Online native HIC-MS analysis of cys-linked ADCs

For the more detailed determination of the drug-to-antibody ratio (DAR) of antibody-drug-conjugates (ADCs) a coupling of hydrophobic interaction chromatography (HIC) and mass spectrometry (MS) is highly desirable as this eliminates the intermediate isolation step. Due to high concentrations of non-volatile salts, which are usually used in HIC mode, a coupling to MS would seem impossible and the use of volatile salts requires an even higher concentration to achieve the same salting-out effect.

To overcome this obstacle and to enable simultaneous UV and MS detection a post-column makeup flow and a splitter have to be used. The makeup flow decreases the salt concentration while the splitter reduces the flow rate to enable the coupling to MS. A nanospray ionisation mass spectrometer (NSI-MS) was chosen because of its high sensitivity and salt tolerance.

In this application note the DAR of SigmaMAb ADC-mimic was determined using YMC's BioPro HIC BF column. The analysis was performed using 3 M ammonium acetate in water and a 2-propanol/water mixture (30/70) as eluents at a gradient elution.

The SigmaMAb ADC-mimic is an IgG1 conjugated with dansyl fluorophores at the cysteine residues of the inter-chain disulfide bonds. This results in payloads of 0–4 pairs. The DAR as well as degraded forms could be determined.

Table 1: Chromatographic conditions

Column:	BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Part number:	BHB00S04-1046WT
Eluent:	A) 3 M ammonium acetate in water
	B) 2-propanol/water (30/70)
Gradient:	10% B (0–2 min)
	10–97% B (2–18 min)
	97% B (18–22 min)
Flow rate:	0.3 mL/min
Sample:	SigmaMAb ADC-mimic
Injection:	10 µg
Detection:	UV at 280 nm, NSI-MS
Setup:	Post-column makeup flow: 100% water at 1.5 mL/min (reducing salt conc. 6-fold)
	Splitter to reduce the flow rate to 1–5 µL/min

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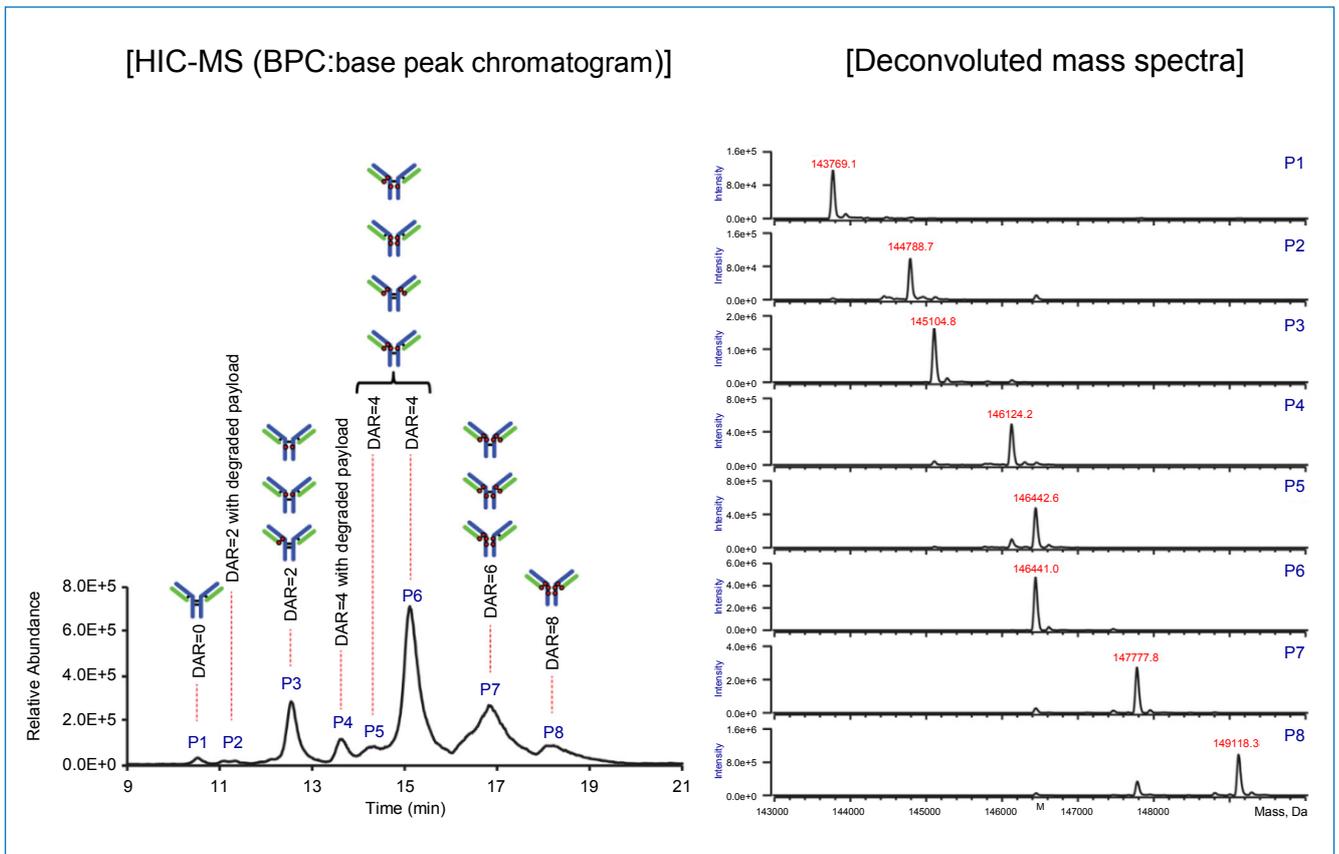
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Figure 1: HIC-MS characterisation of an ADC-mimic.

Courtesy by S. Wang, Regeneron Pharmaceuticals Inc.

Reference:
Y. Yan, T. Xing, S. Wang, T. J. Daly, N. Li, Online coupling of analytical hydrophobic interaction chromatography with
native mass spectrometry for the characterization of monoclonal antibodies and related products,
J. Pharm. Biomed. Anal. 186 (2020) 113313.

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