

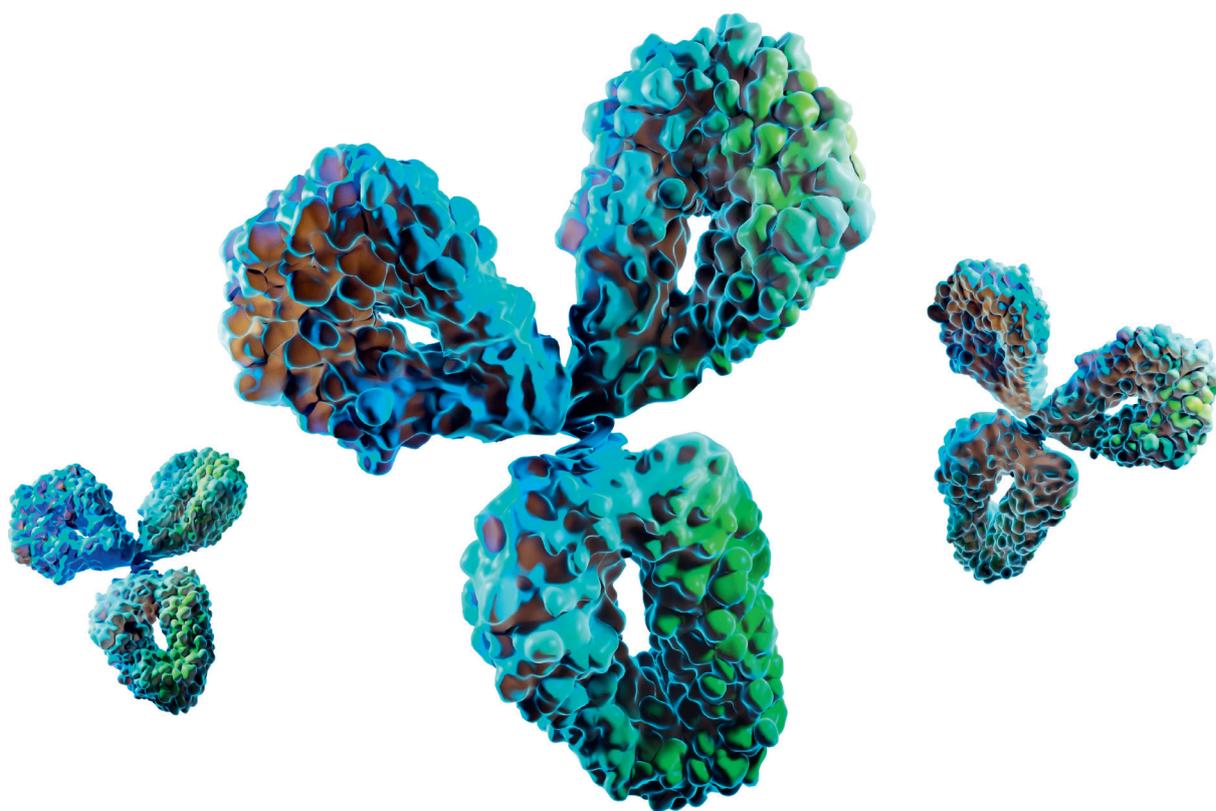


Native IEX-MS analysis of monoclonal antibodies using YMC Accura BioPro IEX SF columns

Direct coupling of ion exchange chromatography (IEX) to mass spectrometry (MS) is a game-changer for simplifying workflows and minimising artefacts caused by lengthy isolation steps. While volatile buffers and salts make coupling possible, the high salt concentrations typically used in IEX pose a challenge for MS compatibility.

To overcome this, nano-electrospray ionisation (nanoESI)

sources, which tolerate high salt concentrations, can be combined with systematic setups like flow splitters (reducing flow to sub- $\mu\text{L}/\text{min}$). However, even greater performance gains can be achieved using IEX columns with smaller inner diameters (ID) and bioinert coated hardware. This setup yields sharper peaks, higher resolution, and optimal recovery—even from the first injection.



This application note, based on data from Roche Diagnostics, demonstrates that using a 2.1 mm ID bioinert coated YMC Accura BioPro IEX SF column delivers superior resolution compared to a standard 4.6 mm ID PEEK column when coupled to MS.



Table 1: Chromatographic conditions.

Columns:	YMC Accura BioPro IEX SF (5 µm) 150 x 2.1 mm ID (bioinert coated stainless-steel hardware) BioPro IEX SF (5 µm) 100 x 4.6 mm ID (PEEK hardware)
Part Nos.:	SF00S05-15Q1PTC SF00S05-1046WP
Eluent:	A) 50 mM ammonium acetate*, 2% acetonitrile, pH 5.0 B) 160 mM ammonium acetate*, 2% acetonitrile, pH 8.5
Gradient:	0–100 %B in 55 min (NISTmAb) 47–58 %B in 55 min (trastuzumab)
Flow rate:	250 µL/min
Temperature:	45 °C
Injection:	5 µl (c=5 mg/ml)
Sample:	NISTmAb Trastuzumab
Detection:	UV at 280 nm nanoESI-MS positive mode
Systems:	Vanquish Flex UHPLC Vanquish Horizon UHPLC

*MS grade and usual HPLC grade used

UV detection of NISTmAb and trastuzumab

Before MS integration, the method's effectiveness was validated using UV detection. With detectors installed in series, UV analysis was performed first, followed by MS.

High resolution using the YMC Accura BioPro IEX SF column was confirmed by analysing the reference standard NISTmAb (Figure 1) and commercially available trastuzumab (Herceptin®), shown in Figure 2.

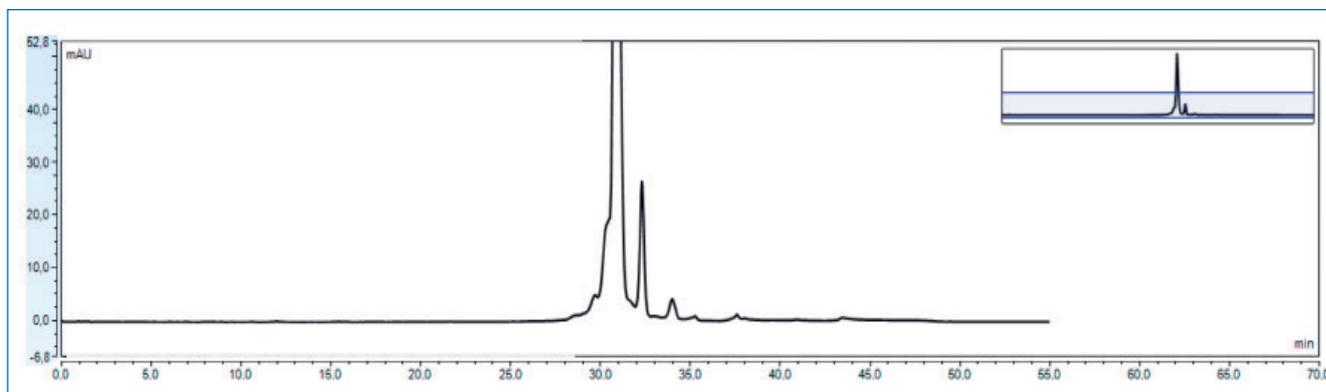


Figure 1: Chromatogram obtained for NISTmAb using the YMC Accura BioPro IEX SF column detected with UV.

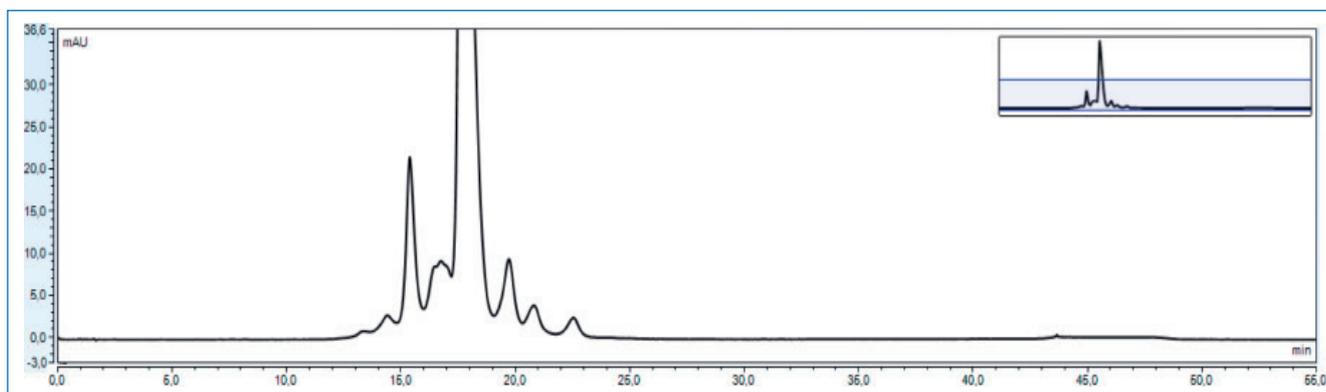


Figure 2: Chromatogram obtained for trastuzumab using the YMC Accura BioPro IEX SF column detected with UV.



LC-MS analysis of trastuzumab

The total ion chromatograms (TICs) for trastuzumab analysed with both columns are presented in Figure 3. The YMC Accura BioPro IEX SF column (top) consistently provided sharper peaks and significantly higher resolution compared to the larger-ID PEEK column.

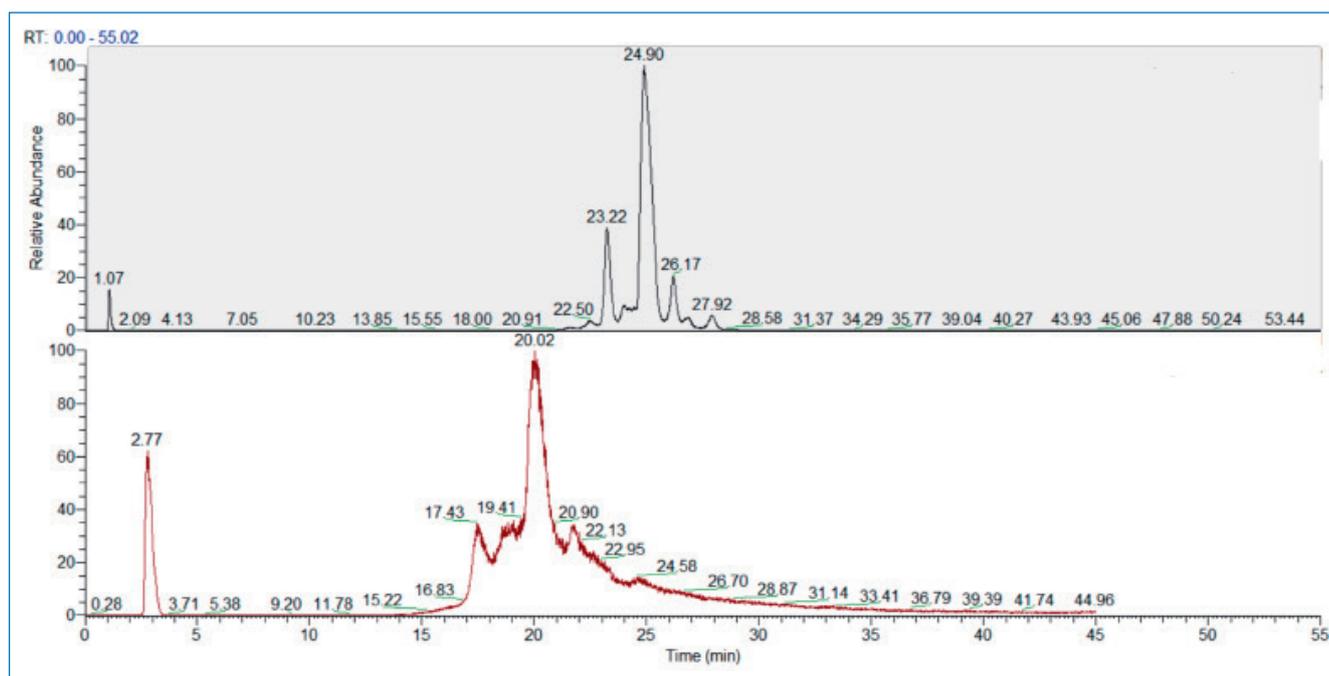


Figure 3: TICs of the analysis of trastuzumab using a YMC Accura BioPro IEX SF column (top) and the corresponding PEEK column (bottom) obtained on different HPLC systems.

Conclusion

The results demonstrate that the YMC Accura BioPro IEX SF column (2.1 mm ID) is ideally suited for native IEX-MS analysis of monoclonal antibodies, offering superior performance, sharper peaks, and high resolution for demanding workflows.

**Application data by courtesy of Oksana Tyshchuk, Roche Diagnostics GmbH, Penzberg, Germany*