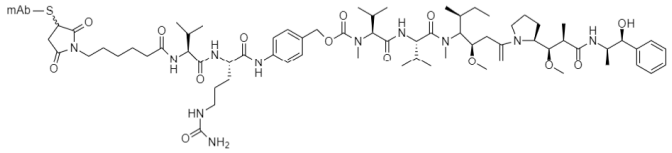
Total antibody quantification of an ADC after plasma incubation

Measurement of antibody concentration of a trastuzumab-vcE ADC after incubation in plasma

A key feature of monoclonal antibody (mAb) proteins is their stability in plasma due to their resistance to proteases. It is this combined with their highly targeting nature that make them attractive as biopharmaceuticals. Antibody drug conjugates (ADCs) have an added payload which may or may not include a cleavable linker. When measuring these samples for stability either in plasma or during storage it is important to measure that the mAb scaffold is stable as well as the conjugated drug. VVSVLTVLHQDWLNGK has been identified as a peptide that is consistent in human mAb Fc regions and be used to quantitate protein concentration.

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 depletedrat serum (Sprague Dawley (BioIVT) for time periods up to seven days. The ADC was then captured on Protein A magnetic beads (BioRad), before enzymatic digestion with trypsin/Lys-C. Data was collected over two pooled biological replicates and three technical replicates. All data was collected on a SCIEX X500B Mass Spectrometer with an EXION HPLC monitoring the peptide VVSVLTVLHQDWLNGK 4+ ion with a heavy labelled internal standard. The chromatography was performed with a bioZen XB-C18 Column (1.7 µm, 150 x 2.1 mm).



MMAE cytotoxic payload conjugated through cysteine residues

Time (days)	Adjusted amount of peptide (ng)
0	469 ± 16
1	339 ± 5
2	177 ± 4
5	162 ± 10
7	111

Table 1: Adjusted VVSVLTVLHQDWLNGK concentration 20 µg of ADC were incubated and digested. 10 µL of sample were injected on column after a 10x dilution.

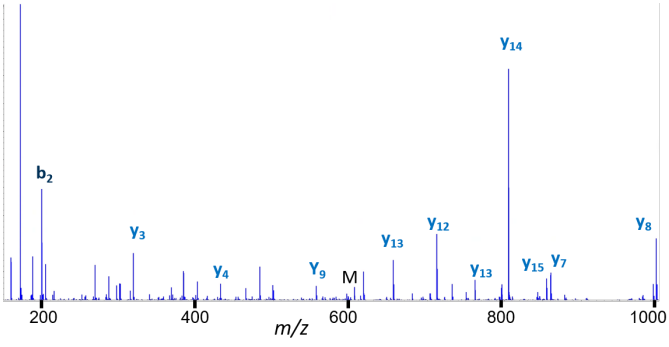


Figure 1: MSMS fragmentation of VVSVLTVLHQDWLNGK 2+ 805.3 m/z was used as the quantifier ion with 171 m/z as the qualifier.

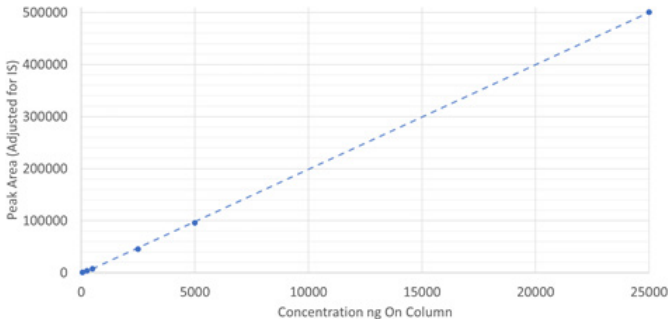


Figure 2: Calibration curve of VVSVLTVLHQDWLNGK over three orders of magnitude r^2 was >0.99 with an internal heavy labelled standard.

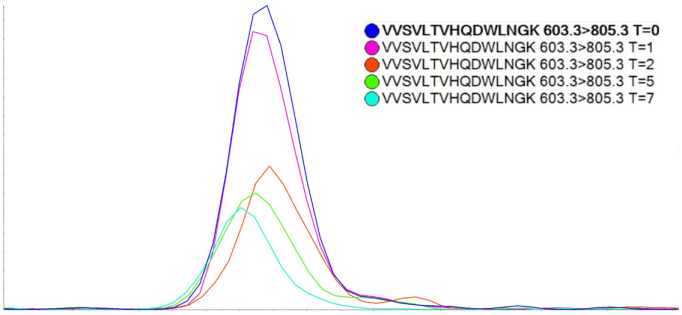


Figure 3: XIC of VVSVLTVLHQDWLNGK quantifier ion y14 805.3 m/z Intensity of peak reduces over time as the antibody protein degrades in serum

The payload was observed in a single peak eluting at 3.1 minutes on a twenty-minute gradient of water and acetonitrile (both with 0.1% formic acid). Figure 1 shows the MSMS spectrum of the payload with a number of fragments which could be positively assigned as b and y ions.

A calibration curve was generated using SCIEX OS software and serum incubated samples were compared to this. At time zero the concentration of VVSVLTVLHQDWLNGK from the ADC was 469 ± 16 ng which is expected at near 100% capture rate from 20 µg of ADC.

Figure 3 shows the XIC peak area of VVSVLTVLHQDWLNGK over the five time points. We are able to observe a decrease in response over time due to degradation or aggregation of the protein in serum.