



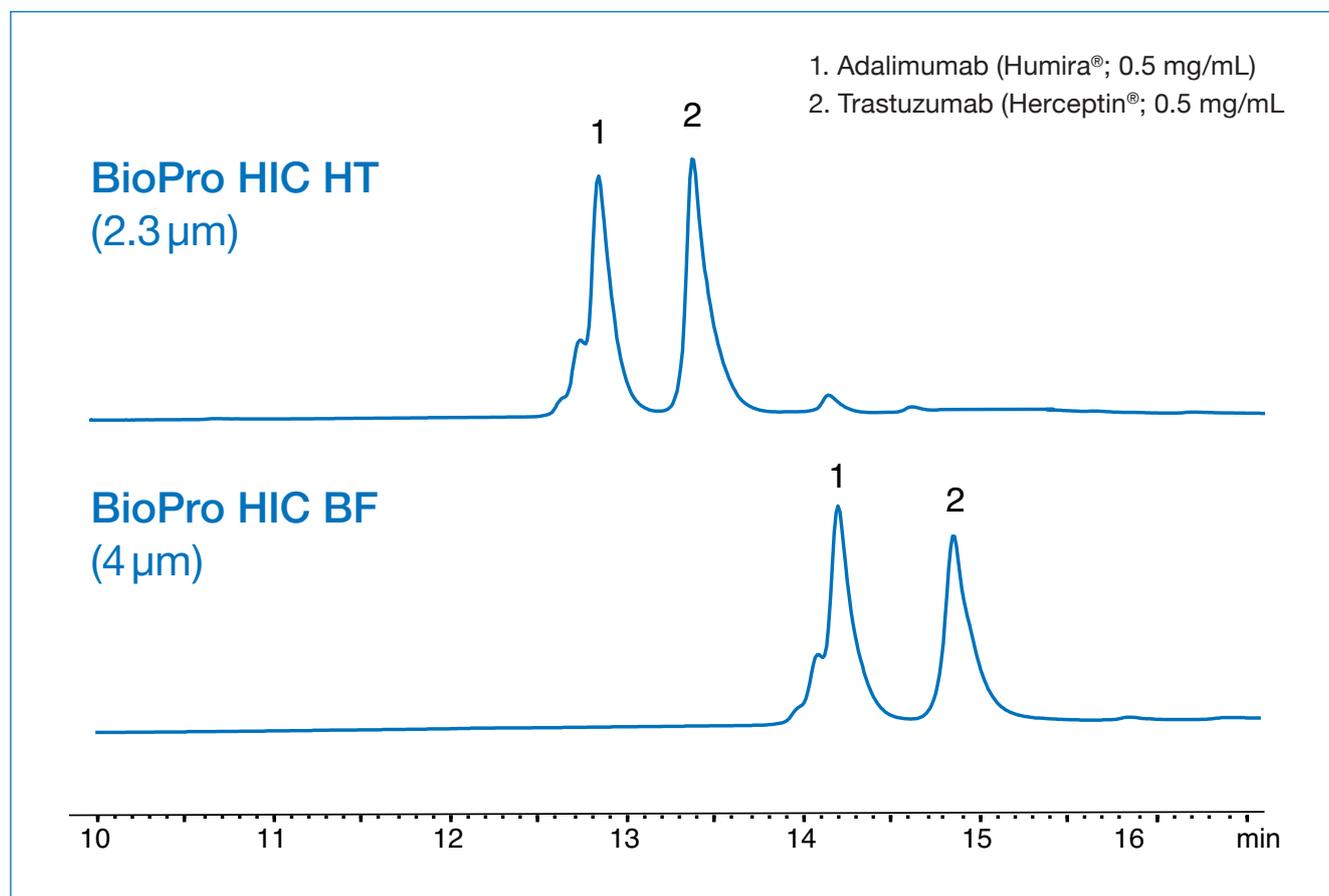
The right HIC column for each separation: BioPro HIC HT and BioPro HIC BF

Hydrophobic interaction chromatography (HIC) is used to analyse monoclonal antibodies (MAbs), proteins and also for the specific determination of the drug-to-antibody ratio (DAR) of antibody-drug-conjugates (ADCs). In order to address the separation requirements of these compounds, YMC offers two different HIC columns, BioPro HIC HT and BioPro HIC BF. They both have in common their non-porous polymethacrylate particle, while their properties and chromatographic behaviours are quite different.

BioPro HIC HT, YMC's latest hydrophobic interaction chromatography column, is designed for the analysis of biopharmaceuticals, such as ADCs or MAbs. It provides high resolution at very fast runs and therefore high throughput. This makes BioPro HIC HT the first-

choice column for HIC analyses of ADCs and MAbs. BioPro HIC BF columns show a stronger retention of proteins than BioPro HIC HT columns due to their higher hydrophobicity. They can therefore be used for the separation of low hydrophobic proteins. The strong hydrophobic retention of BioPro HIC BF columns is especially useful for the analysis of oxidised MAbs since the oxidised variants elute earlier than the non-oxidised MAb.

In this application note the retention behaviour of BioPro HIC HT and BioPro HIC BF is compared. A mobile phase of sodium phosphate buffer at neutral pH with a decreasing gradient of the lyotropic salt ammonium sulphate was used to separate two commercial monoclonal antibodies from each other.





Due to its optimised hydrophobicity for ADCs and MAbs, the BioPro HIC HT column provides much lower retention times, while the antibodies elute about 1.5 min later from the more hydrophobic BioPro HIC BF column.

The already shorter analysis time of the BioPro HIC HT column can further be reduced due to its rigid 2.3 µm non-porous polymer particles, which are pressure tol-

erant up to 400 bar. This allows a more rapid analysis through increased flow rates without loss of resolution.

In contrast, BioPro HIC BF shows low back pressures and high loadability, allowing lab scale purifications and detection of minor constituents by injecting large volumes.

Table 1: Chromatographic conditions

Column size:	100 x 4.6 mm ID
Part No:	BHH00SQ3-1046PTH BHB00S04-1046WT
Eluent:	A) 100 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 7.0) containing 2.0 M (NH ₄) ₂ SO ₄ B) 100mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 7.0)
Gradient:	0%B (0-1 min), 0-100%B (1-11 min), 100%B (11-15 min)
Flow rate:	0.5 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Injection:	15 µL
Sample:	1. Adalimumab (Humira®; 0.5 mg/mL) 2. Trastuzumab (Herceptin®; 0.5 mg/mL)

Ordering Information

Phase	Particle size [µm]	Column ID [mm]	Column Length [mm]	Part number	Precolumn filter 2 µm* (pack of 5)
BioPro HIC HT	2.3	4.6	100	BHH00SQ3-1046PTH	XRPRCS35
BioPro HIC BF	4	4.6	100	BHB00S04-1046WT	XRPRCS35

*Holder required, part no. XRPRCS03