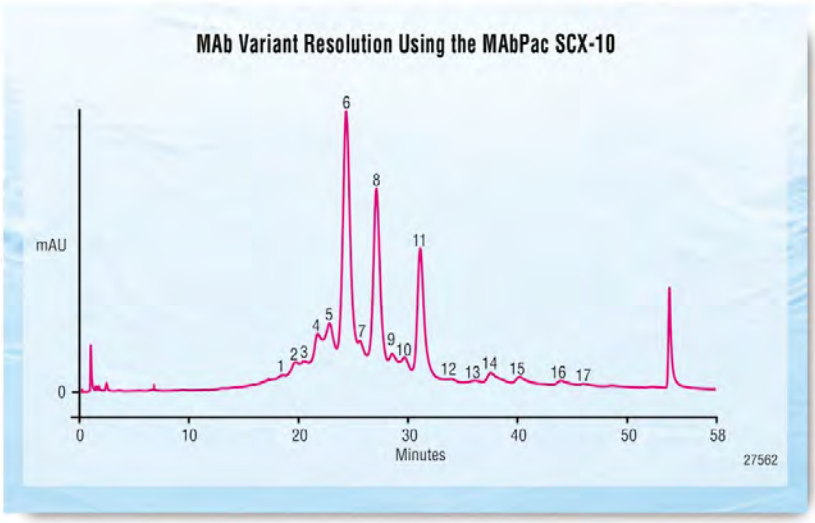
MAbPac SCX-10 Column for Monoclonal Antibody Variant Analysis and Characterization



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

- **Highest resolution of monoclonal antibody variants**
- **Exceptionally high efficiency**
- **Fast Analysis**
- **Available in 3, 5 or 10 µm particle size**
- **Excellent column-to-column and lot-to-lot reproducibility**
- **Hydrophobic interactions essentially eliminated**
- **Ideal for stability and QA/QC testing**

High-Resolution, High-Efficiency Fast 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 exceptionally high resolving power, permitting the separation of monoclonal antibody variants that differ by as little as one charged residue. Hydrophobic interactions with the resin are essentially eliminated, resulting in highly efficient peaks.

While maintaining the same high resolution of longer MAbPac SCX-10 column formats, significantly faster analysis of monoclonal antibody variant samples can be achieved on MAbPac SCX-10 columns of shorter format and smaller particle size. The MAbPac SCX-10, 3 and 5 µm particle size columns in a 4 × 50 mm format provide this exceptional capability.

The MAbPac 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 hydrophilic layer surrounds the polymeric beads, preventing hydrophobic interactions between proteins and the resin, also contributing to peaks with high efficiency. A proprietary grafted cation-exchange surface provides pH selectivity control, resulting in high-resolution separations.

The 4 × 250 mm 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 most monoclonal antibody samples. The columns are designed to address regulatory requirements for biopharmaceutical characterization. Consistent manufacturing processes ensure reproducibility in methods development and data analysis.

Applications

Use of the MAbPac SCX-10 column has been demonstrated for analysis and characterization of monoclonal antibodies requiring high resolution and efficiency (Figure 1A).

Monoclonal antibodies are currently developed by pharmaceutical and biotechnology companies for various therapeutic applications. Monoclonal antibodies undergo several post-translational 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 exceptionally high efficiencies and high resolution of monoclonal antibody variants.

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 exceptionally high resolution for acidic and basic variant analysis of monoclonal antibodies. Figure 1B shows the high resolution separation of monoclonal antibody acidic and basic variants, and the C-terminal lysine variants. Five acidic and eight basic variant peaks are resolved. Another example is shown in Figure 1C, which displays four acidic and seven basic monoclonal antibody variant peaks resolved using the MAbPac SCX-10 column.

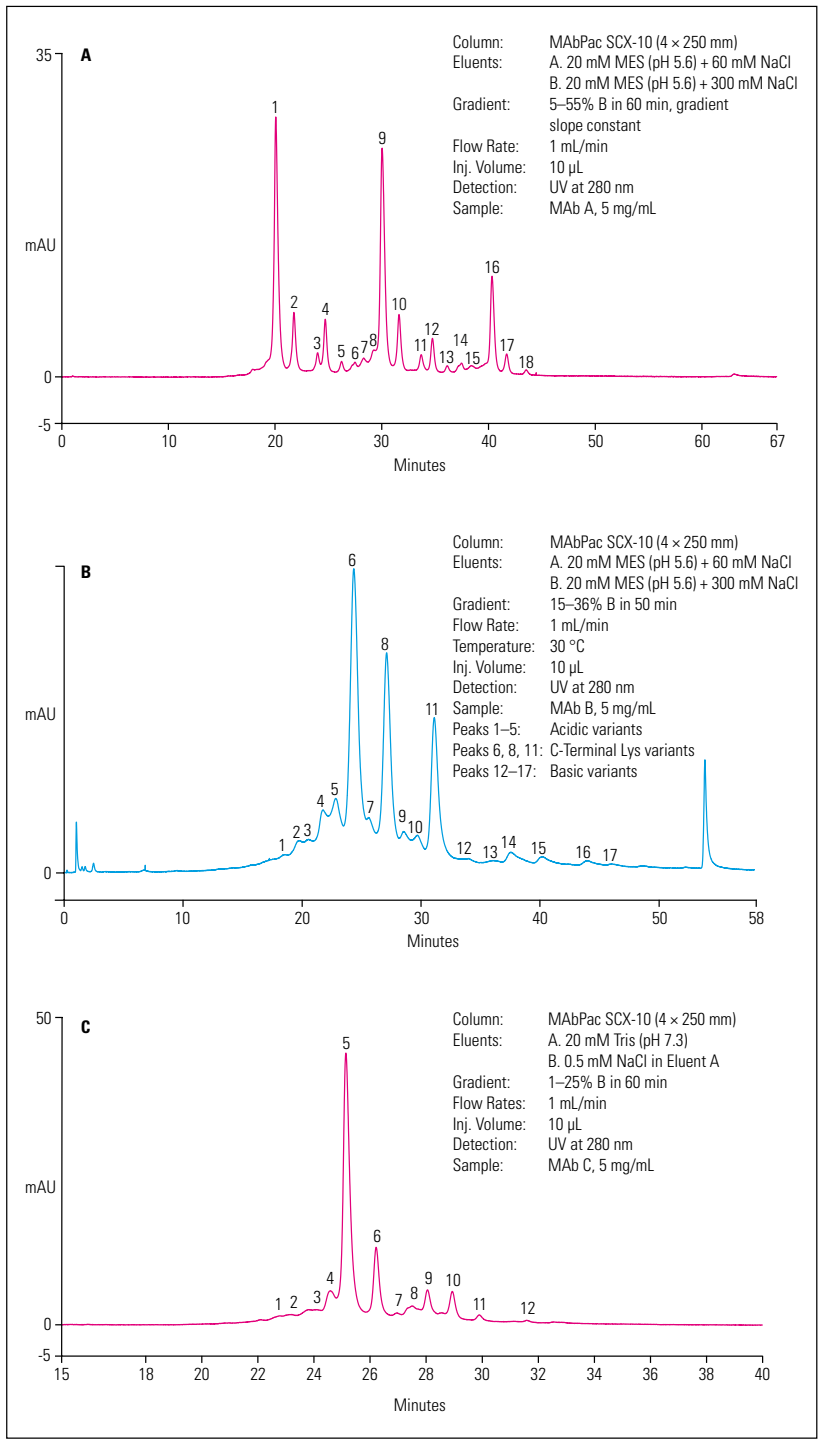


Figure 1. The MAbPac SCX-10 column provides excellent peak efficiencies and exceptional resolution of monoclonal antibody variants. A) MAb A separation showing excellent peak efficiencies; B) Separation of MAb B acidic, basic and C-terminal lysine variants; C) MAb C variant separation.

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 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 variations in C-terminal lysine presence.

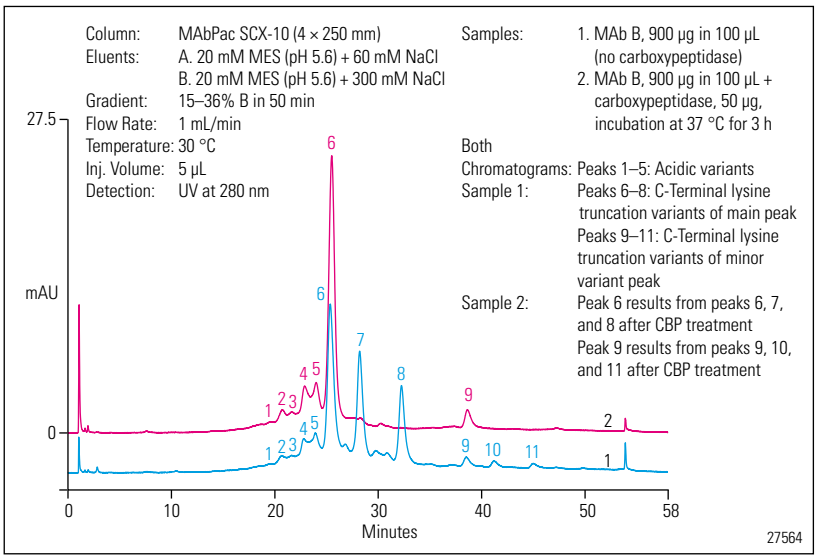


Figure 2. Baseline resolution of C-terminal lysine variants of a monoclonal antibody sample. A second chromatogram verifies that the three major peaks are due to variations in C-terminal content: after the treatment with carboxypeptidase B, only one major peak remains.

Analysis of MAb Fragments After Digestion with Papain and Carboxypeptidase

The MAbPac SCX-10 column can successfully provide high resolution and efficiency for monoclonal antibodies that have been treated with papain and carboxypeptidase enzymes. 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.

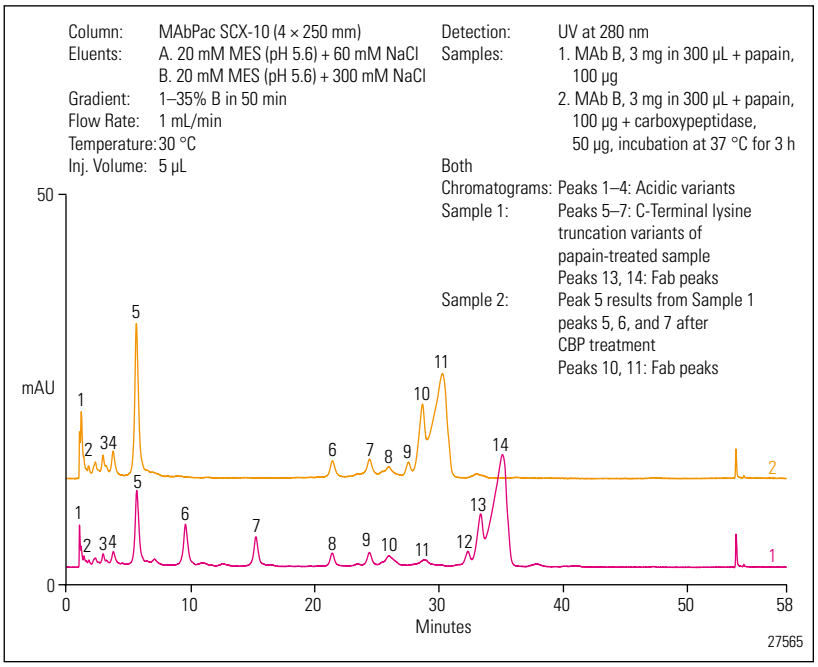


Figure 3. High resolution analysis of monoclonal antibody fragments after treatment with papain or papain and carboxypeptidase enzymes. The expected acidic, C-terminal lysine vatiants containing Fc, and Fab fragment peaks are well resolved.

Optimization

The MAbPac SCX-10 resin technology provides the ability to optimize high-resolution monoclonal antibody variant separations. High-resolution 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 5 shows the use of a pH gradient on a 4 × 50 mm MAbPac SCX-10, 3 µm column for the fast high resolution analysis of a monoclonal antibody variant sample which takes only 15 minutes.

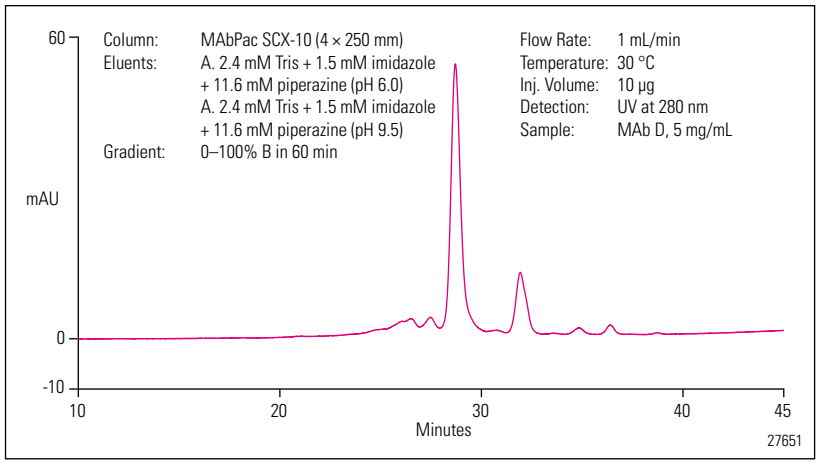


Figure 4. With the use of a pH gradient, the MAbPac SCX-10 column provides high resolution of monoclonal antibody variants.

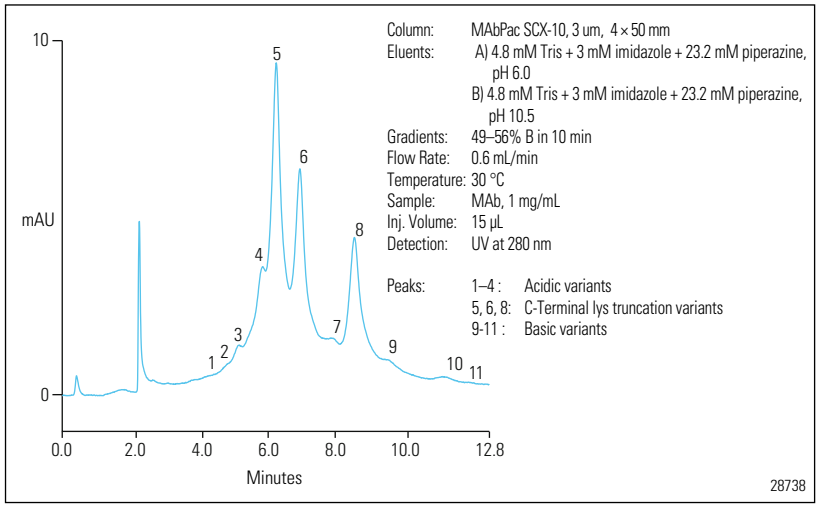


Figure 5. Fast pH-gradient elution: MAb separation using 3 µm MAb SCX-10 columns.

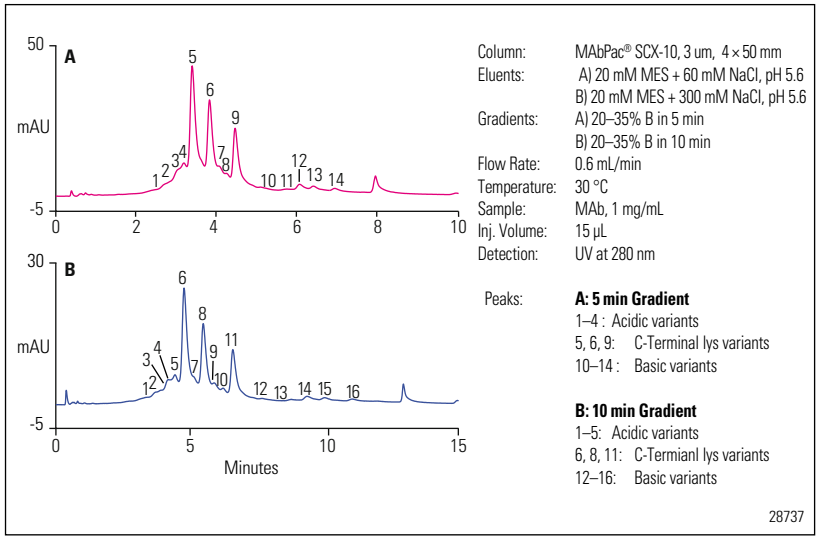


Figure 6. Fast salt-gradient elution: MAb separations using 3 µm MAb SCX-10 columns.

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 4 × 50 mm, 3 µm MAbPac SCX-10 column Figures 5 and 6 show high resolution monoclonal antibody variant separations with exceptionally short separation times which were accomplished with pH and salt gradients, respectively. Figure 7 demonstrates the 5 µm MAbPac SCX-10, 4 × 50 mm 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, 4 × 50 mm MAbPac SCX-10 columns provide the same high resolution as the MAbPac SCX-10, 10 µm, 4 × 250 mm columns but with significantly faster analysis time. Figure 8 shows the 3 µm MAbPac SCX-10, 4 × 50 mm column providing significantly faster analysis while maintaining the comparable high resolution for monoclonal antibody variant analysis as the MAbPac SCX-10, 10 µm, and ProPac™ WCX-10, 4 × 250 mm columns.

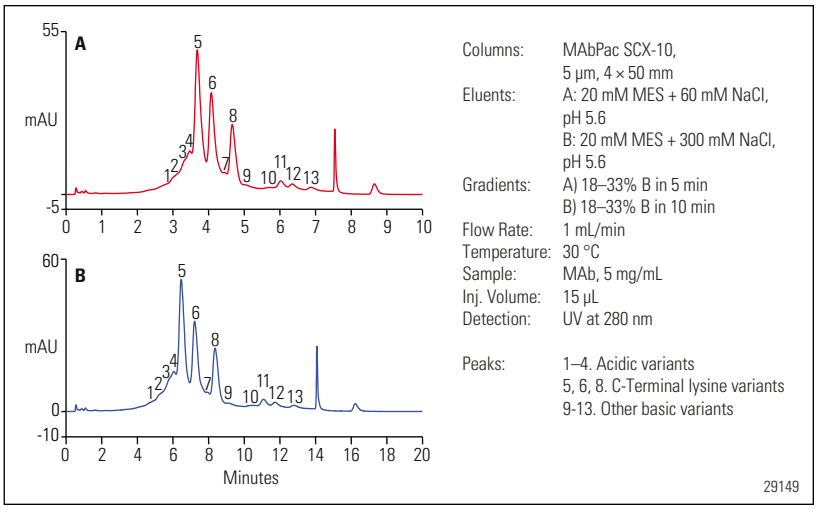


Figure 7. The 5 µm MAbPac SCX-10, 4 × 50 mm column provides fast, high resolution monoclonal antibody variant analysis.

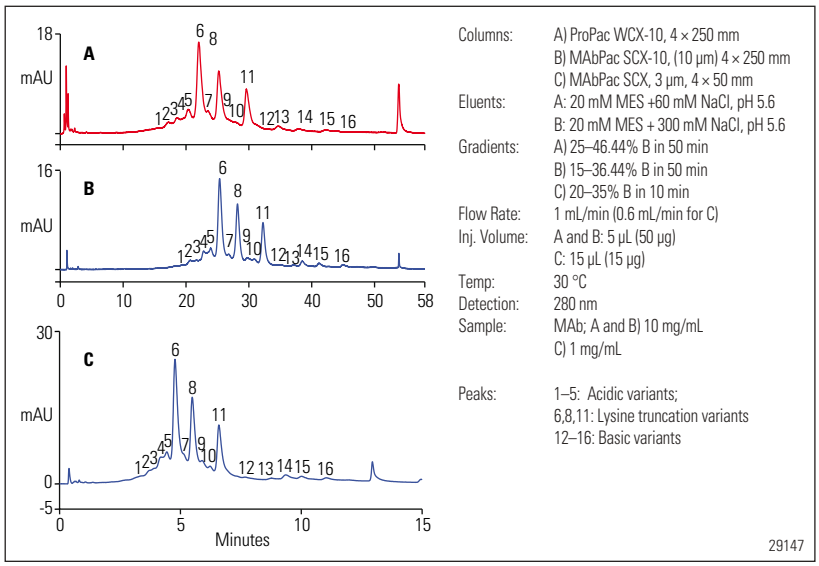


Figure 8. The 3 µm MAbPac SCX-10, 4 × 50 mm column provides significantly faster analysis while maintaining the similar high resolution as that obtained using the MAbPac SCX-10 and ProPac WCX-10, 4 × 250 mm columns.

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 9 shows column reproducibility with virtually no change in performance for four different columns.

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

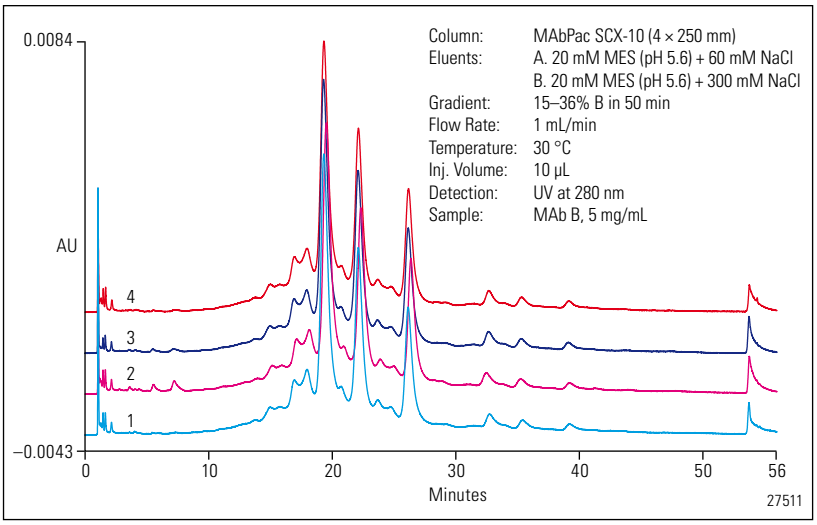


Figure 9. Demonstration of column-to-column reproducibility with virtually no change in performance for four different MAbPac SCX-10 columns tested.

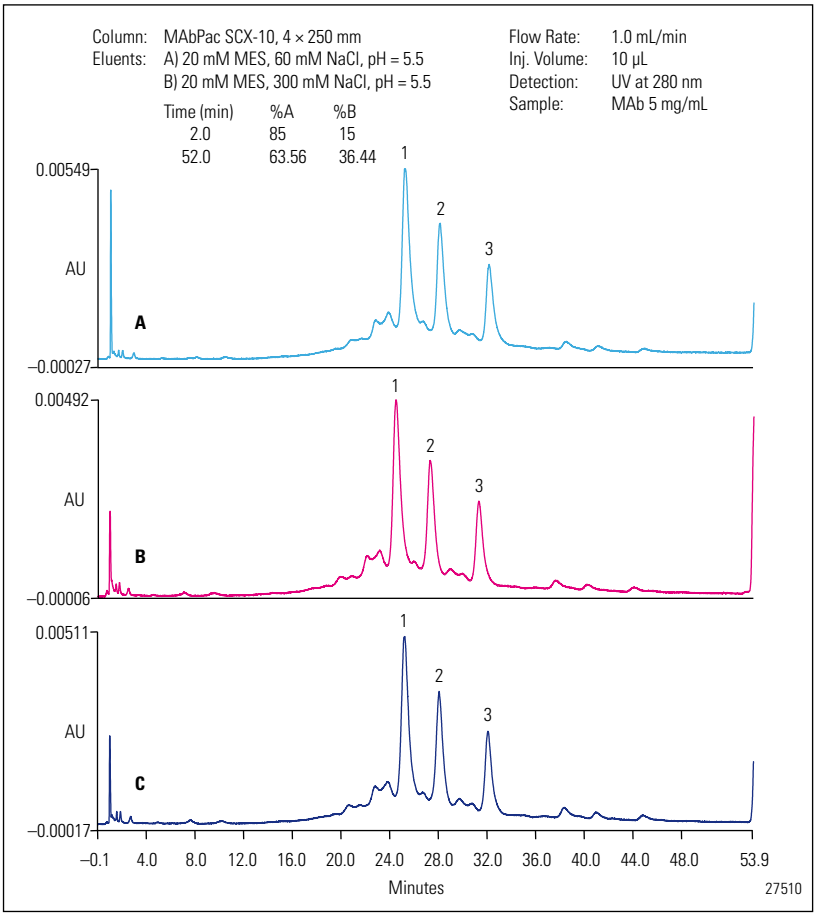


Figure 10. Excellent lot-to-lot reproducibility of the MAbPac SCX-10 column is shown. Columns from each of the three different lots (A, B, C) were used for MAb analysis.

SPECIFICATIONS

Column Construction	PEEK™
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
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

To order in the U.S., call 1-800-346-6390, or contact the Thermo Fisher Scientific office nearest you. Outside the U.S., order through your local Thermo Fisher Scientific office or distributor. Refer to the following part numbers.

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 × 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
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

LPN 2567-06 01/12

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