APPLICATION NOTE

High resolution analysis of monoclonal antibodies using the cation exchange column BioPro IEX SF

Cation exchange chromatography (CEX) is perceived to be the gold standard for the charge sensitive characterisation of monoclonal antibodies (MAbs). Acidic and basic variants caused by chemical or enzymatic modifications can be separated from the main isoform of the MAb. These antibody variants have to be critically evaluated as differences in impurities and/or degradation products could lead to severe undesirable side effects.

In this application note, based on a comparative study performed by the University of Geneva, School of Pharmaceutical Sciences, four therapeutic MAbs of different species, isotypes and isoelectric point (pI) together with their variants were analysed using pH and salt gradient mode[1]. The non-porous YMC column BioPro IEX SF used for this study is an outstanding stationary phase for the characterisation of charge variants MAbs in CEX mode.

Table 1: Antibodies analysed

	Antibody	Species	Isotype	pI
1	Natalizumab (Tysabri®)	Humanised	IgG4	7.3
2	Cetuximab (Erbitux®)	Chimeric	IgG1	7.9
3	Adalimumab (Humira®)	Human	IgG1	8.4
4	Denosumab (Prolia®; XGEVA®)	Human	IgG2	8.8

Table 2: General chromatographic conditions

Column:	BioPro IEX SF (5 µm) 100 x 4.6 mm ID
Part No:	SF00S05-1046WP
Flow rate:	0.6 mL/min
Temperature:	30 °C
Detection:	Fluorescence: ex 280 nm, em 350 nm
Injection:	2 µL (100 µg/mL each)

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Separation of monoclonal antibodies in pH and salt gradient mode

Table 3: Special conditions in pH and salt gradient mode

	pH gradient mode	Salt gradient mode
Eluent A	CX-1 pH Gradient Buffer A* (pH 5.6)	10 mM MES**-NaOH (pH 5.7)
Eluent B	CX-1 pH Gradient Buffer B* (pH 10.2)	10 mM MES**-NaOH (pH 5.7) containing 1 M NaCl
Gradient	0–100 % B (0–20 min)	0–20 % B (0-20 min)

*commercially available from Thermo Fisher Scientific; 10 times diluted; **MES: (N-morpholino)ethanesulfonic acid

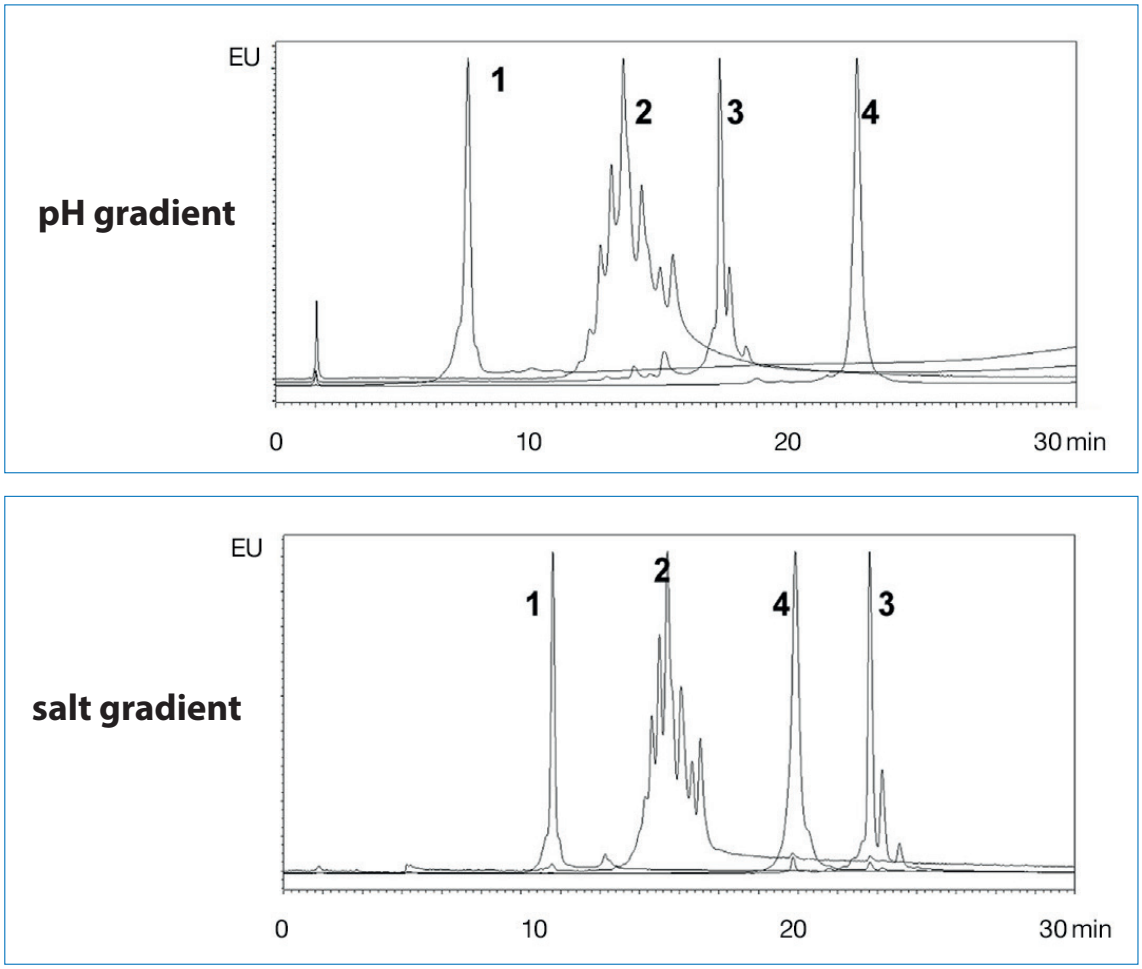

Figure 1: pH and salt gradient separations of the four monoclonal antibodies [1].

High resolution can be obtained for the separation of natalizumab, cetuximab, adalimumab and denosumab as well as their variants in both, pH and salt gradient mode. The only difference observed when changing from pH to salt gradient was a peak reversal between adalimumab and denosumab.

Higher resolution by column coupling

The BioPro IEX SF already shows high resolution for the separation of natalizumab, cetuximab, adalimumab and denosumab. Extending the column length by a coupling approach can help to improve antibody cation exchange analysis. By coupling two columns together, extending the gradient time (40 min, 80 min and 120 min) and reducing the severity of the gradient (0.5 %, 0.25 % and 0.17 %B/min) greater resolution can be obtained.

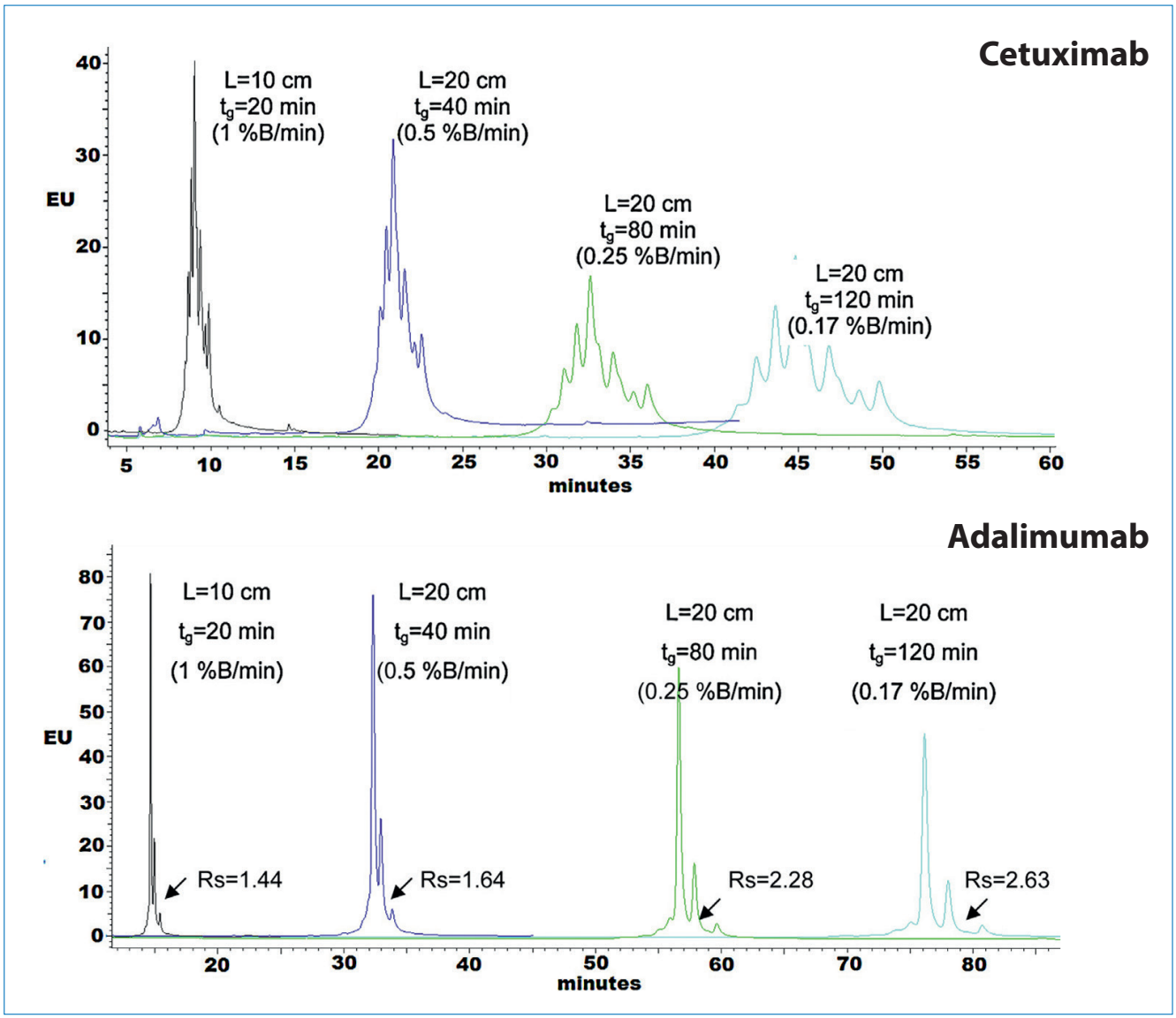

Figure 2: Improved resolution of the different variants through the extension of column length and gradient; L = 10 cm: 100 x 4.6 mm ID; L=20 cm: 100 x 4.6 mm ID x2 [1].

The resolution was drastically improved by increasing column length and gradient time for the cetuximab and adalimumab variants. The resolution power for adalimumab is nearly doubled when using the two coupled columns and the 120 min long gradient. The column coupling allows comparison of chromatographic profiles of different batches of biosimilar products of intact antibodies

[1] S. Fekete, A. Beck, D. Guillarme, Characterisation of cation exchanger stationary phases applied for the separations of therapeutic monoclonal antibodies, J. Pharm. Biomed. Anal., 2015, 111, 169–176.