DAR Analysis of Antibody-Drug Conjugate by HIC

The antibody drug market has continued to expand in recent years, and antibody drugs held 7 of the top 10 blockbuster drug spots for 2012. The most promising antibody drug candidates for next-generation biopharmaceuticals are ADCs (antibody-drug conjugates). ADCs have a structure in which a low-molecular drug is chemically bonded to an antibody (IgG). Because there are numerous binding sites for a low-molecular drug on an antibody (Cys, Lys residues, etc.), heterogeneity arises with respect to the number of bonds and binding sites. Consequently, it is necessary to study in detail the effect that these heterogeneity have on the medicinal effects and safety of ADCs. Since low-molecular drugs are strongly hydrophobic compared with antibodies, differences arise in hydrophobicity when the bonding number of low-molecular drug differs. This property can be utilized to determine the drug-to-antibody ratio (DAR) by hydrophobic interaction chromatography (HIC).

Introduced here is an application in which an ADC was separated using a TSKgel Butyl-NPR column.

An ADC (Trastuzumab-vcMMAE) in which an antineoplastic drug (monomethyl auristatin E, MMAE) is bonded via a linker to Trastuzumab was used. The results of HIC analyses using common ammonium sulfate gradient elution conditions showed that ADC could not be suitably eluted. Hence, an organic solvent (2-propanol) was added to eluent B, and by optimizing the organic solvent concentration, peaks exhibiting different DARs (DAR = 0 to 8) could be well separated (for the DAR, each peak fractionated and attributed by LC-MS/MS).

Reference

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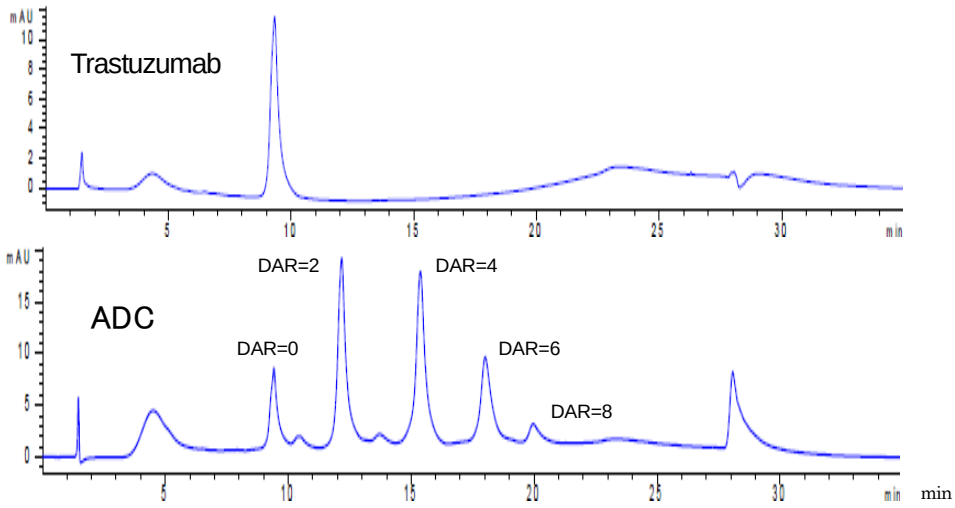

Figure 1 Chromatograms of Trastuzumab and ADC

Table 1 Conditions

Column:	TSKgel Butyl-NPR (4.6 mm I.D. x 10 cm)
Eluent:	A) 25 mmol/L phosphate buffer (pH 7.0) including 1.5 mol/L ammonium sulfate
	B) 25 mmol/L phosphate buffer (pH 7.0) / 2-propanol = 8 / 2
Gradient:	0 → 100 % B (20 minutes)
Flow rate:	0.5 mL/min
Detection:	UV 280 nm
Injection vol.:	10 µL
Sample Conc.:	Herceptin; 0.24 g/L, ADC(Herceptin-vcMMAE); 2.2 g/L