APPLICATION NOTE

MS compatible charge variant analysis of 28 commercial monoclonal antibodies by CEX

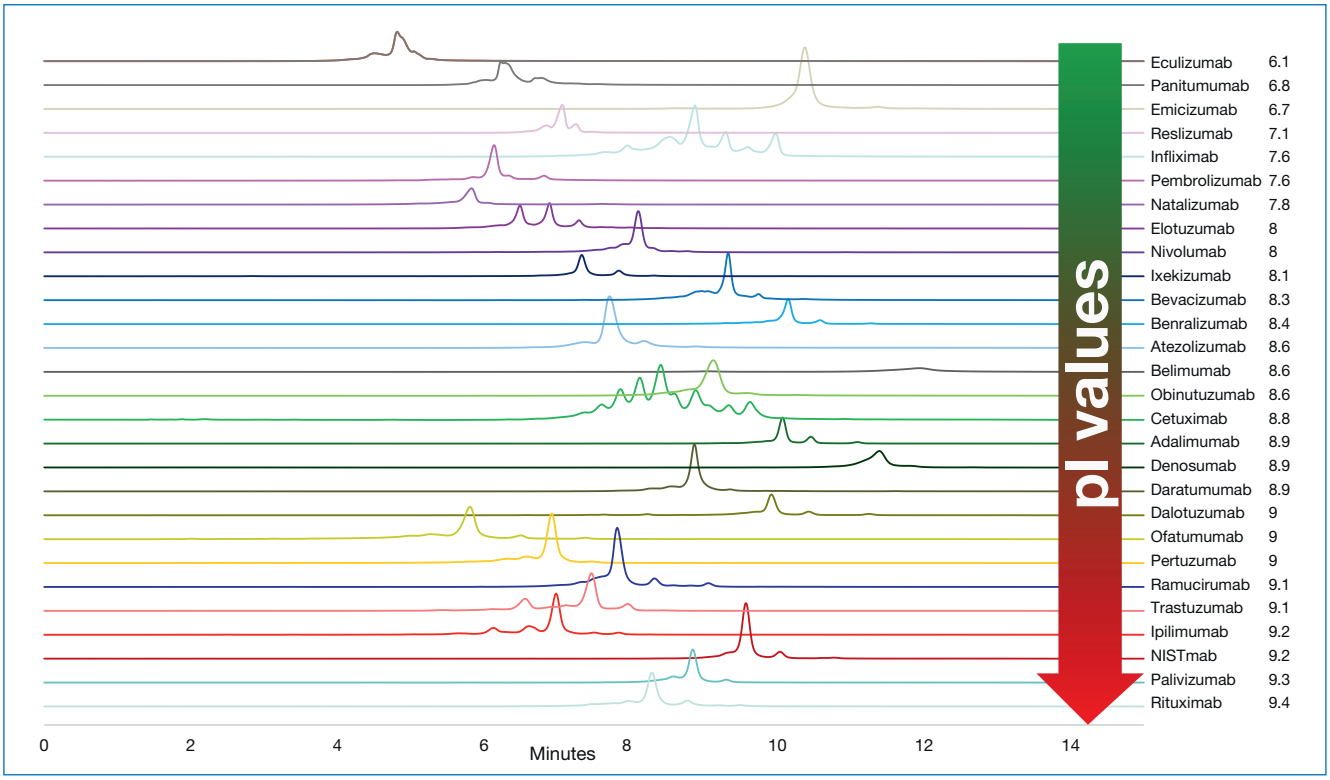
Cation exchange chromatography (CEX) is perceived to be the gold standard for the charge sensitive characterisation of monoclonal antibodies (MAbs). Acidic and basic variants caused by chemical or enzymatic modifications can be separated from the main isoform of the MAb. These antibody variants have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

This application note is based on data produced by the University of Geneva, School of Pharmaceutical Sciences according to the method described by Yan et al. [1]. It shows the analysis of 28 MAbs with different isoelectric points (pI 6.1–9.4) using YMC's BioPro IEX SF column. Each MAb could be separated from acidic and basic variants which can be further characterised if coupled to a mass spectrometer (possible setup described by Yan et al.).

To achieve an acceptable elution window the initial and final ratio of mobile phase B was tuned for each MAb depending on its isoelectric point starting with an initial ratio of 20–30 % B (higher ratio for MAbs with higher pI).

Chromatographic conditions

Column:	BioPro IEX SF (5 µm) 100 x 4.6 mm ID
Part No:	SF00S05-1046WP
Eluent:	A) 20 mM CH_3COONH_4-CH_3COOH (pH 5.6)
	B) 140 mM CH_3COONH_4-10 mM NH_4HCO_3 (pH 7.4)
Gradients:	Depending on the pI of MAb starting with 20–30 % B
	Inital %B (0–2 min), inital–100 %B (2–18 min), 100 %B (18–22 min)
Flow rate:	0.4 mL/min
Temperature:	ambient
Detection:	Fluorescence: ex 280 nm, em 360 nm



[1] Y. Yan, A. P. Liu, S. Wang, T. J. Daly und N. Li, "Ultrasensitive Characterization of Charge Heterogeneity of Therapeutic Monoclonal Antibodies," Anal. Chem., 2018, 90, 13013 - 13020.

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