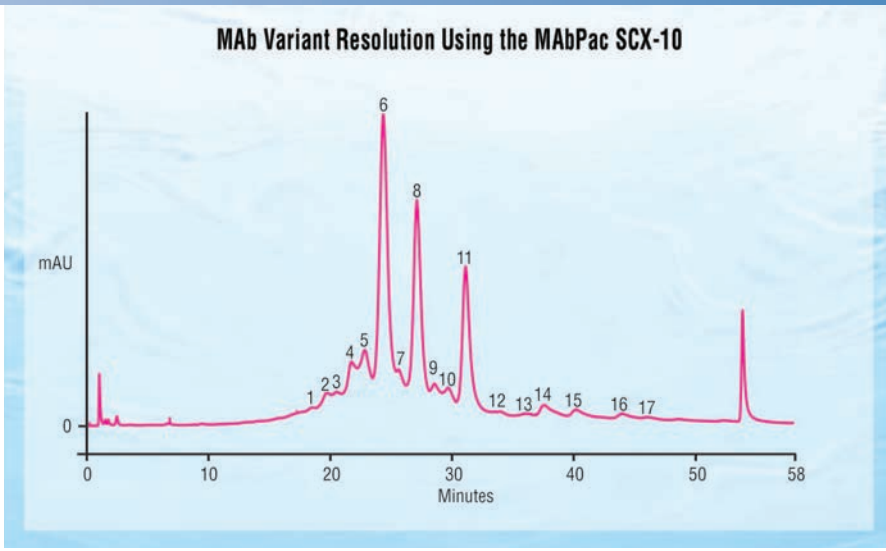
# MAbPac SCX-10 Column for Monoclonal Antibody Variant Analysis and Characterization

The Thermo Scientific™ MAbPac™ SCX-10 columns separate closely-related monoclonal antibody variants for characterization and quality control assessment.

- High resolution of monoclonal antibody variants
- Exceptionally high efficiency
- High throughput capability
- Available in 3, 5 or 10 µm particle size
- UHPLC column formats
- Excellent lot-to-lot and column-to-column reproducibility
- No Hydrophobic interactions
- Ideal for R&D and QA/QC development




## High-Resolution, High-Efficiency Analysis of Monoclonal Antibody Variants

The MAbPac SCX-10 column is a strong cation-exchange column designed specifically for the high resolution, high efficiency analysis of monoclonal antibodies and associated variants. The unique nonporous pellicular resin provides high resolving power, permitting the separation of monoclonal antibody variants that differ by as little as one charged residue.

The MAbPac SCX-10 columns are available in 10, 5, and 3 µm particle sizes in standard and UHPLC formats. Significantly faster analysis can be achieved on MAbPac SCX-10 columns with smaller particle sizes and in UHPLC formats. The MAbPac SCX-10, 3 and 5 µm particle size columns in a shorter format provide this exceptional capability.

The MAbPac SCX resin technology is the basis for the superior performance of monoclonal antibody variant analysis. The nonporous core particle provides high rates of mass transfer, which results in high efficiency separations. A proprietary hydrophilic layer surrounds the polymeric beads preventing hydrophobic interactions between proteins and the core of the resin. A proprietary grafted cation-exchange surface provides pH selectivity control and high-resolution separations.

The MAbPac SCX-10 column is complementary to the industry-leading Thermo Scientific ProPac WCX-10 column for monoclonal antibody variant analysis, offering an alternative selectivity and providing higher resolution and efficiency for variant analysis of monoclonal antibody samples.

## Characterization of Monoclonal Antibody Heterogeneity

Monoclonal antibodies are currently developed by pharmaceutical and biotechnology companies for various therapeutic applications. They undergo several posttranslational modifications including oxidations, deamidations, glycosylation, incomplete C-terminal processing, and others. These modifications cause antibody microheterogeneity or variants. Variations in a monoclonal antibody's composition can impact its activity and stability as a biotherapeutic. Monitoring stability of therapeutic monoclonal antibodies is regarded as essential for demonstrating safety and efficacy of a monoclonal antibody drug, and is expected by the FDA and other regulatory agencies. With its ability to characterize monoclonal heterogeneity, the MAbPac SCX-10 column can be used for stability testing and other monoclonal antibody applications by providing high efficiencies and high resolution of monoclonal antibody variant analysis.

## Acidic and Basic Variant Analysis

One of the most important and common analyses of monoclonal antibody heterogeneity is the monitoring and determination of acidic and basic variants. The MAbPac SCX-10 column provides excellent peak efficiencies and high resolution for acidic and basic variant analysis of monoclonal antibodies as shown in Figure 1, where three different monoclonal antibodies (A, B, C) are separated.

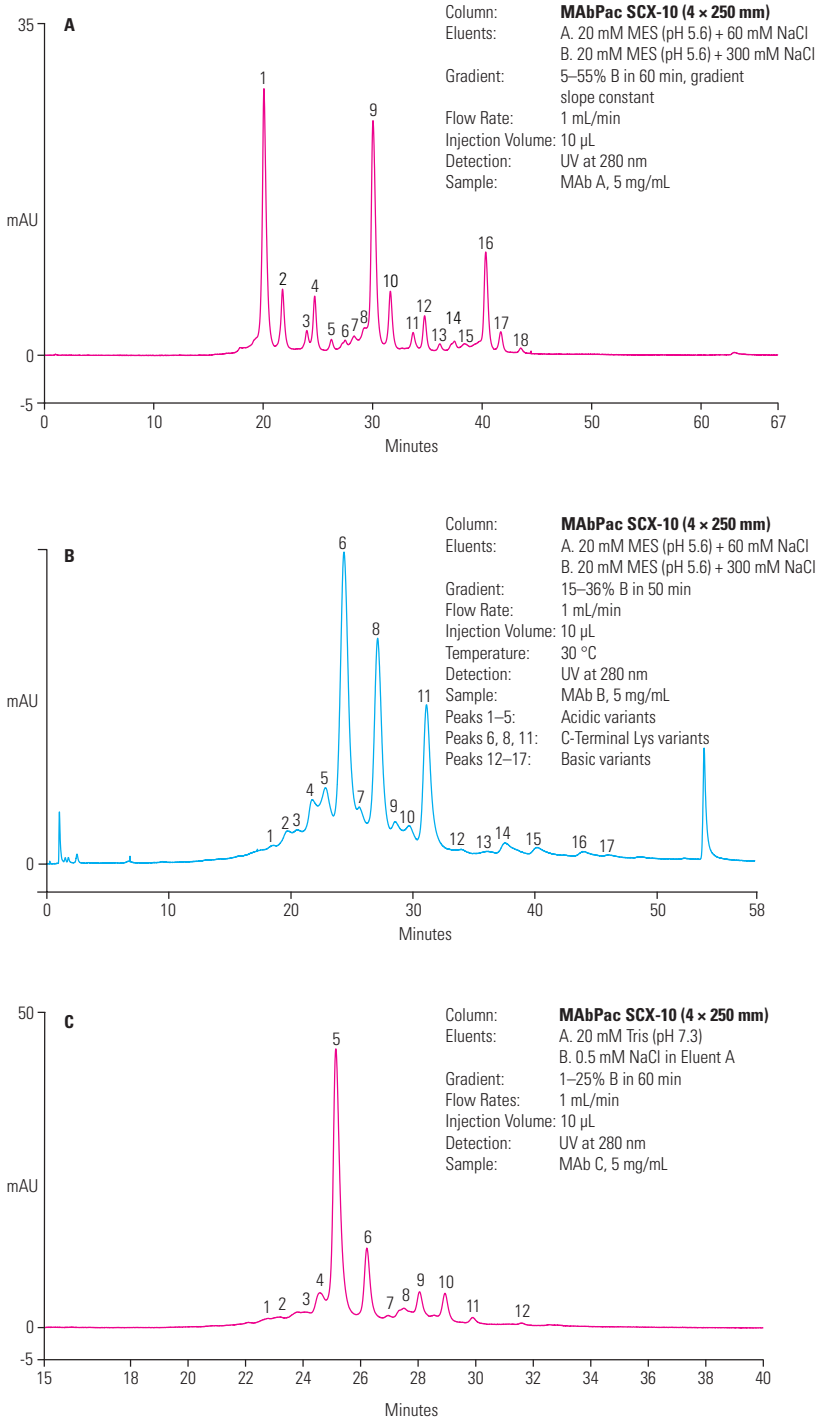



Figure 1: The MAbPac SCX-10 column provides excellent peak efficiencies and exceptional resolution of monoclonal antibody variants. A: MAb A separation showing excellent peak efficiencies; B: Separation of MAb B acidic, basic and C-terminal lysine variants; C: MAb C variant separation

## C-Terminal Lysine Variant Analysis

During the development and the production of therapeutic monoclonal antibodies, characterization of structural variants is a critical challenge. C-terminal processing of lysine residues on the heavy chain of monoclonal antibodies is a common structural variation that demands analysis. Incomplete monoclonal antibody processing results in charge heterogeneity, which is readily identified using the MAbPac SCX-10 column. Figure 2 illustrates this with the baseline resolution of C-terminal lysine truncation variants, and many other acidic and basic variants of a monoclonal antibody sample. After treatment with carboxypeptidase B, only one major peak remains, verifying that the three major peaks were due to different lysine truncations.

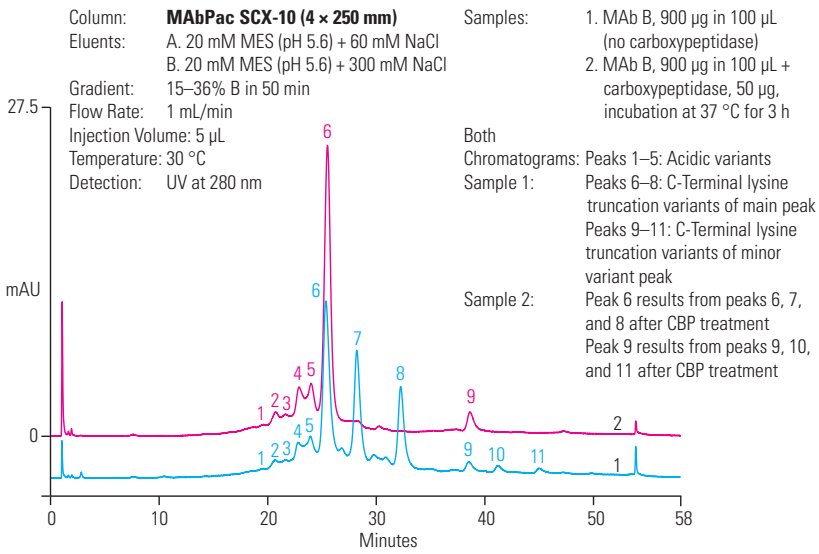



Figure 2: Baseline resolution of C-terminal lysine variants of a monoclonal antibody sample. A second chromatogram verifies that the three major peaks are due to variations in C-terminal content: after the treatment with carboxypeptidase B, only one major peak remains

## Analysis of MAb Fragments After Digestion with Papain and Carboxypeptidase

Monitoring stability of therapeutic proteins is regarded as essential for demonstrating safety and efficacy. Sometimes monoclonal antibodies are treated with papain and carboxypeptidase enzymes in order to monitor charge heterogeneity. Monoclonal antibodies treated with papain enzyme are separated into their Fab and Fc fragments. Figure 3 shows the well resolved Fab and Fc fragments after a monoclonal antibody and its variants are treated with papain alone, or with papain and carboxypeptidase together. The expected acidic, C-terminal lysine truncation-containing Fc fragments and Fab fragment peaks are determined and well resolved. Lysine truncation variant peaks collapse into one main peak with the carboxypeptidase treatment.

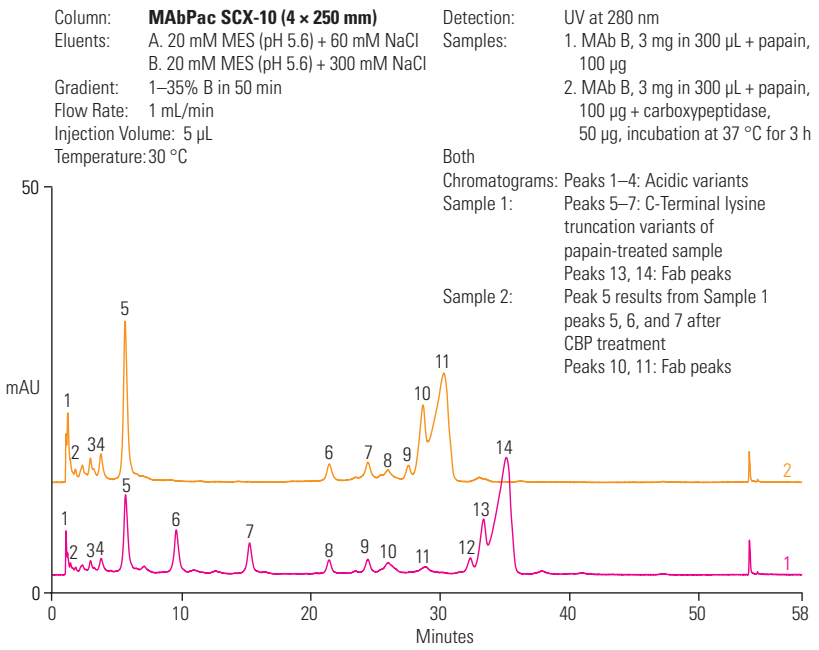



Figure 3: High resolution analysis of monoclonal antibody fragments after treatment with papain or papain and carboxypeptidase enzymes. The expected acidic, C-terminal lysine variants containing Fc, and Fab fragment peaks are well resolved

## Optimizing the Separation with pH Gradients

High-resolution monoclonal antibody variant separations are achieved through the optimization of different buffers, gradient changes, and pH selectivity control. Monoclonal antibody chromatographic separations can be achieved and optimized using gradients based on changing salt or pH conditions. Figure 4 demonstrates a high-resolution monoclonal antibody variant separation using a pH gradient.

The use of a pH gradient for the analysis of monoclonal antibody samples on MAbPac SCX-10 columns offers some key advantages. A single pH method can be used for the analysis of monoclonal antibody samples with varying iso-electric points. Also, pH gradients can provide high resolution separations on short length 50 mm columns thus providing short separation times or fast analysis. These attributes provide fast high-throughput analysis of multiproduct monoclonal antibody samples.

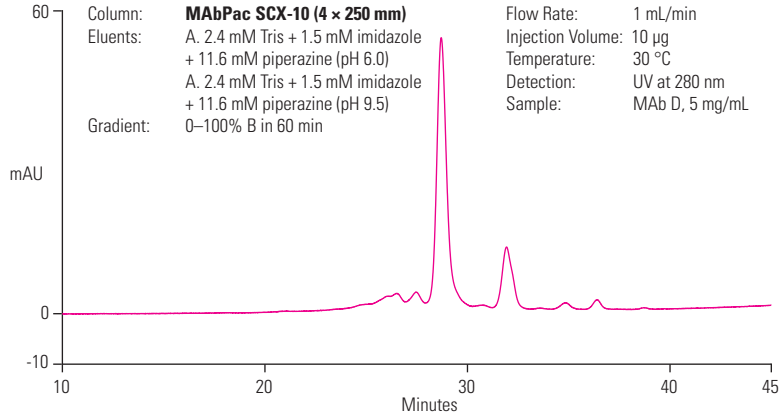



Figure 4: With the use of a pH gradient, the MAbPac SCX-10 column provides high resolution of monoclonal antibody variants

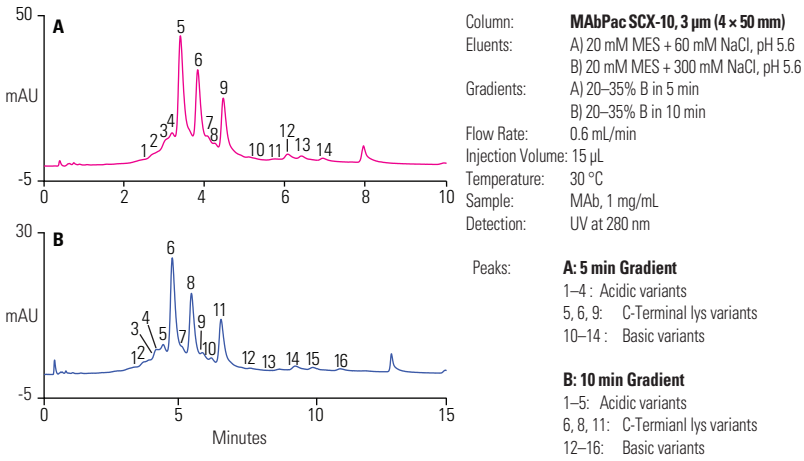
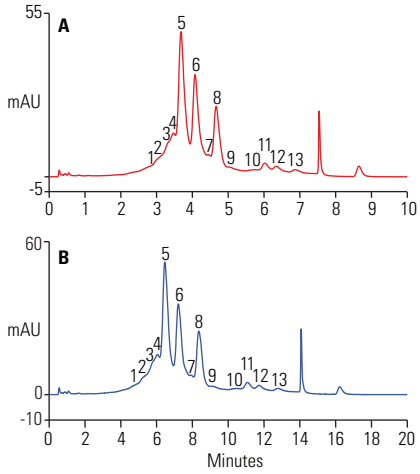



Figure 5: Fast salt-gradient elution: MAb separations using 3 µm MAb SCX-10 columns

## Fast MAB Characterization Analysis

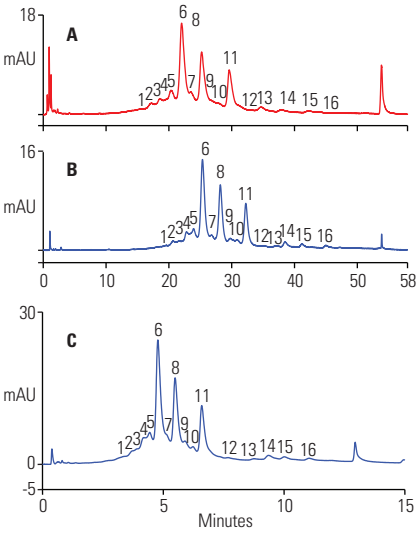
Fast high-resolution analysis of monoclonal antibody samples with exceptionally short separation run times can be achieved on MAbPac SCX-10 columns. This can be accomplished through the use of columns with 3 or 5 µm particle sizes and a column length of 50 mm. Using a 3 µm MAbPac SCX-10 column Figure 5 shows high resolution monoclonal antibody variant separations with exceptionally short separation times which were accomplished with pH and salt gradients, respectively. Figure 6 demonstrates the 5 µm MAbPac SCX-10 column providing fast, high resolution monoclonal antibody variant analysis using MES-based salt gradients. Panel A shows a 5-minute gradient and Panel B shows a 10-minute gradient. High resolution is achieved with a longer, shallow gradient.

The 3 and 5 µm MAbPac SCX-10 columns provide the same high resolution as the MAbPac SCX-10, 10 µm, longer columns but with significantly faster analysis time Figure 7 shows the 3 µm MAbPac SCX-10 column providing significantly faster analysis while maintaining the comparable high resolution for monoclonal antibody variant analysis as the MAbPac SCX-10, 10 µm, and Thermo Scientific™ ProPac™ WCX-10, longer columns.




| | |
|---|---|
| Columns: | **MAbPac SCX-10,** 5 µm, 4 × 50 mm |
| Eluents: | A: 20 mM MES + 60 mM NaCl, pH 5.6<br>B: 20 mM MES + 300 mM NaCl, pH 5.6 |
| Gradients: | A) 18–33% B in 5 min<br>B) 18–33% B in 10 min |
| Flow Rate: | 1 mL/min |
| Injection Volume: | 15 µL |
| Temperature: | 30 °C |
| Sample: | MAb, 5 mg/mL |
| Detection: | UV at 280 nm |
| Peaks: | 1–4. Acidic variants<br>5, 6, 8. C-Terminal lysine variants<br>9-13. Other basic variants |

Figure 6: The 5 µm MAbPac SCX-10, 4 × 50 mm column provides fast, high resolution monoclonal antibody variant analysis




| | |
|---|---|
| Columns: | A) ProPac WCX-10, 4 × 250 mm<br>B) MAbPac SCX-10, (10 µm) 4 × 250 mm<br>C) MAbPac SCX, 3 µm, 4 × 50 mm |
| Eluents: | A: 20 mM MES + 60 mM NaCl, pH 5.6<br>B: 20 mM MES + 300 mM NaCl, pH 5.6 |
| Gradients: | A) 25–46.44% B in 50 min<br>B) 15–36.44% B in 50 min<br>C) 20–35% B in 10 min |
| Flow Rate: | 1 mL/min (0.6 mL/min for C) |
| Injection Volume: | A and B: 5 µL (50 µg)<br>C: 15 µL (15 µg) |
| Temperature: | 30 °C |
| Detection: | 280 nm |
| Sample: | MAb; A and B) 10 mg/mL<br>C) 1 mg/mL |
| Peaks: | 1–5: Acidic variants;<br>6,8,11: Lysine truncation variants<br>12–16: Basic variants |

Figure 7: The 3 µm MAbPac SCX-10, 4 × 50 mm column provides significantly faster analysis while maintaining the similar high resolution as that obtained using the MAbPac SCX-10 and ProPac WCX-10, 4 × 250 mm columns

## UHPLC MAb Separation

Higher resolution MAb charge variant UHPLC separations can be achieved using the small particle MAbPac cation-exchange phase, with higher flow rate compatibility, together with specially developed bio-inert PEEK lined stainless steel column hardware. These columns take advantage of smaller resin size as well as longer column length to maximize the resolution of MAb variant separation, and are suitable for operation up to 10,000 Psi. Figure 8 shows an example of MAb separation comparing MAbPac SCX-10, 10 µm, 4 × 250 mm and UHPLC compatible MAbPac SCX-10, 5 µm, 4.6 × 250 mm columns. Both columns resolved acidic and basic variants from the main lysine truncation peaks. The resolution values of lysine truncation peaks are better on 5 µm column when compared to the 10 µm column, as expected. Higher pressure compatibility of the column hardware allows to use high flow rates for faster separation (See Figure 9).

**A.** Column: **MAbPac SCX, 10 µm**
Dimension: 4 × 250 mm
Sample: MAb 5 mg/mL
Injection Volume: 15 µL
Eluent A: 20 mM MES pH 5.6 + 60 mM NaCl
Eluent B: 20 mM MES pH 5.6 + 300 mM NaCl
Flow Rate: 0.76 mL/min
Gradient: 15-40% B in 30 min

**B.** Column: **MAbPac SCX, 5 µm**
Dimension: 4.6 × 250 mm
Sample: MAb 5 mg/mL
Injection Volume: 20 µL
Eluent A: 20 mM MES pH 5.6 + 60 mM
Eluent B: 20 mM MES pH 5.6 + 300 mM NaCl
Flow Rate: 1 mL/min
Gradient: 15-40% B in 30 min

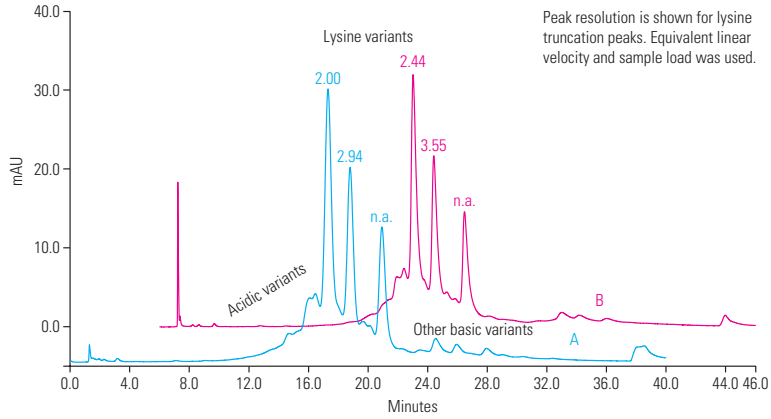
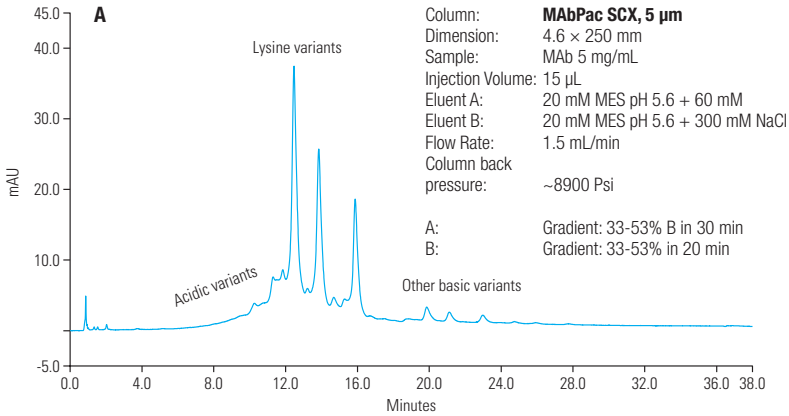



Figure 8: Comparison MAb Separation on UHPLC, BioRS MAbPac SCX-10, 5 µm with MAbPac SCX-10, 10 µm column



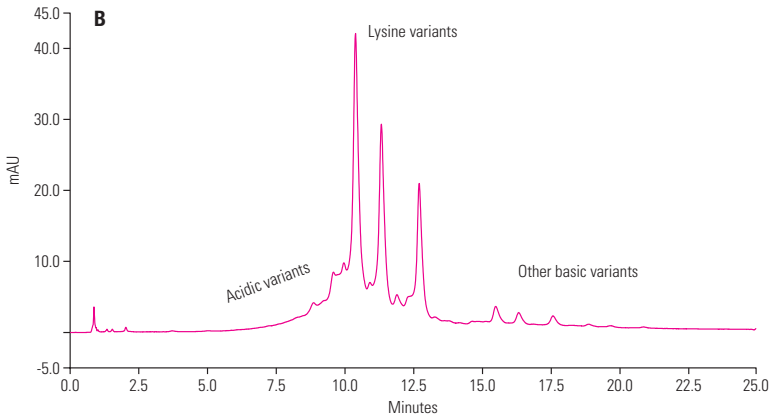




Figure 9: MAb Separation on UHPLC, BioRS MAbPac SCX-10, 5 µm, 4.6 × 250 mm at 1.5 mL/min flow rate

## Reproducibility

The MAbPac SCX-10 column's reproducible manufacturing process eliminates column and lot variability as a concern in methods development and data analysis. MAbPac columns are manufactured and tested under the strictest specifications resulting in unmatched column and lot reproducibility. Figure 10 shows column reproducibility with virtually no change in performance for four different columns.

In addition, Figure 11 demonstrates MAbPac SCX-10 lot reproducibility with virtually no change in performance for three different lots.

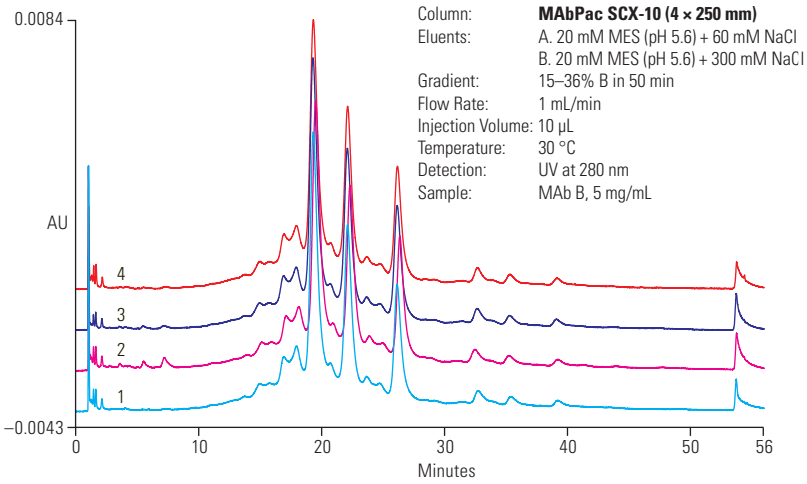



Figure 10: Demonstration of column-to-column reproducibility with virtually no change in performance for four different MAbPac SCX-10 columns tested

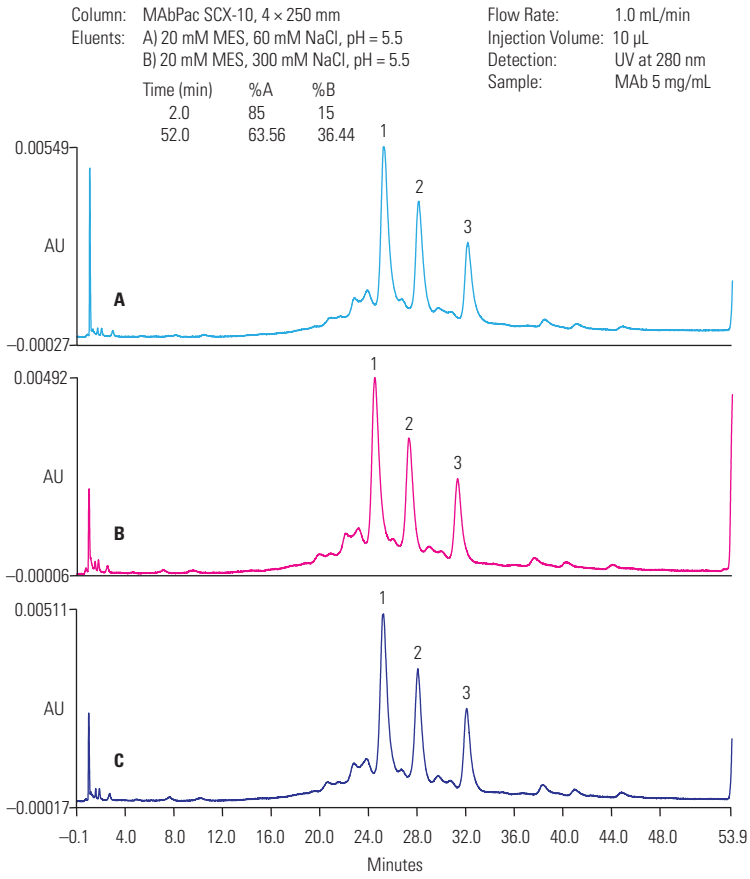



Figure 11: Excellent lot-to-lot reproducibility of the MAbPac SCX-10 column is shown. Columns from each of the three different lots (A, B, C) were used for MAb analysis

## Specifications and Operational Parameters

| | |
|---|---|
| Column construction | PEEK™ and PEEK lined Stainless Steel BioRS columns |
| Bead size | 3 µm, 5 µm, 10 µm |
| Substrate | Highly crosslinked DVB media |
| Pellicular layer | Proprietary hydrophilic |
| Functional group | Sulfonic |
| Pressure limit | 7000 psi for 3 µm, 3000 psi for 10 µm, 10,000 psi for BioRS column |
| Temperature range | Ambient to 60 °C |
| pH range | 2–12 |
| Dynamic loading capacity | Up to 100 µg, depending on the monoclonal antibody sample (10 µm, 4 × 250 mm) |
| Typical buffers | MES, or other Good's buffers, tris, phosphate |
| Solvents | 50% acetonitrile if needed for cleaning |
| Detergent compatibility | Nonionic, anionic, or zwitterionic detergents |

## Ordering Information

| MAbPac SCX-10 Analytical Column | Part Number |
|---|---|
| MAbPac SCX-10, 3 µm, Analytical Column (4 × 50 mm) | 077907 |
| MAbPac SCX-10, 5 µm, Analytical Column (4 × 50 mm) | 078656 |
| MAbPac SCX-10, 5 µm, Analytical Column (4 × 150 mm) | 085198 |
| MAbPac SCX-10, 5 µm, Analytical Column (4 × 250 mm) | 078655 |
| MAbPac SCX-10, 10 µm, Analytical Column (4 × 150 mm) | 075602 |
| MAbPac SCX-10, 10 µm, Analytical Column (4 × 250 mm) | 074625 |
| MAbPac SCX-10, 10 µm, Analytical Column SCX-10HT (4 × 50 mm) | 075603 |
| MAbPac SCX-10, 10 µm, Analytical Column SCX-10 (2 × 250 mm) | 075604 |

| UHPLC, BioRS MAbPac SCX-10 Analytical Column | Part Number |
|---|---|
| MAbPac SCX-10 RS, 5 µm, Analytical Column (4.6 × 50 mm) | 082674 |
| MAbPac SCX-10 RS, 5 µm, Analytical Column (4.6 × 150 mm) | 085209 |
| MAbPac SCX-10 RS, 5 µm, Analytical Column (4.6 × 250 mm) | 082673 |

| Lot Select Column Set | Part Number |
|---|---|
| Lot Select Column Set—Three columns from one resin lot (4 × 250 mm) | SP6864 |
| Lot Select Column Set—One column from each of three resin lots (4 × 250 mm) | SP6865 |

| MAbPac SCX-10 Semipreparative Column | Part Number |
|---|---|
| MAbPac SCX-10, 10 µm, Semipreparative Column (9 × 250 mm) | SP6866 |

| MAbPac SCX-10 Guard Column | Part Number |
|---|---|
| MAbPac SCX-10 Guard Column (2 × 50 mm) | 075749 |
| MAbPac SCX-10 Guard Column (4 × 50 mm) | 074631 |

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