Online native HIC-MS analysis of monoclonal antibodies and their molecular variants

Monoclonal antibodies (MAbs) are often analysed using hydrophobic interaction chromatography (HIC) because of their high hydrophobicity. The direct coupling of hydrophobic interaction chromatography to mass spectrometry (MS) is highly desirable for further characterisation without the need for an isolation step beforehand. Due to high concentrations of non-volatile salts, which are usually used in HIC mode, a coupling to MS seems impossible. Using volatile salts requires an even higher salt 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.

This application note shows how a mixture of seven in-house MAbs as well as the molecular variants of two MAbs can be characterised via HIC-MS using YMC's BioPro HIC BF column. Both analyses were performed using 3 M ammonium acetate in water and 100 % water as eluents as well as the same gradient.

The analysis of the seven different MAbs resulted in six well separated peaks (see fig. 1). Only two MAbs (5 and 6) could not be separated, but the deconvolution spectra also revealed that MAb 5 could be separated from its oxidised form.

A variant peak from each MAb, 8 and 9, which were eluting slightly earlier, could be separated and characterised (see fig. 2).

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) 100 % water
Gradient:	0 % B (0–2 min)
	0–90 % B (2–18 min)
	90 % B (18–22 min)
Flow rate:	0.3 mL/min
Sample:	Mixture of 7 in-house MAbs at 1–2 mg/mL each
	2 in-house MAbs with molecular variants
Injection:	MAb mixture: 3 µL (3–6 µg)
	MAb 8 and MAb 9: 10 µg each
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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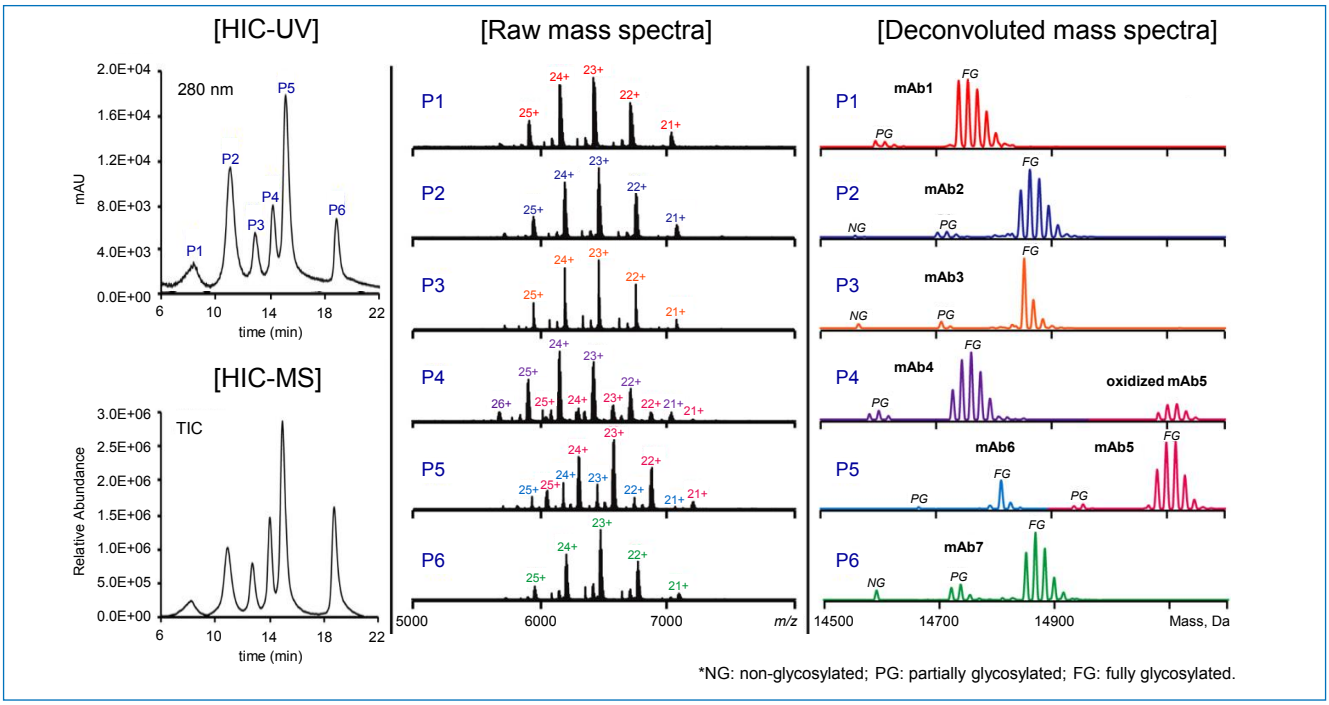

Figure 1: Separation of an antibody mixture of seven different MAbs.

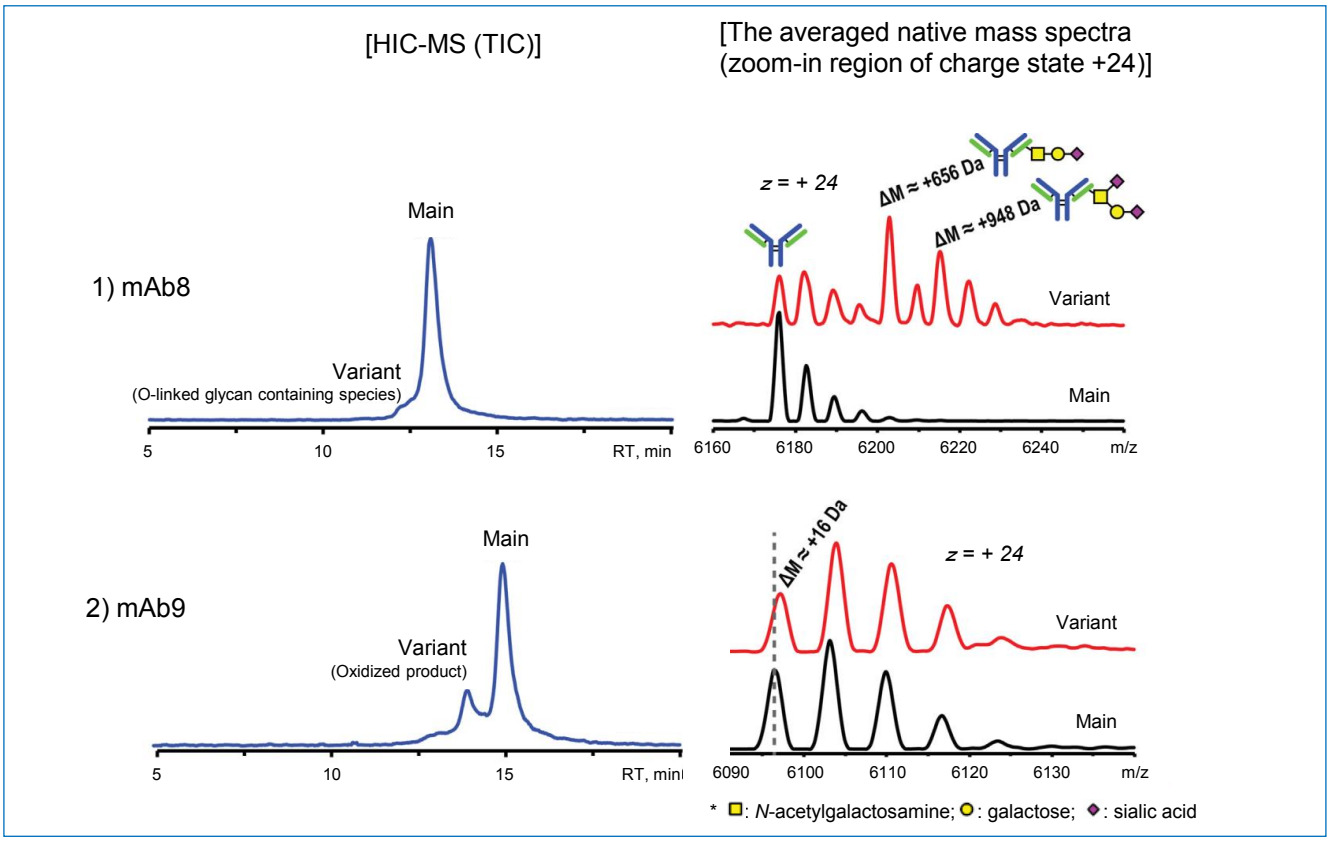

Figure 2: Separation of two MAbs from their molecular variants.

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.