

Analysis of Monoclonal Antibody Charge Heterogeneity Using Ion-Exchange Chromatography on a Fully Biocompatible HPLC System

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ABSTRACT

Dionex has developed a fully biocompatible analytical HPLC, the UltiMate® 3000 Titanium system, for analysis of proteins and protein-based drugs like therapeutic monoclonal antibodies (MAbs). The new biocompatible system was used with our ProPac® ion-exchange column to resolve closely related protein variants. These include C-terminal truncated lysine variants, N-terminal pyroglutamate capped variants, and phosphorylated sialylated and internal asparagine containing deamidation variants.

The UltiMate 3000 Titanium system is available in two versions, quaternary LPG and x2 DGP. Titanium pumps and PEEK fluidic path on the system ensure full compatibility with all biological buffers and labile compounds and aids longevity of the system, particularly for use with harsh salts and pH solutions, while delivering high day-to-day reproducibility and robust operation. Biocompatibility is also critical in maintaining protein integrity and labile post-translational modifications during separation.

ProPac columns are pellicular polymeric supports with hydrophilic coatings and grafted surface chemistry, exhibiting minimal hydrophobic character. In addition, these particles exhibit a wide range of pH stability with high selectivity and minimal band spreading. Using columns packed with weak cation-exchange (WCX) particles, baseline separations were achieved for MAb immunoglobulin G (IgG) variants.

In this study, we separated MAbs using ProPac WCX columns on two different HPLC systems. One system contained a stainless steel (SST) pump and the other system was equipped with an all biocompatible pump and fluidic path. The data generated over several hundred runs of MAb separations on these systems show the advantages of using an all biocompatible system over an SST system.

INTRODUCTION

MAB microheterogeneity can be attributed to glycosylation, oxidation, mutation, phosphorylation, amino terminal modifications (e.g., pyroglutamate), incomplete processing of the C-terminus, and asparagine (Asn) deamidation. These variations in protein composition occur in many types of proteins and can impact the activity and stability of biotherapeutics. Monitoring stability of therapeutic proteins and peptides is regarded as essential for demonstrating safety and efficacy of these drugs and is required by the FDA and other regulatory agencies. These variations are routinely monitored—preferably by cation-exchange chromatography.

The ProPac WCX-10 weak cation-exchange column, is very well suited to resolve closely related protein charge variants. ProPac packings are pellicular polymeric supports with hydrophilic coatings and grafted surface chemistry which exhibited minimal hydrophobic character. In addition, these particles exhibit a wide range of pH stability with high selectivity and minimal band spreading.

RESULTS

In this study we presented data using the new fully biocompatible UltiMate 3000 Titanium system and ProPac WCX column on MAbs variant analysis. Baseline separations were achieved for the majority of the IgG charge variants 1–3. We tested the effects of NaCl gradients and loading on the MAb separation.

We studied MAb separation on a fully biocompatible HPLC system and compared the data with an all SST HPLC system. Our results show excellent reproducibility of MAb separations on the biocompatible HPLC system. In contrast, MAb separation on the SST system exhibited initial seasoning effect (binding of protein to SST surface) as well as tendency to corrode with time which eventually could result in loss of performance of the column.

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Materials

Chromatographic components

All Biocompatible System:

Dual gradient titanium pump with PEEK™ fluidic path (Dionex Corporation)

All SST System:

Dual gradient SST pump with SST fluidic path (Dionex Corporation)

Chromatography was controlled by Chromeleon® chromatography management software(Dionex Corporation)

Chemicals

MES, HEPES, and all other analytical grade chemicals were obtained from Sigma.

Columns

ProPac WCX-10 (Product No 054993) analytical columns (Dionex Corporation).

These columns consist of a polymer support coated with a hydrophilic polymer (Figure 3).



Figure 1. The UltiMate 3000 Titanium system features titanium pumps and PEEK fluidic path.

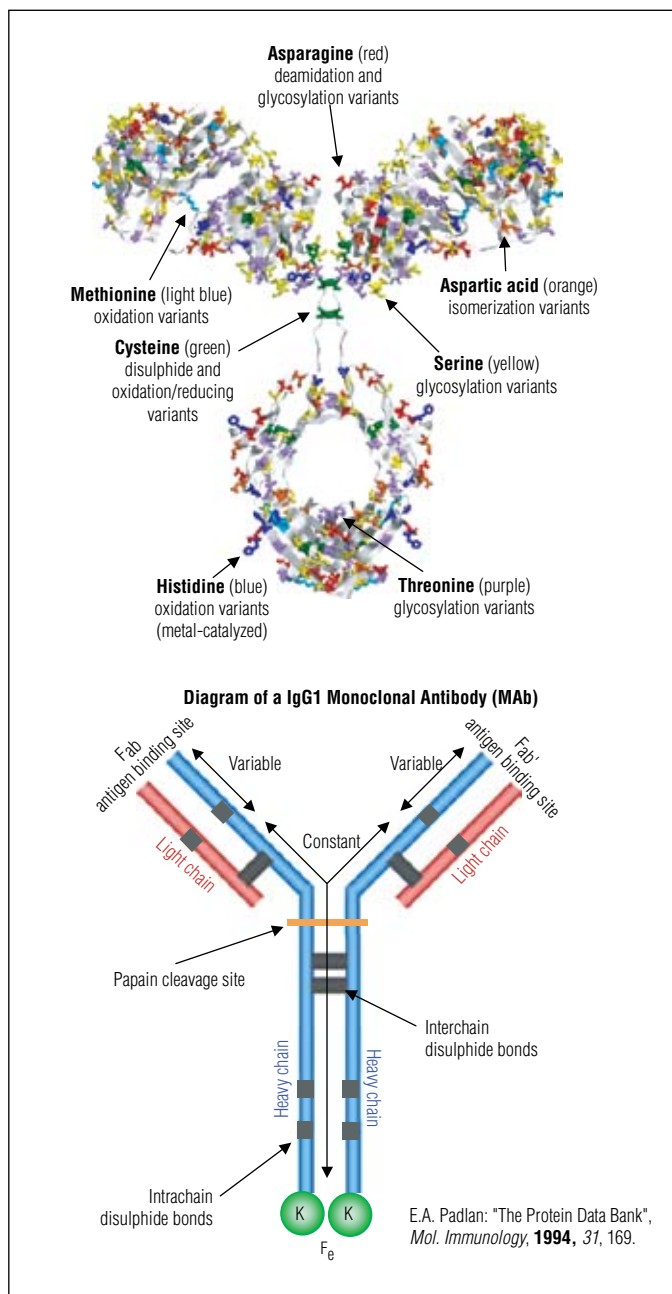


Figure 2. Crystal structure of a human IgG1 MAb.

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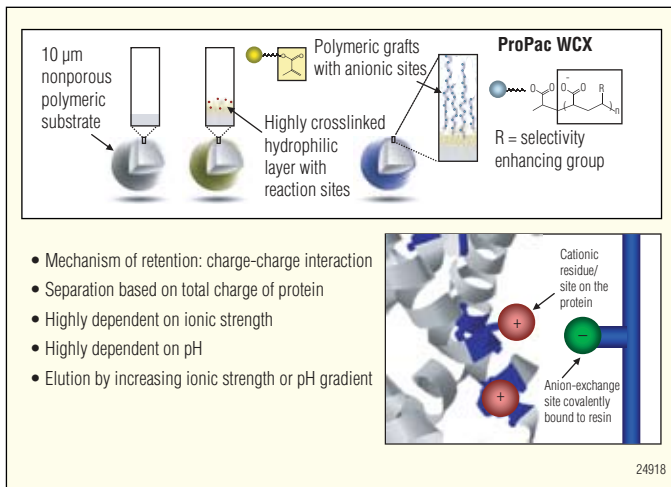


Figure 3. ProPac WCX phase design and mechanism of retention.

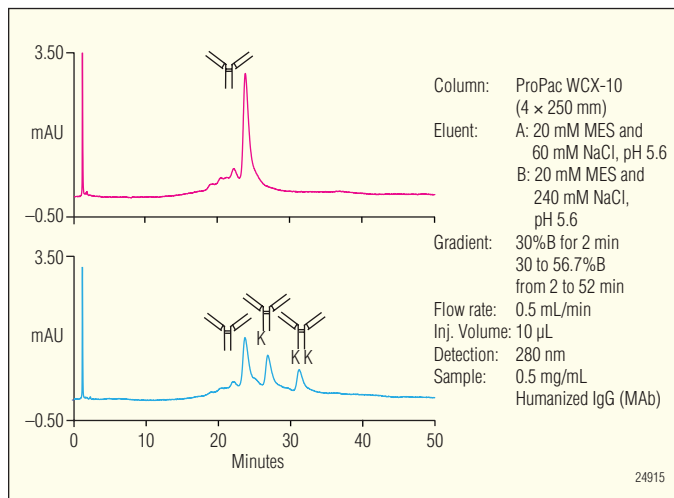


Figure 4. Identification of MAb variants. Humanized IgG¹ MAb variants differing in heavy chain C-terminal lysine content were resolved using a shallow NaCl gradient. Differences in C-terminal lysine were verified by treatment of the MAb with carboxypeptidase B, an enzyme that cleaves C-terminal lysine residues.

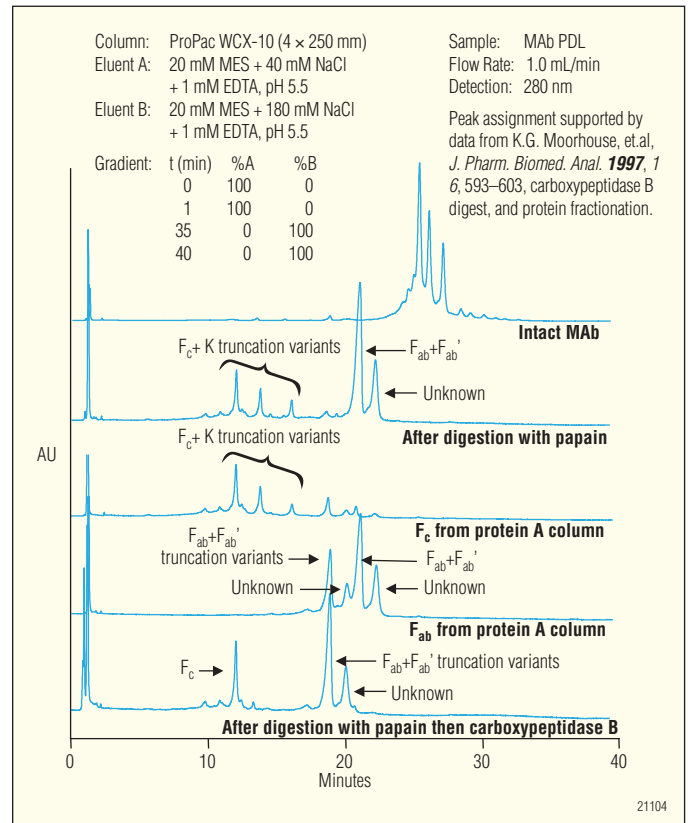


Figure 5. Separation of the papain digest fragments on the ProPac WCX-10.

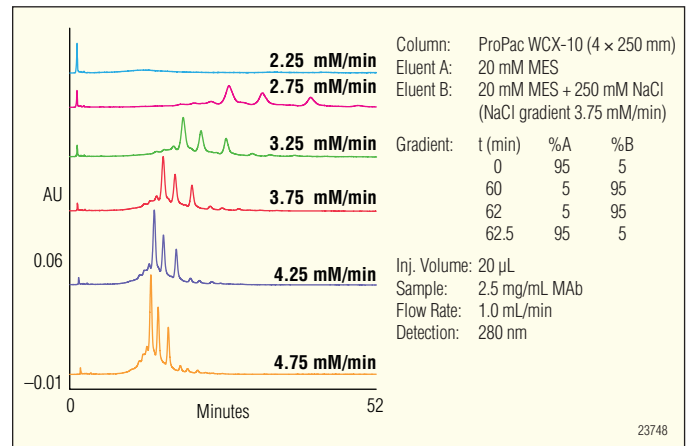


Figure 6. Effect of salt gradients on MAb separation (pH 5.5).

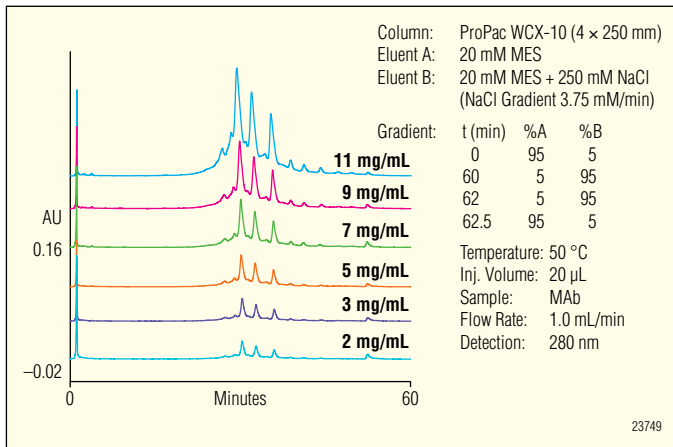


Figure 7. Effect of sample loading on MAb separation (pH 5.5).

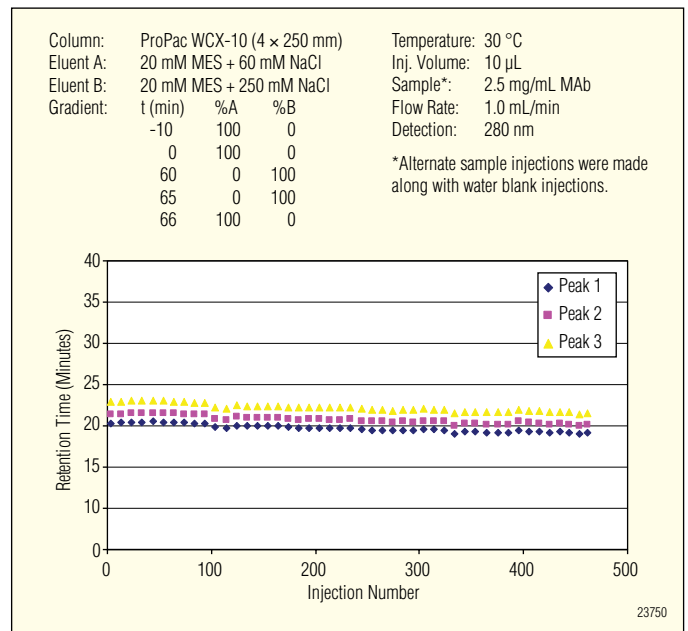


Figure 10. MAb separation on the biocompatible HPLC system.

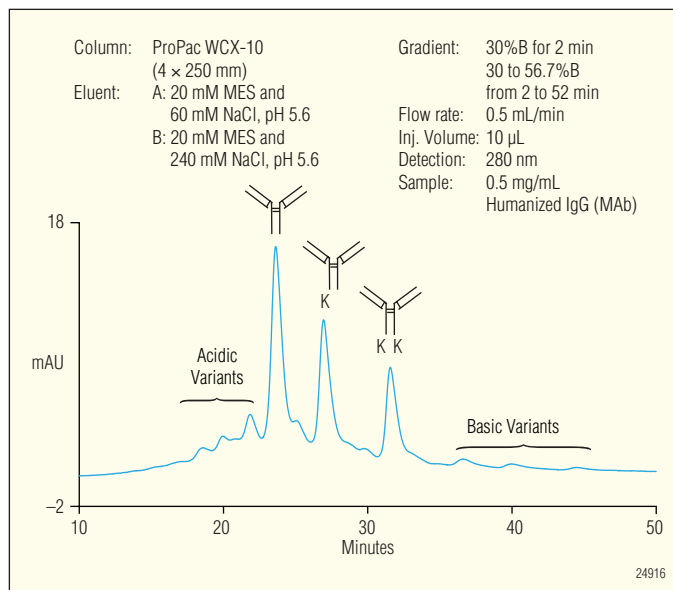


Figure 8. The separation of acidic and basic terminal lysine MAb variants using the ProPac WCX-10 column and the UltiMate 3000 Titanium system.

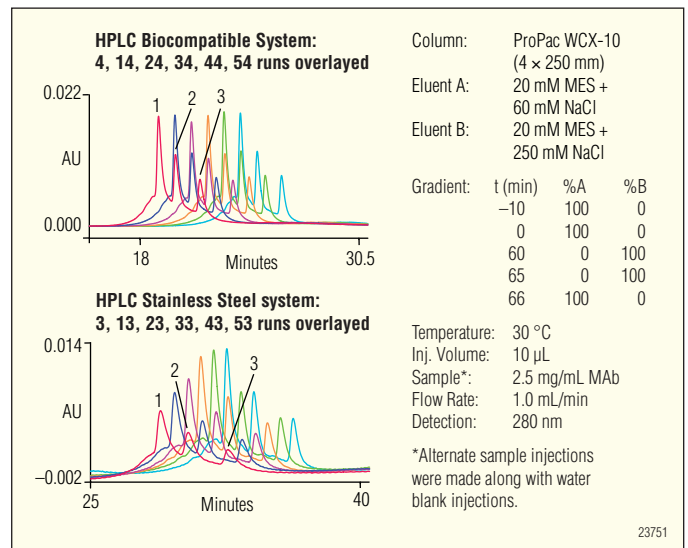


Figure 11. MAb separation on biocompatible vs stainless steel HPLC systems.

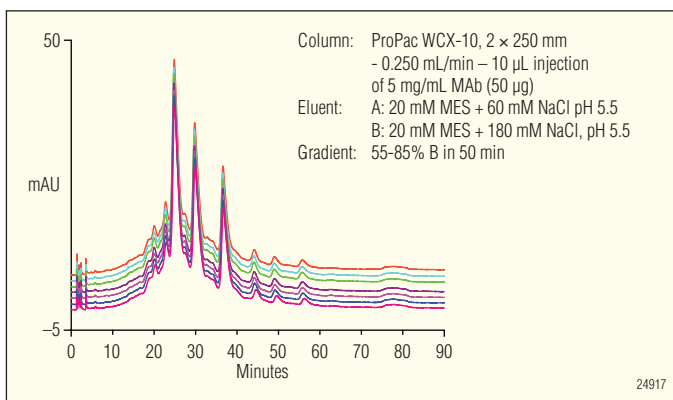


Figure 9. MAb separation—repeatability test on the ProPac WCX-10 column.

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CONCLUSION

Ion-exchange chromatography with ProPac WCX-10 is routinely used in applications such as the separation of MAb variants, papain digested MAb components, and various other distinct protein separation applications.

We have studied effects of different salt gradients and loading on MAb separation using the ProPac WCX-10 column. These parameters appear to influence MAb separation and therefore play an important role in method development.

We compared the MAb separations on a full-biocompatible HPLC system with an SST HPLC system. Our results show that excellent chromatography was achieved on the biocompatible HPLC system. MAb chromatography performed on the SST HPLC system showed initial seasoning effect with a loss of efficiency and peak areas in the first 40 chromatography runs. This may be due to the binding of MAb to the stainless steel pump and/or tubing.

High salt eluents used in chromatography are well known to corrode stainless steel, which will eventually leach onto the column and cause loss of chromatography performance of ProPac WCX-10 columns. This can be prevented to some extent by following periodic passivation techniques. We strongly recommend using a fully biocompatible HPLC systems to perform ion-exchange chromatography.

REFERENCES

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3. Moorhouse K. G., et al. *J. Pharm. and Biomed Anal.* **1997**, 16, 593–603.

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