

Analysis of Monoclonal Antibody Heterogeneity by Cation-Exchange Chromatography: Separation of C-Terminal Lysine Variants

INTRODUCTION

During the development and production of therapeutic proteins, characterization of structural variants is a critical challenge. C-terminal processing of lysine residues on the heavy chains of monoclonal antibodies (MAbs) is a common structural variation that demands careful analysis.¹⁻⁶ As a result of this processing, C-terminal lysine or arginine residues are often absent in proteins isolated from mammalian cell cultures, even though their presence may be expected on the basis of gene sequence. This discrepancy, which is common in plasma-derived proteins, results from the activity of one or more basic carboxypeptidases.

Incomplete protein processing results in charge heterogeneity which is readily identified by cation-exchange chromatography on the Dionex ProPac™ WCX-10 column. The packing in this column is a unique pellicular resin with a hydrophilic coating and carboxylate functional groups on grafted linker arms. The physicochemical properties of this support eliminate secondary (nonionic) interactions between the protein analytes and the stationary phase. In practice, the ProPac WCX-10 weak cation exchange resin affords minimal band spreading and high selectivity.⁷

This Application Note describes a method for analyzing a humanized IgG₁ MAb for C-terminal lysine variants. These variants are baseline-resolved from the native antibody using the ProPac WCX-10 column.

EQUIPMENT

Dionex DX-500 BioLC® Liquid Chromatograph

GP50 Gradient Pump

AD20 Variable Wavelength Absorbance Detector

LC25 Liquid Chromatography Enclosure

AS50 Autosampler (with a 50- μ L sample loop)

PeakNet Chromatography Workstation

REAGENTS

High-purity deionized water prepared with a Milli-Q system (Millipore, Bedford, MA, USA)

Carboxypeptidase B (Boehringer Mannheim, Indianapolis, IN, USA)

Sodium phosphate, monobasic and dibasic (J. T. Baker, Phillipsburg, NJ, USA)

Sodium chloride (Fluka, Ronkonkoma, NY, USA)

Humanized monoclonal IgG₁ antibody (a generous gift from a biotechnology company)

PREPARATION OF SOLUTIONS AND REAGENTS

Two eluents are used for this chromatography: 10 mM sodium phosphate (pH 7.0) and 10 mM sodium phosphate (pH 7.0) with 1.0 M sodium chloride (NaCl). The sodium phosphate buffer system was prepared by diluting appropriate quantities of monobasic and dibasic sodium phosphate concentrate solutions with water to attain the desired pH 7.0. The following procedure is a recommended starting point for obtaining the desired eluents, but some deviation from this formula may be necessary after checking the pH, when using reagents in other labs. If the pH is not 7.0, then adjust the proportions of monobasic and dibasic solutions added. The combined total volume of monobasic and dibasic solutions should remain at 100 mL to produce 10 mM sodium phosphate for 2 L of eluent.

2 M Sodium Chloride

Dissolve 116.90 g sodium chloride in water, and fill to a final volume of 2.0 L. Filter through a 0.45- μ m filter.

200 mM Sodium Phosphate, Dibasic

Dissolve 28.38 g anhydrous dibasic sodium phosphate (Na_2HPO_4) in 1000 mL of water. Filter through a 0.45- μ m filter. Store frozen until needed.

200 mM Sodium Phosphate, Monobasic

Dissolve 27.60 g monohydrate monobasic sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$) in 1000 mL of water. Filter through a 0.45- μ m filter. Store frozen until needed.

10 mM Sodium Phosphate, pH 7.0

Combine 61.0 mL of 200 mM dibasic sodium phosphate, 39.0 mL of 200 mM monobasic sodium phosphate, and 1900 mL water.

10 mM Sodium Phosphate with 1.0 M Sodium Chloride, pH 7.0

Combine 61.0 mL of 200 mM dibasic sodium phosphate, 39.0 mL of 200 mM monobasic sodium phosphate, 1000 mL of 2 M sodium chloride, and 900 mL water.

CONDITIONS

Column: ProPac WCX-10, 4 x 250 mm
Flow Rate: 1 mL/min
Detection: Absorbance, 280 nm
Mobile Phase: A: 10 mM sodium phosphate, pH 7.0
B: 10 mM sodium phosphate,
1 M sodium chloride, pH 7.0
Gradient: Linear, 4–15% B in 30 min

Method

Time (min)	A (%)	B (%)	Comments
Initial	96.00	4.00	Equilibration
0.00	96.00	4.00	Injection
0.10	96.00	4.00	
30.00	85.00	15.00	End Gradient
31.00	25.00	75.00	Regeneration
40.00	25.00	75.00	
42.00	96.00	4.00	Re-equilibration

SAMPLE PREPARATION

Protein samples were prepared for injection by dilution in Mobile Phase A to a final concentration of 0.5 mg/mL.

RESULTS AND DISCUSSION

The ProPac WCX-10 weak cation-exchange column was assessed for its ability to separate humanized IgG₁ variants suspected of having lysine residue variations at the C-terminal of their heavy chains. As shown in Figure 1, a linear gradient of sodium chloride (40–150 mM over 30 min) at neutral pH resolved three variant forms differing in the presence of lysine (zero, one, or two residues) at the C-terminal of the heavy chains

To verify that the different retention times of the three peaks were due to variations in C-terminal lysine content, we treated the IgG₁ sample with carboxypeptidase B, an exopeptidase that specifically cleaves C-terminal lysine residues. The result was a complete disappearance of peaks 2 and 3, which contained one and two terminal heavy chain lysine residues, respectively. The disappearance of peaks 2 and 3 was accompanied by a corresponding quantitative increase in the area under peak 1 (the variant with no terminal lysine, Figure 1). This confirms that peaks 2 and 3 differed from peak 1 in that they contained IgG₁ with terminal heavy chain lysine residues.

CONCLUSION

The ProPac WCX-10 weak cation-exchange column produced baseline separation of C-terminal variants of an IgG₁ MAb. These results demonstrate that this column is well-suited for analyses such as quality control monitoring of protein-based therapeutics that require high efficiency, high resolution separation of closely related proteins.

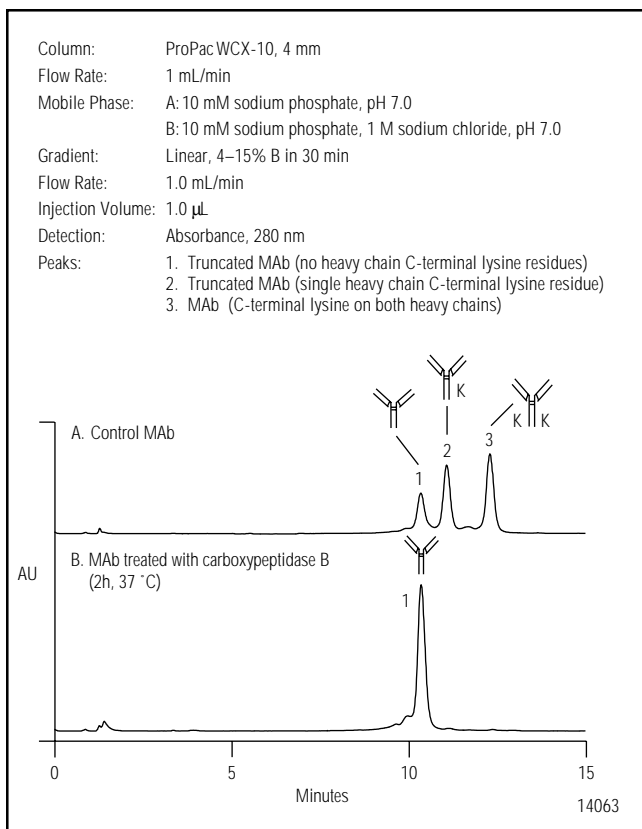


Figure 1 Analysis of an IgG, MAb on the ProPac WCX-10 analytical column (4 x 250 mm) before (upper trace, A) and after (lower trace, B) treatment with carboxypeptidase B for 2 h at 37 °C.

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