

# Characterisation of monoclonal antibodies and related products via HIC-MS

## Summary

The work by Yan et al. demonstrates how hydrophobic interaction chromatography (HIC) and native mass spectrometry (MS) can be coupled directly. The combination of HIC, using BioPro HIC BF by YMC, and native MS enables a highly sensitive method even for samples with low concentrations. Especially molecular species and drug-to-antibody ratios (DAR) can be characterised with this technique.

## BioPro HIC BF:

- **High separation performance**
- **Excellent lot-to-lot reproducibility**
- **Long term stability**
- **Virtually no carryover effects**

## Reference:

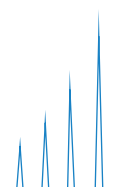
[1] Y. Yan, T. Xing, S. Wang, T. J. Dali, N. Li, "Online coupling of analytical hydrophobic interaction chromatography with native mass spectrometry for the characterization of monoclonal antibodies and related products", J. Pharmaceut. Biomed., 2020, 186, 113313.

## 1. Challenges of HIC applications coupled with MS

Hydrophobic interaction chromatography (HIC) is one of the standard techniques for the LC analysis of monoclonal antibodies (MAbs) and antibody-drug-conjugates (ADCs). The native conditions provide crucial advantages for the analysis of proteins. Usually non-volatile salts such as ammonium sulphate are used in HIC mode at high concentrations (1–2 M). Therefore, a coupling with MS is virtually impossible. If volatile salts such as ammonium acetate are used in HIC mode, the salt concentration needs to be increased to achieve the same salting-out effect. Instead of 2 M ammonium sulphate 5–5.5 M ammonium acetate needs to be applied, so a direct coupling to MS is still challenging but feasible.

## 2. Innovative solution for HIC-MS

In the studies of Yan et al. HIC was coupled directly to online native MS. The coupling is enabled by a combination of a makeup flow and a splitter (s. Fig. 1). Thereby the salt concentration as well as the flow rate are reduced to enable nanospray ionisation (NSI).



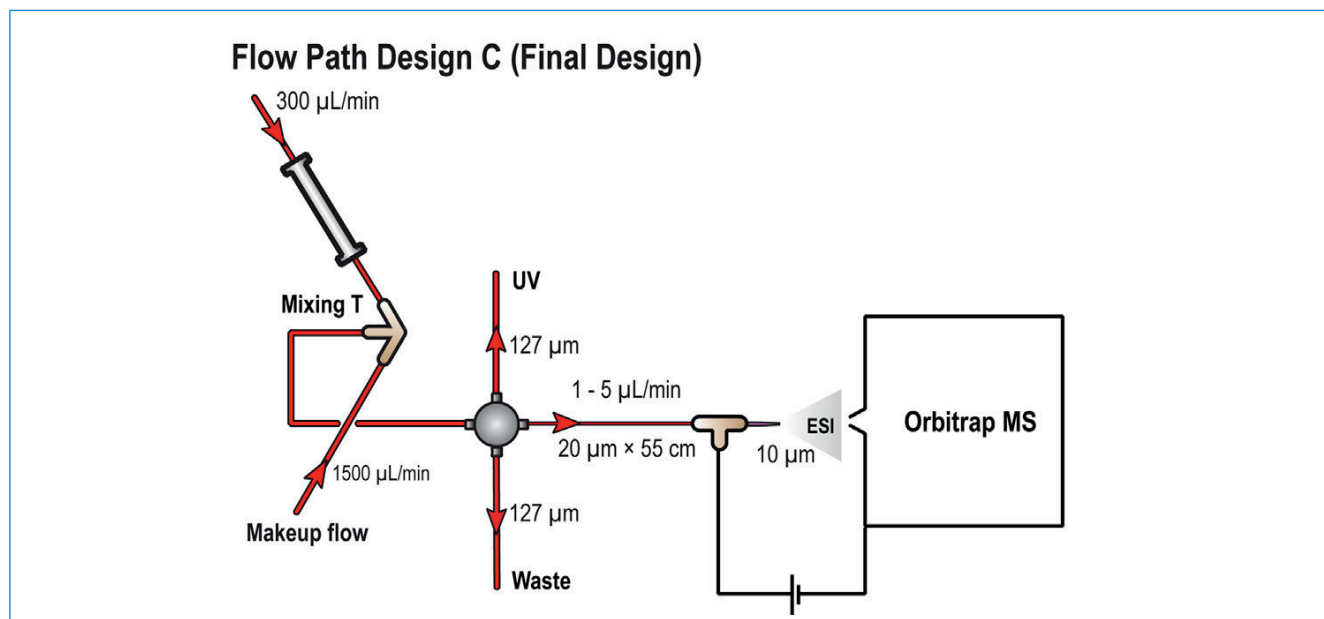


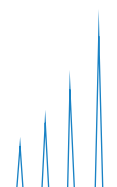
Figure 1: Makeup flow and splitter setup. [1]

BioPro HIC BF, a non-porous column with butyl ligands from YMC, was used for the separation of seven in-house MABs, two MAB samples with post translational modification (PTM) induced molecular variants and a cysteine-linked ADC mimic.

The study was published in April 2020 in the *Journal of Pharmaceutical and Biomedical Analysis* under the title **Online coupling of analytical hydrophobic interaction chromatography with native mass spectrometry for the characterization of monoclonal antibodies and related products.** [1]

### 3. 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 for analysis of MABs and water/isopropanol (70/30) for the ADC mimic
Gradient:	MAB samples: 0–90 % B in 16 min ADC mimic: 10–67 % B in 16 min Followed by a 4 min isocratic hold
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 SigmaMAB ADC mimic (Sigma-Aldrich)
Injection:	MAB mixture: 3 µL (3–6 µg) MAB 8, MAB 9 and SigmaMAB ADC mimic 10 µg each
Detection:	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



## 4. Results

### HIC-MS of a MAb mixture

Seven different MAbs were analysed using a 16 min gradient (from 3M to 300mM of ammonium acetate). Six well separated peaks were obtained. The injection of 3–6 µg for each MAb was sufficient to also detect low-abundance molecular variants present in each MAb such as non-glycosylated and partially glycosylated species.

Only two MAbs (MAb 5 and MAb 6, see Fig. 2) could not be separated. The deconvolution spectra also show MAb 5 and its oxidised form could be separated (Peak 4 and Peak 5).

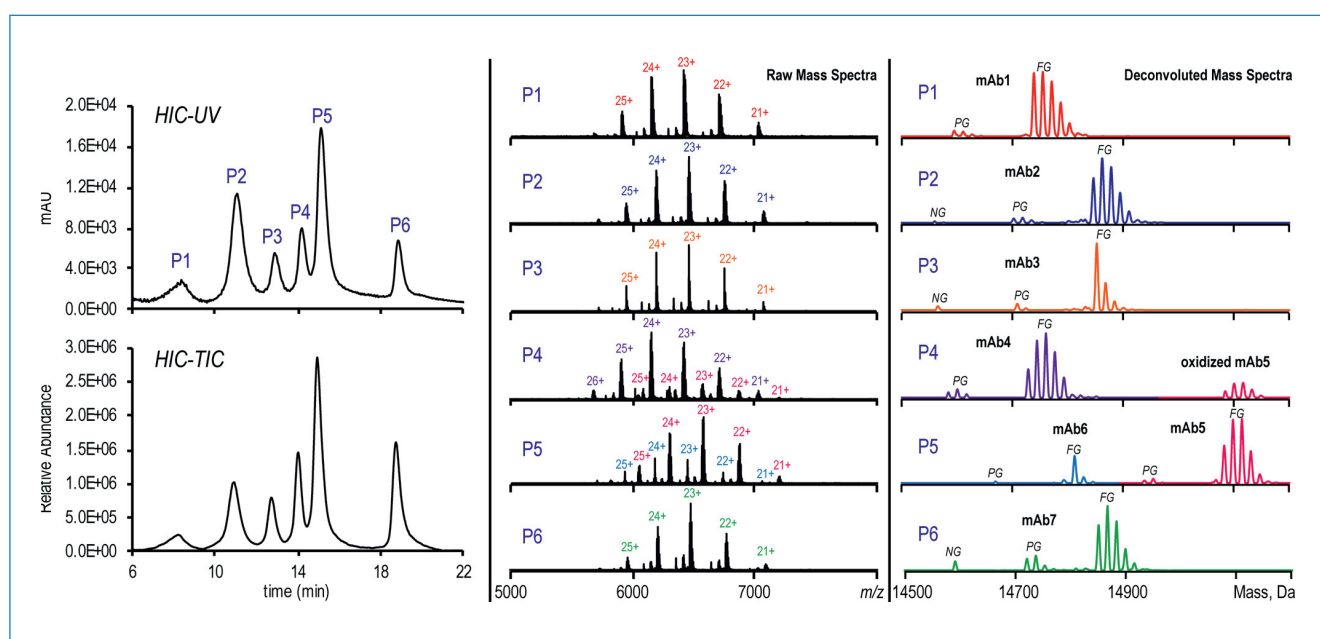
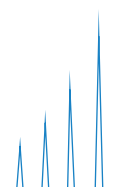


Figure 2: Chromatogram and mass spectra of MAb mixture. [1]

### MAb molecular variants

Two MAb variants with post translational modifications (PTMs) located at or near the complementarily determining regions (CDRs) were characterised. The variants could, at least, be partially separated, a 10 µg injection of MAb 8 and MAb 9 each showed a minor peak eluting slightly earlier, so that the presence of molecular variants with reduced hydrophobicity can be assumed. The mass measurement revealed two major species of MAb 8 and an oxidised product of MAb 9 (see Fig. 3).



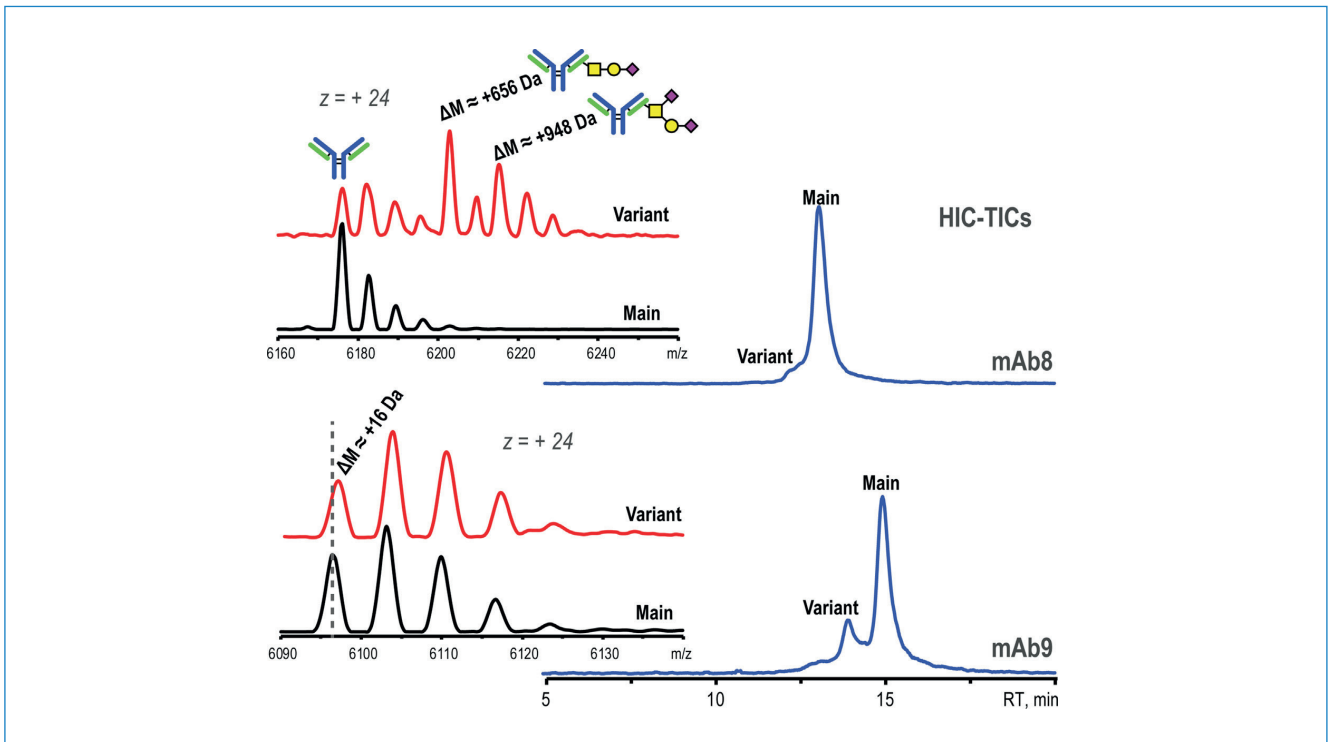


Figure 3: Chromatograms and mass spectra of MAb variants due to PTM. [1]

### DAR determination of ADC mimic

The MAb IgG1 was conjugated with dansyl fluorophores at the cysteine residues of the inter-chain disulfide bonds. This results in payloads of 0–4 pairs. The achieved separation profile is similar to the HIC-UV profile provided by the vendor where a conventional gradient containing ammonium sulphate and isopropanol was used. The DAR as well as degraded forms could be determined.

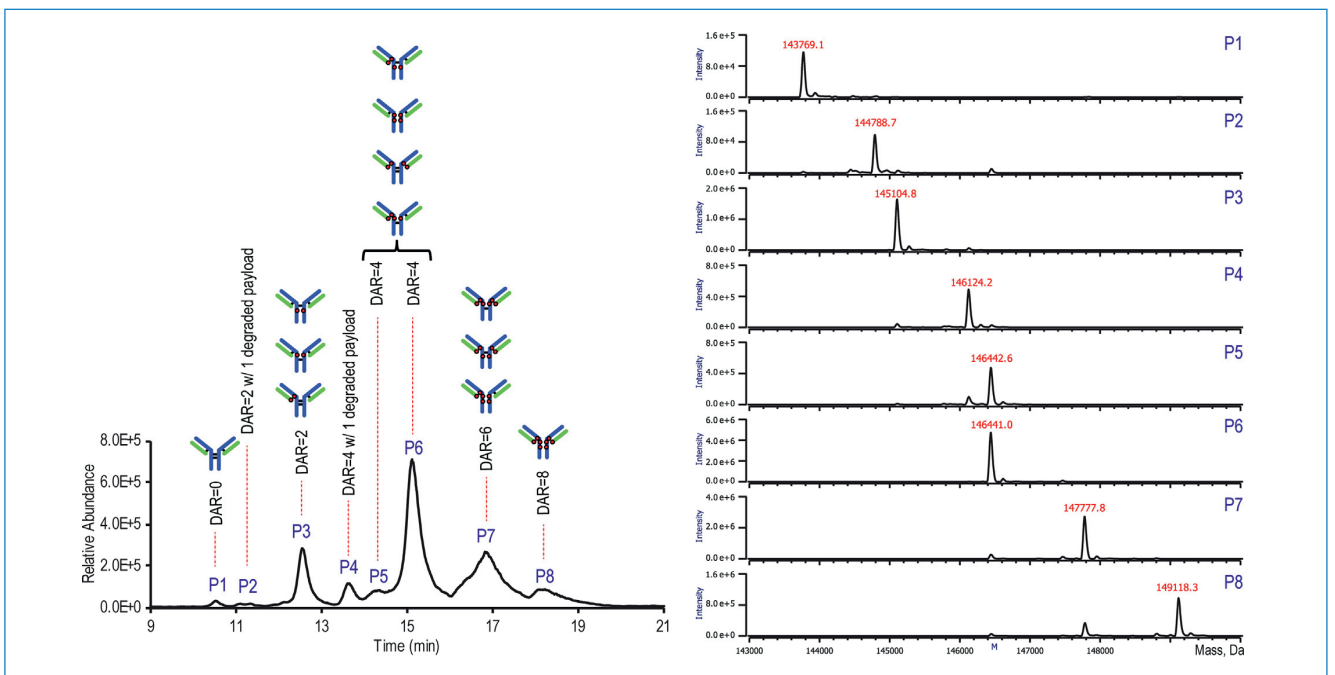


Figure 4: DAR determination and mass spectra of the SigmaMAb ADC mimic. [1]