

Intact Analysis of Antibody Drug Conjugates

Trastuzumab Emtansine Analysis using Benchtop X500B QTOF Mass Spectrometer

Wen Jin¹, Suya Liu¹, Doug Simmons¹, Ian Moore¹, Sean McCarthy² and Sibylle Heidelberger¹
 71 Four Valley Dr. Concord, ON L4K 4V8, Canada
 500 Old Connecticut Path, Framingham, MA, 01701, USA

Introduction

Antibody-drug conjugates (ADCs) are an emerging class of biotherapeutics. They offer the specificity of monoclonal antibodies while incorporating cytotoxic payloads to efficiently target and kill infected cells. By their nature, ADCs are highly complex as they use an antibody backbone which must be well characterized. Adding to this complexity is conjugation of the cytotoxic payloads or drugs to the antibody. The number of drugs attached to the antibody has been shown to impact the safety and efficacy of the resulting ADC, and as such, must be characterized and monitored through development. [1-3]

Presented here is a streamlined approach for the analysis of ADCs to rapidly and accurately calculate the drug-to-antibody ratio (DAR). We will discuss the use of the new, compact X500B QTOF, powered by SCIEX OS, with data processing using BioPharmaView™ 2.0.1 for routine characterization of ADCs and calculation of DAR using both the glycosylated and deglycosylated forms.

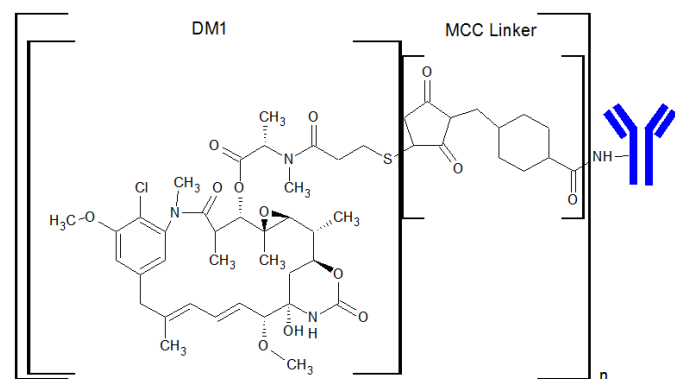


Figure 1: Trastuzumab emtansine. T-DM1 consists of a trastuzumab backbone with MCC linker binding DM1, a cytotoxic drug to the antibody via lysine residues.

Experimental

Samples were prepared either neat for the glycosylated form or using PNGase F (New England BioLabs (Ipswich, MA, USA) using their standard protocol.

LCMS analyses were conducted using a benchtop X500B QTOF mass spectrometer using SCIEX OS equipped with an Exion LC™ system. Table 1 lists the LCMS conditions used in these analyses. Data was processed using BioPharmaView™ for reconstruction of the intact protein and calculation of DAR.

Table 1 Exion LC™ conditions

Column	Agilent Poroshell 300SB-C8 1.0 x 75mm 5 μm
Mobile phase A	0.1 % Formic acid in water
Mobile Phase B	0.1% Formic acid in acetonitrile
Flow rate	0.2 mL/min
Column Temperature	75 °C

Table 2 X500B mass spectrometry conditions

Source parameters:	
CUR	30
GS1	50
GS2	50
Ion Spray Voltage	5000 V
Source Temperature	400°C
TOFMS mass range	900 – 4000 m/z
DP	250 V
Accumulation time	0.5 s
Time bins to sum	80
Intact Protein Mode (IPM)	On
Large Proteins (>70kDa)	On
Decrease Detector Voltage	On
DP	250 V

Results and Discussion

Glycosylated T-DM1

For this study, we used trastuzumab emtansine (T-DM1), a lysine conjugated ADC for the treatment of HER-2 positive metastatic breast cancer. T-DM1 is comprised of an antibody, trastuzumab, covalently linked via lysine residues to cytotoxic drug molecules which are liberated following internalization by target cells (Figure 1). As drug molecules are attached to the antibody following expression, assessment of the drug-to-antibody ratio (DAR) must be determined regularly as part of the drug development process.

We began our study by determining the DAR of the intact ADC. As shown in Figure 2A, the resulting raw spectrum is highly complex. Using the BioPharmaView reconstruction algorithm, we generated high quality reconstructed spectra clearly showing the different DAR species as well as the glycoprofile for each DAR. The resulting reconstruction of the raw data gives us a range of between 0-8 drugs attached to the trastuzumab (Figure 2B). As expected, the glycoprofile for each DAR is consistent across each of the DAR species.

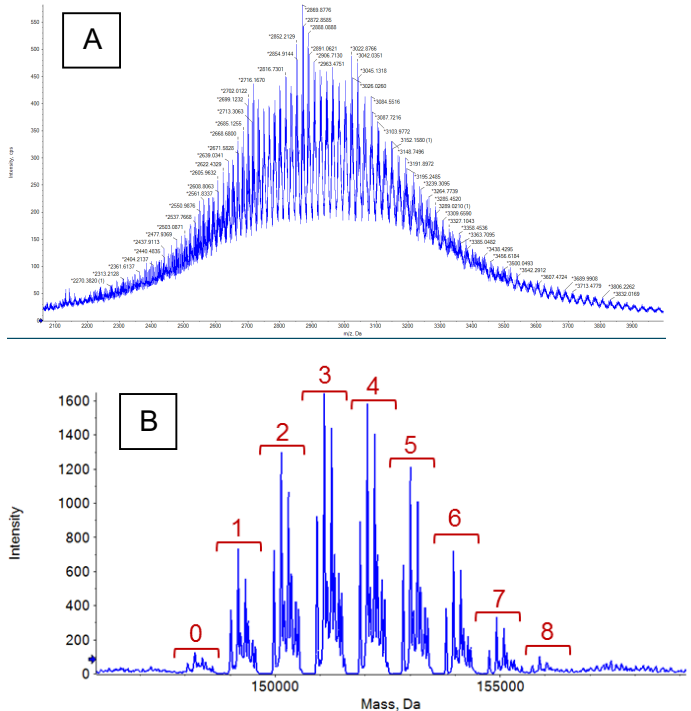


Figure 2: T-DM1 raw (A) and reconstructed (B) data. Raw data (2A) includes glycosylations and ADC which increases the normal complexity of an antibody spectrum while 2B shows the reconstruction using BioPharmaView and calculating 0-8 drugs attached to the trastuzumab.

Looking closely at each reconstructed DAR separately, we clearly see evidence for each of the main glycoforms as well as a corresponding set of species with a mass shift of 221 Da and not 219 Da which would be the mass of the linker itself. (Figure 3). This has previously been reported to be due to a reaction with lysine residues, which results in chemical crosslinking. The initial reaction of trastuzumab to the MCC linker produces an intermediate which, in some cases, due to the proximal location of lysines involves a second reaction causing the inter-chain cross linking and a linker species of 221 Da without the DM1. [2]

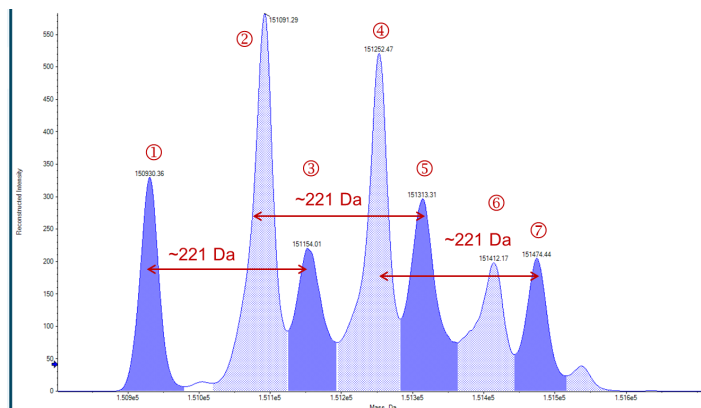


Figure 3: Trastuzumab emtansine with 3 drugs attached. A closer look at the reconstructed DAR shows evidence for the main glycoforms: 1 G0F/G0F, 2: G0F/G1F, 3:G1F/G1F and 4: G1F/G2F with corresponding additions of one MCC linker without DM1.

Using BioPharmaView, (DAR) can be calculated based on the reconstructed peak area distribution of the different drug conjugated antibodies. DAR was calculated to be 3.49 (Figure. 5), which agrees well with previously reported value of 3.5 [1-3].

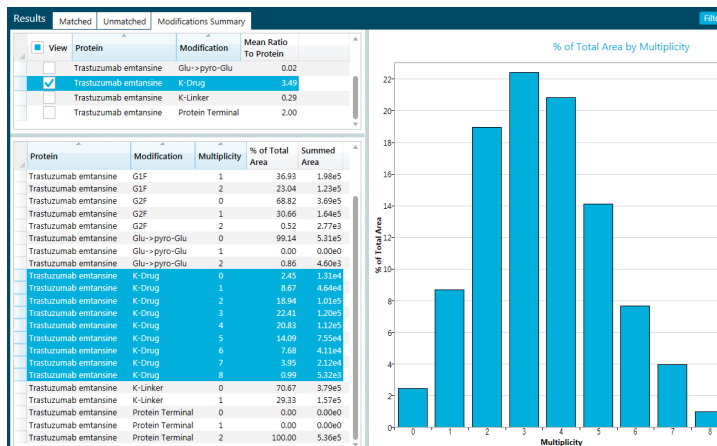


Figure 4: Calculation of DAR for trastuzumab emtansine using BioPharmaView™. Calculation of DAR using the software gives a simple table and graph, showing the median multiplicity of the ratio. Highlighted in the top right is the ratio calculated which was 3.49.

Deglycosylated T-DM1

We then removed the N-linked glycans using PNGaseF to provide a spectrum with reduced complexity as the peaks attributed to the glycoforms would be removed. As shown in Figure 5A, we can clearly see that the glycoform complexity has been reduced. In addition, the presence of the species with a 221 Da higher than each corresponding DAR species is evident confirming our previous findings.

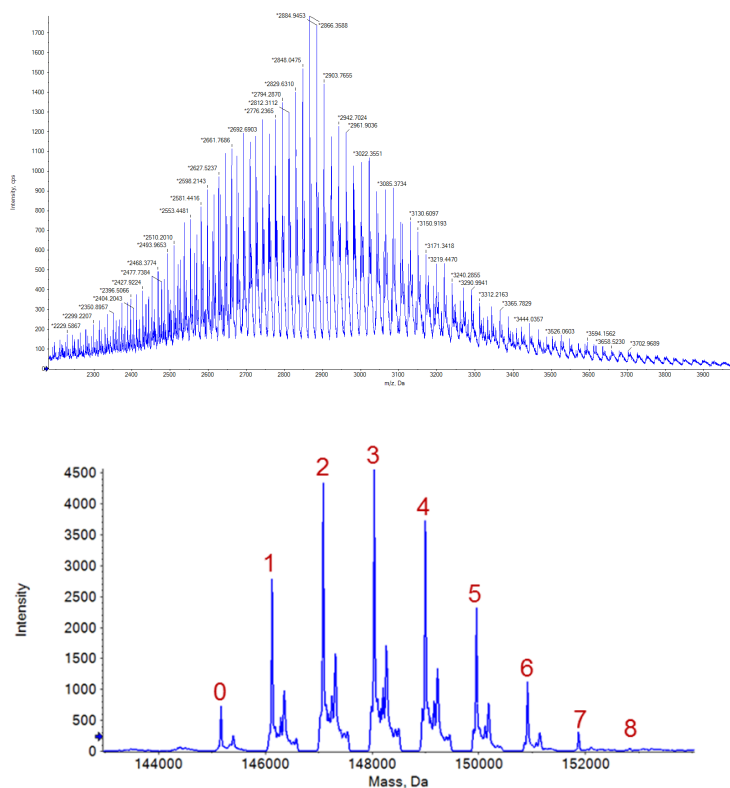


Figure 5: Deglycosylated T-DM1 raw (A) and reconstructed (B) spectrum. Raw data (5A) from the deglycosylated is used to reconstruct the intact protein mass (5B).

The reconstructed spectrum for the deglycosylated trastuzumab emtansine confirms the results observed in figure 2 of the glycosylated form. Both confirm from 0 – 8 drugs attached to the trastuzumab backbone. DAR calculations were performed using the software, and the resulting DAR value is consistent with previously reported values. [2]

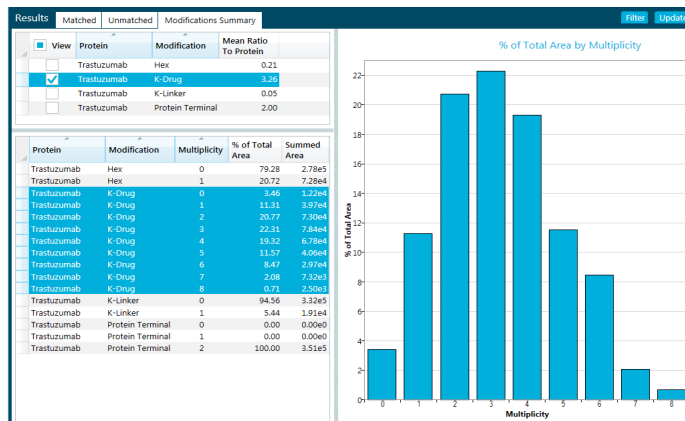


Figure 6: DAR calculation for deglycosylated trastuzumab emtansine. The 8 drug conjugates (highlighted bottom left) are then used to calculate the DAR ratio (highlighted top left) to give a DAR ratio of 3.26.

Conclusion

We have shown that the benchtop X500B QTOF system produces data with such quality that it can be used for routine analysis of such complex biologics such as ADCs which require high resolution data to identify glycosylations and the number of drugs bound to the antibody. We have shown that the mass accuracy is such that we are able to identify a prominent 221 Da mass shift that has been reported by Jacobson et al as a conjugation of the trastuzumab-MCC linker intermediate with a proximal lysine residue. Processing of the data and reconstruction was performed using BioPharmaView resulting in fast reconstruction and accurate DAR calculations. The resulting software was able to calculate the DAR ratio to be 3.46 on the glycosylated form compared to the 3.5 listed in literature. [2]

References

- [1] Michael T. Kim, Yan Chen, Joseph Marhoul and Fred Jacobson. Statistical Modeling of the Drug Load Distribution on Trastuzumab Emtansine (Kadcyla), a Lysine-Linked Antibody Drug Conjugate. *Bioconjugate Chem.* 2014, 25, 1223–1232
- [2] Yan Chen, Michael T. Kim, Laura Zheng, Galahad Deperalta, and Fred Jacobson. Structural Characterization of Cross-Linked Species in Trastuzumab Emtansine (Kadcyla). *Bioconjugate Chem.* 2016, 27, 2037–2047
- [3] Liuxi Chen, Lan Wang, Henry Shion, Chuanfei Yu, Ying Qing Yu, Lei Zhu, Meng Li, Weibin Chen, and Kai Gao. In-depth structural characterization of Kadcyla (ado-trastuzumab emtansine) and its biosimilar candidate. *MABS*, 2016, VOL. 8, NO. 7, 1210–1223

AB Sciex is doing business as SCIEX.

© 2018 AB Sciex. For Research Use Only. Not for use in diagnostic procedures. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.

Document number: RUO-MKT-02-5468-C