

Monoclonal Antibody Heterogeneity Characterization Using Cation-Exchange Columns

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ABSTRACT

Monoclonal antibodies (MAbs) have been approved to treat various diseases, such as cardiovascular and inflammatory diseases and cancer, among others. More than 20 MAbs have been approved, and an additional 150 MAbs are currently in preclinical or clinical trials, or awaiting FDA approval. In the last decade, the MAbs market has grown exponentially, resulting in multibillion dollar annual sales revenue. Accordingly, therapeutic proteins and monoclonal antibodies now form the largest part of the growing biologics drug market and have transformed the biotechnology and biopharmaceutical industries.

Monoclonal antibodies generally exhibit charge heterogeneity from oxidation, asparagine deamidation, aspartic isomerization, lysine truncation, glycan modifications, and other modifications. Therefore, the manufacture and testing procedures of MAbs involve routine analyses and monitoring of impurities resulting from these situations. Weak cation-exchange (WCX) columns are well suited and widely used for the characterization of MAb heterogeneity. ProPac® WCX columns packed with nonporous 10 µm particles with carboxylic acid functional groups are well suited for high-resolution analytical separations of target MAbs from both acidic and basic variants. The authors recently developed a new, strong cation-exchange (SCX) column for MAb analysis with sulfonate functionality and an alternative selectivity, that is complementary to the ProPac WCX column.¹

The new SCX column is built with a newly designed hydrophilic phase with a well-controlled, uniform, and stable ion-exchange separation surface on highly crosslinked polymeric nonporous resin. Here, the authors demonstrate heterogeneity characterization of MAbs using both the ProPac WCX and the new SCX columns, emphasizing the utility and applications of this new SCX column. Further, reproducibility and robustness of the new column are also demonstrated.

INTRODUCTION

Monoclonal antibodies (MW 150 kDa) are composed of one heavy chain (MW 50 kDa) and two types of light chains (kappa and lambda, MW 25 kDa).² They generally exhibit charge heterogeneity from oxidation, asparagine deamidation, aspartic isomerization, lysine truncation, glycan modifications, and other modifications. Therefore, manufacturing and subsequent quality assurance and stability testing procedures of MAbs involve routine analyses and monitoring of the impurities resulting from these situations. Ion-exchange columns (IEX), in general, and weak cation-exchange (WCX) columns, in particular, are well suited for the characterization of MAb heterogeneity.

ProPac weak cation-exchange (WCX) columns are routinely used to characterize MAb heterogeneity as they are well suited for high-resolution analytical separations of both acidic and basic monoclonal antibody variants. ProPac packings are pellicular polymeric supports with hydrophilic coatings and grafted surface chemistry, which exhibit minimal hydrophobic character. In addition, these particles exhibit a wide range of pH stability with high selectivity and minimal band broadening.

Here, the authors discuss the latest development of a new MAb column—the MAbPac™ SCX-10—that is designed as a complementary addition to the ProPac WCX column, providing high resolution and orthogonal selectivity for various proteins and MAb charge variant characterization. Using this column, several applications including characterization of lysine truncation variants, Fab, and Fc fragment analysis are presented. Also, lot-to-lot and column-to-column reproducibility and ruggedness of the new MAbPac column are demonstrated.

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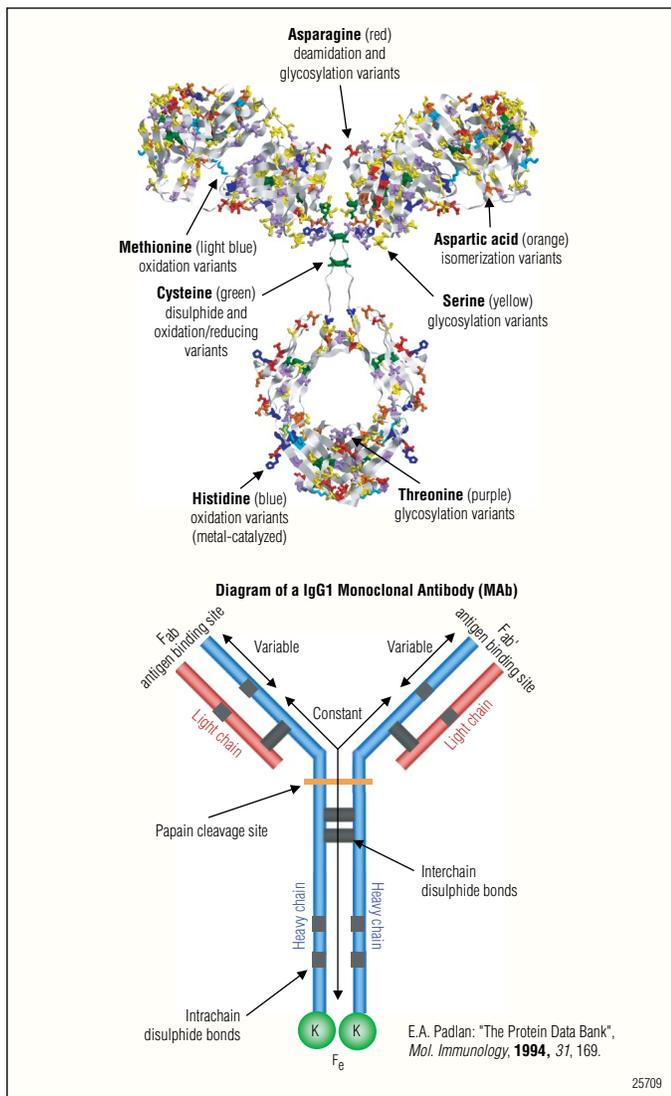


Figure 1. Crystal structure of a human IgG1 MAb.

MATERIALS

Chromatographic Components

MAb Separations

A PEEK™/Inert system including: ICS-3000 DP gradient pump, VWD absorbance detector, UltiMate® Autosampler and thermostatted column compartment (TCC-100) from Dionex Corporation.

Chromeleon® Chromatography Data System software (Dionex Corporation).

Chemicals, MAb

MES, Tris, and all other analytical grade chemicals were obtained from Sigma. MAb was a gift from a local biotech company.

Columns

MABPac SCX-10, 4 × 250 mm (P/N 074625) and ProPac WCX-10, 4 × 250 mm (P/N 054993) columns from Dionex Corporation.

SEPARATION MEDIA AND MECHANISM

Substrate

10 µm nonporous, highly crosslinked styrene-type polymeric media was used.

ProPac WCX Column

1. A proprietary surface modification process to create a highly hydrophilic coating. No hydrophobic interactions are present.
2. Random polymerization approach used for functional grafts.
3. Functional group: carboxylic acid.

MABPac SCX Column

1. A proprietary, different one-step surface modification process yielding uniform hydrophilic coating. No hydrophobic interactions are present.
2. ATRP-based grafting approach to control functional group chain length and density of functional groups.
3. Functional group: sulfonic acid.

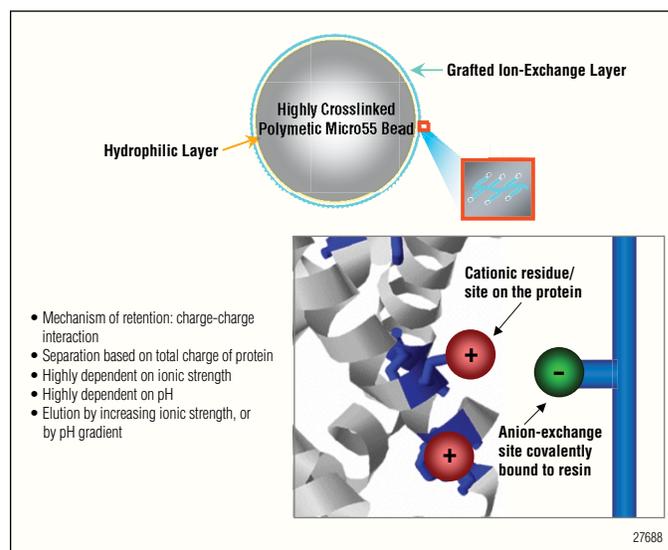


Figure 2. Separation media and mechanism of cation-exchange columns.

SELECTIVITY DIFFERENCES

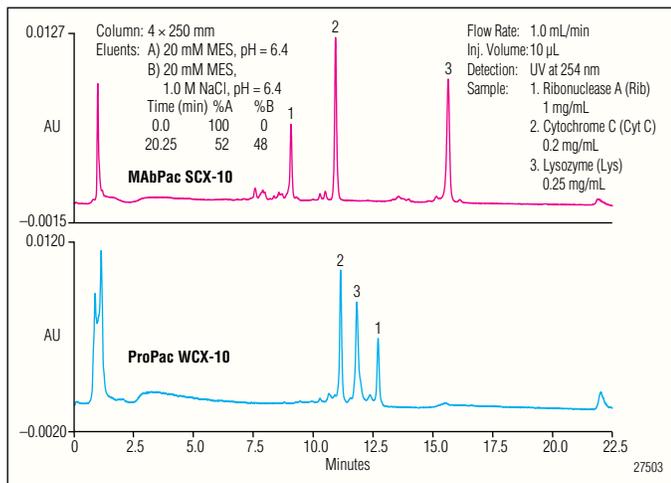


Figure 3. Selectivity differences: MABPac SCX-10 vs ProPac WCX-10 columns. Ribonuclease A elutes prior to other proteins in MABPac SCX-10 as compared to ProPac WCX-10 showing a selectivity change. Such orthogonal selectivity differences are quite helpful in protein heterogeneity characterization.

ProPac WCX-10 vs MABPac SCX-10 COLUMN

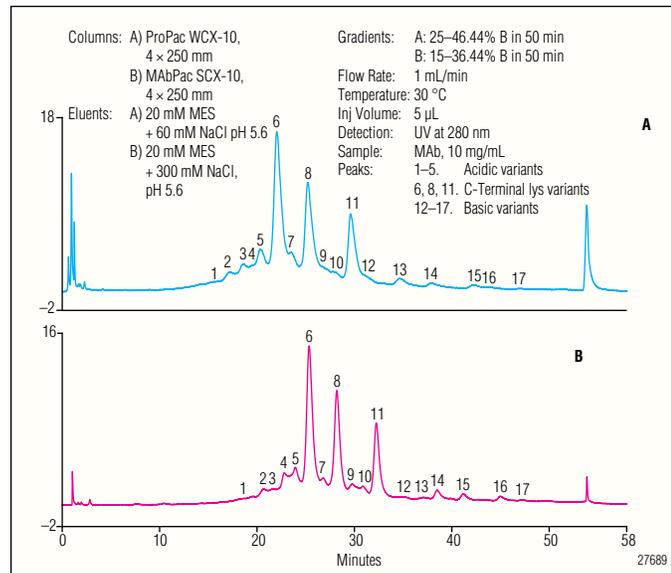


Figure 4. MAb separation: Comparison of ProPac WCX-10 vs MABPac SCX-10 columns. The ProPac WCX-10 and MABPac SCX-10 columns provide high resolution and peak efficiencies of monoclonal antibody acidic, basic, and C-terminal lysine variants.

APPLICATIONS OF THE MABPac SCX-10 COLUMN

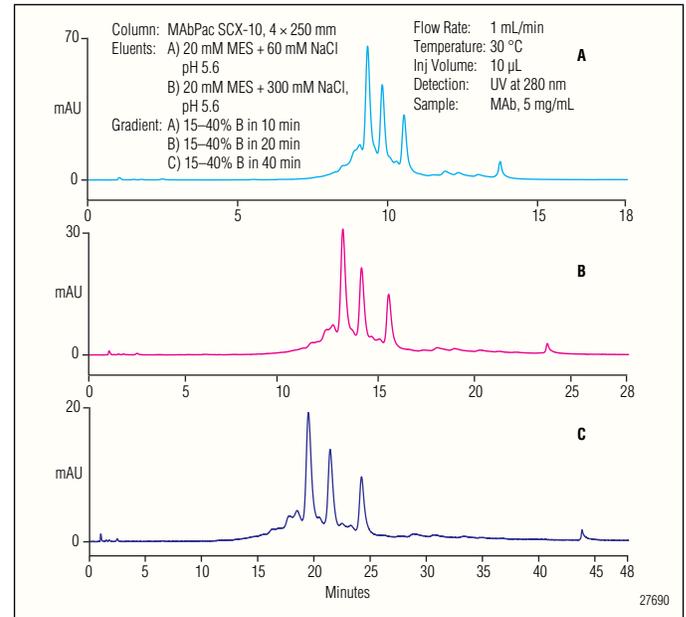


Figure 5. MAb separation on the MABPac SCX-10 column using different gradients. MAb analysis was achieved under 15 min with high resolution.

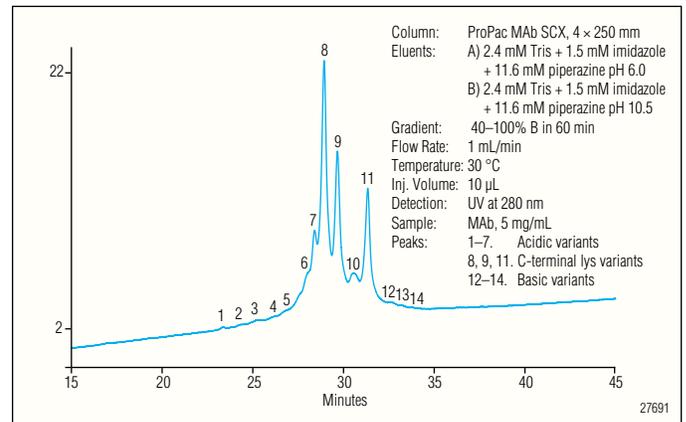


Figure 6. pH gradient-based MAb separation.³ With the use of a pH gradient, the MABPac SCX-10 column provides high-resolution separation of monoclonal antibody variants.

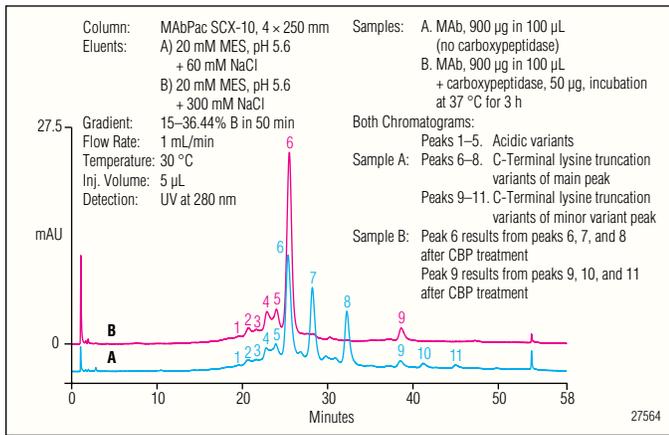


Figure 7. Analysis of MAb lysine truncation variants on the MAbPac SCX-10 column. A second chromatogram (B) verifies that the three major peaks are due to variations in the presence of C-terminal lysine. After carboxypeptidase B treatment, C-terminus lysine is removed and only one major peak remains.

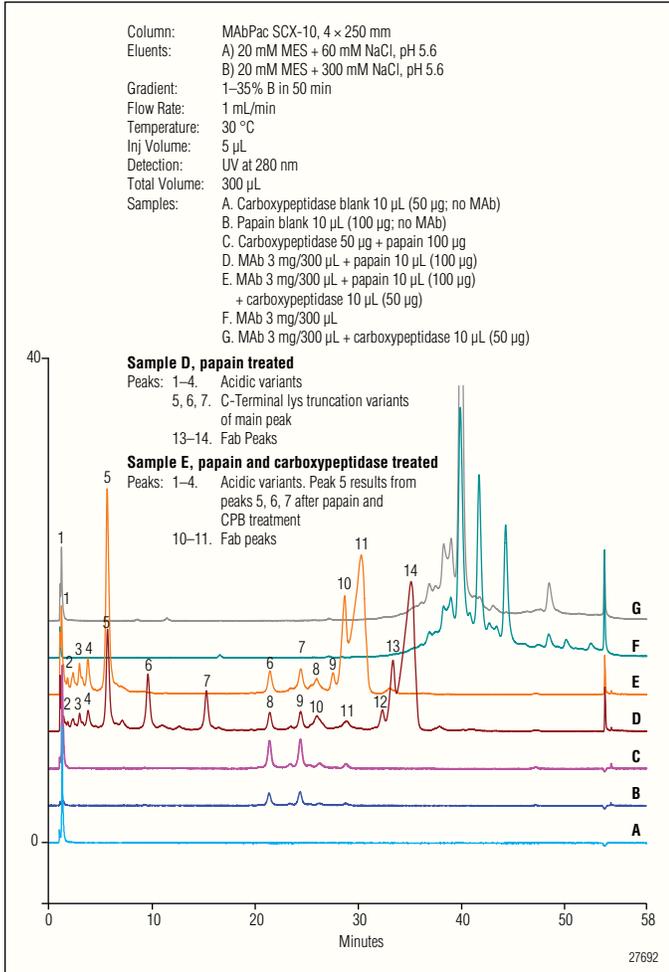


Figure 8. Analysis of Fab and Fc fragments of a MAb after digestion with carboxypeptidase and papain using the MAbPac SCX-10 column.

LOT-TO-LOT-REPRODUCIBILITY

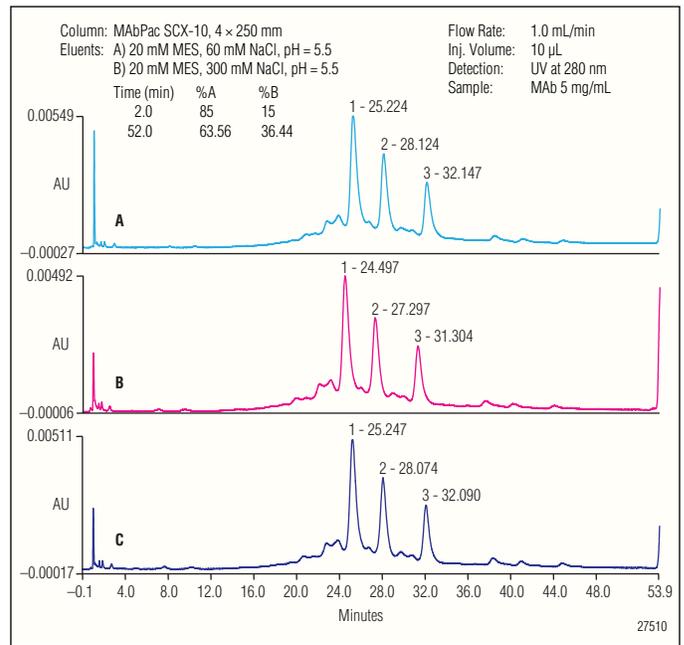


Figure 9. Lot-to-lot reproducibility of the MAbPac SCX-10 column. Columns from each of the three different lots (A, B, C) were used for MAb analysis. Retention times for the C-terminal lysine variants are shown.

COLUMN-TO-COLUMN REPRODUCIBILITY

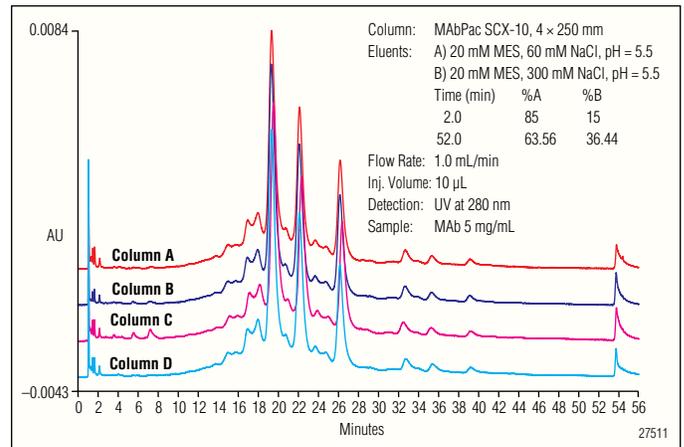


Figure 10. Column-to-column reproducibility of the MAbPac SCX-10 column. No change in performance was noted for 4 different MAbPac SCX-10 columns (A, B, C, D) from the same lot.

RUGGEDNESS OF THE MAbPac SCX COLUMN

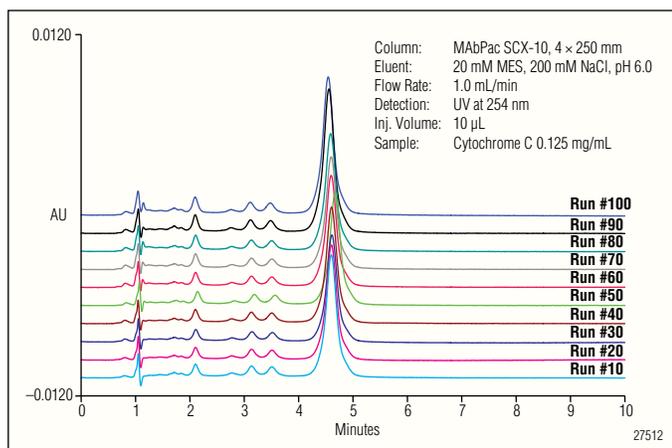


Figure 11. Ruggedness of the MAbPac SCX-10 column. More than 100 isocratic runs were performed to assess the reproducibility, ruggedness, and column performance. The chromatography profiles remain unchanged.

CONCLUSIONS

- A new MAbPac SCX column was introduced for monoclonal antibody separations. This is a complementary addition to existing ProPac WCX columns, providing high resolution and orthogonal selectivity for various proteins and MAb charge variant characterization.
- A controllable and robust ATRP surface grafting technique was applied that provided a uniform functional layer for the ion-exchange process.
- High resolution and high efficiency were obtained for monoclonal antibody variants analysis as well as various MAb fragmentation analysis.
- Columns exhibited lot-to-lot and column-to-column reproducibility.
- Ruggedness of the column was proved within broad pH and temperature ranges.

REFERENCES

1. MAbPac SCX-10 manual and data sheets available at www.dionex.com.
2. Bennett, K. L.; Smith, S. V.; Truscott, R. J. W.; Sheil, M. M. *Anal. Biochem.* **1997**, *245*, 17–27.
3. Farnan, D.; Moreno, G. T. *Anal. Chem.* **2009**, *81*, 8846–8857.

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