

Proteomix® SCX NP5: A Complementary CEX phase to Antibodix™ WCX NP5 for MAb Variant Characterization

APPLICATION NOTES

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.

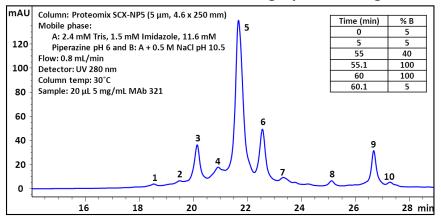
Sepax's Proteomix® SCX is a complementary option to the Antibodix™ WCX phase for the high resolution, high efficiency and high recovery analysis of antibodies and their variants.

Highlighted FACTS:

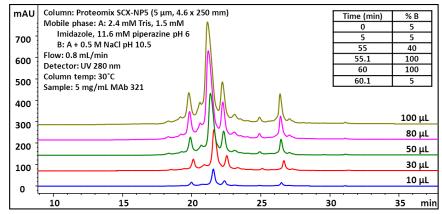
- ► Proteomix® SCX NP5 4.6 x 250 mm can successfully separate monoclonal antibody variants under a variety of different mobile phase systems such as pH and salt gradients.
- ► Monoclonal antibody purity, heterogeneity and stability can be monitored using Proteomix® SCX NP5.
- ► The 5 μ m particle size in Proteomix® SCX NP5 offers superior resolution.
- ► High stability packing material allows for analyses in wide pH and temperature ranges.

Analysis of MAb 321 on Proteomix® SCX NP5

MAb 321 on Proteomix® SCX NP5 using a pH and salt gradient

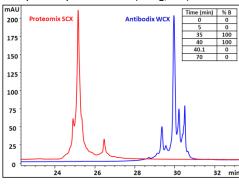


MAb 321 loading test on Proteomix® SCX NP5 4.6 x 250 mm

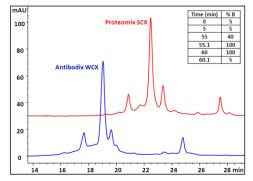


Proteomix® SCX NP5 compared to Antibodix™ WCX NP5 using a pH gradient for the analysis of MAb 321

Mobile phase: A: 2.4 mM Tris, 1.5 mM Imidazole, 11.6 mM piperazine pH 6 and B: A at pH 10.5; Flow rate: 0.8 mL/min; Column temperature: 30° C; Sample: 10μ L MAb 321 (5 mg/mL)



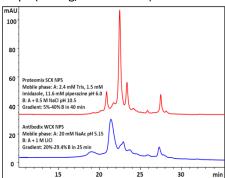
Proteomix® SCX vs. Antibodix™ WCX using a salt and pH gradient for the analysis of MAb 321



Mobile phase: A: 2.4 mM Tris, 1.5 mM Imidazole, 11.6 mM piperazine pH 6 and B: A + 0.5 M NaCl pH 10.5; Flow rate: 0.8 mL/min; Column temperature: 30°C; Sample: 10 μL MAb 321 (5 mg/mL)

Analysis of MAb 321 on Proteomix® SCX compared to the analysis on Antibodix™ WCX

Column: Proteomix SCX NP5 and Antibodix WCX NP5, Flow rate: 0.8 mL/min, Detector: UV 280 nm, Column temperature: 30° C, Sample: $10 \,\mu$ L ($5.0 \,m$ g/mL MAb 321)



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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 (-SO₃H) 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)

Pore size (Å) Non-porous

pH stability 2 - 12Backpressure (psi) ~ 3.500 Maximum backpressure ~ 6,000

Maximum temperature ~ 80 °C

Mobile phase Aqueous or a mixture compatibility

of water and acetonitrile, acetone

or methanol

Sepax Technologies, Inc.

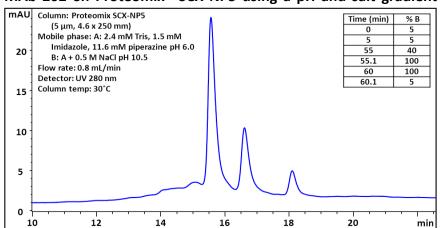
5 Innovation Way

Newark, Delaware 19711, USA

E-mail: info@sepax-tech.com

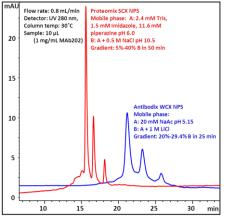
Analysis of MAb 202 on Proteomix® SCX NP5

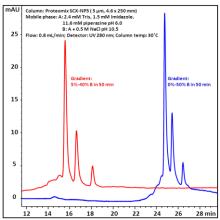
MAb 202 on Proteomix® SCX NP5 using a pH and salt gradient



Analysis of MAb 202 on Proteomix® SCX NP5 compared to Antibodix™ WCX NP5

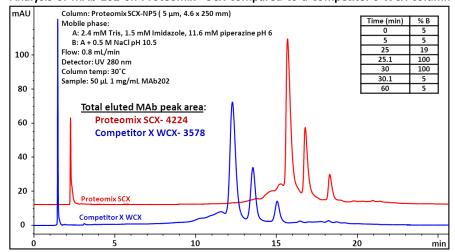
Gradient modification for improved resolution of MAb 202 on Proteomix® SCX





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Analysis of MAb 202 on Proteomix® SCX compared to a competitor's WCX column



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