

Intact mass analysis of a therapeutic lysine linked antibody drug conjugate

DAR of Trastuzumab Emtansine (Kadcyla) using a SCIEX X500B Mass Spectrometer

Biologics represent a growing class of pharmaceuticals being developed to target a variety of disease and illness vectors. Antibody Drug Conjugates (ADCs) combine a monoclonal antibody (mAb) with a cytotoxic drug via a linker. This allows the mAb to target a specific cell type or antigen and deliver a potent molecule without affecting surrounding cells. There are currently twelve approved ADCs with a variety of linkage chemistries of varying specificities. Lysine conjugations are non-specifically conjugated meaning that a heterogenous distribution of payload molecules are present on the final ADC. Measurement of DAR during manufacture is important to ensure correct dosing to the patient.

Here we outline an analysis strategy to confirm the intact molecular weight and DAR for a Lysine-DM 1 conjugated ADC. All data was collected on a SCIEX X500B Mass Spectrometer with an EXION HPLC. The chromatography was performed with a bioZen Intact XB-C8 Column (3.6 μ m, 50 x 2.1 mm).

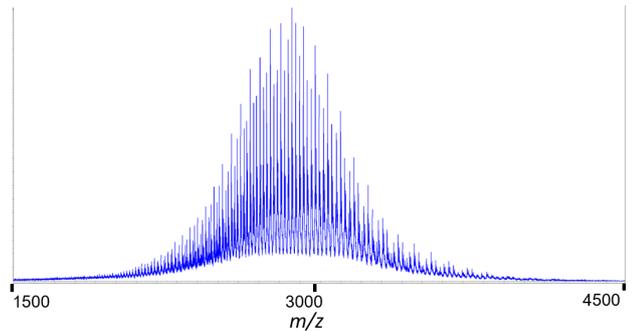


Figure 1: RAW MS of trastuzumab emtansine. Charge envelope is complex with dense overlapping charge states.

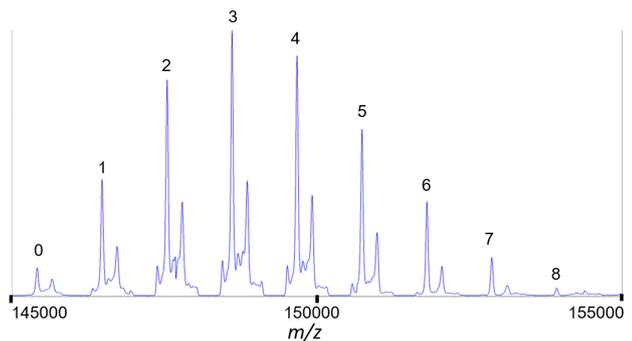
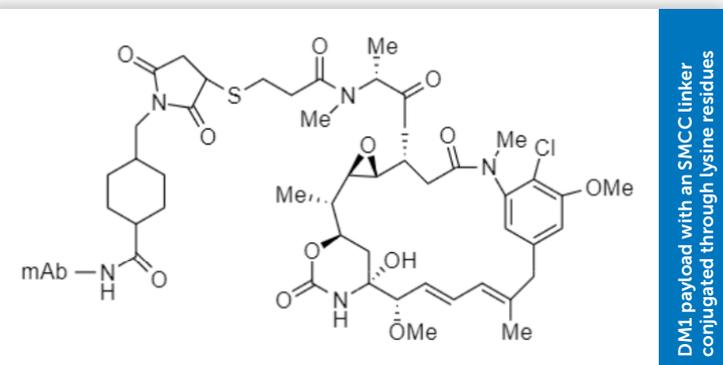


Figure 2: Reconstructed Spectra of trastuzumab emtansine after Endo-S treatment. Up to 8 DM1 conjugates are observed with some MCC linker.



DAR Trastuzumab Emtansine	
Published	MS
3.5	3.43

Figure 2 shows the reconstructed mass spectrum and the assigned number of conjugated payloads. The DAR for this ADC was calculated at 3.43 versus the expected DAR 3.5. The payload DM1 is known to undergo hydrolysis events, this can result in linker crosslinking within the protein or free linker without a warhead present. Approximately 37% of the protein has at least one of these linker species.

Figure 3 details a zoom in on the peak series with six drug conjugates with the shown mass shift owing to the extra linker species.

BioPharmaView Flex software from SCIEX to match the intact masses of the protein and calculate the DAR.

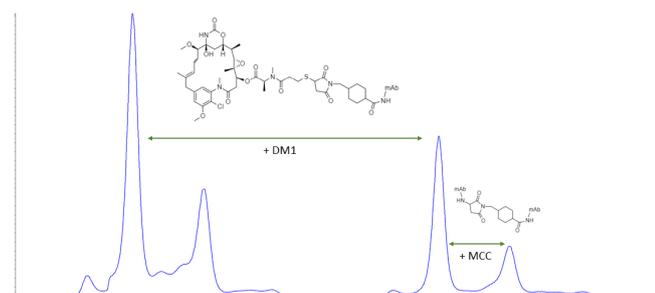


Figure 3: Reconstructed Spectra of trastuzumab emtansine after Endo-S treatment Dar 6-7. Mass shifts between species are correlated to a full DM1 addition and an MCC linker in a free or crosslinked form.