Next Generation Conjugates – Process and Analytical Challenges

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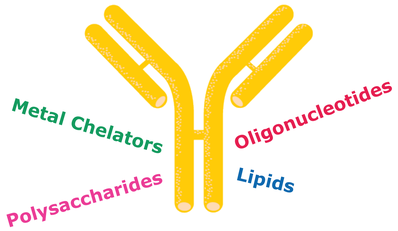
Introduction

Recent advances in Bioconjugation technology have significantly improved selectivity and efficacious outcomes for many diseases previously untreatable or poorly treated by traditional antibody drug conjugate (ADC) therapies.

These new technologies, now termed 'Next Generation Conjugates' increasingly represent different development stages for various indications, highlighting demand for clinical supplies.

To deliver GMP quality clinical product, new challenges have been identified for both process and analytical development. Process chemistry operations must ensure specific distribution of linker-payload to antibody is achieved, and must deliver pure, formulated drug substance at kilogram scale. Robust analytical testing is essential to define key quality attributes and determine product stability.

Merck is an industry leader, supplying early and late-phase clinical supplies for these next generation conjugates. Shown here, specific examples of process and analytical development challenges and corresponding solutions highlight our robust development offerings and vibrant scientific expertise.



Example Conjugates

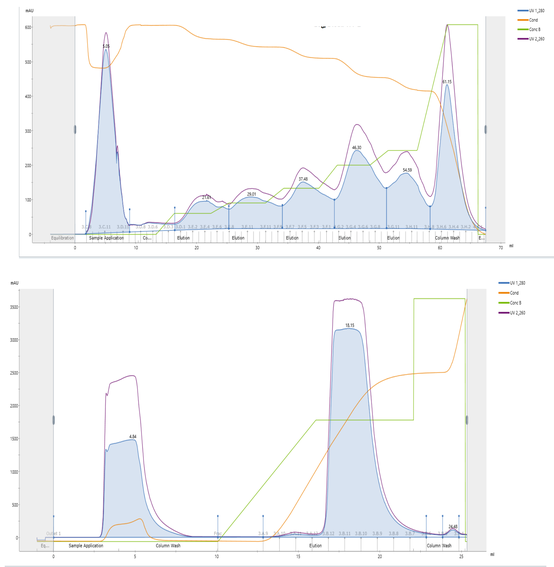
Downstream Process

After conjugation of the protein to the novel payload, isolation and purification steps are required before final formulation.

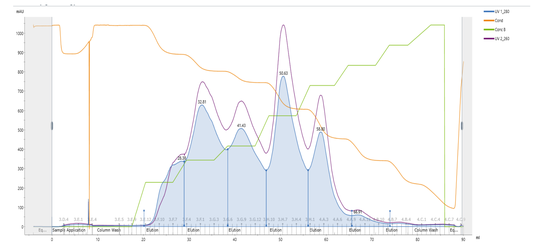
Unlike traditional ADCs, it is a challenge to remove free payload using straightforward Tangential Flow Filtration (TFF) due to the variable physicochemical properties. Different modes of chromatography can be used to purify these conjugates.

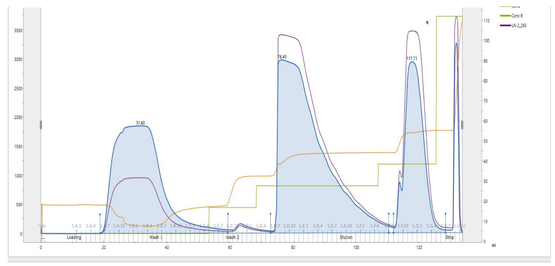
Chromatography

Some examples of purification and fractionation via chromatography have been visualized below:



- Customer specification determines process strategy:
  - BDS purity specification (%D0 content)
  - Removal of residual impurities and aggregates





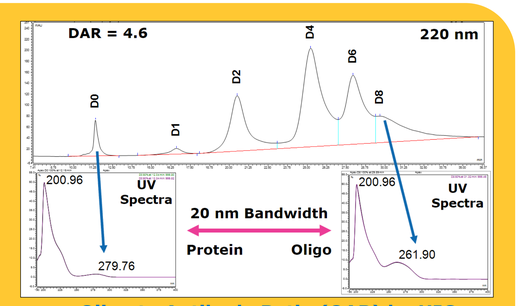
- Preparative chromatography can also achieve fractionation and isolation of DAR species
  - Final yield targets limit this option

Analytical Challenges

Challenge 1: Oligonucleotide Conjugate

Antibody and oligonucleotide raw materials have overlapping UV specificity

- Method development to achieve reasonable peak resolution
- Cannot use 280 or 260 nm signals for DAR evaluation; 220 nm provides normalized response of raw material components
- Spectral evaluation facilitates HIC peak identification and calculated drug load

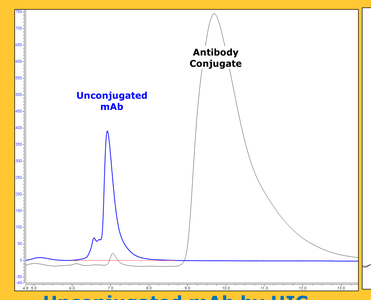


Oligo to Antibody Ratio (OAR) by HIC

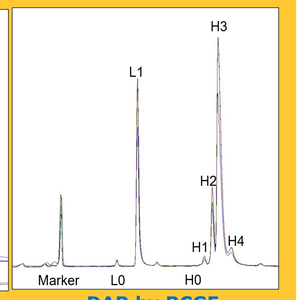
Challenge 2: STING™ Conjugate

Novel payload challenges process development

- Difficult analyte to investigate via traditional HPLC methods
- Multiple analytical methods required to determine product specifications
  - No resolution by HIC for DAR analysis; reducing CGE provides DAR assessment
  - Process control validation derived from quantifying unconjugated mAb via HIC; ensures raw material stoichiometry is achieved.



Unconjugated mAb by HIC



DAR by RCGE

Challenge 3: Carbohydrate Protein Conjugate

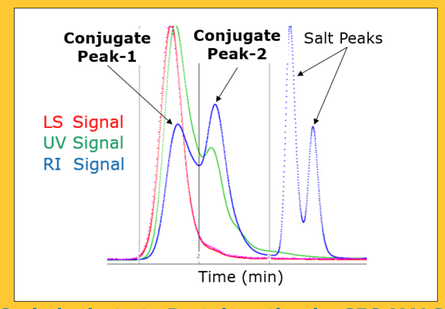
Non-traditional protein constructs with heterogeneous payloads

- Carbohydrates/Polysaccharides tend to lack homogeneity, which presents challenges to accurately measure payload stoichiometry.
- SEC-MALS provides multi-attribute assessment (Light Scattering (LS), Refractive Index (RI), and UV response) to accurately measure extent of protein conjugation to payload.

$$LS_{ADC} = M_{ADC} c_{ADC} \left(\frac{dn}{dc_{ADC}}\right)^2$$

$$dRI_{ADC} = c_{mAb} \frac{dn}{dc_{mAb}} + c_{drug} \frac{dn}{dc_{drug}}$$

$$dUV_{ADC} = c_{mAb}\, \varepsilon_{mAb} + c_{drug}\, \varepsilon_{drug}$$



Carbohydrate to Protein ration by SEC-MALS

Conclusions

The myriad protein-payload composition ranges pose unique challenges and necessitate development of novel unit operations for purification and formulation.

The typically higher dosing strategy of oligo-conjugates, or the lower dosages for vaccine conjugates impact scale-up, as the need for much smaller or much bigger batches using existing infrastructure necessitates further process innovation.

Iterative process development is dependent on robust and reliable analytical techniques:

Although traditional analytical methods are transferrable to next generation conjugates, the nature of the raw materials, intermediates, and final product requires a novel approach towards data interpretation.

Merck has demonstrated successful production of next generation conjugates across different proteins and payloads.

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