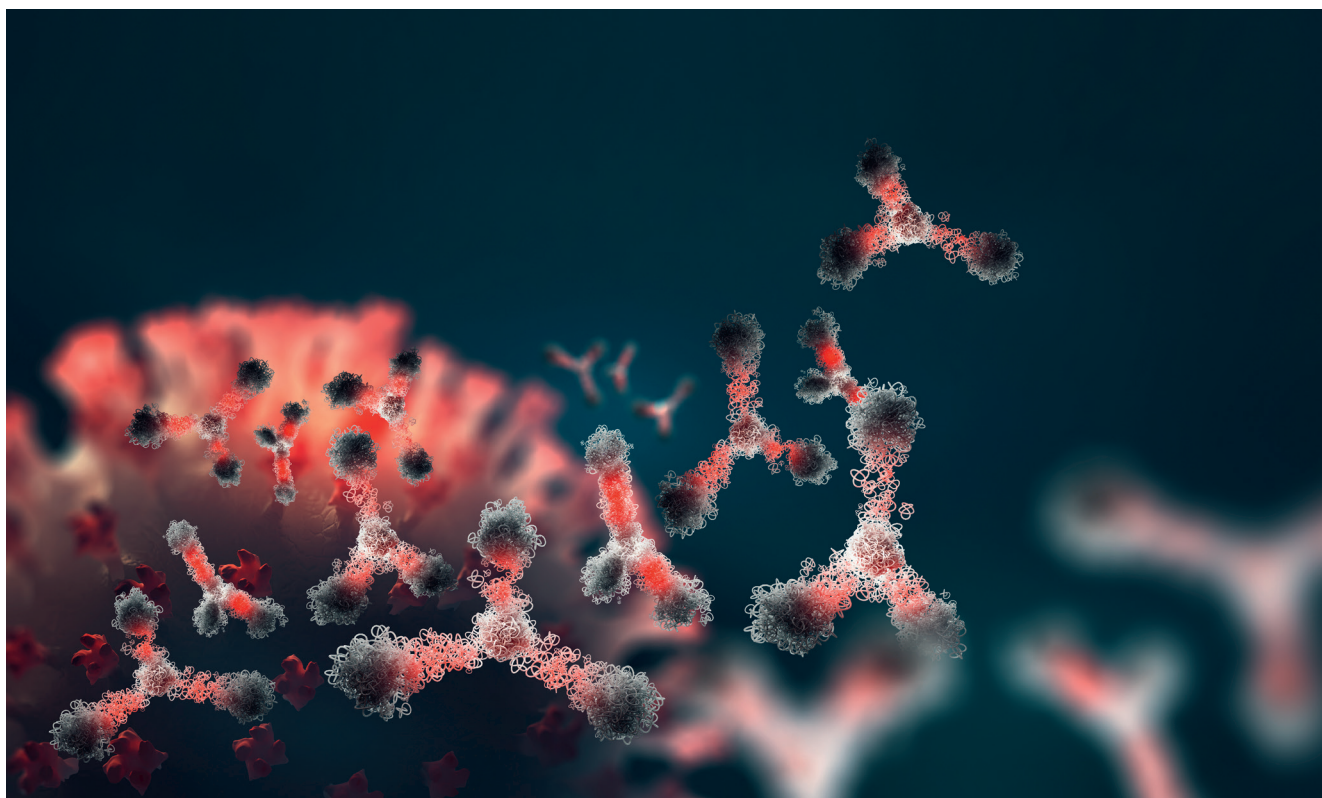


High recoveries from IEX analysis of mAbs with the first injection – no preconditioning required!

Ion exchange chromatography (IEX) is one of the standard methods for the characterisation of charge-sensitive biomolecules such as monoclonal antibodies (mAbs). Typically, bioinert polyether ether ketone (PEEK) is used for the column hardware.

Using bioinert coated stainless steel column hardware is an alternative approach because the bioinert coating has different surface properties. For example, the bioinert coating on YMC Accura columns is less hydrophobic compared to PEEK.



The influence can be demonstrated when analysing bevacizumab. For this purpose, a standard PEEK column and a bioinert coated YMC Accura BioPro IEX SF column were used. Detailed chromatographic conditions can

be found in Table 1. Since preconditioning is usually required for complete recovery, consecutive injections of 25 µg (5 µL) of bevacizumab were performed on both columns (see Figure 1).

Table 1: Chromatographic conditions.

Columns:	YMC Accura BioPro IEX SF (5 µm) 100 x 4.6 mm ID (bioinert coated hardware) BioPro IEX SF (5 µm) 100 x 4.6 mm ID (standard hardware)
Part Nos.:	SF00S05-1046PTC SF00S05-1046WP
Eluent:	A) 20 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 6.8) B) 20 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 6.8) containing 0.2 M NaCl
Gradient:	0–50%B (0–30 min), 0%B (30–45 min)
Flowrate:	0.5 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Injection:	5 µL
Sample:	Bevacizumab (5 mg/mL)
System:	bioinert UHPLC

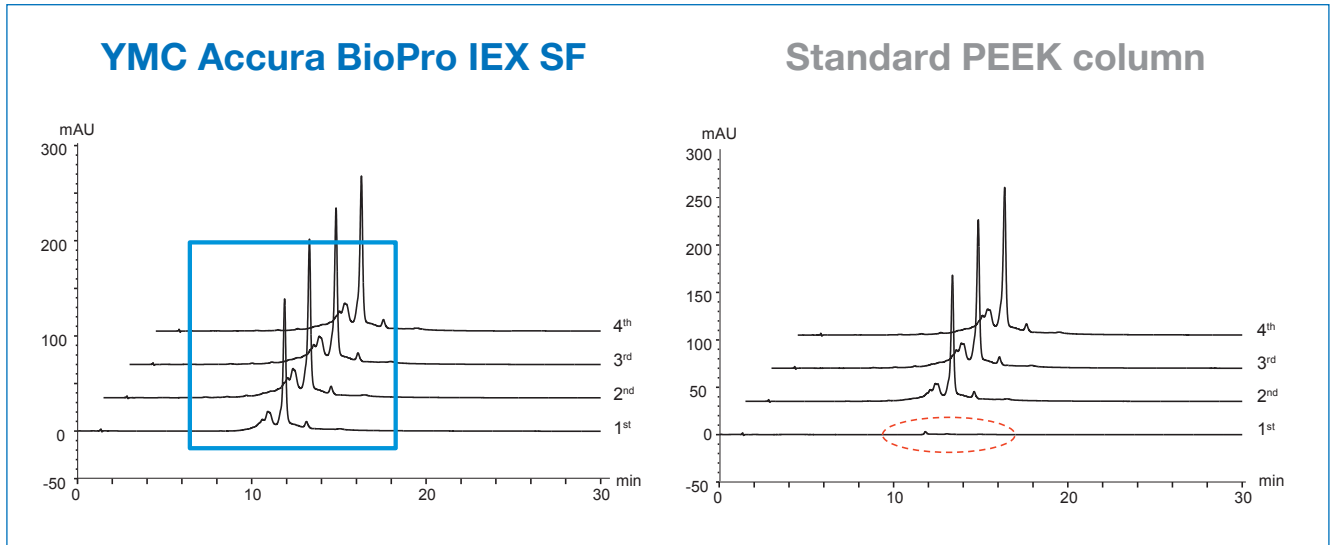


Figure 1: Comparison of the analysis of bevacizumab using the bioinert coated YMC Accura BioPro IEX SF column (left) and the corresponding PEEK column (right).

By using the YMC Accura BioPro IEX SF column, high recovery is achieved with the first injection. In contrast, adsorption of the mAb can be observed with the standard PEEK column.

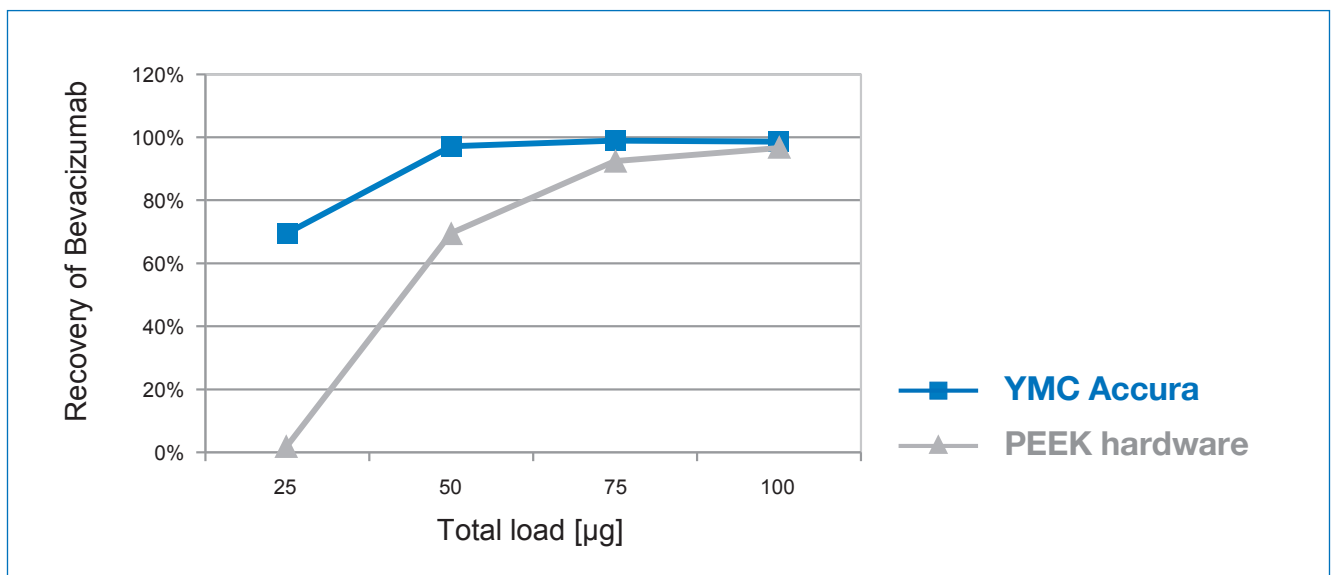


Figure 2: Relative recovery of bevacizumab using a YMC Accura BioPro IEX SF column (blue) and the corresponding PEEK column (grey) according to the total loading amount after consecutive 25 µg injections.

The PEEK column requires 4 injections for conditioning. By using the YMC Accura BioPro IEX SF column, virtually complete recovery is achieved with the second injection (see Figure 2).

Therefore, YMC Accura BioPro IEX SF columns are the ideal choice for a reliable and reproducible mAb analysis. This saves valuable samples by virtually eliminating the need for preconditioning.

