 4.6 mm
- Injection Volume: 75 μL (150 μg)
- Flow Rate: 0.350 mL/min
- Time: 7 minutes
 - Salt from sample diverted at 4.5 minutes
- Sample Prep:
 - None – Glycan Analysis
 - PNGaseF Digestion in 50 mM Ammonium Bicarbonate, pH 8 – Deglycosylated Mass Analysis



Key Takeaways from SEC-MS

19

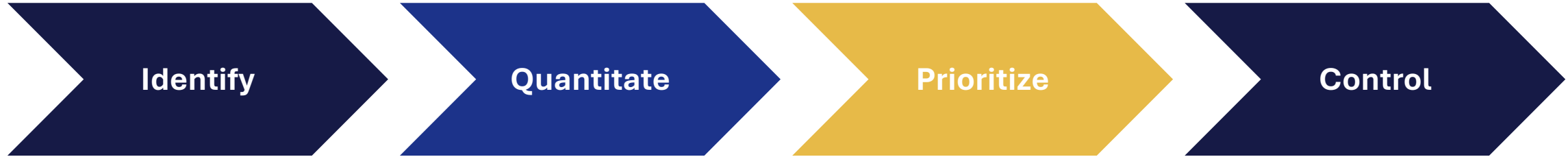
- SEC-MS allows native mass spec to be collected on non-covalently bond antibodies like cysteine ADCs.
- Better, more modern mass specs, will have better sensitivity than the Q Exactive, and therefore will require less sample injected, generating better chromatography.
- Additionally, a better mass spec will allow for a buffer closer to neutral pH, which increases the odds of maintaining the native state of the antibody.

Precise Quantitation of Critical HCPs Using Customized MS Methods



HCP Analysis Deserves More Than a Total Number

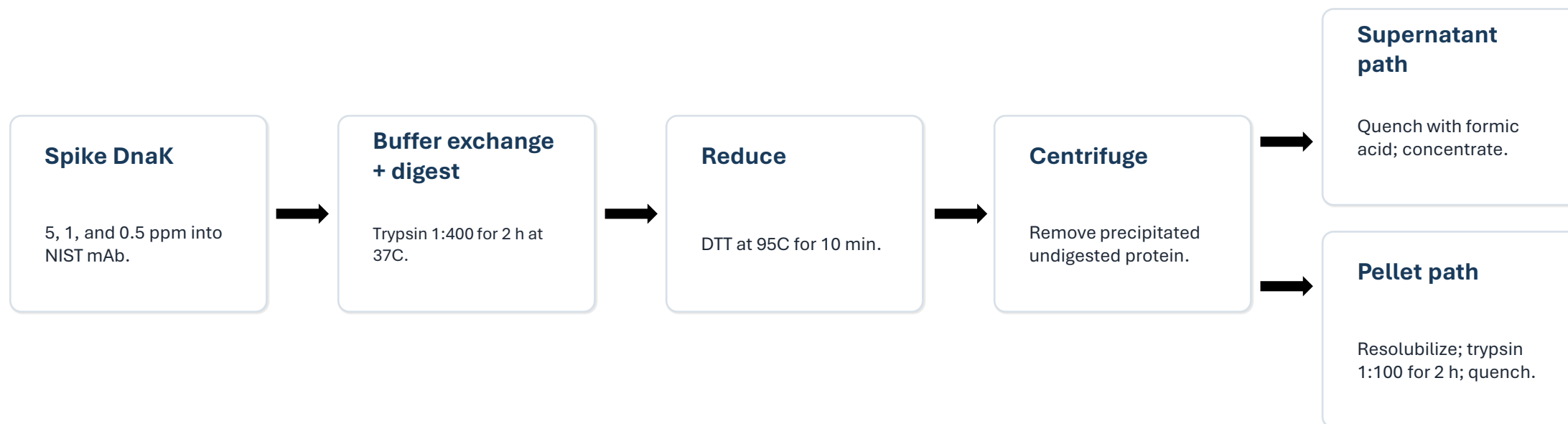
- Residual HCPs are not all equal. Total HCP methods can be useful for broad process control, but decisions often hinge on whether specific high-risk proteins are present and whether they persist through purification.
- Targeted MS can help move HCP discussions from bulk impurity measurement to protein-specific risk mitigation and process understanding
- Immunogenic proteins
 - Heat shock proteins such as DnaK, DnaJ, and GrpE are examples of residual HCPs that can pose elevated patient-safety concern.
- Enzymatic proteins
 - Lipases are HCPs of concern because enzymatic activity can impact product stability, including polysorbate-related issues.



- Identify
 - Using untargeted LC-MS/MS to discover HCPs that are present in the sample. Performed by comparing LC-MS/MS peptide mapping data to existing databases of HCPs depending on the cell type.
- Quantitate & Prioritize
 - Using the coverage from peptide mapping data to estimate which HCPs are likely to be of highest abundance and/or risk.
- Control
 - Use untargeted LC-MS/MS data to develop a quantitative targeted LC-MS/MS method to monitor prioritized HCPs

Customized HCP-MS Workflow for DnaK Detection

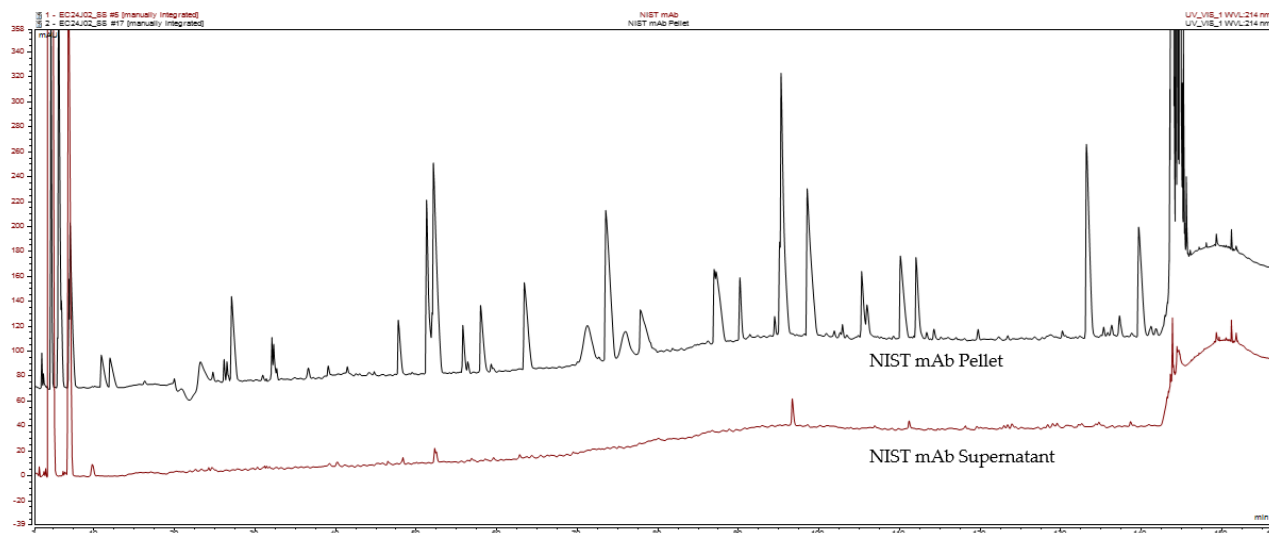
23



- This methodology builds upon the standard HCP-MS workflows, incorporating targeted customization to enable sensitive and specific quantitation of high-risk HCPs. DnaK is used as a representative example to demonstrate the effectiveness of this approach.
- A two-step, tryptic digestion of the native Monoclonal Antibody (mAb) was performed using NISTmAb as a representative sample.
- Native digestion takes advantage of the mAb's resistance to tryptic digestion in the native condition (non-denaturing). Undigested mAb is removed from the sample by thermal precipitation.
- The pellet and supernatant are analyzed for overlapping and unique peptides (DnaK peptides were not observed in the pellet at 5.0 ppm).

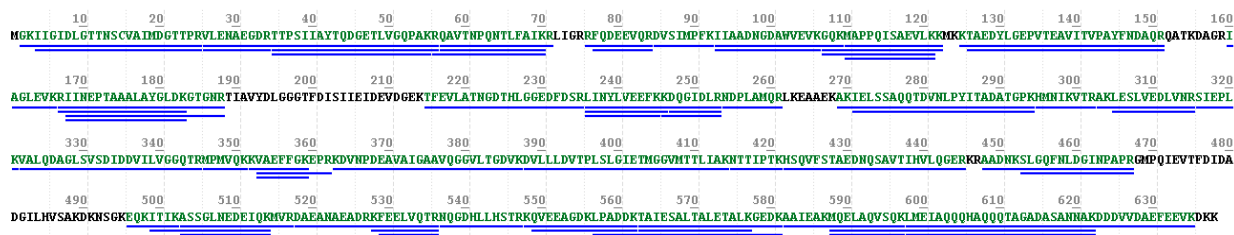
Matrix Reduction and Sequence Coverage

24



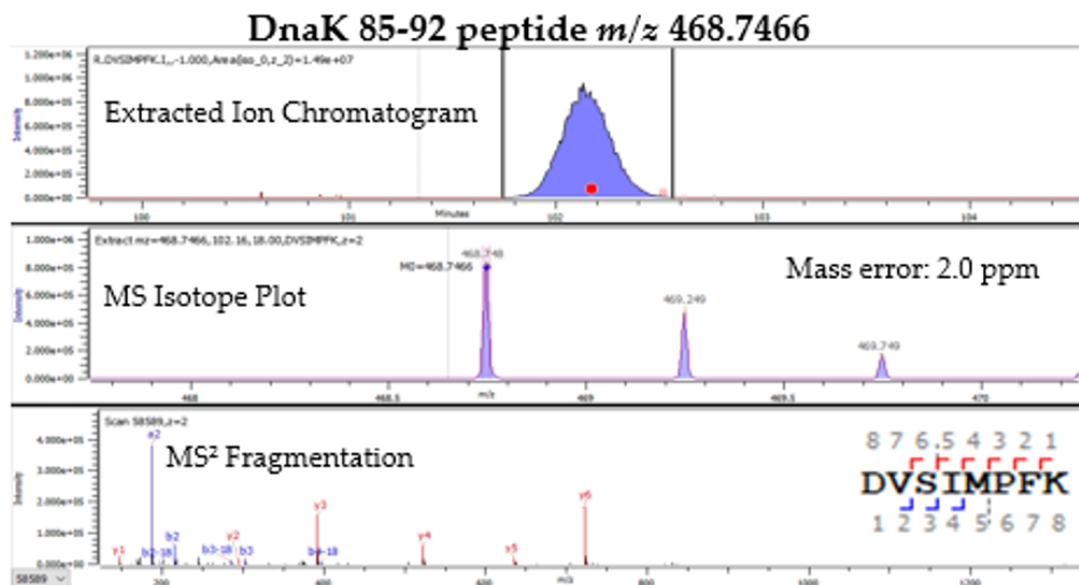
- The chromatogram comparison between pellet and supernatant demonstrates the reduction of signal afforded by the native digest with thermal precipitation of the mAb. The decreased mAb signal decreases interference for lower abundant HCPs.
- Without matrix interference, we got good sequence coverage observed for DnaK in the digest after thermal precipitation of the mAb.

DnaK

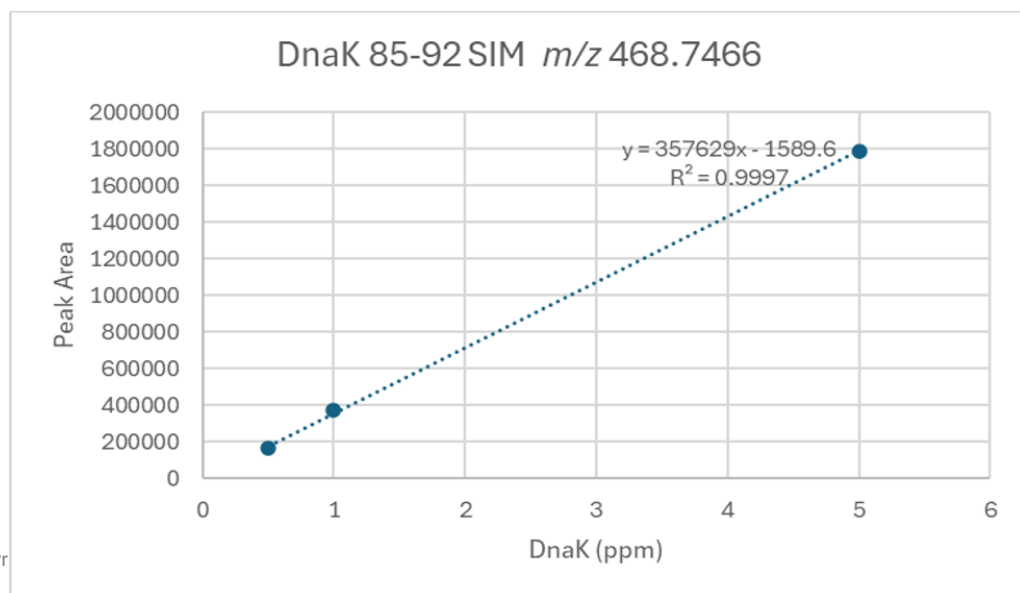


Sensitivity and Linearity

25



- MS signal for a representative DnaK peptide (residues 85-92) with good Extracted Ion Chromatogram (EIC), full scan MS (2.0 ppm), and near complete MS/MS amino acid sequencing.
- We also demonstrate the MS signal DnaK peptide (residues 85-92) for Calibration Standards.
 - very strong signal at 0.5 ppm and good linearity at sub ppm levels and intercept near the origin.



Key Takeaways

- HCP by LC-MS/MS can support sensitive, specific quantitation of priority HCPs at sub-ppm levels.
- DnaK can be detected to much lower levels, and current efforts are to improve low-level accuracy.
- Follow-on work for this methodology will focus on the use of synthesized peptides for cases in which the full intact HCP may not be available – which is true for many important HCPs. Such work may also include stable-isotope labeled peptides.
- The same procedure is currently being extended to lipases to aid with the instances where Polysorbate stability is found to be a problem.
- Another interesting study would be a comparison of this MS methodology with a commonly implemented ELISA procedure.

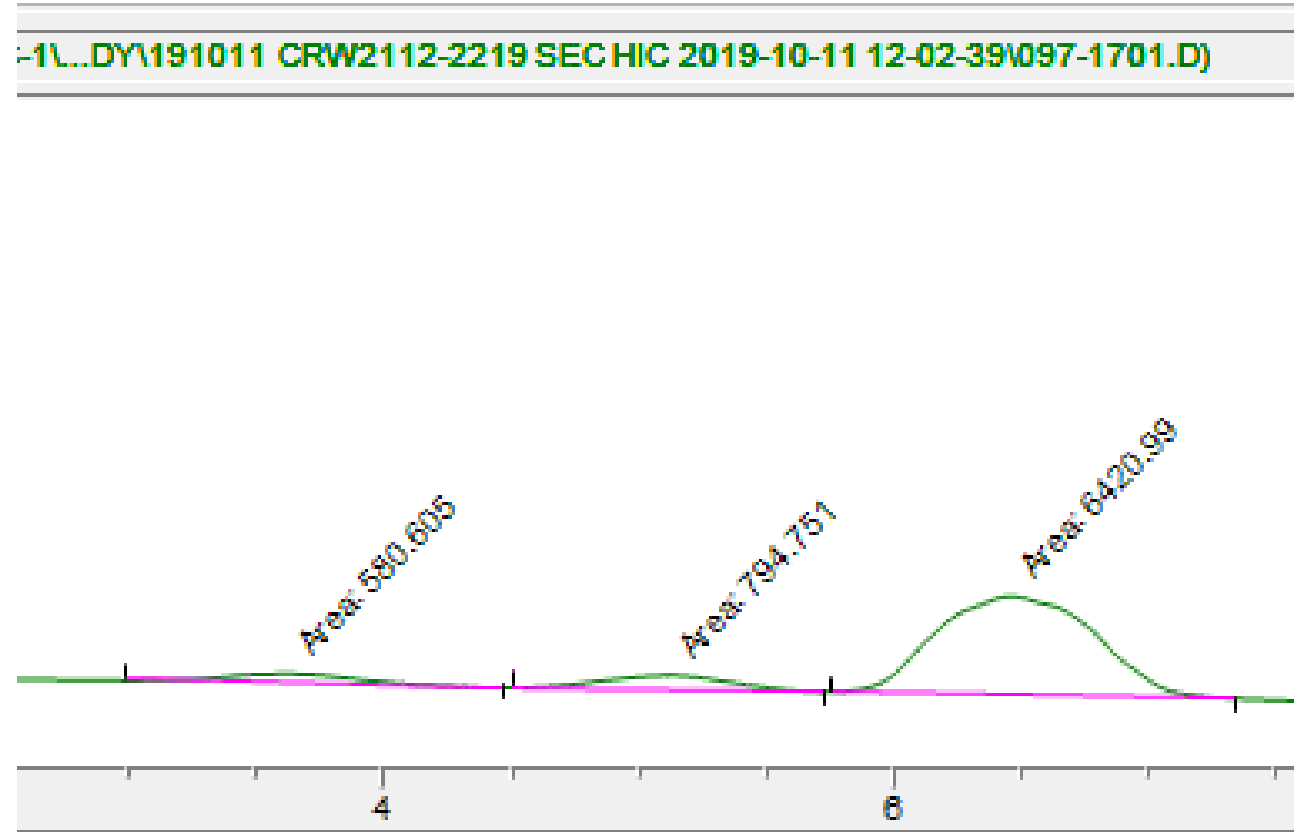
Inline HIC-MS for Measuring HCP Effect on Linker Technology



HIC Chromatogram of Typical Tandem-Cleavage DAR2 ADC

28

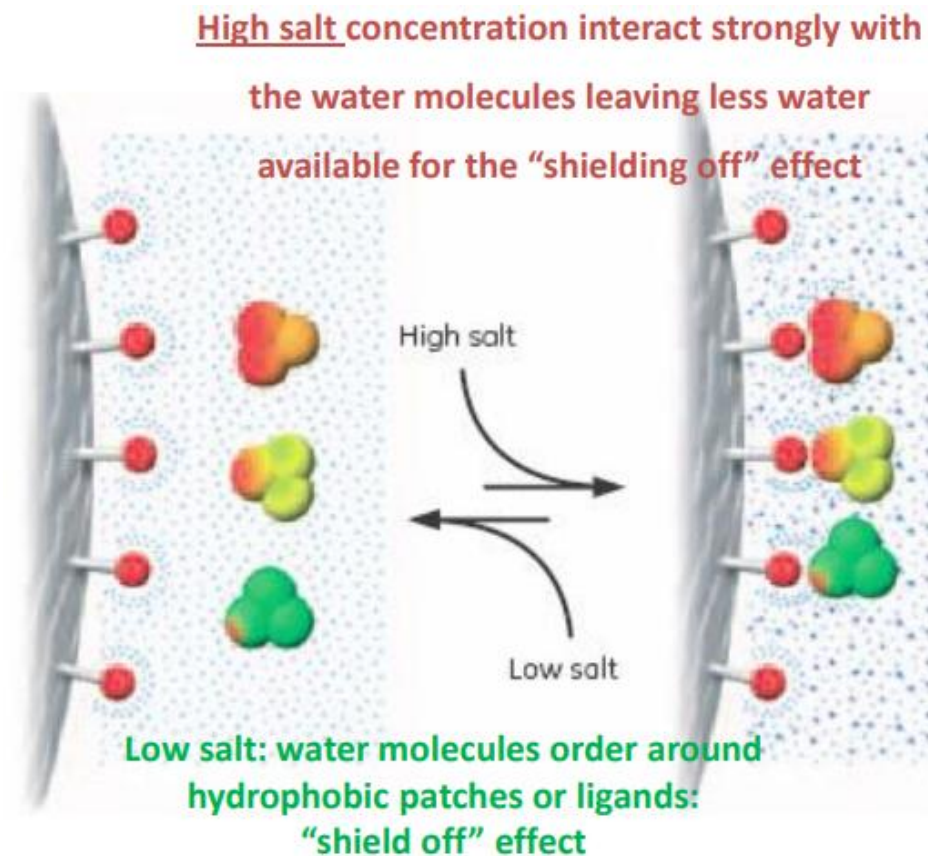
- 3 DAR2 variants were observed in the HIC chromatogram but were initially ignored.
- Initial thoughts were these were relating to antibody-related impurities.
- Research partners always asked for LC-MS connected to HIC
- Also, for a typical non-tandem-cleavage ADC, the DAR2 peak comes out smudged. So another thought was that chromatography was “too good”
- Offline purification by FPLC was also inconclusive



Challenges for HIC-MS Applications

29

- HIC uses a gradient from high salt to low salt to separate molecules by hydrophobicity.
- Usually, the salt is Ammonium Sulfate which is incompatible with LC-MS.
- Heartcutting 2D-LC provides a way to separate with HIC and buffer-exchange the diverted peak into a mass-spec friendly solvent.
- Even with heartcutting, some salt may still leak into the second separation dimension and contaminate the MS



Tosoh HIC Method – Pre-Existing Platform Method

Column	Tosoh TSKgel Butyl-NPR 4.6mmx3.5cm
Mobile Phase A	1.5 M Ammonium Sulfate + 25 mM Sodium Phosphate in Water
Mobile Phase B	25% Isopropanol + 18.75 mM Sodium Phosphate in Water
Flow Rate	1.0 mL/min
Temperature	Room Temperature

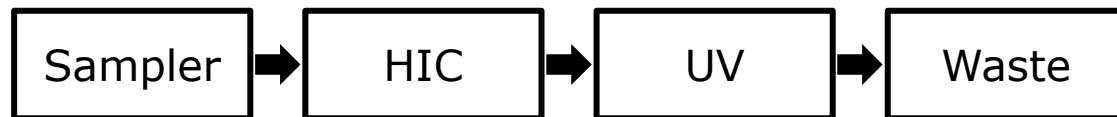
Time (m)	% Mobile Phase B
0.0	12
5.5	42
7.5	70
8.5	100
9.5	100
10.0	12
12.0	12

- Ammonium Sulfate and Sodium Phosphate are incompatible with LC-MS. Any mistake in desalting would lead to contamination.
- 200 bar pressure limit (per manufacturer) on column will inhibit ability to flow into a second column.

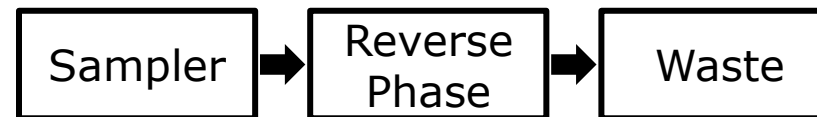
The 2D-HPLC-MS Plan

31

Step 1 – Run HIC Like Normal



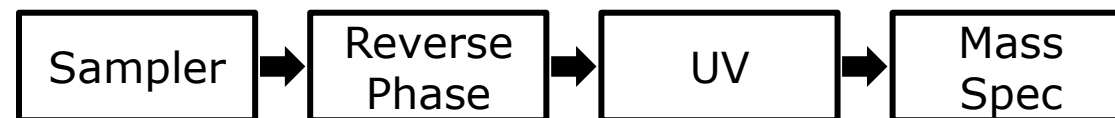
Step 3 – Wash Reverse Phase to Remove Salt



Step 2 – Divert Peak of Interest to RP

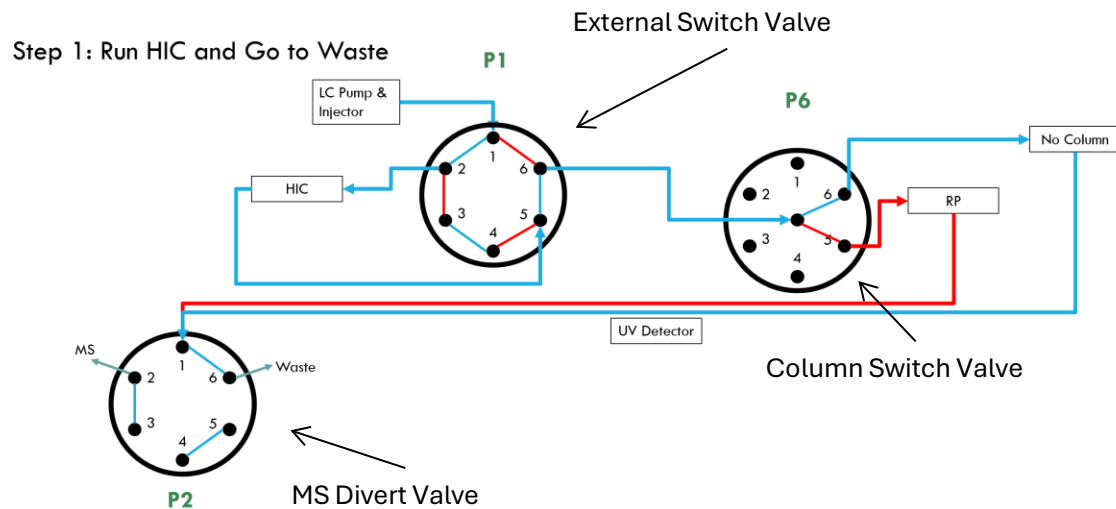


Step 4 – Analyze Retained Peak with Mass Spec

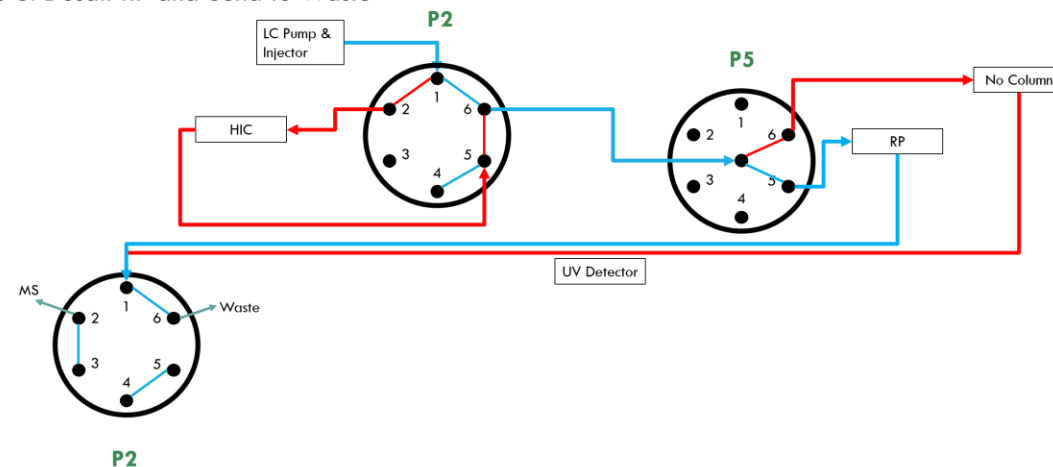


Building an “Unofficial” 2D-LC - Schematic

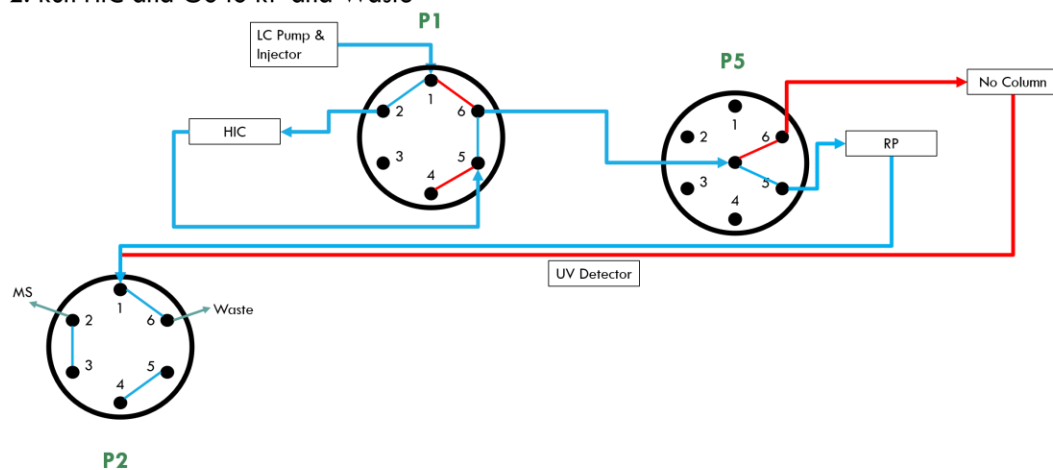
32



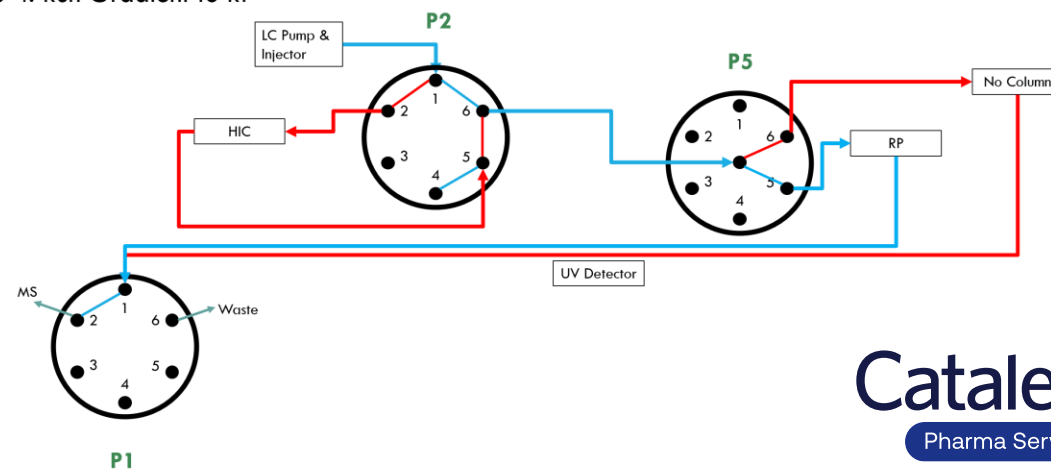
Step 3: Desalt RP and Send to Waste



Step 2: Run HIC and Go to RP and Waste



Step 4: Run Gradient to RP



YMC HIC Method – Mass Spec-Compatible Method

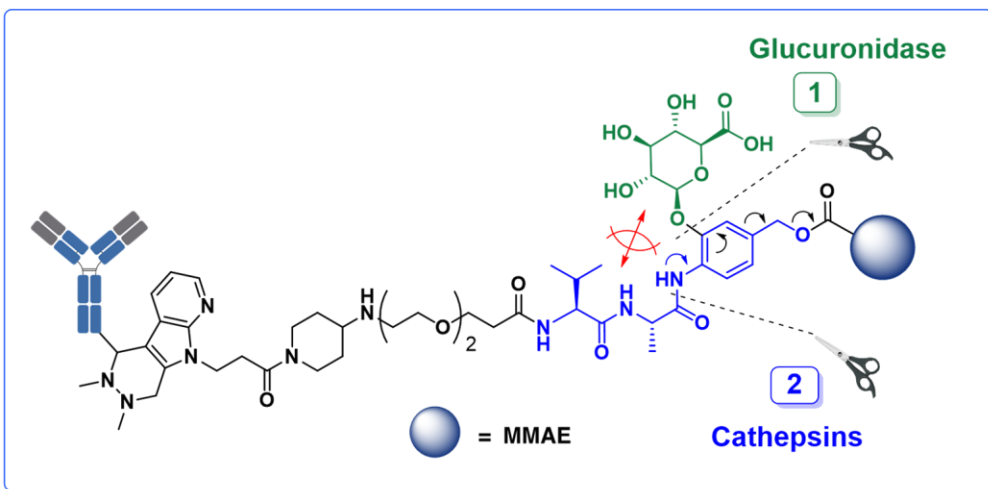
Column	YMC BioPro HIC HT 2.3 µm, 100 X 4.6 mm
Mobile Phase A	3 M Ammonium Acetate in Water
Mobile Phase B	25% Isopropanol in Water
Flow Rate	0.5 mL/min
Temperature	Room Temp

Time (m)	% Mobile Phase B
0.0	0
1.0	0
6.0	100
7.0	100
7.1	0
8.0	0

- At this concentration, Ammonium Acetate will suppress LC-MS signal. Will still require desalting on RP column to achieve best results.
- 400 bar pressure limit (per manufacturer) on column will allow the sample to flow into a second column.
- Ammonium Acetate suppresses UV signal but does not damage mass specs.

SMARTag® Tandem-Cleavage Technology Background

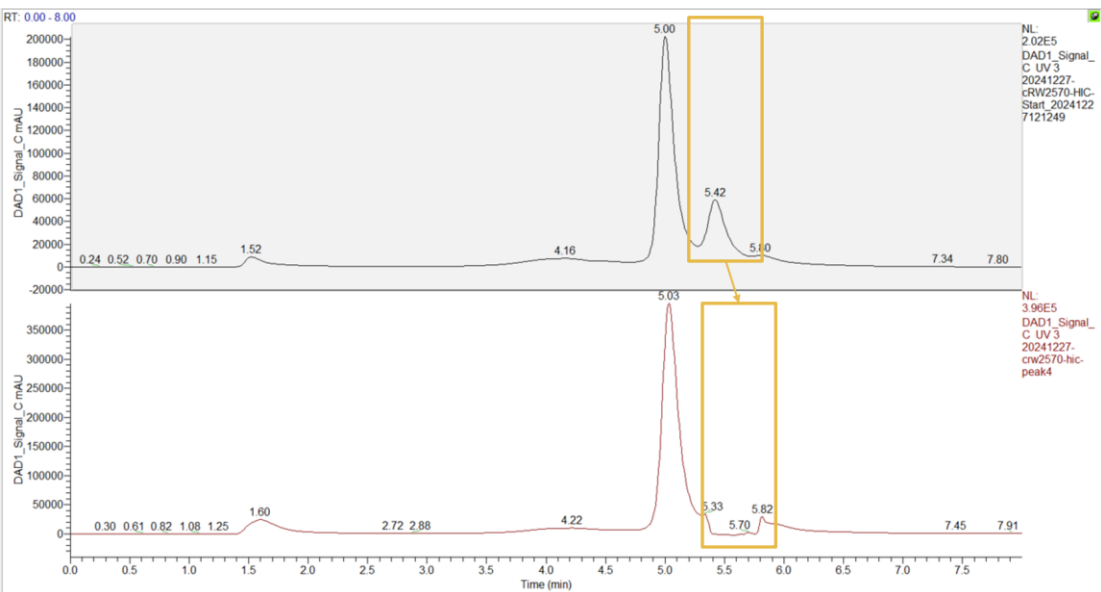
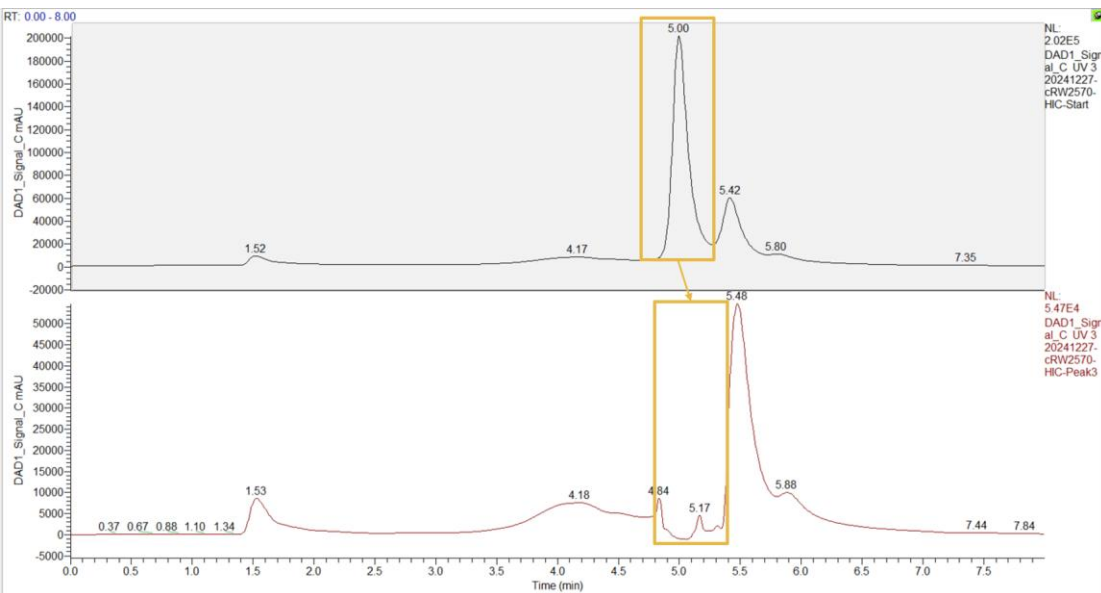
34



- This linker technology relies on two enzymes to release the toxic payload.
- Both enzymes are known to be active at lysosomal pH and tumor microenvironment pH ~5-6.
- The conjugation buffer conditions were also around pH ~5-6.
- Any residual HCP glucuronidase would likely result in the premature loss of the glucuronide piece during conjugation.

Heartcutting Method Results

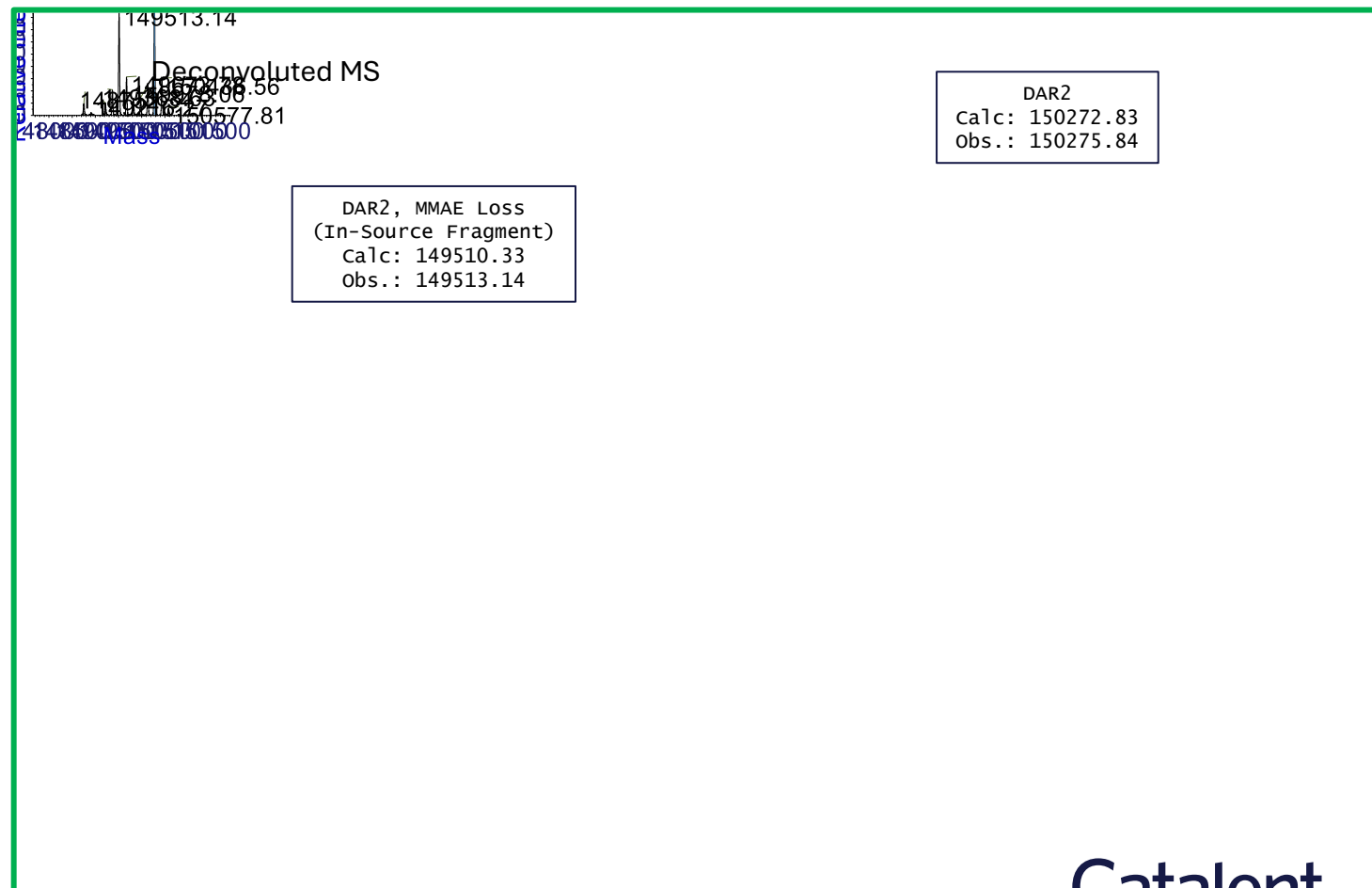
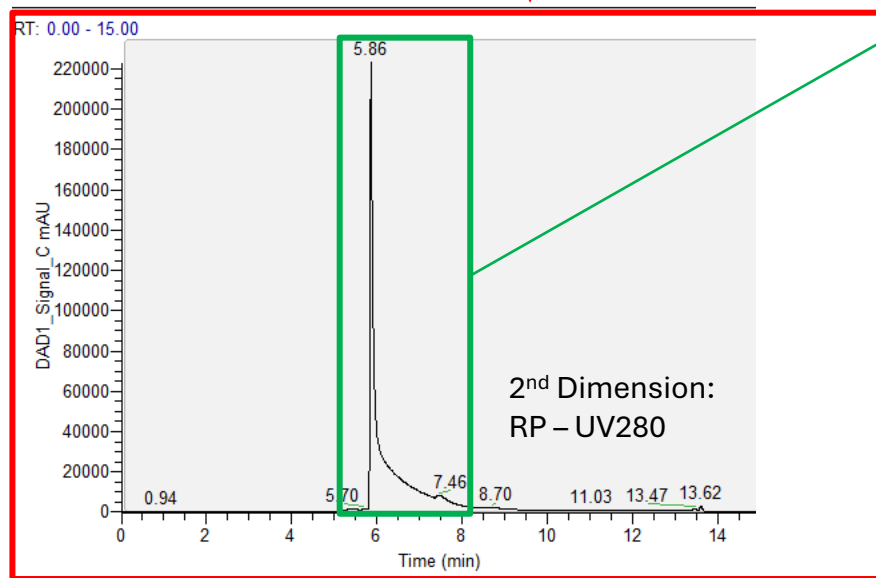
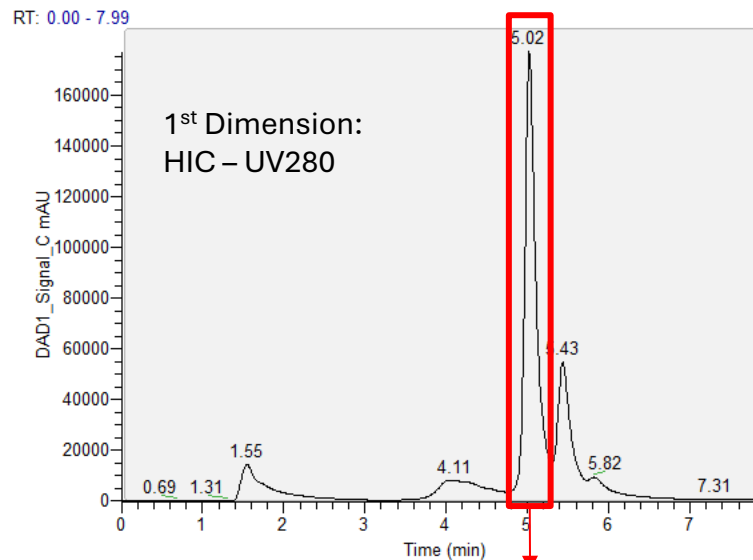
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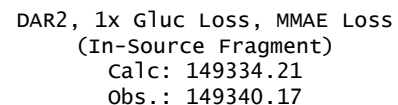
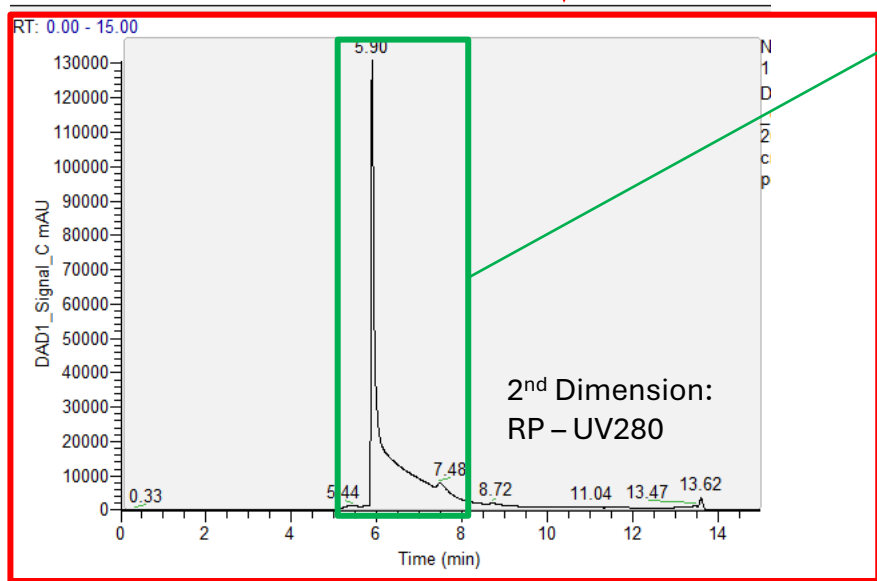


- Due to pressure constraints in our makeshift 2D-LC, the UV detector could not be in-line with the HIC and RP columns.
- This gave us an opportunity to see how effective the valve switching was at “removing” peaks from the UV chromatogram.

DAR2-1 Species, No Gluc Loss Detected

36

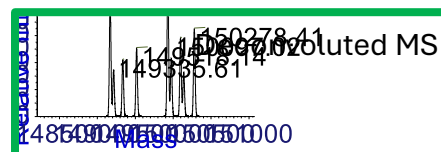
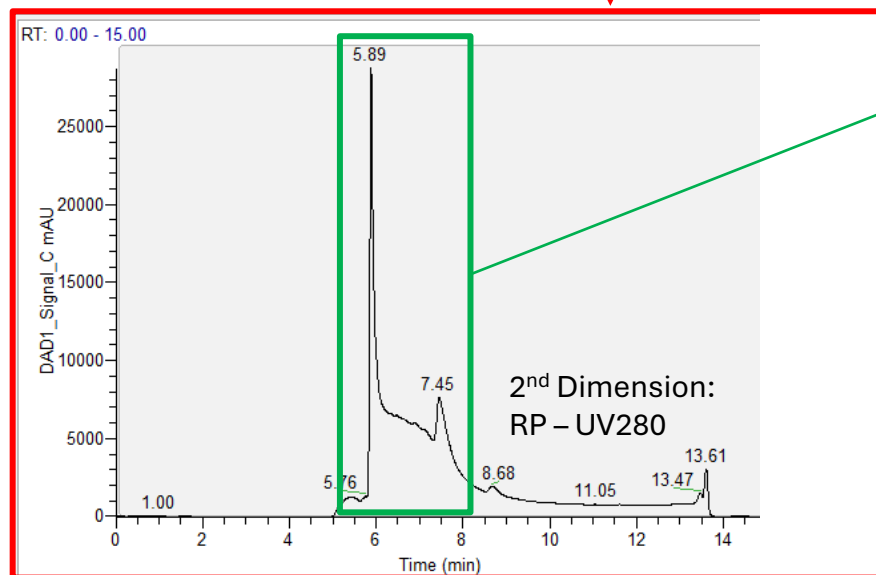
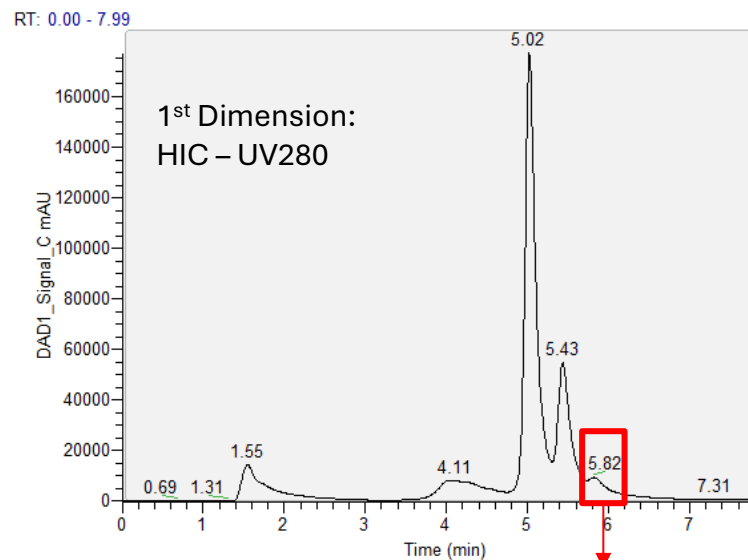




DAR2, 1x Gluc Loss
Calc: 150096.71
Obs.: 150101.22

DAR2-3, 2x Gluc Loss Detected

38



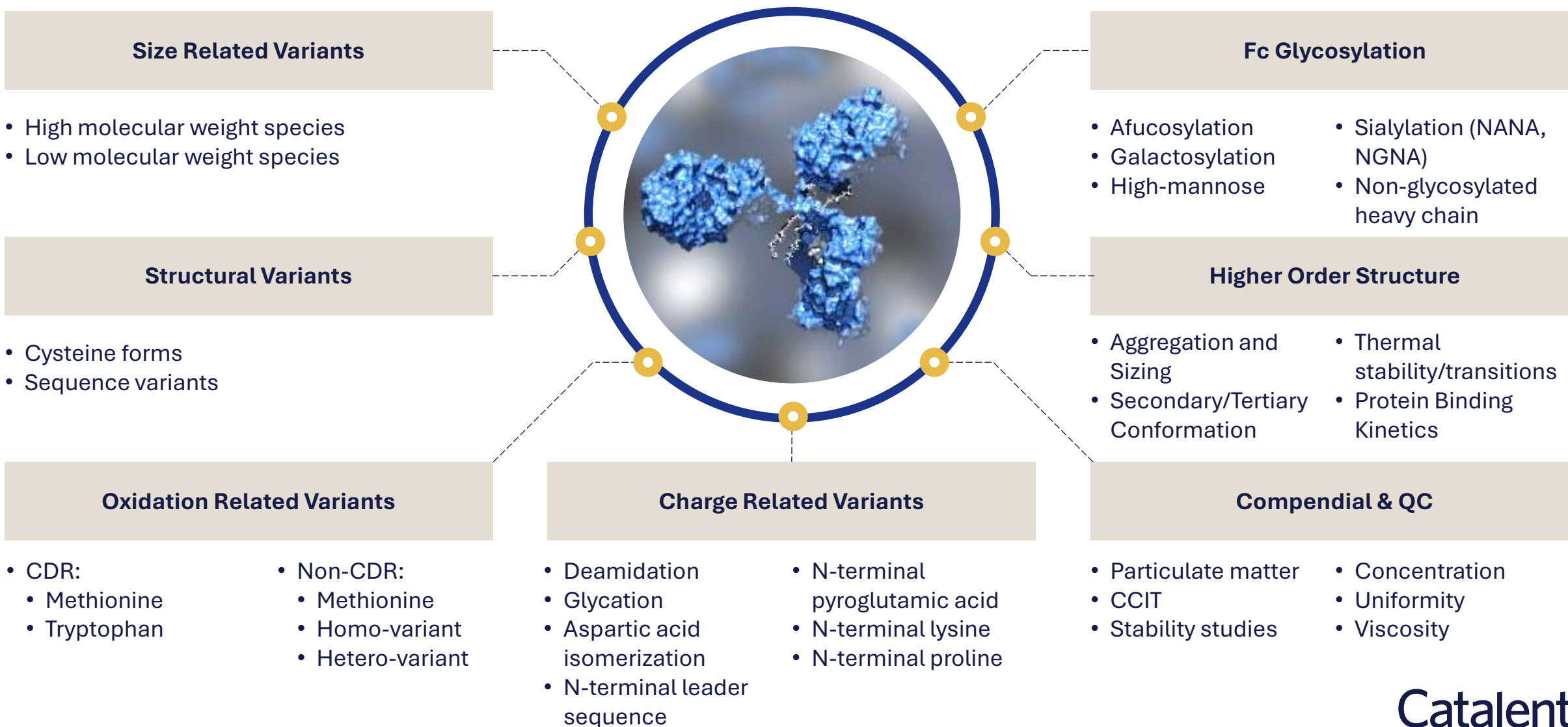
DAR2, 2x Gluc Loss, MMAE Loss
(In-Source Fragment)
calc: 149158.08
obs.: 145168.06

DAR2, 2x Gluc Loss
calc: 149920.58
obs.: 149929.41

- Using 2D-HIC-MS, we were able to find that glucuronide was being lost during the conjugation process.
- Further investigation revealed that the enzymatic HCP, glucuronidase, was being insufficiently removed during the purification process.
- 2D-HIC-MS was able to provide the bioconjugation team confidence that their platform HIC identifications were correct.

Expertise & Capabilities For Antibody, Linker, and Payload

40



Thank you

Any questions?



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