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

Liu Ying1, Min Du1, Henry Shao1, Ying-Qing Yu1, Lan Wang2, Kai Gao2, Weimin Chen1
1Waters Corporation, Milford, USA; 2National Institutes for food and drug control, Beijing, China.

INTRODUCTION

The recent clinical success of antibody-drug conjugates (ADCs) has ingratiated research in the biotherapeutic field. 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 QToF mass spectrometer and a targeted informatics system, to understand the critical quality attributes of lysine-conjugated ADCs.

METHODS

Liquid Chromatography

System: ACQUITY UPLC H-Class Bio System
Detector: ACQUITY UPLC Tunable UV (TUV)
Column: ACQUITY UPLC BEH300 C18, 300Å, 1.7 µm, 2.1 mm x 100 mm

Mass Spectrometry

System: Xevo G2-XS QTof
Detector: ACQUITY UPLC Tunable UV Operating modes: ECD and ESI
Column: ACQUITY UPLC BEH300 C18, 300Å, 1.7 µm, 2.1 mm x 100 mm

Mass Spectrometer: Xevo G2-XS QTof
Acquisition mode: MS2

Informatics

UNIFI Scientific Information System (Waters Corporation)

Data Analysis Type in UNIFI:
1. Peptide Mapping (MSE) workflow
2. Accurate Mass Screening (MSE) workflow

Sample Information

Multiple Antibody drug-conjugated samples (Tmab-DM1, Tmab-DM2, etc.) were digested by trypsin and Asp-N to digest each sample at a final concentration of 50 µM as an internal standard.

ADC Peptide Analysis Workflow

Figure 1. Surface exposed lysine residues on IgG1.

Figure 2: ADC Tmab-DM1 Structure Illustration

Figure 3. UPLC H-Class Bio with Xevo Q2-XS system controlled by UNIFI Informatics software.

DAR Measurement on Intact ADC

The distribution of the drug load is determined by MS intact analysis. The deconvoluted mass spectra contain 15 major peaks with mass differences of 572 Da between adjacent peaks, which is in agreement to the mass of cyclized linked DM1 drug with one MCC linker. In both the intact and conjugation site identification mode, 8 major peaks were quantified for Tmab with +0, +1, +2, +3, +4, +5, +6, +7 DM1 respectively (labeled as +0 drug, +1 drug, etc). The less abundant peaks right next to the major peaks with 219 Da, which attributes to the unreacted linkers that modified the antibody but do not react with DM1.

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

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

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

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

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.

Table 2. Numbers of conjugations sites identified in different regions of Tmab using HCD and MS2 methods.

Table 3. The ID and MS/MS data of tryptic peptides were used to identify the major conjugations sites. The site occupancy was determined by accurate mass screening workflow in UNIFI.

Relative Site Occupancy Quantification

UNIFI Accurate Mass Screening Workflow enables the site occupancy quantitation of ADC proteins (from Asp-N digests).

Figure 8: Site occupancy quantification and cross sample comparison (2 ADC sample with duplicated injections were compared). The data was further elaborated and illustrated here. The percentage of the least abundant site occupancy was calculated in the relative abundance calculation. The peak areas were corrected in the relative abundance calculation. The peak areas were corrected in the relative abundance calculation. The peak areas were corrected in the relative abundance calculation.

CONCLUSIONS

1. For Tmab-DM1, 80 out of 92 conjugation sites were observed.
2. UNIFI provided automated workflow for:
   a. Identification of ADC conjugations (Peptide Mapping Workflow).
   b. Quantification of relative site occupancy (Accurate Mass Screening Workflow).
3. While this presentation has focused on lysine-conjugated ADCs, these UNIFI workflows are directly applicable to other classes of ADC therapeutics.

Reference: