ADCs' DAR Measurement

Analysis of Antibody-Drug Conjugates (ADCs)
By Intact Protein Analysis using Mass Spectrometry

Introduction

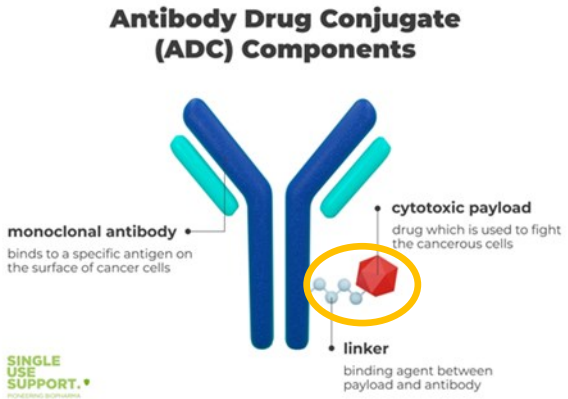
Antibody drug conjugates (ADCs) are a rapidly growing field of interest in Biopharmaceutics. Several ADCs are already successfully used in the treatment of myeloid leukemia or refractory metastatic breast cancer. An ADC is a protein — typically a monoclonal antibody (mAb) — covalently bound (conjugated) to a small-molecule drug using an ADC linker. In comparison to conventional chemotherapy treatments, ADCs have the potential to destroy cancerous cells without damaging healthy cells in the process.

Drug-to-Antibody Ratio (DAR) is a critical quality attribute (CQA) for ADCs because it directly affects their therapeutic efficacy and pharmacokinetics. Determination (and monitoring) of DAR is essential across the ADC development process and within commercial manufacturing operations. The ADCs structural analysis involves using liquid chromatography-mass spectrometry (LC-MS) to characterize the complex structure of an ADC molecule, including the antibody, linker, and cytotoxic payload, by evaluating parameters like drug-to-antibody ratio (DAR), conjugation site distribution, and potential impurities, to ensure its quality and safety for therapeutic use. LC-MS is the most commonly used technique for identification of different ADC species based on their mass-to-charge ratio and fragmentation patterns.
Our powerful technologies, including nano-UHPLC system and high-resolution mass spectrometry, are designed to identify the different ADC species present due to their intricate structure and potential heterogeneity.

Our analyses include, but are not limited to:

1) Analysis of intact protein mass and Drug-to-Antibody Ratio (DAR) (via Lys and Cys conjugated ADCs) by intact protein LC/MS

2) Verification of sequences of HC and LC, conjugation site(s) and glycan profile by peptide mapping



A diagram showing a typical ADC structure

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Specifications

- → **Method –** Intact protein mass spectrometry analysis
- → **Key Instrument –** Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer, Thermo Scientific™ Vanquish™ Neo UHPLC System
- → **Turnaround Time –** Typically, reports will be available within 5 business days of receipt of samples
- → **Mass Specification –** Mass Accuracy: < 20 ppm; Mass Range: 40 to 8000 m/z
- → **Acceptable Samples –** Purified protein in solution or dried (>10 µg/sample, purity >90%)

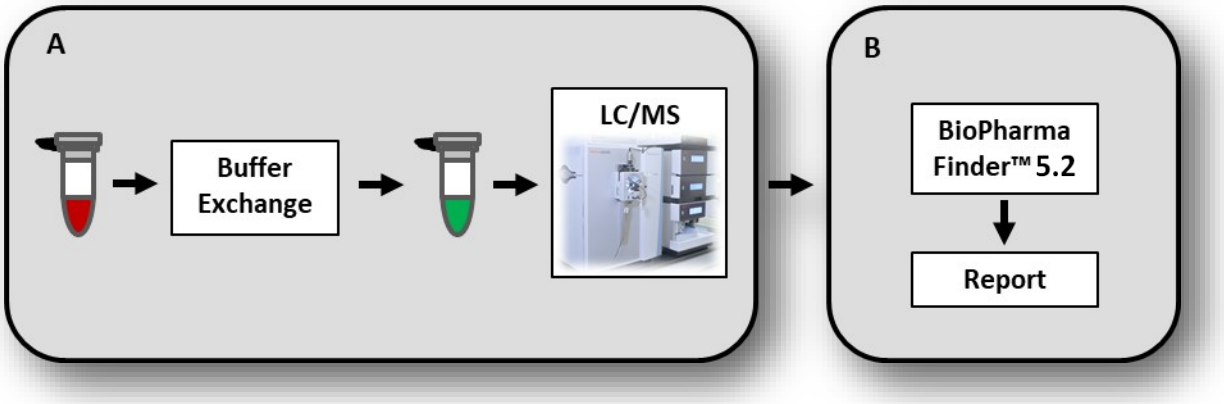

Figure 1:

A) Workflow for intact protein mass spectrometry analysis. *Note: LC/MS = liquid chromatography and mass spectrometry.*

B) Analytical approach used for mass analyses of datasets.

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Data and Results Example:

DAR Analysis of a mAb ADC Sample

Measured Average DAR = 1.99

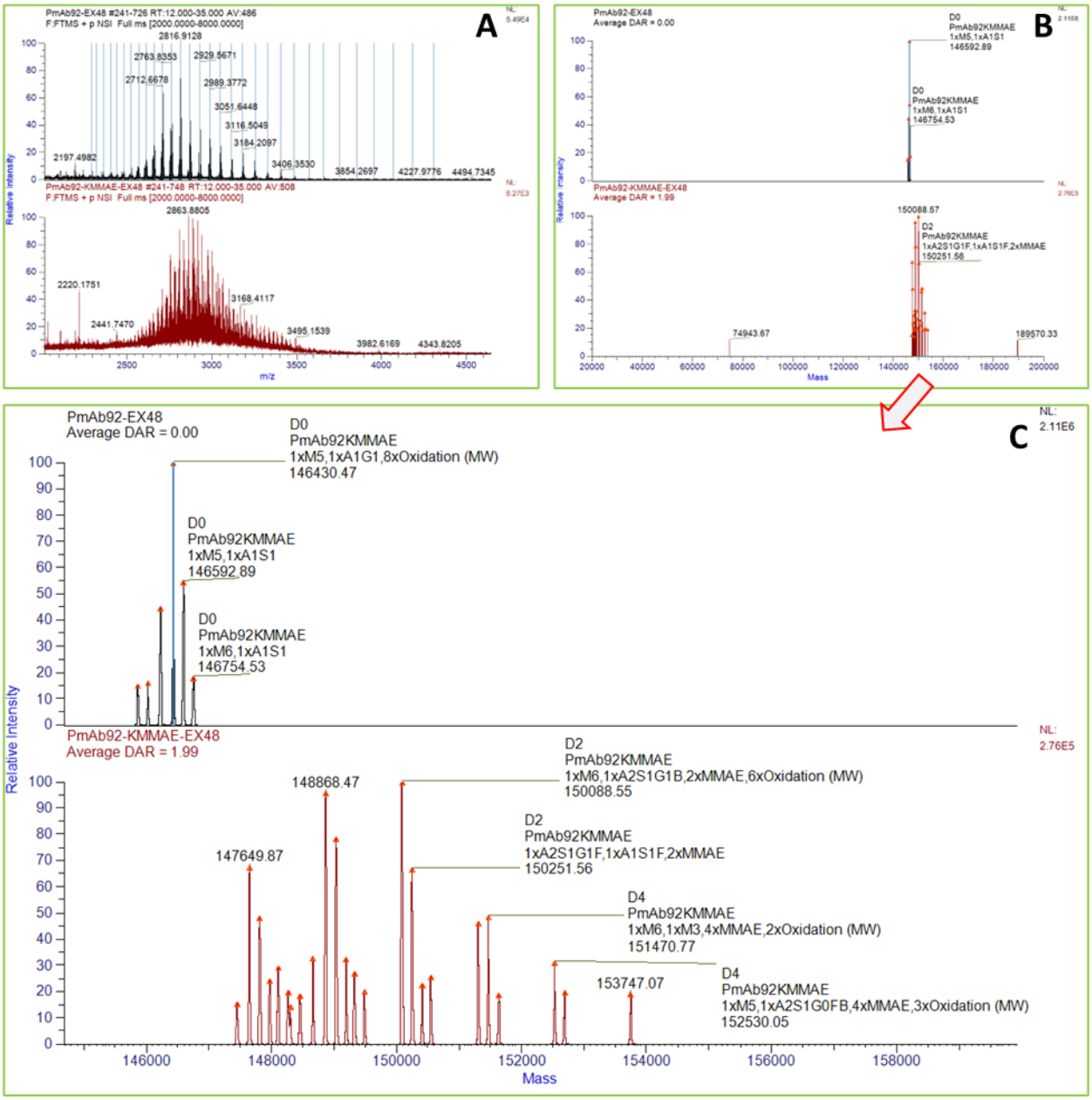

Figure 2: Analysis of ADCs by Native intact LC-MS method. Native intact LC-MS analysis of native unmodified PmAb-92 and modified PmAb-92 by Lysine-linked MMAE. (A) Intact MS spectrum including all DAR forms (DAR 0-8). (B) Deconvoluted Spectrum range from 20 kDa to 200 kDa showing the high purity of the samples and different forms detected. (C) Enlargement of Deconvoluted Spectrum of B to the MW range of 143 kDa to 160 kDa, showing the major forms of lysine-linked ADCs (MMAE) and glycan forms detected. *Note: upper panel = unmodified; lower panel = MMAE modified.* **The DAR of the PmAb92 with Lysine-linked MMAE is 1.99.**

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