New ProSwift Monolith Ion-Exchange Columns and Their Applications

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ABSTRACT

Monolith columns offer significant advantages over conventional porous columns. These advantages include fast mass transfer, high loading capacity, improved resolution even at elevated flow rates with low back pressures, and wide pH stability. These exclusive characteristics support versatile performance in a wide range of biomolecule separations. Recently, we introduced reversed-phase, anion-exchange and cation-exchange phases of ProSwift™ monoliths (4.6 × 50 mm). ProSwift ion-exchange phases include weak anion-exchange (WAX-1S), strong anion-exchange (SAX-1S) and weak cation-exchange (WCX-1S) columns for biomolecule separations. We now introduce 1 mm WAX-1S and WCX-1S columns for high-resolution microanalytical biomolecule separations. The new 1 × 50 mm format columns offer improved sensitivity and reduce solvent consumption. These columns are rugged and exhibit excellent performance. Using these columns, we have developed various applications that will be presented.

INTRODUCTION

ProSwift columns, available with reversed-phase and ion-exchange surface chemistries, provide fast protein separations using conventional HPLC. ProSwift monolithic columns are specifically designed to provide high-resolution and high-efficiency separations of proteins. ProSwift media are based on polymeric monoliths prepared by an in situ polymerization process. They are a new generation of separation media, which are uniquely designed and engineered for separation of biomolecules. The monolith is a single cylindrical polymer rod containing an uninterrupted, interconnected network of through pores, that are also called channels.

MORPHOLOGY OF PROSWIFT ION-EXCHANGE MONOLITHS

The morphologies of the ProSwift SAX-1S, WAX-1S, and WCX-1S monoliths are shown in Figure 1. The monoliths consist of aggregates of globules shaped like cauliflower. The open spaces between the large aggregates are large flow-through channels allowing flow without high back pressure. The spaces among the smaller globules are open or through-pores allowing fast access of the samples to the functionalized surface of the media. The mass transfer of the samples is primarily driven by convective flow through these open pores instead of much slower molecular diffusion. These pores are large enough for even large molecules to flow through freely. Most of the small globules are engineered to be less than 500 nm. Therefore, the path lengths for mass transfer through these small globules are much shorter than the path lengths in conventional bead-based chromatographic phases. In addition, the globules are essentially non-porous based on nitrogen adsorption (BET) measurements and scanning electron microscopy (SEM) examinations. Diffusion-controlled mass transfer is minimized because these globules are non-porous. This is in contrast to porous beads where diffusion-controlled mass transfer predominates.

Features of ProSwift Monoliths

- High speed and high resolution
- Fast mass transfer
- Low backpressures
- Wide range of operational flow rates
- High throughput and improved productivity
- Large loading capacity
- Excellent stability over a wide pH range
- Outstanding reproducibility
- Optimal performance

Protein Society 2007 Presentation

MATERIALS

Chromatographic Components

ICS-3000 DP gradient pump, VWD Absorbance detector (or, UVD 340), AS autosampler, and TCC-100 thermostatted column compartment were from Dionex Corporation. Chromatography was controlled by Chromeleon® chromatography management software (Dionex Corporation).

Proteins used in standards, MES, Tris and all other analytical grade chemicals were obtained from Sigma.

Columns from Dionex Corporation

ProSwift SAX-1S 4.6 × 50 mm, PEEK-lined stainless steel, P/N 064293

ProSwift WAX-1S 4.6 × 50 mm, PEEK-lined stainless steel, P/N 064294

ProSwift WAX-1S 1 × 50 mm, PEEK, P/N 066642

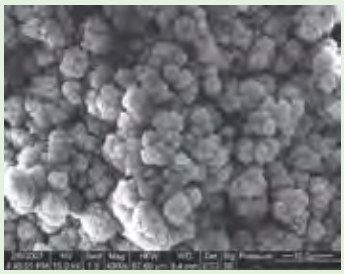
ProSwift WCX-1S 4.6 × 50 mm, PEEK-lined stainless steel, P/N 064295

ProSwift WCX-1S 1 × 50 mm, PEEK, P/N 066643

MORPHOLOGY OF MONOLITH

Figure 1. Morphology of ProSwift Monoliths: SEM Images

ProSwift WAX-1S Monolith (Magnification 5000×)



ProSwift SAX-1S Monolith (Magnification 4000×)

ProSwift WCX-1S Monolith (Magnification 4000×)

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Figure 2. The ProSwift Family

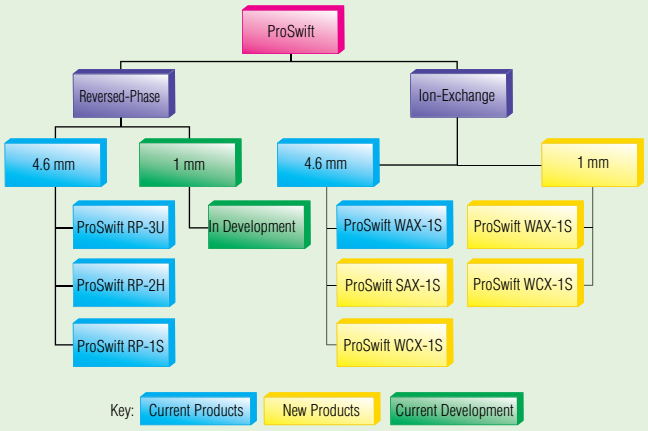

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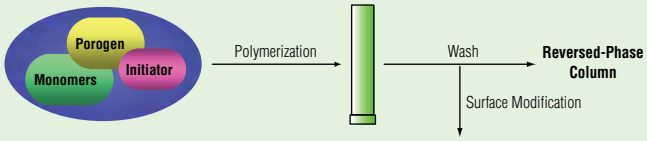
Figure 3. Monolithic Columns: Robust Manufacturing Process



- Simple manufacturing process
- Mixture of functional monomers
- Porogen system controls pore size
- In-situ bed formation during polymerization
- Secondary functionalization

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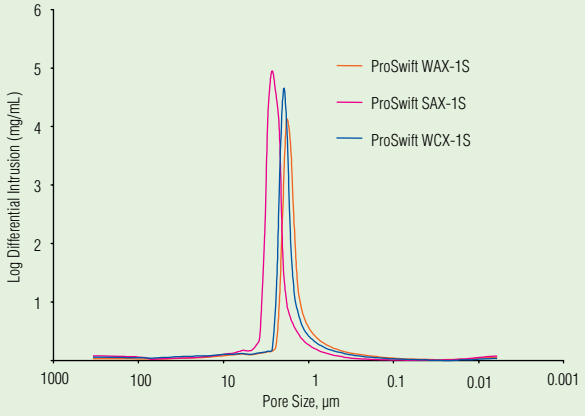
Figure 4. Modal Pore Size Distribution of ProSwift Ion-Exchange Family by Mercury Porosimetry



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PROSWIFT WAX-1S

Figure 5. Comparison of Sensitivity of WAX-1S 4.6 mm and 1 mm Formats

Column:	ProSwift WAX-1S, (4.6 × 50 mm and 1 × 50 mm)	Inj. Volume:	1.3 µL	
Eluents:	A. 10 mM Tris pH 7.6	Temperature:	30 °C	
	B. 1 M NaCl in 10 mM Tris, pH 7.6	Detection:	UV, 280 nm	
Gradient:	5 to 55% of B in 13 min, Hold for 2 min	Sample:	Protein mixture	
Flow Rate:	0.2 or 2.1 mL/min	Peaks:	1. Ovalbumin	1 mg/mL
			2. Trypsin inhibitor	1
			3. Insulin chain A	0.25

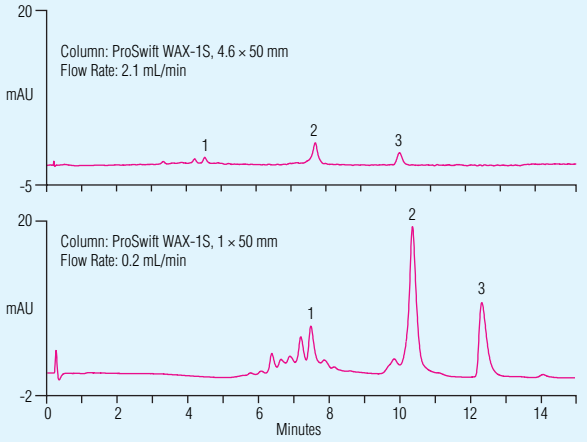


Figure 6. Effect of Flow on WAX-1S

Column:	ProSwift WAX-1S (1.0 × 50 mm)	Inj. Volume:	4.4 µL
Eluents:	A. 10 mM Tris, pH 7.6	Temperature:	30 °C
	B. 1.25 M NaCl in eluent A	Detection:	UV, 215 nm
Gradient:	1–52% B in 1 mL	Sample:	Ovalbumin, 1 mg/mL
Flow Rate:	As indicated		Insulin Chain A 0.25 mg/mL

Peak width at half height is shown

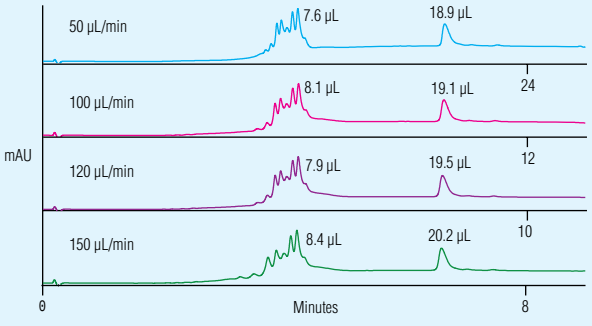


Figure 7. Protein Loading on WAX-1S

Column:	ProSwift WAX-1S (1 × 50 mm)	Inj. Volume:	2–24 µL
Eluents:	A. 10 mM Tris, pH 7.6	Temperature:	30 °C
	B. 1M NaCl in eluent A	Detection:	UV, 214 nm
Gradient:	5–55% B in 7.5 min	Sample:	1. Ovalbumin, 1 mg/mL
Flow Rate:	0.2 mL/min		2. Trypsin Inhibitor, 1 mg/mL
			3. Insulin Chain A, 0.25 mg/mL

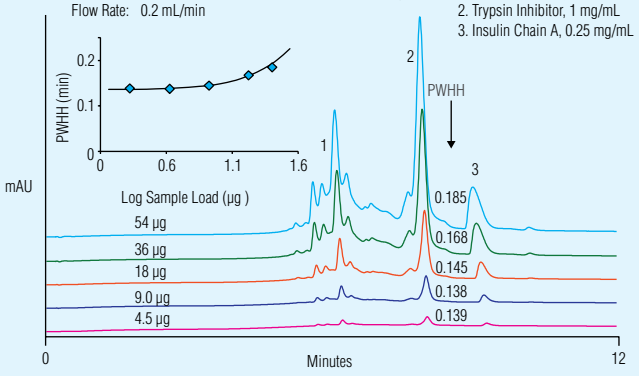
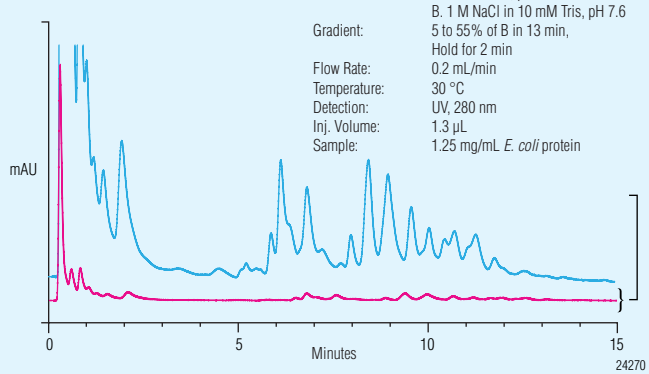


Figure 8. Separation of *E. coli* Proteins on the WAX-1S

Column:	ProSwift WAX-1S (1 × 50 mm)
Eluents:	A. 10 mM Tris, pH 7.6
	B. 1 M NaCl in 10 mM Tris, pH 7.6
Gradient:	5 to 55% of B in 13 min, Hold for 2 min
Flow Rate:	0.2 mL/min
Temperature:	30 °C
Detection:	UV, 280 nm
Inj. Volume:	1.3 µL
Sample:	1.25 mg/mL *E. coli* protein



PROSWIFT SAX-1S

Figure 9. Separation of Protein Mixture on SAX-1S

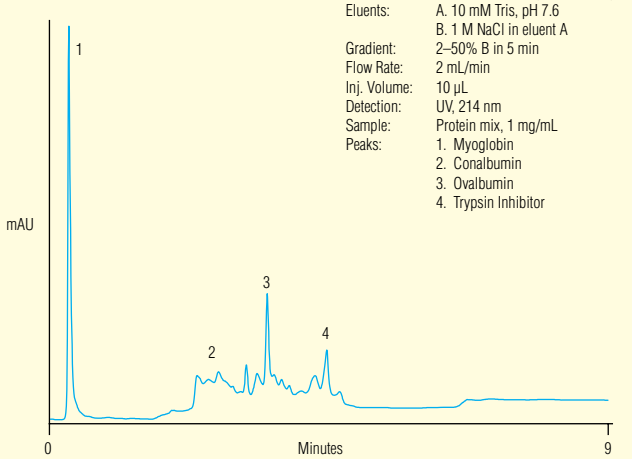


Figure 10. Protein Loading Capacity of SAX-1S

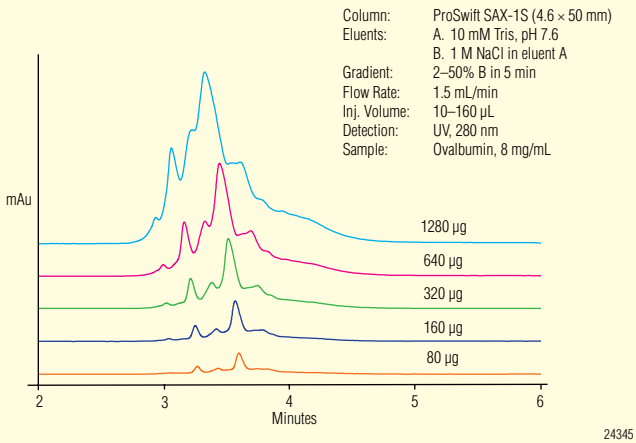


Figure 11. Separation of Pancreatin on SAX-1S

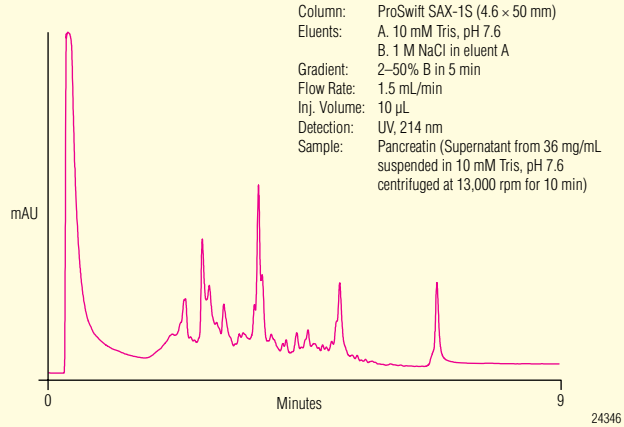
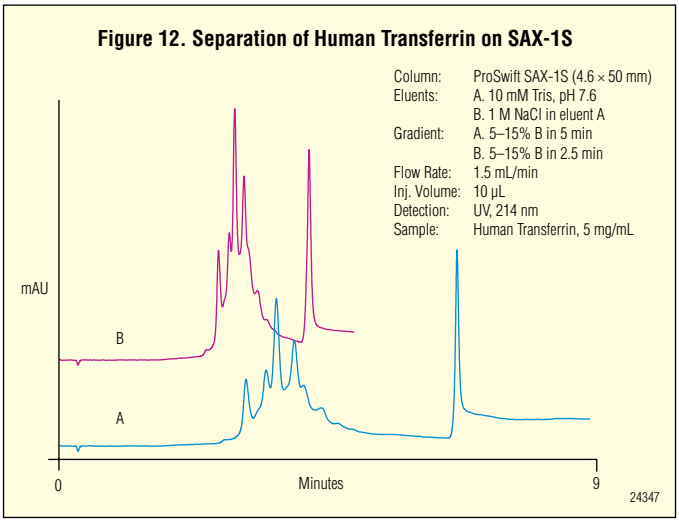


Figure 12. Separation of Human Transferrin on SAX-1S



PROSWIFT WCX-1S

Figure 13. Separation of Monoclonal Antibody (MAb) on WCX-1S

Column: ProSwift WCX-1S (4.6 × 50 mm)
Eluents: A. 10 mM Sodium phosphate (pH 7.6)
B. 1 M Sodium chloride in eluent A
Gradient: 5–10% B in 10 min, 100% B for 4 min
Flow Rate: 1 mL/min
Inj. Volume: 10 µL
Temperature: 30 °C
Detection: UV, 214 nm
Sample: MAb 5 mg/mL

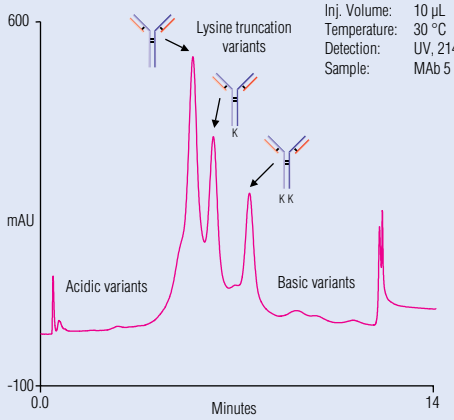


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Figure 14. Protein Loading Capacity of WCX-1S

Column: ProSwift WCX-1S (1 × 50 mm)
Eluents: A. 10 mM Sodium phosphate, pH 7.6
B. 1 M NaCl in eluent A
Gradient: 0–85% B in 7.5 min
Flow Rate: 0.2 mL/min
Inj. Volume: 1–24 µL
Detection: UV, 280 nm
Sample: Protein mix, 1 mg/mL each
Peaks: 1. Ribonuclease A
2. Cytochrome C
3. Lysozyme

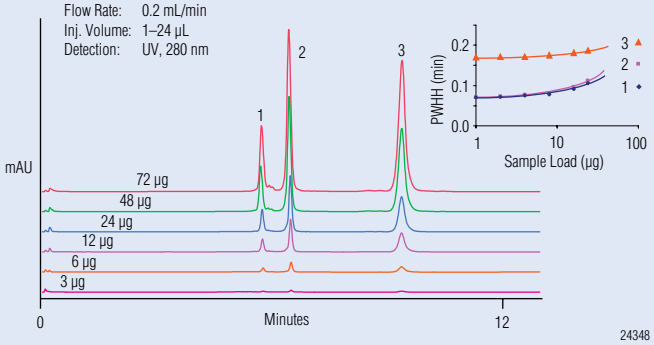

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Figure 15. Loading Capacity of MAb on WCX-1S

Column: ProSwift WCX-1S (1 x 50 mm)
Eluents: A. 10 mM Sodium phosphate pH 7.6
B. 1 M NaCl in eluent A
Gradient: 1–15% B in 7.5 min
Flow Rate: 0.2 mL/min
Inj. Volume: Variable 2-24 µL
Temperature: 30 °C
Detection: UV, 280 nm
Sample: MAb 5 mg/mL

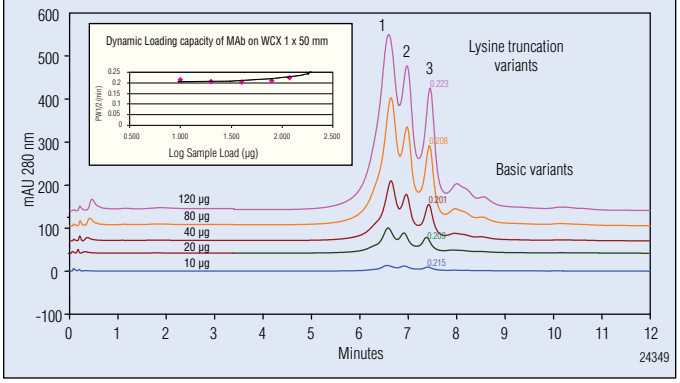

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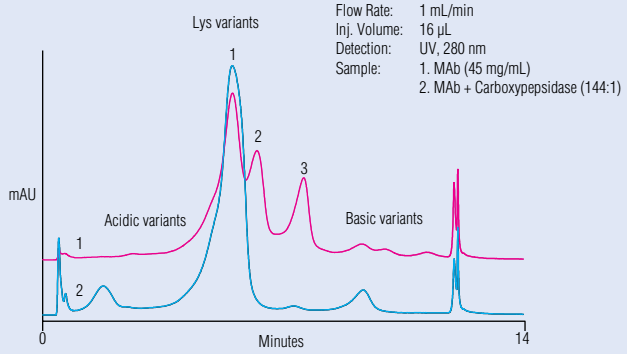
Figure 16. Assay of MAb Variants ± Carboxypeptidase on WCX-1S

Column: ProSwift WCX-1S (4.6 × 50 mm)
Eluents: A. 10 mM Sodium phosphate pH 7.6
B. 1 M NaCl in eluent A
Gradient: 5–10% B in 10 min
Flow Rate: 1 mL/min
Inj. Volume: 16 µL
Detection: UV, 280 nm
Sample: 1. MAb (45 mg/mL)
2. MAb + Carboxypepsidase (144:1)



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STABILITY OF PROSWIFT IEX COLUMNS

Figure 17. Stability of WAX-1S

Column: ProSwift WAX-1S (1 × 50 mm)
Eluents: A. 10m M Tris, pH 7.6
B. 1 M NaCl in eluent A
Gradient: 5–55% B in 15 min
Flow Rate: 0.2 mL/min
Inj. Volume: 1.3 µL
Temperature: 30 °C
Detection: UV, 280 nm

Sample: Protein mix
Peaks: 1. Ovalbumin 1 mg/mL
2. Trypsin Inhibitor 1
3. Insulin chain A 0.25

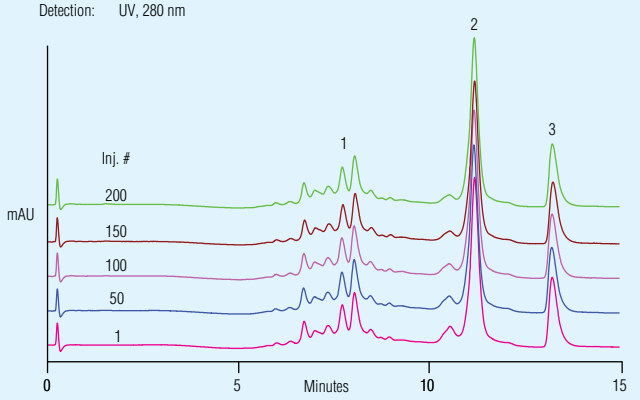

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Figure 18. Stability of SAX-1S

Column: ProSwift SAX-1S (4.6 × 50 mm)
Eluents: A. 10 mM Tris, pH 7.6
B. 1 M NaCl in eluent A
Gradient: 0–50% B in 5 min
Flow Rate: 1.5 mL/min
Inj. Volume: 10 µL

Temperature: 30 °C
Detection: UV, 214 nm
Sample: Protein mix 1 mg/mL
Peaks: 1. Myoglobin
2. Conalbumin
3. Ovalbumin
4. Trypsin Inhibitor

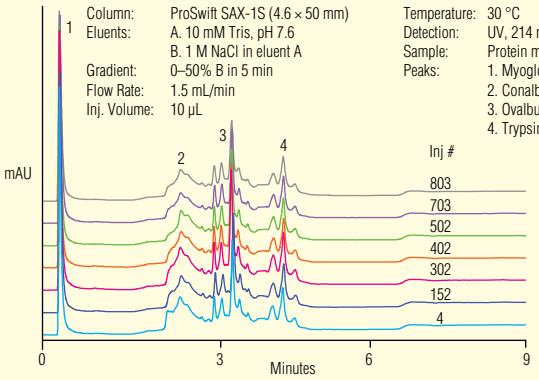

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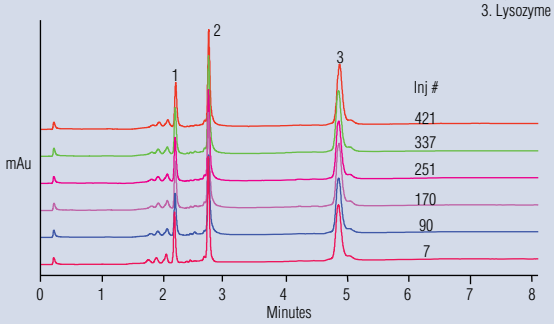
Figure 19. Stability of WCX-1S

Column: ProSwift WCX-1S (4.6 × 50 mm)
Eluents: A. 10 mM Sodium phosphate, pH 7.6
B. 1 M NaCl in eluent A
Gradient: 0–100% B in 5 min
Flow Rate: 2 mL/min

Inj. Volume: 10 µL
Detection: UV, 214 nm
Sample: Protein mix, 1 mg/mL
Peaks: 1. Ribonuclease A
2. Cytochrome C
3. Lysozyme



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CONCLUSION

- ProSwift monoliths are a new family of columns for protein separations.
- ProSwift monoliths are available with reversed phase and ion-exchange surface chemistries.
- They offer high speed, high resolution, and high loading capacity.
- Due to the high loading capacity of ProSwift ion-exchange columns, they can be used in the first dimension of multidimensional chromatography.
- The low backpressure of ProSwift columns enables the use of high flow rates resulting in fast chromatographic separations.
- ProSwift ion-exchange columns include WAX-1S, SAX-1S and WCX-1S.
- Available in different formats including 4.6 × 50 mm (all) and 1 × 50 mm (WAX-1S and WCX 1S) dimensions.
- ProSwift 1 × 50 mm columns offer improved sensitivity and reduced solvent consumption.
- ProSwift columns exhibit excellent stability, reproducibility, and overall performance.

ACKNOWLEDGEMENTS

We thank Ilze Birznieks, Nathan Bach, Doug Jamieson, and Asavari Kale for their help.