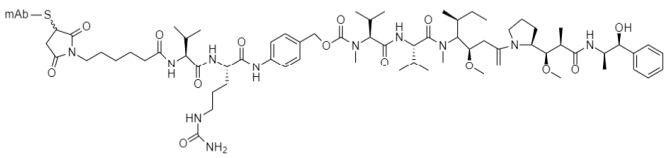
Measurement of ADC free drug after plasma incubation

Measurement of payload loss from a trastuzumab-vcE ADC after incubation in plasma

Antibody drug conjugates (ADCs) combine a monoclonal antibody (mAb) scaffold with a number of cytotoxic drugs. Release of this payload at the target site can vary between ADCs with some having a linker which is cleaved at the target site, whereas some linkers are non-cleavable and rely upon lysosomal degradation after internalisation. When exposed to plasma, some ADCs may prematurely release their cytotoxic warhead, whereas others may lose the entire payload via reverse Michael reaction. It is important to measure how much toxic drug is lost over time as this may be a critical quality attribute (CQA).

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 rat plasma (Sprague Dawley BioIVT) for time periods up to seven days. The ADC was then captured on Protein A beads (mabselect LX), the supernatant was collected and treated with an enzyme to release free MMAE. The sample was crashed with acetonrile before the organic fraction was collected and diluted for analysis. 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 718 m/z ion with an internal standard of MMAF (925 m/z). The chromatography was performed with a Kinetix XB-C18 Column (1.7 µm, 50 x 2.1 mm).



MMAE cytoxic payload conjugated through cysteine residues

Time (days)	Adjusted amount of MMAE (ng)
0	55.7 ± 2
1	197 ± 2
2	281 ± 2
5	529 ± 4
7	645 ± 12

Table 1: Adjusted MMAE concentration
50 µL of sample were injected on column after a 10x dilution

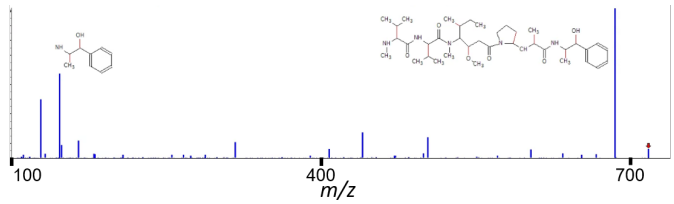


Figure 1: MSMS fragmentation of MMAE payload
ion 686 m/z was used as the quantifier ion with 152 m/z as the qualifier.

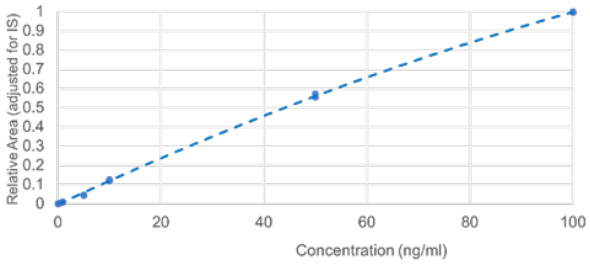


Figure 2: Calibration curve of MMAE over four orders of magnitude
r2 was >0.99 with an internal standard of MMAF.

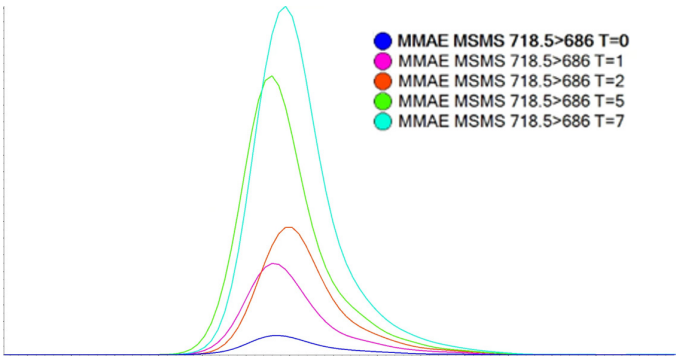


Figure 3: XIC of MMAE payload ion 686 m/z
Intensity of peak is increased over time as payload is lost during incubation in plasma.

The payload was observed in a single peak eluting at 1.25 minutes on a five-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.

A calibration curve was generated using SCIEX OS software and plasma incubated samples were compared to this.

Figure 3 shows the XIC peak area of MMAE over the five time points. We can observe an increase in response over time due to loss of the linker by enzyme activity in the plasma. The peak areas were compared to the calibration curve to give the values shown in table 1. Amounts of free drug over time track well with our previous application note measuring bound drug concentration in plasma (AN-007) and track with 75% loss of drug over seven days.