AN1609: ADC drug-antibody ratio (DAR) determined by SEC-MALS

Introduction

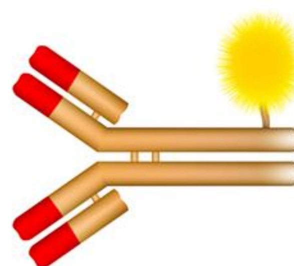
There has been a significant resurgence in the development of antibody-drug conjugates (ADC) as target-directed therapeutic agents for cancer treatment. Among the factors critical to effective ADC design is the drug-antibody ratio (DAR), which describes the degree of drug appended to the antibody and directly impacts both potency and potential toxicity of the therapeutic. In addition to therapeutic/physiological effects, DAR can also affect drug product properties such as stability and aggregation. Determination of DAR is, therefore, of critical importance in the development of novel ADC therapeutics.

DAR is typically assessed by mass spectrometry (MALDI-TOF or ESI-MS) or UV spectroscopy, both of which are subject to certain limitations: 1) calculations based on UV absorption are often complicated by similarities in extinction coefficients of the antibody and small molecule; 2) mass spectrometry, though a powerful tool for molecular weight determination, depends on uniform ionization and recovery between compounds—which is not always the case for ADCs. We present here a method for DAR determination based on SEC-MALS in conjunction with UV absorption and differential refractive index (dRI) detection, which overcomes these limitations for many ADCs.

Materials and Methods

Two model ADCs based on the same monoclonal antibody and drug-linker system, but different conjugation processes, were analyzed. In this example the antibody has been alkylated with a compound having a nominal molecular weight of 1250 g/mol. Each ADC was passed through size-exclusion chromatography (SEC) followed by the HPLC's UV detector, a DAWN® multi-angle light-scattering (MALS) detector and an Optilab® differential refractive index (dRI) detector. Protein molar mass (M_{prot}), molar mass of the drug and linker (M_{drug}) and total molar mass ($M_{complex}$) of the ADC were determined at each elution volume by analysis of signals from the three detectors.

Data collection and analysis were performed in the ASTRA® software using ASTRA's *Protein Conjugate Analysis* method. Conjugate analysis is automated within ASTRA using the differential refractive index increments (*dn/dc*) and UV extinction coefficients, which are determined empirically for each species or mined from the literature.



Results and Discussion

Figure 1 shows UV traces for the two model ADCs along with the calculated values of $M_{complex}$, M_{drug} and M_{prot} of each. As expected, the molar masses of the antibody components are quite similar; but M_{drug} differs considerably. Despite the drug constituting just a small fraction of overall molar mass, this highly sensitive system shows robust M_{drug} values which are well-distinguished from each other and vary little over the peak.

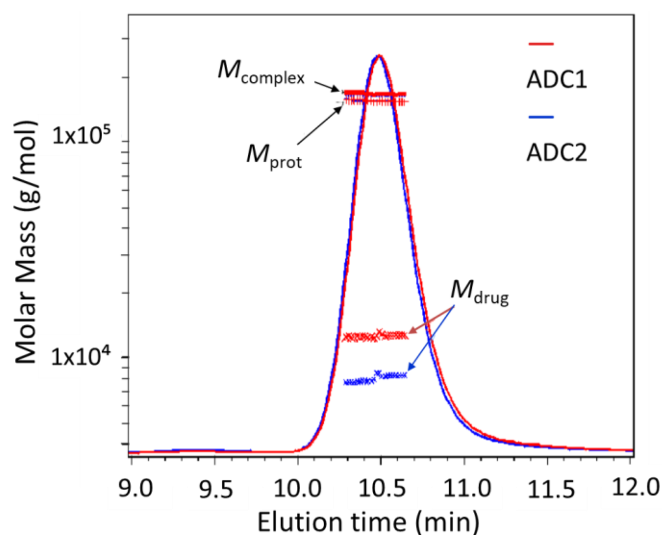


Figure 1. UV chromatograms of ADC1 and ADC2, overlaid with the molar masses of the complex as well as protein and drug components determined by MALS-UV-dRI analysis.

	M_w (kDa)			DAR
	Complex	Antibody	Drug	
ADC1	167.8	155.2	12.6	10.1
ADC2	163.7	155.6	8.1	6.5

Table 1. Peak-average results and final DAR for ADC1 and ADC2

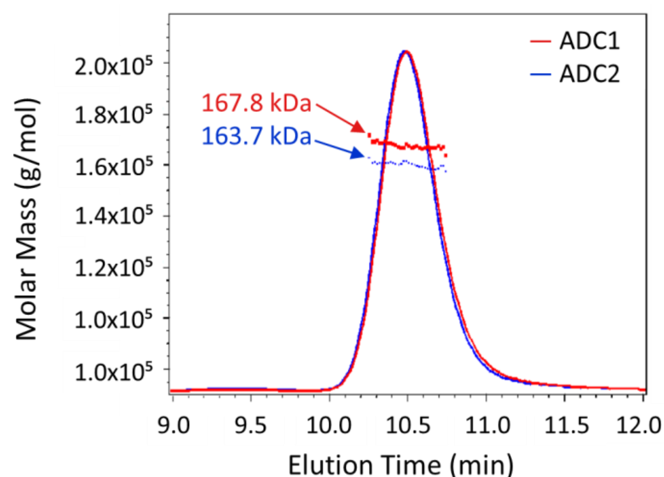


Figure 2. UV Chromatograms of ADC1 and ADC2 with total molar masses overlaid. The 2.5% difference between the two ADCs is robust.

This analysis was successful thanks to the relatively large total appended mass, about 5% (ADC2) to 8% (ADC1) of the total molar mass of the complex. In general, for accurate SEC-MALS-UV-dRI protein conjugate analysis, the modifier mass should be at least 3% of the total. Hence a lower degree of conjugation might not have been amenable to analysis by this method.

Conclusions

SEC-MALS-UV-dRI provides an easy, accurate means to determine DAR of ADCs in the course of product development and quality control. It is suitable for relatively high DAR values where the total drug-linker mass is at least 3% of the overall complex mass.

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