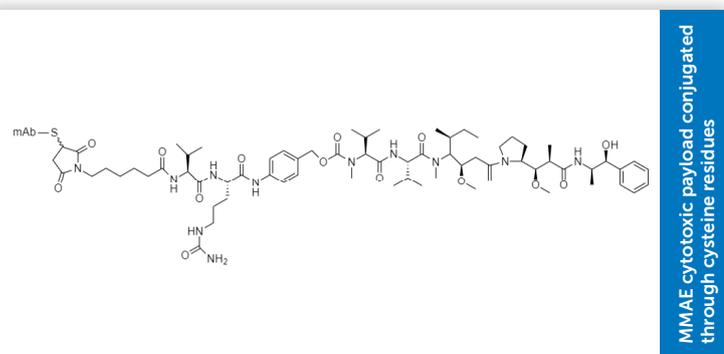


# Measurement of ADC stability in serum via subunit DAR analysis

Measurement of DAR of trastuzumab-vcE ADC after incubation in IgG depleted serum for seven days

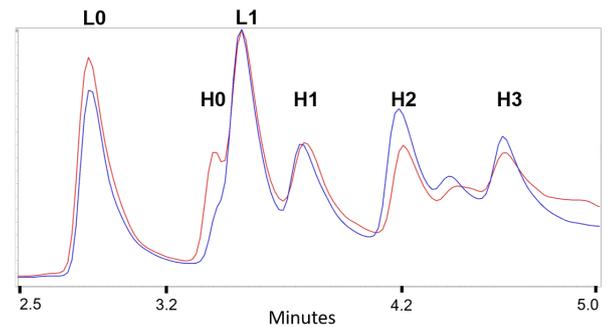
Stability of biologics is a critical quality attribute (CQA) affecting the levels of dosing and efficacy. Measurement of this can be complicated by the inherent heterogeneity present within the protein in addition to linker and payloads of ADCs. Mass Spectrometry (MS) is an appealing technique for these measurements as loss of payload can be measured on the protein level or as a released drug. Intact measurements have the benefit of minimising sample handling after incubation compared to enzymatic release methods.

Here we outline an analysis strategy to determine the stability of a trastuzumab val-cit PABC MMAE conjugate (DAR 4.5). The samples were incubated in IgG depleted rat (Sprague Dawley (BioIVT)) serum for time periods up to seven days. The ADC was then captured on Protein A magnetic beads (BioRad), before release and reduction. Data was collected over two pooled biological replicates and three technical replicates. All data was collected on a SCIEX X500B Mass Spectrometer with a bioZen Intact XB-C8 Column (3.6  $\mu\text{m}$ , 50 x 2.1 mm).

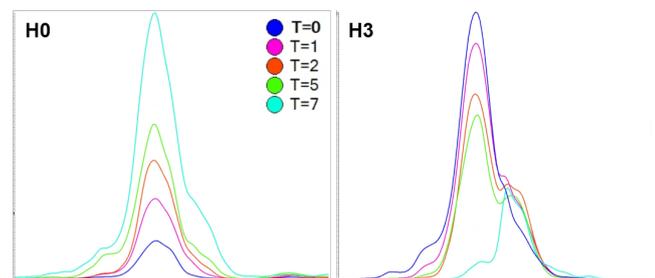


The ADC at t=0 was observed in H1 through to H3 with very little H0, DAR was calculated at just over 4.5 matching Hydrophobic Interaction Chromatography. Figure one shows the TIC of all the subunit peaks after reduction at t=0 (blue) and after seven days incubation in IgG depleted serum (red). Overall, a decrease in all drug bound species and an increase in L0 and H0 was observed.

The overall DAR of both light and heavy chains, including glycoforms and modifications, was recorded in BPV Flex and the output is displayed in Figure 3 where we see a decrease from DAR ~4.5 to DAR ~3 over seven days.

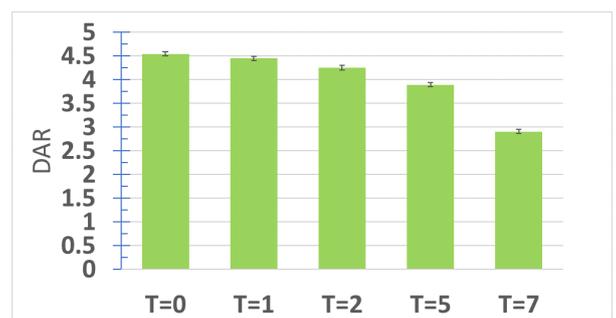


**Figure 1: TIC of reduced trastuzumab vcE conjugate before (blue) and after (red) incubation.** Separation between each conjugated form is observed with changes in extremes after incubation.



**Figure 2: Mass Spectrum of trastuzumab vcE H0 and H3 main glycoforms.** Overlaid spectra show changes in relative intensity and oxidation corresponding with incubation in serum.

Figure 2 shows the mass spectrum of the first glycoform of H0 and H3 normalised against L1. We can clearly track the decrease and oxidation of H3 and the corresponding increase of H0.



**Figure 3: Average DAR versus incubation time.** Overall heavy and light chain DAR decreases after incubation in serum. Error bars from technical replicates.