Comparability Risk Mitigation: Identification of a New Low-Level Heavy-Heavy Chain Variant in an ADC

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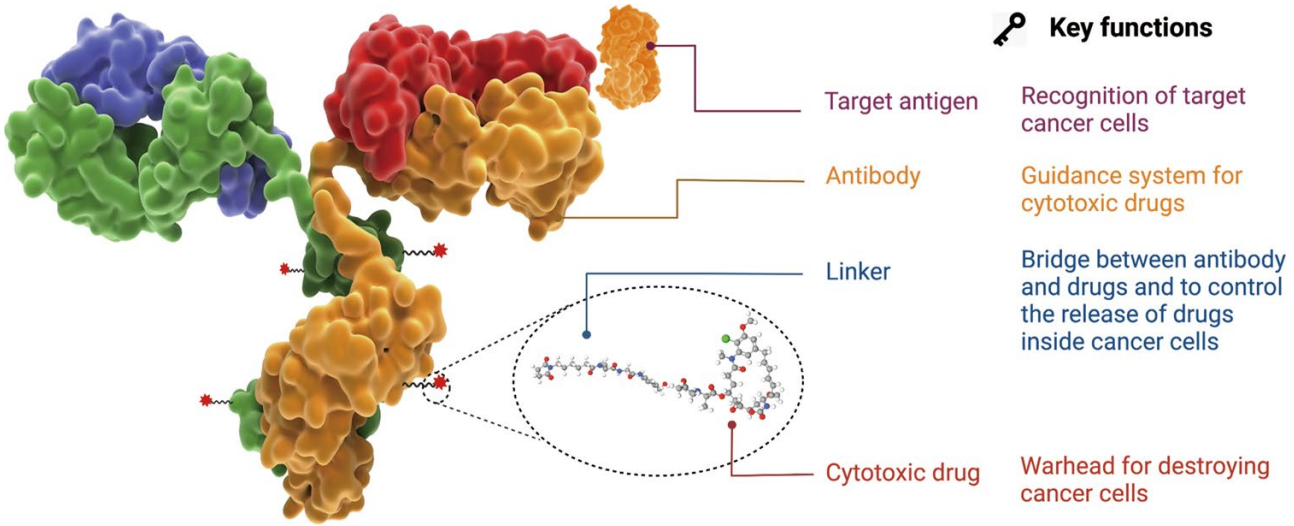
Antibody-Drug-Conjugate (ADC)



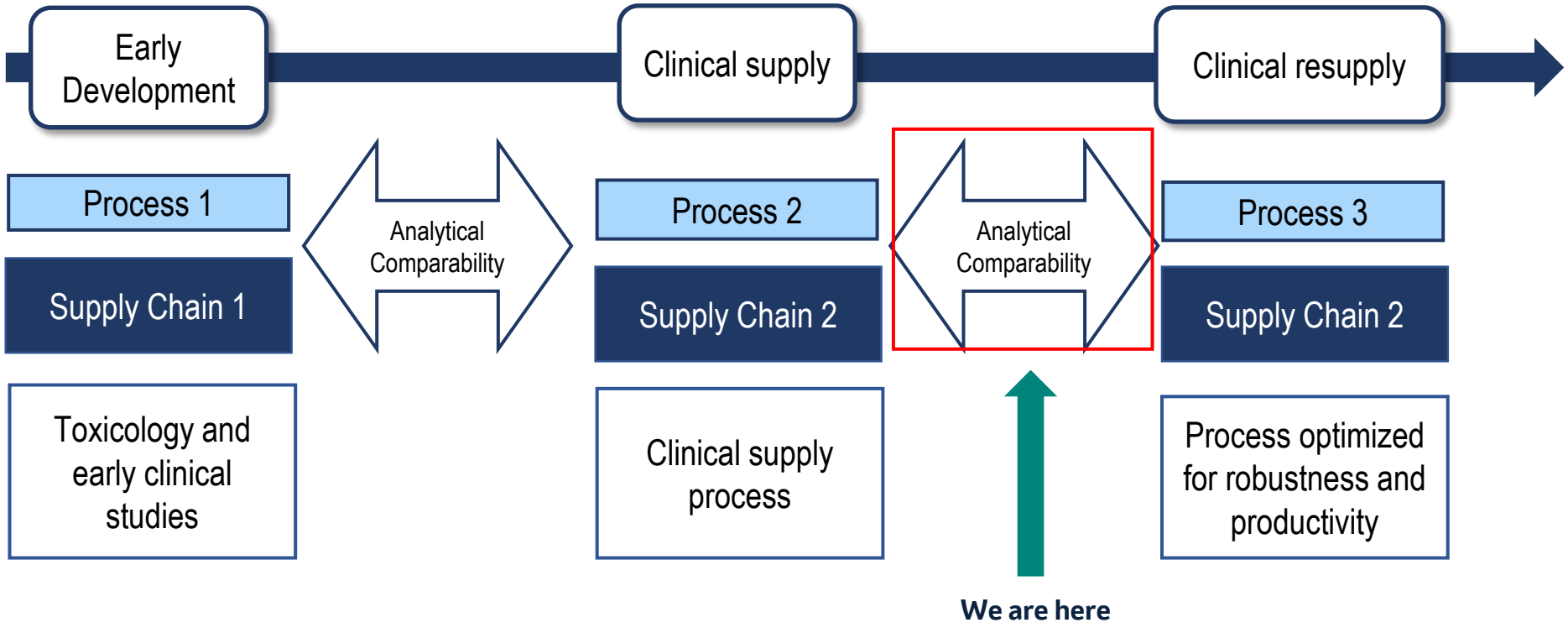
"Antibody drug conjugate: the “biological missile” for targeted cancer therapy." *Signal transduction and targeted therapy* (2022).

- Composed of a monoclonal antibody (mAbs) covalently conjugated to a cytotoxic drug via a chemical linker
- Harness the highly specific targeting capabilities of an antibody to deliver a cytotoxic payload to specific cell types
- Approved ADCs by FDA including Mylotarg, Kadcyla, Adcetris, Polivy, Enhertu, Trodelvy, etc.

"Site-selective modification strategies in antibody–drug conjugates." Chemical Society Reviews (2021).

- Lysine or cysteine residue are common conjugation sites on native antibodies
- ADCs currently approved and in clinical trials vary significantly in their DAR: 1.8-8.0
- Case study: high DAR ADC

Program CMC Strategy



Comparability Study Overview

The study included comparison of Pr3 to Pr2 at the mAb, DL, DS, and DP levels:

- At all four levels, the comparisons included batch release and stability assessments
- The mAb, DS, and DP comparisons included EC
- The mAb and DS comparisons included FD

❖ All comparability assessments met the pre-defined AC

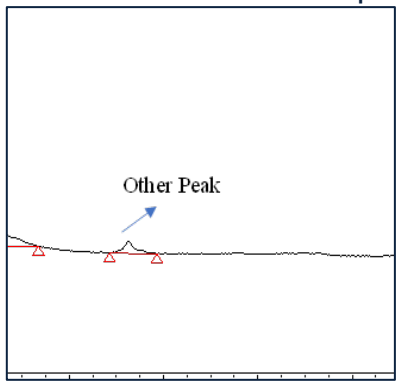
❖ Results submitted in IND amendment

❖ One result identified as risk: a slightly elevated peak in Pr3 DS/DP in the R CE-SDS electropherograms

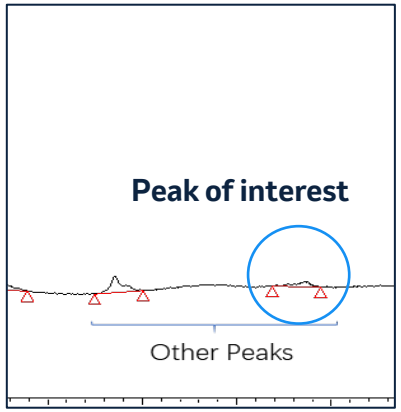
❖ A thorough assessment of this peak was initiated

Differences between Pr2 and Pr3: DS Release Impurities

Process 2 DS R CE-SDS electropherogram



Process 3 DS R CE-SDS electropherogram



Levels of R-CE-SDS "New" Peak

Drug Substance	
Range of Experience	
Process 2 (n>20)	Process 3 (n>10)
ND	<0.3%

ICH Q5E:

"Where the change results in the appearance of new impurities, the ***new impurities should be identified and characterized*** when possible. Depending on the impurity type and amount, it might be appropriate to conduct nonclinical or clinical studies to confirm that there is no adverse impact on safety or efficacy of the drug product."

Question:

- Is the peak of interest a "new impurity" in Pr3? What is the safety impact?
- Is Pr3 comparable to Pr2?

Risk Mitigation Strategy

Peak identity

- Establish orthogonal method for peak characterization and structure elucidation

New impurity or not

- Mine historical batch release data to assess RoE
- Establish investigative CE-SDS method with greater detectability

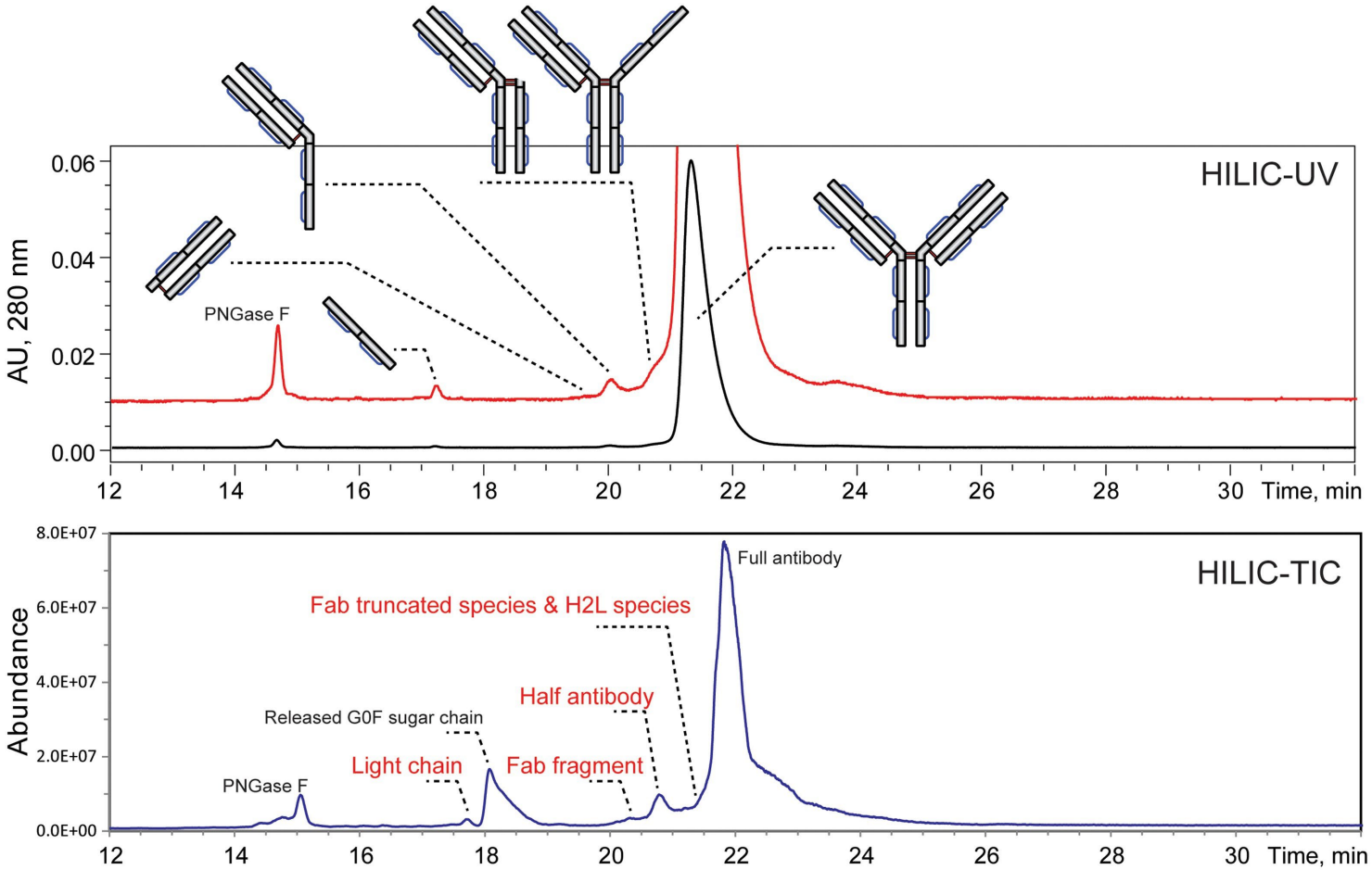
Risk assessment

- Evaluate safety limit based on clinical dose and peak RoE

Agency alignment

- Health authority interaction meeting to align comparability conclusion

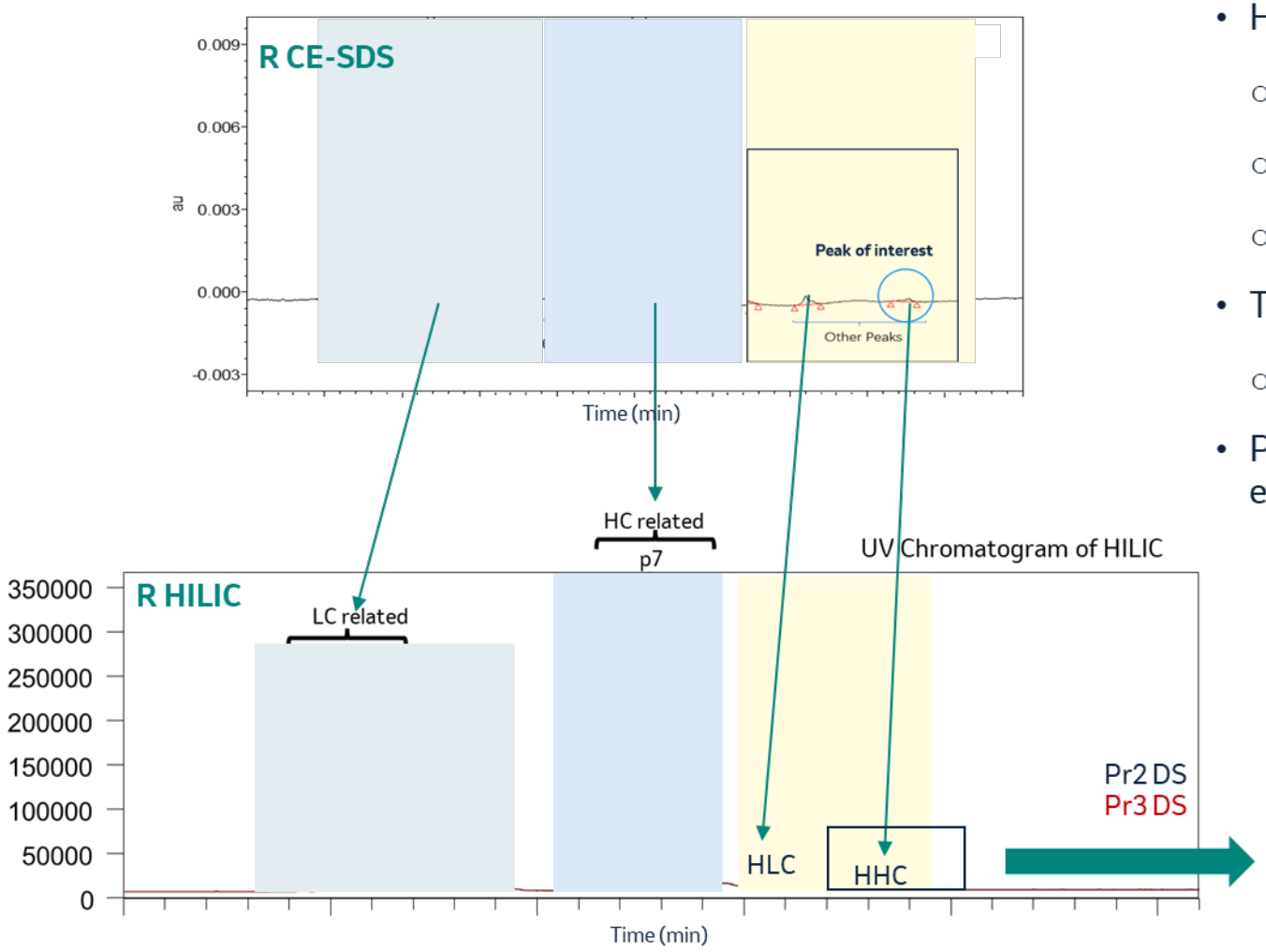
HILIC-UV Coupled with MS for Impurity Characterization



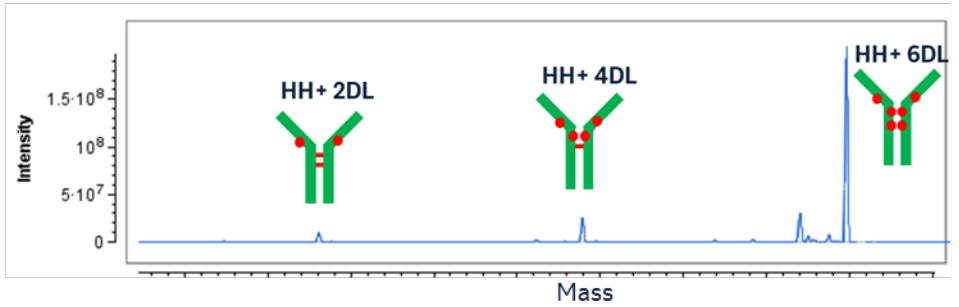
- Hydrophilic interaction liquid chromatography coupled with mass spectrometry (HILIC-MS) characterization method
  - Utilizes an orthogonal mode of separation
  - **Preserves the same peak order**
  - Mobile phase compatible to MS analysis

*"Characterization of product-related low molecular weight impurities in therapeutic monoclonal antibodies using hydrophilic interaction chromatography coupled with mass spectrometry." *Journal of pharmaceutical and biomedical analysis* (2018).

Peak of Interest Identification

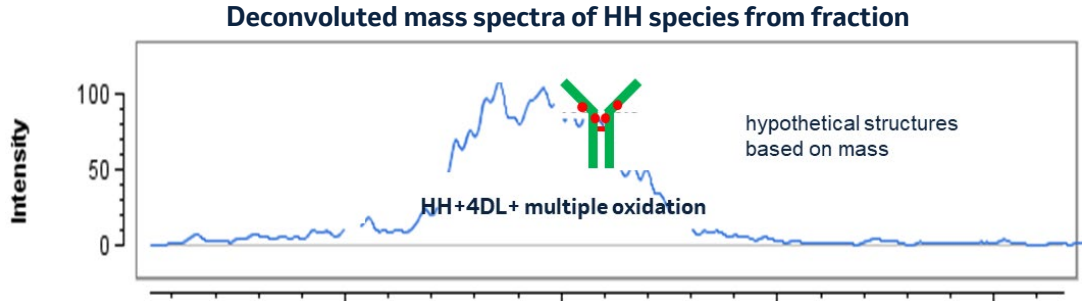


- HILIC-MS characterization method
  - Utilizes an orthogonal mode of separation
  - **Preserves the same peak order**
  - Mobile phase compatible to MS analysis
- The peak of interest is identified as
  - HHC with two, four, or six conjugated DL
- Peak correlation between CE-SDS and HILIC-MS need to be established



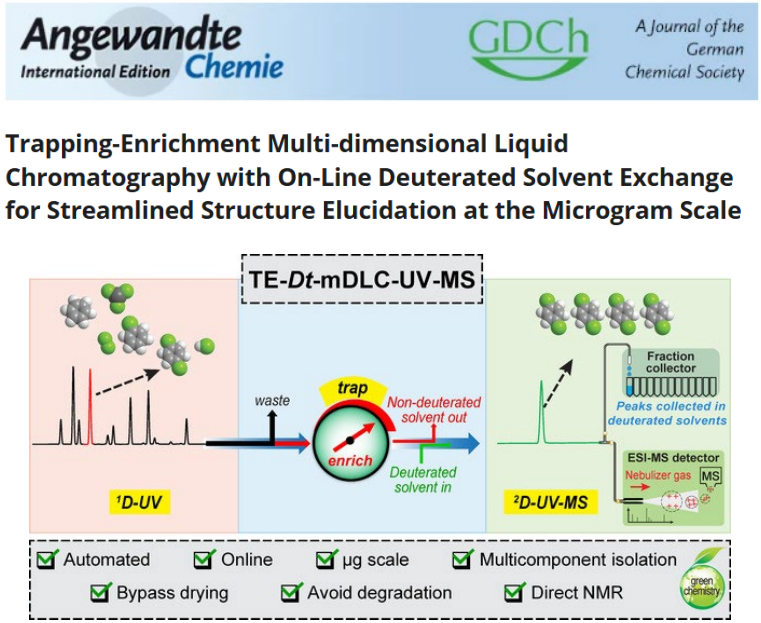
Challenges of Traditional Peak Isolation Approach: Abundance & Stability Issue

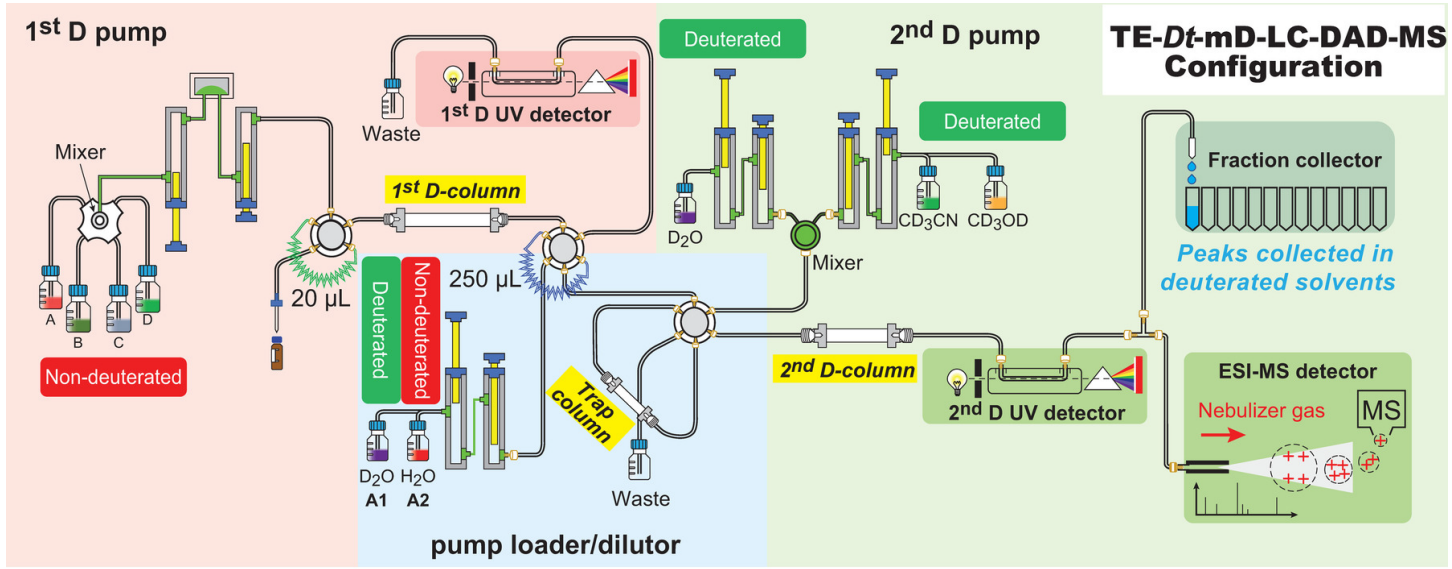
- HILIC method optimization
  - Scale-up loading and shorten run time
- Feasibility test under NR condition : 120 injections (~1D) was collected for purity check and ID
- CE-SDS profiles are similar under reducing and non-reducing condition
- HHC peak% is ~10x lower under reducing condition



- Numerous minor peaks appeared at +16 Da or +32 Da intervals, indicating **significant oxidation**.
- The most abundant species exhibited an approximate 160 Da mass increase relative to the theoretical MW of HH+4DL, corresponding to 10 oxidation.

Introduce TE-mDLC into Biologics





Key features of TE-mDLC

- ❑ *Minimized fraction manipulation*
- ❑ *Fraction of target analyte trapped as many times as needed*
- ❑ *Fraction eluted in the smallest volume as possible (concentration in situ)*
- ❑ *Convenient choices of buffer/eluent ready for analytical testing (NMR, MS, others)*

Ahmad, Imad A. Haidar, et al. "Trapping-enrichment multi-dimensional liquid chromatography with on-line deuterated solvent exchange for streamlined structure elucidation at the microgram scale." *Angewandte Chemie* 134.21 (2022).

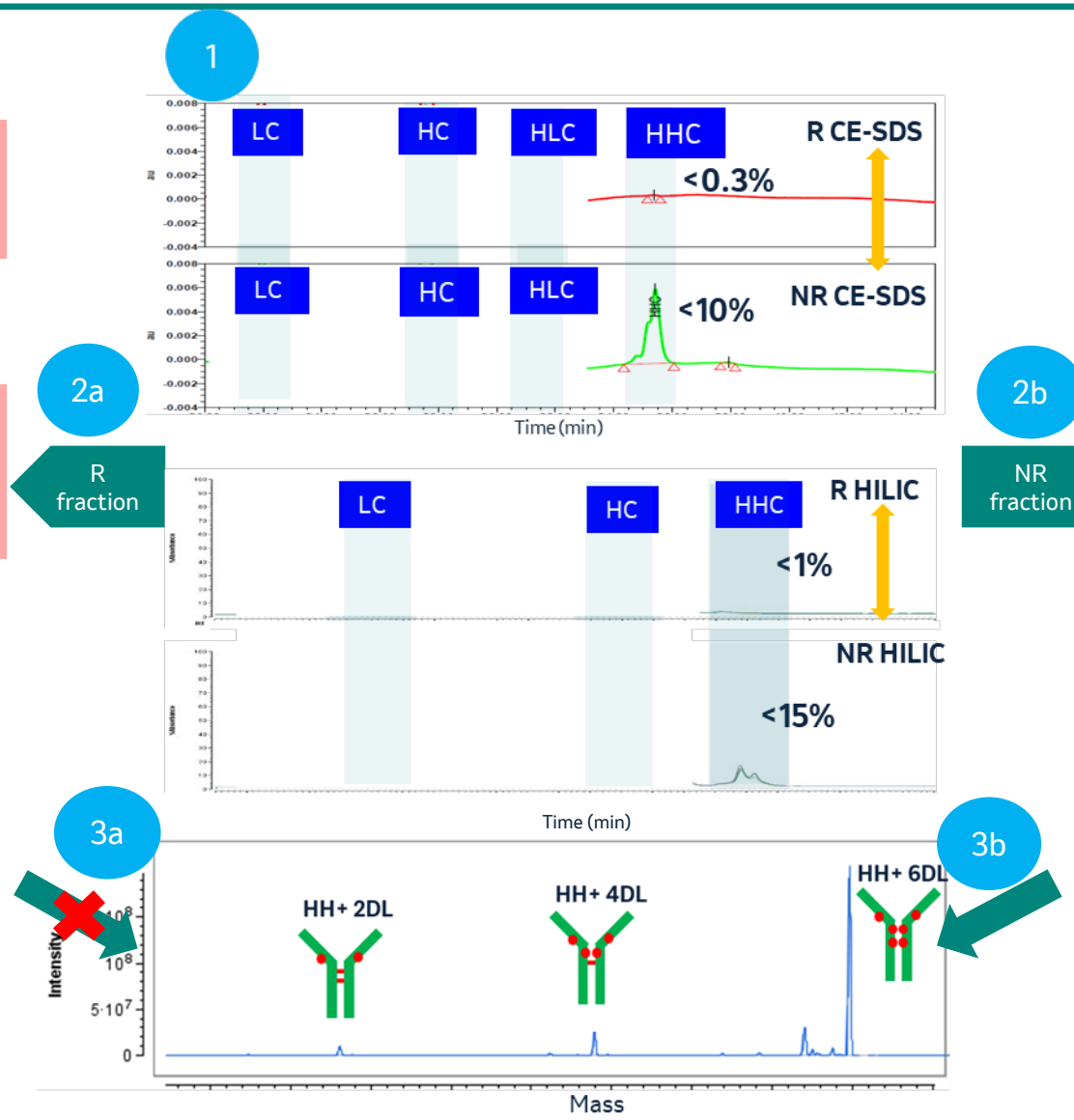
Correlation of CE-SDS and HILIC-UV

R CE-SDS

- Migration by CE-SDS **NOT** correlating

R HILIC-UV/MS

- Lower intensity in HILIC-UV/MS
- Mass could **NOT** be deconvoluted



Correlation **NOT** established under R condition

- Inconsistent RT in R HILIC
- Less pure fraction in R CE-SDS
- Fraction unstable

Correlation established under NR condition

- Consistent migration time
- 100% pure fraction
- ID confirmed as HHC containing varying DL

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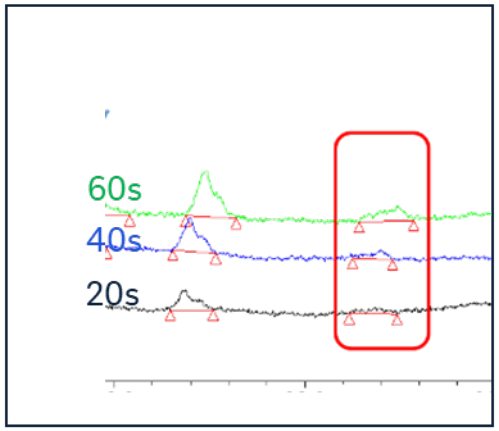
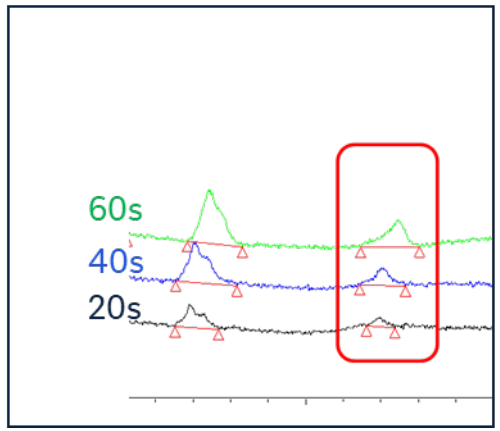
Establishment of Investigative CE-SDS Method

Process 2 DS



Process 3 DS



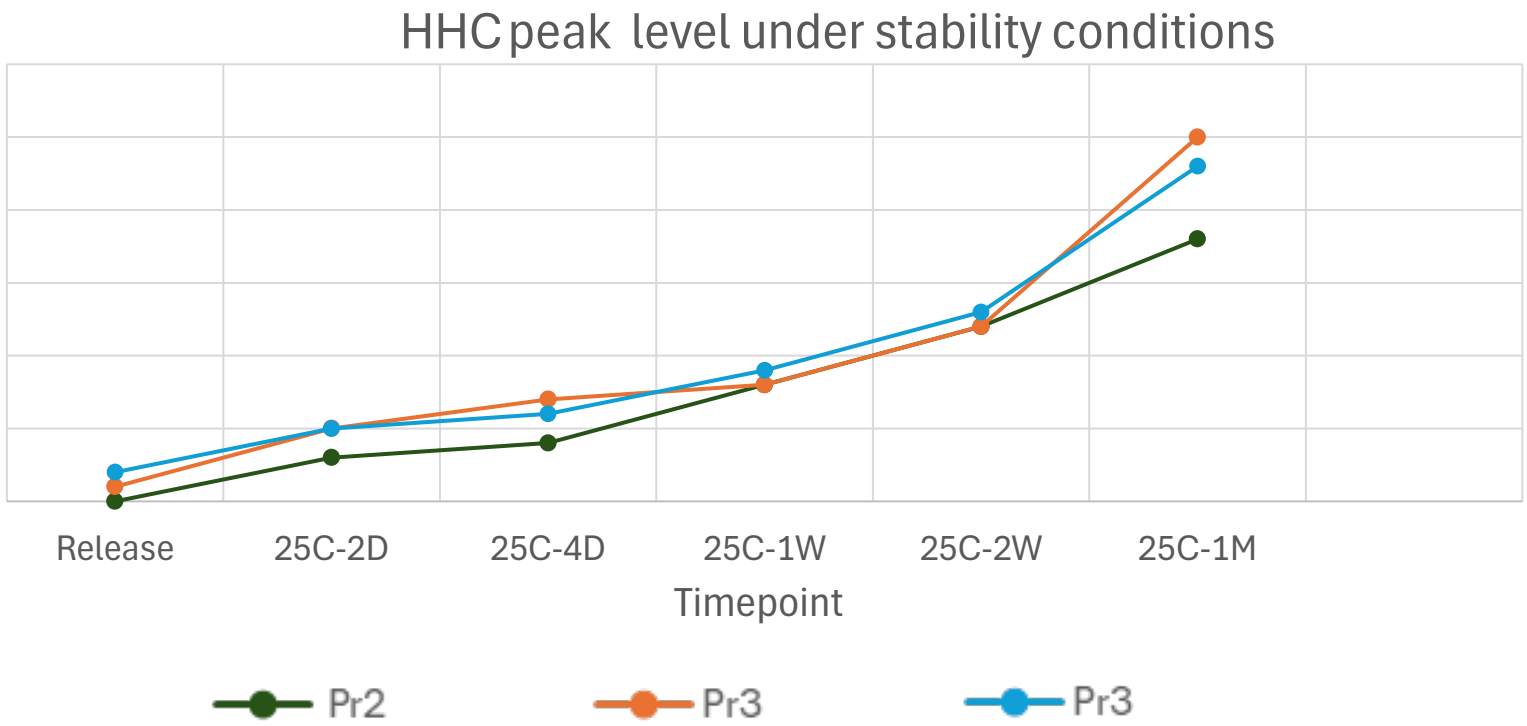
- By increasing the sample injection time from 20s to 60s, greater detectability of peaks at or below the detection limit was achieved.
- Other parameters of the release method remained unchanged.
- Investigative testing of Pr2 batches demonstrated that HHC is not observed under release testing condition (20s), but it becomes observable at higher injection times.

Investigation of Historical Levels of the HHC Peak

- This investigative method enabled quantitative comparison of the peak levels throughout development.
- Process 1 batches used in preclinical toxicology and early clinical studies were also tested for comparison.
- When tested by the modified investigative method, peak of interest is detected in all Pr1 and Pr2 batches evaluated.
- These results demonstrate the HHC peak is **not a new peak** in Pr3 but rather has been present historically in Pr1 and Pr2 batches at lower levels.

R CE-SDS Method	Drug Substance		
	Range of Experience		
Release method	Process 1 (N>5)	Process 2 (N>20)	Process 3 (N>10)
	ND to <0.3%	ND	<0.3%
Investigational method	Process 1 (N>5)	Process 2 (N>20)	Process 3 (N>10)
	<0.3%	<0.3%	<0.3%

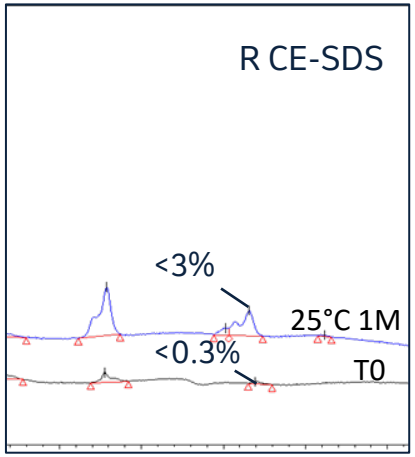
Peak of Interest Grows under Accelerated Stability



- The HHC peak increases under stability conditions in both Pr2 and Pr3 DS batches.

HHC Peak Profile under Stability Conditions

- HHC species observed on stability is different from the HHC species observed at release
- Heterogenous MS peak profile of HHC species reflecting drug loss and oxidation on stability



Peak No.	Major detected species by HILIC-UV/MS	
	Pr3 DS-T0	Pr3 DS-25C-1M
Peak 1	HH+6DL	Unknown
Peak 2	ND	HH+4 DL+2 linker HH+3 DL+3 linker HH+2 DL+4 linker HH+1 DL+ 5 linker HH+6 linker

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Safety Evaluation of the HHC Peak

- An analysis was conducted by non-clinical team to confirm the amounts dosed to patients receiving Pr3 are within the estimated amounts dosed in the preclinical toxicology study.
- The level of HHC observed in the parent DS batch for tox study: 0.09% by release method.
  - The qualified level of HHC was determined based on max dose in the tox study.
- The max level observed in Pr3 batches (<0.3%) is less than the qualified level, providing further assurance there is no risk to patient safety

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Summary

Characterization testing identified the peak as product related, consisting of HHC-xDL.

The HHC peak was present in historical Pr1 and Pr2 batches and is not a new impurity.

The HHC levels present in Pr3 batches are below the qualified level calculated from preclinical toxicology studies.

The HHC peak in Pr3 has no impact on the analytical comparability study.

The peak investigation work was submitted to health authority for feedback

Favorable agency feedback on our comparability Pr2 vs Pr3 assessment

Agreed in principle to the introduction of Process 3 into the registration and into clinical studies

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Acknowledgement

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Thank you