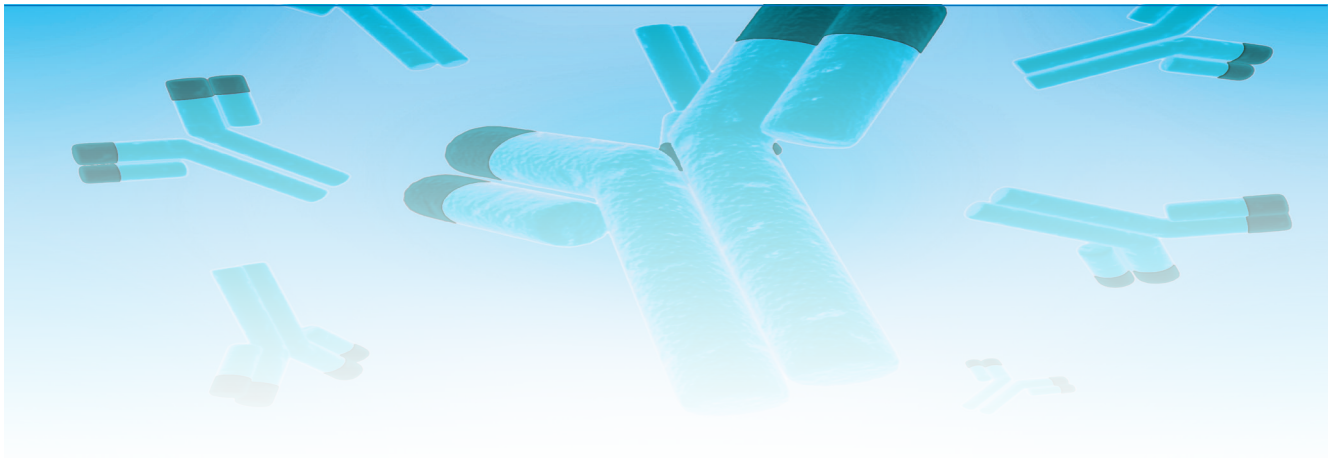

Characterisation of charge heterogeneity of therapeutic monoclonal antibodies via IEX-MS using BioPro IEX SF from YMC

Characterisation of different monoclonal antibodies in SCX-MS mode

1. Introduction

IEX is a nondenaturing technique used for separation and isolation of protein charge variants before characterisation via MS. This is a two-step process, where the specific charge variant peak of interest has to be isolated first for the following characterisation via mass spectrometry because in IEX mostly nonvolatile buffers are used, which are not compatible with MS. The coupling of MS to IEX would save time and exclude possible artefacts of the long isolation process. In addition, it is possible to overlook minor species that do not exhibit distinctive UV peaks in this two-step approach.

In this study by Yan et al. high resolution SCX was coupled directly to an ultrasensitive online native MS. A combined pH and salt gradient based on a MS compatible buffer system was utilised.
They used BioPro IEX SF, a non-porous strong cation exchange phase from YMC for the characterisation of five monoclonal antibodies with different isoelectric points and NISTmAb for the evaluation of this method.

This study was published in October 2018 in the journal ***Analytical Chemistry*** under the title ***Ultrasensitive characterization of charge heterogeneity of therapeutic monoclonal antibodies using strong cation exchange chromatography coupled to native mass spectrometry.*** [1]

2. Results

Yan et al. chose the BioPro IEX SF column (5 µm; formerly YMC-BioPro SP-F) over other tested columns because the non-porous polymer bead packing provides several advantages including fast elution properties, peak sharpness, high resolution and high recovery rate. These characteristics make the BioPro IEX SF especially suitable for micro-scale analysis.

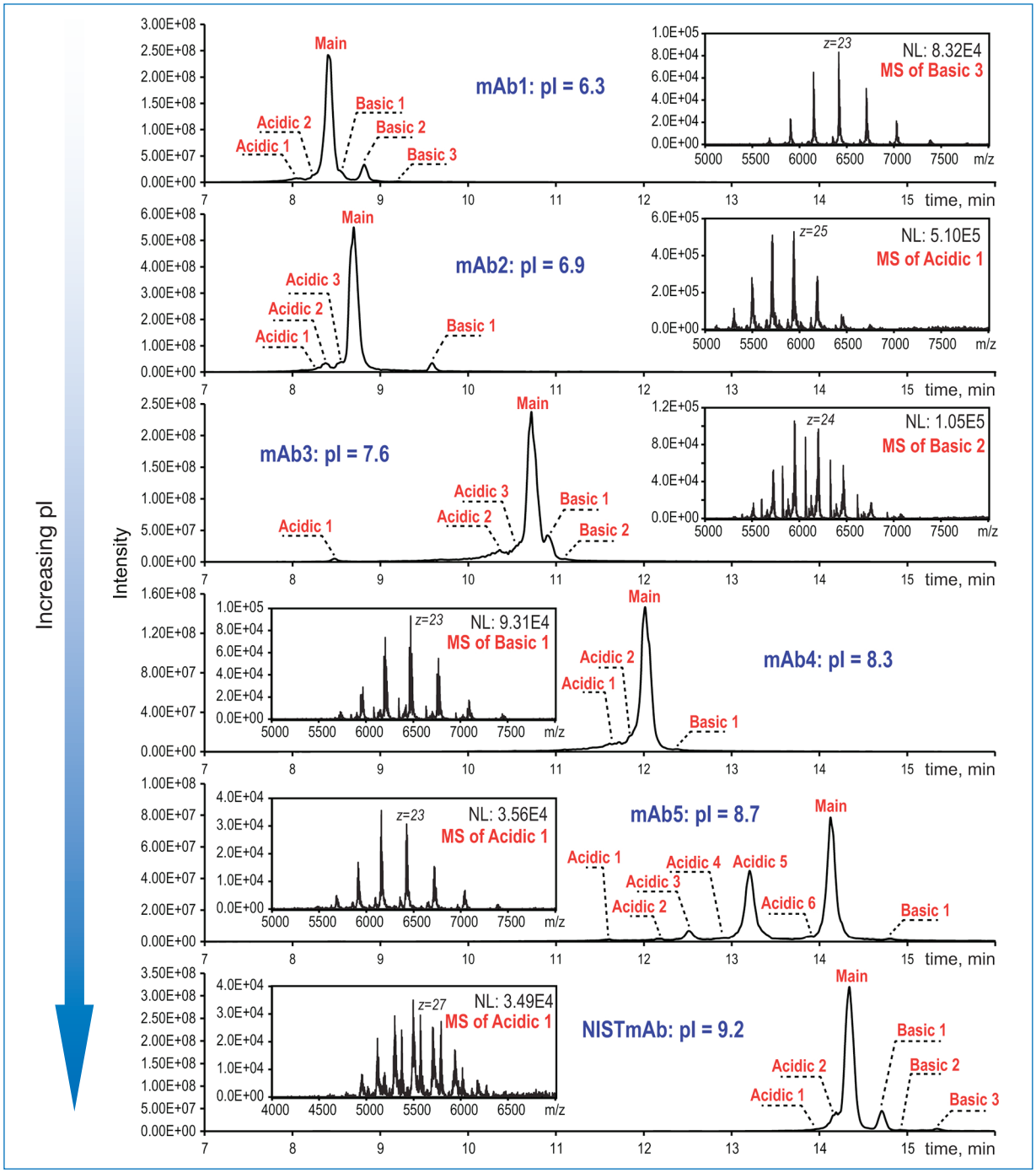

Figure 1: TICs of the SCX-MS analysis of 6 different mAbs with pI values ranging from 6.3 to 9.2. [1]

Separation of charge heterogenic mAbs via SCX-MS

Table 1: SCX-MS method parameters

Column	BioPro IEX SF (100 x 4.6 mm ID, 5 µm particle size)
Part. No.	SF00S05-1046WP
Mobile phase	A: 20 mM ammonium acetate pH 5.6 adjusted with 20 mM acetic acid B: 140 mM ammonium acetate, 10 mM ammonium carbonate, pH 7.6
Flow rate	0.4 mL/min
Gradient	0 % B (0 – 2 min), 0 – 100 % B (2 – 16 min), 100 % B (16 – 20 min), 100 % A (20 – 27 min)
Temperature	45 °C
Equipment	Post column analytical splitter (~ 400:1) to reduce analytical flow to ca. 1 µL/min
Detector	PDA (UV) PicoTip Emitter (NSI-MS)

The mobile phase composition and gradient were optimised to achieve an efficient separation of charge variants by changing the pH (5.6 – 7.4) and the salt concentrations (20 – 150 mM) in a range where mAbs are maintained in native states. A post column splitter was used so that nanospray ionisation could be performed while the separation was performed on an analytical scale with an ideal flow rate of 0.4 mL/min.
They used five in-house mAbs and NISTmAb with an isoelectric point pI between 6.3 and 9.2 to test the method. The retention time in general correlates with the pI of the mAbs. Several mAbs with a pI of 8 and higher eluted at a pH below 7.4, but each mAb exhibited a different retention time. In Figure 1 the chromatograms of the six mAbs are shown. All mAbs showed a good chromatographic separation of charge variants. It is apparent that the 2 – 6 acidic and 1 – 3 basic charge variant peaks were separated or partially separated from the main peak. Figure 1 also shows the mass spectrum of a minor peak of each mAb. The mass spectrum gives an indication of the modification of the charge variant, which is shown in Table 2.
Furthermore, with this SCX-MS method it is possible to detect and measure minor charge variant species present at levels below 1 % in all six mAbs.

This illustrates the high resolution of the BioPro IEX SF and the high sensitivity of the mass detector.

Table 2: Minor charge variants of the six different mAbs observed by SCX-MS analysis [1]

mAb sample	peak #	Δmass, Da	proposed identity	relative abundance[1]
mAb1	basic 3	+ 39.4	2× N-term Gln	0.2 %
mAb2	acidic 1	+ 176.7	1× glucuronyl	0.5 %
mAb3	basic 2	+ 254.4	2× C-term Lys	0.5 %
mAb4	basic 1	+ 129.0	1× C-term Lys	0.2 %
mAb5	acidic 1	+ 352.4	2× glucuronyl	0.4 %
NISTmAb	acidic 1	+ 2238.1	unknown	0.05 %

[1] *The relative abundance is calculated using a representative glycoform.*

3. Summary

With BioPro IEX SF a SCX-MS method that achieves both good chromatographic resolution and high MS data quality for mAbs has been developed. It is possible to analyse the charge heterogeneity of mAbs with a wide range of pI values.

Advantages:

- simple modifications to mobile phase pH and salt concentrations can be made without significantly impacting MS data quality
- pI ranges spanning from 6.3 to 9.2

The non-porous BioPro IEX SF offers:

- high efficiency
- very high resolution
- high recovery rate
- low operating pressures
- excellent batch-to-batch reproducibility

4. Literature

[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.