

Monoclonal Antibody and Fragments Separation on Antibodix™ Weak Cation Exchange Chromatography



Haiying Chen and Katherine McLaughlin

Sepax Technologies, Inc., 5-100 Innovation Way, Newark DE 19711

INTRODUCTION

Recombinant monoclonal antibodies (MAbs) have become a very important segment of protein drug therapeutics. Post-translational modifications including glycosylation, oxidation, deamidation and incomplete C-terminal processing can contribute to antibody heterogeneity. Degradation reactions during manufacturing, formulation, and storage can also cause structural and functional changes in MAbs. It is important to characterize and evaluate the heterogeneities and monitor the stability of MAbs for shelf-life studies. Antibodix™ weak cation exchange (ABX WCX) is designed to separate antibodies with minor forms. ABX particle surface is grafted with a highly hydrophilic, neutral polymer layer with a thickness in the range of nanometers. The hydrophobic PS/DVB resin surface is completely covered by a hydrophilic coating which eliminates non-specific bindings with antibody proteins, leading to high efficiency and high recovery separations. In this study, we applied ABX WCX NP5 for a MAb molecule, MAb 321, separation for variants and stability studies. Different mobile phase systems such as pH and salt gradients were applied to the separations. MAb 321, Fab/Fc and F(ab')₂ fragment separations were also achieved. Comparing the data from columns with different particle sizes, 5 μm offers more improved resolution than 10 μm WCX.

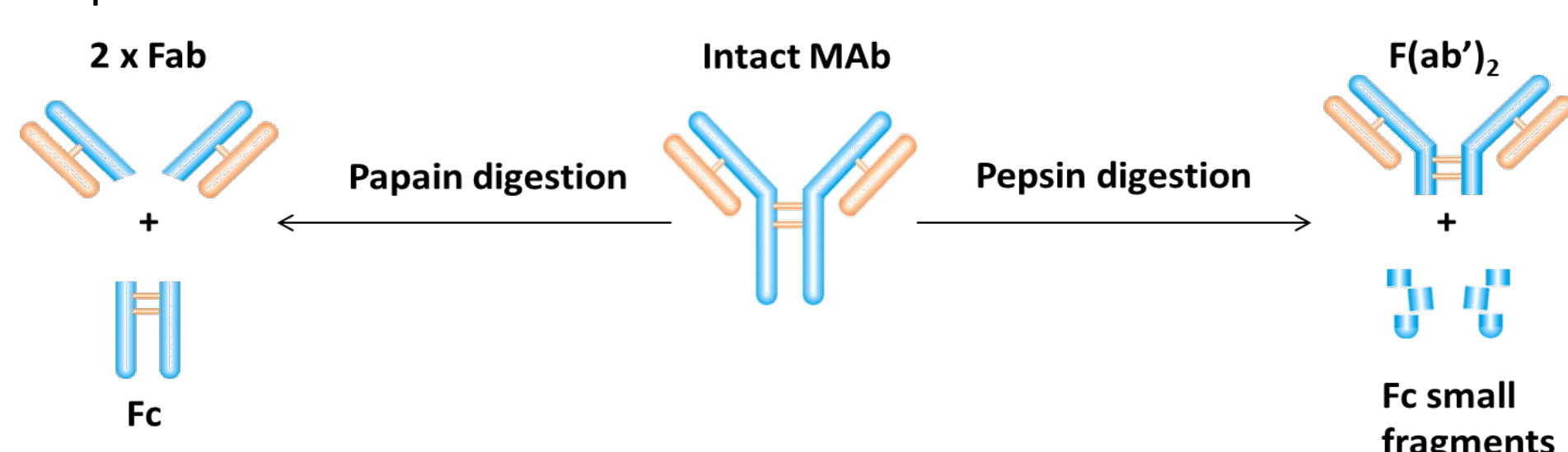
EXPERIMENTAL

Columns: Antibodix™ WCX NP5 (5 μm, non-porous, 4.6 x 250 mm) and Antibodix™ WCX NP10 (10 μm, non-porous, 4.6 x 250 mm)

HPLC System: Agilent 1200 HPLC with quaternary pump

Detection: UV 280 nm and UV 214 nm, Flow: 0.8 mL/min, Column temperature: 30 °C

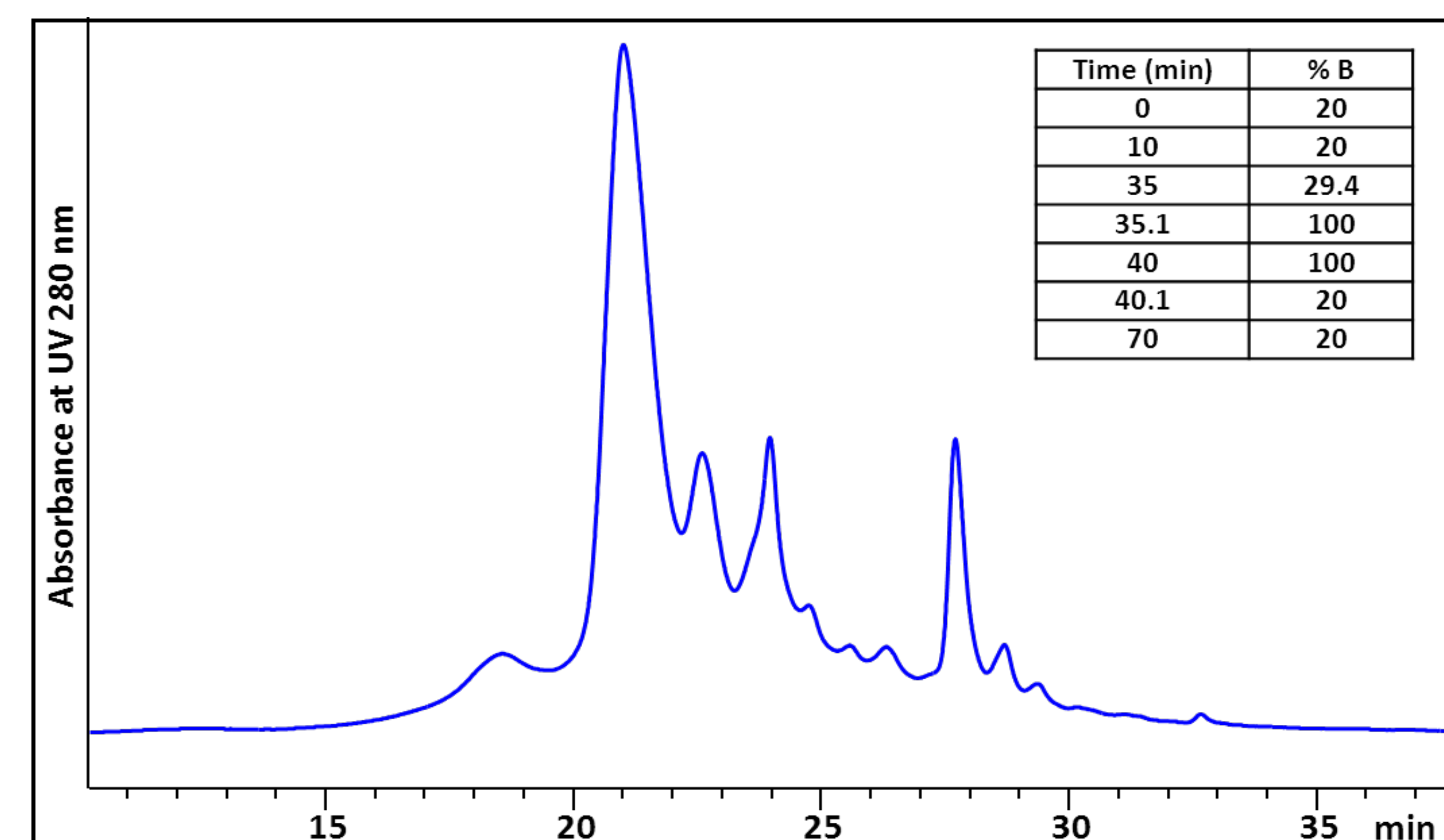
Sample Preparation:



Papain digestion: MAb 321 (1 mg/mL) was incubated in 100 mM Tris-HCl, pH 7.6, 2 mM EDTA and 5 mM Cysteine. The digestion was started by adding 1 mg/mL papain. The papain/MAb ratio was at 1:100. The digestion mixture was incubated for 2, 3, 3.5 and 4 hours at 37 °C.

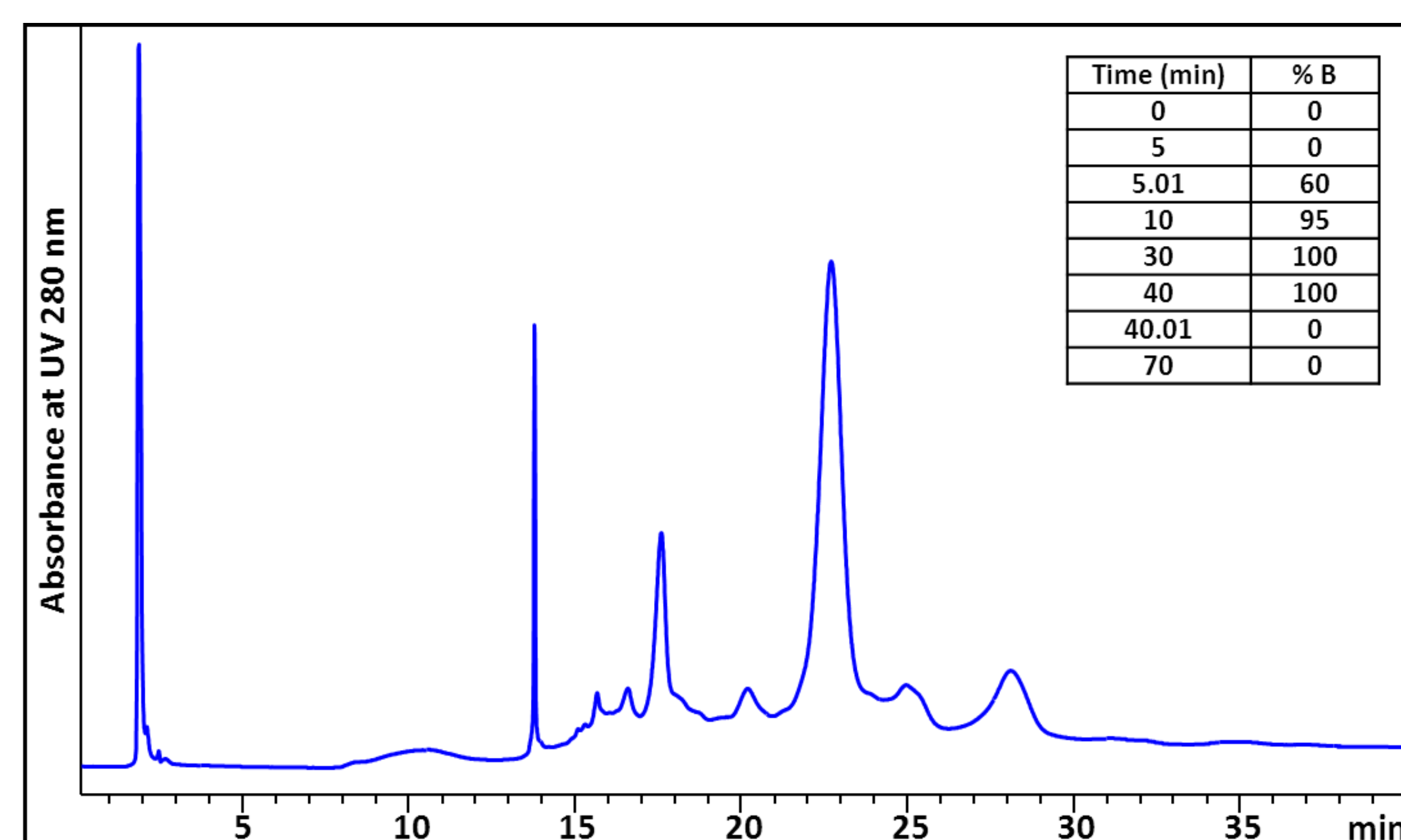
Pepsin digestion: MAb 321 was incubated at a final concentration of 1 mg/mL in 20 mM sodium acetate, pH 4.0 with a pepsin to MAb 321 ratio of 1:40. The digestion was carried out at 37 °C for 15.5 hours. The reaction was stopped by adding 2 M TRIS to increase the pH to 8.0.

ANALYSIS OF MAb 321 USING AN LiCl GRADIENT



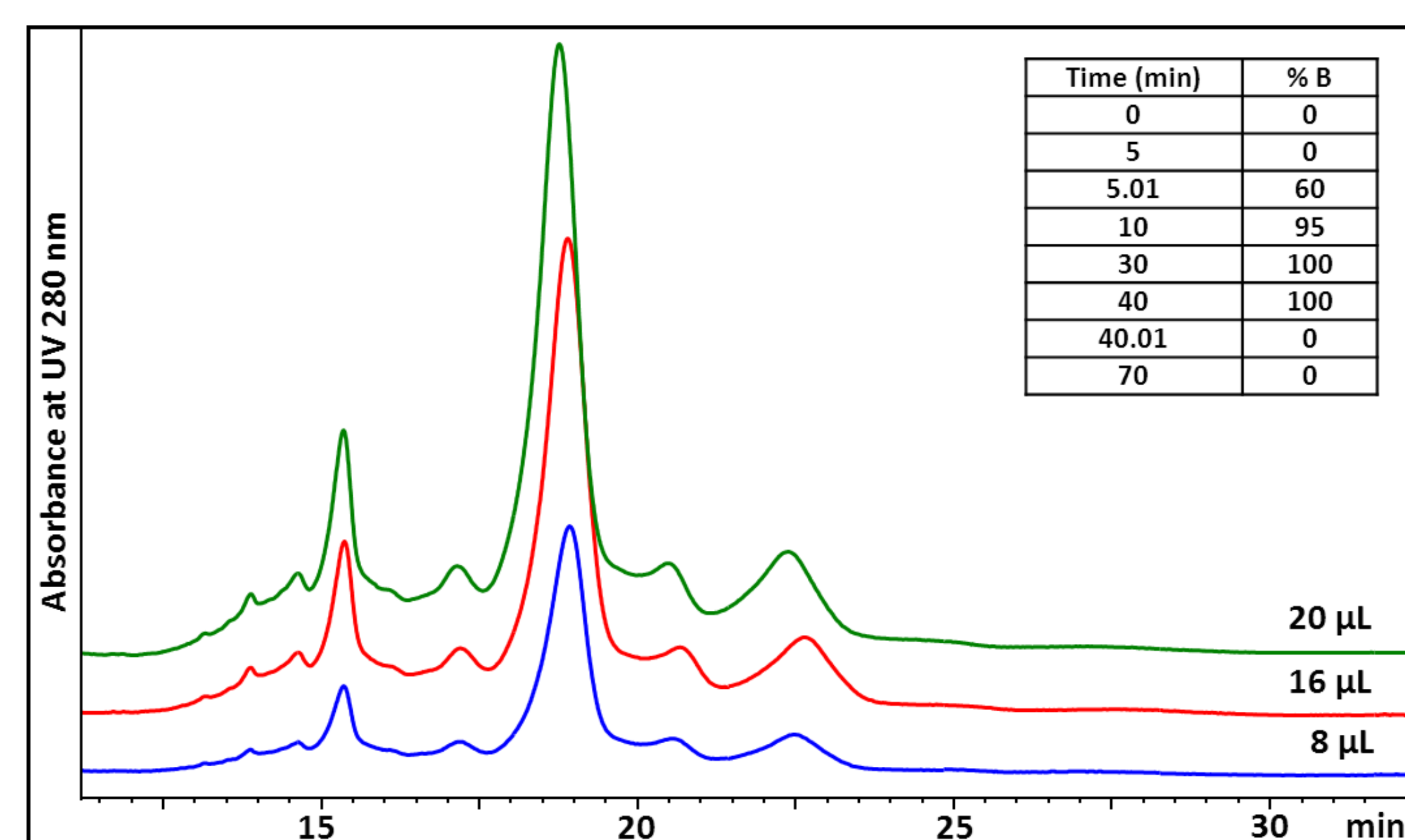
MAb 321 analysis on Antibodix™ WCX NP5, 4.6 x 250 mm. Mobile phase was A: 20 mM sodium acetate pH 5.15 and B: A + 1 M LiCl. Flow rate was 0.8 mL/min and the column temperature was at 30°C. 100 μg of intact MAb 321 was injected.

ANALYSIS OF MAb 321 USING AN NaCl and pH GRADIENT



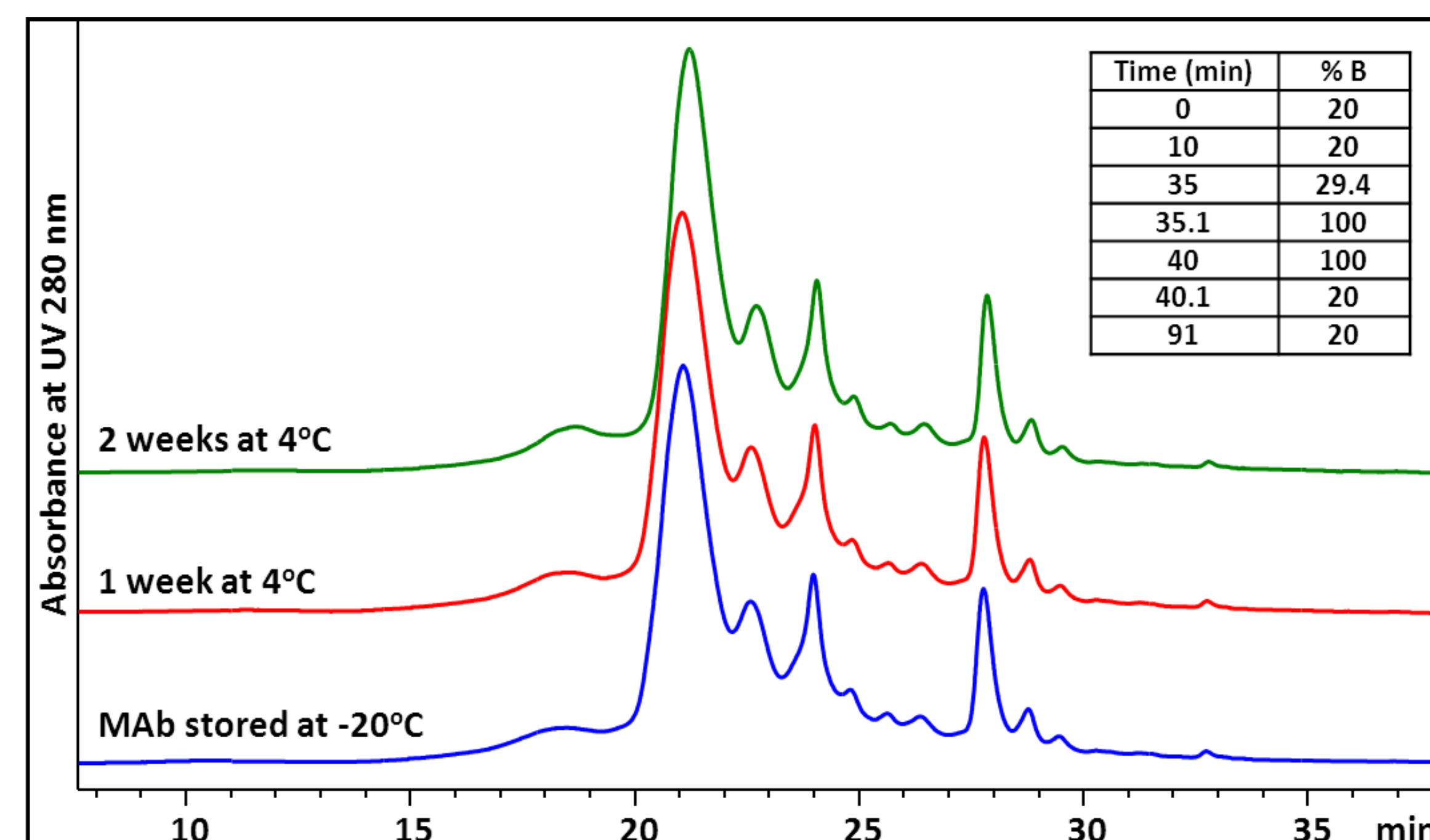
MAb 321 analysis on Antibodix™ WCX NP5, 4.6 x 250 mm. Mobile phase was A: 20 mM phosphate buffer pH 5 and B: A + 10 mM NaCl pH 7.5. Flow rate was 0.8 mL/min and the column temperature was at 30°C. 100 μg of intact MAb 321 was injected.

MAb LOADING TEST ON ANTIBODIX WCX NP5



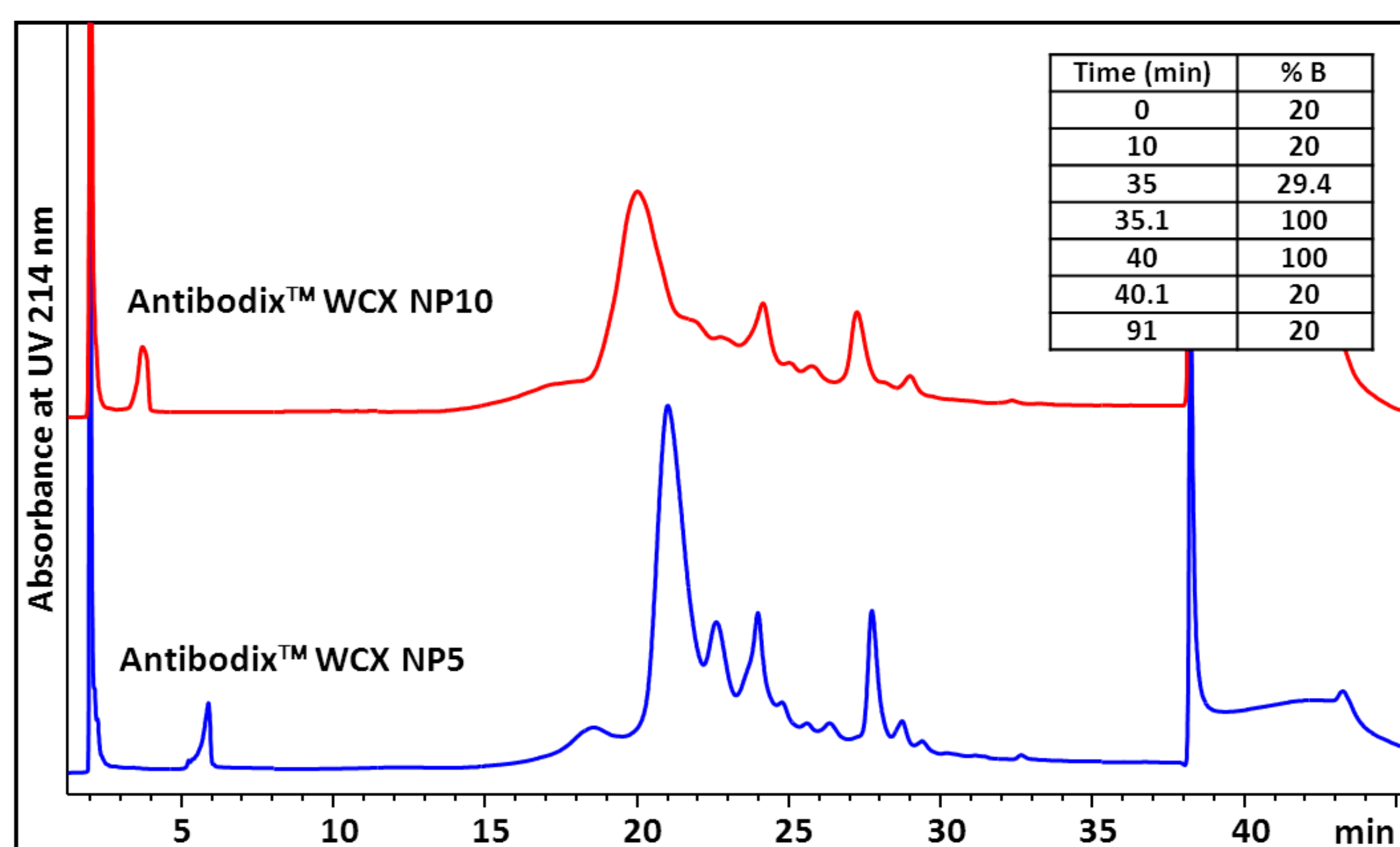
MAb 321 analysis on Antibodix™ WCX NP5, 4.6 x 250 mm. Mobile phase was A: 20 mM phosphate buffer pH 5 and B: A + 10 mM NaCl pH 7.5. Flow rate was 0.8 mL/min and the column temperature was at 30°C.

MAb 321 STABILITY TEST ON ANTIBODIX WCX NP5



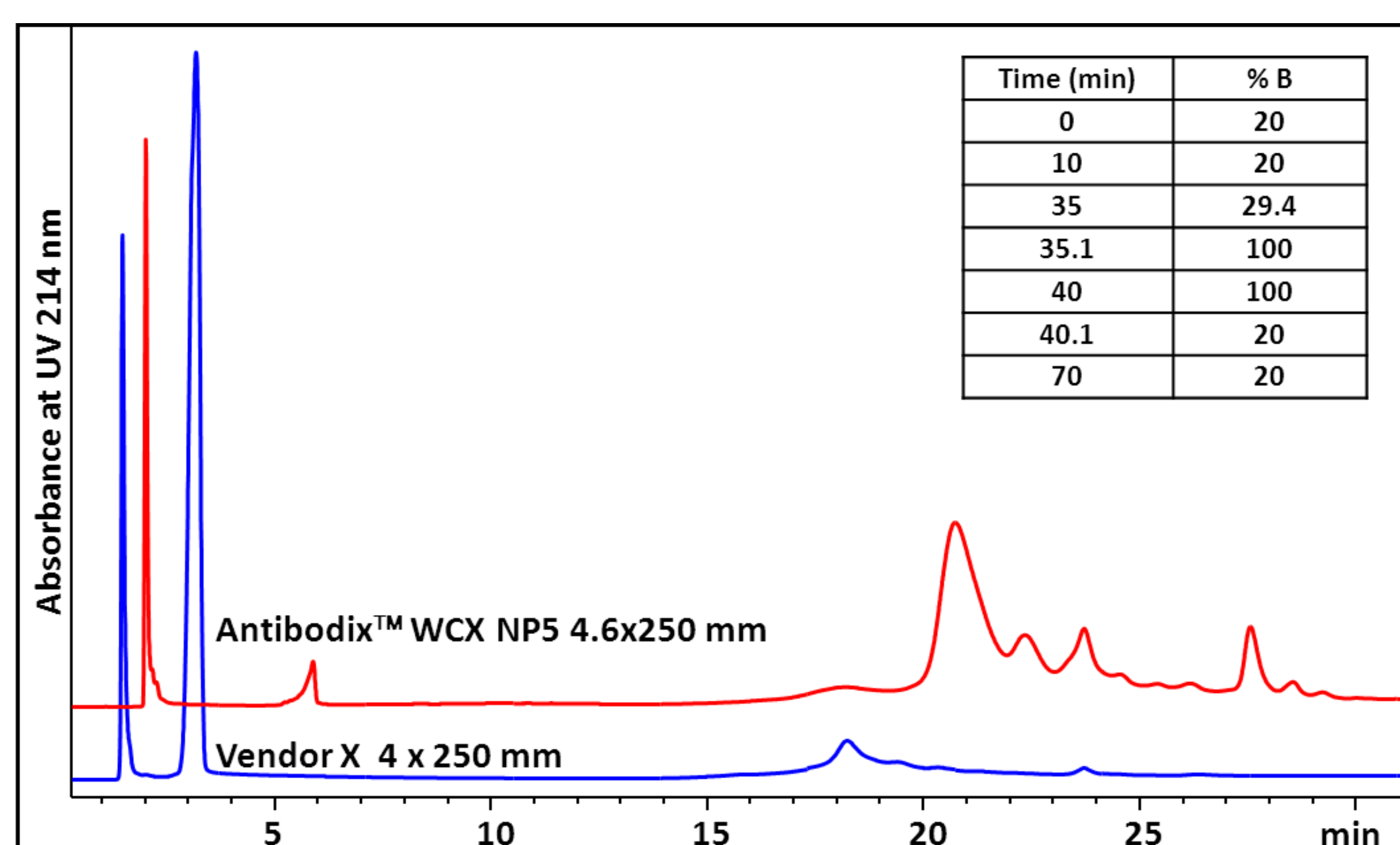
MAb 321 analysis on Antibodix™ WCX NP5, 4.6 x 250 mm. Mobile phase was A: 20 mM sodium acetate pH 5.15 and B: A + 1 M LiCl. Flow rate was 0.8 mL/min and the column temperature was at 30°C.

MAb 321 COMPARISON ON ANTIBODIX WCX NP5 AND NP10



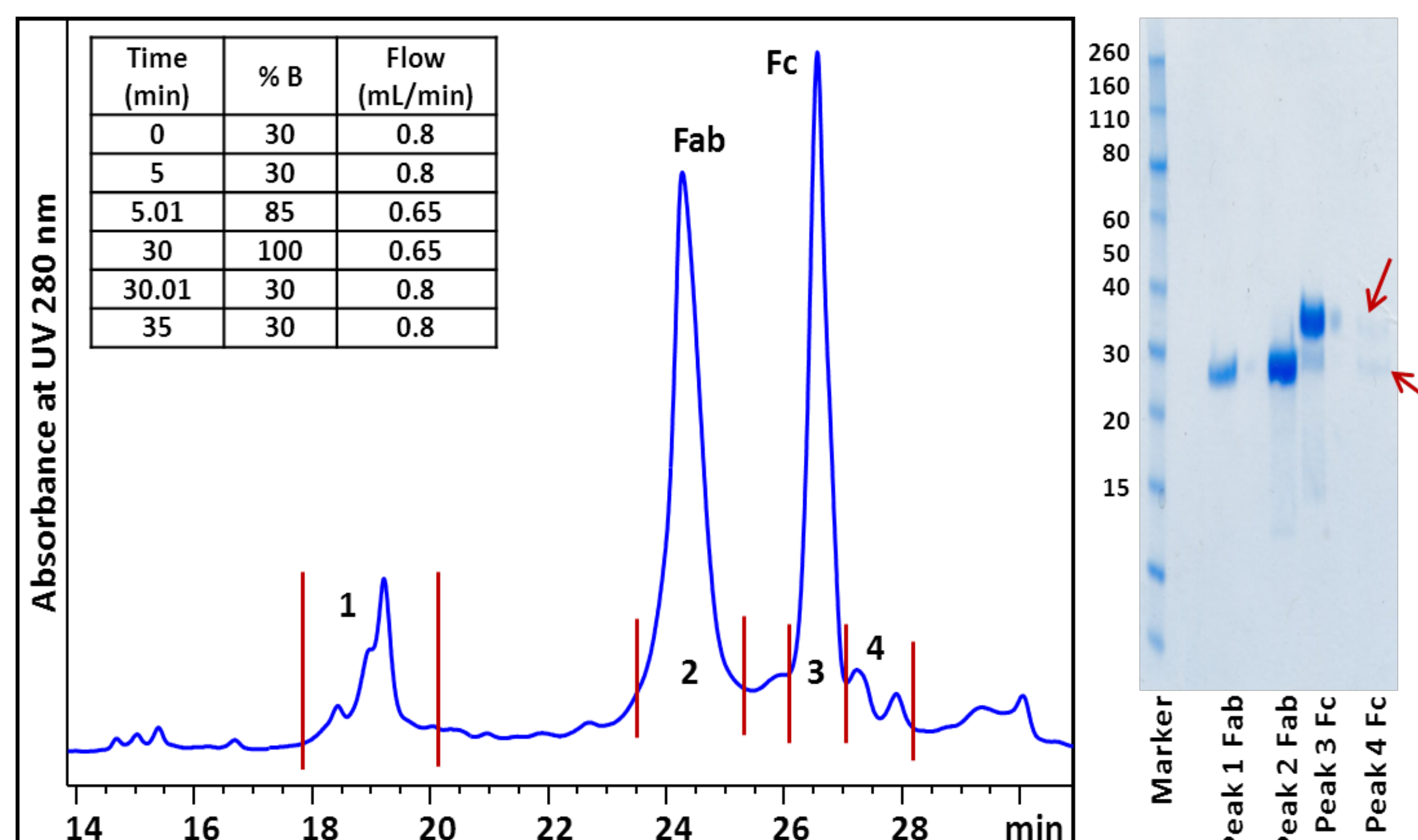
MAb 321 analysis on Antibodix™ WCX NP5, 4.6 x 250 mm. Mobile phase was A: 20 mM sodium acetate pH 5.15 and B: A + 1 M LiCl. Flow rate was 0.8 mL/min and the column temperature was at 30°C. 100 μg of intact MAb 321 was injected.

COMPARISON OF ANTIBODIX WCX NP5 TO A COMPETITOR



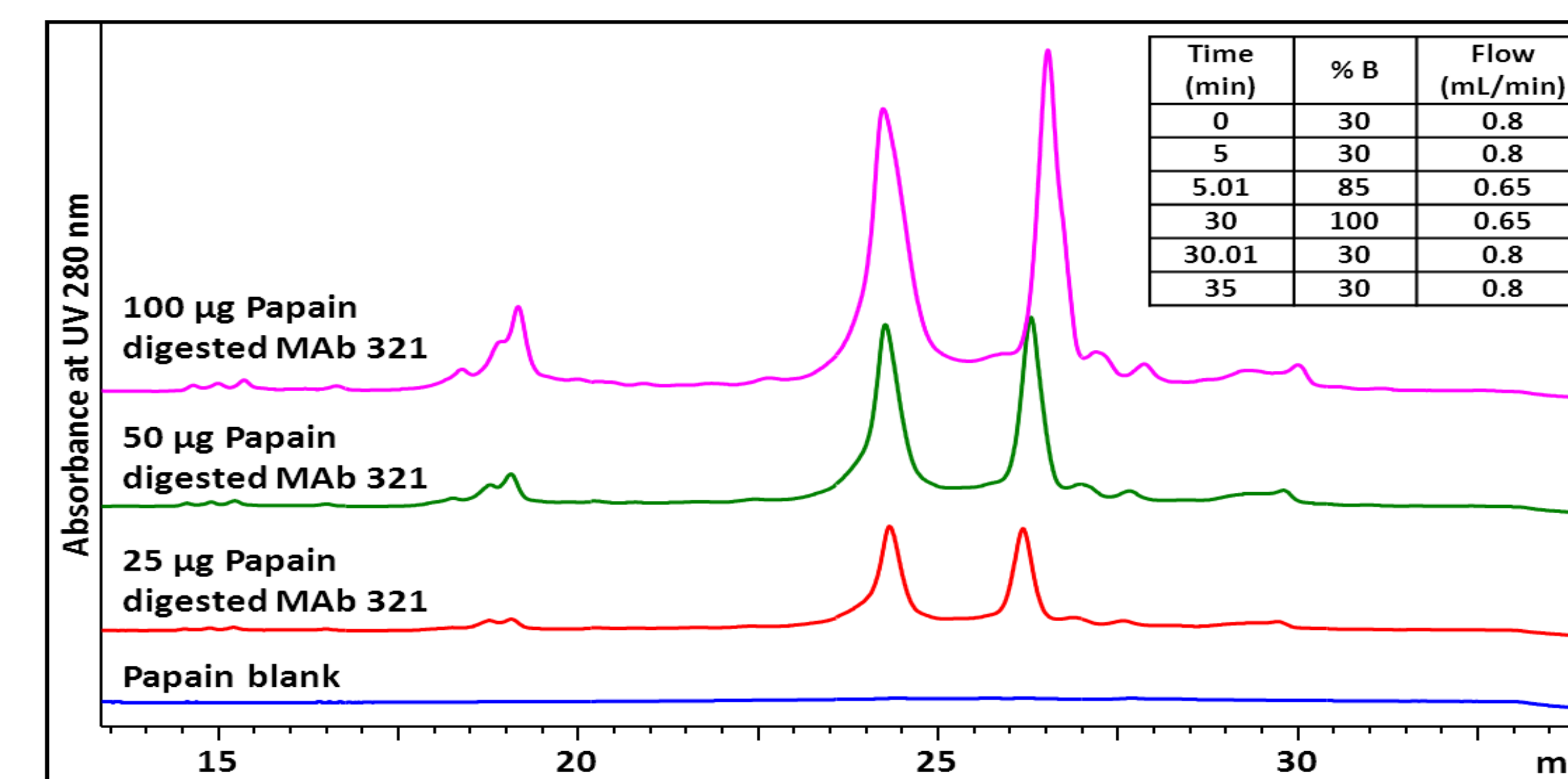
MAb 321 analysis on Antibodix™ WCX NP5, 4.6 x 250 mm. Mobile phase was A: 20 mM sodium acetate pH 5.15 and B: A + 1 M LiCl. Flow rate was 0.8 mL/min and the column temperature was at 30°C. 100 μg of intact MAb 321 was injected.

FAB AND Fc ANALYSIS USING AN NaCl and pH GRADIENT



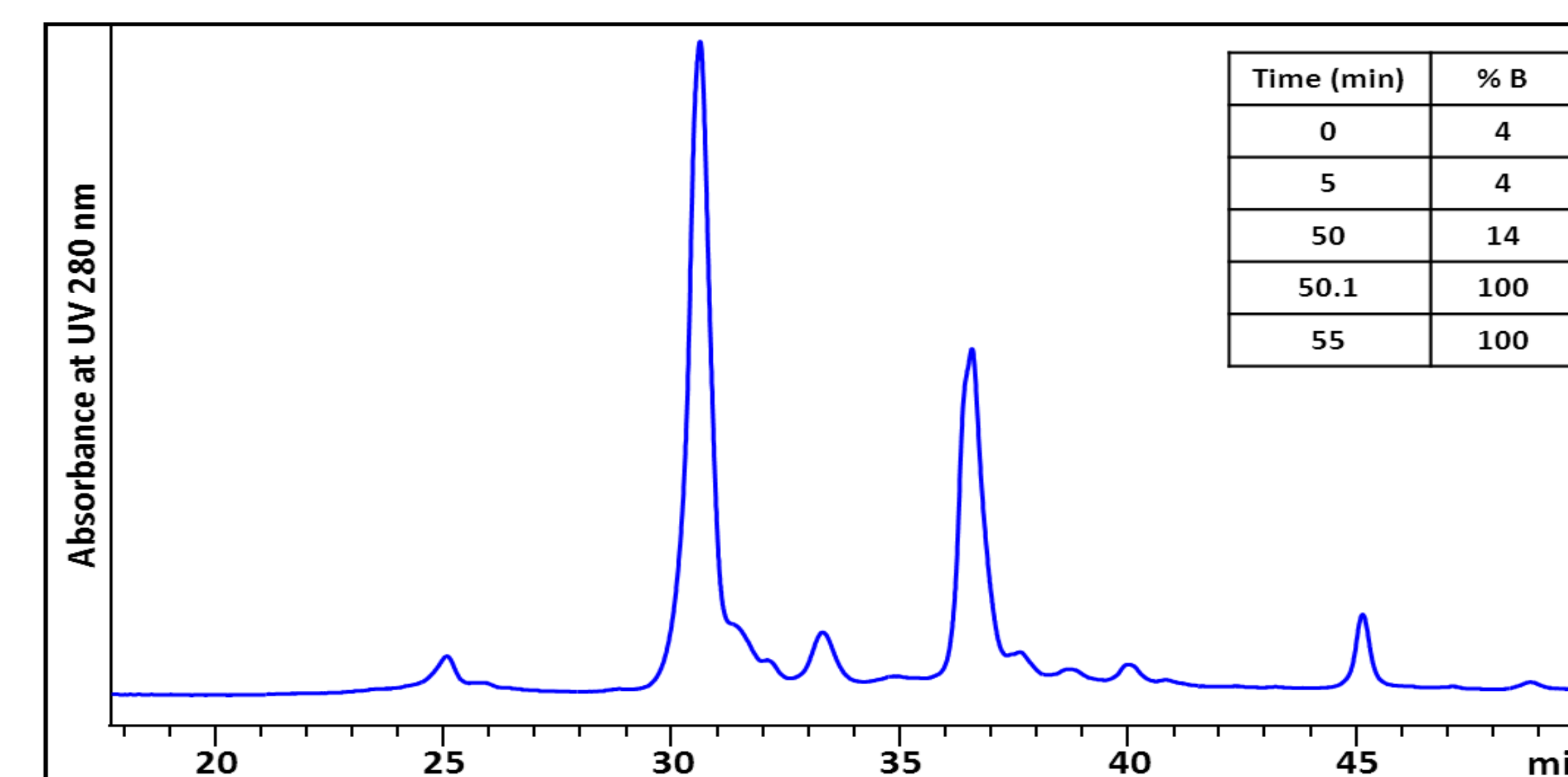
Fab and Fc fragment analysis on Antibodix™ WCX NP5, 4.6 x 250 mm. Mobile phase was A: 20 mM acetic acid + 50 mM NaCl pH 3.5 and B: 20 mM sodium succinate + 50mM NaCl pH 6.0. Flow rate was 0.8 mL/min and the column temperature was at 30°C. 100 μg of papain digested MAb 321 was injected.

FAB AND Fc LOADING TEST ON ANTIBODIX WCX NP5



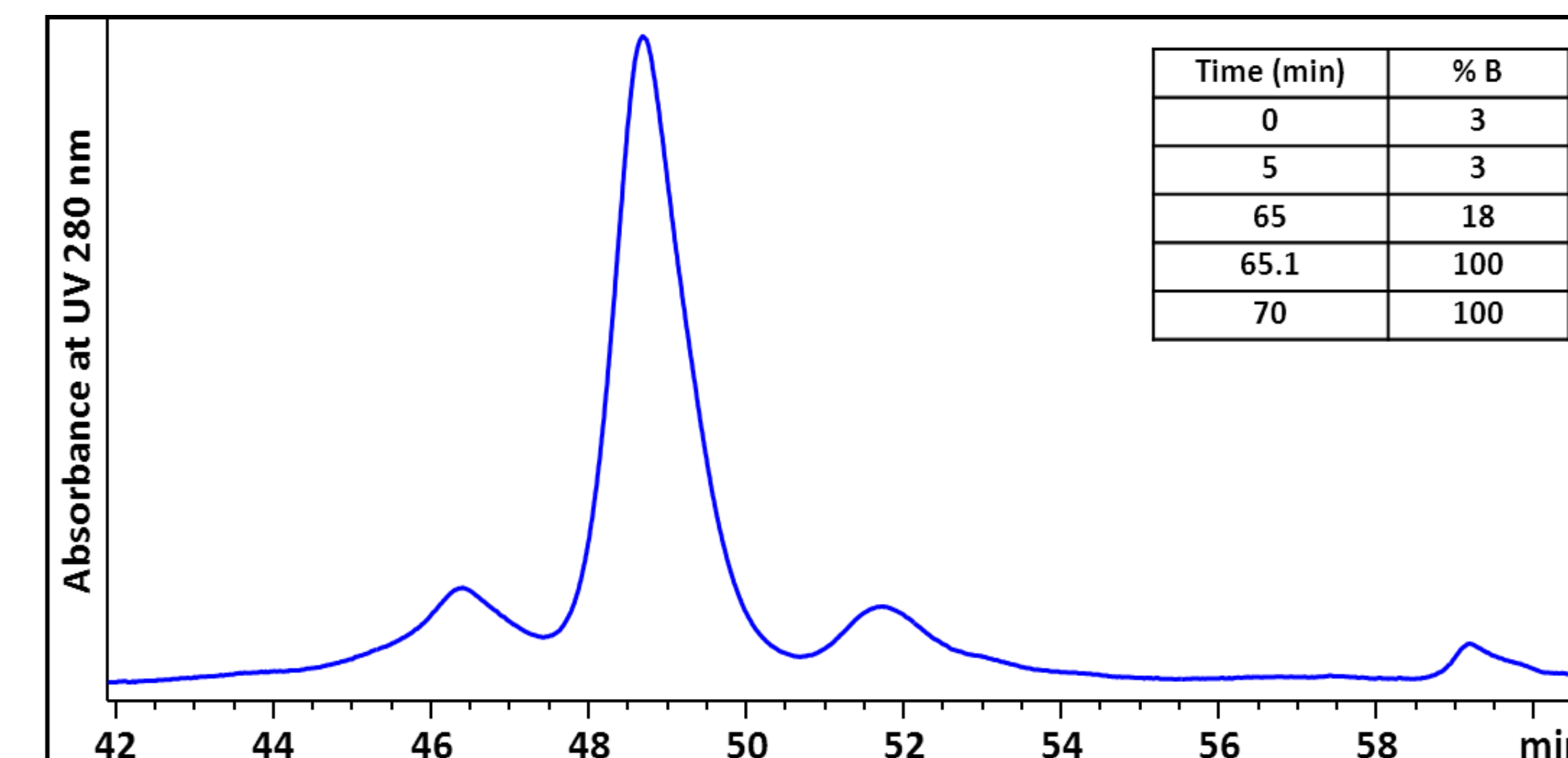
Fab and Fc fragment analysis on Antibodix™ WCX NP5 4.6 x 250 mm. Mobile phase was A: 20 mM acetic acid + 50 mM NaCl pH 3.5 and B: 20 mM sodium succinate + 50mM NaCl pH 6.0. Flow rate was 0.8 mL/min and the column temperature was at 30°C.

FAB AND Fc ANALYSIS USING AN NaCl GRADIENT



Fab and Fc fragment analysis on Antibodix™ WCX NP5, 4.6 x 250 mm. Mobile phase was A: 20 mM phosphate buffer pH 5.5 and B: A + 1 mM NaCl. Flow rate was 0.8 mL/min. 25 μg of papain digested MAb 321 was injected.

F(ab')₂ ANALYSIS USING AN NaCl GRADIENT



F(ab')₂ fragment analysis on Antibodix™ WCX NP5, 4.6 x 250 mm. Mobile phase was A: 20 mM phosphate buffer pH 5.5 and B: A + 1 mM NaCl. Flow rate was 0.8 mL/min. 50 μg of pepsin digested MAb 321 was injected.

CONCLUSION

- Sepax's Antibodix WCX NP5 4.5 x 250 mm can successfully separate monoclonal antibody variants under different mobile phase systems such as pH and salt gradients.
- Monoclonal antibody purity, heterogeneity and stability can all be monitored using Antibodix WCX NP5.
- The smaller particle size of Antibodix WCX NP5 offers higher resolution than Antibodix WCX NP10.