

Antibody Solution Kit for the Separation and Characterization of Monoclonal Antibodies



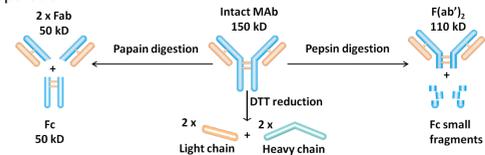
Haiying Chen and Katherine McLaughlin
Sepax Technologies, Inc., 5-100 Innovation Way, Newark DE 19711

INTRODUCTION

Monoclonal antibodies (MAbs) have increasingly been becoming drug candidates for disease therapeutics. Due to the molecular complexity of MAbs, the characterization remains a challenge and required step throughout the development and manufacturing process. In order to determine the efficacy of the molecules, aggregation, heterogeneity such as charge variants, C-terminal lysine processing, deamidation, glycosylation, MAbs must be screened for their structural and biological changes. The antibody solution kit offers a complete separation solution for MAb analysis. In the kit, the Zenix™ size exclusion chromatography (SEC) column is designed for high efficiency and resolution separation of monoclonal antibody monomers, aggregates, fragments such as heavy/light, fab/fc and f(ab')₂ fragments. With its uniform, hydrophilic, and neutral nanometer thick films chemically bonded on high purity silica, non-specific interactions between proteins and the column surface is minimized to result in high resolution of MAb separation. With volatile mobile phase systems, Zenix™ SEC-300 is able to separate MAb fragments, which can be analyzed by on-line mass spectrometry. Molecular weight information of MAb fragments is then obtained with SEC-LC/MS work flow. The antibody solution kit provides Antibodix™ NP5 weak cation exchange (WCX) column to separate the charge variants. Multiple mobile phase systems were investigated for optimum charge variant separation. With its non-porous polymer bead, the Antibodix™ WCX is suitable for resolving slightly different structures of MAbs within a wide pH range of 2-12. With Zenix™ SEC-300 and Antibodix™ WCX NP5, the antibody solution kit offers a complete set of tools for monoclonal antibody analysis.

EXPERIMENTAL

Columns: Zenix™ SEC-300 (3 μm, 300 Å, 7.8 x 300 mm),
Zenix™ SEC-300 (3 μm, 300 Å, 4.6 x 300 mm) and
Antibodix™ WCX NP5 (5 μm, non-porous, 4.6 x 250 mm)
HPLC System: Agilent 1200 HPLC Detection: UV 280 nm and UV 214 nm
Sample Preparation:

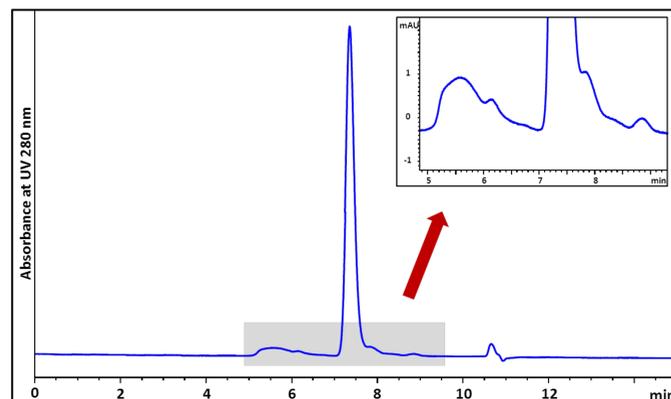


Dithiothreitol (DTT) reduction: MAb 321 was diluted to 1 mg/mL with 150 mM phosphate buffer, pH 7.0. Antibodies were reduced with a final concentration of 20 mM DTT and incubated at 65 °C for 15 minutes.

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.

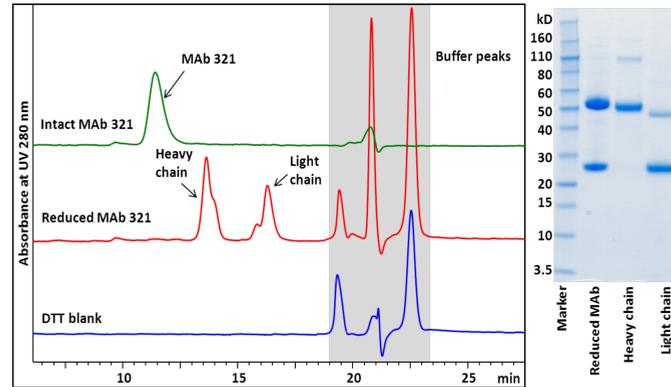
Pepsin digestion: MAb 321 was incubated at a final concentration of 1 mg/mL in 20 mM sodium acetate, pH 4.0 with a pepsin to MAb 321 ratio of 1:40. The digestion was carried out at 37 °C for 15.5 hours. The reaction was stopped by adding 2 M TRIS to increase the pH to 8.0.

ANALYSIS OF INTACT MAb ON ZENIX™ SEC-300



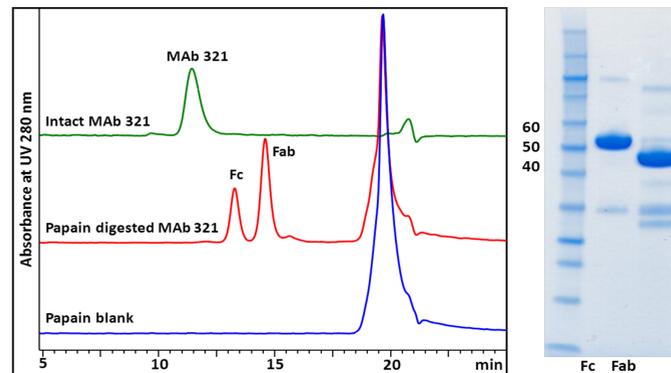
Intact MAb 321 analysis on Zenix™ SEC-300, 4.6 x 300 mm. Mobile phase was 150 mM sodium phosphate buffer, pH 7.0. Flow rate was 0.35 mL/min. 2 μg of intact MAb 321 was injected.

ANALYSIS OF HEAVY AND LIGHT CHAINS ON ZENIX™ SEC-300



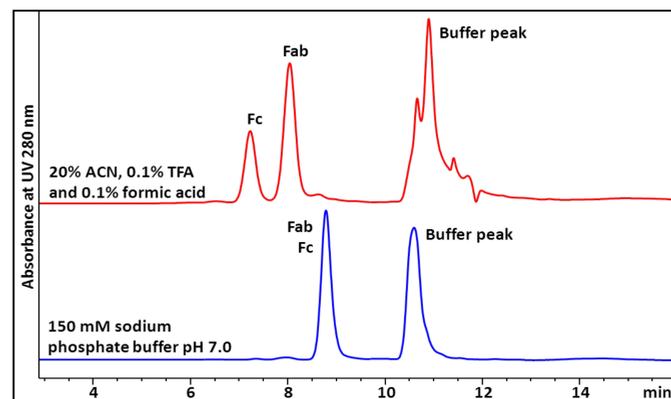
Reduced MAb 321 heavy and light chain separation on Zenix™ SEC-300, 4.6 x 300 mm. Mobile phase was 0.1% TFA, 0.1% formic acid with 20% acetonitrile. Flow rate was 0.2 mL/min. 5 μg of intact MAb 321 and 20 μg of DTT reduced MAb 321 were injected. The 4-12% Bis-Tris gel image (right) of reduced MAb sample, light chain and heavy chain fractions.

ANALYSIS OF Fab/Fc FRACTIONS ON ZENIX™ SEC-300



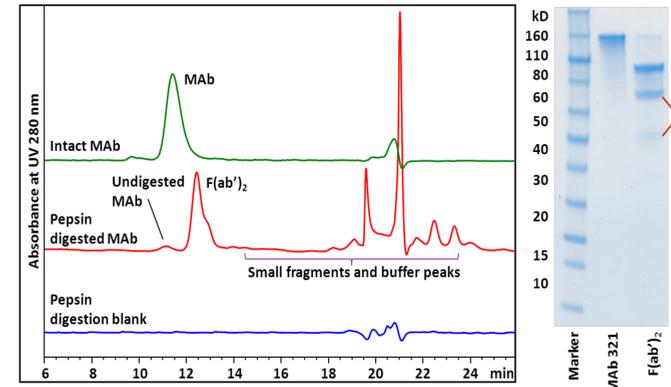
Fab/Fc fragment separation on Zenix™ SEC-300, 4.6 x 300 mm. Mobile phase was 0.1% TFA, 0.1% formic acid with 20% acetonitrile. Flow rate was 0.2 mL/min. 5 μg of intact MAb 321 and 5 μg of papain digested MAb 321 were injected. The 4-12% Bis-Tris gel image (right) of collected Fc and Fab fractions.

MOBILE PHASE EFFECT FOR Fab/Fc ON ZENIX™ SEC-300



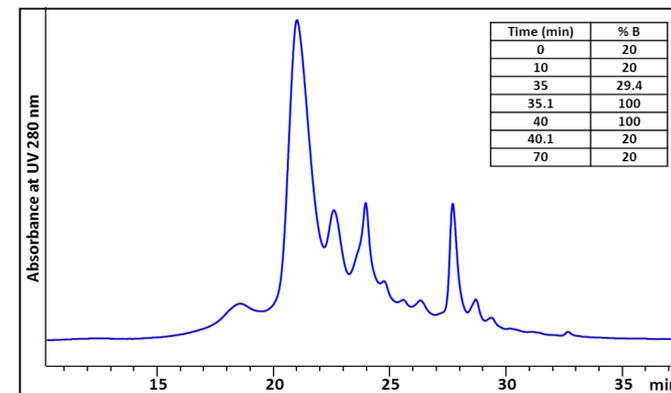
Organic mobile phase vs. salt mobile phase for Fab/Fc separations on Zenix™ SEC-300, 4.6 x 300 mm. Flow rate was 0.35 mL/min and 5 μg of papain digested MAb 321 was injected for both runs. Mobile phases were as indicated.

ANALYSIS OF F(ab')₂ FRAGMENT ON ZENIX™ SEC-300



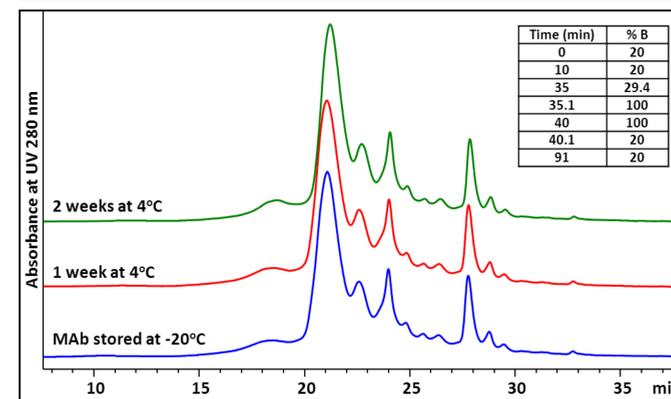
F(ab')₂ separation on Zenix™ SEC-300, 4.6 x 300 mm. Mobile phase was 0.1% TFA, 0.1% formic acid with 20% acetonitrile. Flow rate was 0.2 mL/min. 5 μg of intact MAb 321 and 15 μg of pepsin digested MAb 321 were injected. On the right is the 4-12% Bis-Tris gel image. 5 μg of each sample were loaded. Band (a) is undigested MAb, band (b) is F(ab')₂, and bands (c) are smaller fragments from the digestion.

INTACT MAb ON ANTIBODIX™ WCX USING AN LiCl GRADIENT



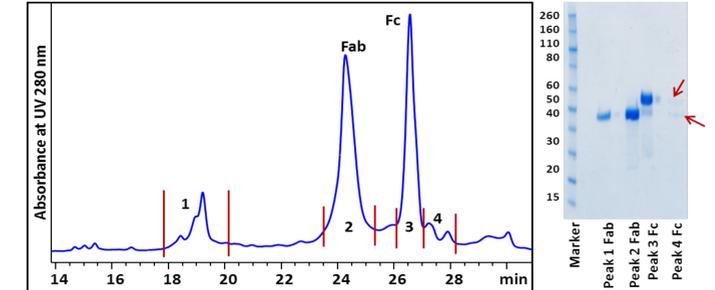
MAb 321 analysis on Antibodix™ WCX NP5, 4.6 x 250 mm. Mobile phase was A: 20 mM sodium acetate pH 5.15 and B: A + 1 M LiCl. Flow rate was 0.8 mL/min and the column temperature was at 30 °C. 100 μg of intact MAb 321 was injected.

MAb 321 STABILITY TEST ON ANTIBODIX™ WCX NP5



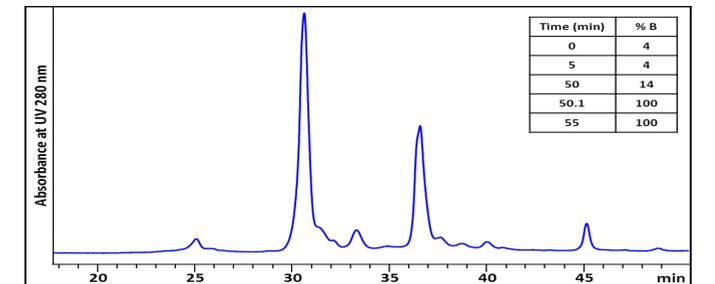
MAb 321 analysis on Antibodix™ WCX NP5, 4.6 x 250 mm. Mobile phase was A: 20 mM sodium acetate pH 5.15 and B: A + 1 M LiCl. Flow rate was 0.8 mL/min and the column temperature was at 30 °C.

FAB/FC ON ANTIBODIX™ WCX USING AN NaCl AND pH GRADIENT



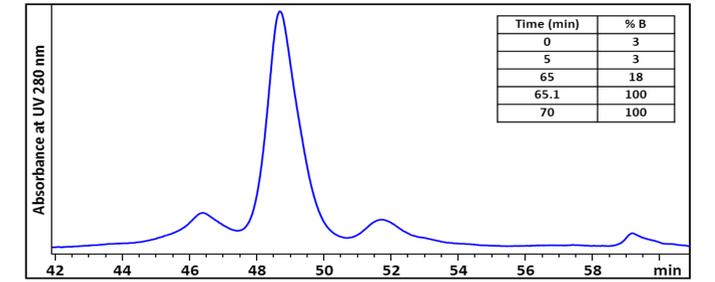
Fab and Fc fragment analysis on Antibodix™ WCX NP5, 4.6 x 250 mm. Mobile phase was A: 20 mM acetic acid + 50 mM NaCl pH 3.5 and B: 20 mM sodium succinate + 50 mM NaCl pH 6.0. Flow rate was 0.8 mL/min and the column temperature was at 30 °C. 100 μg of papain digested MAb 321 was injected.

FAB AND FC ON ANTIBODIX™ WCX 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 ON ANTIBODIX™ WCX 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

- Zenix™ SEC-300 4.6x300 can analyze intact MAb with the separation of aggregates, monomers and fragments. Zenix™ SEC-300 can be applied to monitoring MAb lot to lot consistency and MAb stability during the manufacturing and storage process.
- Zenix™ SEC-300 4.6x300mm can successfully separate MAb fragments including heavy/light chains, Fab/Fc and F(ab')₂ from their reaction mixture, respectively.
- With volatile mobile phases and a reduced flow rate, online SEC-MS can successfully generate accurate mass information for intact MAbs and MAb fragments.
- Sepax's Antibodix™ WCX NP5 4.5x250mm can successfully separate MAb variants under different mobile phase systems such as pH and salt gradients.
- MAb purity, heterogeneity and stability can all be monitored using Antibodix™ WCX NP5.
- The antibody solution kit offers a complete set of tools for MAb analysis.