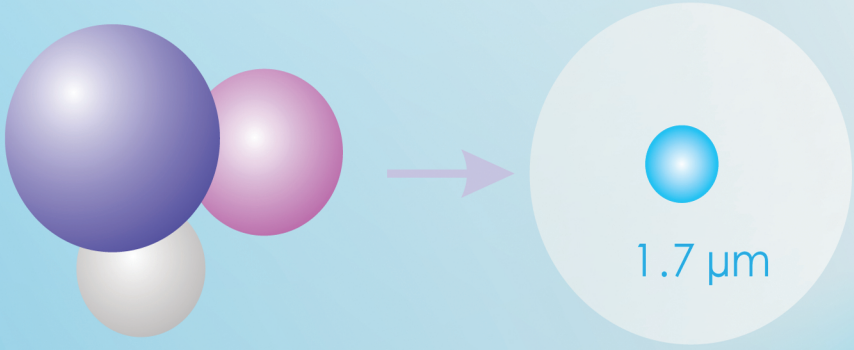
Cation Exchange Chromatography



Smaller Particle

Higher Efficiency

Sepax Technologies

Content

Introduction

Proteomix® CEX phase (WCX and SCX) and Antibodix™ WCX phase

Comprised of a rigid, spherical, highly cross-linked poly (styrene divinylbenzene) (PS/DVB) non-porous bead with particle size of 1.7 µm (3, 5, 10 µm are also available). The PS/DVB resin surface is grafted with a highly hydrophilic, neutral polymer thin layer with the thickness in the range of nanometers. The hydrophobic PS/DVB resin surface is totally covered by a hydrophilic coating that eliminates non-specific bindings with biological analytes, leading to high efficiency and high recovery separations for biological molecules. On the top of the hydrophilic layer, cation-exchange functional groups are attached via a proprietary chemistry, resulting in a high capacity ion-exchange layer.

Stationary Phase Structures

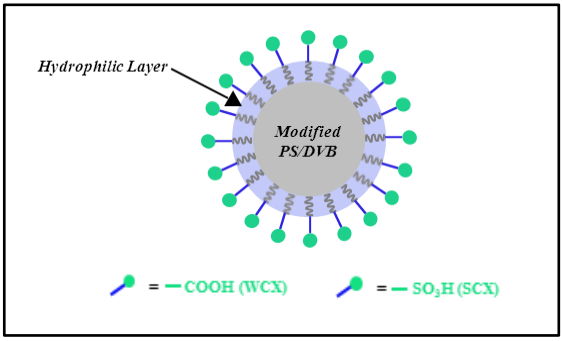


Figure 1. General structure for Sepax's Cation Exchange Phases.

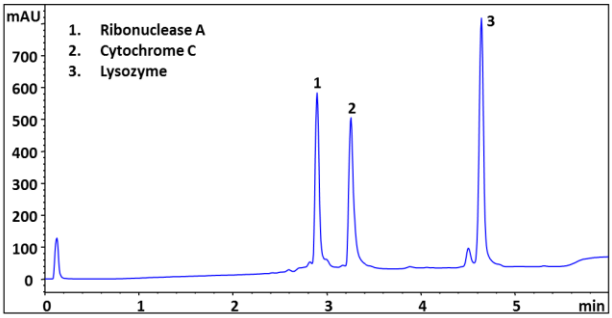
Key features of Proteomix® and Antibodix™ phases

Characteristics	Proteomix® SCX	Antibodix™ WCX Proteomix® WCX
Particle size	1.7 µm	1.7 µm
Pore size (Å)	Non-porous	Non-porous
Surface structure	Strong cation exchange functional groups attached to a hydrophilic coating	Weak cation exchange functional groups attached to a hydrophilic coating

Technical specifications of Proteomix® CEX and Antibodix™ WCX

Phase	Proteomix® SCX, WCX	Antibodix™ WCX
Dimensions	4.6 x 100 mm	4.6 x 100 mm
Material	Non-porous PS/DVB beads grafted with a highly hydrophilic, neutral polymer thin layer.	Non-porous PS/DVB beads grafted with a highly hydrophilic, neutral polymer thin layer.
Particle size	1.7 µm	1.7 µm
Pore size (Å)	Non-porous	Non-porous
pH stability	2-12	2-12
Flow rate	0.5 - 1.0 mL/min	0.30 - 0.75 mL/min
Backpressure	~ 200 - 400 bar	~200 - 400 bar
Maximum backpressure	~ 12,000 psi (828 bar)	~12,000 psi (828 bar)
Maximum temperature (^{o}C)	~ 80	~ 80
Mobile phase compatibility	Aqueous or a mixture of water and acetonitrile, acetone, or methanol	Aqueous or a mixture of water and acetonitrile, acetone, or methanol

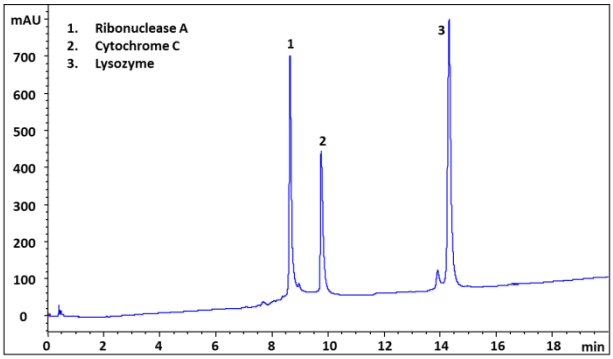
Quality Control Test for Proteomix® SCX NP1.7 4.6 x 30 mm



Compound Name	RT (min)	Area	Plates	Tailing	Resolution
Ribonuclease A	2.89	1867	19185	1.71	---
Cytochrome C	3.25	2049	17838	1.64	3.96
Lysozyme	4.64	3151	41932	0.97	14.74

Figure 2. A standard quality control test on a Proteomix® SCX NP1.7 4.6 x 30 mm. Mobile phase A: 20 mM sodium phosphate buffer pH 6.0 and B: A + 1.0 M NaCl. The gradient was 0-50% B in 5 minutes with a 15 minute prewash. Flow rate was 0.2 mL/min. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL each).

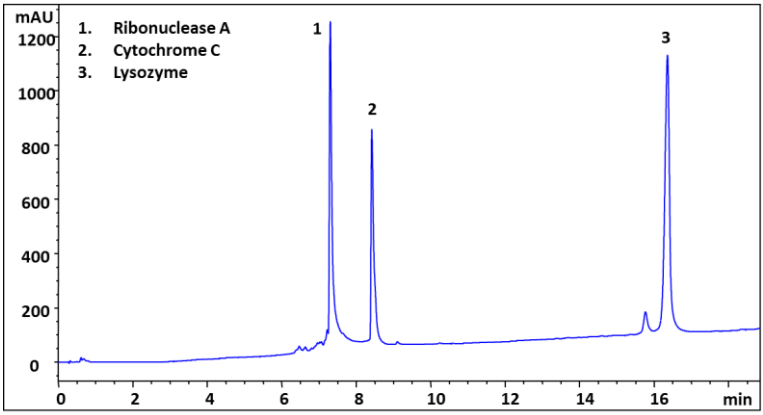
Quality Control Test for Proteomix® SCX NP1.7 4.6 x 50 mm



Compound Name	RT (min)	Area	Plates	Tailing	Resolution
Ribonuclease A	8.64	3875	71970	2.13	---
Cytochrome C	9.75	2549	59402	2.31	7.65
Lysozyme	14.32	5585	92428	1.36	26.17

Figure 3. A standard quality control test on a Proteomix® SCX NP1.7 4.6 x 50 mm. Mobile phase A: 20 mM sodium phosphate buffer pH 6.0 and B: A + 1.0 M NaCl. The gradient was 0-75% B in 25 minutes with a 15 minute prewash. Flow rate was 0.75 mL/min. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL each).

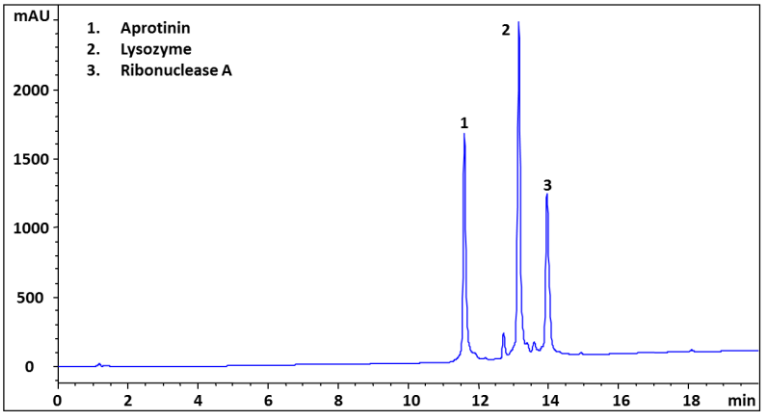
Quality Control Test for Proteomix® WCX NP1.7 4.6 x 50 mm



Compound Name	RT (min)	Area	Plates	Tailing	Resolution
Ribonuclease A	7.31	6052	71850	3.05	---
Cytochrome C	8.42	4212	78343	2.44	9.72
Lysozyme	16.36	9283	83449	0.99	45.70

Figure 4. A standard quality control test on a Proteomix® WCX NP1.7 4.6 x 50 mm. Mobile phase A: 20 mM sodium phosphate buffer pH 6.5 and B: A + 1.0 M NaCl. The gradient was 0-100% B in 25 minutes with a 15 minute prewash. Flow rate was 0.5 mL/min. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL each).

Quality Control Test for Antibodix™ WCX NP1.7 4.6 x 50 mm



Compound Name	RT (min)	Area	Plates	Tailing	Resolution
Aprotinin	9.94	9489	85527	1.44	---
Lysozyme	11.50	14976	101466	1.02	11.14
Ribonuclease A	12.04	8625	51440	1.03	3.03

Figure 5. A standard quality control test on a Antibodix™ WCX NP1.7 4.6 x 50 mm. Mobile phase A: 10 mM sodium phosphate buffer pH 6.0 and B: A + 1.0 M NaCl. The gradient was 10-100% B in 25 minutes with a 15 minute prewash. Flow rate was 0.5 mL/min. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of Ribonuclease A, Aprotinin and Lysozyme (1 mg/mL each).

Particle Size Comparison for Proteomix® SCX 4.6 x 50 mm

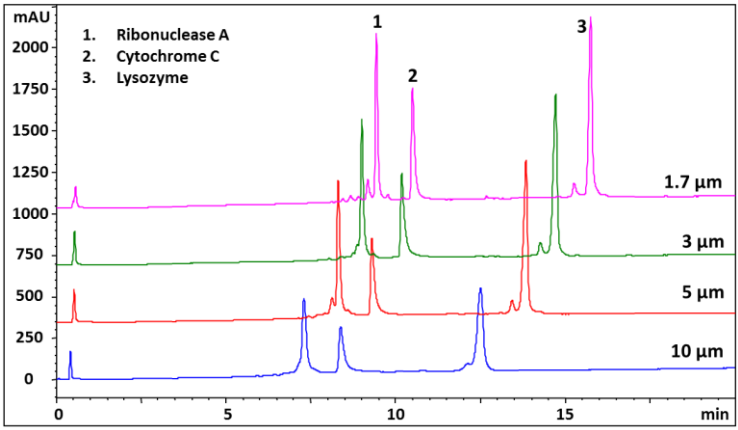


Figure 6. A comparison of different particle sizes for Proteomix® SCX NP 4.6 x 50 mm. Mobile phase A: 20 mM sodium phosphate buffer pH 6.0 and B: A + 1.0 M NaCl. The gradient was 0-75% B in 25 minutes with a 15 minute prewash. Flow rate was 0.75 mL/min. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL each).

Particle Size Comparison for Proteomix® WCX 4.6 x 50 mm

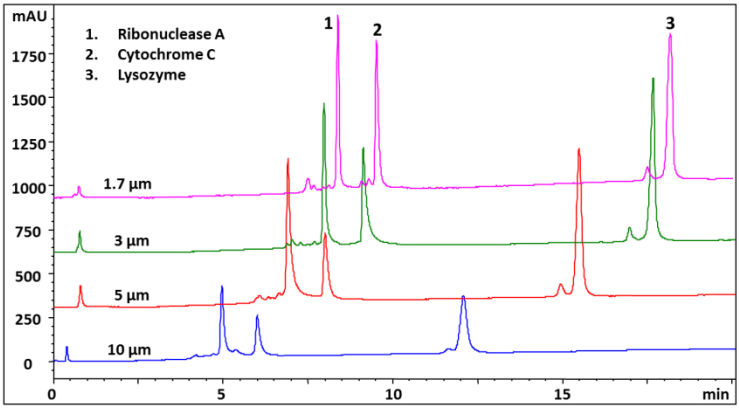


Figure 7. A comparison of different particle sizes for Proteomix® WCX NP 4.6 x 50 mm. Mobile phase A: 20 mM sodium phosphate buffer pH 6.5 and B: A + 1.0 M NaCl. The gradient was 0-75% B in 25 minutes with a 15 minute prewash. Flow rate was 0.5 mL/min. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL each).

Lot to Lot Reproducibility for Proteomix® SCX 4.6 x 50 mm

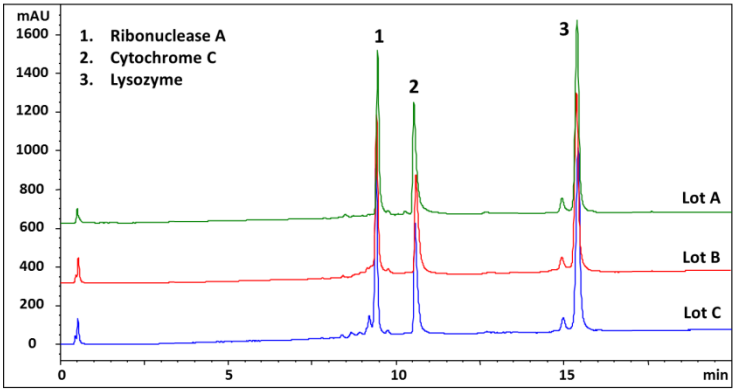


Figure 8. Lot to lot test showing the reproducibility of Proteomix® SCX NP1.7 4.6 x 50 mm. Mobile phase A: 20 mM sodium phosphate buffer pH 6.0 and B: A + 1.0 M NaCl. The gradient was 0-75% B in 25 minutes with a 15 minute prewash. Flow rate was 0.75 mL/min. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL each).

Lot to Lot Reproducibility for Proteomix® WCX 4.6 x 50 mm

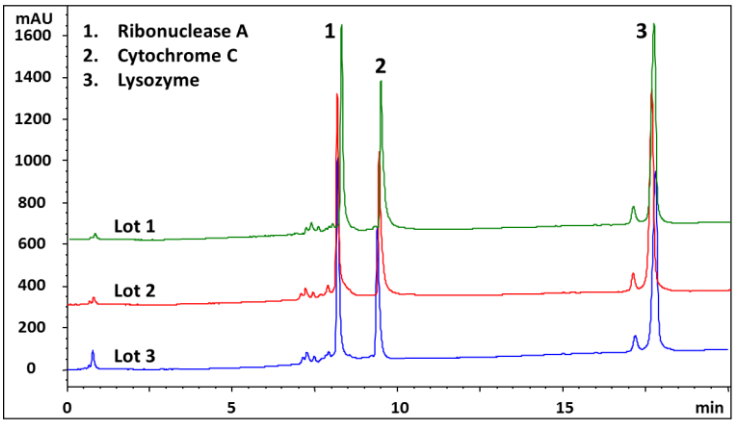


Figure 9. Lot to lot test showing the reproducibility of Proteomix® WCX NP1.7 4.6 x 50 mm. Mobile phase A: 20 mM sodium phosphate buffer pH 6.5 and B: A + 1.0 M NaCl. The gradient was 0-75% B in 25 minutes with a 15 minute prewash. Flow rate was 0.5 mL/min. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL each).

Column Lifetime for Proteomix® SCX 4.6 x 50 mm

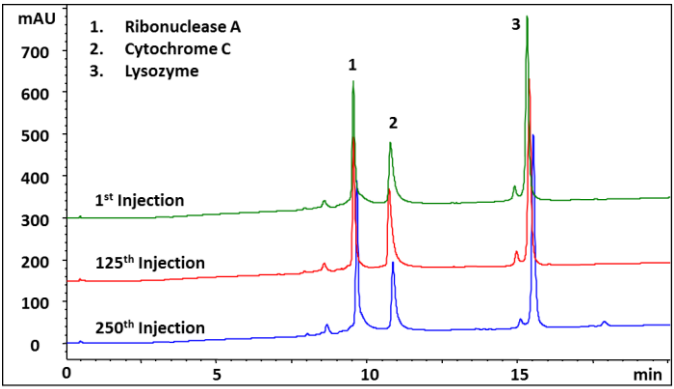


Figure 10. Lifetime test for Proteomix® SCX NP1.7 4.6 x 50 mm with a 4 x 10 mm guard. Mobile phase A: 20 mM sodium phosphate buffer pH 6.5 and B: A + 1.0 M NaCl. The gradient was 0-100% B in 25 minutes with a 15 minute prewash. Flow rate was 0.5 mL/min. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL each).

Column Lifetime for Antibodix™ WCX 4.6 x 50 mm

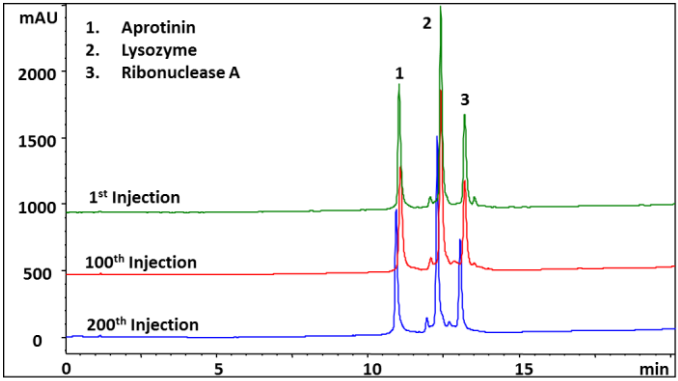


Figure 11. Lifetime test for Antibodix™ WCX NP1.7 4.6 x 50 mm with a 4 x 10 mm guard column. Mobile phase A: 20 mM sodium phosphate buffer pH 6.5 and B: A + 1.0 M NaCl. The gradient was 10-100% B in 25 minutes with a 15 minute prewash. Flow rate was 0.3 mL/min. UV detection was set at 214 nm. 3 µL of sample was injected and the sample is a mixture of Aprotinin, Lysozyme and Ribonuclease A (1 mg/mL each).

MAb separation on Proteomix® SCX NP1.7 4.6 x 100 mm vs. NP5 4.6 x 250 mm

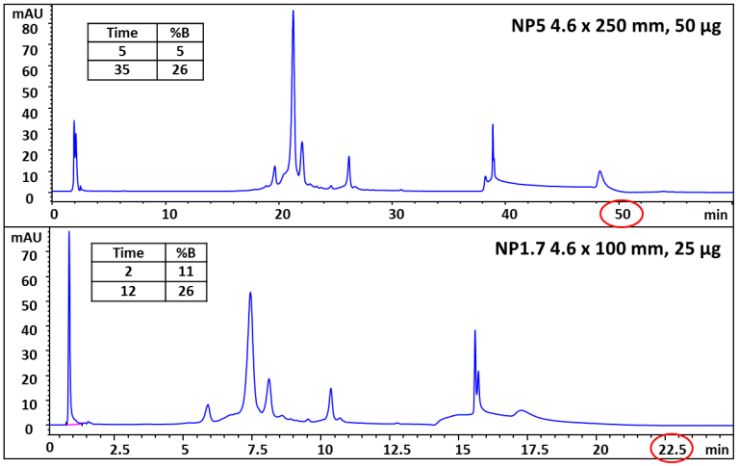


Figure 12. Particle size and column length comparison for Proteomix® SCX NP. Mobile phase A: 2.4 mM Tris, 1.5 mM Imidazole, 11.6 mM piperazine pH 6.0 and B: A + 0.5 M NaCl pH 10.5. The gradient was 0-100% B in 25 minutes with a 15 minute prewash. Flow rate was 0.75 mL/min (NP1.7) and 0.8 mL/min (NP5). UV detection was set at 280 nm. 5 µL of MAb 321 was injected on each column (1 mg/mL).

MAb separation on Proteomix® SCX NP1.7 vs. Proteomix® SCX NP5 (4.6 x 100 mm)

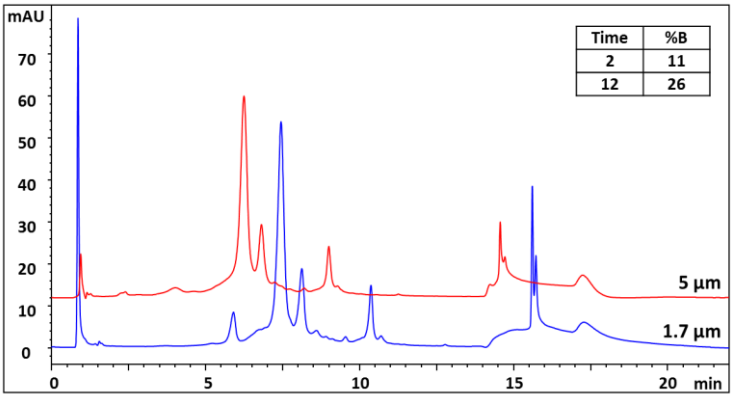


Figure 13. Particle size comparison for Proteomix® SCX NP1.7 4.6 x 100 mm to Proteomix® SCX NP5 4.6 x 100 mm. Mobile phase A: 2.4 mM Tris, 1.5 mM Imidazole, 11.6 mM piperazine pH 6.0 and B: A + 0.5 M NaCl pH 10.5. The gradient was 0-100% B in 25 minutes with a 15 minute prewash. Flow rate was 0.75 mL/min. UV detection was set at 280 nm. 5 µL of MAb 321 was injected on each column (5 mg/mL).

Separation of BSA Digest on Proteomix® SCX NP1.7 2.1 x 30 mm

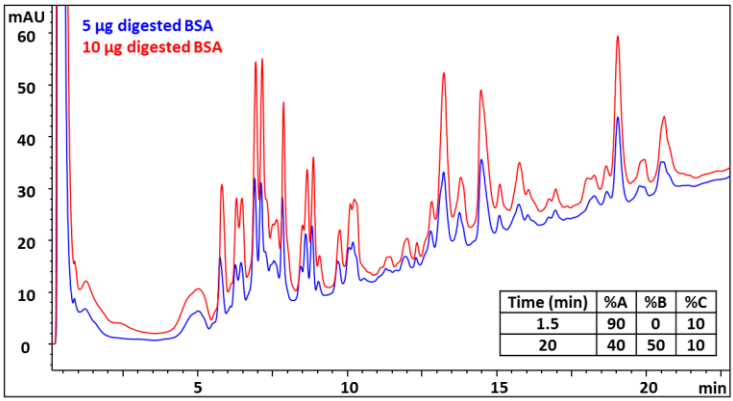


Figure 14. Separation of BSA tryptic digest on Proteomix® SCX NP1.7 2.1 x 30 mm. Mobile phase A: 10 mM phosphate buffer pH 2.5, B: A + 1 M NaCl and C: 100% acetonitrile. Flow rate was 0.35 mL/min. UV detection was set at 214 nm. 5 and 10 µg of trypsin digested BSA was injected on the column.

Separation of Peptides on Proteomix® SCX NP1.7 2.1 x 30 mm

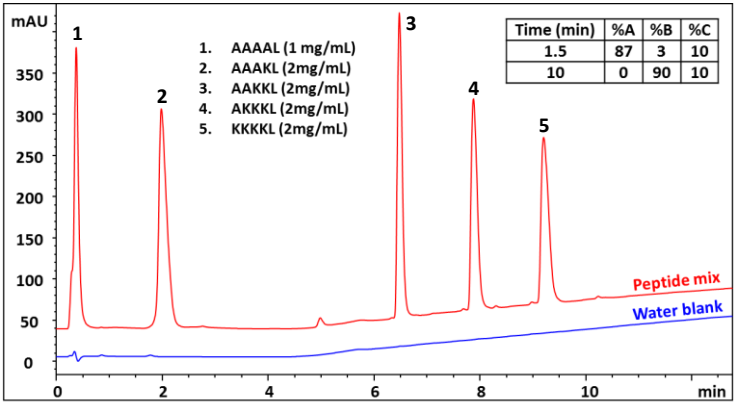


Figure 15. Separation of four Peptides on Proteomix® SCX NP1.7 2.1 x 30 mm. Mobile phase A: 10 mM phosphate buffer pH 2.5, B: A + 1 M NaCl and C: 100% acetonitrile. Flow rate was 0.35 mL/min. UV detection was set at 214 nm. 2 µL of the peptide mixture was injected.

Gradient Optimization for Peptide Separation on Proteomix® SCX NP1.7

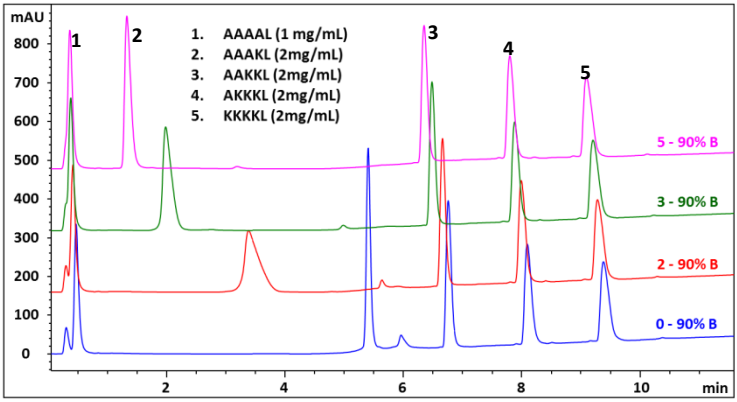


Figure 16. Gradient optimization for the separation of four Peptides on Proteomix® SCX NP1.7 2.1 x 30 mm. Mobile phase A: 10 mM phosphate buffer pH 2.5, B: A + 1 M NaCl and C: 100% acetonitrile. Flow rate was 0.35 mL/min. UV detection was set at 214 nm. 2 µL of the peptide mixture was injected.

Particle Size Comparison for the Separation on Peptides on Proteomix® SCX

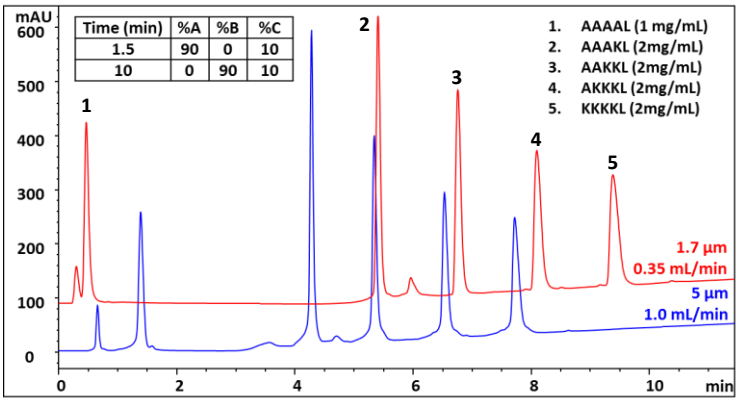


Figure 17. Particle size comparison for Proteomix® SCX NP1.7 2.1 x 30 mm to Proteomix® SCX NP5 4.6 x 100 mm. Mobile phase A: 10 mM phosphate buffer pH 2.5, B: A + 1 M NaCl and C: 100% acetonitrile. Flow rate was 1.0 mL/min (for 5 µm) and 0.35 mL/min (for 1.7 µm). UV detection was set at 214 nm. 2 µL of the peptide mixture was injected.

Shorter Run Time for Peptide Separation on Proteomix® SCX NP1.7

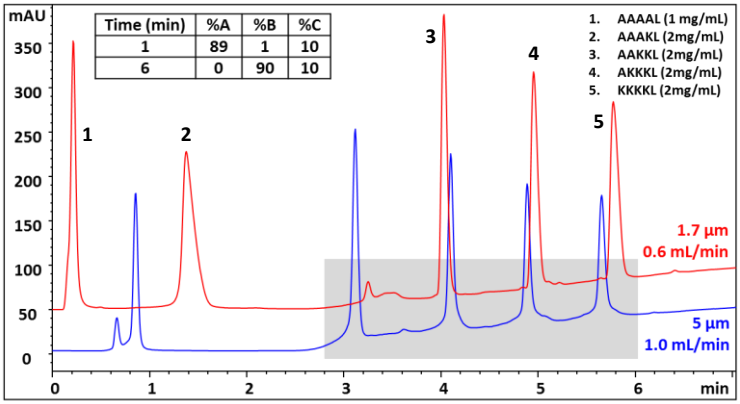


Figure 18. Short run time and high resolution for the separation of five Peptides on Proteomix® SCX NP1.7 2.1 x 30 mm compared to Proteomix® SCX NP5 4.6 x 100 mm. Mobile phase A: 10 mM phosphate buffer pH 2.5, B: A + 1 M NaCl and C: 100% acetonitrile. Flow rate was 1.0 mL/min (for 5 µm) and 0.6 mL/min (for 1.7 µm). UV detection was set at 214 nm. 2 µL of the peptide mixture was injected.

Zoomed View of Short Run for Peptide Separation on Proteomix® SCX NP1.7

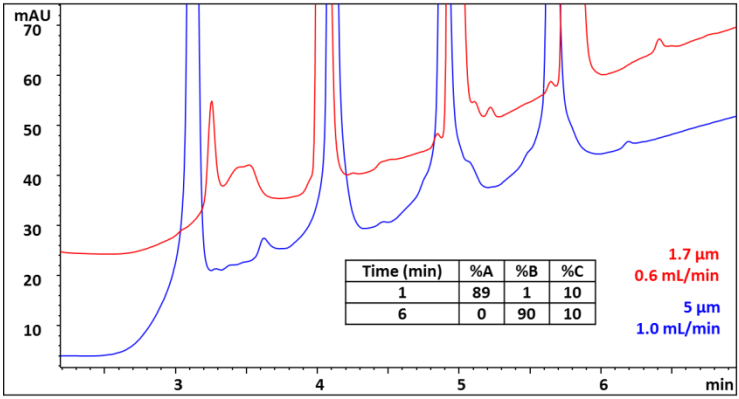


Figure 19. Zoomed view of short run time and high resolution for the separation of five Peptides on Proteomix® SCX NP1.7 2.1 x 30 mm compared to Proteomix® SCX NP5 4.6 x 100 mm. Mobile phase A: 10 mM phosphate buffer pH 2.5, B: A + 1 M NaCl and C: 100% acetonitrile. Flow rate was 1.0 mL/min (for 5 µm) and 0.6 mL/min (for 1.7 µm). UV detection was set at 214 nm. 2 µL of the peptide mixture was injected.

Analysis of Fab and Fc Fragments on Proteomix® WCX NP1.7

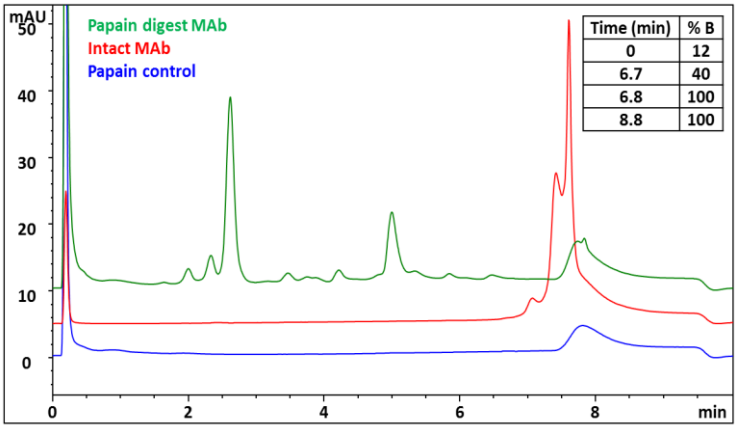


Figure 20. Analysis of papain digested MAb fragments, Fab and Fc, on Proteomix® WCX NP1.7 4.6 x 30 mm. Mobile phase A: 20 mM Sodium Acetate pH 5.15, B: A + 1 M LiCl. Flow rate was 1.5 mL/min. UV detection was set at 280 nm. 25 µL of the papain digested MAb 321 (1 mg/mL) was injected. Digestion condition: 5.0 mM L-Cysteine, 2.0 mM EDTA and 0.1M Tris-HCl pH 7.6 (Papain: MAb=100:1). Incubate at 37 ˚C for 3.5 hours, add 5% TFA to stop the reaction and chill on ice for 15 minutes.

Particle Size Comparison for the Separation of MAb on Antibodix™ WCX

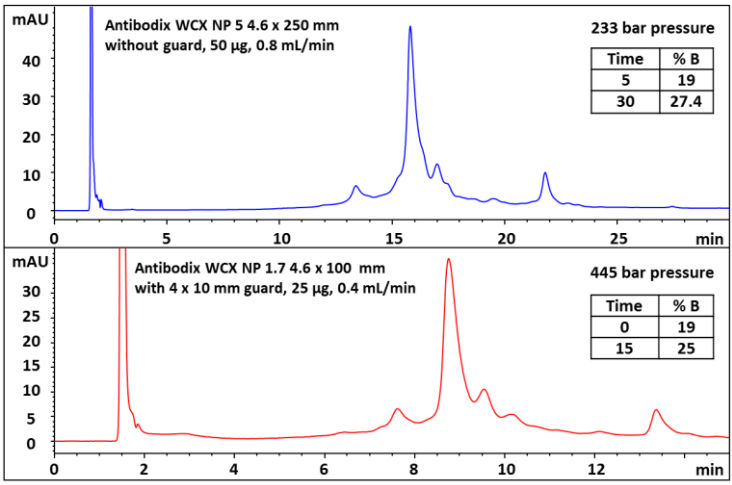


Figure 21. Comparison of Antibodix™ WCX NP5 4.6 x 250 mm (without a guard) to Antibodix WCX NP1.7 4.6 x 100 mm (with a 4 x 10 mm guard). Mobile phase A: 20 mM Sodium Acetate pH 5.15 and B: A + 1 M LiCl. Flow rate was 0.4 mL/min (NP1.7) and 0.8 mL/min (NP5). UV detection was set at 280 nm. MAb 321 was injected on each column for analysis.

MAb Loading Study on Antibodix™ WCX NP1.7 4.6 x 100 mm

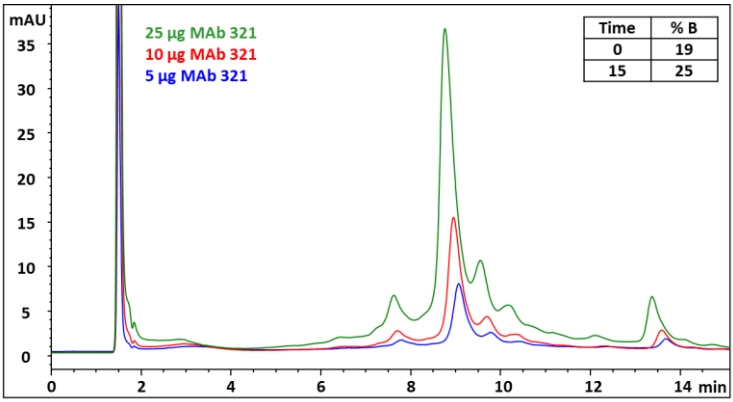


Figure 22. MAb Loading Study on Antibodix™ WCX NP1.7 4.6 x 100 mm. Mobile phase A: 20 mM Sodium Acetate pH 5.15 and B: A + 1 M LiCl. Flow rate was 0.4 mL/min. UV detection was set at 280 nm. 5 µL, 2 µL and 1 µL of MAb 321 (5 mg/mL) were injected for analysis.

Particle Size Comparison for the Separation of MAb on Antibodix™ WCX

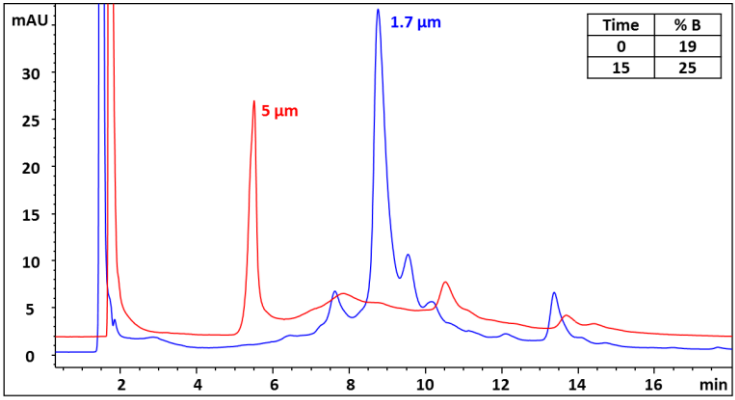


Figure 23. Comparison of Antibodix™ WCX NP5 4.6 x 100 mm (without a guard) to Antibodix WCX NP1.7 4.6 x 100 mm (with a 4 x 10 mm guard). Mobile phase A: 20 mM Sodium Acetate pH 5.15 and B: A + 1 M LiCl. Flow rate was 0.4 mL/min for both columns. UV detection was set at 280 nm. 25 µg of MAb 321 was injected on each column for analysis.

Gradient Optimization for MAb Separation on Antibodix™ WCX NP1.7

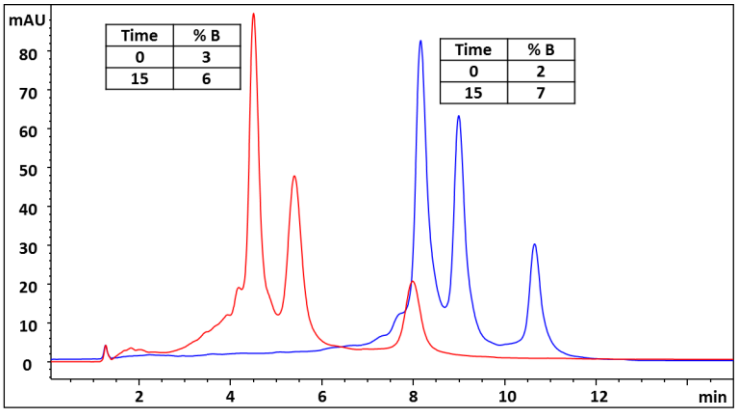


Figure 24. Gradient optimization for the analysis of MAb on Antibodix™ WCX NP1.7 4.6 x 100 mm. Mobile phase A: 20 mM Sodium phosphate pH 7.5 and B: A + 1 M NaCl. Flow rate was 0.4 mL/min. UV detection was set at 280 nm. 2 µL of MAb 016 was injected for each run (11 mg/mL).

Troubleshooting

It is the user's responsibility to determine the optimum sample loading and running conditions to best utilize the Proteomix® and Antibodix™ columns. The following information is provided for reference to troubleshoot your experiments.

High back pressure

A sudden increase in backpressure suggests that the column inlet frit might be blocked. In this case it is recommended that the column be flushed in reverse flow with an appropriate solvent. To prevent the clogging, remove the particulates from samples and mobile phases with filtration.

Poor resolution

1. Column may be overloaded. Reduce sample injection.
2. Try using different mobile phases in order to optimize you running conditions. Vary buffers, concentrations and pHs.

Peak tailing

This indicates that a different starting mobile phase should be used. To promote sample binding to the column try starting conditions at different pHs and at different salt concentrations.

Column cleaning and regeneration

Proteomix® and Antibodix™ columns may be contaminated by strongly adsorbed samples, which results in decreasing column performance. It is usually indicated by an increase in backpressure and a broader peak. When this happens, the general procedure for column cleaning is as follows:

1. Disconnect the column from the detector.
2. Clean your column in the reverse flow direction.
3. Run the column at less than 50% of the maximum recommended flow rate. Monitor the backpressure.
4. 10-15 column volumes of cleaning solution are sufficient. Run 3-5 column volumes of nanopure water between each solution.

In general, the recommended cleaning solution is 50 mM phosphate buffer with 1.0 M NaCl at pH 10.

Note: Separations on ion exchange columns are sensitive to the pH changes in the mobile phases. In order to have good reproducibility of the separations, make sure the pHs of the same buffer in different lots are the same. pH meters need to be calibrated correctly each time for buffer making.

Column Protection

When running a Proteomix® or an Antibodix™ 1.7 µm columns it is important to ensure that the mobile phase is made fresh and filtered through a 0.2 µm filter every day. In addition to filtering the sample and the mobile phase, the best way to protect the separation column is to install a guard column or a pre-column filter in front of it. In most cases a pre-column filter helps to remove the residual particulates that are in the sample, the mobile phase, or leached from the HPLC system, such as pump and injector seals. However, a guard column is highly recommended because it is more effective in trapping highly adsorptive sample components and residual particulates in the sample, the mobile phase or from the HPLC system.

Ordering Information

Proteomix® SCX NP1.7

P/N	ID x Length (mm)	Pore Size (Å)	Particle Size
401NP2-4001	4 x 10 (guard)	Non-porous	1.7
401NP2-4603G	4.6 x 30 with guard	Non-porous	1.7
401NP2-4605G	4.6 x 50 with guard	Non-porous	1.7
401NP2-4610G	4.6 x 100 with guard	Non-porous	1.7

Proteomix® WCX NP1.7

P/N	ID x Length (mm)	Pore Size (Å)	Particle Size
402NP2-4001	4 x 10 (guard)	Non-porous	1.7
402NP2-4603G	4.6 x 30 with guard	Non-porous	1.7
402NP2-4605G	4.6 x 50 with guard	Non-porous	1.7
402NP2-4610G	4.6 x 100 with guard	Non-porous	1.7

Antibodix™ WCX NP1.7

P/N	ID x Length (mm)	Pore Size (Å)	Particle Size
602NP2-4001	4 x 10 (guard)	Non-porous	1.7
602NP2-4603G	4.6 x 30 with guard	Non-porous	1.7
602NP2-4605G	4.6 x 50 with guard	Non-porous	1.7
602NP2-4610G	4.6 x 100 with guard	Non-porous	1.7

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