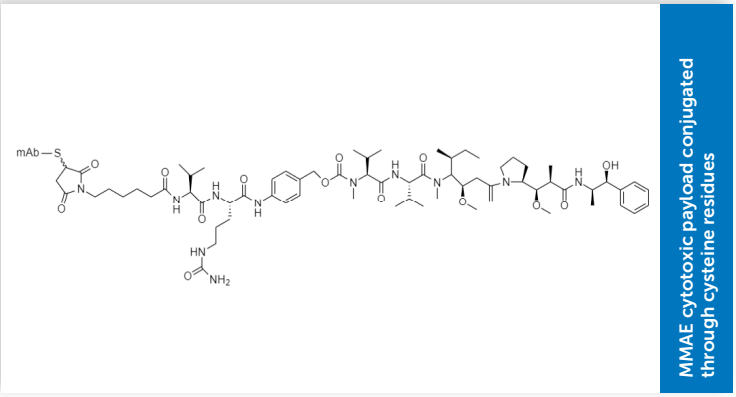
Native Intact Analysis of a Cysteine Linked Antibody Drug Conjugate

Native intact analysis of Brentuximab Vedotin using a SCIEX X500B Mass Spectrometer

Antibody-drug conjugates (ADCs) are an emerging class of biopharmaceutical combining the specific targeting nature of monoclonal antibodies (mAbs) with highly cytotoxic payloads. The field of conjugation strategies is rapidly evolving with innovative methods to control drug-antibody-ratio (DAR). One of these such techniques is the conjugation of payloads to inter-chain cysteines after they have been reduced. This leads to reproducible even numbered species that can be tuned through levels of reduction. The antibody protein complex is held together through non-covalent interactions however this presents an analytical challenge for LC/MS as most methods rely upon denaturing reverse phase chromatography.

Here we outline an analysis strategy to confirm the DAR of a cysteine linked ADC with a SCIEX X500B Mass Spectrometer utilising a native ionisation approach with 400mM ammonium acetate. SEC chromatography was performed with an EXION HPLC using a bioZen dSEC-2 column (1.8 µm, 50 x 2.1 mm).



Calculated DAR Brentuximab Vedotin	
HIC	MS
4.03	3.94

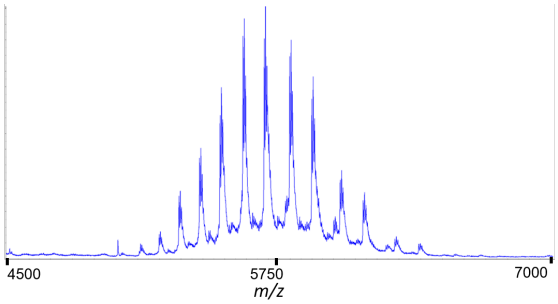


Figure 1: Raw spectra of brentuximab vedotin. The ADC presents in a narrow distribution after native ionisation unlike a broad distribution during reverse phase.

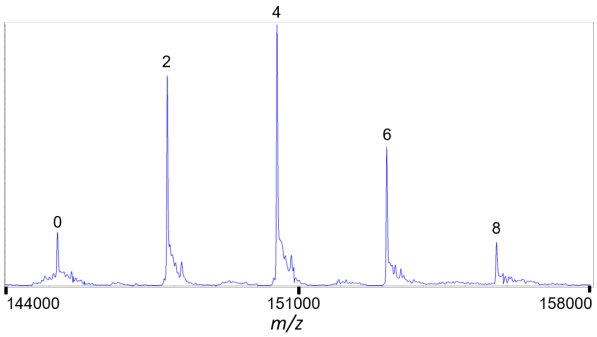


Figure 2: Reconstructed spectra of brentuximab vedotin intact. The DAR was calculated as 3.98 with the majority of the ADC in even numbered species.

Native analysis via Mass Spectrometry requires the use of volatile solvents such as ammonium acetate to balance the conservation of a proteins folded solution-phase conformation with the ionisation efficiency of the protein and transfer to the gas phase. SEC chromatography provides a route to online native analysis with efficient buffer exchange and desalting at MS friendly flow rates.

Figure 1 shows the raw mass spectra for brentuximab vedotin in a narrow charge envelope indicative of folded protein. Though reconstruction of this envelope can be performed, the presence of heterogenous glycan structures combined with varying degrees of drug conjugation can present challenges to ensuring an accurate DAR. Because of this, the sample was also analysed after treatment with PNGase F to remove the N-linked glycans from the heavy chain to simplify the spectra. This ensures minimal spectral overlap leading to improved accuracy in calculating intact molecular weight. Figure 2 shows the reconstructed glycan free spectra showing a distribution from 0 to 8 conjugated species. A small degree of odd DAR species were observed which were difficult to assign via other techniques. The DAR for the intact species was calculated as 3.98 with hydrophobic interaction chromatography conducted on the same sample giving a value of 4.03.