How to develop a method for commercial MAbs using a Dial-a-mix method and YMC's BioPro IEX SF column

Monoclonal antibodies (MAbs) are immunologically active proteins, which bind specifically to certain cells or proteins. The impact of MAbs is becoming increasingly important for the treatment of different types of cancer and autoimmune diseases. Nowadays, a wide variety of therapeutic antibodies are available on the market and several more are in research and development.

How to find the optimum pH and salt gradient

Cation exchange chromatography (CEX) is perceived to be the gold standard for the charge sensitive characterisation of monoclonal antibodies. For charge variant analysis of MAbs the IEX method development requires finding the optimum pH value and salt gradient. A relatively easy way to approach this is to use a quaternary pump HPLC and the dial-a-mix method. This enables the lab scientist to utilise all four mobile phase bottles/lines to very easily (and quickly) screen various pH conditions and salt gradients.

Once the most appropriate conditions are determined, the method can be fine-tuned for optimal performance. The method can then be transferred to a binary HPLC system if desired, for simplicity and improved reliability. Using a buffer with lower pH than the isoelectric point (pI) of the targets will retain the analytes carrying a net positive charge, allowing for proper binding/interaction with the CEX stationary phase.

Identifying optimum conditions for three commercial MAbs

This technical note shows how to run a fast pH screening with YMC's BioPro IEX SF using a dial-a-mix method with a quaternary HPLC pump and a salt gradient. For this work three different commercially available MAbs, Cetuximab (Erbitux®), Bevacizumab (Avastin®) and Rituximab (MabThera®), with isoelectric points (pI) varying from 8.3 to 8.7 were screened with four different potassium phosphate buffers.

Chromatographic conditions

Column:	BioPro IEX SF (5 µm) 100 x 4.6 mm ID
Part number:	SF00S05-1046WP
Eluent:	A: 100 mM KH_2PO_4
	B: 100 mM K_2HPO_4
	C: 500 mM NaCl
	D: Water
Gradient:	0–30% 500 mM NaCl (0–55 min) for exact composition see below
Flow rate:	0.5 mL/min
Temperature:	25 °C
Sample:	Cetuximab, Bevacizumab, Rituximab (in 20 mM phosphate buffer at 1 mg/mL)
Injection:	10 µL
Detection:	UV at 220 nm

YMC Europe GmbH · Schöttmannshof 19 · 46539 Dinslaken · Phone +49 (0)2064 427-0 · Fax +49 (0) 2064 427-222 · Email: info@ymc.de · www.ymc.de

Gradient conditions:

Time (min)	% A + % B	% C	% D
0.0	20	0	80
55.0	20	30	50
55.1	20	0	80
60.0	20	0	80

pH dependent buffer composition:
(final phosphate buffer concentration of 20 mM)

pH value	% A	% B
6.2	16	4
6.4	14	6
6.6	12	8
6.8	10	10

Salt gradient screening

A quick screening of three MAbs was performed to determine a starting point for a salt gradient that would work well for eluting each antibody sample. Using knowledge obtained from previous IEX method development projects, a buffer pH of 6.6 was chosen for this process. All four samples were run under these conditions and it was determined that a shallow gradient of 0–30 % 500 mM NaCl over 55 minutes would enable proper elution for the pH screening analyses.

pH determination

Once the most appropriate NaCl concentration and gradient time were determined, the method was run at various buffer pH to determine which condition would be best for each individual sample. The elution pH was varied by changing the mobile phase composition of the two phosphate buffers (lines A and B) using the dial-a-mix method and quaternary pump for mixing. The ratio of mobile phases A and B remained constant throughout the runs to ensure pH was kept constant. As the salt gradient was increased (line C), the amount of water was decreased (line D) in proportion. Each antibody sample was evaluated at four different pH conditions.

Conclusion

The data shows that YMC's BioPro IEX SF performs well when using the dial-a-mix method for early screening of pH and salt gradient parameters. This method of screening allows faster method development, with sample analysis being completed overnight in some cases.

Once the data is analysed and approximate method parameters are obtained from dial-a-mix screenings, the method can then be converted to a two-mobile phase system using a binary pump HPLC.
This helps minimise any variability from using a quaternary pump for mobile phase mixing, and allows for optimisation of final method parameters.

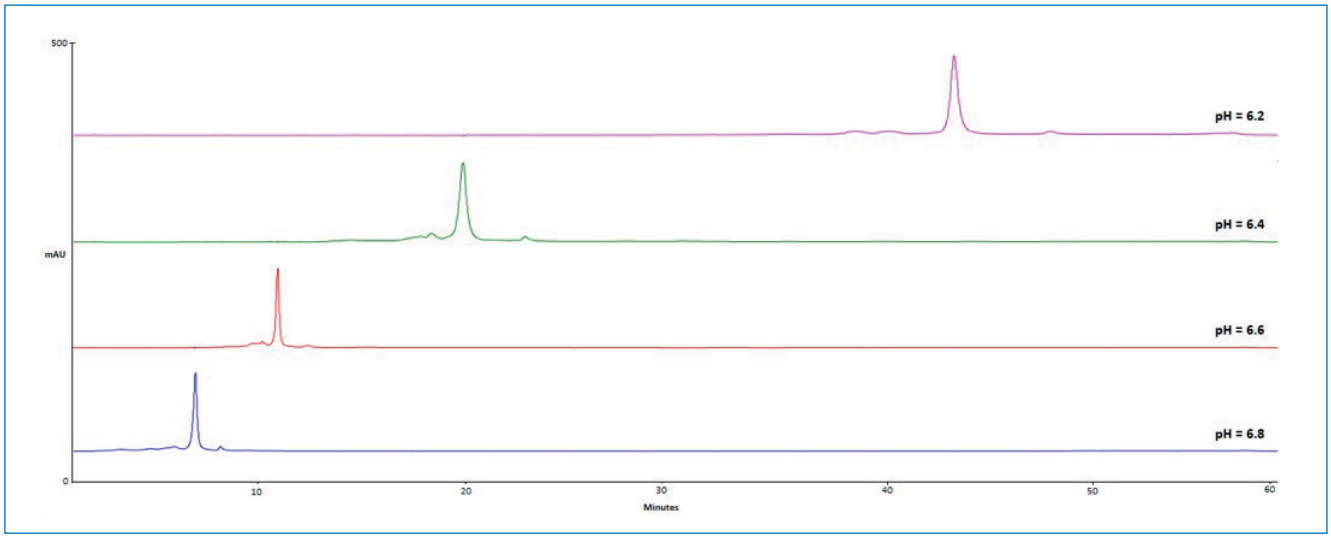

Figure 1: Chromatograms of Bevacizumab pH screening.

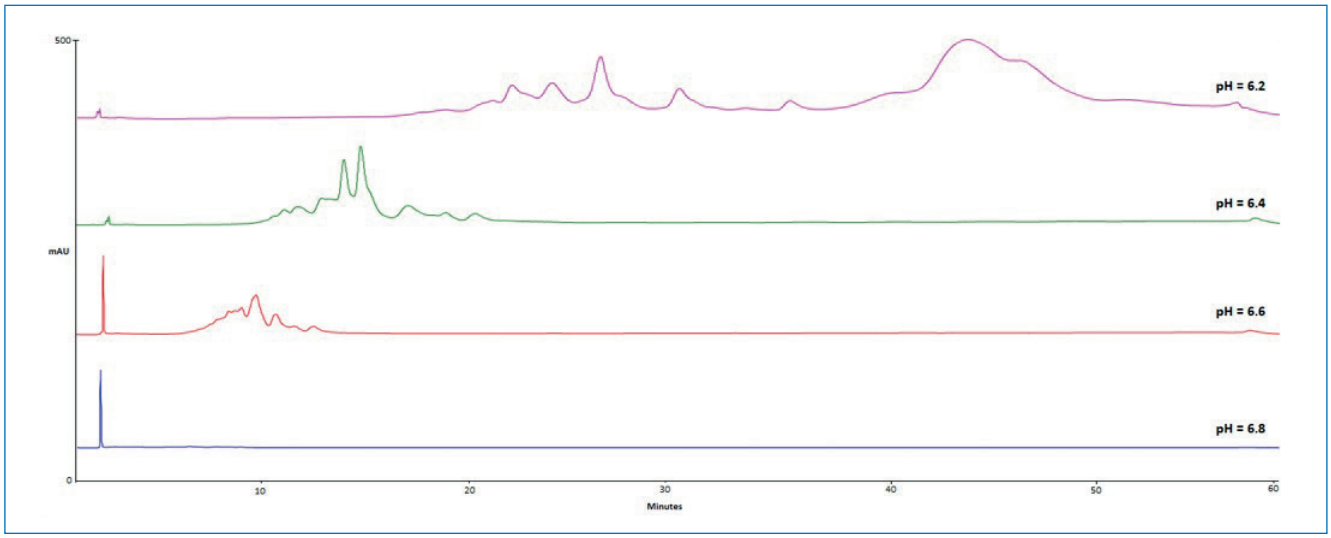

Figure 2: Chromatograms of Cetuximab pH screening.

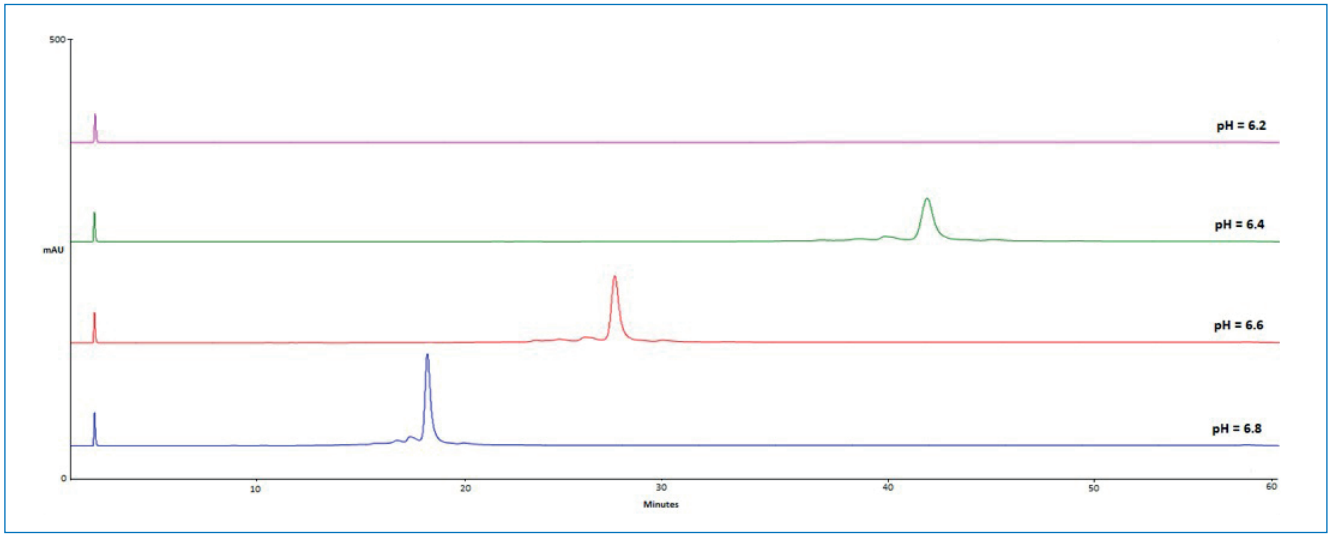

Figure 3: Chromatograms of Rituximab pH screening.

**Application data by courtesy of YMC America, Inc.*