

Reaction monitoring of Antibody-Drug-Conjugates (ADCs) Using YMC's BioPro HIC BF

Drug-antibody ratio (DAR) of antibody-drug conjugates (ADCs) is important for their therapeutic efficacy and pharmacokinetics. Therefore control of **DAR in the synthesis process is a key for ADC quality control**. The ability to monitor this step reliably can also be advantageous for process understanding and control.

To provide a solution for this approach, RIKEN, the Japanese National Research and Development Agency successfully developed a method involving **hydrophilic interaction chromatography (HIC)** linked to UV/Vis spectroscopy (see table 1).

YMC's BioPro HIC BF stationary phase was used

with a mobile phase of sodium phosphate buffer at neutral pH with a decreasing gradient of the lyotropic salt ammonium sulphate (from 1.5 M to 0 M in 15 min). At the same time, the amount of organic modifier was increased (5-20 %) to improve the results and shorten the retention time.

The chromatograms in fig. 1 show the **effective separation of intact Mab from ADCs with their single drug-to-antibody ratios (DARs)**. The high resolution of the separation enables easy detection of differences in reaction conditions. This shows that BioPro HIC BF is suitable for characterisation as well as reaction monitoring of ADCs.

Table 1: Chromatographic conditions

Column:	BioPro HIC BF 4 µm, 100 x 4.6 mm ID
Eluent:	A) 50 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 7.0) containing 1.5 M (NH ₄) ₂ SO ₄ /2-propanol (95/5) B) 50 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 7.0)/2-propanol (80/20)
Gradient:	0%B (0-1 min), 0-100%B (1-15 min), 100%B (15-20 min), 0%B (20-30 min)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Sample:	Antibody Drug Conjugate*

*Courtesy of RIKEN

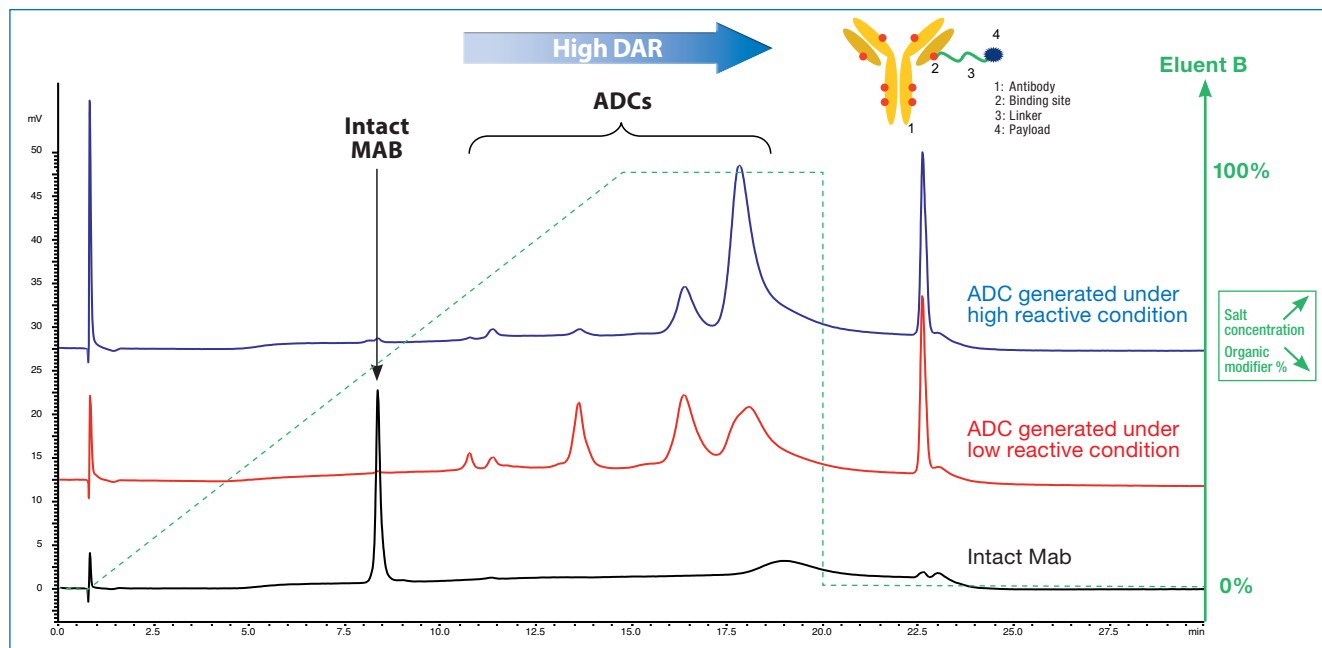


Fig. 1: Separation of drug-to-antibody ratio (DAR) using BioPro HIC BF.

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