Analysis of non-deglycosylated antibody-drug-conjugates by TripleTOF® high resolution quadrupole-time-of-flight instrument and effective reconstruction software

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Introduction

Antibody drug conjugates (ADC's) are an important class of biotherapeutic compounds delivering a targeted, usually cytotoxic drug selectively to the target cell. Lysine –linked ADC's can be very complex with multiple payloads conjugated to the same biotherapeutic protein. The drug-antibody-ratio (DAR) needs to be characterized and monitored from batch-to-batch, to ensure safety and efficacy of the biotherapeutic. Analysis time bottleneck in a conventional set-up would be the deglycosylation step, which is usually required to eliminate complexity in the sample. Here we present data acquired from a non-treated ADC with multiple payloads, and N-glycosylation intact. Despite the complexity, reproducible analysis and processing was achieved. Differential ion mobility was used to further reduce the complexity and the background interferences.

Intact protein analysis for antibodies and ADCs is a rapid method for global observations of changes to product from lot-to-lot. Intact protein analysis is used to control mass of complete protein product, (i.e. to discover possible clipping or truncation), glycosylation heterogeneity, and to assess the DAR of ADCs. These aspects should be vigorously controlled, as biological therapeutics are produced in cells, and glycosylation patterns may vary due to changes in production. The drug conjugation efficiency should also be assessed to ensure safety and efficacy.

Reproducible, reliable and trustworthy data and processing are the most important factors to assess the information from intact protein analysis.

Lysine linked ADC's are a common class of ADCs, with a high number of possible conjugation sites in the structure (IgG amino acids sequence typically has more than 70 Lys –residues). Monitoring and characterization of the DAR calls for strong data quality and confidence in data processing, with automated calculations to allow fast decision making on the quality of the product. The tools allowing for good quality spectra and automated batch processing in the biopharmaceutical industry allow not only for the sample preparation time to be reduced, but also to allow the response time to the synthetic chemist department to be reduced. The speed of the analysis would also allow for the opportunity to look at more synthesis options in the same timeframe, and therefore faster synthesis method optimization.

Total analysis time depends on the sample preparation, the simpler this can be made, the more samples can be analyzed in a reasonable time frame. To reduce the sample preparation time high data quality, and orthogonal separation methods can be used to evaluate product complexity. Differential ion mobility has been shown to effectively reduce the data complexity and to separate features of different sizes and mobility. Here, the interferences in the spectra were separated from the ADC of interest.

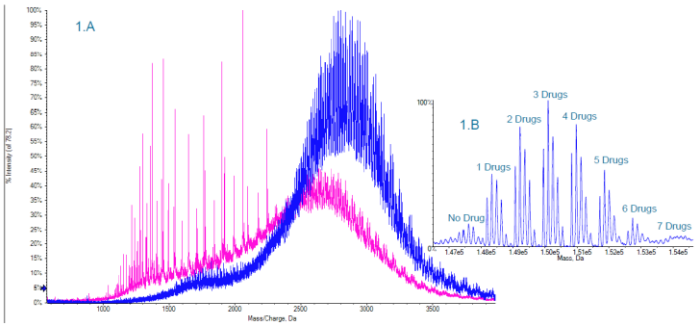

Figure 1 (A) comparison of raw spectra from the chromatographic system 1: reverse phase separation. In Blue: spectra with SelexION® technology cell optimized for the transmission of ADC ions. In Pink: no differential mobility separation applied. (B) Reconstructed spectrum of the SelexION technology-acquired data.

Experimental

Lysine-linked ADC was kindly provided by Sanofi-Vitry (France). The amino acid sequence and drug moiety structure are proprietary information.

The ADC sample was analyzed using TripleTOF 5600 coupled to Shimadzu Nexera UHPLC, by two different chromatographic set-ups:

1. Agilent SB C-8 (5µm 1mmx75mm). Gradient with 20 min run time, 200 µL/min flow rate. Solvents A: 0.1% formic acid (FA) water; B: 0.1% FA Acetonitrile.
2. Waters Acquity UPLC BEH 200 SEC (1,7µm 4,6mmx300mm). Gradient with 35 min run time, 300-400 µL/min flow rate. Solvents A: Ammonium formate 25mM, 1% FA; B: Acetonitrile. Column temperature 60 ºC in both cases. 5-10µg of ADC loaded on column per run.

SelexION® DMS technology was used in chromatographic system 1. Data was acquired with a TOF-MS scan over the mass range (m/z = 400-4500).Peak reconstruction was performed using BioPharmaView™ software.

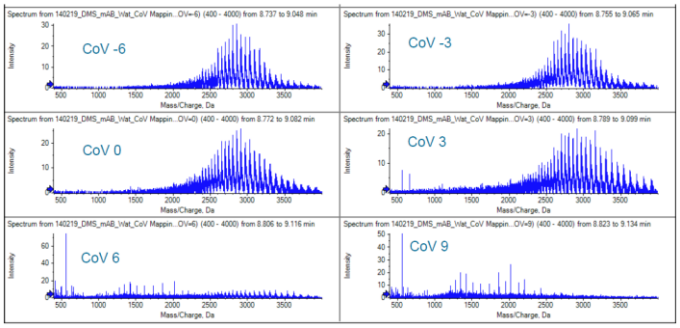

Figure 2 Optimization of the SelexION technology compensation voltages (CoV) on a monoclonal antibody (mAb). Panes have been marked with the applied CoV (here shown from -6 to 9). The separation voltage was optimized to 3500V. For large proteins such as mAbs, the transmission optimum was found with negative CoVs; for the smaller proteins (Ab light chain) and interferences, the transmission optimum was found with positive CoVs.

Results

In the initial (higher through-put) RP (C-8) set-up with SelexION differential mobility separation technology was used to reduce background interference to allow for cleaner spectra and better processing (Figure 1). The signal from the light chain (MW around 25kDa) and the formulation background interference was separated from the signal of the ADC (MW around 150 kDa) by separation voltage (SV) of 3500 V and compensation voltage (CoV) of 3. The SelexION parameters were optimized by multiple injections to ramp through compensation voltages from -12V to +12V, with the lower molecular weight features having transmission optimum at the higher end (6-12V) and the higher molecular weight features having their transmission optimum at lower CoV values (-3 - 0) (Figure 2).

The second chromatographic set-up, with size exclusion type separation, allowed for reconstruction quality spectra to be acquired in normal mode with the TripleTOF system (Figure 3).

Data processing was achieved in BioPharmaView™ software which employs maximum entropy reconstruction, here 100 iterations employed (Figure 4). Batch submission in BioPharmaView™ software demonstrated high level of reproducibility in both, analysis and reconstruction processing.

Non-deglycosylated ADC was identified to carry up to 7 payloads per antibody (Figures 4 and 5). The glycosylation pattern showed three glycan structures to be the most significant ones (combinations of G0F-G0F, GOF-G1F, G1F-G1F, G1F-G2F, G2F-G2F; Figure 4.). The raw data was complex, carrying heterogeneity from both, the drug conjugation and the glycosylation. The charge state envelope showed highest intensity signal for charge states from about +40 to +65 in chromatographic system 1, and +35 to +60 in the chromatographic system 2.

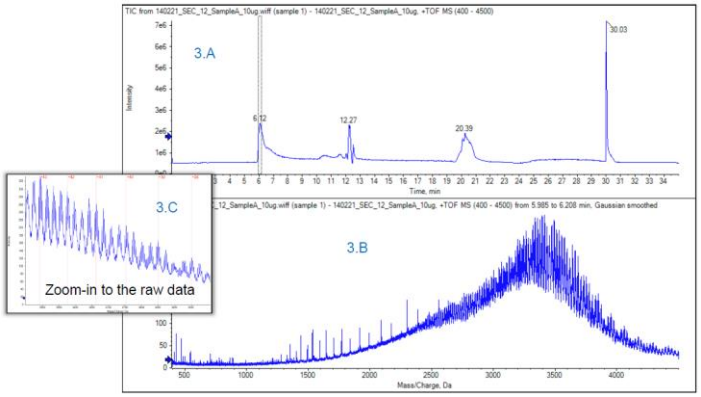

Figure 3 Chromatographic system 2 (SEC separation) Total ion Chromatogram (3A) and the obtained ADC raw spectrum (3B) and a zoom-in of the spectrum (3C) with the charge states highlighted

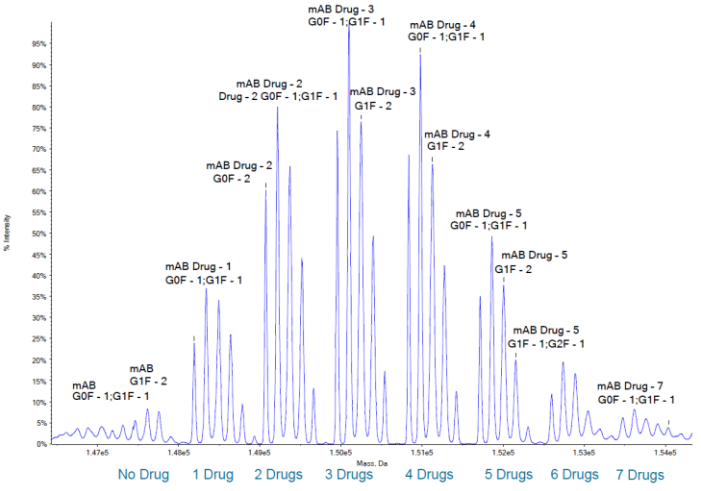

Figure 4 BioPharmaView software reconstruction of the data presented in Figure 3 Drug load was up to 7 per antibody moiety, with all the different drug conjugations demonstrating the same glycosylation pattern of combinations of G0Fs to G2Fs.

In addition to reconstruction, BioPharmaView software performs automated DAR calculations. The calculations for the samples shown in Figure 4 are shown in Figure 5. The batch processing of six injections of the same sample are shown in Figure 6.

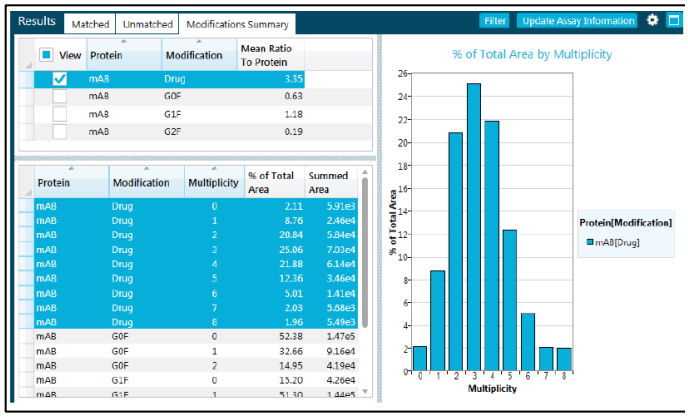

Figure 5 Automated DAR calculations for the non-deglycosylated ADC. The reconstruction area is given as a value, and also as a percentage of the total area per each multiplicity of conjugation. The multiplicity is represented as columns and the final DAR ratio is calculated.

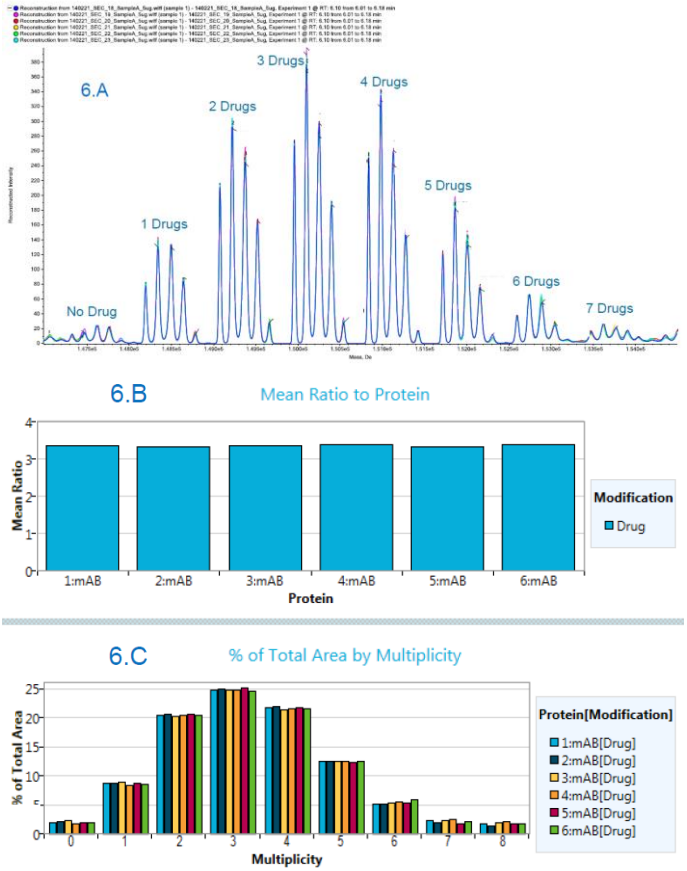

Figure 6 Reconstruction of analysis of six injections of the non-deglycosylated ADC: (6A) the overlay of six reconstructed spectra, (6B) the DAR ratios and (6C) the multiplicity represented as columns for the six injections.

Conclusions

Complex ADC's can be reproducibly analyzed without time consuming sample preparation to deglycosylate the protein. The reconstruction with BioPharmaView software enabled both, the DAR to be calculated and the glycosylation pattern to be monitored within a single sample and single analysis for this complex biotherapeutic. The sample analysis time was reduced to obtain higher through-put by excluding sample pretreatment time, combining shorter analysis time with SelexION technology separation, and by enabling fast data-analysis batch-processing by BioPharmaView software

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