



## High throughput DAR determination of brentuximab vedotin (Adcetris®) by reducing the analysis time

**D**rug-to-antibody ratios (DAR) of antibody-drug-conjugates (ADCs) such as brentuximab vedotin is important for their therapeutic efficacy and pharmacokinetics. Therefore, control of DAR is a key factor for ADC quality control. Given that, it is important for QC purposes to obtain a satisfying resolution of all DARs and the resulting average ratio whilst performing the separation in the shortest time.

Brentuximab vedotin (Adcetris®) is a cysteine conjugated ADC targeting different types of lymphoma with an average DAR of 4. Typically, this 2<sup>nd</sup> generation ADC is analysed by hydrophobic interaction chromatography (HIC).

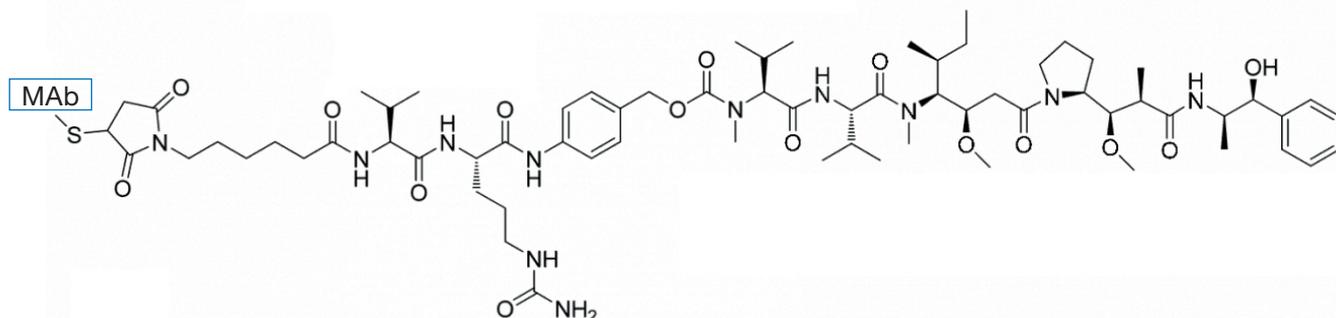
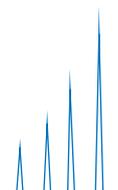


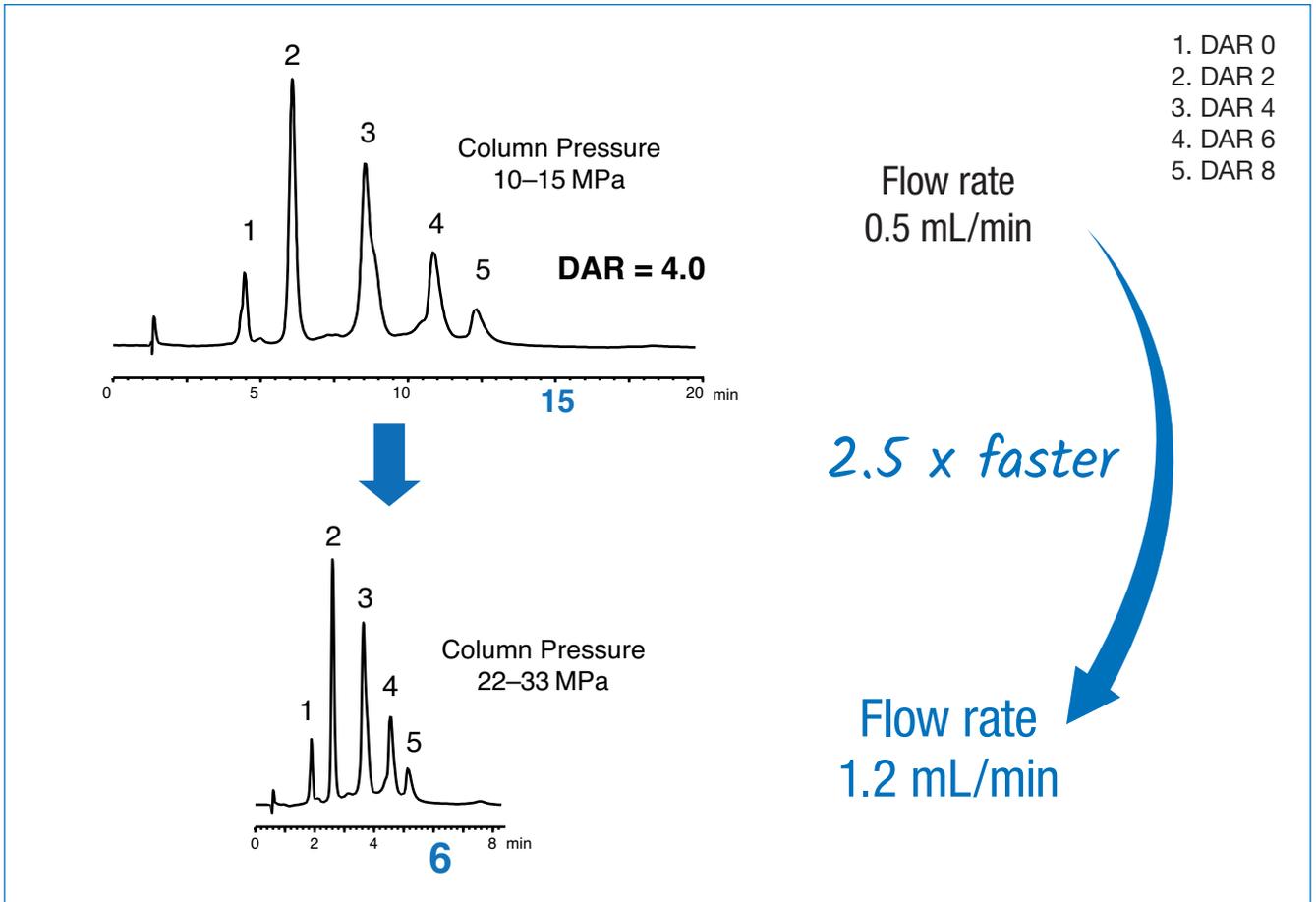
Figure 1: Skeletal formula of brentuximab vedotin [1].

In this application note YMC's latest hydrophobic interaction chromatography column, BioPro HIC HT, was used. A mobile phase of sodium phosphate buffer at neutral pH with decreasing gradients of the lyotropic salt ammonium sulphate was used. The rigid 2.3  $\mu\text{m}$  non-porous polymer particles are pressure tolerant up to 400 bar and allow rapid analyses through increased flow rates without loss of resolution. This allows flow rates to be increased by 2.5 times with savings of about 60% in time.

Table 1: chromatographic conditions

Column size:	100 x 4.6 mm ID
Part No:	BHH00SQ3-1046PTH
Eluent:	A) 20 mM $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$ (pH 7.0) containing 1.0 M $(\text{NH}_4)_2\text{SO}_4$ B) 20 mM $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$ (pH 7.0)/2-propanol (85/15)
Gradient:	0-100% B (0-15 min), 100% B (15-20 min) 0-100% B (0-6.25 min), 100% B (6.25-8.3 min)
Temperature:	25 °C
Detection:	UV at 280 nm
Injection:	10 $\mu\text{L}$
Sample:	Brentuximab vedotin (Adcetris®) (2.5 mg/mL)





## Ordering information for BioPro HIC HT

Particle size [µm]	Column ID [mm]	Column Length [mm]	Part number	Precolumn filter 2 µm* (pack of 5)
2.3	4.6	100	BHH00SQ3-1046PTH	XRPRCS35

\*Holder required, part no. XRPRCS03

[1] <https://www.adcreview.com/brentuximab-vedotin-sgn35/> as of 17/04/2020.

