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Analysis of an Antibody-Drug Conjugate on a Novel Benchtop MALDI-TOF/TOF Platform

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Introduction

Antibody-drug conjugates (ADCs) have become one of the most promising therapeutic strategies for targeted cancer treatment, enabling the delivery of cytotoxic payloads via monoclonal antibodies (mAbs). Due to the heterogeneous nature of drug conjugation—primarily occurring at lysine or cysteine residues—accurate determination of the drug-to-antibody ratio (DAR) and drug distribution is essential for ADC development.

Established analytical techniques such as ultraviolet spectrophotometry, hydrophobic interaction chromatography (HIC), LC-MS, and ion mobility spectrometry provide reliable and well-validated methods for this purpose. However, these approaches can be time-consuming and complex. In contrast, MALDI-TOF MS offers a rapid alternative for antibody analysis, enabling faster DAR measurements compared to LC-MS.

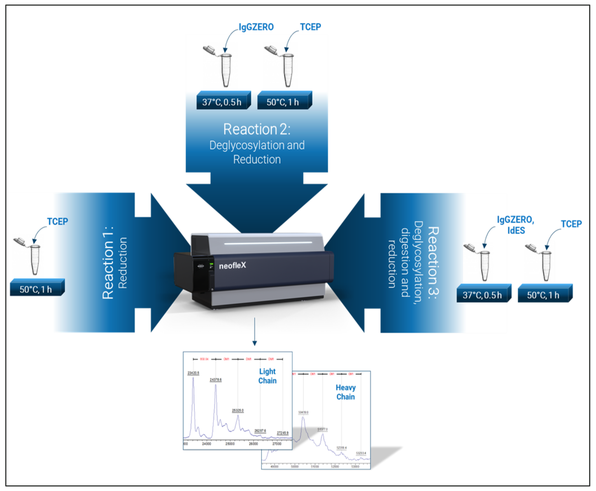

Fig. 1 Workflows for ADC analysis by MALDI-TOF

Methods

Ado-trastuzumab emtansine (KADCYLA®, Genentech) was processed using three workflows (Fig. 1) to analyze drug distribution across antibody subunits:

- W1: Reduction
- W2: Deglycosylation + Reduction
- W3: Deglycosylation + IdeS Digestion + Reduction

The ADC was filtered with a 30 kDa MWCO Amicon® ultracentrifugation filter and treated as follows:

- Deglycosylation: IgGZERO (1 Unit/µg protein), 37 °C, 30 min
- Digestion: FabRICATOR (IdeS) (1 Unit/µg protein), 37 °C, 30 min
- Reduction: 50 mM TCEP, 50 °C, 1 hr

IgGZERO and FabRICATOR were sourced from Genovis Inc. Samples were cleaned (C4 ZipTips®), eluted (30% acetonitrile/0.1% TFA), and spotted on a Bruker MALDI target with 2,5-DHAP. Spectra were acquired using a Bruker neofleX MALDI-TOF/TOF in positive linear mode.

Results

- Singly charged ADC subunits and fragments are analyzable by MALDI-TOF MS in positive linear mode.
- Light chain and heavy chain each conjugate with up to four drug moieties.
- Deglycosylation and reduction of ADC yields a consistent DAR_{avg} = 3.90

This study aims to develop a reliable and reproducible method for determining ADC DAR using MALDI-TOF MS. Unlike earlier studies that used MALDI-MS for rapid DAR assessment of intact ADCs, this approach enables the resolution of specific drug conjugation states.

As such, three workflows were tested to analyze distinct antibody subunits and identify a consistent DAR calculation approach. Each setup was evaluated using technical replicates from two independent sample spots.

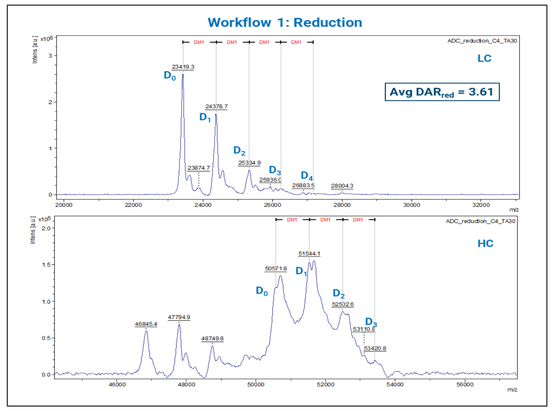

Fig. 2 Workflow 1 (W1) - Reduction of ADC

In W1(Fig. 2), the reduced heavy chain showed incompletely resolved glycosylated peaks, lowering peak area measurement accuracy and resulting in DAR_{red} = 3.61, at the lower end of reported values for trastuzumab-emtansine.

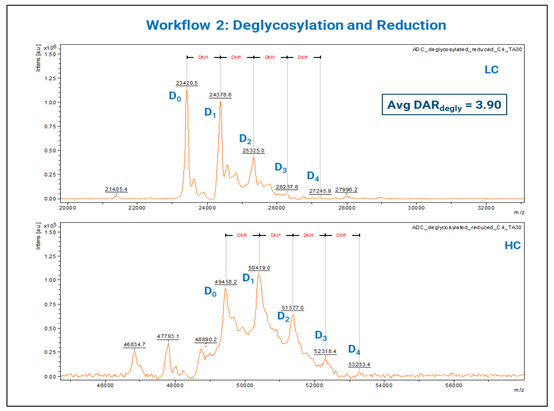

Fig. 3 Workflow 2 (W2) – Deglycosylation and Reduction of ADC

In contrast, W2, with both reduction and deglycosylation, produced well-resolved, highly reproducible peaks (Fig. 3). The resulting average DAR (DAR_{degly} = 3.90; technical replicates = 3.88, 3.92) closely aligned with the reported upper range values, supporting the robustness of this workflow for MALDI-TOF DAR analysis.

Lastly, W3 cleaved the ADC heavy chain into Fc/2' and Fd' fragments (Fig. 4), improving mass accuracy. However, an overlap between high DAR light chain species (D3, D4) with low DAR Fd' fragments (D0, D1) compromised accurate peak determination, making this method unsuitable for accurate DAR determination. Nonetheless, IdeS digestion may still benefit other ADCs with different payloads.

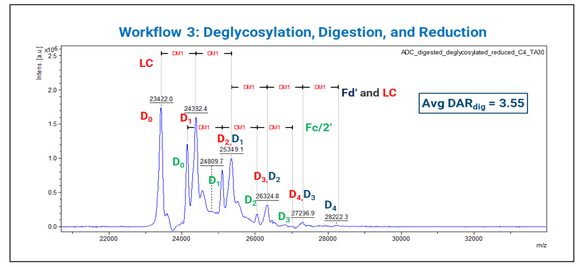

Fig. 4 Workflow 3 (W3) – Deglycosylation, Digestion, and Reduction of ADC

DAR values were calculated based on the peak area ratios of conjugated species, using the formula shown in Fig. 5.

$$DAR = \sum DAR\ of\ the\ nth\ species\ (Dn)\ X\ Peak\ area\ ratio$$

$$Peak\ area\ ratio = \frac{Peak\ area}{Sum\ of\ peak\ areas}$$

$$DAR_{red}\ or\ DAR_{degly} = 2\ (DAR_{LC} + DAR_{HC})$$

$$DAR_{dig} = 2\ (DAR_{LC} + DAR_{Fc/2'} + DAR_{Fd'})$$

Fig. 5 DAR calculations

Conclusions

- W2 (Reduction + Deglycosylation) is the most robust evaluated method for trastuzumab ADCs DAR calculations
- W2 measures accurate and reproducible DAR values with well-resolved peaks and aligns with theoretical expectations.
- Simple and rapid acquisition of ADC-DAR states via MALDI-TOF MS

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