Peptide digestion analysis of an antibody drug conjugate

Trypsin digestion of a trastuzumab val-cit PABC MMAE conjugate, analysed using an IDA MSMS methodology

ADCs can be produced with a variety of different linker chemistries between the payload and the protein. One popular conjugation method utilises partial reduction of the cysteines present in interchain disulfides to create free thiols for addition of the cytotoxic molecules. The use of tryptic digestion allows these modifications to be localised on the particular amino acids to ensure the correct cysteines have been conjugated. High-resolution mass spectrometry (MS) can determine fragmentation events from both the payload, and peptide backbone for assignment of modified peptides.

Here we outline an analysis strategy to determine the conjugate locations of a trastuzumab val-cit PABC MMAE conjugate (DAR 4.5) after a trypsin/Lys-C (Promega) digestion. MS conditions were optimised for conjugate retention in source. All data was collected on a SCIEX X500B mass spectrometer with an EXION HPLC. The chromatography was performed with a bioZen XB-C18 Column (1.7 µm, 150 x 2.1 mm).

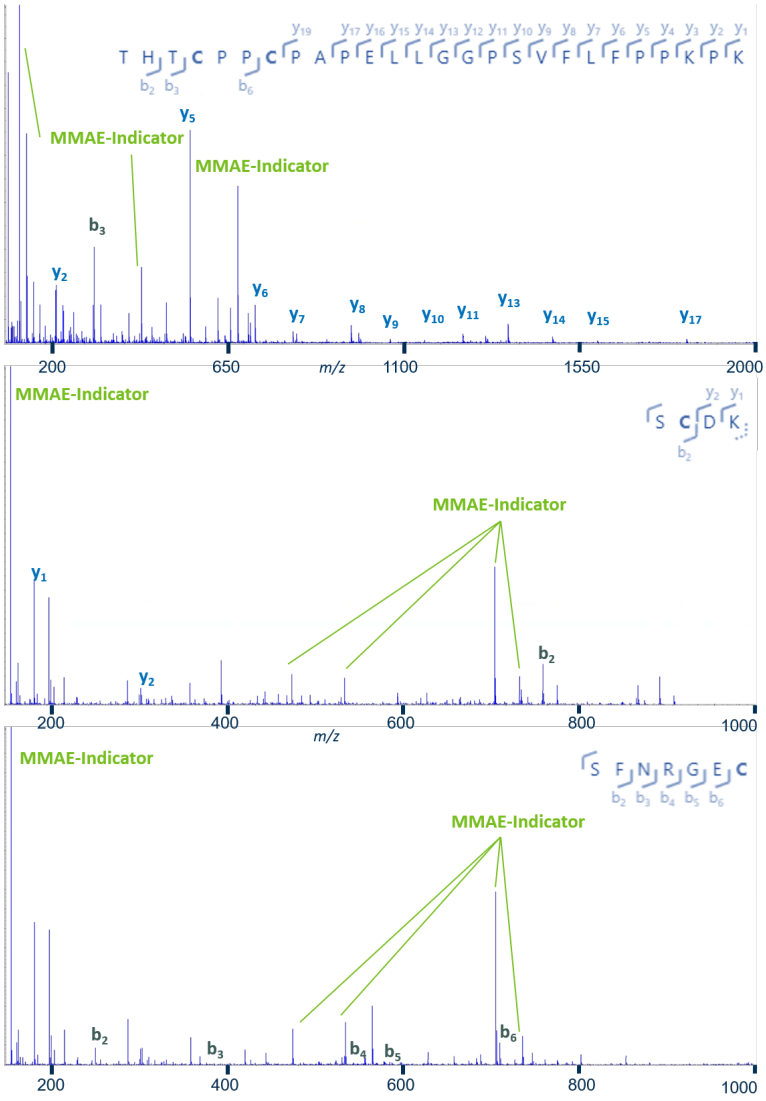
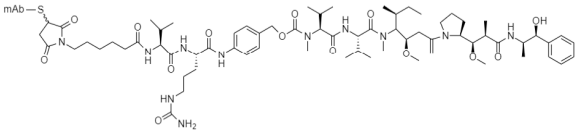


Figure 1: MSMS data from three peptides with a conjugation present on the Cysteine. Due to the addition of the hydrophobic payload, conjugated peptides elute late on the gradient between 57 and 70 minutes. The conjugated side chain is commonly lost during CID making assignment of full b and y series difficult.



MMAE cytotoxic payload conjugated through cysteine residues

The sample was separated on a 70-minute gradient and analysed using BioPharmaView Flex. Figure 1 shows peptides SCDK, SFNRGEC, THTCPPCPAPELLGGPSVFLFPPKPK all present with val-cit PABC MMAE conjugated to their cysteines. Some in source fragmentation was observed with the loss of warhead with a mass loss of 848.75 m/z these were observed as separate peptides at the same retention time. No other cysteines sites were observed with the full conjugate or partial fragment. The hinge peptide was also observed with a single site conjugated, with the other bearing a carbamidomethyl after alkylation.

Due to fragmentation of the small molecule conjugate during collision induced dissociation (CID) when a conjugate is present indicator ions for the payload have been used to strengthen MSMS scoring in addition to the b and y ions. For longer peptides such as the missed cleavage SCDKTHTCPPCPAPELLGGPSVFLFPPKPK when two conjugates are present it can be difficult to assign them to particular residues. The peptides SFNRGEC and SCDK were both observed with high levels of conjugation >95%. The hinge region peptide THTCPPCPAPELLGGPSVFLFPPKPK was observed at 64% intensity with two conjugates present and 5% with a single conjugate and an alkylation. Larger missed cleavages were present at low abundance, however not used in calculations.