Monitoring the Critical Quality Attributes of Antibody Drug Conjugates (ADCs) as Part of Biosimilar Development: Case Studies of ado-trastuzumab emtansine

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INTRODUCTION

The recent clinical success of antibody-drug conjugates (ADCs) has invigorated research in the biotherapeutic field.[1] A broad variety of methods to functionalize antibodies with various payloads are currently available. The conjugation methods can influence the sample heterogeneity, and therefore impact the pharmacokinetic, safety and therapeutic efficacy of the product. As a result, it has become evident that quality attributes such as the site of modification and the drug-to-antibody ratio (DAR) need to be controlled to meet more stringent requirements for medical applications. In this study, we investigated the utility of an integrated high resolution analytics platform, consisting of a new IMS Q-Tof mass spectrometer and a targeted informatics system, to understand the critical quality attributes of Lysine-conjugated ADCs.

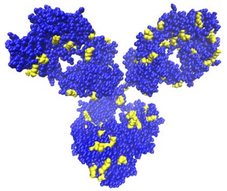


Figure 1. Surface exposed lysine residues on IgG1.

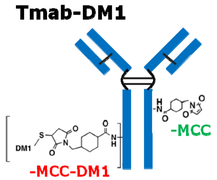


Figure 2: ADC Tmab-DM1 Structure Illustration

METHODS

Liquid Chromatography

System: ACQUITY UPLC H-Class Bio System

Detector: ACQUITY UPLC Tunable UV (TUV)

Column: ACQUITY UPLC Protein BEH C4, 300Å, 1.7 µm, 2.1 mm x 50 mm and ACQUITY UPLC BEH300 C18, 300Å, 1.7 µm, 2.1 mm x 100 mm

Mass Spectrometry

Mass Spectrometer: Xevo G2-XS QTof and Vion IMS QTof

Acquisition mode: MS full scan for intact mass analysis and DDA and MS^E for Peptide mapping

Informatics

UNIFI Scientific Information System (Waters Corporation)

Data Analysis Type in UNIFI:

1. **Intact Mass Analysis with automatic DAR calculation MS-RT window based workflow**
2. **Peptide Mapping (MS^E and DDA) workflow**
3. **Accurate Mass Screening (MS^E) workflow**

Sample Information

Multiple Antibody-Drug-Conjugates (ADCs) samples (Tmab-DM1, Figure 1, 2) were deglycosylated before intact mass analysis for DAR determination. The samples were denatured, alkylated, and digested by Trypsin and Asp-N endoproteinase for peptide mapping with Leucine enkephalin (LeuEnk) was added to each sample at a final concentration of 50 fmol/µl as an internal standard.

Figure 3. UPLC H-Class Bio with Xevo G2-XS system and the Vion IMS QTof system controlled by UNIFI Informatics software. Both systems provide a single platform for instrument control, data processing and reporting.

RESULTS AND DISCUSSION

DAR Measurement on Intact ADC

The distribution of the drug load is determined by MS intact analysis. The deconvoluted mass spectra contain 9 major peaks with mass difference of 958 Da between adjacent peaks, which is in agreement to the mass of covalently linked DM1 drug with one MCC linker. In both the innovator and candidate biosimilar ADCs, major peaks correspond to Tmab with 0-8 DM1 drug and linkers respectively (labelled as +0 drug, +1 drug, etc). The less abundant peaks (labelled as *) right next to the major peaks with 221 Da, which attributes to the unreacted linkers that modified the antibody but do not react with DM1.

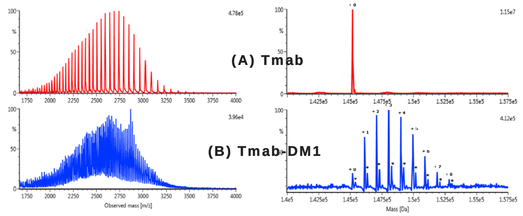


Figure 4. Combined raw spectra and deconvoluted spectra for Tmab(A) and Tmab-DM1 (B).

ADC Peptide Analysis Workflow

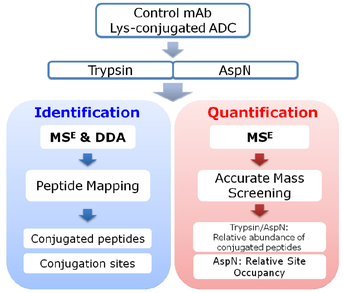
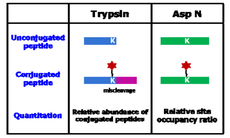


Figure 5. ADC Peptide level analysis identification and the quantification workflows. Lysine-conjugated ADC and unconjugated control mAb were digested by trypsin and Asp N respectively, followed by MS^E and DDA modes. UNIFI Peptide Mapping workflow was used to identify the conjugated peptides and pinpoint the conjugation sites. The same set of MS^E data were further analyzed using Accurate Mass Screening workflow in UNIFI to quantify the relative site occupancy and relative abundance of conjugated peptides across different samples.

Table 1. The enzyme of choice for different quantification purposes. Trypsin digest was used to calculate relative abundance of conjugated peptides, while Asp-N digest was used to determine the relative site occupancy of individual site.



Conjugation Site Identification

Figure 6. LC/MS^E chromatogram (BPI) of tryptic peptide mapping analysis for Tmab vs Tmab-DM1 in comparison mode.

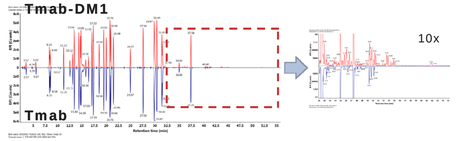


Figure 7. LC/MS^E chromatogram (BPI) of Asp-N peptide mapping analysis for Tmab vs Tmab-DM1 in comparison mode.

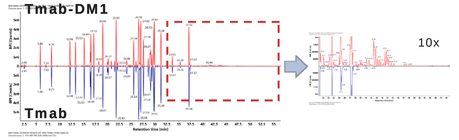
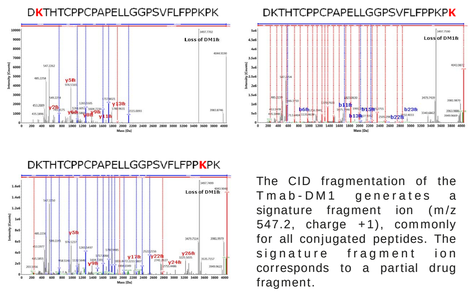


Table 2. Numbers of conjugation sites identified in different regions of Tmab using DDA and MS^E methods.

Region	Total # of Lys	Trypsin		Asp N	
		DDA	MSE	DDA	MSE
Variable Fab	12	9	10	6	8
Constant Fab	14	13	13	9	7
Fc	20	16	17	4	6
Total	46	38	40	19	21

Figure 8. MS/MS spectra to confirm conjugations sites for positional isomers for Asp-N peptide ^{224}DKTHTCPPCPAPELLGGPSVFLFPPKPK^{251}



The CID fragmentation of the Tmab-DM1 generates a signature fragment ion (m/z 547.2, charge +1), commonly for all conjugated peptides. The signature fragment ion corresponds to a partial drug fragment.

Relative Site Occupancy Quantification

UNIFI Accurate Mass Screening Workflow enables the site occupancy quantitation of ADC peptides (from AspN digestion).[2]

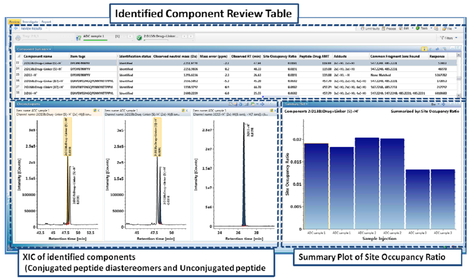


Figure 8. Site occupancy quantitation and cross sample comparison (3 ADC sample with duplicated injections were compared).
—> Peptide 2:D13 with 0 and 1 conjugation was illustrated here. The site occupancy ratio was automatically generated during the data processing. The diastereomeric peaks are combined in the relative% calculation. Trending plot is displayed for 2:D13 peptides across multiple ADC samples. These ADC samples were prepared via different conjugation methods.

CONCLUSIONS

1. For Tmab-DM1, 80 out of 92 conjugation sites were observed.
2. UNIFI provided automated workflow for:
   - In-depth primary structure characterization of lysine-conjugated ADC, including automatic DAR calculation.
   - Site specific localization of ADC conjugation (Peptide Mapping Workflow).
   - Quantification of relative site occupancy (Accurate Mass Screening Workflow).
   - While this presentation has focused on lysine-conjugated ADCs, these UNIFI workflows are directly applicable to other classes of ADC biotherapeutics.

Reference:

1. Wang L, Amphlett G, Blattler WA, Lambert JM, Zhang W, Protein Sci. 2005 Sep;14(9): 2436-46.
1. Chen L, Wang L, Shion H, Yu C, Yu YQ, Zhu L, Li M, Chen W, Gao K., MAbs. 2016 Jul 5:0. [Epub ahead of print]
2. Waters Application Note (PN = 720005603). "Automated Quantitative Analysis of Antibody Drug Conjugates Using an Accurate Mass Screening Workflow in the UNIFI Scientific Information System".

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