

PRODUCT MANUAL

for

ProSwift® IonExchange Monolithic Columns

SAX-1S
WAX-1S
WCX-1S
SCX-1S

Now sold under the
Thermo Scientific brand

Thermo
SCIENTIFIC

 **DIONEX**

IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION



PRODUCT MANUAL

FOR

ProSwift® Ion Exchange Monolithic Columns

ProSwift SAX-1S

4.6x50 mm, PEEK-Lined Stainless Steel P/N 064293

1x50 mm, PEEK P/N 068459

ProSwift WAX-1S

4.6x50 mm, PEEK-Lined Stainless Steel P/N 064294

1x50 mm, PEEK P/N 066642

ProSwift WCX-1S

4.6x50 mm, PEEK-Lined Stainless Steel P/N 064295

1x50 mm, PEEK P/N 066643

ProSwift SCX-1S

4.6x50 mm, PEEK-Lined Stainless Steel P/N 066765

1x50 mm, PEEK P/N 071977



TABLE OF CONTENTS

1. INTRODUCTION.....	6
1.1. MORPHOLOGY OF PROSWIFT® ION EXCHANGE MONOLITHS.....	6
1.2. BACKPRESSURES AND PORE SIZE DISTRIBUTIONS.....	7
1.3. FORMAT COMPARISON.....	8
1.4. CHEMISTRY COMPARISON.....	10
2. PROSWIFT WAX-1S.....	11
2.1. RESOLUTION AND SPEED OF SEPARATION.....	11
2.2. LOADING CAPACITY.....	12
2.3. ROBUSTNESS AND RUN STABILITY.....	15
2.4. BATCH-TO-BATCH REPRODUCIBILITY.....	16
2.5. PH STABILITY.....	17
2.6. TEMPERATURE STABILITY.....	18
2.7. SOLVENT STABILITY OF PROSWIFT.....	19
2.8. APPLICATIONS.....	19
2.8.1. <i>Protein Test Mix</i>	19
2.8.2. <i>Separation of Pancreatin</i>	20
2.8.3. <i>Separation of Tubulin</i>	21
2.8.4. <i>Separation of E. Coli Proteins</i>	21
2.8.5. <i>Oligonucleotide Separations</i>	22
2.8.5.1. Effect of Oligonucleotide (ON) Length on Retention.....	22
2.8.5.2. Effect of pH on Oligonucleotide Retention.....	22
2.8.5.3. Effect of Temperature on Oligonucleotide Retention.....	23
2.8.5.4. Effect of Fluorophores and Capture Probes on Oligonucleotide Retention.....	23
2.8.5.5. Effect of Oligonucleotide Loading Quantity on Peak Shape and Resolution.....	24
3. PROSWIFT SAX-1S.....	25
3.1. RESOLUTION AND SPEED OF SEPARATION.....	25
3.2. LOADING CAPACITY.....	25
3.3. ROBUSTNESS AND RUN STABILITY.....	27
3.4. BATCH-TO-BATCH REPRODUCIBILITY.....	28
3.5. PH STABILITY.....	29
3.6. TEMPERATURE STABILITY.....	30
3.7. SOLVENT STABILITY.....	31
3.8. APPLICATIONS.....	32
3.8.1. <i>Separation of protein mixture</i>	32
3.8.2. <i>Separation of Pancreatin</i>	33
3.8.3. <i>Separation of Caseins</i>	34
3.8.4. <i>Separation of Human Transferrin</i>	35
4. PROSWIFT WCX-1S.....	36
4.1. RESOLUTION AND SPEED OF SEPARATION.....	36
4.2. LOADING CAPACITY.....	38
4.3. ROBUSTNESS AND RUN STABILITY.....	39
4.4. BATCH-TO-BATCH REPRODUCIBILITY.....	40
4.5. PH STABILITY.....	41
4.6. TEMPERATURE STABILITY.....	42
4.7. APPLICATIONS.....	43
4.7.1. <i>Separation of Protein mixture</i>	43
4.7.2. <i>Separation Snake Venom proteins</i>	45
4.7.3. <i>Monoclonal Antibody (MAB) Separations</i>	46
4.7.3.1. Separation of acidic and basic variants in of monoclonal antibodies.....	46
4.7.3.2. MAb Loading Capacity.....	47
4.7.3.3. Characterization of MAb C-terminal Lysine Variants.....	48
5. PROSWIFT SCX-1S.....	50







5.1.	SPEED AND RESOLUTION.....	50
5.2.	LOADING CAPACITY	50
5.3.	ROBUSTNESS AND RUN STABILITY	54
5.4.	BATCH-TO-BATCH REPRODUCIBILITY	55
5.5.	pH STABILITY	56
5.6.	TEMPERATURE STABILITY	58
5.7.	APPLICATIONS	59
5.7.1.	<i>Hemoglobin</i>	59
5.7.2.	<i>Separation Snake Venom proteins</i>	61
6.	SPECIFICATIONS.....	63
7.	INSTALLATION.....	64
7.1.	SYSTEM REQUIREMENTS	64
7.2.	SYSTEM VOID VOLUME	64
7.3.	FLOW RATE EQUIVALENCY.....	64
7.4.	GRADIENTS	65
7.5.	OPERATIONAL PARAMETERS.....	65
7.6.	ELUENT LIMITATIONS	65
7.7.	CHEMICAL PURITY REQUIREMENTS	65
7.7.1.	<i>Inorganic Chemicals</i>	65
7.7.2.	<i>Buffer Components</i>	65
7.7.3.	<i>Deionized Water</i>	66
7.8.	ELUENT PREPARATION.....	66
7.8.1.	<i>Adjusting the pH of the Eluent</i>	66
7.8.2.	<i>Filtering the Eluent</i>	66
7.8.3.	<i>Degassing the Eluent</i>	66
7.8.4.	<i>10 mM Tris Buffer Preparation</i>	66
7.8.4.1.	<i>10mM Tris Buffer (pH 7.6) Preparation (Eluent A):</i>	66
7.8.4.2.	<i>1M Sodium Chloride (NaCl) in 10mM Tris Buffer Preparation (Eluent B)</i>	66
7.9.	<i>10 mM SODIUM PHOSPHATE BUFFER PREPARATION</i>	67
7.9.1.	<i>10 mM Sodium Phosphate, dibasic (Na₂HPO₄) Preparation</i>	67
7.9.2.	<i>10 mM Sodium Phosphate, monobasic (NaH₂PO₄) Preparation</i>	67
7.9.3.	<i>10mM Sodium Phosphate Buffer, pH 7.6 Preparation (Eluent A)</i>	67
7.9.4.	<i>1M Sodium Chloride (NaCl) 10 mM Sodium Phosphate Buffer, pH 7.6 Preparation (Eluent B)</i>	67
8.	OPERATION.....	68
8.1.	QUICKSTART FOR PROSWIFT SAX-1S, WAX-1S, SCX-1S, AND WCX-1S	68
8.2.	INCREASE FLOW RATE	69
8.3.	SAMPLE PREPARATION.....	69
8.4.	COLUMN EQUILIBRATION	69
9.	TROUBLESHOOTING.....	70
9.1.	HIGH BACK PRESSURE	71
9.1.1.	<i>Finding the Source of High System Pressure</i>	71
9.1.2.	<i>Clogged Column Bed Support (Frit) Assemblies</i>	71
9.2.	HIGH BACKGROUND OR NOISE	71
9.2.1.	<i>Contamination of Eluents</i>	71
9.2.2.	<i>Contaminated Column</i>	71
9.2.3.	<i>Contaminated Hardware</i>	71
9.3.	POOR PEAK RESOLUTION	71
9.3.1.	<i>Loss of Column Efficiency</i>	72
9.3.2.	<i>Poor Resolution Due to Shortened Retention Times</i>	72
9.3.3.	<i>Loss of Front End Resolution</i>	72
9.4.	SPURIOUS PEAKS.....	73
9.5.	SMALL PEAK AREAS	73
10.	APPENDIX A - QUALITY ASSURANCE REPORTS	74
10.1.	<i>ProSwift SAX-1S (4.6x50 MM)</i>	74



10.2.	PROSWIFT SAX-1S (1x50 MM).....	75
10.3.	PROSWIFT WAX-1S (4.6x50 MM)	76
10.4.	PROSWIFT WAX-1S (1x50 MM)	77
10.5.	PROSWIFT WCX-1S (4.6x50 MM)	78
10.6.	PROSWIFT WCX-1S (1x50 MM)	79
10.7.	PROSWIFT SCX-1S (4.6x50 MM).....	80
10.8.	PROSWIFT SCX-1S (1x50 MM).....	81
11.	APPENDIX B - COLUMN CARE.....	82
11.1.	NEW COLUMN EQUILIBRATION	82
11.2.	COLUMN CLEANUP	82
11.2.1.	<i>Cleanup Solution</i>	82
11.2.2.	<i>Column Cleanup Procedure</i>	82
11.3.	COLUMN STORAGE.....	83
11.3.1.	<i>Short Term Storage:</i>	83
11.3.2.	<i>Long Term Storage:</i>	83
12.	APPENDIX C - REFERENCES	84

GUIDE TO IMPORTANT INFORMATION

 SAFETY	<i>Safety information can help prevent bodily harm.</i>
 WARNING	<i>Warning information can help prevent equipment harm.</i>
 CAUTION	<i>Caution information can help prevent problems.</i>
 NOTE	<i>Note information can help with tips for improved use.</i>

1. INTRODUCTION

1.1. Morphology of ProSwift® Ion Exchange Monoliths

ProSwift monolithic columns are specifically designed to provide high-resolution, high efficiency separations of proteins at elevated linear velocities with sample loads exceeding the capacities of non-porous beads.

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, which are also called channels. Its unique morphology, pore structure and pore size distribution offer the optimum performance for separation of proteins and other biomolecules.

The morphologies of the ProSwift SAX-1S, SCX-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 the large flow-through channels allowing flow without high back pressure. The spaces among the smaller globules are the open or through-pores allowing fast access of the samples to the functionalized surface of the media. Mass transfer is primarily driven by convective flow through these open pores instead of much slower molecular diffusion. This allows faster flow rates to be used with little effect on resolution. 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 and band broadening occurs as the flow rate is increased.

In summary, flow-through pores, short mass transfer paths, and non-porous globules are characteristics unique to ProSwift monolith morphology. These monolith features enable much faster analyte mass transfer than porous beads.

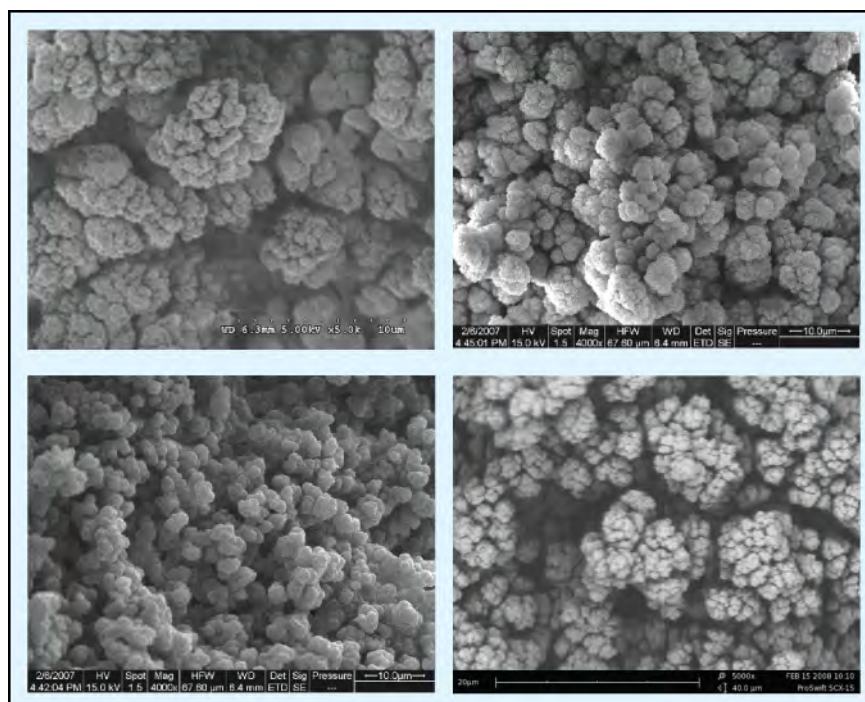


Figure 1
SEM Images of the ProSwift Monoliths
 (Top left) ProSwift WAX-1S (5000x), (Top right) ProSwift SAX-1S (4000x),
 (Bottom left) ProSwift WCX-1S (4000x), (Bottom right) ProSwift SCX-1S (5000x)

1.2. Backpressures and Pore Size Distributions

ProSwift monoliths have very high permeability. The pore volume is about 60% of the column volume, which is much higher than the porous beads. There are two types of pores: large pores (approximately a micron or larger) for eluent to flow through and the small pores (ten to hundreds of nanometers) for most of the separations to take place. The modal pore sizes for these monoliths are shown in Figure 2. When comparing the pore size with the SEM images you can see how the pore structure can relate to pore size. The monolith columns have large channels running through them that are generally in the same size range. This is reflected in the porosimetry data. The range of modal pore size is from approximately 1.6 to 2.7 μm . These large pores allow the eluent to flow through with moderate back pressure, and allow higher flow rates for faster separations. The backpressure generated at different flow rates of ProSwift WAX-1S 4.6x50 mm is shown in Figure 3. The other ion-exchange monoliths have similar back pressure correlations. At any given flow rate, the backpressures generated on ProSwift columns are typically lower than those of bead-based analytical columns.

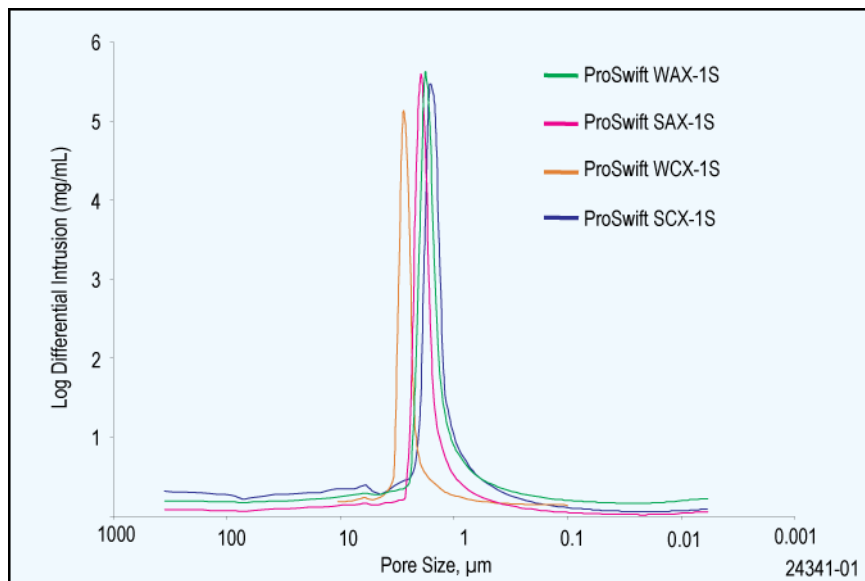


Figure 2
Pore Size Distribution of ProSwift Ion Exchange Family
by Mercury Porosimetry

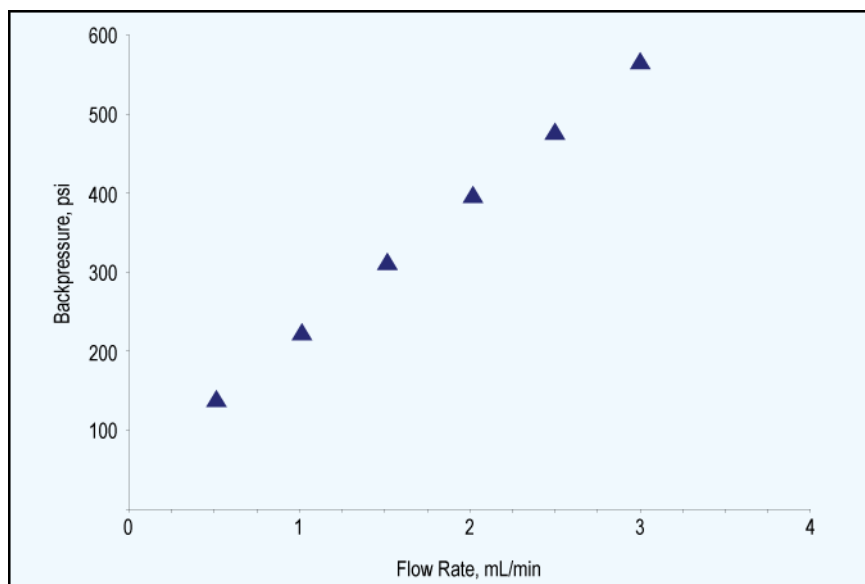


Figure 3
Flow Rate and Back Pressure Correlations for 4.6x50 mm ProSwift Ion Exchange Family

1.3. Format Comparison

The ProSwift columns are available in a 4.6x50mm and a 1x50mm format. This allows similar separations to be carried out in different formats.

The high loading capacities allow research scale semi-preparative analysis to be carried out using the 4.6 mm. Large amounts of sample can be loaded and resolution is maintained to allow detection and/or collection of protein low in abundance.

Where sample is limited, the 1mm format is ideal. The better mass sensitivity of the smaller i.d. allows better detection with lower loading. Like the 4.6mm version, these columns have high capacity allowing relatively large amount to be loaded.

Figure 4 demonstrates the effect of sample loading. The same mass of sample was injected onto both the 4.6mm i.d. and the 1mm i.d. ProSwift WAX-1S. Figure 5 shows how much less sample needs to be injected onto the 1mm i.d. column in order to achieve the same response.

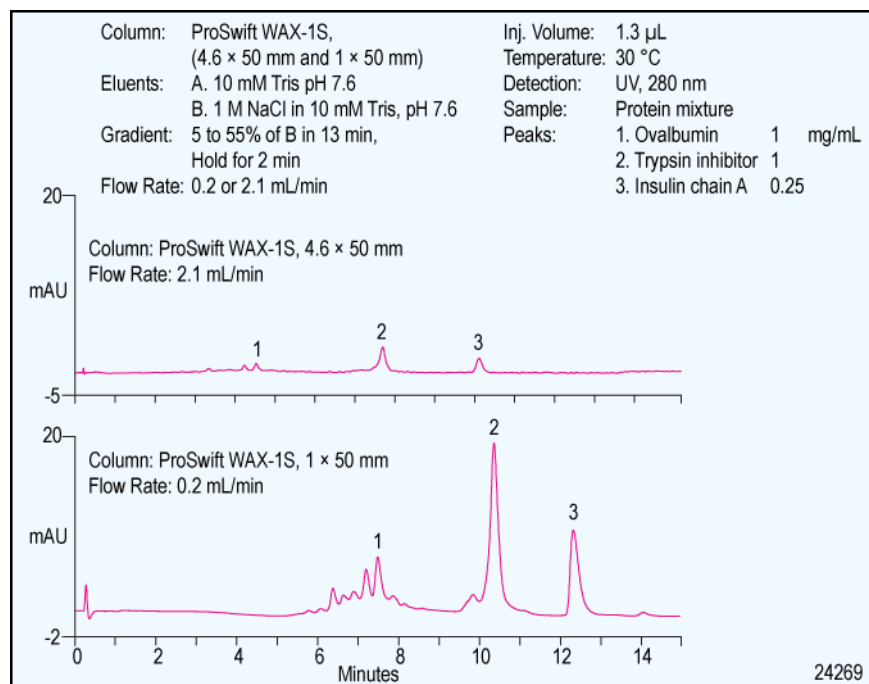


Figure 4

Comparison of ProSwift WAX-1S 4.6x50mm and 1x50mm with equal sample loading

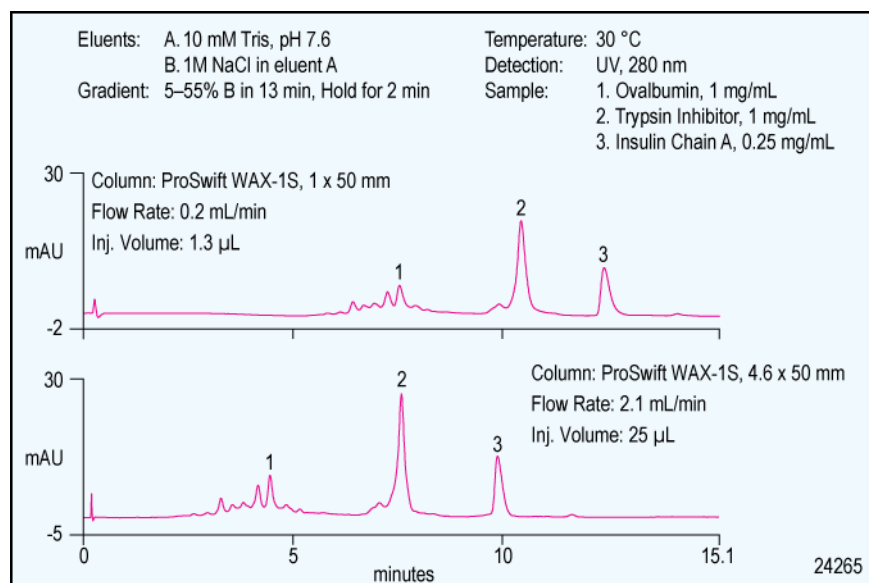


Figure 5

Comparison of ProSwift WAX-1S 4.6x50mm and 1x50mm with equivalent mass loading

The 1-mm columns can be operated within the flow rate range of most standard analytical HPLC pumps without the requirement for micro systems. However it should be noted that standard systems have significant delay volumes compare to micro systems therefore this should be minimized by reducing the amount and diameter of the tubing. Post column tubing can also be responsible for band broadening; the volume of this tubing should also be reduced as much as possible. In Figure 6 the ICS-3000 DP gradient pump has a smaller delay volume than the DX-600 GP50 gradient pump. Band broadening can also result due to larger UV cell volumes. The peaks in the ICS-3000 example are sharper as a 2.5 μ L cell is used.

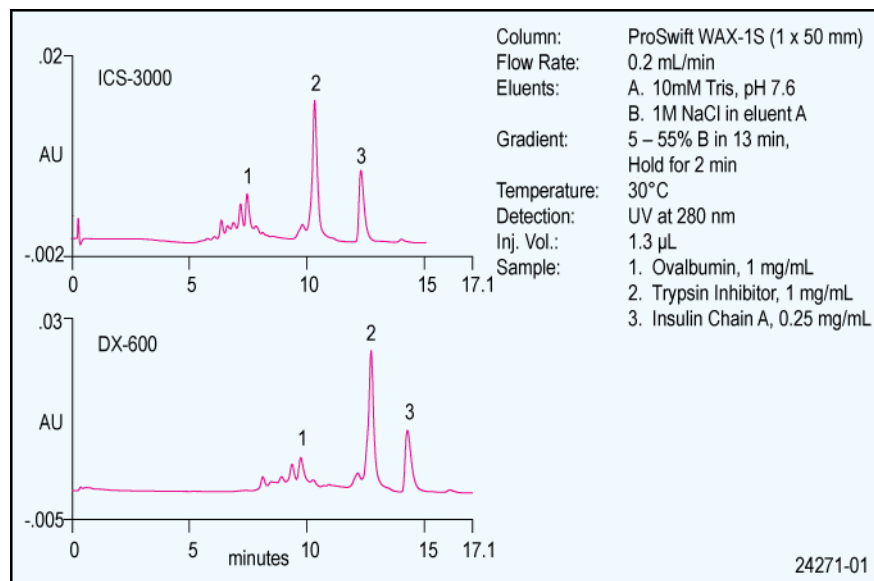


Figure 6
Comparison of System Delay Volume using the ProSwift WAX-1S 1x50mm

The ProSwift 1-mm ion exchange columns can be run at higher linear velocities than the 4.6 mm column. Using the same gradient (~2X CV compared to the 4.6 mm) excellent resolution is achieved and run time is reduced when they are compared on the same system. This is shown in Figure 7.

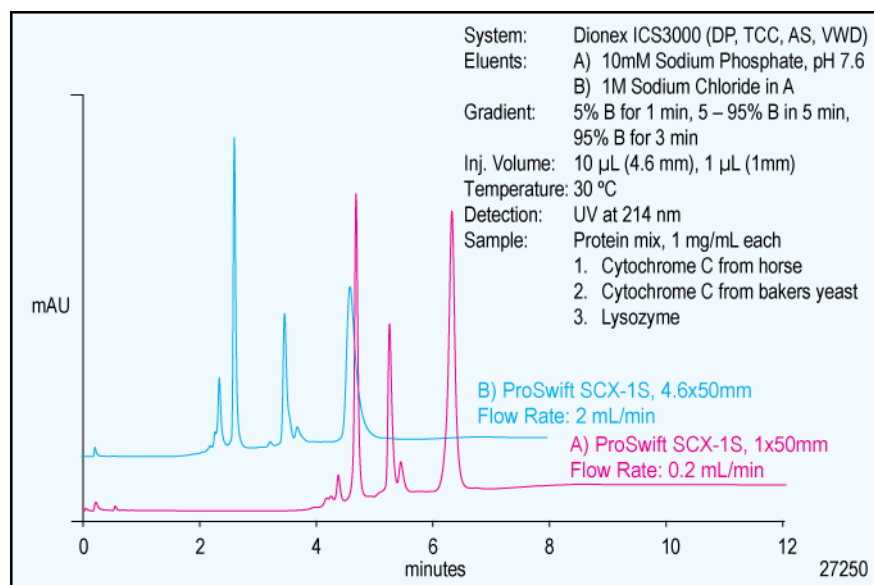


Figure 7
Comparison of ProSwift SCX-1S 1x50 mm and 4.6x50 mm

1.4. Chemistry Comparison

Weak and strong ion exchanges are designed to allow you to choose columns that are more specifically suited towards your application.

Depending on the pKa of your protein it will be charged or neutral at the pH of your buffer solution. If the net protein charge is positive then it will be retained on a cation exchange column. It will elute in the void of an anion exchange column.

The inverse is true also. A net negative charge will be retained on an anion exchanger.

The difference between the strong and weak phases of each type is related to the pH range at which the phase is charged. Typically with a strong anion or cation column the functional groups of the column will remain charged at high or low pH values whereas with a weak anion or cation column the pH range in which the functional groups remains charged will be reduced. The actual pH range of a particular column varies depending on the functional group.

Figure 8 shows how the selectivity of the column can also be used to obtain the desired separation. The functional group not only contributes to the operational pH of the phase but can also affect the selectivity. In the case shown below there is much improved resolution between ribonuclease A and cytochrome C using the ProSwift SCX-1S but lysozyme is retained less.

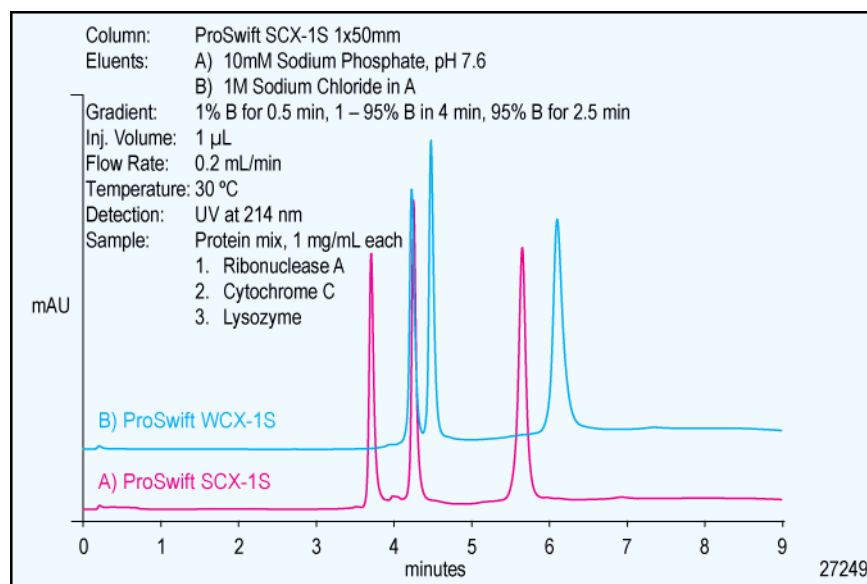


Figure 8

Comparison of the ProSwift WCX-1S and SCX-1S 1x50mm columns under the same conditions

2. PROSWIFT WAX-1S

2.1. Resolution and Speed of Separation

As described in Section 1.1, the uniquely designed morphology of ProSwift monoliths allows fast analyte mass transfer. This property minimizes band broadening upon flow rate increases, thus resulting in higher resolution than conventional beads over a wide range of flow rates. Fast analyte mass transfer is especially beneficial for large molecules, whose diffusivities are much lower than small molecules. This emulates an advantage of non-porous beads. In combination with their low back pressure, ProSwift monoliths offer excellent separations using fast gradients at low and high flow rates, which improve productivity. Figure 9 compares the chromatography of a protein sample using a 1 mL gradient on a 1x50 mm ProSwift WAX-1S monolith at 50, 100, 120 and 150 $\mu\text{L}/\text{min}$. These flow-rates would be equivalent to 1, 1.5, 2, and 3.15 mL/min on a 4.6 mm ID column. Resolution of the ovalbumin variants and the peak width (measured in μL) changes very little over this flow range, demonstrating that mass transfer is not limiting at these increasing flow rates.

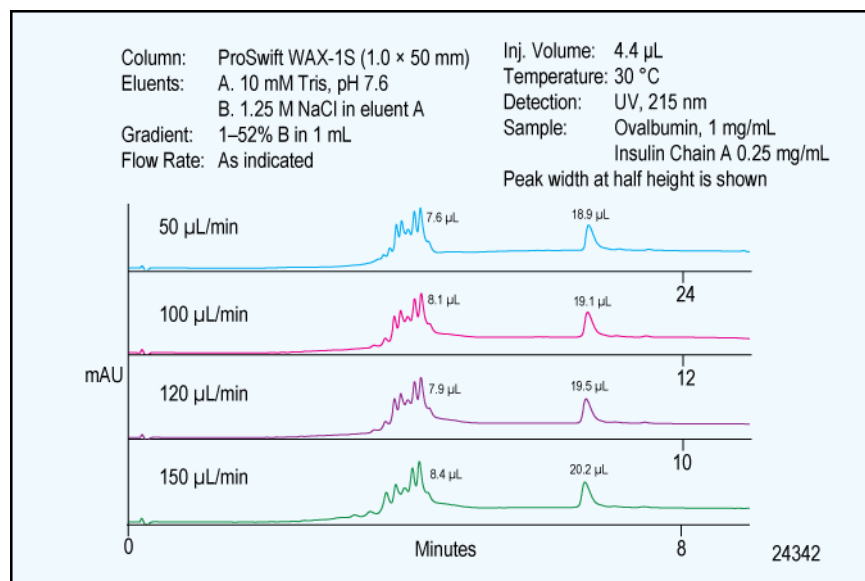


Figure 9
Protein Separation at Different Flow Rates on the ProSwift WAX-1S (1x50 mm)

2.2. Loading Capacity

ProSwift monolith surfaces are irregular as shown by the SEM images in Figure 1. This produces loading capacities comparable to porous beads. This optimized morphology supplies the best features of non-porous beads and porous beads: high resolution and high capacity: “The best of both worlds!”

Figure 10 shows the relative capacities of the nonporous DNAPac PA100 4x50 mm column and the ProSwift WAX-1S 4.6x50 mm monolith. The figure shows the two columns to give comparable peak shapes when 0.5 µg or less is injected. However, when more sample is injected the peak width on the DNAPac column increases while that of the ProSwift column remains stable up to ~ 50 µg. At a peak width of ~ 0.5 min, the ProSwift column exhibits 15 times the capacity of the DNAPac.

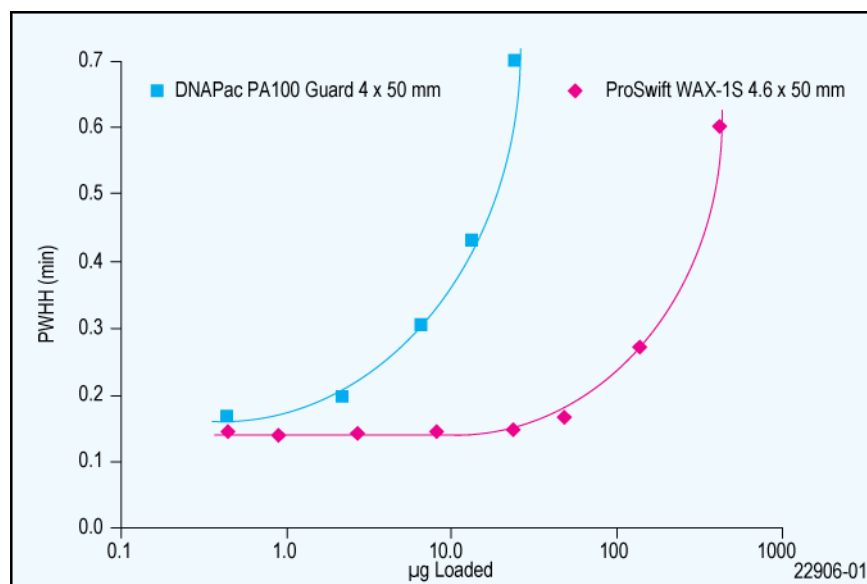


Figure 10
Comparison of Peak Width at Half Height versus Sample Load
of ProSwift WAX-1S (4.6x50 mm) and DNAPac PA100 (4x50 mm)

Figure 11 compares the loading capacity of these columns. When 120 µg of the 25-base oligonucleotide (with and without the trityl protecting group) was injected on each column at pH 8 and eluted using a salt gradient, the ProSwift monolith delivers 10-15 fold more capacity than the DNAPac column. This produces analytical resolution with purification capacity as shown.

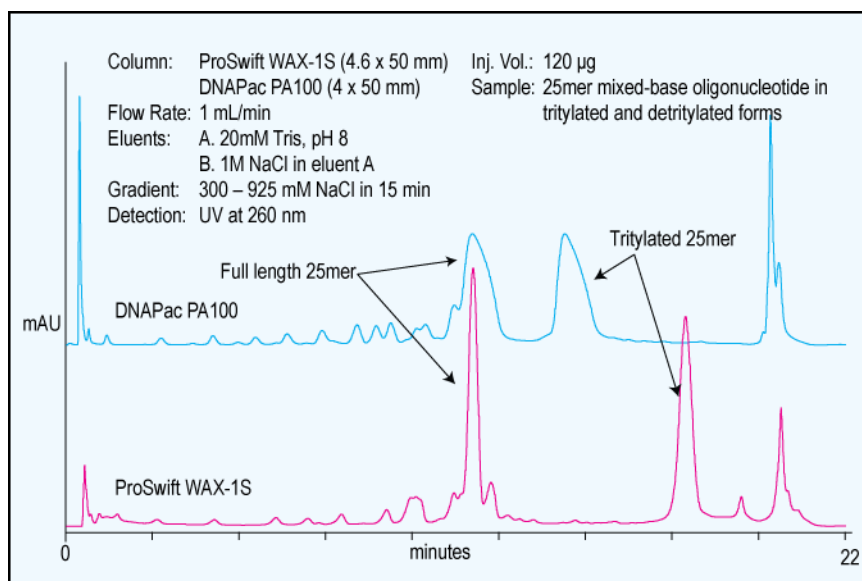


Figure 11
Loading Capacity of ProSwift and DNAPac Columns

The protein loading capacity of ovalbumin on ProSwift WAX-1S monolith is shown in Figure 12. The phosphorylation variants are separated, even when 1120 µg of ovalbumin was loaded on to the column.

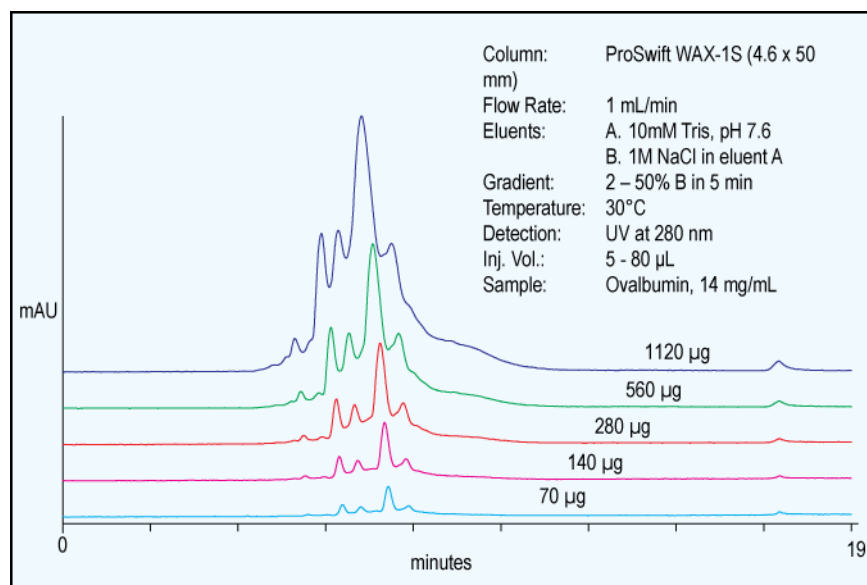


Figure 12
Protein Loading Capacity of ProSwift WAX-1S (4.6x50 mm)

The ProSwift WAX-1S also shows high loading capacity in a 1 mm format. Figure 13 shows up to 8 µg protein loaded on to the column without compromising resolution.

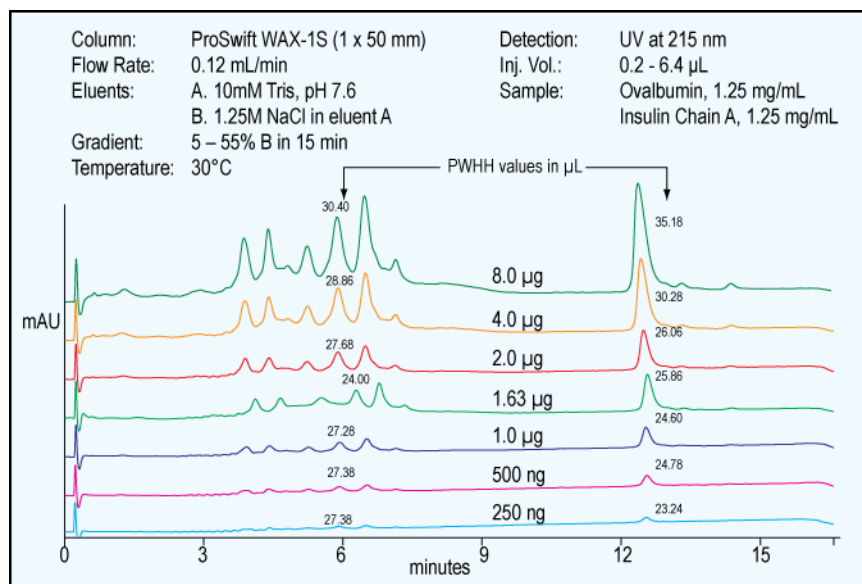


Figure 13
Protein Loading Capacity of ProSwift WAX-1S (1x50 mm)

At 54 µg (Figure 14) lower abundant proteins can be visible and the column still shows good resolution of variants.

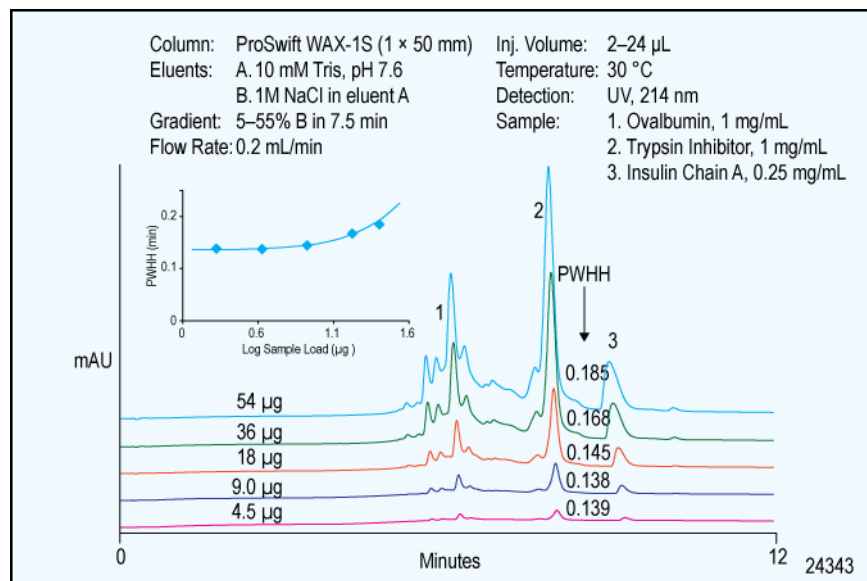


Figure 14
Protein Loading Capacity of ProSwift WAX-1S (1x50 mm)

2.3. Robustness and Run Stability

Durability and robustness are characteristic of the ProSwift monolithic columns. The ProSwift monoliths exhibit stability and reproducibility even after hundreds of runs.

Figure 15 and Figure 16 show the ProSwift WAX-1S columns retain high resolution over 200 cycles of operation and was assessed by intermittently injecting ovalbumin on a WAX-1S 4.6x50 mm and a protein mix on a WAX-1S 1x50 mm.

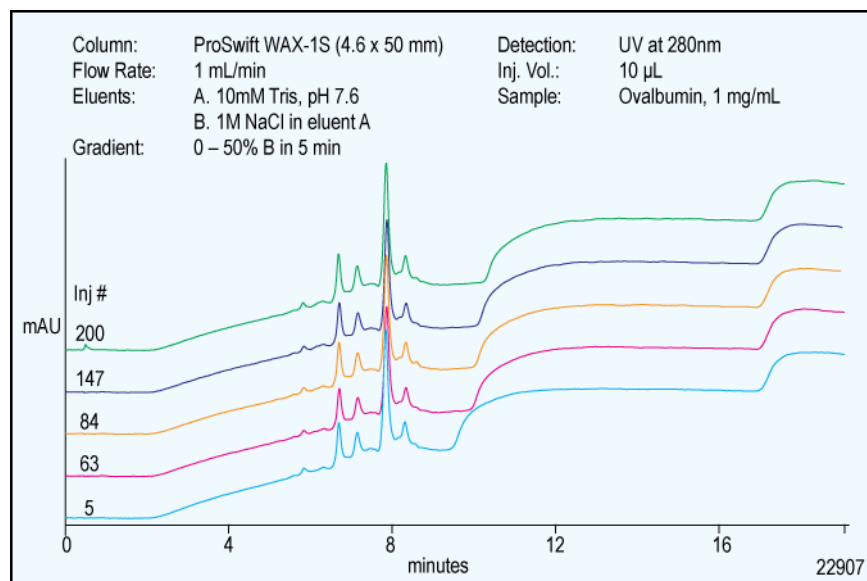


Figure 15
Stability of ProSwift WAX-1S 4.6x50mm Column

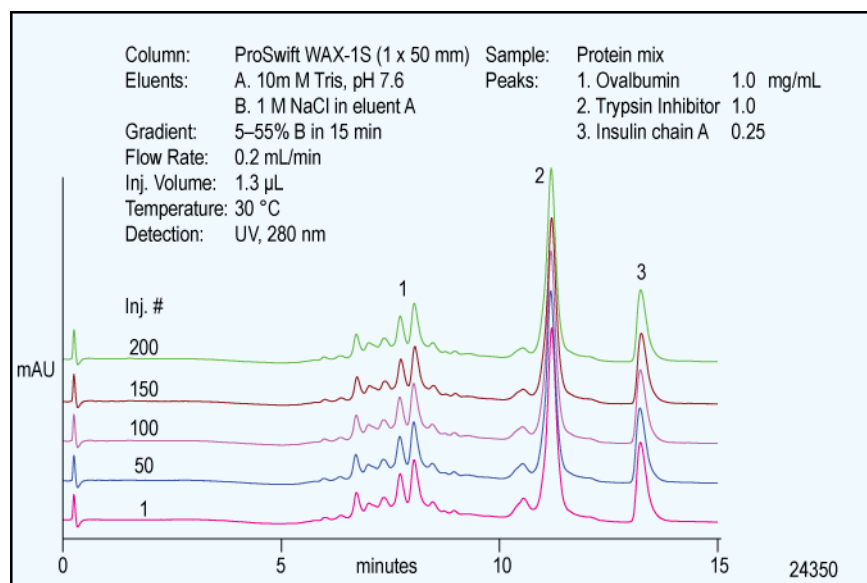


Figure 16
Stability of ProSwift WAX-1S 1x50mm Column

2.5. pH Stability

The ProSwift WAX-1S anion exchange monoliths have a polymethacrylate support with amine functional groups on the surface. During the regeneration process, the column is stable at temperatures up to 30 °C with the treatment of 1 M NaOH and 0.1 M HCl. The column is stable at pH 2 to 12 under operating conditions for long periods. Figure 19 and Figure 20 demonstrate that washing at both pH 1 and 14 (sufficient for sterilization) has no effect on chromatographic performance provided the columns are adequately equilibrated after washing.

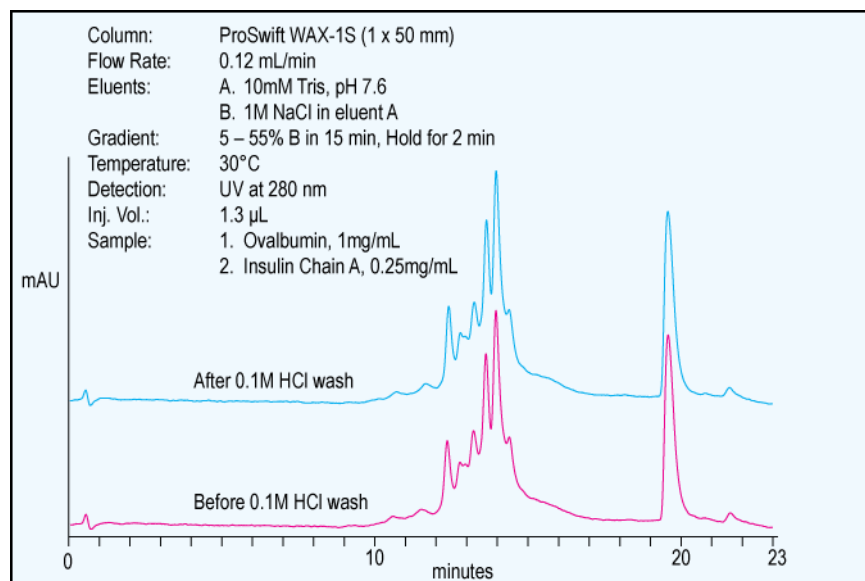


Figure 19
Effect of Transient (3 Hours) Hydrochloric Acid Washing
on the Performance of the ProSwift WAX-1S

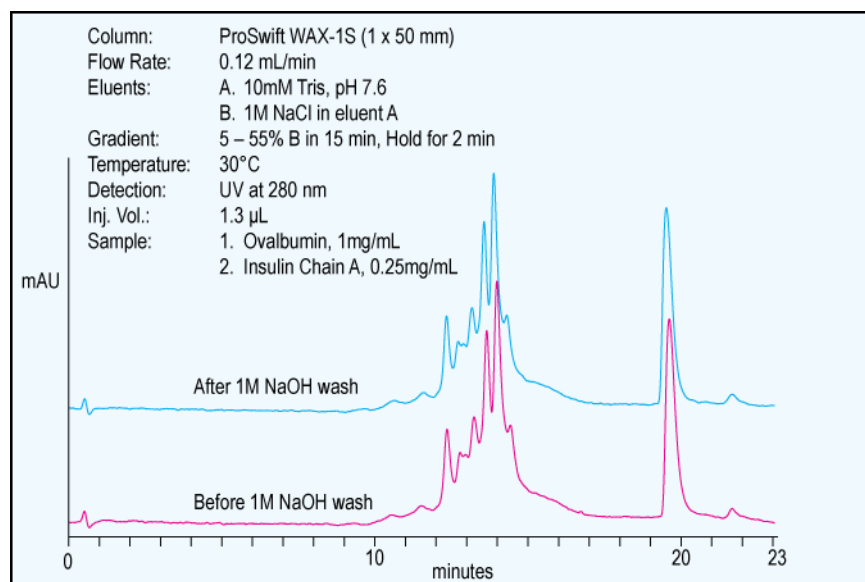


Figure 20
Effect of Transient (3 Hours) Sodium Hydroxide Washing
on the Performance of the ProSwift WAX-1S

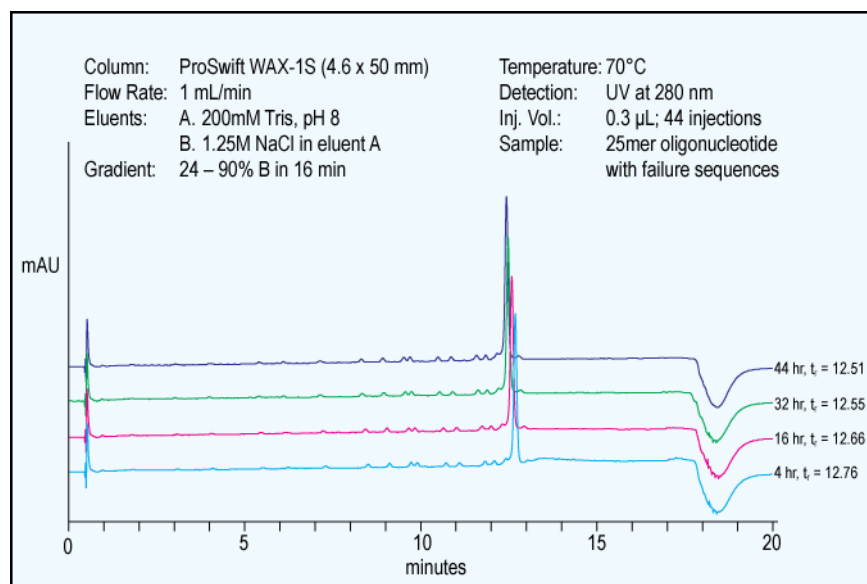
2.6. Temperature Stability


WARNING

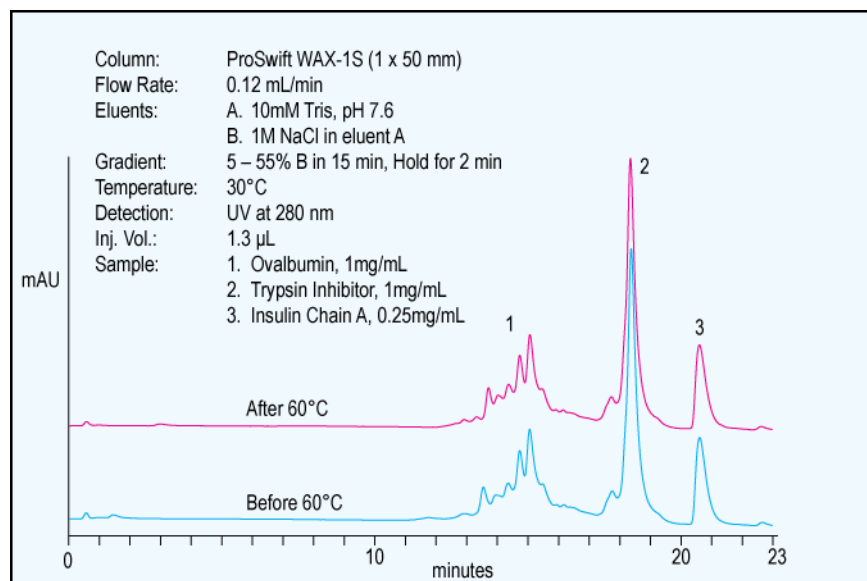
*Caution should be taken when using elevated temperatures and elevated pH.
See Section 1.10 for additional information regarding temperature and pH limits.*

Anion exchange phases produced with methacrylate polymers are stable at ambient temperatures from pH 2 to 12 using normal separation conditions. Temperature stability decreases at elevated pH. The ProSwift WAX-1S columns tolerate temperatures to 70 °C for short periods, and are quite stable at temperatures up to 60 °C at pH from 6 to 8. At pH between 8.0 and 9.5, the maximum operating temperature decreases linearly to ambient temperature.

Oligonucleotide peak shape on the ProSwift WAX-1S monolith is shown in Figure 21 to be reproducible during a 44 hour exposure to 70 °C at pH 8. Peak retention diminished by only 0.25 minutes during the test. Figure 22 also shows that the 1x50 mm format is stable to running at elevated temperatures for long periods of time.


Figure 21

Retention of a Mixed-Base 25mer Oligonucleotide on the ProSwift WAX-1S Monolith at 70 °C


Figure 22

Before and After Running Column at 60 °C for 18 Hours on the ProSwift WAX-1S Monolith

2.7. Solvent Stability of ProSwift

Anion exchange phases produced with methacrylate polymers are stable when used with 100% commonly used organic solvents such as acetonitrile and methanol.

2.8. Applications

2.8.1. Protein Test Mix

Figure 23 and Figure 24 show the separation of protein mixture on both the ProSwift WAX-1S columns. Both protein mixtures contained ovalbumin which contains two phosphorylation sites that result in closely related variants.

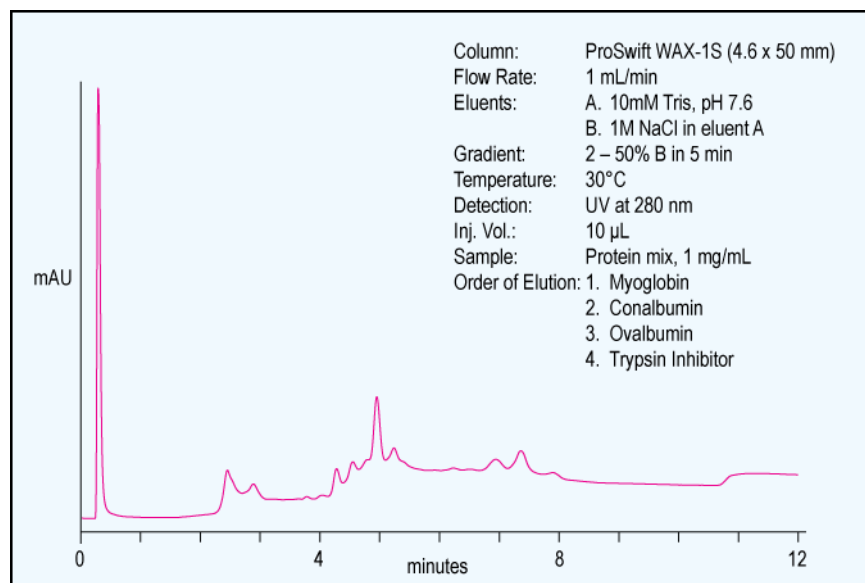


Figure 23

Separation of a Protein Mixture on ProSwift WAX-1S Monoliths

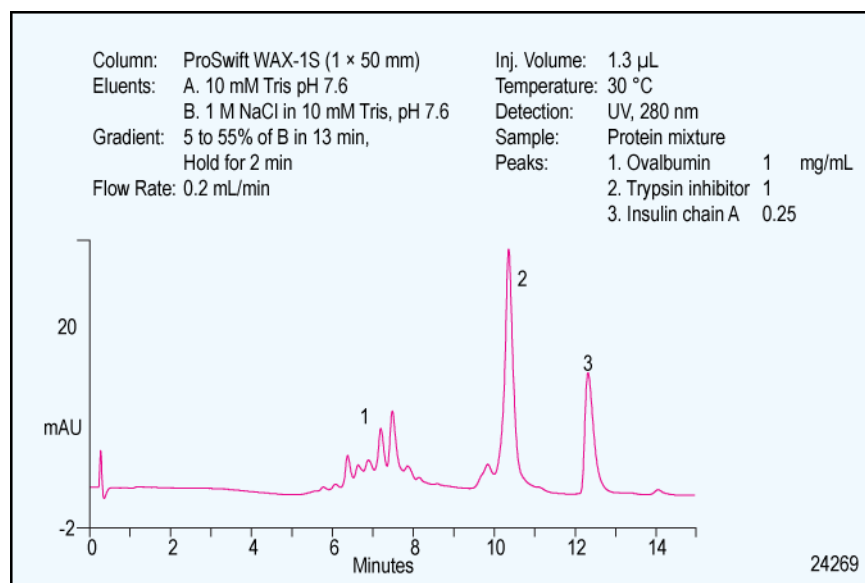


Figure 24

Separation of a Protein Mixture on ProSwift WAX-1S Monoliths

2.8.2. Separation of Pancreatin

Pancreatin is a complex sample containing a variety of proteases (protein digesting enzymes like trypsin and pepsin), amylases (carbohydrate digesting enzymes), and lipases (fat digesting enzymes). Figure 25 shows the separation of Pancreatin on a 4.6x50 mm ProSwift WAX-1S monolith into an unbound and a bound fraction. The bound fraction contained several well resolved peaks. Figure 26 shows a similar result on a 1x50 mm WAX-1S column.

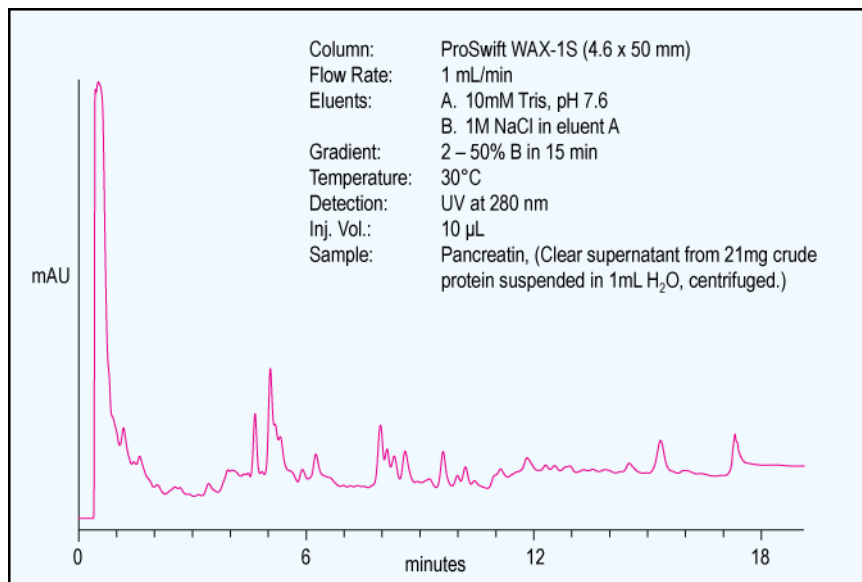


Figure 25

Separation of a Pancreatin on ProSwift WAX-1S (4.6x50 mm) Monolith

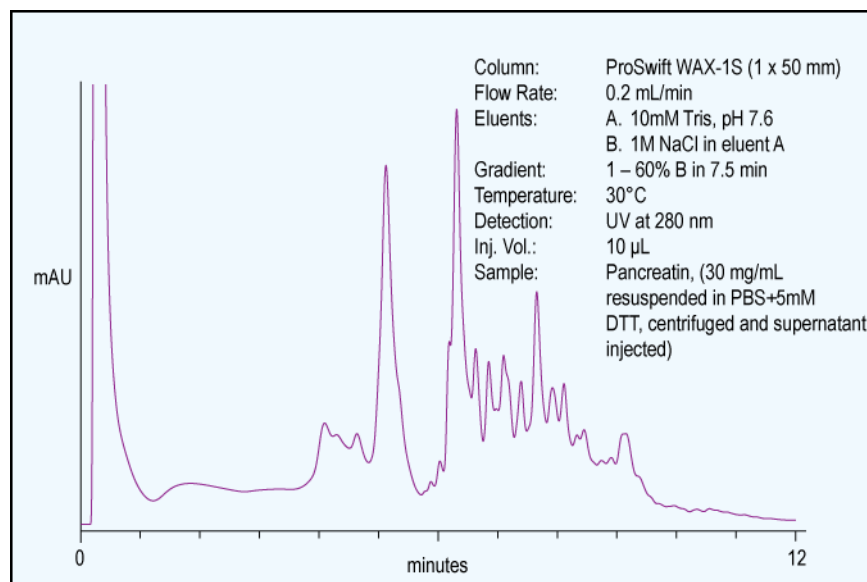


Figure 26

Separation of a Pancreatin on ProSwift WAX-1S (1x50 mm) Monolith

2.8.3. Separation of Tubulin

Tubulin is composed of a heterodimer of closely related 55kDa proteins called alpha and beta tubulin. Tubulin polymerizes to form microtubules. Microtubules serve as structural components within cells and are involved in many cellular processes including mitosis, cytokinesis, and vesicular transport. Irreversible damage of microtubules causes cell death. Purified mammalian brain tubulin contains selected microtubule-associated proteins (MAPs) consisting of high molecular weight MAPs around 300 kDa (MAP1 and MAP2) and low molecular weight group of tau proteins (between 55 and 70 kDa). Figure 27 shows the separation of MAP-rich tubulin components on a 1x50 mm ProSwift WAX-1S monolith.

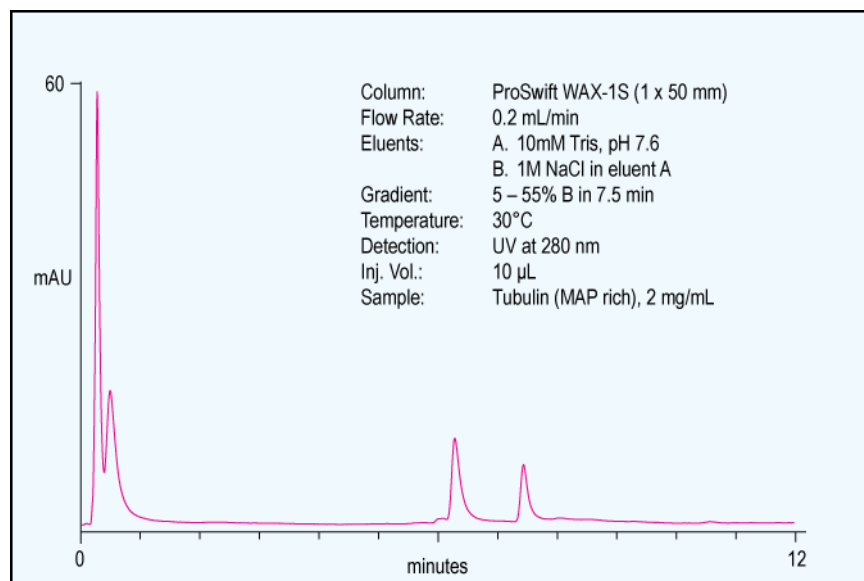


Figure 27

Separation of MAP-Rich Tubulin on ProSwift WAX-1S (1x50 mm) Monolith

2.8.4. Separation of E. Coli Proteins

In the analysis of highly complex protein mixtures the use of multidimensional chromatography is often necessary. For top-down proteomics the use of a high capacity column in the first dimension is an early step towards the identification of individual proteins. Figure 28 shows how the 1x50 mm ProSwift WAX-1S monolith can be used to separate a complex mixture of proteins obtained from the lysate of E. Coli cells. Low column volume in combination with the high capacity makes this column excellent for use in the first dimension of multi-dimensional chromatography.

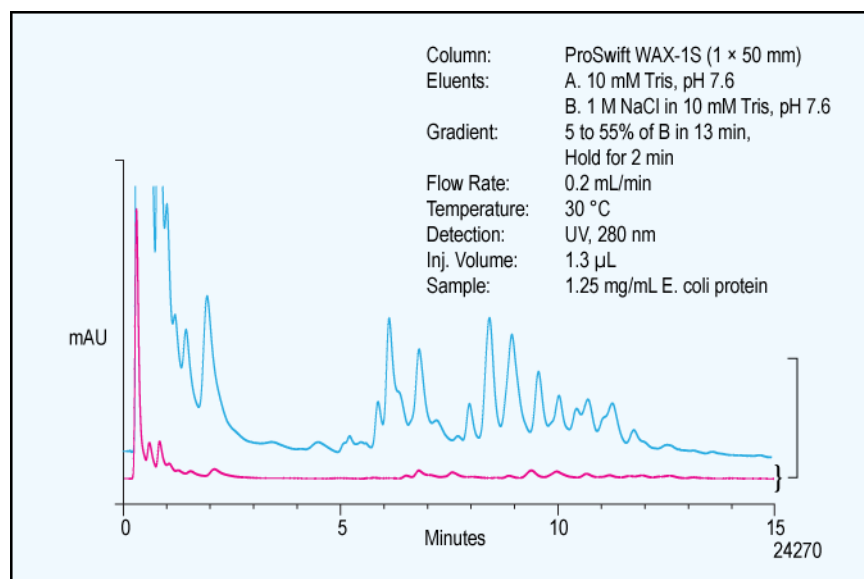


Figure 28

Separation of E. Coli Proteins on the ProSwift WAX-1S (1x50 mm) Monolith

2.8.5. Oligonucleotide Separations

The anion-exchange functionality of the ProSwift WAX-1S makes it suitable for the separation of oligonucleotides. Dionex highly recommends the use of the DNAPac PA100 and PA200 for high resolution separations and the DNASwift column for lab scale purification.

2.8.5.1. Effect of Oligonucleotide (ON) Length on Retention

Homopolymer oligonucleotides (phosphorylated deoxy-Adenyl oligos) elute in order of length as shown in Figure 29).

However, base composition also influences retention, so identical length mixed-base oligonucleotides with different base compositions may be fully resolved. Here, oligonucleotides from 12 to 30 bases long are fully resolved, and those from 40 to 60 bases are at least partially resolved in this 35 minute curved gradient of NaCl, at 1.0 mL/min.

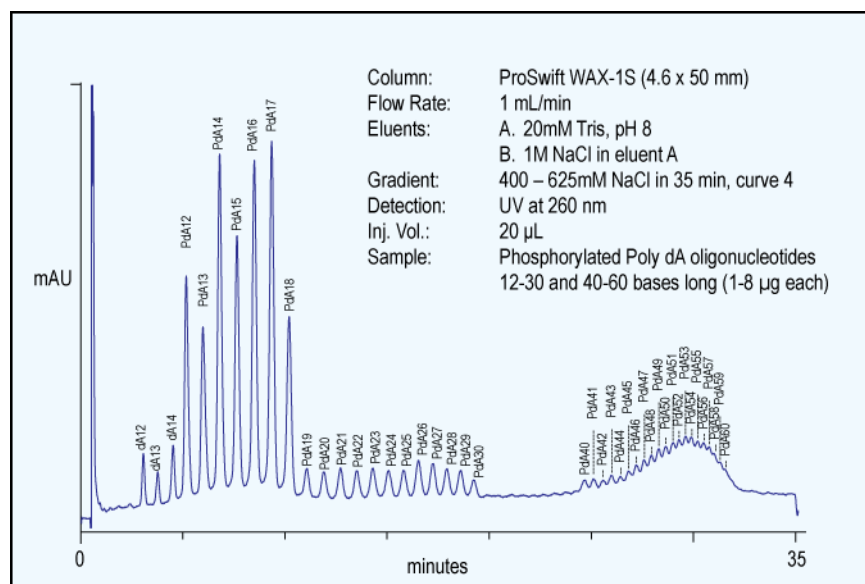


Figure 29
Elution of PdA Oligonucleotides on ProSwift WAX-1S (4.6x50 mm)

2.8.5.2. Effect of pH on Oligonucleotide Retention

The ProSwift WAX-1S is a weak anion exchanger, so its net charge diminishes as the pH rises above pH 8. As the net charge on the phase diminishes so does retention. In Figure 30, elution of two 25-base oligonucleotides is shown to occur earlier at pH 8.0 than pH 6.5, and significantly earlier at pH 9.5 than at pH 8.0.

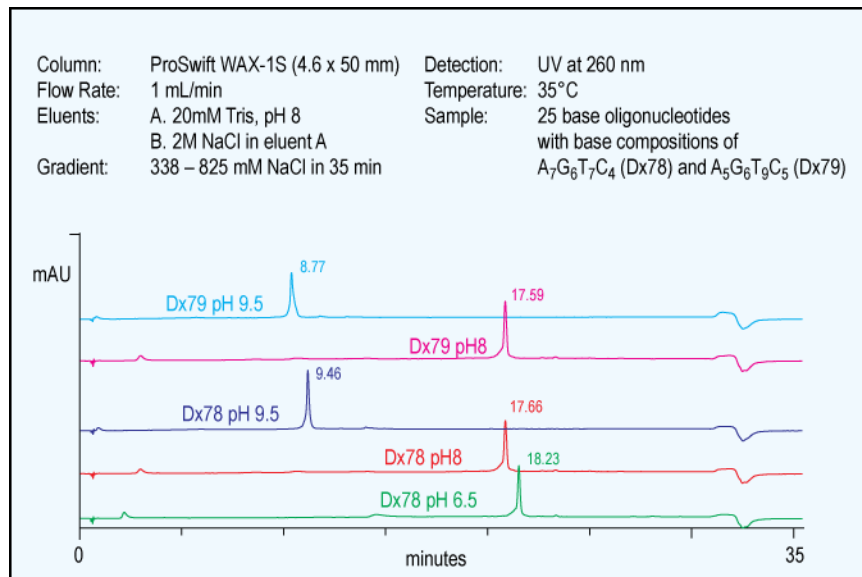


Figure 30
Effect of pH on Oligonucleotide Retention on ProSwift WAX-1S (4.6x50 mm)

2.8.5.3. Effect of Temperature on Oligonucleotide Retention

Here, a partially detritylated synthetic oligonucleotide was analyzed at temperatures ranging from 10-60 °C using the same gradient. With an increase in temperature, retention of both the tritylated and detritylated oligonucleotides increases. Retention of the tritylated oligonucleotide increases to a greater degree than the retention of the detritylated oligonucleotide. In the example shown in Figure 31, the tritylated oligonucleotide retention has increased to the point where it is not eluted, even at 1.125 M sodium chloride. Note that tritylated oligonucleotide peak shape improves as the temperature increases above 20 °C.

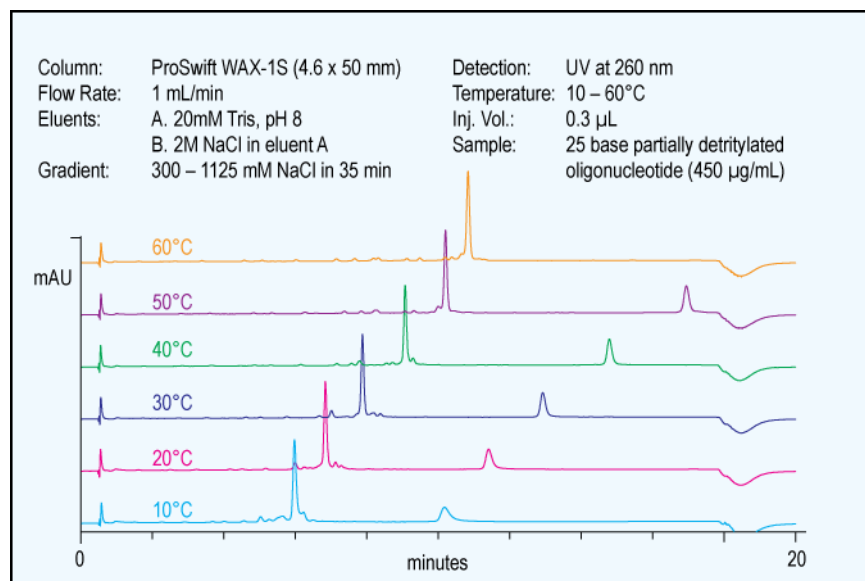


Figure 31

Effect of Temperature on ProSwift WAX-1S (4.6x50 mm) Oligonucleotide Elution

2.8.5.4. Effect of Fluorophores and Capture Probes on Oligonucleotide Retention

Oligonucleotides derivatized with different fluorescent or affinity probes can be resolved from one another on the ProSwift WAX-1S column (Figure 32). Thus, post-labeling purification of labeled oligonucleotides from their unlabeled parents is readily accomplished on the ProSwift WAX-1S monolith.

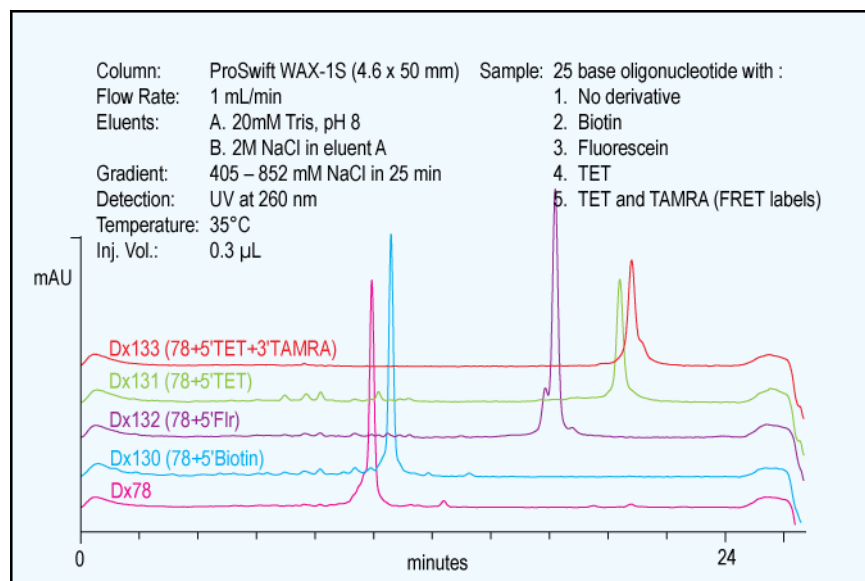


Figure 32

Effect of Derivatization on Retention Using ProSwift WAX-1S (4.6x50 mm)

2.8.5.5. Effect of Oligonucleotide Loading Quantity on Peak Shape and Resolution

Synthetic oligonucleotides removed from the synthesizer before removal of the trityl protecting group are readily separated from their detritylated counterparts (Figure 33). This example shows also the effect of ON load on peak response and demonstrates that overloading the monolith still allows good separation of the full length ON from its detritylated failure sequences.

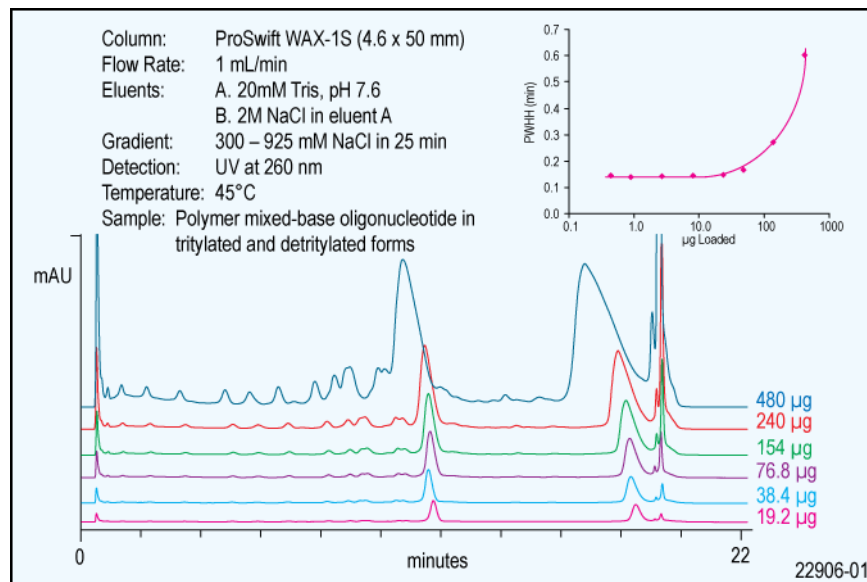


Figure 33
Ar₂₅±T Loading Study Using ProSwift WAX-1S (4.6x50 mm)

Based on sample loading studies Figure 33 shows the effect of oligonucleotide sample load on peak width when separated on the ProSwift WAX-1S column. The ProSwift monolith shows good peak width, up to ~ 120 µg, per injection and can be used for purification at significantly higher sample loads.

3. PROSWIFT SAX-1S

3.1. Resolution and Speed of Separation

As described in Section 1.1, the uniquely designed morphology of ProSwift monoliths allows fast analyte mass transfer. This property minimizes band broadening upon flow rate increases, thus resulting in higher resolution than conventional beads over a wide range of flow rates. Fast analyte mass transfer is especially beneficial for large molecules, whose diffusivities are much lower than small molecules. This emulates an advantage of non-porous beads. In combination with their low back pressure, ProSwift monoliths offer excellent separations using fast gradients at low and high flow rates, which improve productivity.

3.2. Loading Capacity

Figure 34 shows the dynamic loading capacity of ProSwift SAX-1S. Up to 1280 µg of protein was loaded on to the column, where phosphorylation variants were still partially resolved.

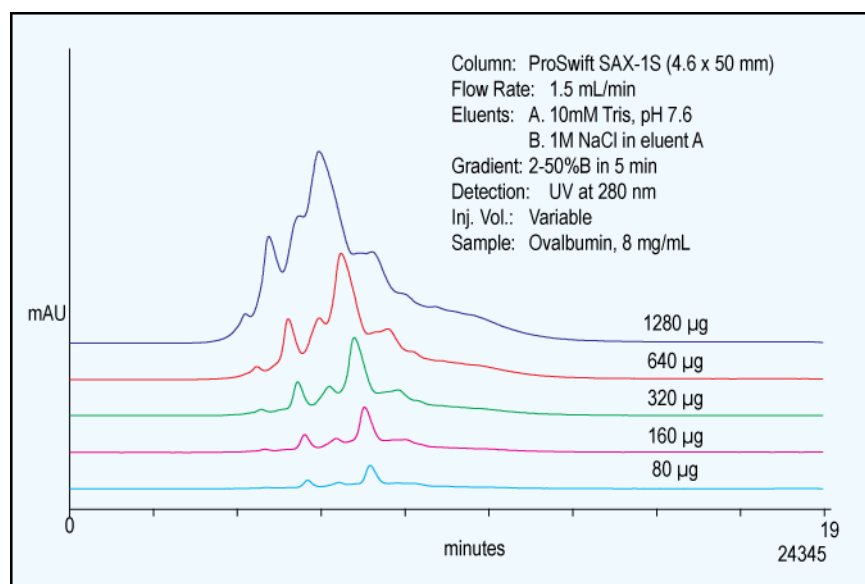


Figure 34
Protein Loading Capacity of ProSwift SAX-1S (4.6x50 mm)

Figure 35 shows the effect of loading of a protein/oligo mixture on the peak shape and resolution of ProSwift SAX-1S 1 x 50 mm column. Even at 54 μg of loading, the phosphorylation variants of ovalbumin are clearly resolved. The peak widths at half height values are given for trypsin inhibitor.

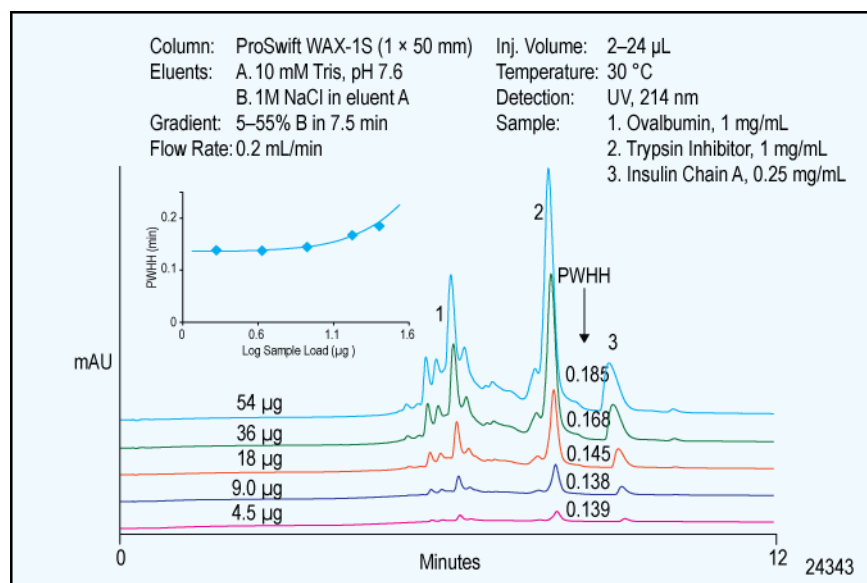


Figure 35
Protein Loading Capacity of ProSwift SAX-1S (1x50 mm)

Further, the dynamic loading capacity for ProSwift SAX 1 x 50 mm column was determined using trypsin inhibitor, Figure 36. 50% increase in peak width at half height was measured and used as the dynamic loading capacity of ProSwift SAX 1 x 50 mm.

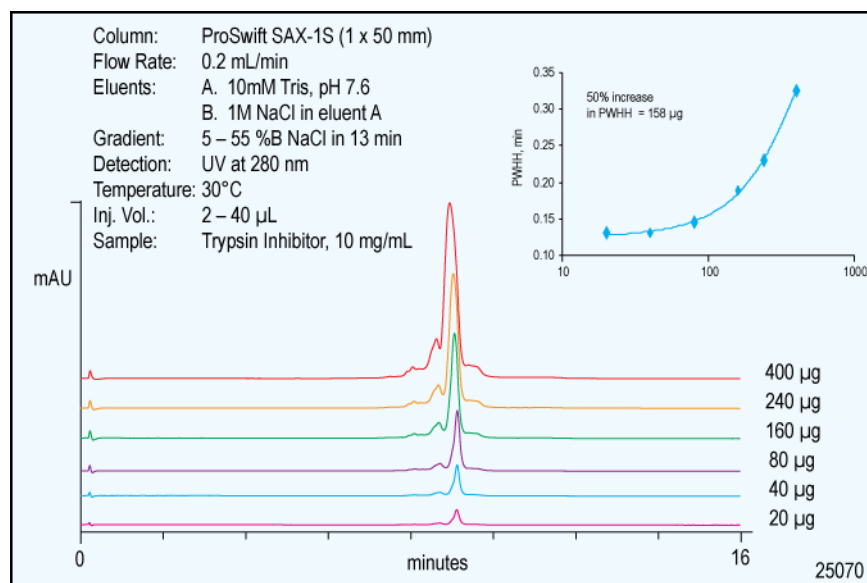


Figure 36
Dynamic Loading of Trypsin Inhibitor on ProSwift SAX-1S (1x50 mm)

3.4. Batch-to-Batch Reproducibility

ProSwift ion exchangers are manufactured by a patented *in situ* manufacturing process, which has the least number of variables affecting the reproducibility when compared to other technologies. The manufacturing process includes a single step polymerization followed by (in some cases) a single step surface modification. It does not require additional sieving, coating, multiple surface modification and packing processes.

Excellent batch to batch reproducibility of ProSwift SAX-1S, both 4.6 and 1 mm i.d., is shown in Figure 39 and Figure 40 respectively.

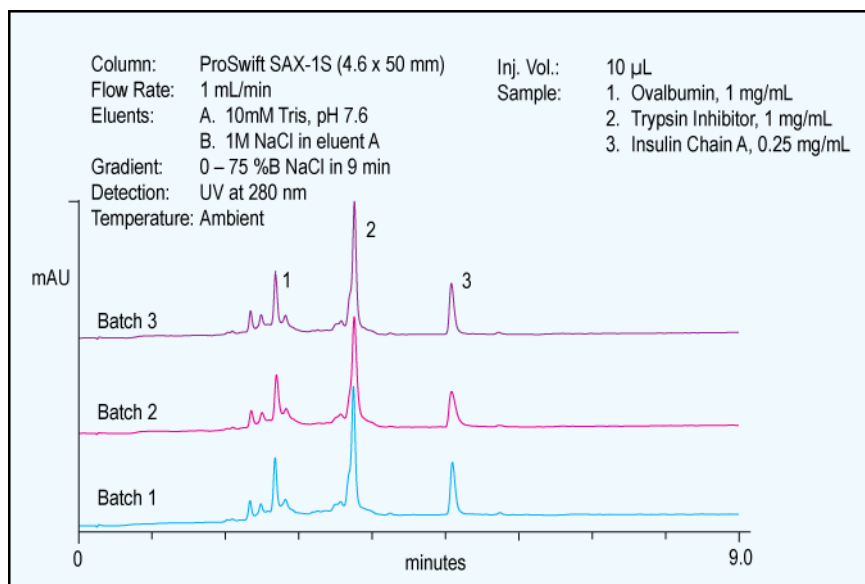


Figure 39
Batch-to-Batch Reproducibility of ProSwift SAX-1S (4.6x50 mm)

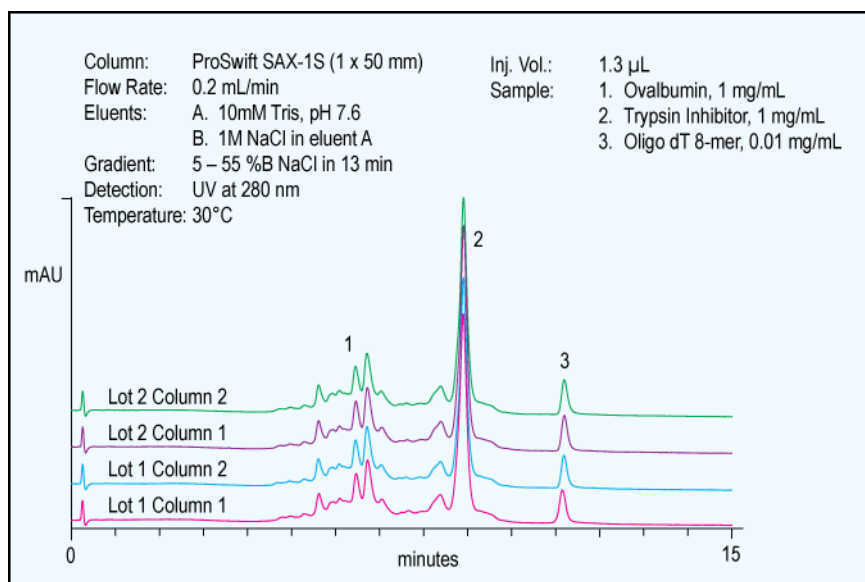


Figure 40
Batch-to-Batch and Column to Column Reproducibility of ProSwift SAX-1S (1x50 mm)

3.5. pH Stability

The ProSwift SAX-1S anion exchange monoliths have a polymethacrylate support with amine functional groups on the surface. During the regeneration process, the column is stable at temperatures up to 30 °C with the treatment of 1 M NaOH and 0.1 M HCl. The column is stable at pH 2 to 12 under operating conditions for long periods. Figure 41 and Figure 42 demonstrate that washing at both pH 1 and 14 (sufficient for sterilization) has no effect on chromatographic performance provided the columns are adequately equilibrated after washing.

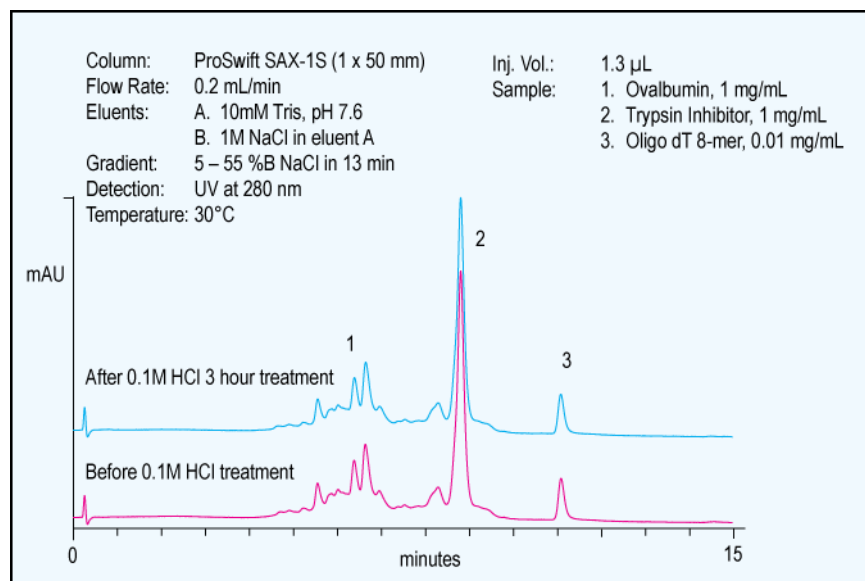


Figure 41
Effect of Transient (3 Hours) Hydrochloric Acid Washing
on the Performance of the ProSwift SAX-1S

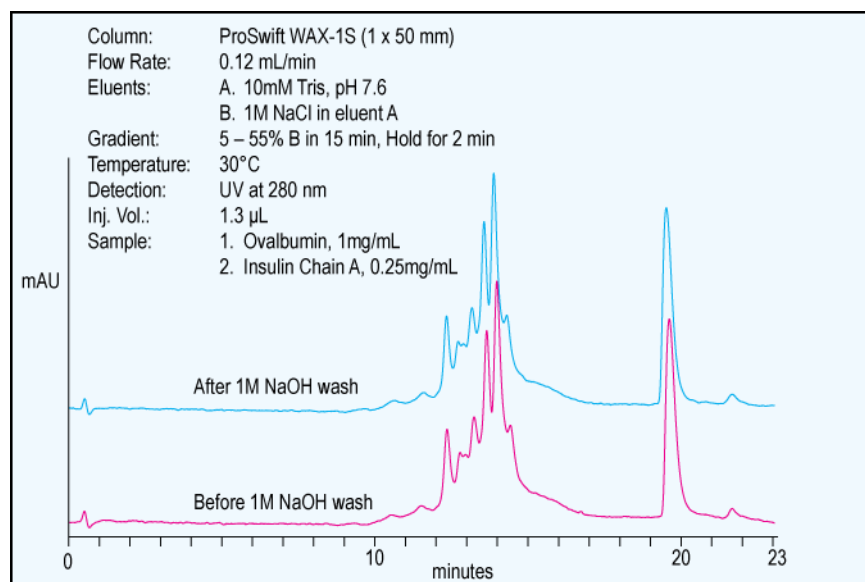


Figure 42
Effect of Transient (3 Hours) Sodium Hydroxide Washing
on the Performance of the ProSwift SAX-1S

3.6. Temperature Stability


WARNING

*Caution should be taken when using elevated temperatures and elevated pH.
See Section 1.10 for additional information regarding temperature and pH limits.*

Anion exchange phases produced with methacrylate polymers are stable at ambient temperatures from pH 2 to 12 using normal separation conditions. Temperature stability decreases at elevated pH. The ProSwift WAX-1S and SAX-1S columns tolerate temperatures to 70 °C for short periods, and are quite stable at temperatures up to 60 °C at pH from 6 to 8. At pH between 8.0 and 9.5, the maximum operating temperature decreases linearly to ambient temperature.

Figure 43 shows that the ProSwift SAX-1S 1x50 mm format is stable to running at 70 °C for 18 hours.

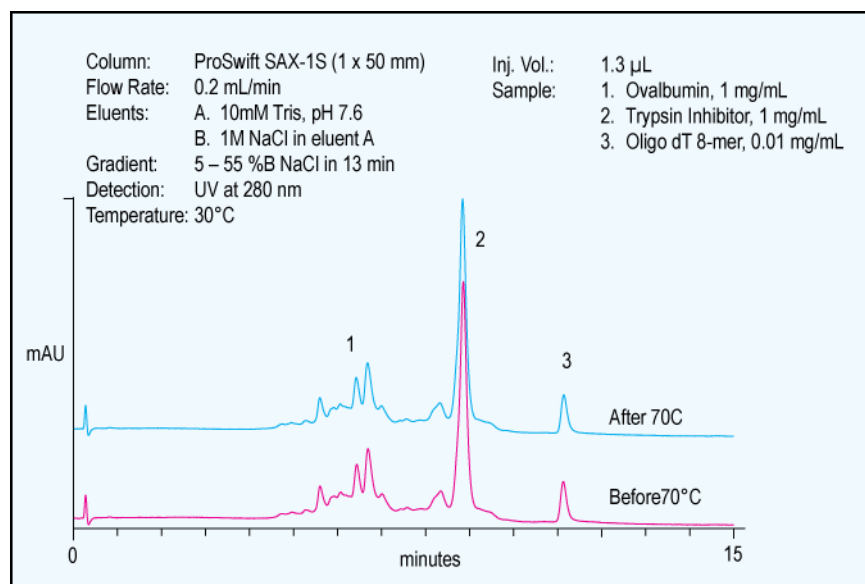


Figure 43

Before and After Running Column at 70°C for 18 Hours on the ProSwift SAX-1S Monolith

3.8. Applications

3.8.1. Separation of protein mixture

Figure 45 shows the separation of a protein mixture on ProSwift SAX-1S 4.6x50 mm column. The protein mixture contained myoglobin, conalbumin, ovalbumin (which contains two phosphorylation sites that result in closely related variants) and trypsin inhibitor.

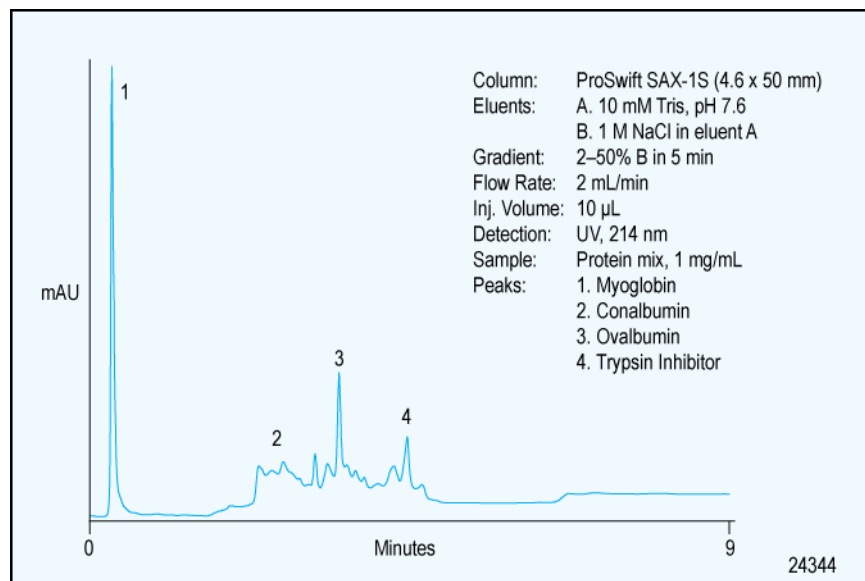


Figure 45
Separation of Protein Mixture

Figure 46 shows the separation of a protein/oligo mixture on ProSwift SAX-1S 1x50 mm column. The proteins include ovalbumin and trypsin inhibitor. The oligonucleotide is a dT 8mer.

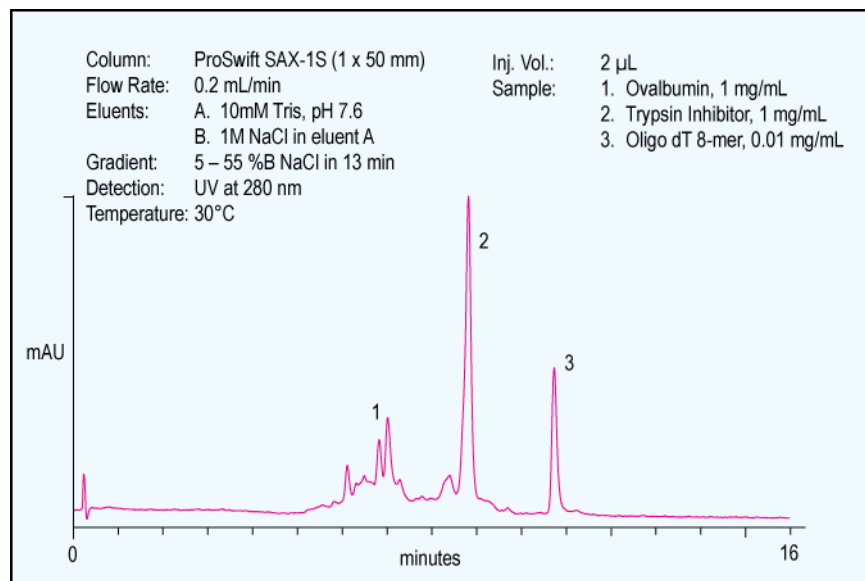


Figure 46
Protein/Oligo Separation on ProSwift SAX-1S (1x50 mm)

3.8.3. Separation of Caseins

The casein content of milk represents about 80% of milk proteins. The principal casein fractions are alpha caseins, beta-casein, and kappa-casein. Caseins are conjugated proteins, most with phosphate group(s) esterified to serine residues. These phosphate groups are important to the structure of the casein micelle. Calcium binding by the individual caseins is proportional to the phosphate content. Figure 49 and Figure 50 shows the retention of casein proteins (α , β , κ) on a ProSwift SAX-1S column. However, by varying buffer/gradient conditions suitable separation of variants can be achieved.

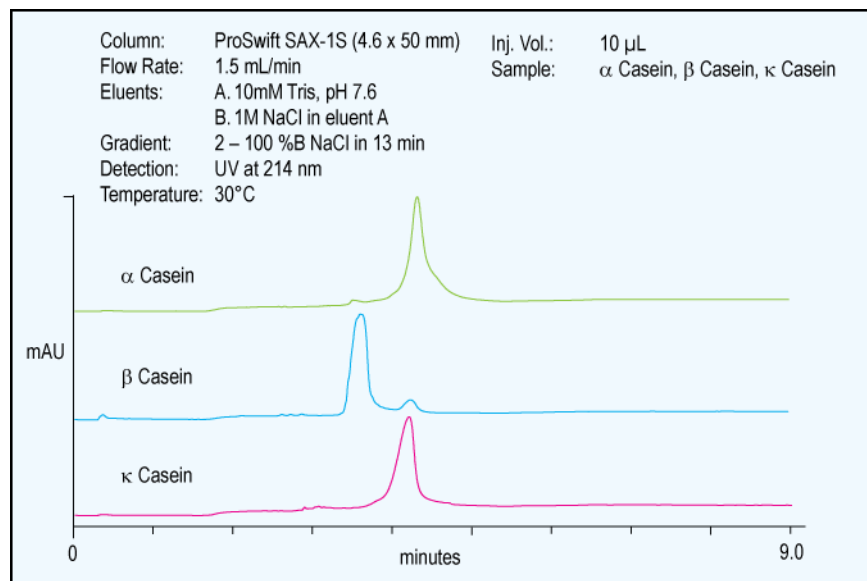


Figure 49
Separation of Caseins on ProSwift SAX-1S (4.6x50 mm)

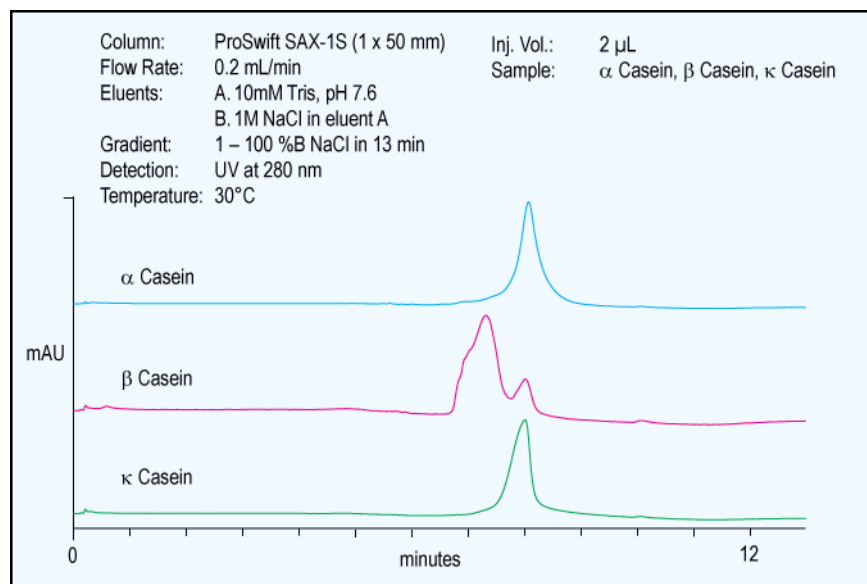


Figure 50
Separation of Caseins on ProSwift SAX-1S (1x50 mm)

3.8.4. Separation of Human Transferrin

Transferrin is a blood plasma glycoprotein, which binds iron very tightly but reversibly. Transferrin has a molecular weight of around 80,000 Da and contains two specific high affinity Fe(III) binding sites. The affinity of transferrin for Fe(III) is extremely high but decreases progressively with decreasing pH below neutrality. Human transferrin is separated into multiple peaks representing differences in the degree of sialylation of its oligosaccharides (Figure 51). Increasing the slope of the gradient (1,2) shows these variants can still be separated with reduced analysis time. Figure 52 shows the separation of Human transferrin on ProSwift SAX-1S 1x50 mm column.

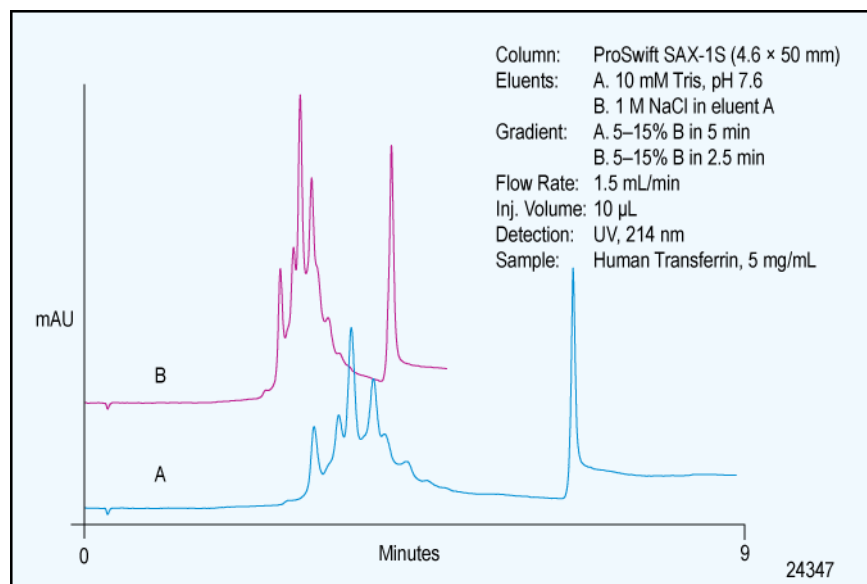


Figure 51

Separation of Human Transferrin on ProSwift SAX-1S (4.6x50 mm)

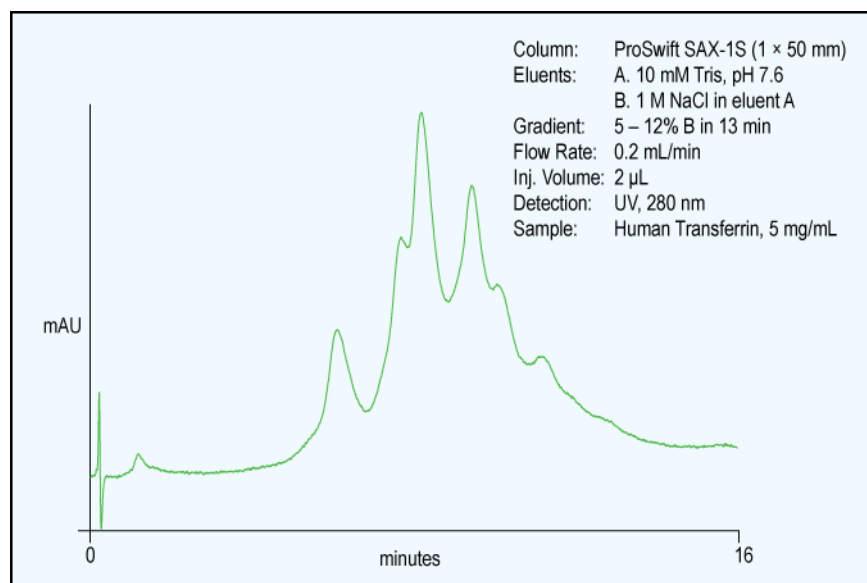


Figure 52

Separation of Human Transferrin on ProSwift SAX-1S (1x50 mm)

4. PROSWIFT WCX-1S

4.1. Resolution and Speed of Separation

As described in Section 1.1, the uniquely designed morphology of ProSwift monoliths allows fast analyte mass transfer. This property minimizes band broadening upon flow rate increases, thus resulting in higher resolution than conventional beads over a wide range of flow rates. Fast analyte mass transfer is especially beneficial for large molecules, whose diffusivities are much lower than small molecules. This emulates an advantage of non-porous beads. In combination with their low back pressure, ProSwift monoliths offer excellent separations using fast gradients at low and high flow rates, which improve productivity.

Use of 1 mm monoliths will result in substantially less eluent consumption than with a 4.6 mm monolith. For example flow rates in the 1 mm column should be 21 times less than in the 4.6 mm monolith. Thus, 0.25 mL/min flow rate in a 1 mm column will be equivalent to 5.0 mL/min in a 4.6 mm format. To obtain similar chromatography at various flow rates, the gradient times in a method or eluent program must be adjusted in proportion to the ratio of the flow rates.

As can be seen from Figure 53, total run-to-run time can be reduced from 38 to about 15 minutes when the flow rate is increased from 0.10 to 0.20 mL/min, without significant change in resolution (see inlay).

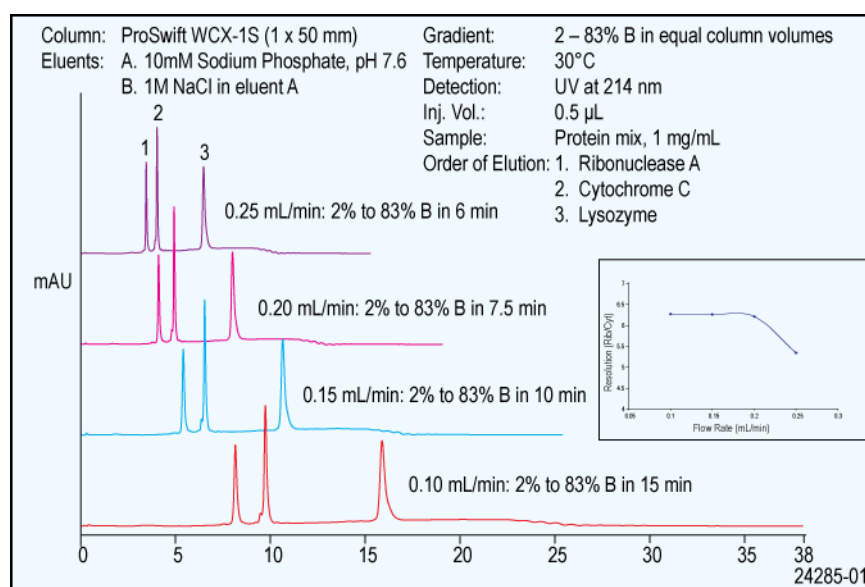


Figure 53
Protein Separation at Different Flow Rates with the ProSwift WCX-1S (1x50 mm)

Effects of flow rate on peak parameters with the 1x50 mm ProSwift WCX-1S monolith are shown in Figure 53, Figure 54 and Figure 55. These plots were obtained from the chromatograms shown in Figure 53.

Conventional packed bed columns (where mass transfer is dominant) follow the Van Deemter curve, and faster flow rates cause poorer peak efficiencies and consequently a decrease in resolution among peaks. Furthermore, fast flow rates can irreversibly damage the bed.

In the case of the WCX-1S 1 mm monolith, peak volume remains stable across the flow rate range shown in Figure 55; as expected peak width (minutes) decreases with increasing flow rate. Resolution between ribonuclease A and cytochrome C, shown in the inlay of Figure 53, is independent of flow rate up to 0.20 mL/minute, and then it decreases slightly at 0.25 mL/minute. However, all peaks are still well resolved. This is a clear advantage of the monoliths over packed bed columns. As Figure 54 shows the asymmetry is not dependent on flow rate, indicating that the monolith is not disturbed or damaged at the higher flow rates.

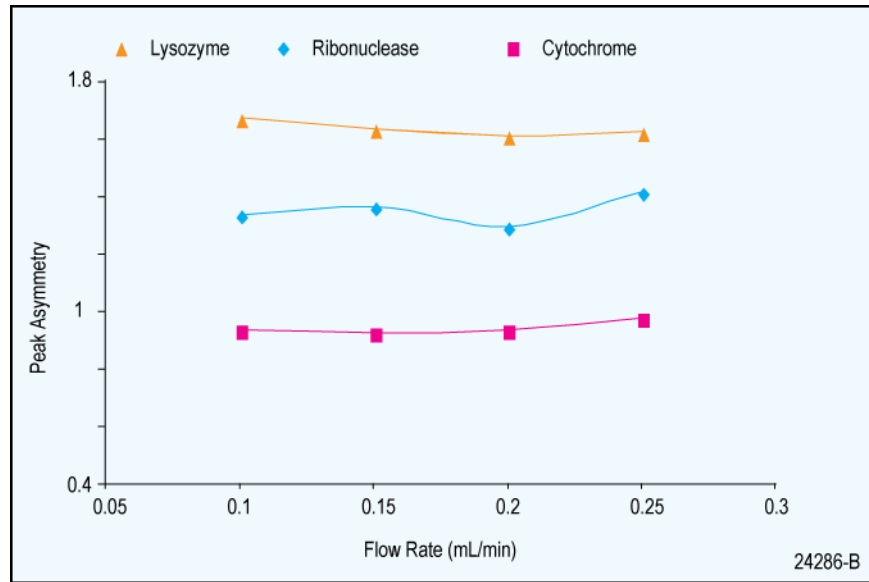


Figure 54

Effect of Flow Rate on Peak Asymmetry with the ProSwift WCX-1S (1x50 mm)

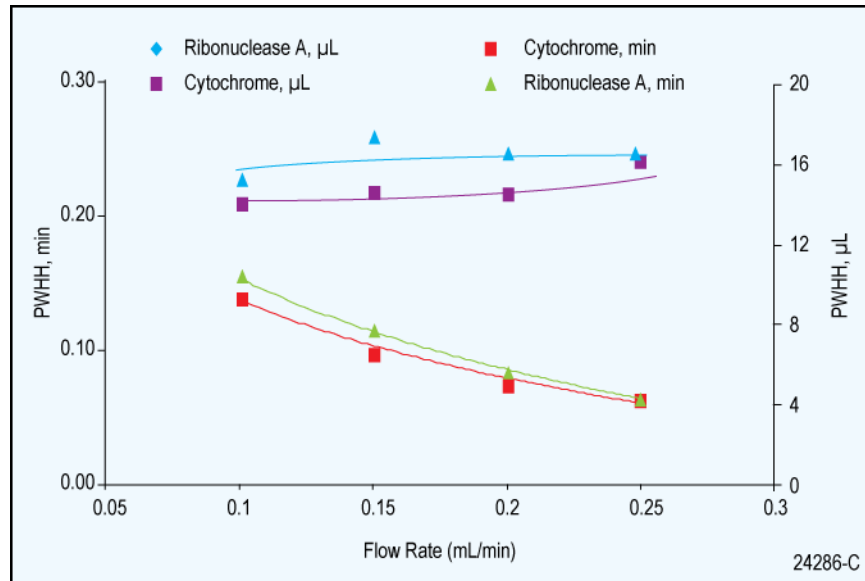


Figure 55

Effect of Flow Rate on Peak Volume and Width with the ProSwift WCX-1S (1x50 mm)

4.2. Loading Capacity

Figure 56 shows the dynamic loading capacity of ProSwift WCX-1S column. Ribonuclease A and lysozyme were used as model proteins to establish loading limits for this column. As much as 1600 µg of protein was loaded on to the column in this study. Dynamic Loading Capacity is defined as sample load resulting in a 1.5 times increase in peak width at half height (PWHH), which is 665 µg for ribonuclease A, and 820 µg for lysozyme. This is shown in Figure 57.

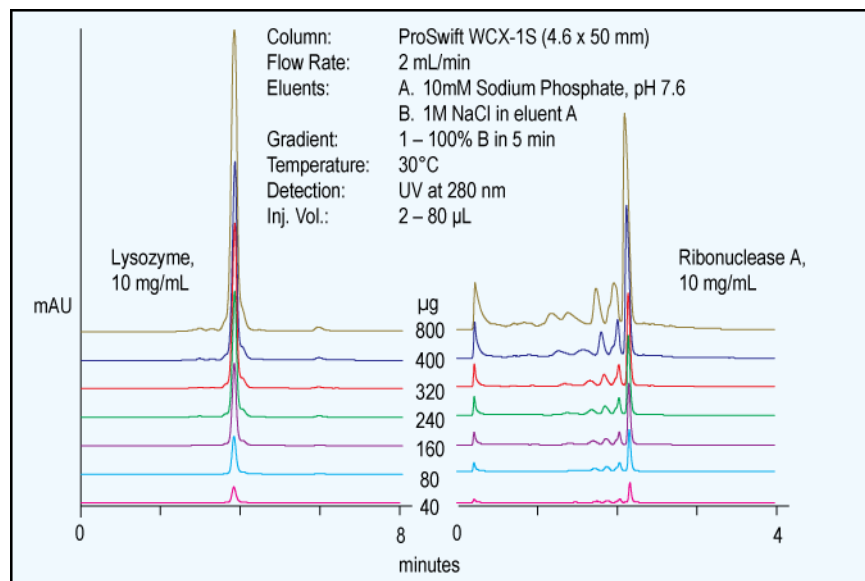


Figure 56

Dynamic Loading of Ribonuclease A and Lysozyme on ProSwift WCX-1S (4.6x50 mm)

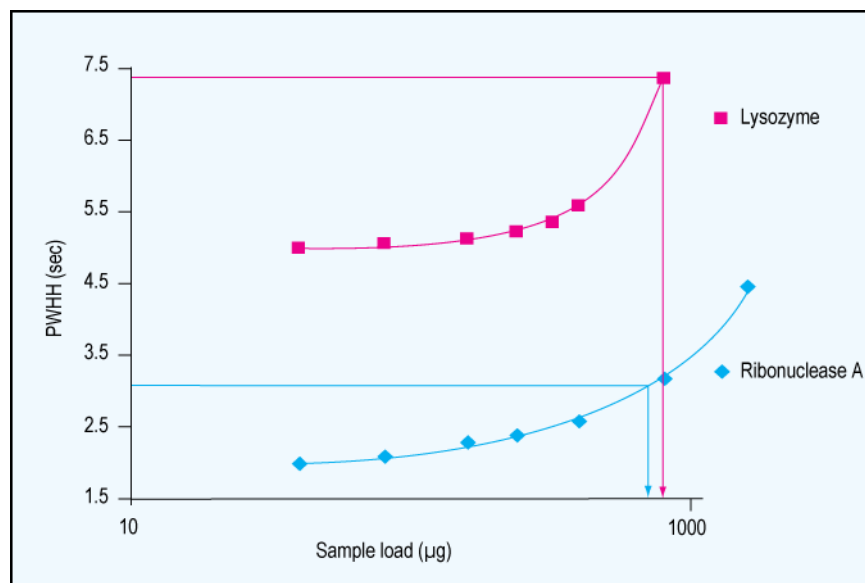


Figure 57

Peak Width at Half Height as a Function of Sample Load

Figure 58 shows that when a mixture of three proteins is loaded onto the 1x50 mm ProSwift WCX-1S monolith more than 100 µg protein can be loaded.

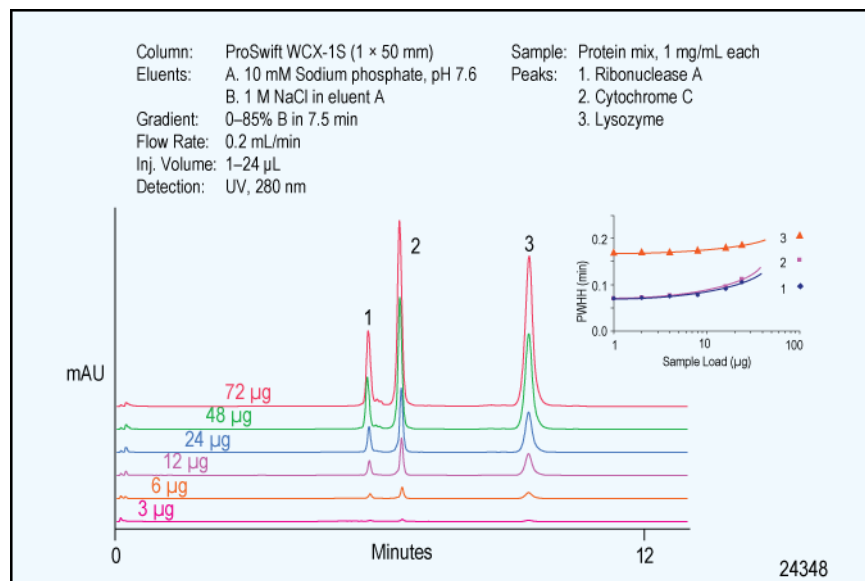


Figure 58
Dynamic Sample Loading on the ProSwift WCX-1S (1x50 mm)

4.3. Robustness and Run Stability

Durability and robustness are characteristic of the ProSwift monolithic columns. The ProSwift monoliths exhibit stability and reproducibility even after hundreds of runs.

Figure 59 shows the stability of ProSwift WCX-1S column. The stability was assessed by testing the separation of a protein mixture intermittently over more than 400 cycles.

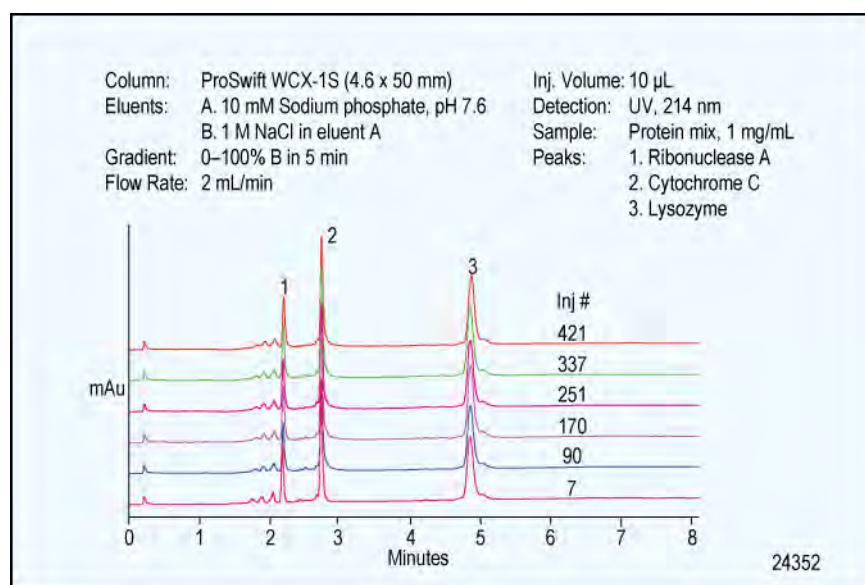


Figure 59
Stability of ProSwift WCX-1S (4.6x50 mm)

4.4. Batch-to-Batch Reproducibility

ProSwift ion exchangers are manufactured by a patented *in situ* manufacturing process, which has the least number of variables affecting the reproducibility when compared to other technologies. The manufacturing process includes a single step polymerization followed by (in some cases) a single step surface modification. It does not require additional sieving, coating, multiple surface modification and packing processes.

Excellent batch to batch reproducibility of ProSwift WCX-1S 4.6 is shown in Figure 60.

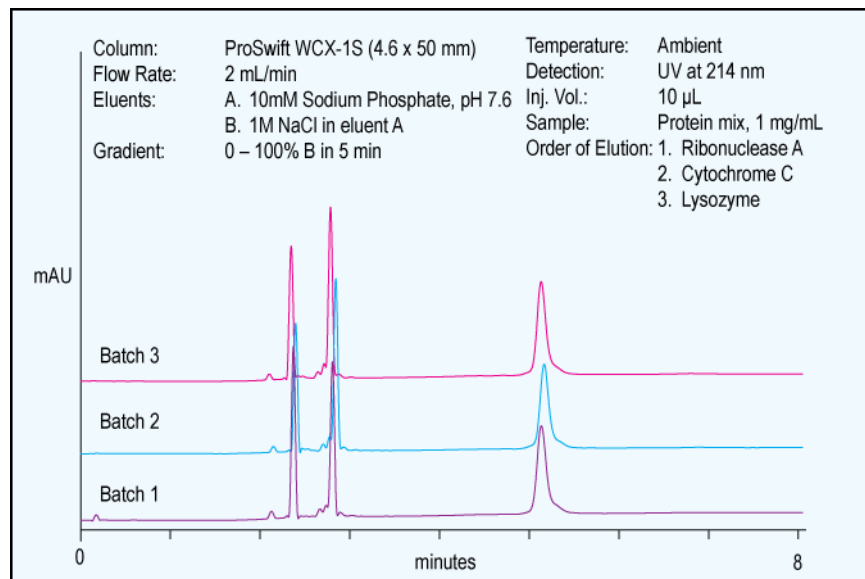


Figure 60
Batch-to-Batch Reproducibility of ProSwift WCX-1S (4.6x50 mm)

4.5. pH Stability

The ProSwift weak (WCX) cation exchange monoliths have an acrylate/methacrylate copolymer support with carboxylate functional groups. During cleaning, the column is stable at temperatures up to 30 °C with a transient treatment of 1 M NaOH or 0.1 M HCl. Figure 61 shows that the performance of the 1x50 mm ProSwift WCX-1S monolith is unaffected by washing for 1 hour at 0.05 mL/min with 1 M NaOH.



For faster equilibration after a NaOH treatment, wash the column with 500 mM NaCl buffered at pH 7.6.

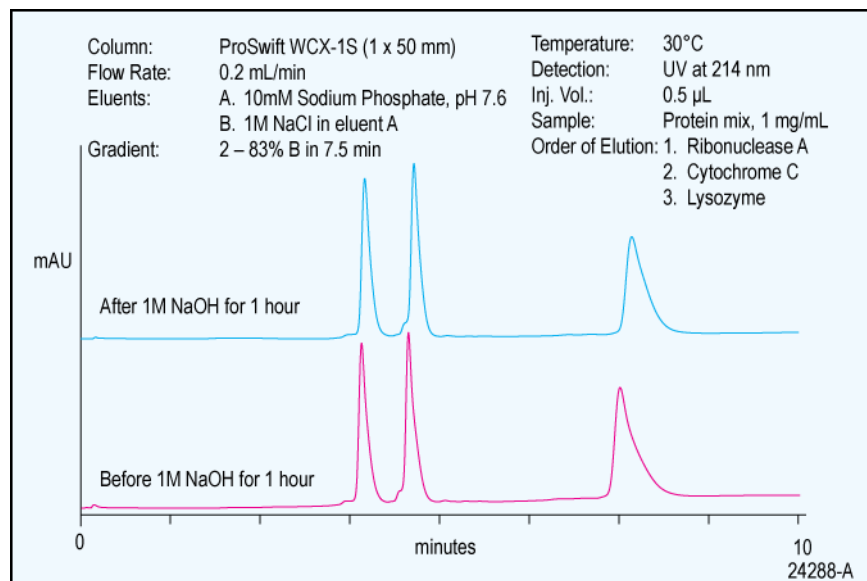


Figure 61
Effect of Transient (1 Hour) Sodium Hydroxide Washing
on the Performance of the ProSwift WCX-1S

4.6. Temperature Stability


WARNING

*Caution should be taken when using elevated temperatures and elevated pH.
See Section 1.10 for additional information regarding temperature and pH limits.*

Cation exchange phases produced using methacrylate and acrylate polymers show temperature stability similar to that of the other ProSwift ion exchange columns. Figure 62 shows a comparison before and after 24 hours exposure at 60 °C at pH 7.6 on the ProSwift WCX-1S.

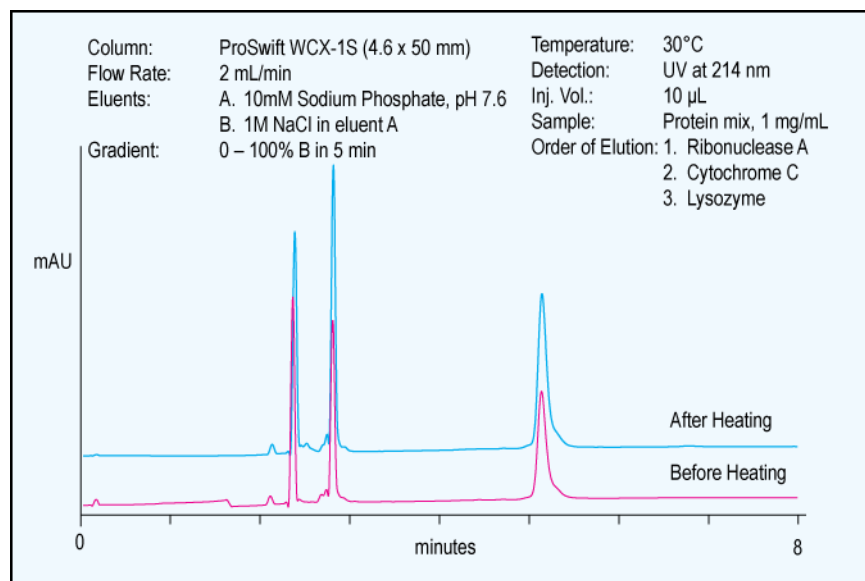


Figure 62

Protein Separation on the ProSwift WCX-1S Before and After 24 Hours of Use at 60 °C

4.7. Applications

4.7.1. Separation of Protein mixture

Figure 63 and Figure 64 shows the separation of a protein mixture on the 4.6x50 mm and 1x50 mm ProSwift WCX-1S columns. The protein mixture contained ribonuclease A, cytochrome C and lysozyme. The high capacity of this column is demonstrated in Figure 65, showing a loading of up to 160µg of protein shows no effect on peak width.

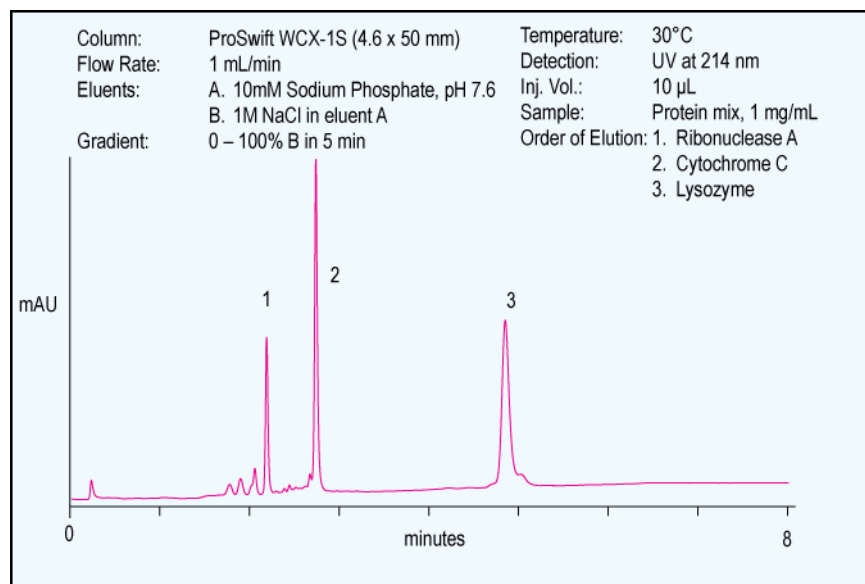


Figure 63
Separation of Protein Mixture on ProSwift WCX-1S (4.6x50 mm)

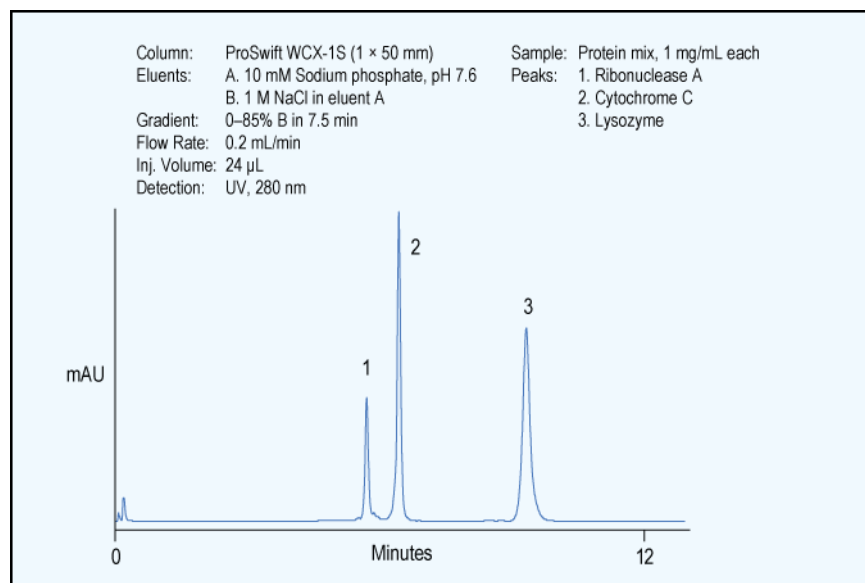


Figure 64
Separation of Protein Mixture on ProSwift WCX-1S (1x50 mm)

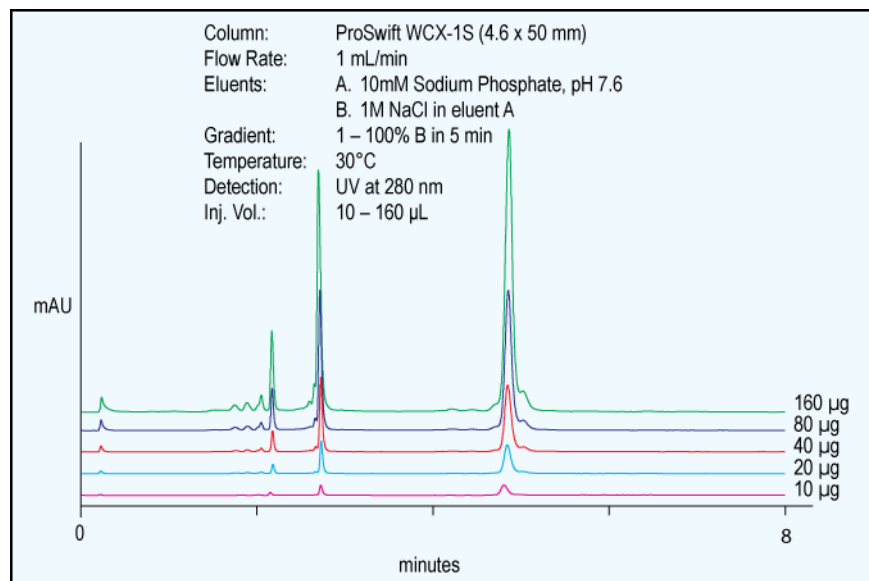


Figure 65
Effect of Loading on the Separation of Protein Mixture on ProSwift WCX-1S (4.6x50 mm)

Figure 66 shows that peak selectivity is influenced by eluent composition and pH. The use of Tris and phosphate buffers at identical pH values reveals that retention of ribonuclease A and cytochrome C responds differently to these buffers. Use of MES buffer at a lower pH (pH 6.5) confirms that retention of ribonuclease A and cytochrome C are also influenced by pH.

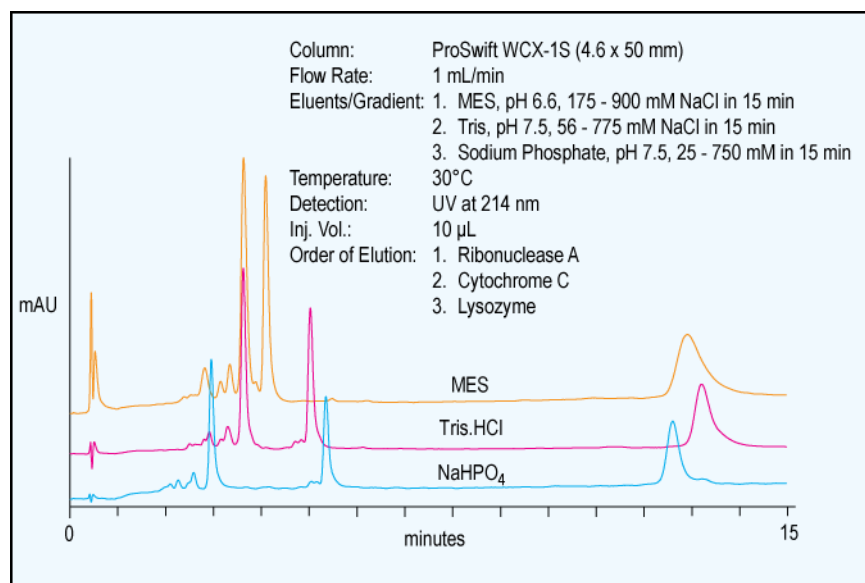


Figure 66

Separation of Proteins Using Different Buffer Conditions on ProSwift WCX-1S (4.6x50 mm)

4.7.2. Separation Snake Venom proteins

The separation of snake venom proteins/peptides by ProSwift WCX-1S is shown in Figure 67. Snake venoms are composed of many hydrolytic enzymes, a complex mixture of proteases, peptidases, and nucleases. Some of these components contribute to the toxicity of the venom. These can be separated and purified to develop anti-venom therapies.

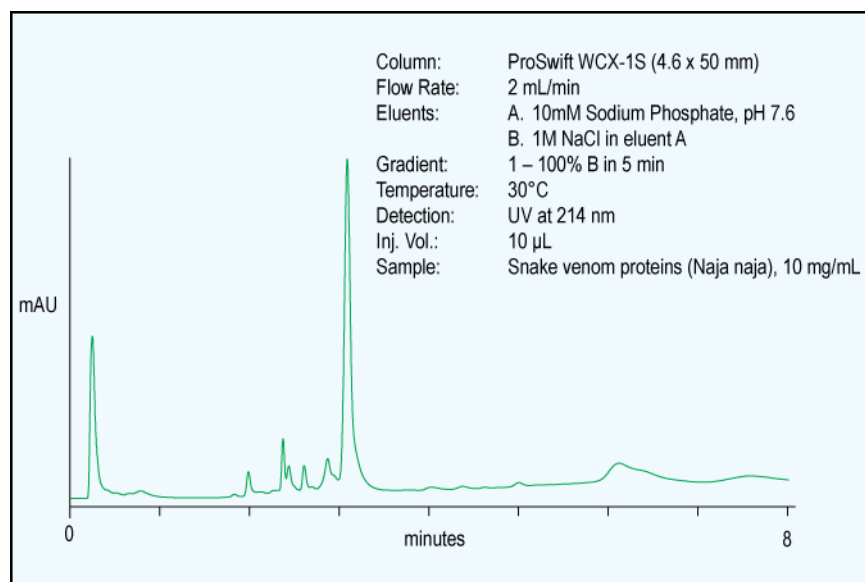


Figure 67

Separation of Snake Venom Proteins on ProSwift WCX-1S (4.6x50 mm)

4.7.3. Monoclonal Antibody (MAb) Separations

Monoclonal antibodies (MAbs) are currently developed by pharmaceutical and biotechnology companies for various therapeutic applications. MAbs undergo several post-translational modifications including oxidations, deamidations, and truncations as well as glycan modifications. Manufacturing of MAbs and subsequent stability testing procedures involve monitoring of the impurities resulting from asparagine deamidation, aspartic isomerization, disulfide interchange, peptide bond cleavage and oxidation.

4.7.3.1. Separation of acidic and basic variants in of monoclonal antibodies

ProSwift WCX-1S was used to characterize MAb heterogeneity. One of the example applications is shown in Figure 68. Selectivity can vary when using different buffer compositions. Variations in buffer composition and pH, as shown in Figure 69, seem to have little effect on the relative selectivity of these MAb variants.

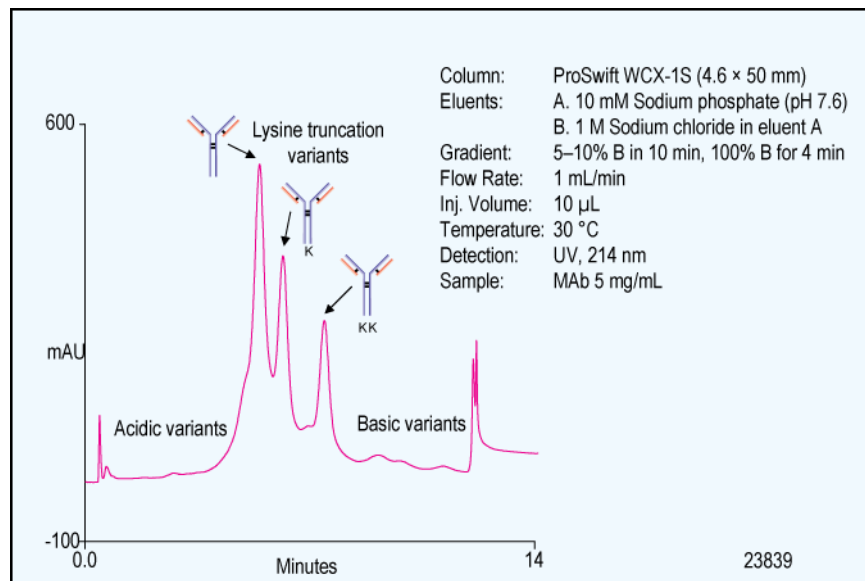


Figure 68
Separation of MAb Variants

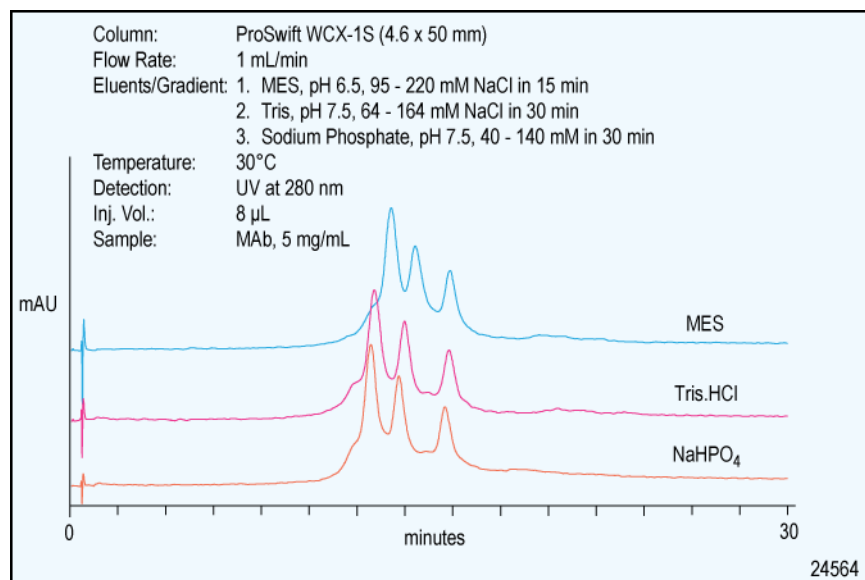


Figure 69
MAb Variants Separated Using Different Buffer Conditions

4.7.3.3. Characterization of MAb C-terminal Lysine Variants

In this example, the ProSwift WCX-1S column was used to separate variants of a humanized IgG, containing lysine residue variation at the C-terminal of the heavy chains. As shown in Figure 71, it resolves three variant forms differing by the presence of lysine at the C-terminal of the heavy chains (with either 0, 1, or 2 lysine residues).

To verify that the reason for the different retention times of the three peaks was the different content of heavy chain C terminal lysine, the IgG preparation was treated for 3 hours at 37 °C with carboxypeptidase B, an exopeptidase that specifically cleaves basic C terminal residues. This treatment of the IgG preparation resulted in the quantitative disappearance of peaks 2 and 3 (containing 1 and 2 terminal lysine residues, respectively, on their heavy chains). The decreased peak areas in peaks 2 and 3 were accompanied by a corresponding quantitative increase in peak area 1 (variant with no terminal lysine) confirming that peaks 2 and 3 differed from peak 1 in that they contained IgG with 1 and 2 terminal heavy chain lysine residues, respectively. The advantage of using ProSwift WCX for this application lies in column's capacity. In the example a sample of 720 µg of protein was loaded onto the 4.6x50 mm monolith and compared with and without the addition of carboxypeptidase B. This capability also helps collection of isolated acidic and basic variants.

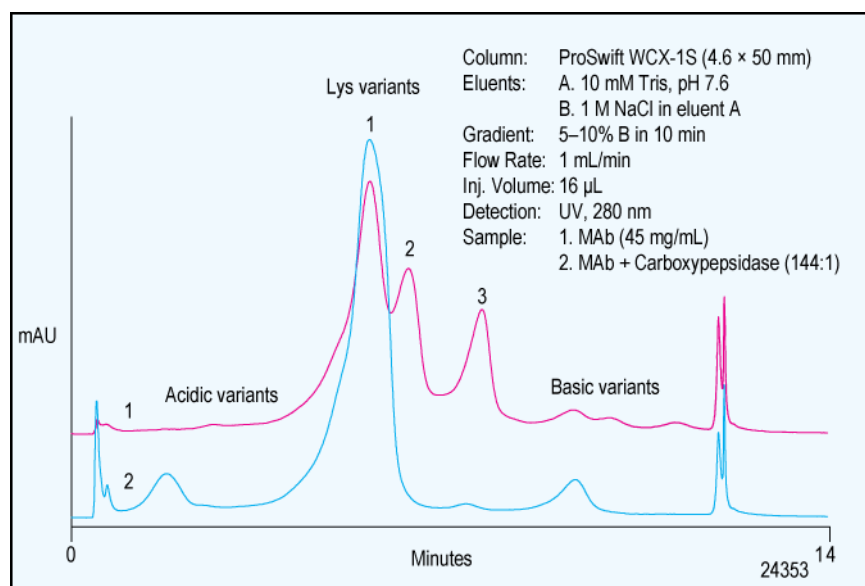


Figure 71

Assay of MAb Variants ± Carboxypeptidase on ProSwift WCX-1S (4.6x50 mm)

A repeat of the previous test on a 1x50 mm monolith with a different buffer mixture yielded similar results (Figure 72). However, use of the new buffer at a lower pH resolves more acidic variants or other impurities.

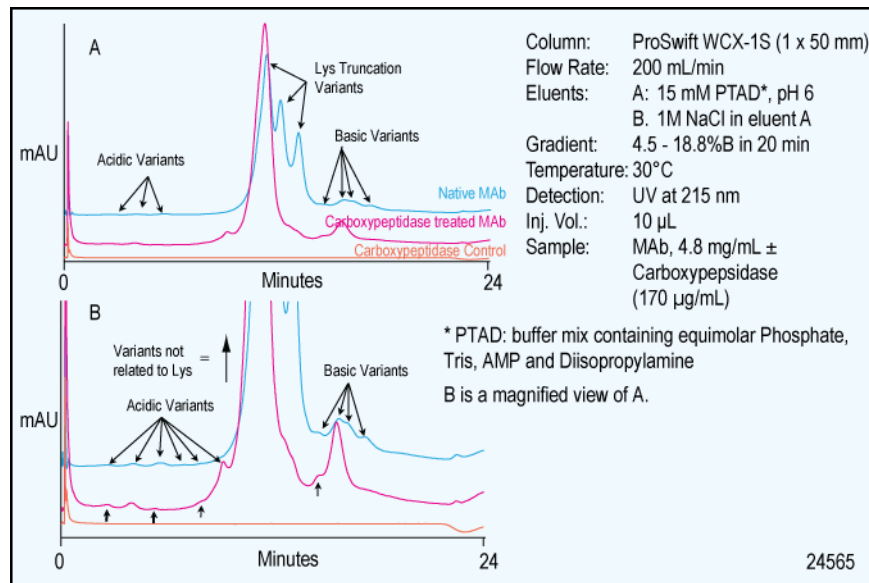


Figure 72
Assay of MAb Variants ± Carboxypeptidase on ProSwift WCX-1S (1x50 mm)

5. PROSWIFT SCX-1S

5.1. Speed and Resolution

As described in Section 1.1, the uniquely designed morphology of ProSwift monoliths allows fast analyte mass transfer. This property minimizes band broadening upon flow rate increases, thus resulting in higher resolution than conventional beads over a wide range of flow rates. Fast analyte mass transfer is especially beneficial for large molecules, whose diffusivities are much lower than small molecules. This emulates an advantage of non-porous beads. In combination with their low back pressure, ProSwift monoliths offer excellent separations using fast gradients at low and high flow rates, which improve productivity. Figure 67 shows the separation of protein mixture at increased flow rates under normalized gradient conditions and the corresponding resolution values were shown in the table. Even at high flow rates (0.3mL/min i.e. >6mm/s) high resolution is maintained.

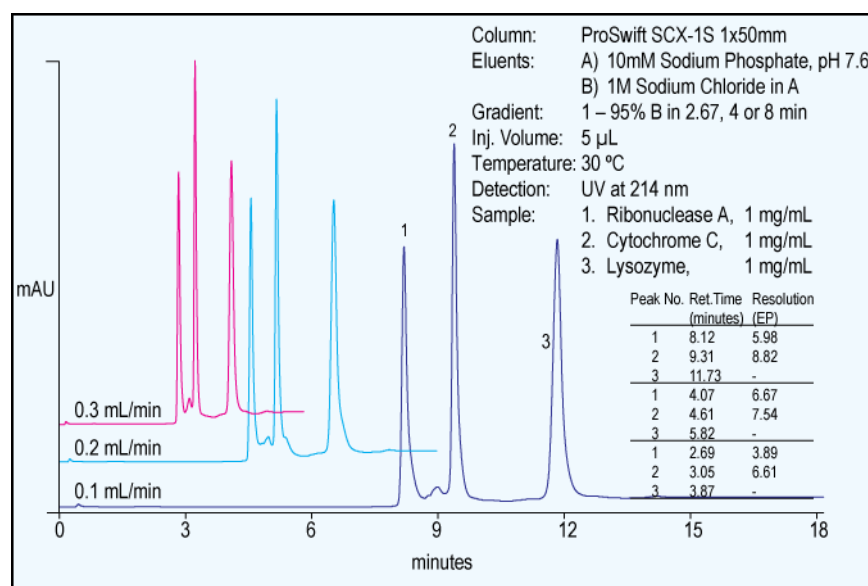


Figure 73

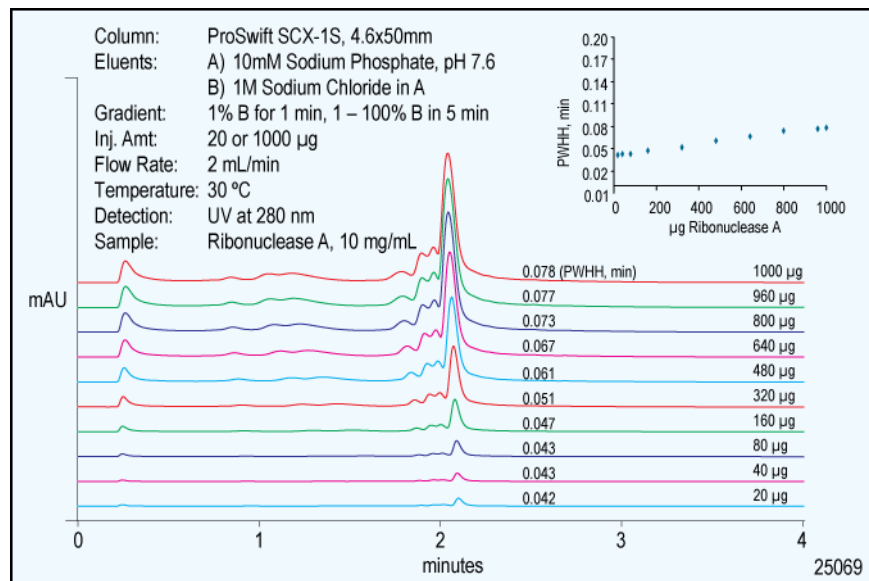
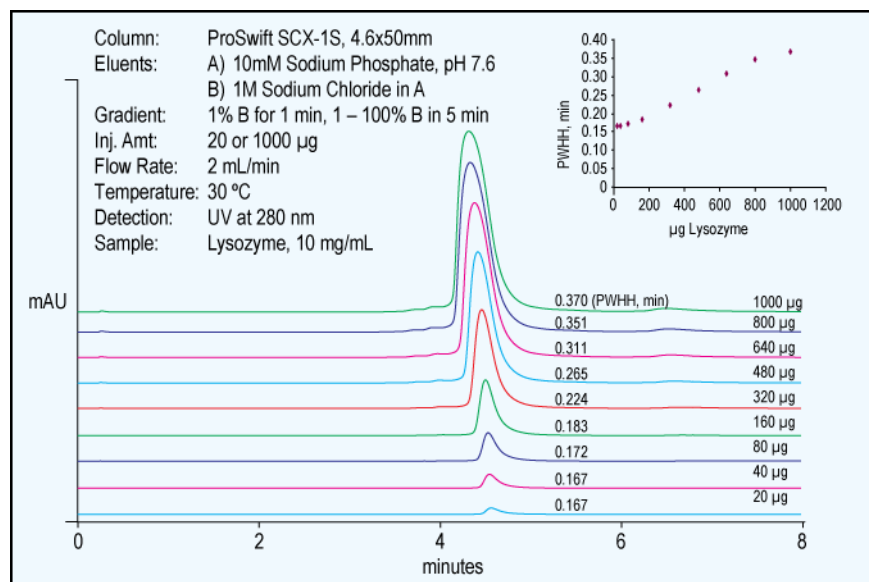
Effect of flow rate on run time and resolution on the ProSwift SCX-1S 1x50mm

5.2. Loading Capacity

ProSwift monolith surfaces are irregular as shown by the SEM images in Figure 1. This produces loading capacities comparable to porous beads. This optimized morphology supplies the best features of non-porous beads and porous beads: high resolution and high capacity: “The best of both worlds!”

Figure 74 and Figure 75 show the dynamic loading capacity of ProSwift SCX-1S 4.6 x50 mm column. Ribonuclease A and lysozyme were used as model proteins to establish loading limits for this column. Up to 1mg each of protein in separate experiments was loaded on to the column in this study. The peak width at half height (PWHH) was measured and presented. Since there is no significant loss of resolution for the variants up to 1mg, even higher amounts may be loaded for purification purposes.

Dynamic loading capacity of ProSwift SCX 1x 50 mm using a 3 protein mixture (Figure 70), cytochrome C (Figure 71) and lysozyme (Figure 71) is shown. Three protein mixture (Ribonuclease A, cytochrome C and lysozyme) was loaded at different amounts (2-24 µg each protein) and PWHH values for cytochrome C peak were derived (Figure 70). Similarly, different amounts of either cytochrome C or lysozyme were loaded and the PWHH is determined at each of these concentrations and plotted. Dynamic loading capacity is defined as 50% increase in PWHH and was extrapolated for cytochrome C (~40 µg, Figure 71) and for lysozyme (42 µg, Figure 72).


Figure 74
Dynamic Sample Loading on the ProSwift SCX-1S (4.6x50 mm)

Figure 75
Dynamic Sample Loading of Lysozyme on the ProSwift SCX-1S (4.6x50 mm)

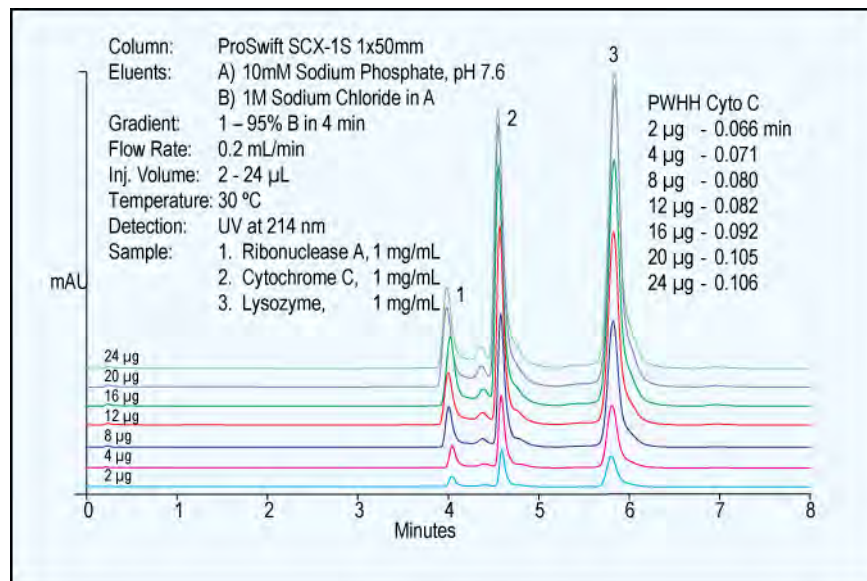


Figure 76

Dynamic Sample Loading of a 3 component protein mix on the ProSwift SCX-1S (1x50 mm)

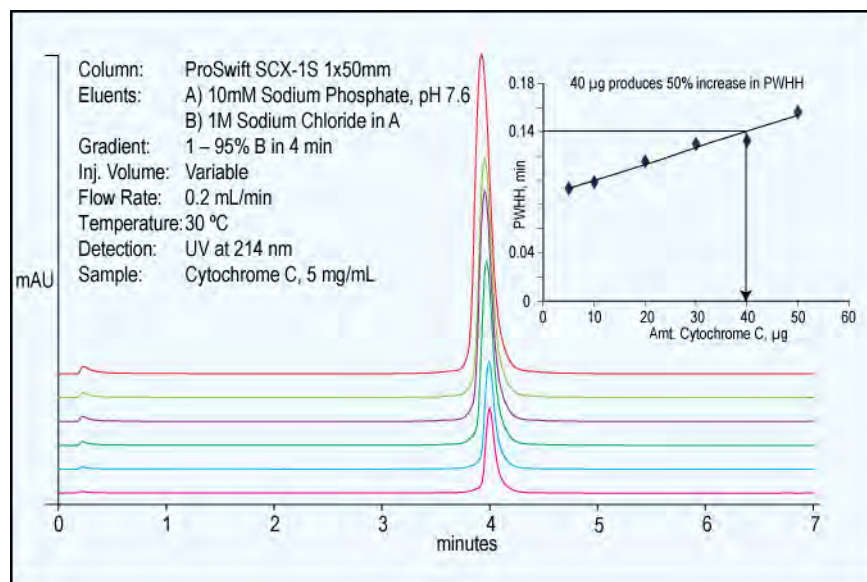


Figure 77

Dynamic Sample Loading of Cytochrome C on the ProSwift SCX-1S (1x50 mm)

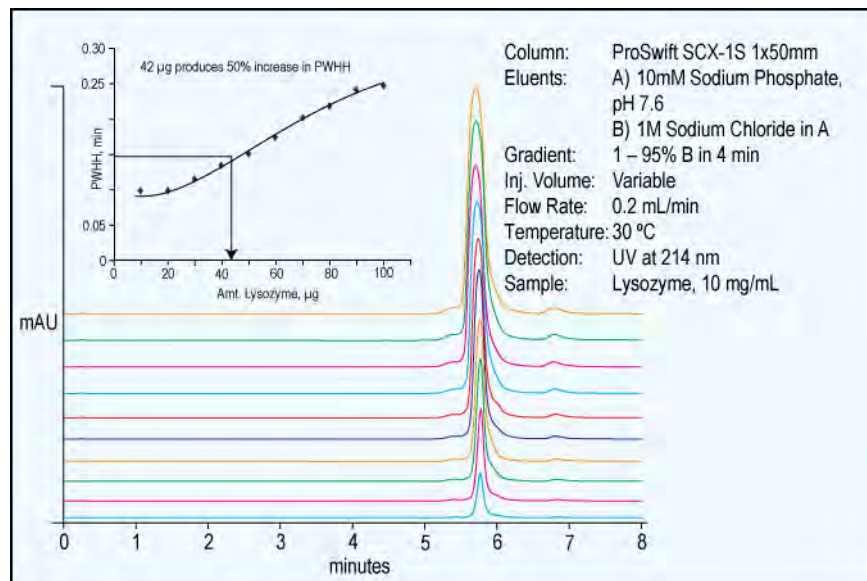


Figure 78
Dynamic Sample Loading of Lysozyme on the ProSwift SCX-1S (1x50 mm)

5.3. Robustness and Run Stability

Durability and robustness are characteristic of the ProSwift monolithic columns. The ProSwift monoliths exhibit stability and reproducibility even after hundreds of runs.

Figure 79 and Figure 80 show the stability of ProSwift SCX-1S columns. The stability was assessed by testing the separation of a protein mixture intermittently over more than 150 runs for 4.6x50 mm column (Figure 73) at 2.0 mL/min and more than 500 runs at 0.3 mL/min were performed for 1x 50 mm column and selected runs were overlaid (Figure 74).

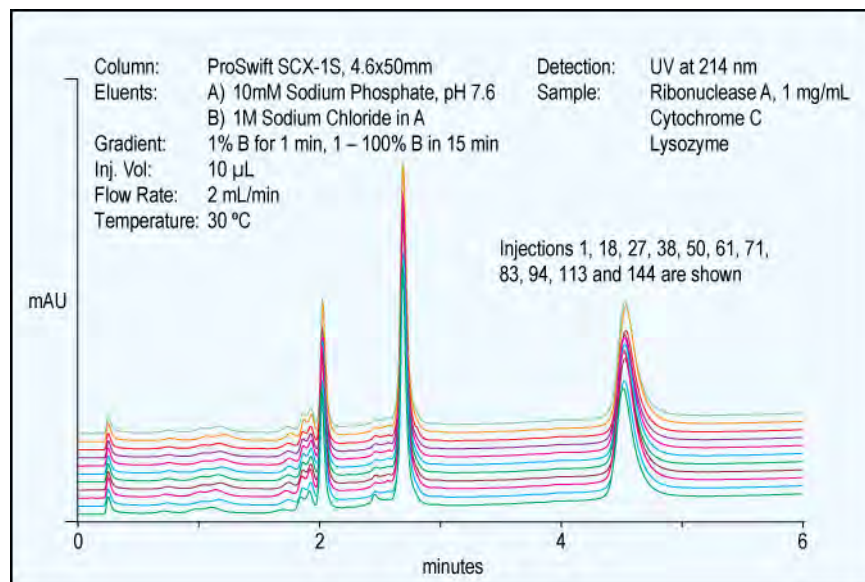


Figure 79
Stability of ProSwift SCX-1S (4.6x50 mm)

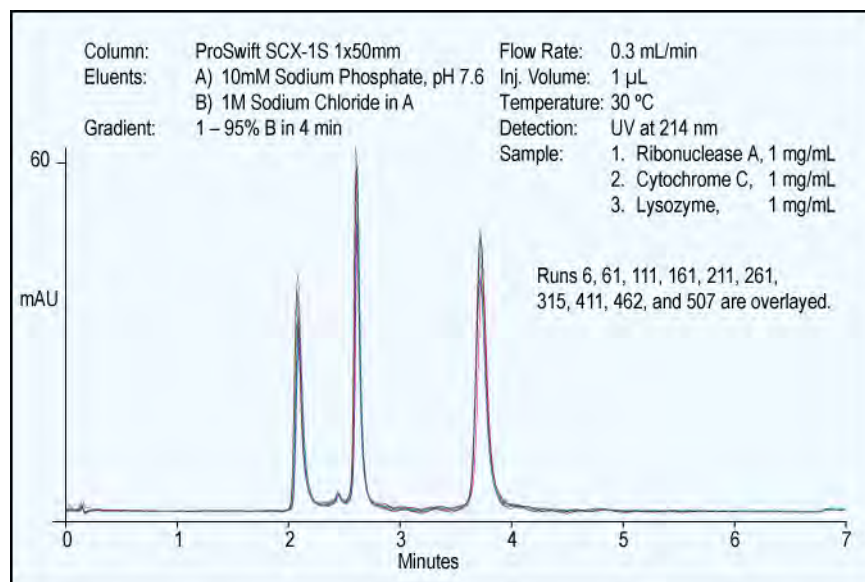


Figure 80
Stability of ProSwift SCX-1S (1x50mm)

5.4. Batch-to-Batch Reproducibility

ProSwift ion exchangers are manufactured by a patented *in situ* manufacturing process, which has the least number of variables affecting the reproducibility when compared to other technologies. The manufacturing process includes a single step polymerization followed by (in some cases) a single step surface modification. It does not require additional sieving, coating, multiple surface modification and packing processes.

Excellent batch to batch reproducibility of ProSwift SCX-1S is shown in Figure 81.

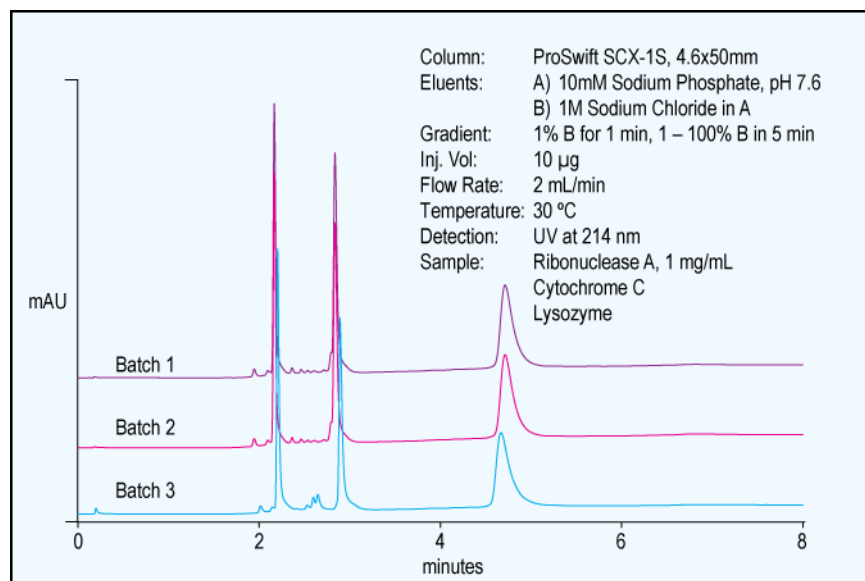


Figure 81
Batch-to-Batch Reproducibility of ProSwift SCX-1S (4.6x50 mm)

5.5. pH Stability

The ProSwift strong (SCX) cation exchange monolith has an acrylate/methacrylate copolymer support with sulfonate functional groups respectively. During cleaning, the column is stable at temperatures up to 30 °C with a transient treatment of 1 M NaOH or 0.1 M HCl. Figure 82, Figure 83 and Figure 85 show how the 4.6x50mm and 1x50mm ProSwift SCX-1S monolith is not affected by ten one hour washes with either 1M NaOH or 0.1M HCl.



For faster equilibration after a NaOH treatment, wash the column with 500 mM NaCl buffered at pH 7.6.

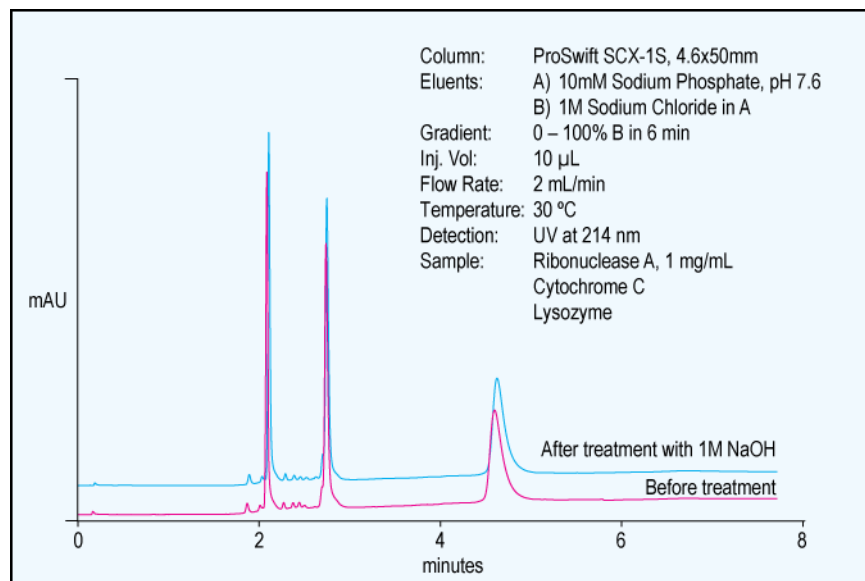


Figure 82
Effect of Transient (1 Hour) Sodium Hydroxide Washing
on the Performance of the ProSwift SCX-1S

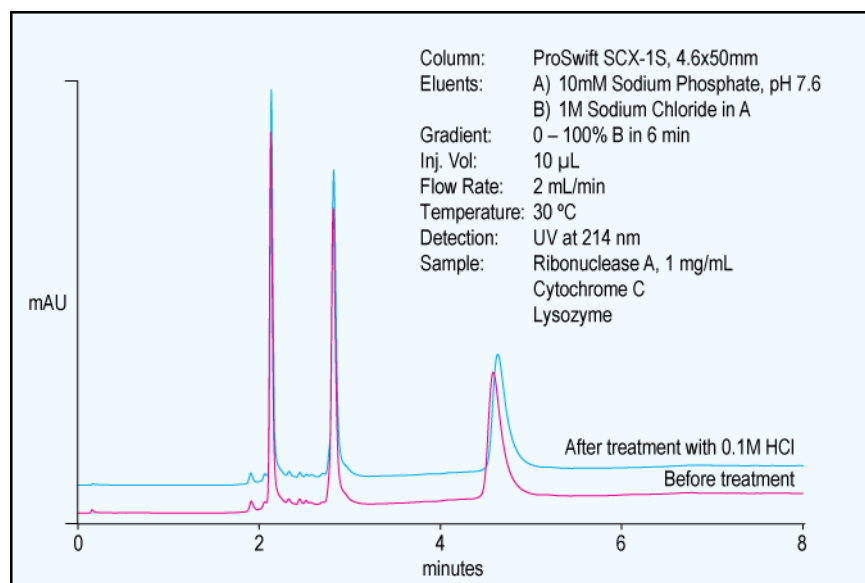


Figure 83
Effect of Transient (1 Hour) Hydrochloric Acid Washing
on the Performance of the ProSwift SCX-1S

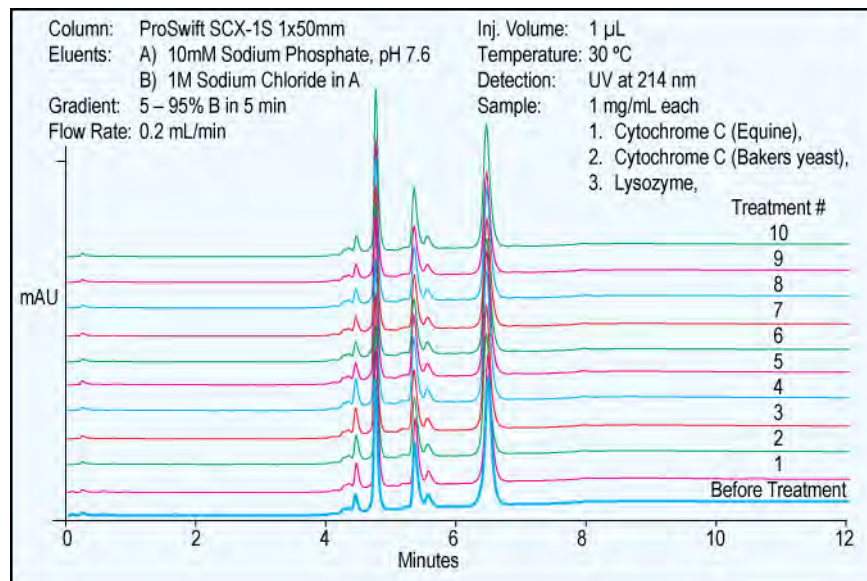


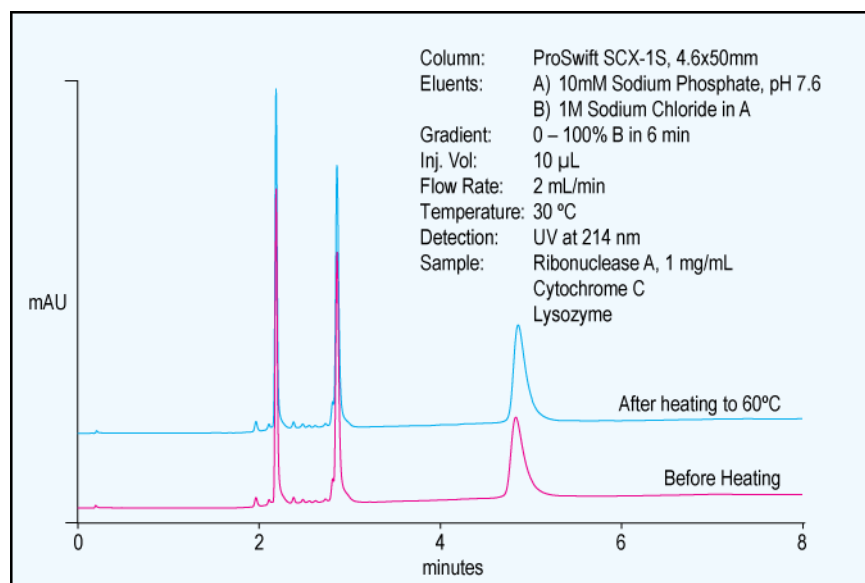
Figure 84
Effect of Multiple Transient (1 Hour) Hydrochloric Acid Washing
on the Performance of the ProSwift SCX-1S

5.6. Temperature Stability

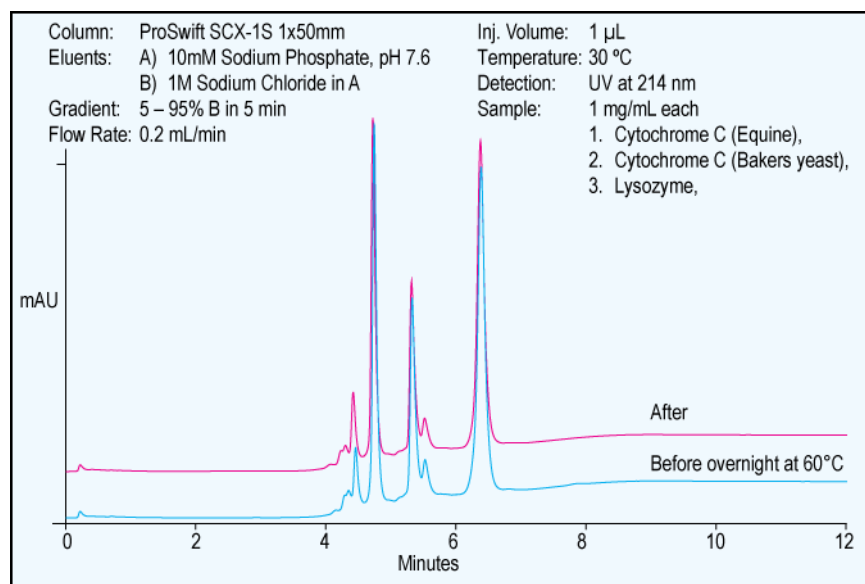

WARNING

*Caution should be taken when using elevated temperatures and elevated pH.
See Section 1.10 for additional information regarding temperature and pH limits.*

Cation exchange phases produced using methacrylate and acrylate polymers show temperature stability similar to that of the ProSwift WAX-1S column. Figure 85 and Figure 91 shows a comparison before and after extended exposure at 60 °C at pH 7.6 on the ProSwift SCX-1S.


Figure 85

Protein Separation on the ProSwift SCX-1S Before and After 24 Hours of Use at 60 °C


Figure 86

Protein Separation on the ProSwift SCX-1S Before and After overnight of Use at 60 °C

5.7. Applications

5.7.1. Hemoglobin

Clinical laboratories routinely separate and quantify the levels of hemoglobin variants in blood samples. These include glycosylated hemoglobins as well as sequence variants. ProSwift SCX-1S column is useful for such analyses. Separation of Hemoglobin A2 control variants by ProSwift SCX-1S column is shown in Figure 87 (4.6 x 50 mm) and in Figure 88 (1.0 x 50 mm). It should be noted that the ProSwift SCX-1S 1x50 mm column is more hydrophilic than the 4.6x50 mm column therefore requires a lower ionic strength (10mM) sodium phosphate concentration. Figure 89 shows the separation of different human hemoglobins such as fetal, adult, sickle cell, abnormal beta chain mutant proteins and Figure 90 shows the difference between human, bovine and porcine hemoglobins.

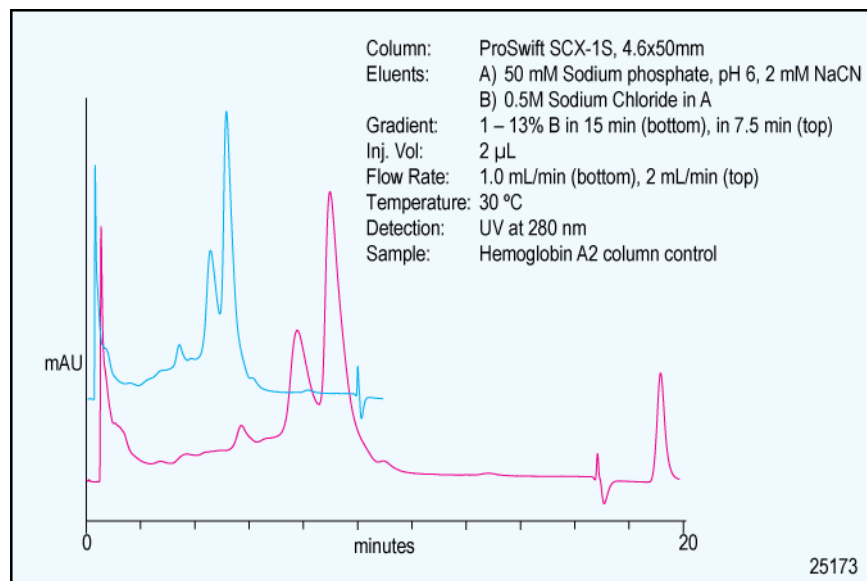


Figure 87
Separation of Hemoglobin on ProSwift SCX-1S (4.6x50 mm)

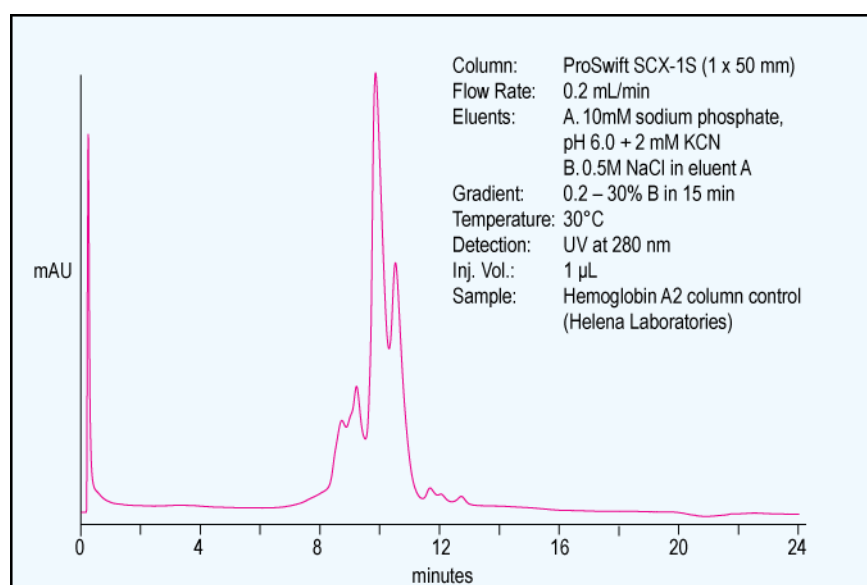


Figure 88
Analysis of Hemoglobin A2 column control on ProSwift SCX-1S (1x50 mm)

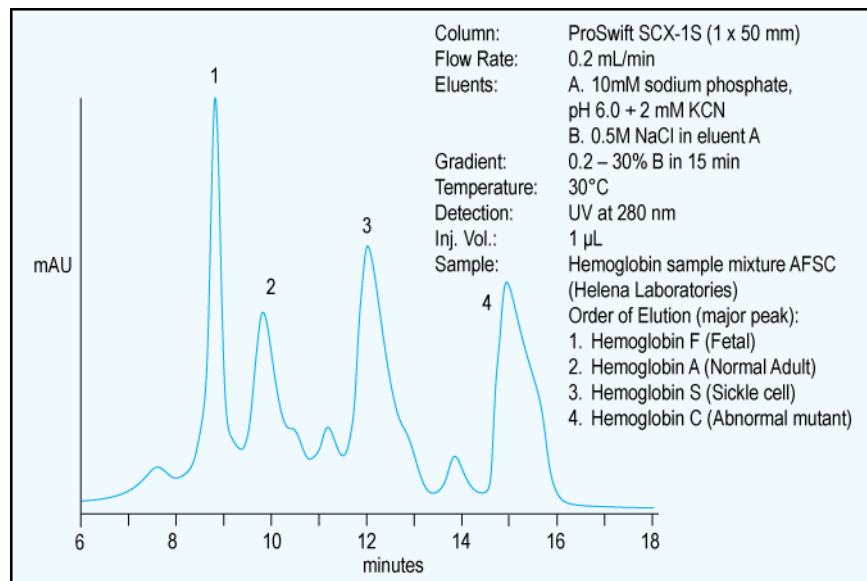


Figure 89
Separation of Hemoglobins AFSC on ProSwift SCX-1S (1x50 mm)

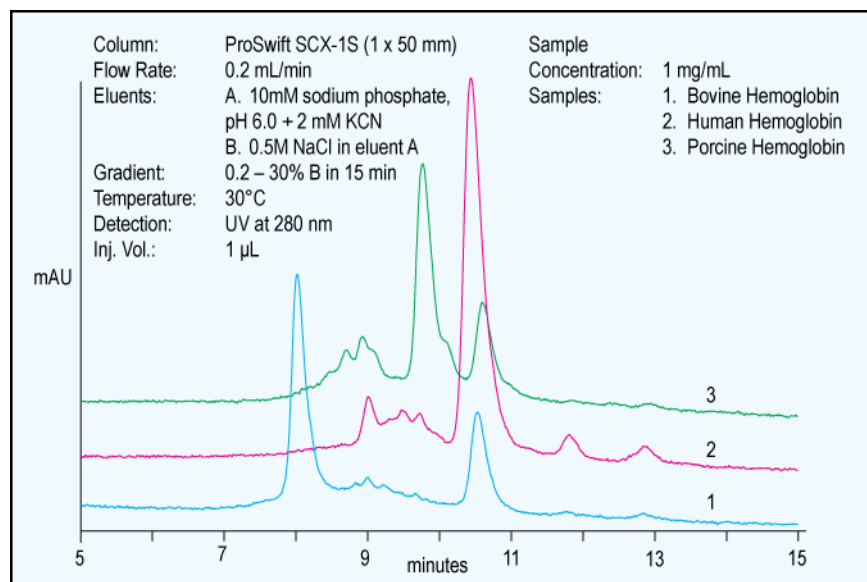


Figure 90
Separation of Hemoglobin on ProSwift SCX-1S (1x50 mm)

5.7.2. Separation Snake Venom proteins

The separation of snake venom peptides and proteins by the ProSwift SCX-1S is shown below. Venoms from Naja (Figure 91 and Figure 92) and Viper (Figure 93 and Figure 94) snakes can be compared. This application was shown using both 4.6 x 50 mm and 1 x 50 mm columns. Snake venoms are composed of many hydrolytic enzymes, a complex mixture of proteases, peptidases, and nucleases. Some of these components contribute to the toxicity of the venom. These can be separated and purified to develop anti-venom therapies.

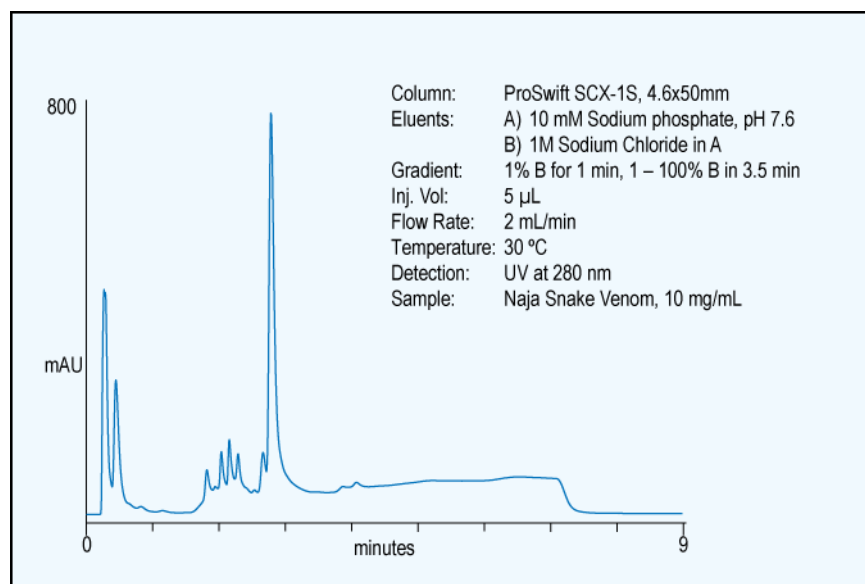


Figure 91
Separation of Naja Naja Venom on ProSwift SCX-1S (4.6x50mm)

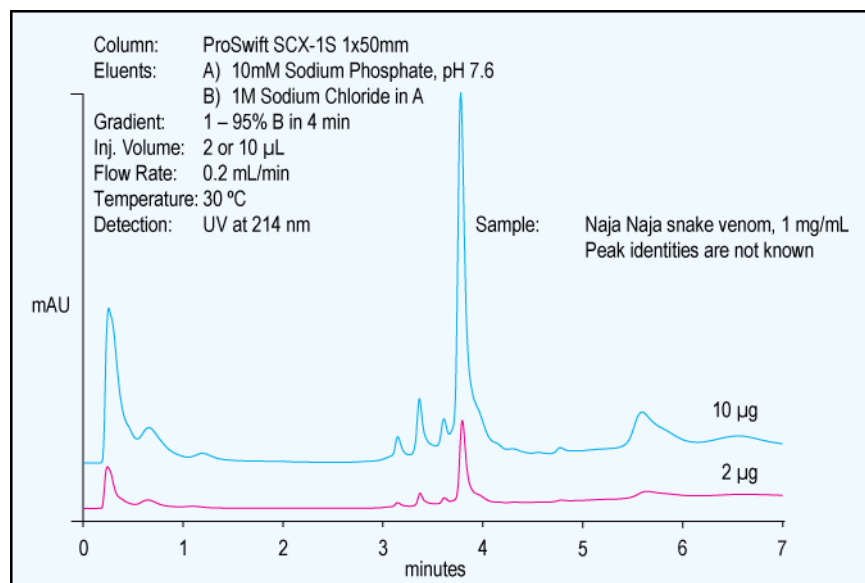


Figure 92
Separation of Naja Naja Venom on ProSwift SCX-1S (1x50mm)

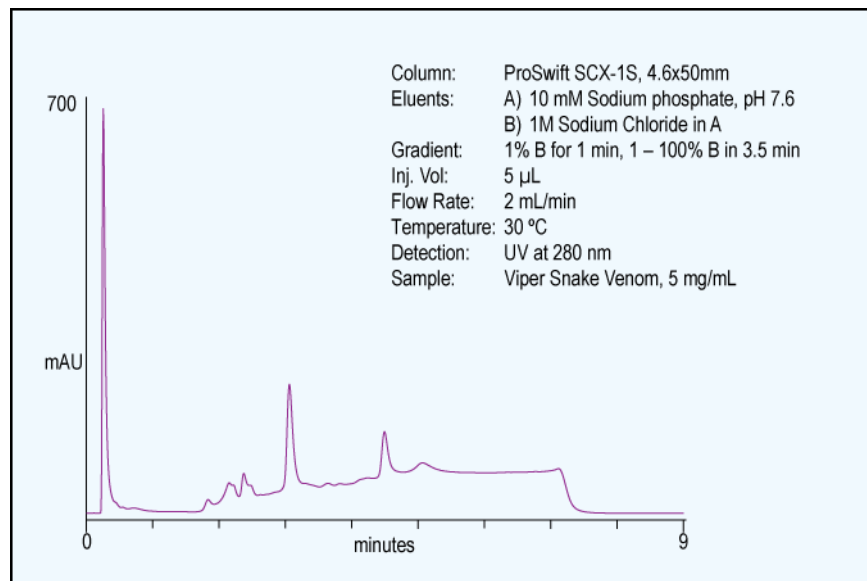


Figure 93
Separation of Viper Venom on ProSwift SCX-1S (4.6x50 mm)

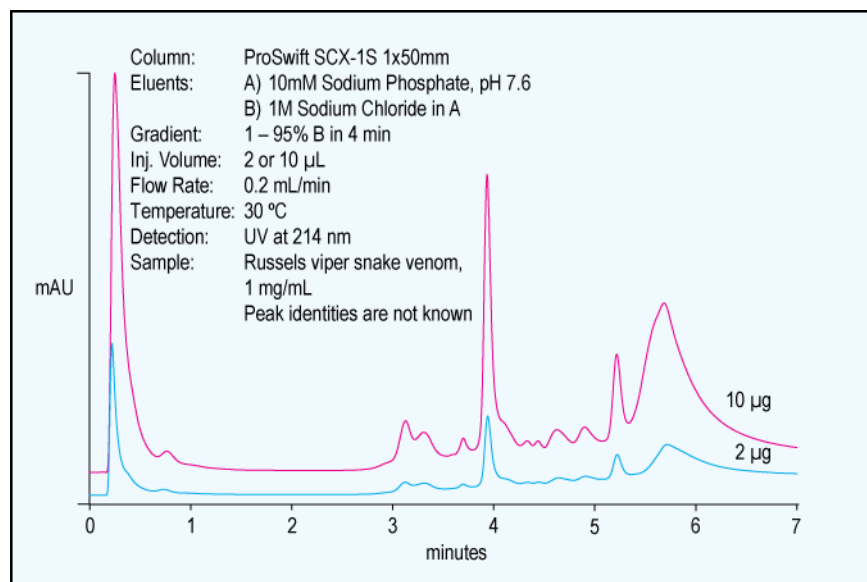



Figure 94
Separation of Viper Venom on ProSwift SCX-1S (1x50 mm)

**6. SPECIFICATIONS****Table 1**


Parameter	ProSwift WAX-1S		ProSwift WCX-1S		ProSwift SAX-1S		ProSwift SCX-1S	
<i>Column dimension</i>	4.6x50 mm	1x50 mm	4.6x50 mm	1x50 mm	4.6x50 mm	1x50 mm	4.6x50 mm	1x50 mm
<i>Base Matrix Material</i>	Polymethacrylate							
<i>Surface chemistry</i>	Tertiary amine		Carboxylic acid		Quaternary amine		Sulfonic acid	
<i>Protein binding capacity per mL of polymer</i>	18 mg/mL BSA	18 mg/mL BSA	23 mg/mL Lysozyme	23 mg/mL Lysozyme	18 mg/mL BSA	18 mg/mL BSA	13 mg/mL Lysozyme	13 mg/mL Lysozyme
<i>Protein binding capacity per column</i>	13 mg BSA	0.7 mg BSA	16 mg Lysozyme	0.9 mg Lysozyme	13 mg BSA	0.7 mg BSA	9.1 mg Lysozyme	0.5 mg Lysozyme
<i>Bed Height</i>	44 mm	50 mm	42 mm	50 mm	45 mm	50 mm	42 mm	50 mm
<i>Bed volume</i>	0.73 mL	0.039 mL	0.70 mL	0.039 mL	0.75 mL	0.039 mL	0.70 mL	0.039 mL
<i>pH Range</i>	2 - 12							
<i>Recommended Flow rate</i>	0.5 – 1.5 mL/min	0.05 – 0.25 mL/min	0.5 – 1.5 mL/min	0.05 – 0.20 mL/min	0.5 – 1.5 mL/min	0.05 – 0.25 mL/min	0.5 – 1.5 mL/min	0.05 – 0.25 mL/min
<i>Maximum Flow rate</i>	2 mL/min	0.30 mL/min	2 mL/min	0.25 mL/min	2 mL/min	0.3 mL/min	2 mL/min	0.3 mL/min
<i>Operating pressure</i>	< 500 psi 3.4 MPa	< 1500 psi 10.4 MPa	< 500 psi 3.4 MPa	< 1000 psi 6.9 Mpa	< 500 psi 3.4 MPa	< 1500 psi 10.4 MPa	< 500 psi 3.4 MPa	< 1500 psi 10.4 MPa
<i>Maximum Pressure</i>	1000 psi 6.9 MPa	2000 psi 13.8 MPa	1000 psi 6.9 Mpa	2000 psi 13.8 Mpa	1000 psi 6.9 MPa	2000 psi 13.8 MPa	1000 psi 6.9 Mpa	2000 psi 13.8 MPa
<i>Temperature</i>	60 °C				70 °C		60 °C	
<i>Solvent Compatibility</i>	Most common organic solvents							

7. INSTALLATION

7.1. System Requirements

 WARNING	<p><i>ProSwift ion exchange monoliths are prepared in PEEK bodies with polymeric frits to minimize unnecessary exposure to metal ions that negatively impact their chromatographic performance. A major source of decreasing column performance is metal leaching from stainless steel HPLC systems. The use of halide salt mobile phases always causes corrosion of metal components leading to denaturation of protein samples and column fouling. We strongly recommend the use of metal-free systems and components, including pump heads, tubing, frits and in-line filters.</i></p>
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For 4.6 mm format: ProSwift monoliths are designed to operate with standard HPLC systems that include inert gradient pumps, flow paths, and injection valve materials.

 CAUTION	<p><i>There may be an increase in back pressure when using smaller internal diameter tubing. The upstream back pressure generated after the column outlet (including the connecting tubing between the column outlet and detector cell inlet, the cell, and cell waste line) MUST not exceed the maximum allow pressure limits. Excessive back pressure after the column may cause irreversible damage to the column. See Section 1, Table 1 for pressure limits.</i></p>
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For 1 mm format: The ProSwift 1mm formats will deliver the best results when all connecting tubing between the pump and the detector is replaced with 0.003" ID 1/16 inch OD tubing (P/N 049715). This may increase the system pressure. Record the system pressure at intended flow with the column removed (do not connect the post column components for this measurement). This pressure is separate from that experienced by the column. When evaluating the back pressure on the column, this pressure value should be subtracted from the total pressure when all components are installed.

The injection loop size may affect the delay time (a 100µL injection loop will introduce a 1 minute delay when operating at 100µL/min).

Eluents high in salts may evaporate and clog the narrow bore (≤ 0.005 ") tubing when the chromatograph is idle. This may produce very high pressure values when the system is restarted. Take the precaution of clearing all eluent lines before restarting the system with the column installed.

A further alteration worth considering is the use of a microbore flow cell. Typically standard flow cells enclose illuminated volumes of 9-11 µL. Peaks from 1 mm column operating at 100-150 µL/min may elute in that volume, or even a little less. Hence, the peak dispersion in a standard flow cell may broaden and otherwise deform a peak eluting from a 1 mm column.

7.2. System Void Volume

For 4.6 mm format monoliths, the tubing between the injection valve and detector is recommended to be ≤ 0.010 " ID PEEK tubing (P/N 0426910). For 1 mm monoliths, this tubing should be replaced with 0.003" ID tubing (P/N 049715). Minimize the length of all liquid lines, especially the tubing between the column and the detector. The use of larger diameter and/or longer tubing may decrease peak efficiency and peak resolution.

Use of 0.003" ID tubing for 1mm columns at 100-200µL/min will generate different delay times than the same pump components with 0.010" ID tubing at 1-2 mL/min. Due to the pump and proportioning volumes, delay time with 0.003" ID tubing will be longer than that with 0.010" ID tubing at higher flow rates. Where normal-bore applications employ a 1-2 minute isocratic section before initiation of gradients, use of the same delay in 1mm column applications will cause the component to elute significantly later. Hence, we recommend elimination of the isocratic steps at the beginning of 1mm monolith methods. Similarly, regeneration and equilibration times after sample analyses and column wash steps will be longer. We recommend that users increase their re-equilibration times by 10-15 minutes for these applications.

7.3. Flow Rate Equivalency

When using a 1 mm monolithic column compared to a 4.6mm monolith a flow of 100µL/min is equivalent to 2.15 mL/min on 4.6 mm monolith. See maximum allowed flow rate and pressure specification in Section 1, exceeding these maximum allowed parameters may cause irreversible damage to the monolith.

7.4. Gradients

When changing the operational flow rate in a method remember to modify the gradient times if the intention of the change is to maintain the separation performance. Thus, gradient times are proportional to the flow rate.


7.5. Operational Parameters

Table 2

pH Range:	See Specification Chart in Section 1.10
Temperature Limit:	See Specification Chart in Section 1.10
Pressure Limit:	See Specification Chart in Section 1.10
Organic Solvent Limit:	100% acetonitrile if required for cleaning.
Detergent Compatibility:	For all Ion Exchange columns, Nonionic detergents are recommended. Anionic detergents are suitable for cation exchange monoliths. Cationic detergents are suitable for anion exchange monoliths.
Typical Buffer and Salts:	Tris, AMP, ammonium, sodium and potassium salts of chloride, and perchlorate, MES, or acetate.

7.6. Eluent Limitations

The ProSwift anion exchange columns are compatible with typical eluents such as sodium or potassium chloride or sulfate salts in Tris, phosphate or acetate buffers, up to their limit of solubility. Use of organic solvents in the eluent may be helpful for very hydrophobic analytes, but is typically unnecessary. If you employ solvents, test their solubility with the salt-containing eluents prior to use. Some combinations of eluent salts and organic solvents are not miscible.

 CAUTION	<p><i>For all Ion Exchange columns, NONIONIC detergents are recommended.</i></p> <p><i>Do not use anionic surfactants, which will irreversibly bind to anion exchange monoliths.</i></p> <p><i>Do not use cationic surfactants, which will irreversibly bind to cation exchange monoliths.</i></p>
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7.7. Chemical Purity Requirements

Obtaining sensitive, consistent, and accurate results requires eluents that are free of impurities. Chemicals, solvents and deionized water used to prepare eluents must be the highest purity available. Low trace impurities and low particle levels in eluents will extend the life of your ion exchange columns and system components. Dionex cannot guarantee proper column performance when the quality of the chemicals, solvents, and water used to prepare eluents is substandard.

7.7.1. Inorganic Chemicals

Always use reagent grade or better inorganic chemicals to prepare eluents. Whenever possible, use inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity. These chemicals will detail the purity with an actual lot analysis on each label.

When using solvents, HPLC Grade products or equivalent should be used to prepare eluents.

7.7.2. Buffer Components

Many chemicals may be used as pH buffering components in eluents. For protein and peptide separations, some will serve better than others, when detection is considered. Most organic buffers (e.g., Tris, Acetate, etc.) will contribute to significant baseline drift at detection wavelengths below 225 nm. Alternatively, phosphate, which buffers at pH values between 6 and 7.6, will contribute very little to baseline disturbances during low wavelength detection.

The buffering components used may affect long-term changes in pressure during chromatography. Buffers that bind to the stationary phase (anions for anion-exchange, and cations for cation-exchange chromatography) will buffer the stationary phase quickly, producing more stable pressures. Buffers that do not bind to the stationary phase (cations for anion-exchange, and anions for cation-exchange chromatography) will not efficiently buffer the stationary phase. Thus, stationary phase ionization will occur very slowly, and may result in pressure changes over time. In addition, since these buffers do not effectively buffer the stationary phase, their use may also alter the apparent selectivity of ion-exchange columns. This can be an advantage as different buffers may produce slightly different selectivity for certain proteins.

7.7.3. Deionized Water

Deionized water used to prepare eluents should be Type I Reagent Grade Water with a specific resistance of ≥ 18 megohm-cm. Ensure that it is free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 μm . Bottled HPLC-Grade Water from Burdick & Jackson is acceptable, but most other bottled water contains an unacceptable level of ionic impurities.

7.8. Eluent Preparation

7.8.1. Adjusting the pH of the Eluent

Add all eluent electrolytes to eluent solutions before adjusting the pH. To make sure that the pH reading is correct, calibrate the pH meter prior to use. Stir the solutions during adjustment and employ temperature correction. Take care to ensure the accuracy of the pH electrode when using Tris buffers because some electrodes give erroneous results with Tris.

7.8.2. Filtering the Eluent

To extend the lifetime of your column as well as your HPLC pump, filter all eluent buffers using a 0.2 μm membrane filter to remove insoluble contaminants from the eluents.

7.8.3. Degassing the Eluent

Before use, degas the eluents. Degassing can be done either by using Dionex pump degas functions as described in the pump manuals or by placing the eluent reservoir in a sonic bath and drawing vacuum on the filled reservoir with a vacuum pump for 5 minutes while operating the sonic bath.

7.8.4. 10 mM Tris Buffer Preparation



NOTE

All eluents should be filtered through a 0.2 μm pore filter prior to use.

7.8.4.1. 10mM Tris Buffer (pH 7.6) Preparation (Eluent A):

1. Use a clean and dry 1 liter bottle (eluent/mobile phase bottle) to prepare this solution,
2. Using a plastic weigh boat, weigh 1.21 ± 0.01 grams of tris base,
3. Transfer the tris base into the 1 liter bottle,
4. Add 998.79 ± 0.05 grams with deionized water,
5. Add a stirring bar and stir until the tris base is completely dissolved,
6. Using 2M HCl and a standardized [calibrated] pH meter, adjust the pH of the solution to $\text{pH } 7.60 \pm 0.02$,
7. Cap and label the bottle.

7.8.4.2. 1M Sodium Chloride (NaCl) in 10mM Tris Buffer Preparation (Eluent B)

1. Use a clean and dry 1 liter bottle (eluent/mobile phase bottle) to prepare this solution,
2. Use a large capacity balance (≥ 4 Kg), tare the balance, weigh the 1 liter bottle and record the weight,
3. Using a plastic weigh boat, weigh 58.44 ± 0.02 grams of NaCl,
4. Transfer the NaCl into the 1 liter bottle,
5. Add approximately 0.5 liters of 10mM Tris Buffer, pH 7.6 to the 1 liter bottle,
6. Cap and swirl the bottle by-hand until the NaCl salt is dissolved,
7. Place the 1 liter bottle with the dissolved NaCl on the balance and add 10 mM Tris Buffer, pH 7.6 to a final weight of 1031.33 ± 0.02 grams,
8. Add a stirring bar to the 1 liter bottle,
9. Cap and gently stir the solution for 5 minutes,
10. Label the bottle.

7.9. 10 mM Sodium Phosphate Buffer Preparation

7.9.1. 10 mM Sodium Phosphate, dibasic (Na₂HPO₄) Preparation

1. To prepare 2 liters, weigh out 2.89 ± 0.02 grams **sodium phosphate, dibasic, anhydrous** into a 3-4 liter flask, a flask with a volume larger than 2 liter is needed to complete a forthcoming step in the process.
2. Add 1990.44 ± 0.02 grams of water,
3. Stir to dissolve,
4. Cap and label the flask.

7.9.2. 10 mM Sodium Phosphate, monobasic (NaH₂PO₄) Preparation

1. To prepare 1 liter, weigh out 1.38 ± 0.02 grams **sodium phosphate, monobasic, monohydrate** into a 1 liter flask,
2. Add 993.88 ± 0.02 grams of water,
3. Stir to dissolve,
4. Cap and label the flask.

**NOTE**

Adjusting pH of Phosphate Buffers:

- Lower pH – Add sodium phosphate monobasic solution
- Raise pH – Add sodium phosphate dibasic solution

7.9.3. 10mM Sodium Phosphate Buffer, pH 7.6 Preparation (Eluent A)

1. To the 2 liters of **10 mM sodium phosphate, dibasic solution**, add ~500 grams of the **sodium phosphate, monobasic solution**,
2. Using a standardized [calibrated] pH meter, add sufficient **sodium phosphate, monobasic buffer** to adjust the pH to 7.60 ± 0.05 ,
3. Cap and label the bottle.

7.9.4. 1M Sodium Chloride (NaCl) 10 mM Sodium Phosphate Buffer, pH 7.6 Preparation (Eluent B)

1. To prepare 1 liter, weigh out 58.44 ± 0.02 grams of **sodium chloride** into a 1 liter flask,
2. Add 1037.60 ± 0.02 grams of **10mM Sodium Phosphate Buffer, pH 7.6**,
3. Stir to dissolve the sodium chloride,
4. Cap and label the bottle.

8. OPERATION

8.1. QuickStart for ProSwift SAX-1S, WAX-1S, SCX-1S, and WCX-1S

ProSwift Monolith columns offer superior separation of large biomolecules. Conditioning of the column bed is **required** prior to initial use and after long-term storage. The QuickStart process will ensure extended column lifetime and reproducibility.

I. Preparation:


a. Eluent Preparation

The following eluents are recommended, but the column may be used with any eluents suitable for analysis. Typically Eluent A is a low salt buffer and Eluent B is a high salt buffer.

<u>Eluent</u>	<u>Anion Exchange</u>	<u>Cation Exchange</u>
A	0.01 M Tris•HCl buffer pH 7.6	0.01M Na ₂ PO ₄ buffer, pH 7.6
B	1 M NaCl in 0.01 M Tris •HCl buffer pH 7.6	1 M NaCl in 0.01 M Na ₂ PO ₄ buffer, pH 7.6

b. Column Installation

Install the column on the instrument in the correct flow direction.

 WARNING	<p><i>Sudden increases in flow rates may damage monolithic columns. Always increase the flow rate slowly using a linear flow gradient or stepwise increments in flow rate.</i></p> <p><i>If the eluent composition generates back pressure in excess of the maximum pressure, reduce the flow rate to ensure the upstream back pressure is less than the maximum pressure.</i></p> <p><i>The maximum pressure limit for 4.6mm is 1000 psi (6.9 MPa) and for 1mm is 2000 psi (13.8 MPa)</i></p>
---	--

II. Flow Rate Start-Up (Ramping)

Using a linear or stepwise flow gradient, increase the flow rate of Eluent A starting from 0.00 mL/min to the desired flow rate using the flow rates given below.

- 4.6 mm Column: Use a rate of ≤0.50 mL/min, every 30 seconds
- 1mm Column: Use a rate of ≤0.10 mL/min every minute.

III. Column Conditioning - Use the guidelines below to determine the proper startup conditions::

4.6 mm:

Removal of Storage Solution:

- Using the desired flow rate, run a 8 mL (~10 column volume) binary gradient from 100% A to 100% B.
- Pump another 8 mL (~10 column volumes) of 100% B through the column.

Column Equilibration:

- Equilibration from 100% B to the starting eluent composition should include at least a 1 minute reverse gradient to the initial conditions.
- Pump at least 8 mL (~10 column volumes) of this eluent composition through the column.

1 mm:

Removal of Storage Solution:

- Using the desired flow rate, run a 0.4 mL (~10 column volume) binary gradient from 100% A to 100% B.
- Pump another 0.4 mL (~10 column volumes) 100% B through the column.

Column Equilibration:

- Equilibration from 100% B to the starting eluent composition should include at least a 1 minute reverse gradient to the initial conditions.
- Pump at least 0.4 mL (~10 column volumes) of this eluent composition through the column.

IV. Storage:

- For short-term storage, <3 days, store the column in the initial buffer compositions.
- For long-term storage, >3 days, use the following solution to avoid microbial growth on the column.

Anion Exchange

0.05 M NaCl in 0.01 M Tris•HCl, pH 7.6 + 0.1% NaN₃

Cation Exchange

0.05 M NaCl in 0.01 M Na₂PO₄, pH 7.6 + 0.1% NaN₃

- In all cases the column should be tightly sealed with end plugs to prevent the column from drying out.

8.2. Increase Flow Rate

Establishing flow rate:

4.6 mm: Slowly increase the flow rate from less than 0.5 mL/min to the desired level (up to 2 mL/min in test chromatogram) over 1-2 minutes.

1mm: Slowly increase the flow rate from less than 0.01 mL/min the desired flow rate over 1-2 minutes.



CAUTION

Sudden extreme increases in flow rates may damage the column. To prolong column life, avoid immediate and dramatic increases in the flow rate.

8.3. Sample Preparation

For best results, dissolve the samples in the initial run buffer or in pure deionized water. The salt concentration should be determined so the column is not overloaded by the injected sample.

Generally, for protein analysis loading of no more than 1 mg protein per mL of column volume is recommended for the ProSwift monolith however, more can be loaded for purification purposes.

For oligonucleotide analyses, Dionex recommends loading no more than 120 µg per mL of column volume, although more can be loaded for oligonucleotide purification (Section 4.1.3.5).

If the sample may contain particulates, filter it through a 0.2 µm syringe filter before loading it into the injection valve.

8.4. Column Equilibration

Before performing a run after storage, wash and equilibrate the column using protocols described in QuickStart (Section 3.1). When switching to a different buffer type, use an eluent volume of 20 times the column volume (approximately 15 mL for 4.6x50 mm and approximately 1mL for 1x50 mm) to ensure the monolith is well equilibrated.

Commonly in chromatography employing gradients front end peak reproducibility can be improved by using adequate column equilibration time. At the end of the gradient the column contains high levels of eluting salt. Prior to injection at least 10 column volumes of starting eluent composition should be passed over the column to equilibrate the stationary phase. This will ensure the stationary phase has the same composition for every injection. See Figure 95.

If the operating flow rate is below that of the max operational flow rate, equilibration time may be decreased by increasing the flow rate during equilibration.

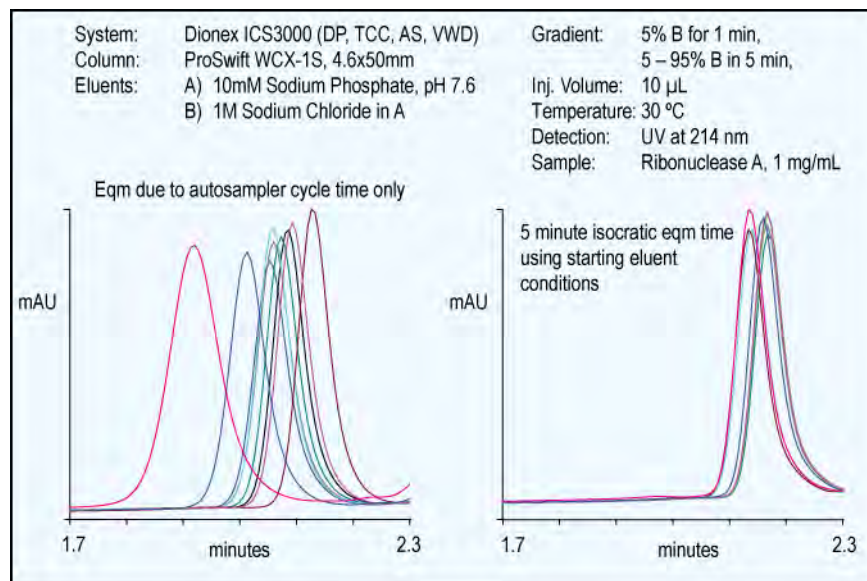


Figure 95
Effect of equilibration time on retention time reproducibility

**9. TROUBLESHOOTING**

The purpose of the Troubleshooting Guide is to help solve operating problems that may arise while using ProSwift columns. If you cannot solve the problem on your own, contact the Dionex North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390).

Table 3
ProSwift Ion Exchange Column Troubleshooting Summary

Observation	Cause	Action	Reference Section
High back pressure	Blocked component	Isolate and clear blocked component.	9.1
	Plugged column bed supports (frit)	Clean, regenerate or replace column, reverse column flow to flush out particles trapped at inlet frit or replace frit. (1mm Frit, Package of 5, Product No. 067083).	
	Other system modules	Disconnect, and replace	System module manual
High background noise	Bad eluents	Remake eluents	7.8
	Contaminated column	Clean column	9.2.2
	Contaminated hardware	Clean component	9.2.3
	Pump lost prime, air bubbles in line Not enough backpressure at cell outlet when going from larger ID to narrow ID column	Re-prime pump, ensure there are no air bubbles. Degas buffers, Add backpressure tubing to cell outlet	
Poor resolution	Poor efficiency due to large system void volumes	Re-plumb system	9.3
	Column headspace	Reverse column orientation.	
	Contamination of column or frit	Clean column, reverse column flow to flush out particles trapped at inlet frit.	
	Inadequate Gradient due to system dead volumes when extrapolating gradients from different column ID	Minimize dead volumes, modify gradient times.	8.4
Short retention times	Un-equilibrated system	Lengthen first eluent time before inject	8.4
	Flow rate too fast	Recalibrate pump or reduce flow	System module manual
	Bad eluents	Remake eluents	7.8
	Column contamination	Clean column	9.2.2
Poor front end resolution	Bad eluents	Remake eluents	7.8
	Column overloading	Reduce sample size or concentration	8.3
	Insufficient column equilibration	Increase pre-equilibration time	8.4
	Sluggish injection valve	Service valve	System module manual
	Large system void volumes	Re-plumb system	7.1, 7.2
Spurious peaks	Sample contamination	Pre-treat samples	8.3, 9.4
	Sluggish injection valve	Service valve	System module manual
	Contaminated eluents	Test contamination, and remake eluents	7.8

9.1. High Back Pressure

9.1.1. Finding the Source of High System Pressure

If the system pressure is very high, it is advisable to find out what is causing the high system pressure.

The system should be used with a high pressure in-line filter eluent filter. The filter should be positioned between the pump and the injection valve. Make sure you have an inert high pressure in-line filter in place and that it is not contaminated.

- A. Make sure that the pump is set to the correct eluent flow rate. Higher than recommended eluent flow rates will cause higher pressure. Measure the actual pump flow at various flow rates, if necessary by collecting the flow of deionized water into a pre-weighed graduated cylinder. Calculate the flow rate based on the collected volume of deionized water.
- B. Find out what part of the system is causing the high pressure. It could be a piece of tubing that has plugged or whose walls have collapsed, an injection valve with a plugged port, a column with particulates plugging the bed support (frit), a plugged high pressure in-line filter, or the detector cell.

To find out which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure. It should not exceed 50 psi (0.34 MPa). Continue adding the system components (injection valve, column, and detector) one by one, while watching the system pressure. The pressure should increase up to a maximum of 1000 psi (6.9 MPa) at a flow rate of 1.0 mL/min when the 4.6 mm ProSwift column is connected. No other components should add more than 100 psi (0.69 MPa) of pressure. Refer to the appropriate manual for cleanup or replacement of the problem component.

In the case of 1 mm columns, the plumbing should be done with 0.003" ID PEEK tubing. In this case, the system backpressure should be similarly tested as explained above, but using a flow rate of 0.1 mL/min. Total system pressure, from the pump to the cell outlet tubing but not including the column, should be between 300 – 500 psi at this flow rate.

9.1.2. Clogged Column Bed Support (Frit) Assemblies

If the column inlet frit or the media is determined to be the cause of the high back pressure, clean the column in the reversed direction, or regenerate the columns using the methods described in "APPENDIX B - COLUMN CARE". Frits used in ProSwift columns are not serviceable. If the column still shows high pressure, replace the column.

9.2. High Background or Noise

9.2.1. Contamination of Eluents

- A. Make sure that all eluents are made correctly, and from chemicals with the recommended purity.
- B. Make sure that the deionized water used to prepare the reagents has a specific resistance of 18.2 megohm-cm.
- C.

9.2.2. Contaminated Column

Remove the ProSwift column from the system. If the background noise decreases, then the column itself is the cause of the high background. Clean the column as instructed in Appendix B - Column Care.

9.2.3. Contaminated Hardware

To eliminate the hardware as the source of the high background signal, bypass the column and pump deionized water with a specific resistance of 18.2 megohm-cm through the system. The background signal should be less than 0.1 mA₂₆₀. If it is not, check the detector cell by injecting deionized water directly into it. See the appropriate manual for further details.

9.3. Poor Peak Resolution

Poor peak resolution can be due any or all of the following factors.

**9.3.1. Loss of Column Efficiency**

- A. Ensure that system void volumes have been minimized. Extra-column volumes can cause sample band dispersion and will decrease peak efficiencies. Make sure you are using the appropriate tubing (see Section 7.1) to make all eluent liquid line connections between the injection valve and the detector cell inlet on the system. Make all tubing lengths as short as possible. Check for leaks.
- B. Contamination of media or frit due to binding of sample or eluent components. This can be responsible for the loss of column efficiency. Please refer to Appendix B.2.2- Column cleanup procedure.
- C. For 1 mm monolith applications ensure that the flow cell is not introducing dispersion (cell volume should be $\leq 2.5 \mu\text{L}$).

9.3.2. Poor Resolution Due to Shortened Retention Times

Even with adequate system and column efficiency, resolution of peaks will be compromised if the analytes elute too early.

- A. Check the eluent flow rate. If it is different from the flow rate specified by the analytical protocol, recalibrate the pump. Measure the eluent flow rate as described in Section 5.1.1.A.
- B. Check to ensure that the eluent compositions and concentrations are correct. For isocratic analysis, an eluent that is too strong will cause the peaks to elute too early. Prepare fresh eluent. If you are using a gradient pump to proportion the final eluent from concentrated eluents in two or three different eluent reservoirs, the composition of the final eluent may not be accurate enough for the application. Use one reservoir containing the correct eluent composition to see if this is the problem. This may be a problem when one of the proportioned eluents is less than 5%.
For gradient analysis, remake the eluents or adjust the times in the gradient program to obtain the required peak resolutions.
- C. Column contamination can lead to a loss of column capacity because fewer of the binding sites will be available for the sample compounds. Polymers or metal ions might be concentrating on the column. Refer to Appendix B.2.2 - Column Cleanup Procedure, for column cleanup recommendations.

Possible sources of column contamination are impurities in chemicals or components in the sample matrix. Be especially careful to make sure that the recommended chemicals are used. The deionized water should have a specific resistance of at least 18.2 megohm-cm, and the solvents should be of HPLC-grade.

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column. If you need assistance in solving resolution problems, contact the nearest Dionex Office.

9.3.3. Loss of Front End Resolution

If poor resolution and efficiency is observed for very early eluting peaks (near the system void volume) compared to the later eluting peaks, check the following:

- A. Improper eluent concentration may be the problem. Remake the eluent as required for your application. Ensure that the water and chemicals used are of the required purity.
- B. Column overloading may be the problem. Reduce the amount of sample injected onto the column by either diluting the sample or injecting a smaller volume onto the column.
- C. The column may not be equilibrated with the first eluent. Increase the amount of time the first eluent runs through the columns before injection.
- D. Sluggish operation of the injection valve may be the problem. Check the valve operation to make sure there are no leaks or partially plugged port faces. Refer to the valve manual for instructions.
- E. Improperly swept out volumes anywhere in the system prior to the columns may be the problem. Swap components (one at a time) in the system prior to the analytical column and test for front-end resolution after every system change.



9.4. Spurious Peaks

- A. The column may be contaminated. If the samples contain an appreciable level of ionic components and the column is used with a weak eluent system, these components may remain on the analytical column. The retention times for the analytes in subsequent injections will then decrease, and spurious, inefficient (broad) peaks can show up at unexpected times. Clean the column as indicated in “APPENDIX B - COLUMN CARE, Column Cleanup.”
- B. The injection valve may be creating a baseline disturbance. This baseline upset can show up as a peak of varying size and shape. It will happen when the injection valve needs to be cleaned or torque reapplied (see valve manual). Check to see there are no restrictions in the tubing connected to the valve. Also, check the valve port faces for blockage and replace them if necessary. Refer to the Valve Manual for troubleshooting and service procedures. Small baseline disturbances at the beginning or at the end of the chromatogram can be overlooked as long as they do not interfere with the quantification of the peaks of interest. If cleaning and reapplication of the torque to the valve does not help, replace the valve.
- C. The eluent may be contaminated. When performing gradient chromatography, contaminants in the eluent may accumulate on the column until the eluent strength is sufficient to elute them. Increasing the equilibration time at low eluent strength will result in more pronounced contaminant peaks. If this is observed, make the eluents again. If the problem persists, prepare the eluents from higher purity chemicals.
- D. If you need assistance in determining the best way to clean strongly retained solutes in your specific sample matrix from the ProSwift WAX-1S columns, please contact Dionex Technical help at 1-800-Dionex-0 (1-800-346-6390).

9.5. Small Peak Areas

Small peak areas can occur when the injection valve is incorrectly installed or controlled. Plumb the injection valve's sample loop so that it is inserted into the flow path when the inject command is issued.

**10. APPENDIX A - QUALITY ASSURANCE REPORTS****10.1. ProSwift SAX-1S (4.6x50 mm)**

ProSwift® SAX-1S

Date: 26-Feb-07 14:21

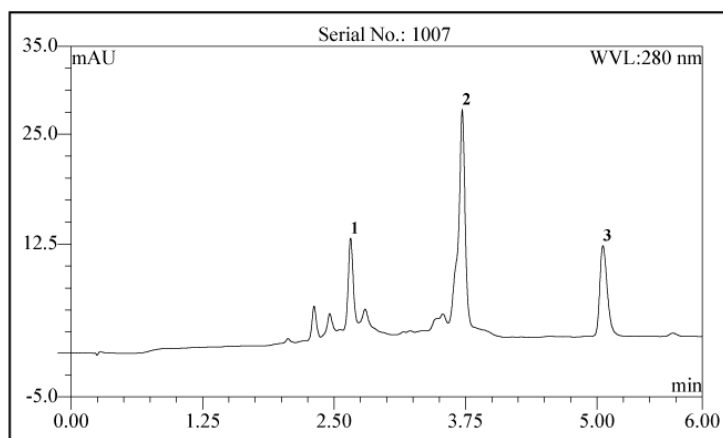
4.6 x 50 mm

Serial No. : 001007

Product No. 064293

Lot No. : 032-06-027

Eluent A: 10 mM Tris, pH 7.6
Eluent B: 1M Sodium Chloride in 10 mM Tris, pH 7.6
Gradient: 0% to 75% B in 9 minutes, Step 100% B, Hold 3 minutes.
Flow Rate: 2.0 mL/min
Temperature: 30 °C
Detection: UV 280 nm
Injection Volume: 10 µL
Storage Solution: 0.05M NaCl in 10 mM Tris, pH 7.6 + 0.1% Sodium Azide



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Resolution (EP)	Peak Width (50%) (min)	Concentration (mg/mL)
1	Ovalbumin	2.66	0.8	10.95	0.05	1.0
2	Trypsin Inhibitor	3.72	0.7	11.70	0.06	1.0
3	Insulin Chain A	5.06	1.3	15.48	0.07	0.25

QA Results:

Component	Parameter	Specification	Results
	Pressure	<=880	502
Insulin Chain A	Asymmetry	1.0-1.8	Passed
Insulin Chain A	PW (50%)	<=0.11	Passed
Insulin Chain A	Ret. Time	4.99-5.21	Passed
T1/T2	Ret.Time Ratio	1.04-1.54	Passed

T1 = Ret.Time of Insulin Chain A minus Trypsin Inhibitor

T2 = Ret.Time of Trypsin Inhibitor minus Ovalbumin

Production Reference:

Datasource: MonoBio
 Directory: Production\MONO1\2_IonEx_Analytical\SAX-1S
 Sequence: 064293_2006-032-027
 Sample No.: 12

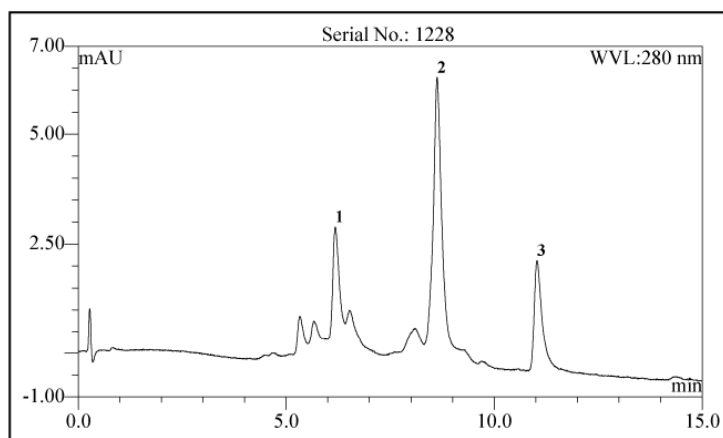
Chromleon® Dionex® Corporation 1994-2010

Doc.No.: 067099-03 (QAR)

6.80 SR8 Build 2623 (156243)

**10.2. ProSwift SAX-1S (1x50 mm)****ProSwift® SAX-1S****Date:** 23-Oct-09 11:36**1 x 50 mm****Serial No. :** 001228**Product No. 068459****Lot No. :** 001-09-150

Eluent A: 10 mM Tris, pH 7.6
Eluent B: 1M Sodium Chloride in 10 mM Tris, pH 7.6
Gradient: 5% to 45% B in 13 minutes, Hold 2 minutes.
Flow Rate: 0.20 mL/min
Temperature: 30 °C
Detection: UV 280 nm
Injection Volume: 1.3 µL
Storage Solution: 0.05M NaCl in 10 mM Tris, pH 7.6 + 0.1% Sodium Azide



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Resolution (EP)	Peak Width (50%) (min)	Concentration (mg/mL)
1	Ovalbumin	6.18	0.9	8.17	0.16	1.0
2	Trypsin Inhibitor	8.63	1.1	7.52	0.19	1.0
3	Oligo dT8-mer	11.03	1.7	n.a.	0.18	0.01

QA Results:

Component	Parameter	Specification	Results
	Pressure	<=1210	873
Oligo dT8-mer	Asymmetry	1.0-2.0	Passed
Oligo dT8-mer	PW (50%)	<=0.22	Passed
Oligo dT8-mer	Ret. Time	10.98-11.42	Passed
T1/T2	Ret.Time Ratio	0.93-1.17	Passed

T1 = Ret.Time of Oligo dT8-mer minus Trypsin Inhibitor

T2 = Ret.Time of Trypsin Inhibitor minus Ovalbumin

Production Reference:

Datasource: MonoBio
Directory: Production\MONO14_ICS3K_#1\SAX-1S_1mm
Sequence: 068459_2009-001-150-2MM OD
Sample No.: 28

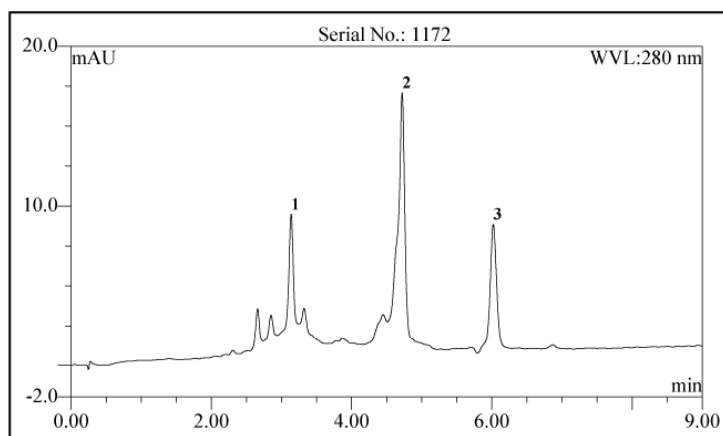
Chromleon® Dionex® Corporation 1994-2010

Doc.No.: 068451-03 (QAR)

6.80 SR8 Build 2623 (156243)

**10.3. ProSwift WAX-1S (4.6x50 mm)****ProSwift® WAX-1S****4.6 x 50 mm****Product No. 064294****Date:** 18-Dec-06 18:15**Serial No. :** 001172**Lot No. :** 006-06-188

Eluent A: 10 mM Tris, pH 7.6
Eluent B: 1M Sodium Chloride in 10 mM Tris, pH 7.6
Gradient: 0% to 75% B in 9 minutes, Step 100% B, Hold 3 minutes.
Flow Rate: 2.0 mL/min
Temperature: 30 °C
Detection: UV 280 nm
Injection Volume: 10 µL
Storage Solution: 0.05M NaCl in 10 mM Tris, pH 7.6 + 0.1% Sodium Azide



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Resolution (EP)	Peak Width (50%) (min)	Concentration (mg/mL)
1	Ovalbumin	3.14	0.8	11.57	0.07	1.0
2	Trypsin Inhibitor	4.72	0.6	8.42	0.09	1.0
3	Insulin Chain A	6.02	1.1	15.78	0.09	0.25

QA Results:

Component	Parameter	Specification	Results
	Pressure	<=660	367
Insulin Chain A	Asymmetry	0.95-1.7	Passed
Insulin Chain A	PW (50%)	<=0.13	Passed
Insulin Chain A	Ret. Time	5.68-6.23	Passed
T1/T2	Ret.Time Ratio	0.76-0.90	Passed

T1 = Ret.Time of Insulin Chain A minus Trypsin Inhibitor

T2 = Ret.Time of Trypsin Inhibitor minus Ovalbumin

Production Reference:

Datasource: MonoBio
Directory: Production\MONO1\2_IonEx_Analytical\WAX-1S
Sequence: 064294_2006-006-188
Sample No.: 60

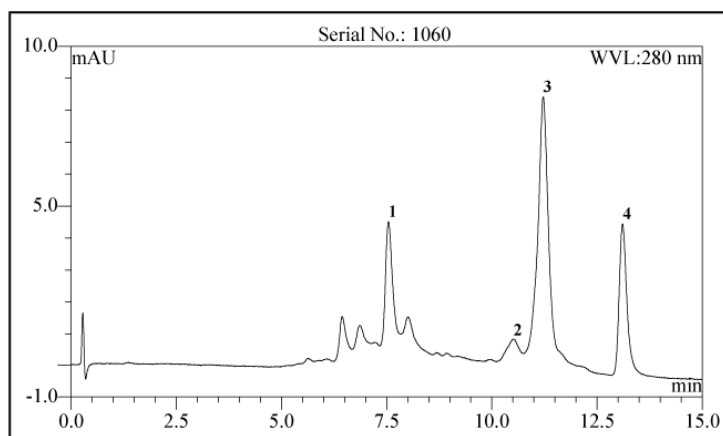
Chromleon® Dionex® Corporation 1994-2010

Doc.No.: 067100-04 (QAR)

6.80 SR8 Build 2623 (156243)

**10.4. ProSwift WAX-1S (1x50 mm)****ProSwift® WAX-1S****1 x 50 mm****Product No. 066642****Date:** 17-Jul-08 08:06**Serial No. :** 001060**Lot No. :** 008-08-024

Eluent A: 10 mM Tris, pH 7.6
Eluent B: 1M Sodium Chloride in 10 mM Tris, pH 7.6
Gradient: 5% to 45% B in 13 minutes, Hold 2 minutes.
Flow Rate: 0.20 mL/min
Temperature: 30 °C
Detection: UV 280 nm
Injection Volume: 1.3 µL
Storage Solution: 0.05M NaCl in 10 mM Tris, pH 7.6 + 0.1% Sodium Azide



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Resolution (EP)	Peak Width (50%) (min)	Concentration (mg/mL)
1	Ovalbumin	7.54	0.8	6.93	0.19	1.0
3	Trypsin Inhibitor	11.22	0.9	5.12	0.25	1.0
4	Oligo dT8-mer	13.10	1.3	n.a.	0.19	0.01

QA Results:

Component	Parameter	Specification	Results
	Pressure	<=1100	787
Oligo dT8-mer	Asymmetry	1.0-2.2	Passed
Oligo dT8-mer	PW (50%)	<=0.28	Passed
Oligo dT8-mer	Ret. Time	12.57-13.23	Passed
T1/T2	Ret.Time Ratio	0.50-0.56	Passed

T1 = Ret.Time of Oligo dT8-mer minus Trypsin Inhibitor

T2 = Ret.Time of Trypsin Inhibitor minus Ovalbumin

Production Reference:

Datasource: MonoBio
Directory: Production\MONO14_ICS3K_#1\WAX-1S_1mm
Sequence: 066642_2008-008-024-575517
Sample No.: 34

Chromleon® Dionex® Corporation 1994-2010

Doc.No.: 067093-02 (QAR)

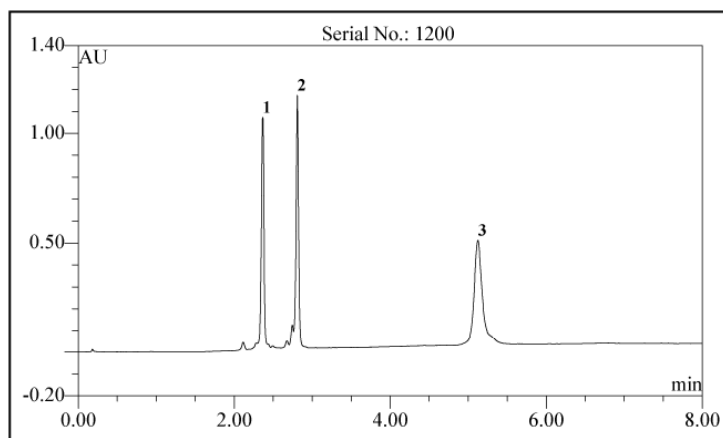
6.80 SR8 Build 2623 (156243)

**10.5. ProSwift WCX-1S (4.6x50 mm)**

ProSwift® WCX-1S
4.6 x 50 mm
Product No. 064295

Date: 19-Jun-07 14:01
Serial No. : 001200
Lot No. : 032-06-117

Eluent A: 10 mM Sodium Phosphate, pH 7.6
Eluent B: 1M Sodium Chloride in 10 mM Sodium Phosphate, pH 7.6
Gradient: 5% B for 1 minute, 5% to 95% B in 5 minutes
Flow Rate: 2.0 mL/min
Temperature: 30 °C
Detection: UV 214 nm
Injection Volume: 10 µL
Storage Solution: 0.05M NaCl in 10 mM Tris, pH 7.6 + 0.1% Sodium Azide



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Resolution (EP)	Peak Width (50%) (min)	Concentration (mg/mL)
1	Ribonuclease A	2.36	1.0	7.85	0.03	1.0
2	Cytochrome C	2.81	1.0	20.05	0.03	1.0
3	Lysozyme	5.12	1.4	n.a.	0.10	1.0

QA Results:

<u>Component</u>	<u>Parameter</u>	<u>Specification</u>	<u>Results</u>
	Pressure	<=660	351
Lysozyme	Asymmetry	1.0-1.9	Passed
Ribonuclease A	PW (50%)	<=0.07	Passed
Lysozyme	Ret. Time	4.99-5.32	Passed
T1/T2	Ret.Time Ratio	3.87-6.16	Passed

T1 = Ret.Time of Lysozyme minus Cytochrome C

T2 = Ret.Time of Cytochrome C minus Ribonuclease A

Production Reference:

Datasource: MonoBio
Directory: Production\MONO1\2_IonEx_Analytical\WCX-1S
Sequence: 064295_2006-032-117_AK_BiMax_AW_System
Sample No.: 13

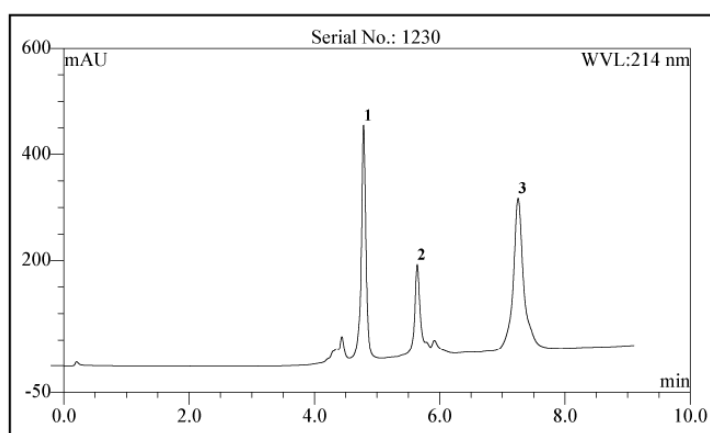
Chromleon® Dionex® Corporation 1994-2010

Doc.No.: 067101-03 (QAR)

6.80 SR8 Build 2623 (156243)

**10.6. ProSwift WCX-1S (1x50 mm)****ProSwift® WCX-1S****Date:** 07-Jul-09 11:44**1 x 50 mm****Serial No. :** 001230**Product No. 066643****Lot No. :** 001-09-082

Eluent A: 10 mM Sodium Phosphate, pH 7.6
Eluent B: 1M Sodium Chloride in 10 mM Sodium Phosphate, pH 7.6
Gradient: 5% B for 0.5 minutes, 5% to 100% B in 7.5 minutes, Hold 2 minutes.
Flow Rate: 0.20 mL/min
Temperature: 30 °C
Detection: UV 214 nm
Injection Volume: 1.0 µL
Storage Solution: 0.05M NaCl in 10 mM Tris, pH 7.6 + 0.1% Sodium Azide



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Resolution (EP)	Peak Width (50%) (min)	Concentration (mg/mL)
1	Cytochrome C Equine	4.79	0.9	6.70	0.07	1.0
2	Cytochrome C S. cerevisiae	5.64	1.3	8.29	0.08	1.0
3	Lysozyme	7.25	1.1	n.a.	0.15	1.0

QA Results:

<u>Component</u>	<u>Parameter</u>	<u>Specification</u>	<u>Results</u>
	Pressure	<=1320	668
Lysozyme	Asymmetry	1.0-2.3	Passed
Lysozyme	PW (50%)	<=0.29	Passed
Lysozyme	Ret. Time	6.97-7.63	Passed
T1/T2	Ret. Time Ratio	1.44-2.20	Passed

T1 = Ret.Time of Lysozyme minus Cytochrome C Saccharomyces cerevisiae

T2 = Ret.Time of Cytochrome C Saccharomyces cerevisiae minus Cytochrome C Equine

Production Reference:

Datasource: MonoBio
 Directory: Production\MONO1\5_IC33K_#2\WCX-1S_1mm
 Sequence: 066643_2009-001-082-2MM OD_Pressure_poly
 Sample No.: 27

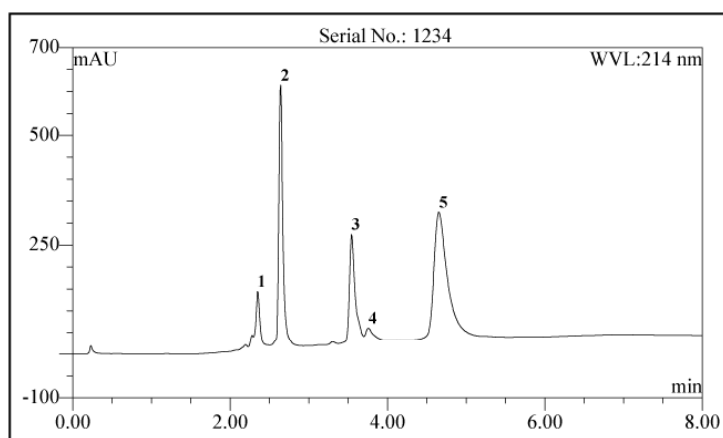
Chromleon® Dionex® Corporation 1994-2010

Doc.No.: 067094-03 (QAR)

6.80 SR8 Build 2623 (156243)

**10.7. ProSwift SCX-1S (4.6x50 mm)****ProSwift® SCX-1S****4.6 x 50 mm****Product No. 066765****Date:** 18-Jul-08 14:54**Serial No. :** 001234**Lot No. :** 008-08-035

Eluent A: 10 mM Sodium Phosphate, pH 7.6
Eluent B: 1M Sodium Chloride in 10 mM Sodium Phosphate, pH 7.6
Gradient: 5% B for 1 minute, 5% to 95% B in 5 minutes
Flow Rate: 2.0 mL/min
Temperature: 30 °C
Detection: UV 214 nm
Injection Volume: 10 µL
Storage Solution: 0.05M NaCl in 10 mM Tris, pH 7.6 + 0.1% Sodium Azide



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Resolution (EP)	Peak Width (50%) (min)	Concentration (mg/mL)
2	Cytochrome C Equine	2.64	1.4	8.90	0.05	1.0
3	Cytochrome C S. cerevisiae	3.54	1.7	5.47	0.07	1.0
5	Lysozyme	4.65	1.8	n.a.	0.17	1.0

QA Results:

Component	Parameter	Specification	Results
	Pressure	<=660	231
Lysozyme	Asymmetry	1.0-2.3	Passed
Cytochrome C Equine	PW (50%)	<=0.08	Passed
Lysozyme	Ret. Time	4.33-4.88	Passed
T1/T2	Ret.Time Ratio	0.81-1.76	Passed

T1 = Ret.Time of Lysozyme minus Cytochrome C S.cerevisiae

T2 = Ret.Time of Cytochrome C S. cerevisiae minus Cytochrome C Equine

Production Reference:

Datasource: MonoBio
Directory: Production\MONO1\5_1CS3K_#2\SCX-1S_46mm
Sequence: 066765_2008-008-035
Sample No.: 18

Chromleon® Dionex® Corporation 1994-2010

Doc.No.: 068450-04 (QAR)

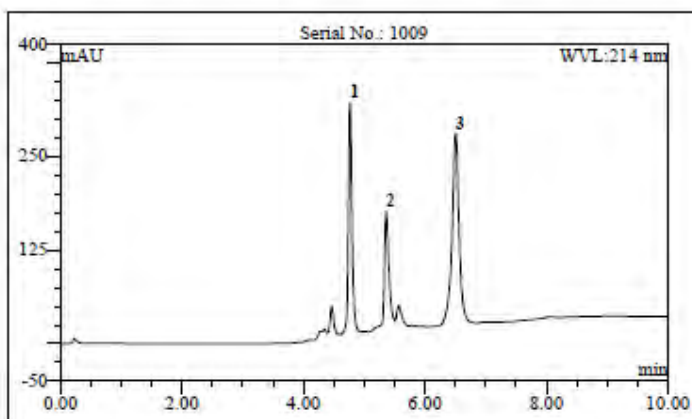
6.80 SR8 Build 2623 (156243)

**10.8. ProSwift SCX-1S (1x50 mm)**

ProSwift® SCX-1S
1 x 50 mm
Product No. 071977

Date: 04-Feb-10 15:38
Serial No. : 001009
Lot No. : 2009-024-014

Eluent A: 10 mM Sodium Phosphate, pH 7.6
Eluent B: 1M Sodium Chloride in 10 mM Sodium Phosphate, pH 7.6
Equilibration: 5% B for 7 minutes.
Gradient: 5% B for 1 minutes, 5% to 95% B in 5 minutes, Hold 6 minutes.
Flow Rate: 0.20 mL/min
Temperature: 30 °C
Detection: UV 214 nm
Injection Volume: 1.0 µL
Storage Solution: 0.05M NaCl in 10 mM Tris, pH 7.6 + 0.1% Sodium Azide



No.	Peak Name	Ret. Time (min)	Asymmetry (EP)	Resolution (EP)	Peak Width (50%) (min)	Concentration (mg/mL)
1	Cytochrome C Equine	4.77	1.2	5.35	0.059	1.0
2	Cytochrome C S. cerevisiae	5.36	1.4	7.32	0.072	1.0
3	Lysozyme	6.50	1.0	n.a.	0.112	1.0

QA Results:

Component	Parameter	Specification	Results
	Pressure	<=550	195
Lysozyme	Asymmetry	1.0-1.7	Passed
Cytochrome C Equine	PW (50%)	<=0.072	Passed
Lysozyme	Ret. Time	6.34-6.67	Passed
T1/T2	Ret. Time Ratio	1.53-2.26	Passed

T1 = Ret. Time of Lysozyme minus Cytochrome C Saccharomyces cerevisiae
T2 = Ret. Time of Cytochrome C Saccharomyces cerevisiae minus Cytochrome C Equine

Production Reference:

Datasource: MonoBio
Directory: Production\Mono1\5_IC33K_#2\SCX-1S_1mm
Sequence: 1SCX-1MM-VALIDATION_AK
Sample No.: 96

Chromleon® Dionex® Corporation 1994-2010

Doc.No.: 071978-02

6.80 SR8 Build 2623 (156243)

11. APPENDIX B - COLUMN CARE

11.1. New Column Equilibration

The columns are shipped in the storage solution containing 0.1% NaN₃ to suppress microbial growth. Before use, please refer to QuickStart (Section 3.1) for cleaning of the storage solution.

11.2. Column Cleanup

If the column inlet frit or the media is fouled by sample or eluent contaminants, (protein precipitates and hydrophobically bound proteins,) these may be removed by a strong solvent, solubilizing agents, acid, base, or pepsin treatment.



CAUTION

Always ensure that the cleanup protocol used does not switch directly between eluents that can react or precipitate when mixed together. Choose a flow rate that will not create higher column back pressure than maximum pressure in column specifications.

11.2.1. Cleanup Solution

Treatment with one or more of these solutions may help clean ion-exchange monoliths

Table 4

Contaminants	Clean up solutions	4.6 mm	1mm
General contaminants	2 M NaCl 0.1 M NaOH (Up to 1 M NaOH for WCX-1S 1mm) 0.1 M HCl	10 CV (7.5 mL)	10 CV (0.40 mL)
Hydrophobic binding proteins	≤ 100% CH ₃ CN ≤ 1 M NaOH ≤ 75% Acetic acid (≤ 100% Ethanol excluding WCX phase.) ≤ 0.5 % non-ionic detergents (in 0.1 M acetic acid solution)	10 CV (7.5 mL)	10 CV (0.40 mL)
Protein Precipitates	6 M guanidine hydrochloride	10 CV (7.5 mL)	10 CV (0.40 mL)

11.2.2. Column Cleanup Procedure

1. Preparation:

a. Eluent Preparation

<u>Eluent</u>	<u>Anion Eluent</u>	<u>Cation Eluent</u>
A	0.01 M Tris•HCl buffer pH 7.6	0.01M Na ₂ PO ₄ buffer, pH 7.6
B	1 M NaCl in 0.01 M Tris •HCl buffer pH 7.6	1 M NaCl in 0.01 M Na ₂ PO ₄ buffer, pH 7.6

b. Column Installation

Install the column on the instrument in the reversed flow direction. For the 4.6 mm column format, install a 1-3 mL injection loop, and for the 1 mm format, a 50-150 µL injection loop.

c. Flow Rate Setup:

For the 4.6 mm column format, set the pump flow rate to ≤0.50 mL/min. For the 1 mm column format, set the flow rate to 0.05 mL/min. for 4.6 mm and 0.1mL/min for 1mm column


WARNING

If your eluent composition generates back pressure in excess of the maximum operation pressure, adjust the flow rate to ensure the backpressure of the column is less than the recommended maximum operation pressure.

2. Column Cleaning Procedures 4.6 mm monoliths:
 - a. Run a method using the following program:
 - Pump 10 CV (ca. 7.5 mL) 100% eluent A.
 - Pump 10 CV (ca. 7.5 mL) gradient from 100% A to 100% eluent B.
 - Pump 10 CV (ca. 7.5 mL) of 100% eluent B.
 - Pump 10 CV (ca. 7.5 mL) reversed gradient from 100% B to 5% eluent B.
 - Pump 10 CV (ca. 7.5 mL) of 5% eluent B.
 - b. Load and Inject Cleaning Solutions:
 - Maintain the flow of 5% eluent B.
 - Load and inject a cleaning solution of your choice (see list of solvents in the above table).
 - Pump 10 CV (ca. 7.5 mL) of 5% eluent B.
 - Continue to load and inject other cleaning solutions of your choices.
 - Pump 10 CV (ca. 7.5 mL) of 5% eluent B.
 - c. Repeat the above cleaning steps with the same or different cleaning solvents until a constant baseline is achieved during the gradient run.
3. Column Cleaning Procedures for the 1.0 mm monoliths:
 - a. Run a method using the following program:
 - Pump 10 CV (ca. 0.40 mL for 1x50 mm column) 100% eluent A.
 - Pump 10 CV (ca. 0.40 mL) gradient from 100% A to 100% eluent B.
 - Pump 10 CV (ca. 0.40 mL) of 100% eluent B.
 - Pump 10 CV (ca. 0.40 mL) reversed gradient from 100% B to 5% eluent B.
 - Pump 10 CV (ca. 0.40 mL) of 5% eluent B.
 - b. Load and Inject Cleaning Solutions:
 - Maintain the flow of 5% eluent B.
 - Load and inject a cleaning solution of your choice (see list of solvents in the above table).
 - Pump 10 CV (ca. 0.40 mL) of 5% eluent B.
 - Continue to load and inject other cleaning solutions of your choices.
 - Pump 10 CV (ca. 0.40 mL) of 5% eluent B.
 - c. Repeat the above cleaning steps with the same or different cleaning solvents until a constant baseline is achieved during the gradient run.
4. Column Re-equilibration:
 - a. Reconnect the ProSwift column in its proper orientation.
 - b. Equilibrate the column with eluent before resuming normal operation.

11.3. Column Storage

11.3.1. Short Term Storage:

For short term storage (less than 3 days), use the low salt concentration eluent (pH = 3 - 10) as the column storage solution.

11.3.2. Long Term Storage:

For long term storage, use the following solution to avoid microbial growth on the column.

Anion Exchange

0.05 M NaCl in 0.01 M Tris•HCl, pH 7.6 + 0.1% NaN₃

Cation Exchange

0.05 M NaCl in 0.01 M Na₂PO₄, pH 7.6 + 0.1% NaN₃

Flush the column with at least 10 mL of the storage eluent. Cap both ends securely using the plugs supplied with the column.



12. APPENDIX C - REFERENCES

1. High Speed Separation of Oligonucleotides using SWIFT WAX monolithic column, B. Kunnummal, S. Xie, T. Jiang, L. Xu and R. W. Allington, *PharmaGenomics*, 8, March/April 2004.
2. Porous Polymer Monoliths: An Alternative to Classical Beads. S. Xie, R. W. Allington, F. Svec, J. M. J. Fréchet, *Advances in Biochemical Engineering/Biotechnology Spec. Vol: Modern Advances in Chromatography*, Ed. Dr. R. Freitag, 2002.