



HPLC Analysis of Drug-to-Antibody Ratio (DAR) Using BioPro HIC BF

Antibody-Drug Conjugates (ADCs) are a recent development of an important class of highly potent biopharmaceutical drugs designed as a targeted therapy for the treatment of people with cancer. ADCs are monoclonal antibodies chemically linked to biologically active small molecule drugs. By combining powerful cell-killing abilities of potent agents with target-specific antibodies, ADCs deliver cytotoxic drugs to the diseased cells, while limiting the toxicity in the non-targeted ones. A significant attribute of ADCs is

the average number of drugs conjugated to the antibodies, the drug-to-antibody ratio (DAR). The DAR value affects the efficiency of the drug, as low drug loading reduces the potency, while high drug loading can negatively affect pharmacokinetics and toxicity.

As an example for ADCs, the unmodified cysteine-conjugated ADC mimic and its DAR with DAR values ranging from 2 to 8 is well resolved on the BioPro BF column (see fig. 1).

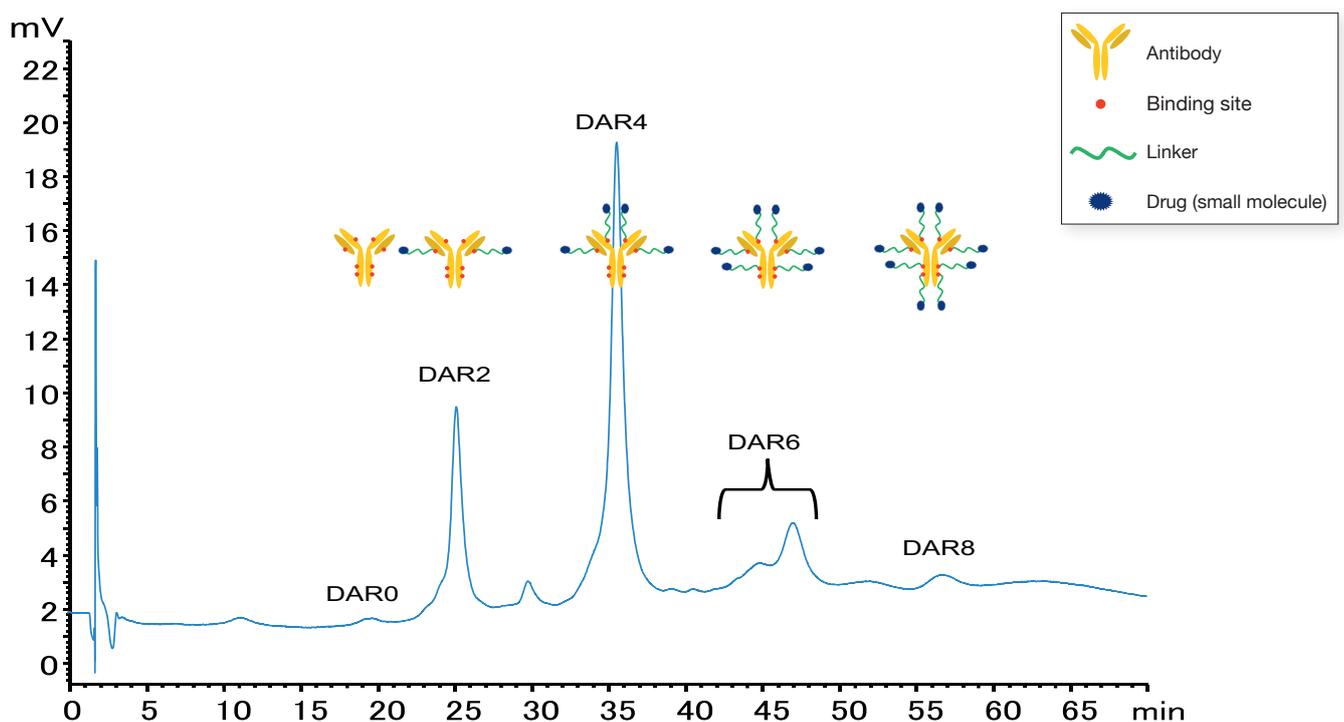


Fig. 1: Separation of antibody conjugates with different drug-to-antibody ratio (DAR) using BioPro HIC BF.

Table 1: Chromatographic conditions

Column:	BioPro HIC BF (4 μ m) 100 x 4.6 mm ID	
Part No.:	BHB00S04-1046WT	
Eluent:	A) 50 mM NaH_2PO_4 - Na_2HPO_4 (pH 6.8) containing 1.5 M $(\text{NH}_4)_2\text{SO}_4$ /IPA (95/5) B) 50 mM NaH_2PO_4 - Na_2HPO_4 (pH 6.8)/IPA (80/20)	
Gradient:	Time [min]	Eluent B [%]
	0	30
	5	30
	45	80
	70	80
Flow rate:	0.4 mL/min	
Temperature:	25 $^\circ\text{C}$	
Detection:	UV at 280 nm	
Injection:	20 μL	



Since the attachment of a drug molecule alters the hydrophobicity of a mAb or ADC, a decreasing gra-

dent of the lyotropic salt ammonium sulfate is required (see fig. 2).

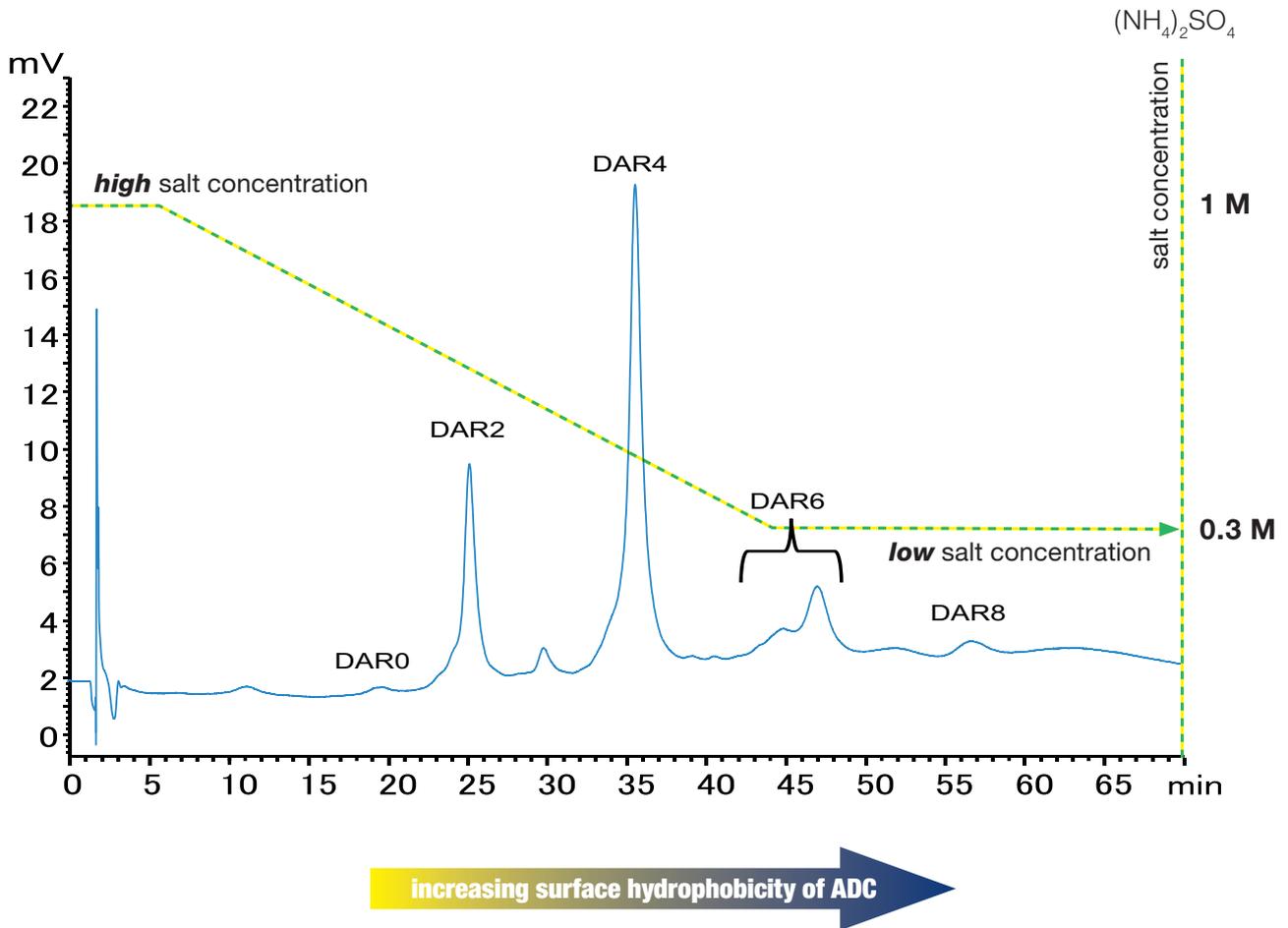


Fig. 2: HIC conditions for the separation of antibody conjugates with different drug-to-antibody ratio (DAR) using BioPro HIC BF.

Ordering information for BioPro HIC BF

Phase	Particle size [μm]	Functional group	Column ID [mm]	Column Length [mm]	Part number
BioPro HIC BF	4	butyl	4.6	100	BHB00S04-1046WT

