Determination of Average DAR of an Antibody Drug Conjugate (ADC) Mimic using a bioZen™ 1.8 µm SEC-2 Column

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Overview

Antibody drug conjugates (ADC) are a rapidly expanding field in the biopharma industry using the specificity of antibodies to deliver cytotoxic payloads. Their appeal lies in the ability to target and kill tumour cells without compromising healthy ones. A stable link between the antibody and the drug is crucial and drugs linked to antibodies through cysteine residues provide a very reliable and reproducible strategy for achieving an even Drug Antibody Ratio (DAR), something which is essential to ensure good immunogenicity and low patient toxicity. Cysteine linked ADCs provide a specific challenge as the disulphide bonds used to hold the protein together are broken making the complexes unable to withstand the conditions of typical reversed phase LC-MS. The protein in this form is held together only through non-covalent interactions and dissociates under reversed phase conditions. Other analytical approaches to these compounds include subunit analysis of ADCs or HIC, however each of these also have their own analytical shortcomings. In this application note we demonstrate that if kept under native conditions it is possible to get Average DAR of a cysteine conjugate. This application note serves to highlight how native MS can be used to get intact mass for non-covalently linked large molecules, including cysteine linked ADCs.

We analyzed a commercially available cysteine linked ADC mimic obtained from Sigma and were able to identify DAR 0 through 8 using the bioZen SEC-2 in tandem with the SCIEX® X500B.

Using XIC (**Figure 1**) each drug antibody proteoform was used to calculate DAR. The average DAR was calculated to be 3.4 which corresponded to the reported Sigma value of 4.0 ± 0.8. We see under 'native' conditions (**Figure 2 and 3**) the ADC remains intact allowing distinct DAR species to be observed and upon reconstruction DAR and glycoforms can be labelled.

LC Conditions

Column:	bioZen 1.8 µm SEC-2
Dimension:	150 x 4.6 mm
Part No.:	00F-4769-E0
Recommended Guard:	SecurityGuard™ ULTRA
Guard Cartridge Part No.:	AJ0-9850
Guard Holder Part No.:	AJ0-9000
Mobile Phase:	100 mM Ammonium Acetate
Flow Rate:	200 µL/min
Temperature:	25 °C
Detector:	QTOF (SCIEX X500B)
Sample:	Sigma ADC Mimic (MSQC8), 100 µg

Figure 1

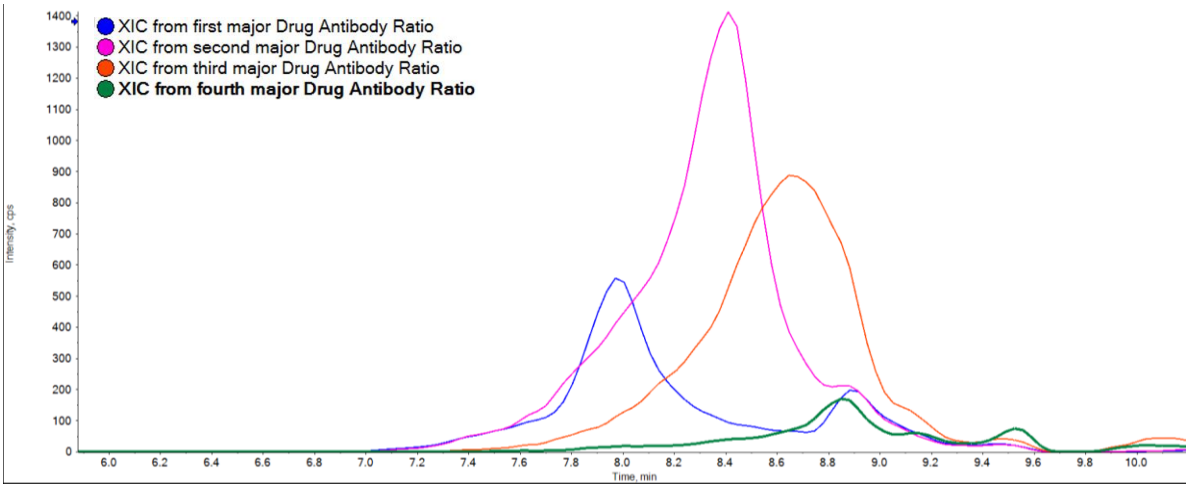

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Figure 2

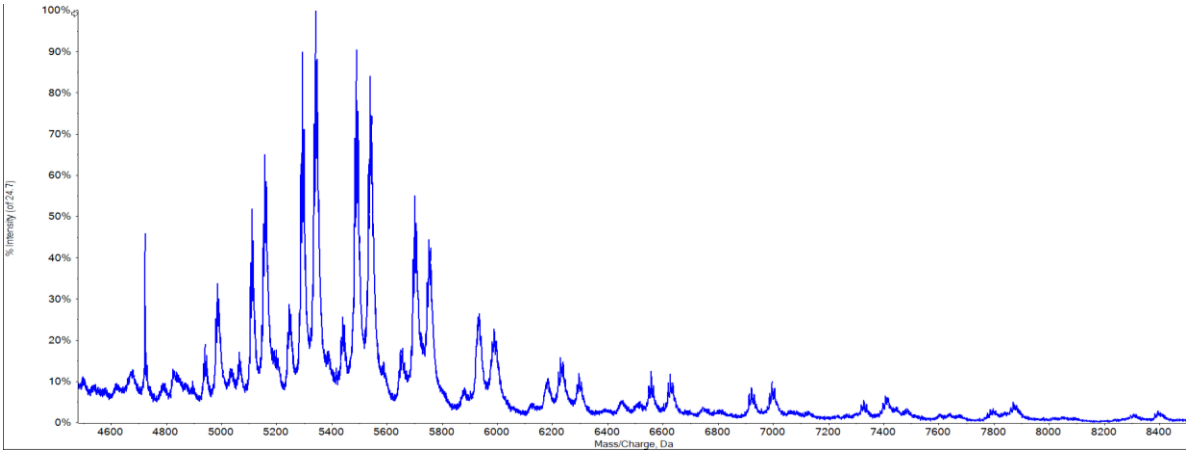

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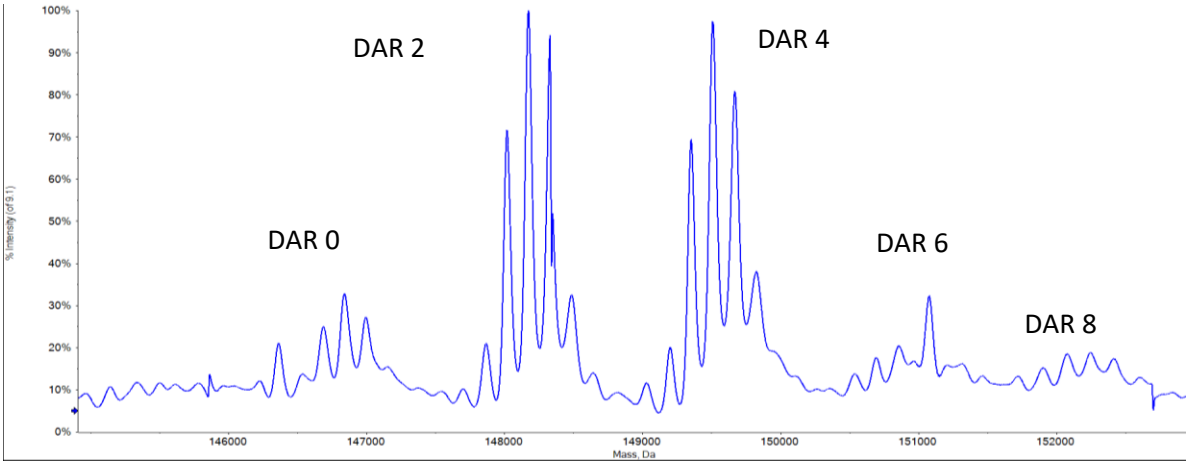
Figure 3



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