APPLICATION NOTE

HIC Analysis of Different Monoclonal Antibodies using Isopropanol as Modifier

Monoclonal antibodies (MAb) are immunologically active proteins. They recognise and bind specifically to certain cells or proteins that contain the specific antigen. After the binding process the immune system is stimulated to attack those cells or proteins.
This behaviour of MAbs is applicable for the treatment of different kinds of cancer and autoimmune diseases. A broad variety of therapeutically antibodies are commercially available and several more are in research.

Antibodies are often analysed in HIC mode (hydrophobic interaction chromatography) because of the non-denaturating conditions used and the high selectivity to changes of protein surface-associated hydrophobicity.

An organic solvent in the mobile phase can lead to the denaturation of the antibodies, though with the addition of 15 % protic isopropanol the denaturation is limited and higher recoveries are achieved.

With the non-porous BioPro HIC BF column from YMC high resolution with narrow peak widths can be achieved for the analysis of MAbs. This application note developed by Department of Analytical Pharmaceutical Chemistry, the University of Geneva shows how to realise robust chromatographic results using commercially available MAbs: Adalimumab (Humira®), Brentuximab (commercially available as ADC "Brentuximab-vedotin"/ Adcetris®), Pembrolizumab (Keytruda®) and Reslizumab (Cinqaero®).
The analysis can be carried out in 12 minutes. In order to maintain non-denaturating conditions the column temperature should be kept at 20 °C.
The eluents used are 20 mM sodium phosphate buffer containing ammonium sulfate and 20 mM sodium phosphate buffer with 15 % isopropanol in order to achieve high recovery.

Table 1: Chromatographic conditions

Column:	BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Part No.:	BHB00S04-1046WT
Eluent:	A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 7.4) containing 1.5 M $(NH_4)_2SO_4$
	B) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 7.4) / 2-propanol (85/15)
Gradient:	0–100 % B (0–20 min)
Flow rate:	1.0 mL/min
Temperature:	20 °C
Detection:	fluorescence: ex 280 nm, em 360 nm
Injection:	3 µL (2 mg/mL)

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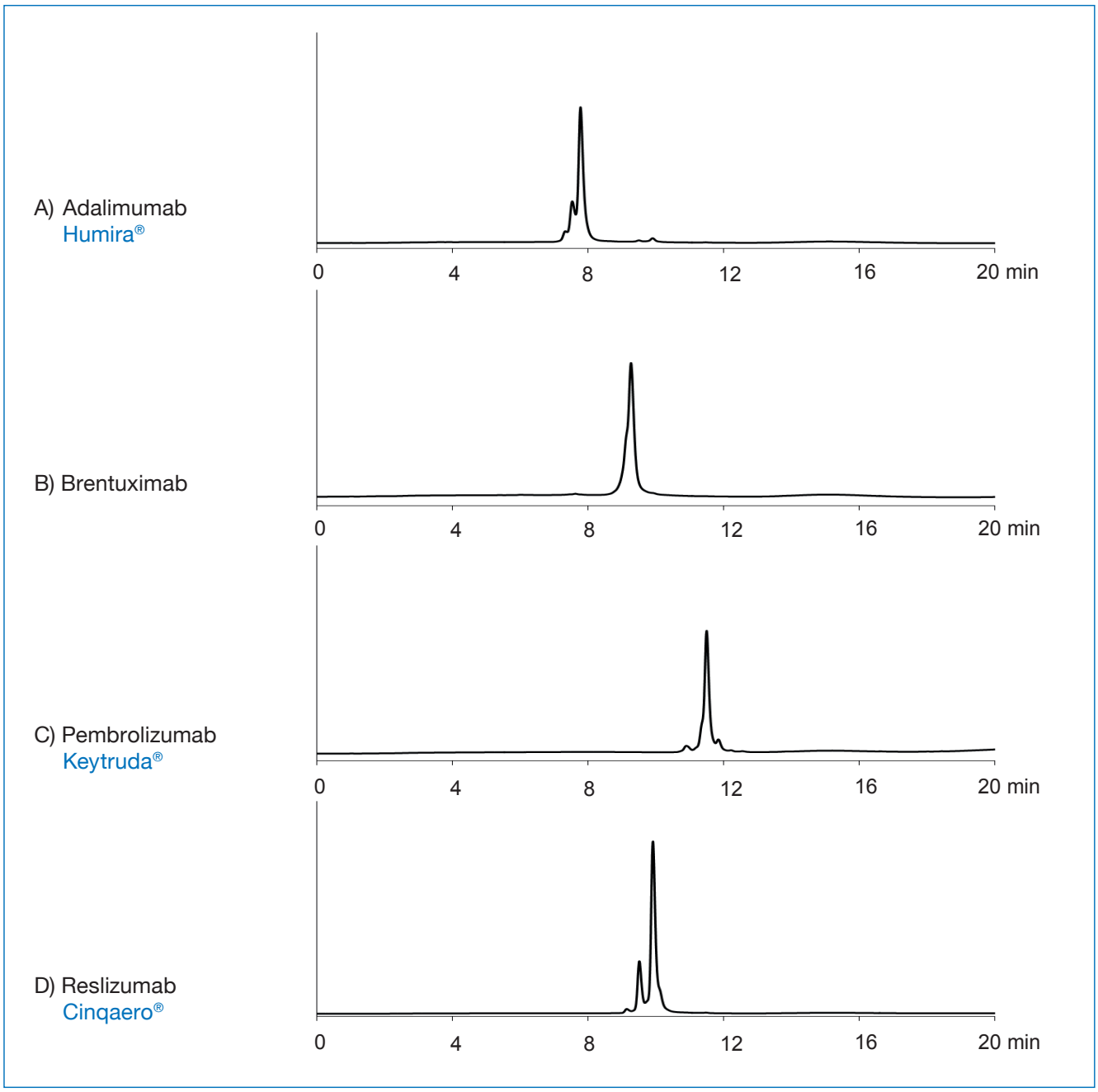

Figure 1. HIC analysis of different monocolonal antibodies using BioPro HIC BF*.

*by courtesy of University of Geneva, School of Pharmaceutical Sciences, Department of Analytical Pharmaceutical Chemistry