Analysis of mAb using a pH Gradient on Proteomix® SCX NP5 4.6 x 250 mm

Ion Exchange Chromatography is frequently used for antibody analysis. Antibodies and antibody fragments can all be separated on cation exchange columns based on their charge states.

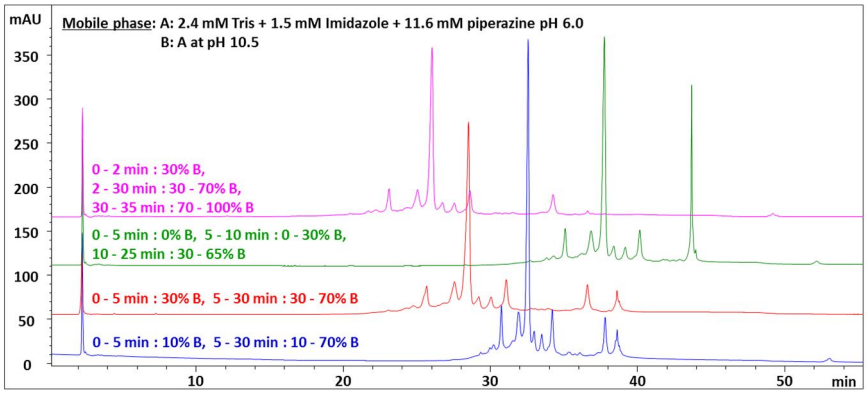
Sepax's Proteomix® SCX is a complementary option to the Antibodix™ WCX phase for the high resolution, high efficiency and high recovery analysis of antibodies and their variants.

Highlighted FACTS:

- ⋙ Proteomix® SCX NP5 4.6 x 250 mm can successfully separate monoclonal antibody variants using a pH gradient.
- ⋙ Monoclonal antibody purity, heterogeneity and stability can be monitored using Proteomix® SCX NP5.
- ⋙ The 5 µm particle size in Proteomix® SCX NP5 offers superior resolution.
- ⋙ High stability packing material allows for analyses in wide pH and temperature ranges.

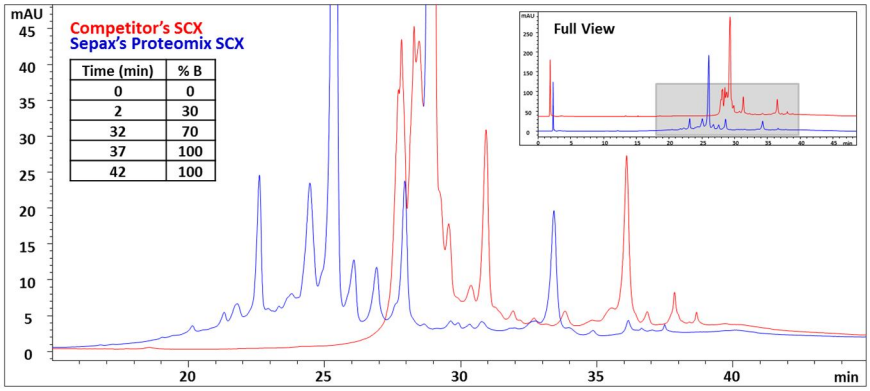
Optimization of pH Gradient for mAb Analysis on Proteomix® SCX

Column: Proteomix® SCX NP5 (5 µm, 4.6 x 250 mm)
Flow rate: 0.8 mL/min, Detector: UV 280 nm, Column temperature: 25 ˚C
Sample: 10 µL mAb 321 (5.0 mg/mL)

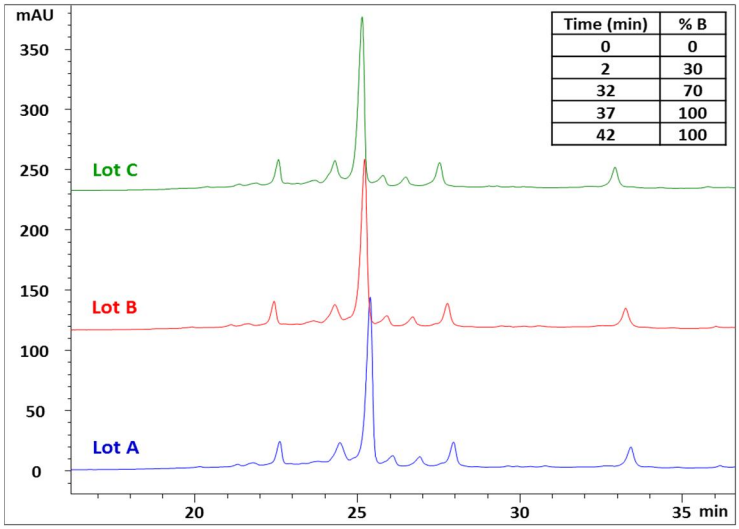


Analysis of mAb on Proteomix® SCX Compared to Competitor's SCX

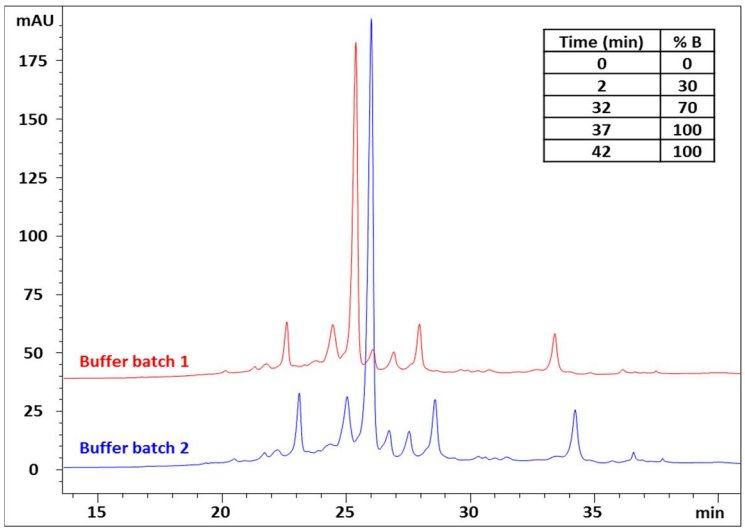
Columns: Proteomix® SCX NP5 (4.6 x 250 mm) and Competitor's SCX (5 µm, 4.0 x 250 mm)
Mobile phase: A: 2.4 mM Tris + 1.5 mM Imidazole + 11.6 mM piperazine pH 6.0 and B: A at pH 10.5; Flow rate: 0.8 mL/min, Detector: UV 280 nm, Column temp: 25 ˚C, Sample: 10 µL mAb 321 (5 mg/mL)



Analysis of mAb 321 on Proteomix® SCX NP5 Lot to Lot Comparison to show Reproducibility



Analysis of mAb 321 on Proteomix® SCX NP5 Reproducibility between batches of Mobile Phase



Column: Proteomix® SCX NP5 (4.6 x 250 mm), Mobile phase: A: 2.4 mM Tris + 1.5 mM Imidazole + 11.6 mM piperazine pH 6.0 and B: A at pH 10.5; Flow rate: 0.8 mL/min, Detector: UV 280 nm, Column temperature: 25 ˚C, Sample: 10 µL mAb 321 (5 mg/mL)

Analysis of mAb using a pH Gradient on Proteomix® SCX NP5 4.6 x 250 mm

What is Proteomix® SCX NP5

Proteomix® SCX NP5 (Strong Cation Exchange): Comprised of rigid, spherical, highly cross-linked non-porous poly(styrene divinylbenzene) (PS/DVB) beads. The PS/DVB particle surface is grafted with a hydrophilic, neutral polymer layer which is nanometers thick. The resin surface is covered by a hydrophilic coating which eliminates non-specific bindings with antibody proteins, leading to high efficiency and high recovery separations. On top of the hydrophilic layer, strong cation-exchange sulfonate (—SO_3H) functional groups are attached via a proprietary chemistry, resulting in a high capacity ion-exchange layer.

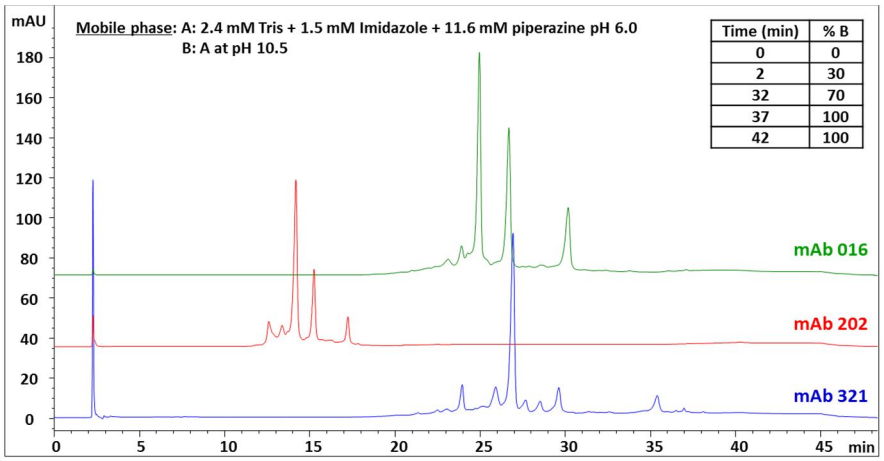
Technical Specifications:

Phase	Proteomix® SCX NP5
Material	Sulfonate strong cation exchange groups bonded to a hydrophilic film grafted on PS/DVB
Particle size (µm)	5
Pore size (Å)	Non-porous
pH stability	2 – 12
Backpressure (psi)	~ 3,500
Maximum backpressure	~ 6,000
Maximum temperature	~ 80 °C
Mobile phase compatibility	Aqueous or a mixture of water and acetonitrile, acetone or methanol

Sepax Technologies, Inc.
5 Innovation Way
Newark, Delaware 19711, USA
Tel: (302) 366-1101 | Fax: (302) 366-1151
E-mail: info@sepax-tech.com

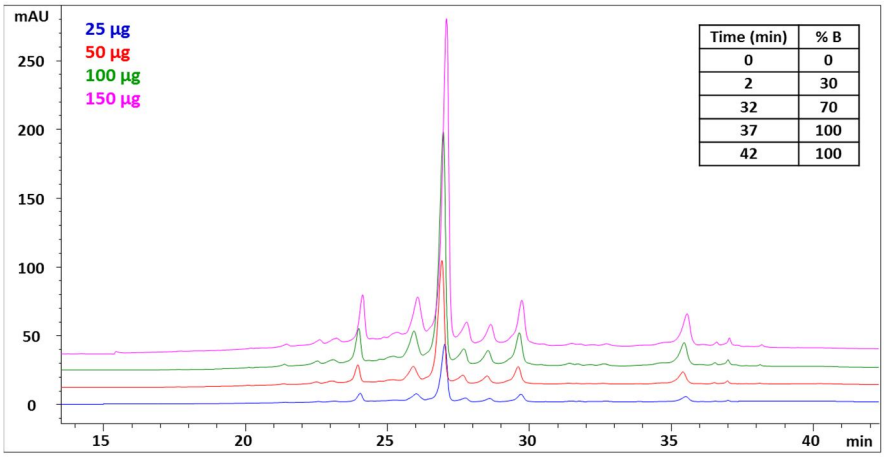
Three Different mAbs on Proteomix® SCX using the same pH Gradient

Column: Proteomix® SCX NP5 (5 µm, 4.6 x 250 mm)
Flow rate: 0.8 mL/min, Detector: UV 280 nm, Column temperature: 25 °C, Sample: 10 µL mAb 321 (5.0 mg/mL), 50 µL mAb 202 (1.0 mg/mL), 10 µL mAb 016 (5.9 mg/mL)



Loading Test for mAb 321 on Proteomix® SCX using a pH gradient

Column: Proteomix® SCX NP5 (5 µm, 4.6 x 250 mm), Mobile phase: A: 2.4 mM Tris + 1.5 mM Imidazole + 11.6 mM piperazine pH 6.0 and B: A at pH 10.5; Flow rate: 0.8 mL/min, Detector: UV 280 nm, Column temperature: 25 °C, Sample: mAb 321



Faster mAb Analysis on Proteomix® SCX with a Higher Flow Rate

Column: Proteomix® SCX NP5 (5 µm, 4.6 x 250 mm), Mobile phase: A: 2.4 mM Tris + 1.5 mM Imidazole + 11.6 mM piperazine pH 6.0 and B: A at pH 10.5; Flow rate: specified, Detector: UV 280 nm, Column temperature: 25 °C, Sample: 10 µL mAb 321 (5.0 mg/mL)