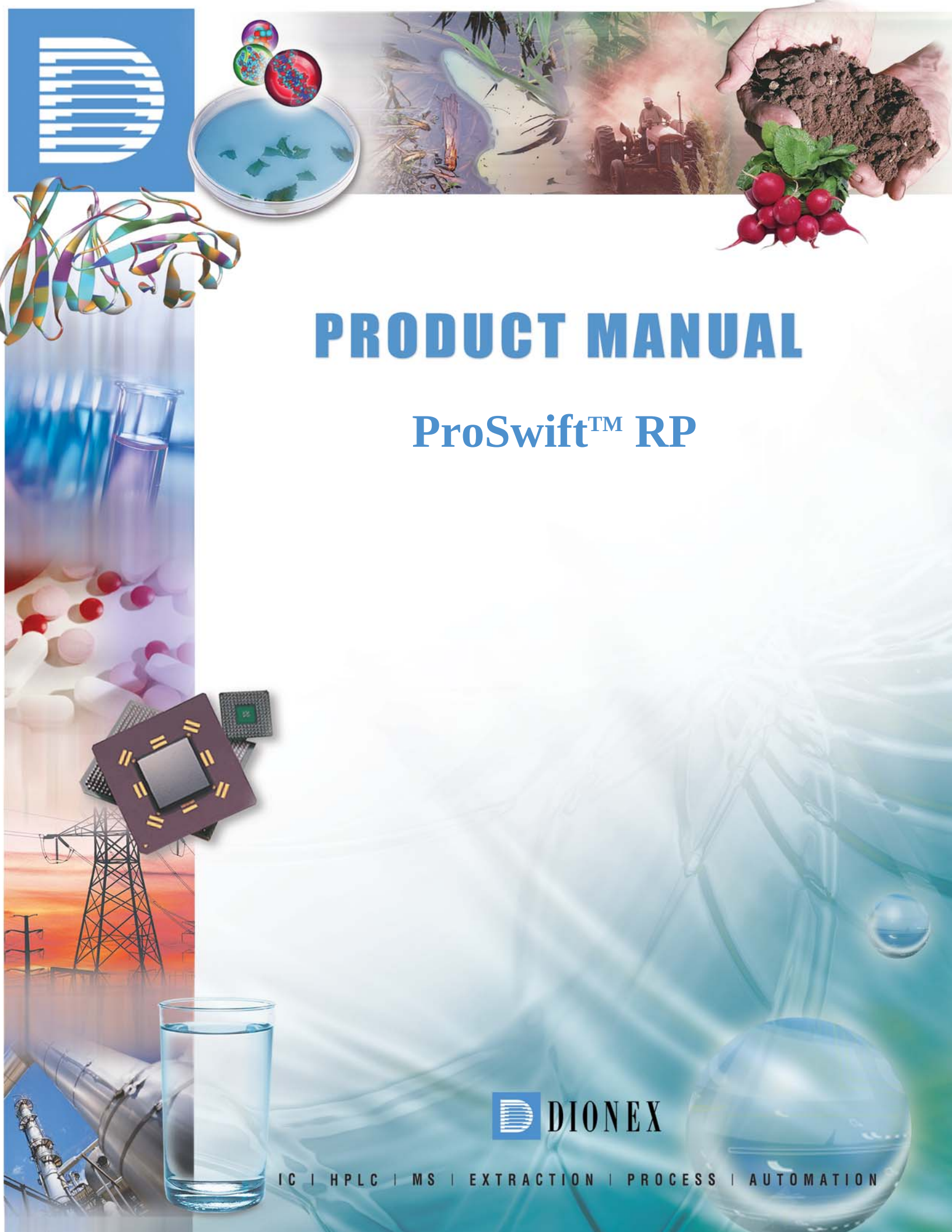

PRODUCT MANUAL

ProSwift™ RP

DIONEX

IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

PRODUCT MANUAL

FOR

PROSWIFT™ RP-1S COLUMN

(Stainless Steel, 4.6 x 50mm, P/N 064297)

PROSWIFT™ RP-2H COLUMN

(Stainless Steel, 4.6 x 50mm, P/N 064296)

PROSWIFT™ RP-3U COLUMN

(Stainless Steel, 4.6 x 50mm, P/N 064298)

Dionex® Corporation, 2006
Document No. 065123
Revision 02
August 2006

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GUIDE TO IMPORTANT INFORMATION

SAFETY ***Safety information can help prevent bodily harm.***

WARNING ***Warning information can help prevent equipment harm.***

CAUTION ***Caution information can help prevent problems.***

NOTE ***Note information can help with tips for improved use.***

1. INTRODUCTION

ProSwift™ reversed-phase monolithic columns are specifically designed to provide high-resolution and high efficiency separations of proteins, peptides, and other biomolecules.

1.1. Morphology of ProSwift Reversed-phase Monoliths

ProSwift reversed-phase media are based on polymeric monoliths prepared by an *in situ* polymerization process. They represent a new generation of separation products which are uniquely designed and engineered for the separation of biomolecules. The monolith is a single cylindrical polymer rod containing an uninterrupted, interconnected network of through pores (channels). Its unique morphology, pore structure, and pore size distribution offer optimum performance for separation of proteins, peptides, oligonucleotides, and other biomolecules.

The morphologies of the ProSwift RP-1S (P/N 064297), RP-2H (P/N 064296), and RP-3U (P/N 064298) monoliths are shown in Figure 1. The monoliths consist of aggregates of globules shaped like cauliflower. The open spaces among 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. The mass transfer of the samples is primarily driven by convective flow through these open pores instead of much slower molecular diffusion. These pores are large enough for 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 mainly non-porous based on BET measurements and SEM examinations. Since they are non-porous, these globules minimize diffusion-controlled mass transfer. This is in contrast to porous beads where diffusion-controlled mass transfer predominates.

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 compared to porous beads.

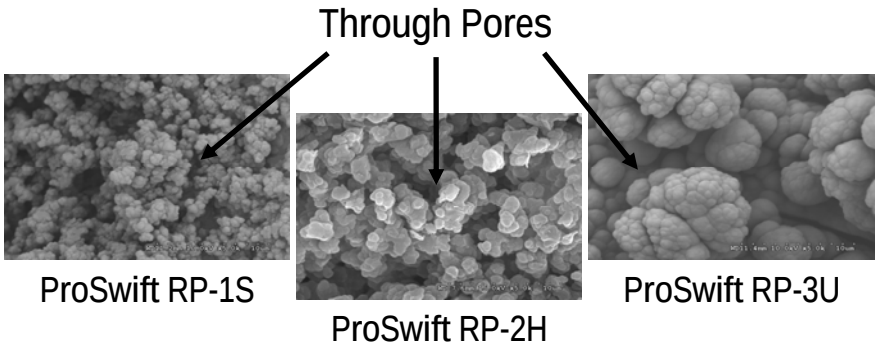


Figure 1
SEM Images of the ProSwift RP-1S, RP-2H, and RP-3U Monoliths

1.2. Backpressures and Pore Size Distributions

ProSwift monolith phases have very high permeability. The pore volume is about 60% of the column volume, which is much higher than porous beads. There are two types of pores: large pores (approximately a micron or larger) for eluent to flow through and small pores (ten to hundreds of nanometers) where most of the separations take place. The modes of the pore size distribution for RP-1S, RP-2H, and RP-3U are 1035, 2230, and 5181 nm, as shown in Figures 2-4. These large pores allow the eluent to flow though with moderate back pressure, allowing high flow rates for faster analyte separations.

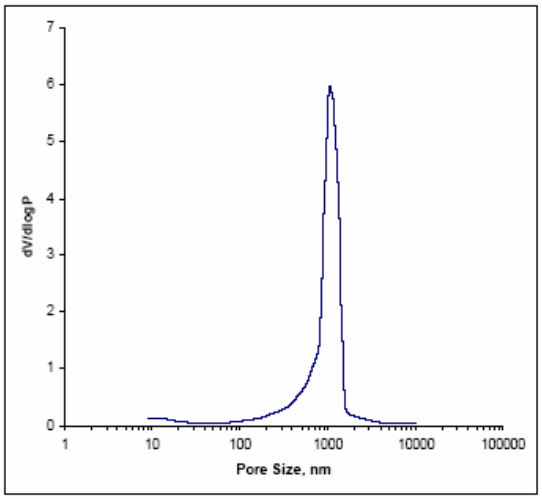


Figure 2
Pore Size Distribution of ProSwift RP-1S by Mercury Porosimetry

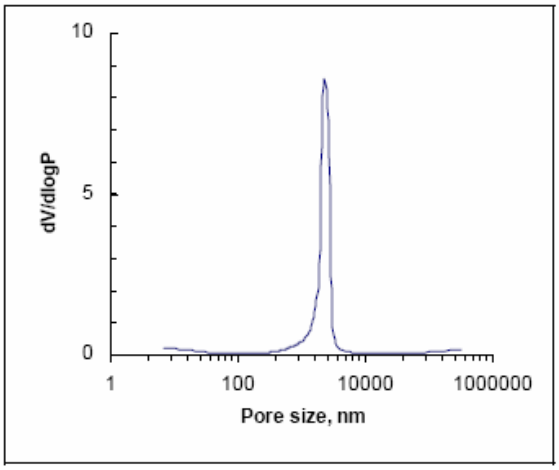


Figure 3
Pore Size Distribution of ProSwift RP-2H by Mercury Porosimetry

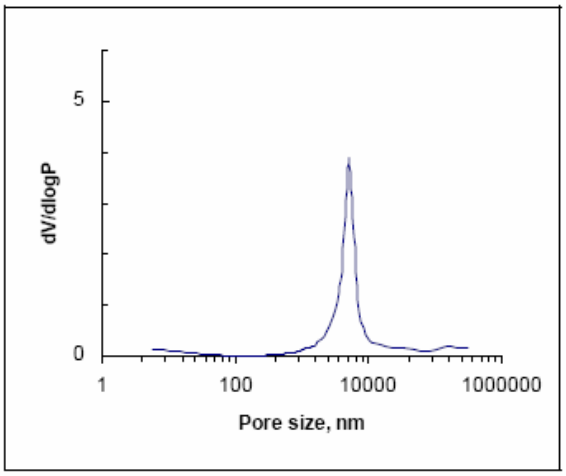


Figure 4
Pore Size Distribution of ProSwift RP-3U by Mercury Porosimetry

The backpressure generated at different flow rates of ProSwift RP are shown below. At any given flow rate, the backpressure generated on ProSwift monoliths is significantly lower than that of currently available bead-based analytical columns.

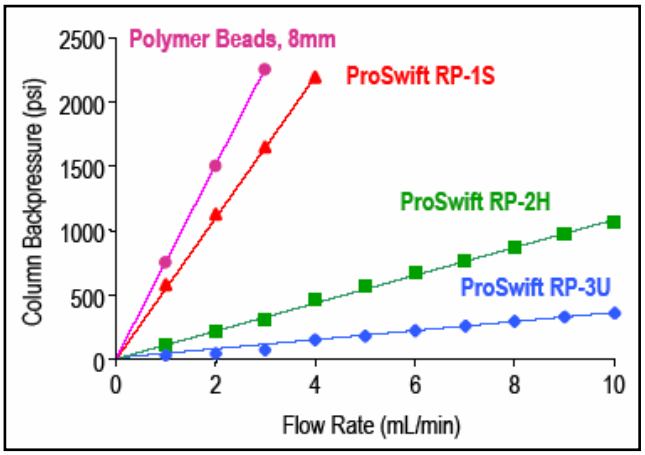


Figure 5
Column Backpressure

1.3. Resolution and Speed of Separation

As described in Section 1.1, the uniquely designed morphology of ProSwift monoliths allows fast analyte mass transfer. This fast mass transfer minimizes band broadening upon flow-rate increases, thus resulting in higher resolution than conventional beads over a wide range of flow rates. This is especially true for large molecules whose diffusivities are much lower than small molecules. This provides the benefits of non-porous beads. In combination with low backpressure, ProSwift monoliths offer excellent separation at low and high flow rates which improves productivity.

NOTE

The loss of resolution at elevated flow rates is much lower in ProSwift monoliths than in conventional packed bead columns. Therefore, when productivity is the most important factor in your separation, higher flow rates are recommended. When resolution is the most important factor in your separation, lower flow rates are recommended.

Column:	RP-2H (4.6 x 50mm)
Flow rate:	1 or 4 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient:	1-75% B in 12 min 1-75% B in 3 min
Detection:	UV at 214 nm
Sample:	Protein Mixture (from Sigma)
Injection volume:	5 µL
Order of elution:	1. Ribonuclease A (1.5 mg/mL) 2. Cytochrome C (0.5 mg/mL) 3. BSA (1.5 mg/mL) 4. Carbonic anhydrase (0.9 mg/mL) 5. Ovalbumin (1.5 mg/ mL)

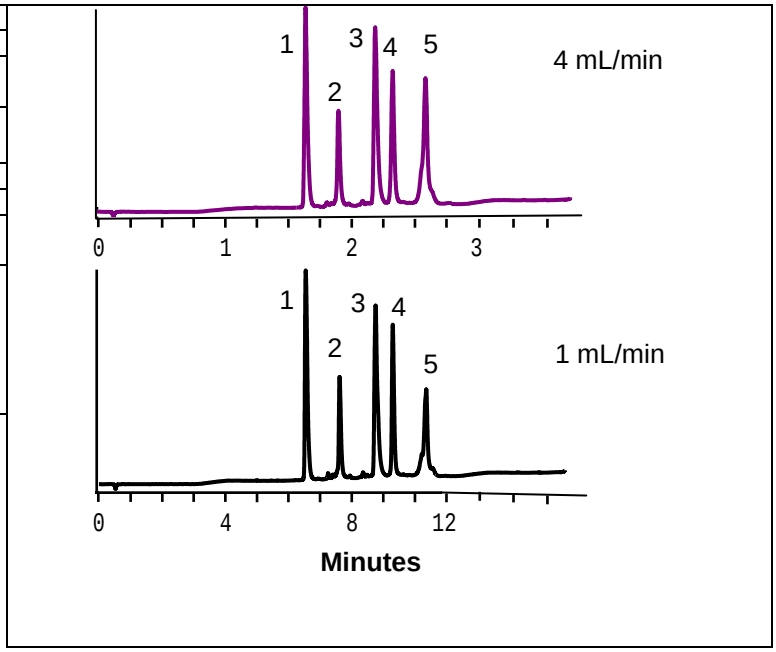


Figure 6
Separation of Five Protein Standards at 1 mL/min and 4 mL/min on RP-2H

1.4. Loading Capacity

ProSwift monolith surfaces are irregular as shown in Figure 1. This produces loading capacity comparable to porous beads. Table 2 shows the frontal loading capacity of each column type.

1.5. Robustness and Run Stability

ProSwift columns are durable and robust. The ProSwift monoliths exhibit stability and reproducibility for hundreds of runs. Figure 7 shows the ProSwift RP-1S retained high resolution and high run stability over 500 cycles of operation. The chromatograms overlayed perfectly from cycles 4, 104, 204, 304, and 503, indicating reproducibility, run stability, and robustness of the column.

Column:	RP-1S (4.6 x 50mm)
Flow rate:	1 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient:	1-75%B in 12 min (Sample) 1-100%B in 4 min, 100%B for 1 min and 100-1%B in 3.9 min (For Each blank cycle)
Detection:	UV at 214nm
Injection volume:	5 µL
Sample:	Mix of 5 Proteins 1. Ribonuclease A (1.5 mg/mL) 2. Cytochrome C (0.5 mg/mL) 3. BSA (1.5 mg/mL) 4. Carbonic anhydrase (0.9 mg/mL) 5. Ovalbumin (1.5 mg/ mL)

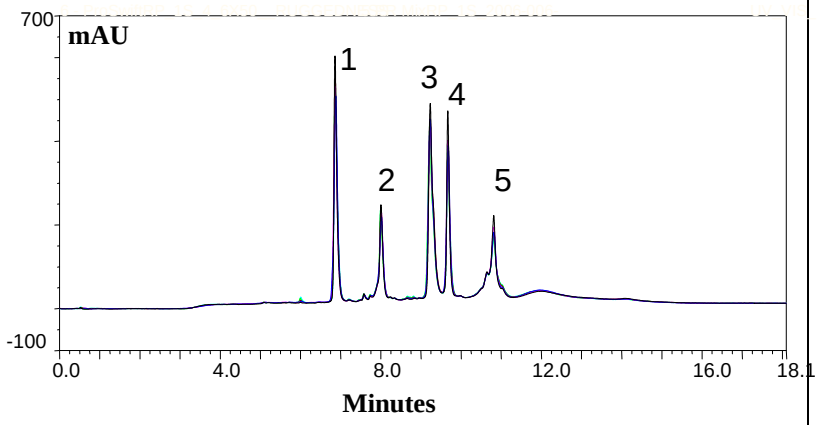


Figure 7
Reproducibility of ProSwift RP-1S: Overlay of 5 Chromatograms

1.6. Batch-To-Batch Reproducibility

ProSwift reversed-phase media are manufactured by a patented *in situ* manufacturing process that does not require additional sieving, coating, multiple surface modifications, or packing processes. Different lots of ProSwift monoliths exhibit virtually identical chromatography, as shown in Figure 8.

Column:	RP-3U (4.6 x 50mm)
Flow rate:	4 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient:	15-80 % B in 3 min.
Detection:	UV at 214nm
Sample:	Standard Protein Mix.
Injection volume:	10 µL
Order of elution:	1. Ribonuclease A (0.1 mg/mL) 2. Cytochrome C (0.05 mg/mL) 3. BSA (0.1 mg/mL) 4. Carbonic anhydrase (0.1 mg/mL)

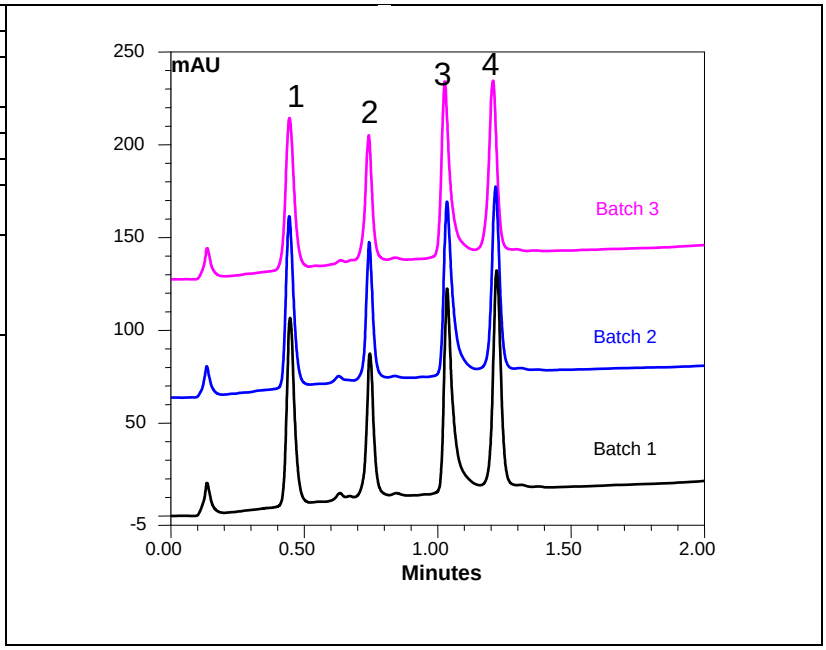


Figure 8
Batch-to-Batch Reproducibility of RP-3U

1.7. pH Stability

The ProSwift RP monolith column is stable with treatment of 1 M NaOH and 0.1 M HCl in the regeneration process. It is shown to be stable at pH 1 - 14 at normal temperature and flow for at least 48 hours. Figure 9 shows the resolution of four protein standards before and after base and acid regeneration treatments.

Column:	RP-3U (4.6 x 50mm)
Flow rate:	4 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient:	15 – 80 % B in 3 min
Detection:	UV at 214nm
Injection volume:	10 µL
Sample:	1. Ribonuclease A (0.1 mg/mL) 2. Cytochrome C (0.05 mg/mL) 3. BSA (0.1 mg/mL) 4. Carbonic anhydrase (0.1 mg/mL)
Acid Treatment:	0.1 M HCl at 0.1 mL/min for 48 hours
Base Treatment:	1 M NaOH at 0.1 mL/min for 48 hours

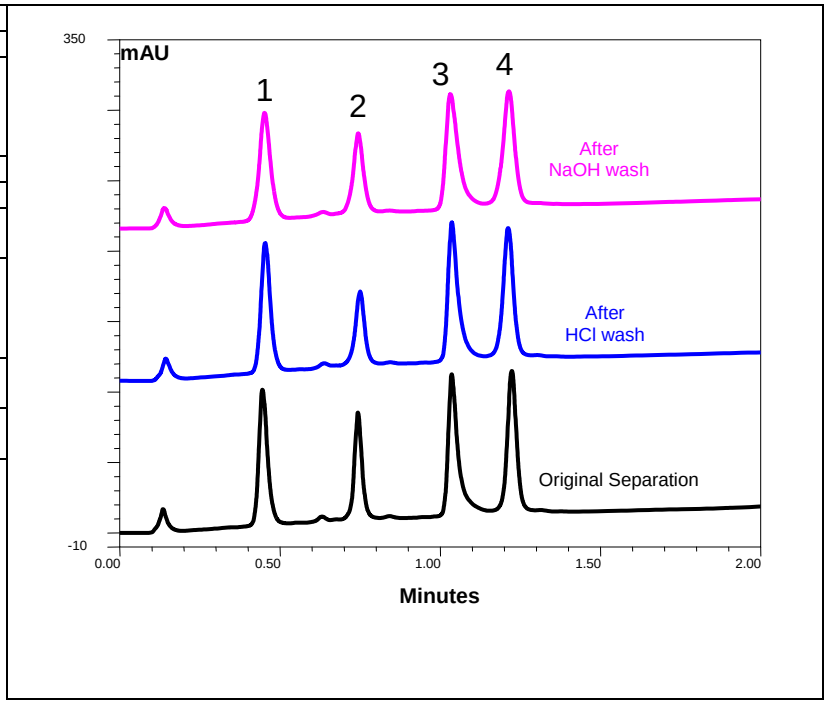


Figure 9
Resolution of ProSwift RP-3U
Before and After Strong Base / Acid Treatments

1.8. Summary of Characteristics of ProSwift Monolithic Media

The following table summarizes the general characteristics of the ProSwift monolithic media.

Table 1
ProSwift Characteristics

Characteristics of ProSwift™ Monolithic Technology			LC Properties
Porous Structure	Flow-through pores	Fast mass transfer driven by convective flow	High efficiency
	No interstitial volume	Fast mass transfer due to short flow path	High resolution
	Large pore & High permeability	High flow velocity with low back pressure	Fast separation
	Mostly non-porous globules	Minimize diffusion controlled mass transfer within pores	High resolution
	Porous structure	Much higher surface area than non-porous beads	Higher capacity
	Chemically bonded aggregates	Rigid polymer structure due to chemical bonding between the aggregates	High run-to-run stability
Manufacturing Process	Single-step in situ polymerization	Minimum number of reaction variables No sieving steps No coating steps No multiple derivatization steps No packing steps	Improved batch reproducibility
	Solution polymerization	Broad choice of monomers & Direct copolymerization of hydrophilic monomers	High selectivity High recovery

1.9. Specifications

The following table summarizes the specifications of the ProSwift monolithic columns.

Table 2
ProSwift Specifications

Parameter	ProSwift RP-1S	ProSwift RP-2H	ProSwift RP-3U
Column Dimension	4.6 x 50mm	4.6 x 50mm	4.6 x 50mm
Base Matrix Material	Polystyrene	Polystyrene	Polystyrene
Surface Chemistry	Phenyl	Phenyl	Phenyl
Protein Binding Capacity per mL of Polymer	5.5 mg/mL Insulin	1 mg/mL lysozyme	0.5 mg/mL lysozyme
Bed Height	46 mm	44 mm	40 mm
Bed Volume (mL)	0.76 mL	0.73 mL	0.66 mL
pH Range	1 -14	1 -14	1 -14
Recommended Flow Rate	1 – 4 mL/min	1 – 10 mL/min	1 – 16 mL/min
Maximum Flow Rate	4 mL/min	14 mL/min	20 mL/min
Maximum Pressure	2800 psi (190 Bar, 19.3 Mpa)	2800 psi (190 Bar, 19.3 Mpa)	2800 psi (190 Bar,19.3 Mpa)
Maximum Temperature	70 ºC	70 ºC	70 ºC

2. CHROMATOGRAPHY SYSTEM

A chromatography system contains a gradient pump, an autosampler, a thermal compartment, and a UV detector. The system is controlled by Chromeleon® data management.

UV detectors from various manufacturers can be used. However, the UVD340U and PDA100 from Dionex are highly recommended.

2.1. UV detection

The UV detector should be connected immediately after the analytical column.

2.2. Pumps

Gradient LC pumps from any manufacturer can be used. However, the Dionex UltiMate HPG-3200A (Stainless Steel) is highly recommended.

2.3. Injectors

Either an autosampler (WPS-3000 from Dionex Corp. is recommended) or a manual injector can be used for sample injection.

2.4. Column Oven

Optimal reproducibility of results is achieved by regulating the temperature of the column using a column oven during chromatography. Therefore, a column oven, such as the Dionex TCC-3100, is highly recommended.

3. INSTALLATION

3.1. System Requirements

Monolith columns can be run on any HPLC or FPLC system, including Dionex HPLC systems. Each of the possible configurations offers multiple sampling options.

3.2. System Void Volume

For best performance, minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers. If you need assistance in properly configuring your system, contact the Dionex North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or the nearest Dionex Office (see “Dionex Worldwide Offices” on the Dionex Reference Library CD-ROM, P/N 053891).

3.3. The Injection Loop

Use a sample loop according to your sample injection volume requirement. For manual injections Dionex recommends full-loop injections. For autosamplers, follow the manufacturer’s recommendation.

3.4. Column Installation

Connect the column with the direction of flow as indicated by the arrow on the column label.

4. OPERATION

4.1. Quick Start

I. Preparation:

a. Eluent Preparation

The following eluents are recommended, but the column may be used with any eluents suitable for your analysis. Typically, Eluent A is a mobile phase with low organic solvent content, and Eluent B is a mobile phase with high organic content.

Eluent A: 0.1% TFA in water

Eluent B: 0.1% TFA in CH_3CN

b. Column Installation

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

WARNING

Sudden increases in flow rates may damage the column.
Always increase the flow rate slowly using a linear flow gradient or stepwise flow increments.
If your eluent composition generates back pressure in excess of the maximum operation pressure, reduce the flow rate to ensure the backpressure of the column is less than the recommended maximum operation pressure.

c. Flow Rate Start-Up (Ramping)

Using a linear flow gradient increase the flow rate of 100% Eluent B from 0.00 to your desired flow rate in 1 minute or by increasing the flow in stepwise increments of ≤2 mL/min every 30 seconds.

II. Column Conditioning:

a. Removing Storage Solution from the Column:

- Pump 15 mL (20 column volumes) of 100% B at your desired flow rate.

b. Column Equilibration:

- Equilibration from 100% B to your starting eluent composition should include at least a 1 minute reverse gradient to initial conditions.
- Pump this eluent composition through the column for at least 15 mL (20 column volumes.)

III. Storage:

Store the column in 100% B.

4.2. General Operating Conditions

Sample Volume:	<1-250 µL
Column:	ProSwift RP-1S, RP-2H, or RP-3U
Eluent:	1–100% solvent
Eluent Flow Rate:	0.5 mL to 4.0 mL/min (RP-1S) 1.0 mL to 10 mL/min (RP-2H) 1.0 mL to 16 mL/min (RP-3U)
Detector:	UV
Storage Solution:	100% Acetonitrile

4.3. ProSwift (RP-1S, RP-2H, and RP-3U) Operation Precautions

CAUTION

Filter and Degas Eluents
Filter Samples
Eluent pH between 1 and 14
Sample pH between 1 and 14
Do not exceed Maximum Operating Flow Rate (RP-1S: 4.0 mL/min, RP 2H: 10 mL/min, RP 3U : 16 mL/min)
Maximum Pressure = 2800 psi (19.3 MPa, 190 Bar)

4.4. Chemical Purity Requirements

Obtaining reliable, consistent and accurate results require eluents that are free of impurities. Chemicals, solvents and deionized water used to prepare eluents must be the highest purity available. Dionex cannot guarantee proper column performance when the quality of the chemicals, solvents, and water used to prepare eluents has been compromised.

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

4.4.1. Deionized Water

The deionized water used to prepare eluents should be Type I Reagent Grade Water with a specific resistance of 18.2 megaohm-cm. The deionized water should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm.

4.4.2. Solvents

CAUTION

The ProSwift (RP-1S, RP-2H, and RP-3U) columns are cross-linked polymeric monoliths with very hydrophobic surfaces. These columns must be operated so that the eluent being pumped over the column contains minimally 1% solvent to ensure that the hydrophobic surfaces are "wetted" and maintain maximum column performance.

The ProSwift (RP 1S, RP-2H, and RP-3U) columns can withstand all common HPLC solvents (see Table 3) at concentrations shown. However, solvents and degassed water should be premixed in concentrations that allow proper mixing by the gradient pump and minimize out-gassing. Therefore, the columns should have an operational organic solvent concentration range of 1 to 95% to ensure proper chromatographic system performance.

Avoid creating high viscosity pressure fronts that may compress the monolith when the eluent is changed. To do this, equilibrate the column for about 10 minutes with an eluent containing only 5% of the current solvent type (e.g., methanol.) Exchange this eluent for an eluent with 5% of the new solvent type (e.g., acetonitrile) and then equilibrate the column and allow the system to stabilize (about 10 minutes more). Next, run a 15-minute gradient from 5% of the new solvent type to the highest percentage used during the new analysis protocol.

The solvents used must be free of ionic impurities. However, since most manufacturers of solvents do not test for ionic impurities, use the highest grade of solvents available. Currently, several manufacturers are making ultrahigh purity solvents that are compatible for HPLC and spectrophotometric applications. These ultrahigh purity solvents will usually ensure that the results are not affected by ionic impurities in the solvent. At Dionex, we have obtained consistent results using High Purity Solvents manufactured by Burdick and Jackson and Optima® Solvents by Fisher Scientific.

When using a solvent in an ionic eluent, column generated back pressures will depend on the solvent used, concentration of the solvent, the ionic strength of the eluent, and the flow rate used. Also, the column back pressure will vary as the composition of water and solvent mixture varies. The maximum backpressure limit for the ProSwift (RP-1S, RP-2H, and RP-3U) columns is 2,800 psi (19.3 MPa, 190 Bar.)

Ensure that all of the inorganic chemicals are soluble in the highest solvent concentration to be used during the analysis.

Table 3
HPLC Solvents for Use with ProSwift (RP-1S, RP-2H, and RP-3U) Columns

Solvent	Maximum Concentration
Acetonitrile	100%
Methanol	100%
2-Propanol	100%
Tetrahydrofuran	80%

4.5. Preparing Eluents Containing Solvents

Remember to mix solvents with water on a volume to volume basis. If a procedure requires an eluent of 95% acetonitrile, prepare the eluent by adding 950 mL of acetonitrile to an eluent reservoir. Then, add 50 mL of deionized water or eluent concentrate to the acetonitrile in the reservoir. Using this procedure to mix solvents with water will ensure that a consistent true volume/volume (v/v) eluent is obtained. Premixing water with solvent will minimize the possibility of out-gassing.

CAUTION

When purging or degassing eluents containing solvents, do not purge or degas the eluent excessively. Volatile solvents may evaporate.

Always degas and store all eluents in glass or plastic eluent bottles pressurized with helium. Because nitrogen is soluble in solvent containing eluents, helium is recommended to purge and degas these eluents.

Acetonitrile (CH_3CN) hydrolyzes to ammonia and acetate when left exposed to basic solutions. To prevent eluent contamination from acetonitrile hydrolysis, always add acetonitrile to basic aqueous eluents by proportioning the acetonitrile into the basic eluent with the gradient pump. Keep the acetonitrile in a separate eluent bottle which contains only acetonitrile and water. Never add the acetonitrile directly to the basic solutions, e.g., carbonate or hydroxide in the eluent bottles.

4.5.1. Eluents for Gradients

Gradient applications are straightforward as long as solvents and water are premixed in concentrations that allow mixing by the gradient pump to give the required gradient ramp for your chromatography. For example, if you want to build a solvent gradient from 5% solvent to 95% solvent, make the following eluents:

- Eluent A: 5% solvent/95% water
- Eluent B: 95% solvent/5% water

Then, by programming the gradient pump properly, you can go from 100% Eluent A to 100% Eluent B. This will avoid out-gassing and refractive index problems associated with mixing neat solvents with water.

5. APPLICATIONS

Before attempting any of the following example applications, verify that your system is properly configured. Ensure that all of the eluents have been made from high purity reagents and deionized water. All water used in the preparation of eluents should be degassed and deionized prior to mixing. For chemical purity requirements, see Section 4.4, "Chemical Purity Requirements." After running synthetic standards to calibrate your system, you may find that real sample matrices foul your columns.

ProSwift monoliths support protein, peptide, and oligonucleotide separations. Several examples are included in this section.

These columns are suitable for a variety of applications including separation of:

1. Standard protein mixtures.
2. Complex mixtures of proteins and other biomolecules (for example; pancreatin, human skeletal muscle proteins (HSMP), cell line extracts, serum, etc.)
3. Standard protein mixture – comparison with other leading columns.

5.1. Separation of Protein Standard Mixture

ProSwift monolith HPLC columns deliver the outstanding resolving power of nonporous media. In addition, their low backpressures allow use of high flow rates for faster chromatographic separations. Figure 10 compares the resolution of a protein mixture at various flow rates. Increasing the flow rate to 8 mL/min decreases the separation times to less then 2 minutes while still providing high resolution. This fast separation demonstrates the capabilities of high throughput and high productivity available with ProSwift columns.

Column:	RP-1S (4.6 x 50mm)
Flow rate:	1or 2 or 4 or 8 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient	1 mL/min: 1-75% B in 12 min 2 mL/min: 1-75% B in 6 min 4 mL/min: 1-75% B in 3 min
Detection:	UV at 214nm
Sample:	Protein Mixture (from Sigma)
Injection volume:	5 µL
Order of elution:	1. Ribonuclease A (1.5 mg/mL) 2. Cytochrome C (0.5 mg/mL) 3. BSA (1.5 mg/mL) 4. Carbonic anhydrase (0.9 mg/mL) 5. Ovalbumin (1.5 mg/ mL)

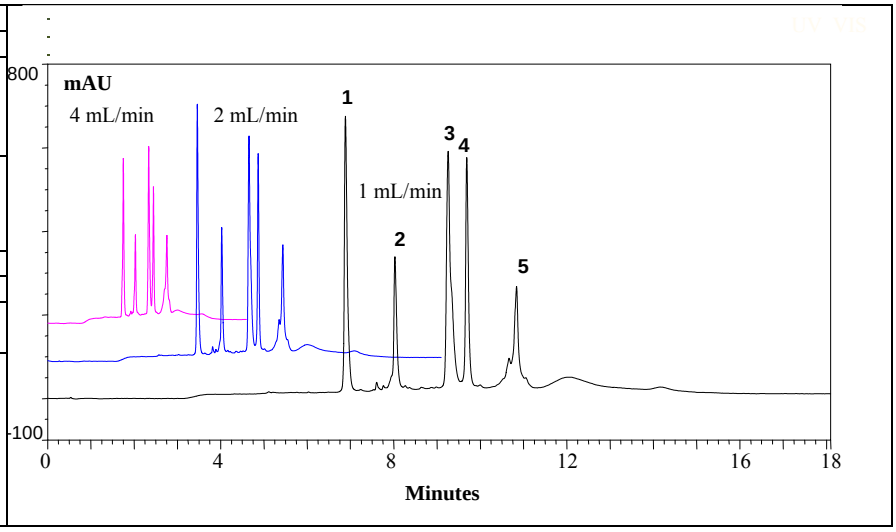


Figure 10
Separation of Protein Standard Mixture on RP-1S with Increasing Flow Rates

Column:	RP-2H (4.6 x 50mm)
Flow rate:	1or 2 or 4 or 8 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient	1 mL/min: 1-75% B in 12 min 2 mL/min: 1-75% B in 6 min 4 mL/min: 1-75% B in 3 min 8 mL/min: 1-75% B in 1.5 min
Detection:	UV at 214nm
Sample:	Protein Mixture (from Sigma)
Injection volume:	5 µL
Order of elution:	1. Ribonuclease A (1.5 mg/mL) 2. Cytochrome C (0.5 mg/mL) 3. BSA (1.5 mg/mL) 4. Carbonic anhydrase (0.9 mg/mL) 5. Ovalbumin (1.5 mg/ mL)

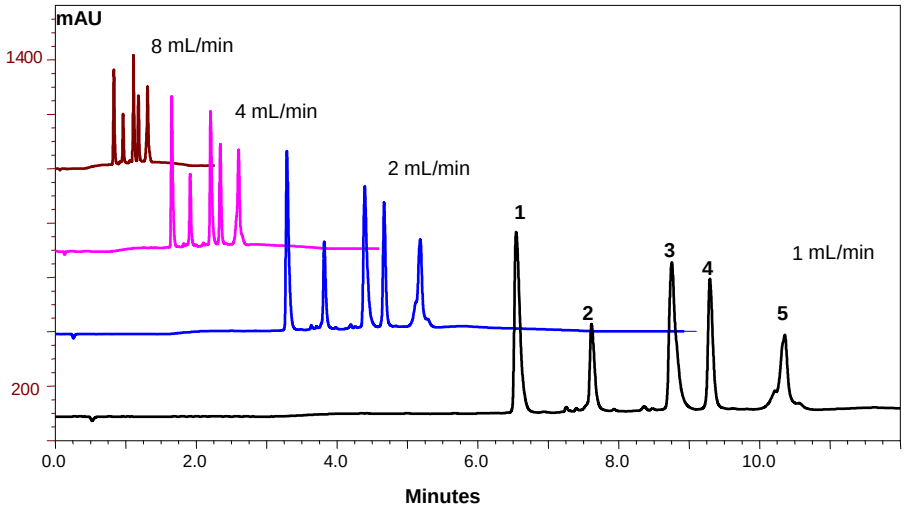


Figure 11
Separation of Protein Standard Mixture on RP-2H with Increasing Flow Rates

Column:	RP-3U (4.6 x 50mm)
Flow rate:	1or 2 or 4 or 8 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient	1 mL/min: 1-75% B in 12 min 2 mL/min: 1-75% B in 6 min 4 mL/min: 1-75% B in 3 min 8 mL/min: 1-75% B in 1.5 min
Detection:	UV at 214nm
Sample:	Protein Mixture (from Sigma)
Injection volume:	5 µL
Order of elution:	1. Ribonuclease A (1.5 mg/mL) 2. Cytochrome C (0.5 mg/mL) 3. BSA (1.5 mg/mL) 4. Carbonic anhydrase (0.9 mg/mL) 5. Ovalbumin (1.5 mg/ mL)

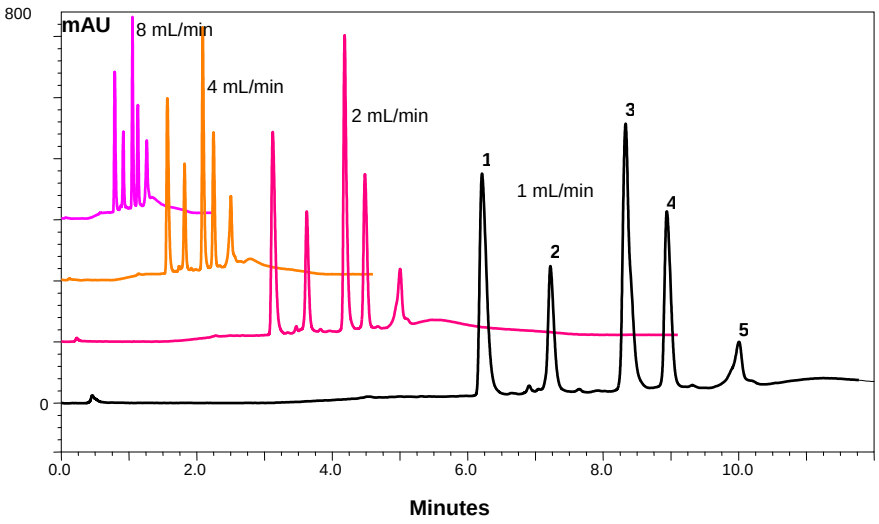


Figure 12
Separation of Protein Standard Mixture on RP-3U with Increasing Flow Rates

Column:	RP-1S (4.6 x 50mm) RP-2H (4.6 x 50mm) RP-3U (4.6 x 50mm)
Flow rate:	1.5 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient	4 mL/min, 1-75%B in 3 min
Detection:	UV at 214 nm
Sample:	Protein Mixture (from Sigma)
Injection volume:	5 µL
Order of elution:	1. Ribonuclease A (1.5 mg/mL) 2. Cytochrome C (0.5 mg/mL) 3. BSA (1.5 mg/mL) 4. Carbonic anhydrase (0.9 mg/mL) 5. Ovalbumin (1.5 mg/ mL)

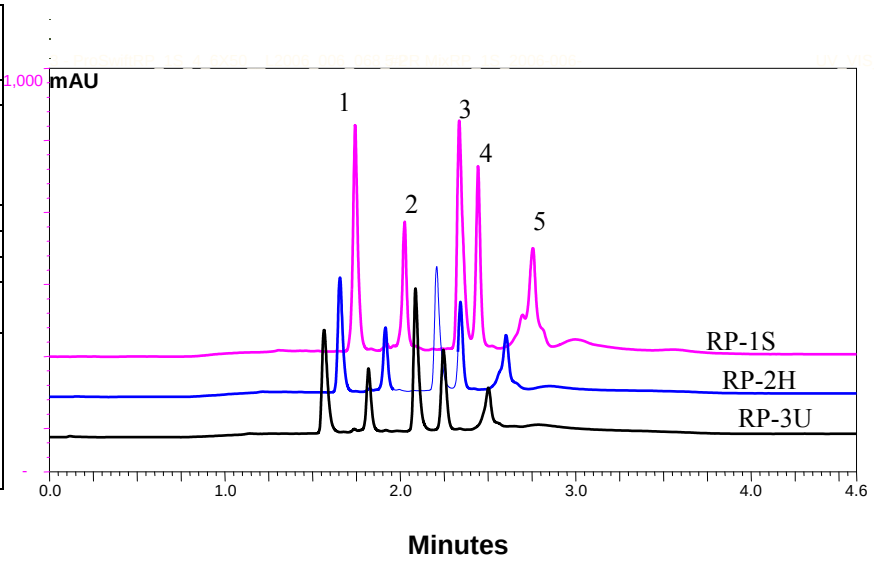


Figure 13
Separation of Protein Mixture on RP Monoliths: Comparison of RP-1S, RP-2H and RP-3U Columns

5.2. Separation of Peptides on the ProSwift RP-1S Column

Figure 14 below shows the separation of peptide standard mixture on the ProSwift RP-1S column at 1 mL and 4 mL flow rates.

Column:	RP-1S (4.6 x 50mm)
Flow rate:	1 mL/min or 4 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) B: DI H_2O / CH_3CN (5:95: v/v)
Gradient	1-25%B in 6 min (1 mL/min) or 1-25%B in 1.5 min (4 mL/min)
Detection:	UV at 214 nm
Sample:	Peptide Mixture (from Sigma)
Injection volume:	5 µL
Order of elution:	1. Gly-Tyr (MW 238.2) 2. Val-Tyr-Val (MW 379.5) 3. Methionine Enkephalin Acetate (Tyr-Gly-Gly-Phe-Met) (MW 573.7) 4. Leucine Enkephalin (Tyr-Gly-Gly-Phe-Leu) (MW 555.6) 5. Angiotensin II acetate (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) (MW 1046.2)

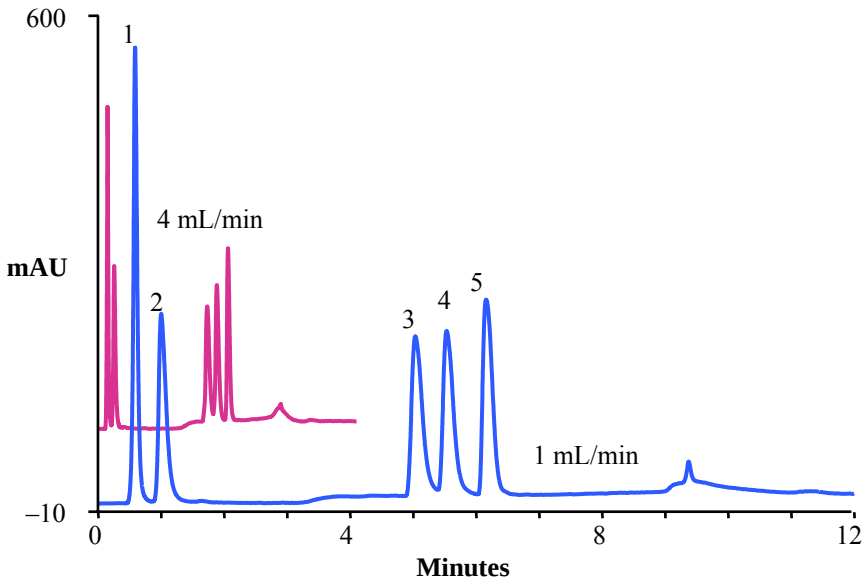


Figure 14
Separation of Peptide Standard Mixture on RP-1S at Different Flow Rates

5.3. Separation of Peptides and Proteins Standards on ProSwift RP Columns

Column:	RP-1S (4.6 x 50mm) RP-2H (4.6 x 50mm) RP-3U (4.6 x 50mm)
Flow rate:	1.5 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) B: DI H_2O / CH_3CN (5:95: v/v)
Gradient	1-64%B in 17 min
Detection:	UV at 214 nm
Sample:	Protein / Peptide Mixture
Injection volume:	5 µL
Order of elution:	1. Methionine enkephalin acetate (tyr-gly-gly-phe-met) (MW 573.7) 2. Leucine enkephalin (tyr-gly-gly-phe-leu) (MW 555.6) 3. Angiotensin II acetate (asp-arg-val-tyr-ile-his-pro-phe) (MW 1046.2) 4. Physalaemin (glu-ala-asp-pro-asn-lys-phe-tyr-gly-leu-met) (MW 1265.4) 5. Substance P acetate (arg-pro-lys-pro-gln-gln-phe-phe-gly-leu-met) (MW 1347.6) 6. Ribonuclease A 7. Cytochrome C 8. Carbonic anhydrase 9. Bovine serum albumin

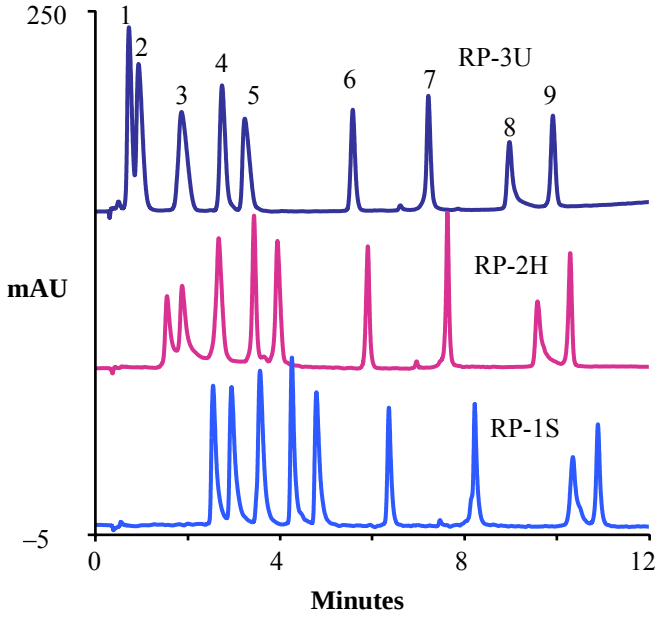


Figure 15
Separation of Peptides and Protein Standards on RP-1S, RP-2H, and RP-3U Columns

5.4. Separation of Peptides: Cytochrome C Trypsin Digest

Column:	RP-1S (4.6 x 50mm)
Flow rate:	1 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient	1-50%B in 12 min
Detection:	UV at 214 nm
Sample:	Cytochrome C-Trypsin digest (2 mg Cytochrome C per mL + 20 µg Trypsin O/N incubation)
Injection volume:	10 µL

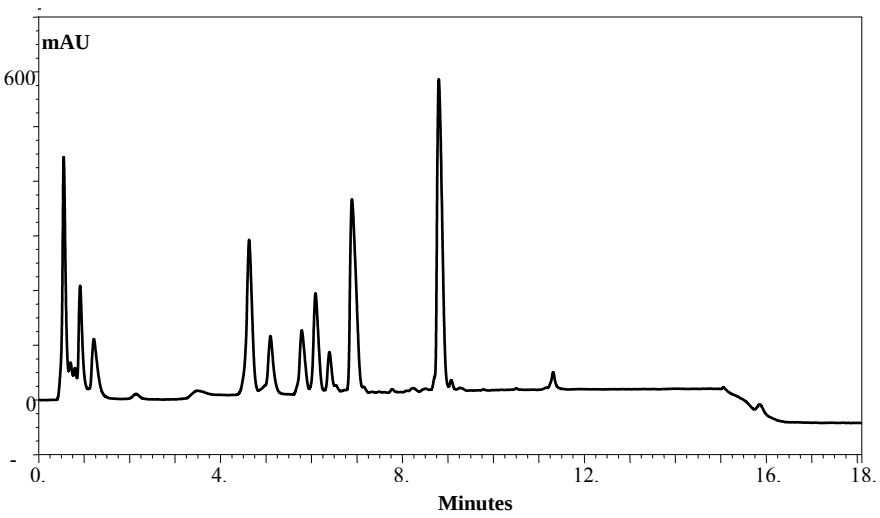


Figure 16
Separation of Peptides: Cytochrome C Trypsin Digest on RP-1S Column

5.5. Separation of Complex Protein Mixtures and other Biomolecules

5.5.1. Separation of Bovine Serum Proteins

The large channels of ProSwift monoliths are particularly suitable for large protein separations. Serum albumin and IgG are major constituents of serum, comprising about 75% of the total protein content. Figure 17 shows the separation of bovine serum proteins using a ProSwift RP-2H monolith. BSA and IgG are separated from each other and from other components. The ProSwift monolith's high permeability supports similar resolution even at much higher flow rates (4 mL/min) with much higher throughput.

Column:	RP-2H (4.6 x 50mm)
Flow rate:	1 or 4 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient:	1 mL/min: 1-75% B in 12 min 4 mL/min: 1-75% B in 3 min
Detection:	UV at 214nm
Sample:	Bovine Serum (1 part + 4 parts of Eluent A).
Injection volume:	5 µL

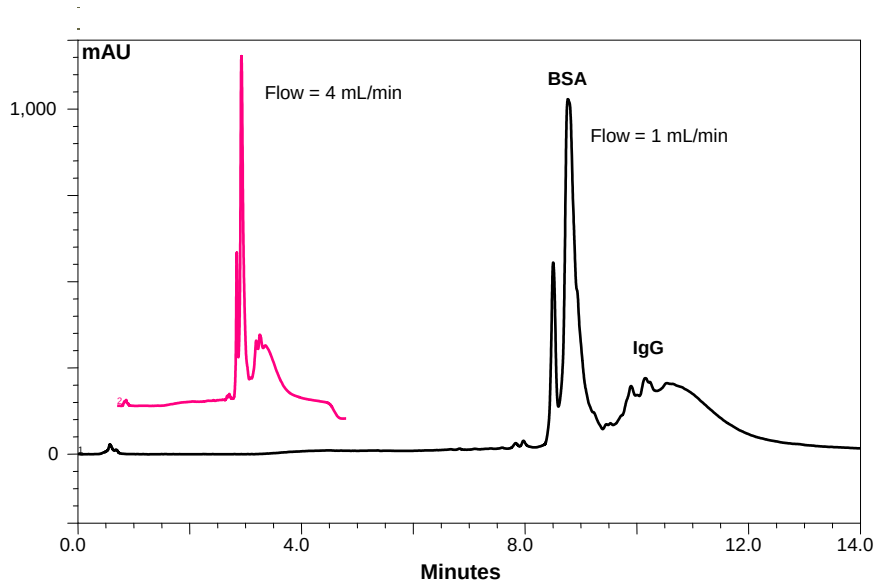


Figure 17
Separation of Bovine Serum Proteins on RP-2H Column

5.5.2. Separation of Snake Venom Proteins and Peptides

The uniquely engineered pore structure of ProSwift monoliths enables resolution of biomolecules with a wide range of molecular weights. The separation of snake venom proteins/peptides by ProSwift RP-1S is shown in Figure 18. RP-2H is shown in Figure 19. Snake venoms are composed of hydrolytic enzymes, a complex mixture of polypeptides (proteases, polypeptidases), 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. Productivity is improved using the higher flow rate supported by the ProSwift monoliths (4 mL/min.).

Column:	RP-1S (4.6 x 50mm)
Flow rate:	1 or 4 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient	1 mL/min: 1-75% B in 12 min 4 mL/min: 1-75% B in 3 min
Detection:	UV at 214nm
Sample:	Viper venom proteins (2.5 mg/mL)
Injection volume:	5 µL

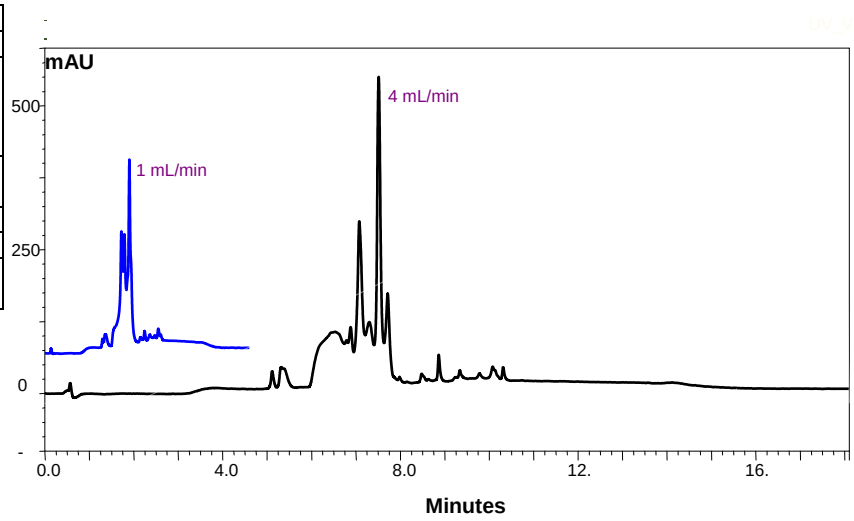


Figure 18
Separation of Snake Venom Proteins on RP-1S Column

Column:	RP-2H (4.6 x 50mm)
Flow rate:	1 or 4 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient:	1 mL/min: 1-75% B in 12 min 4 mL/min: 1-75% B in 3 min
Detection:	UV at 214nm
Sample:	Viper venom proteins (2.5 mg/mL)
Injection volume:	5 µL

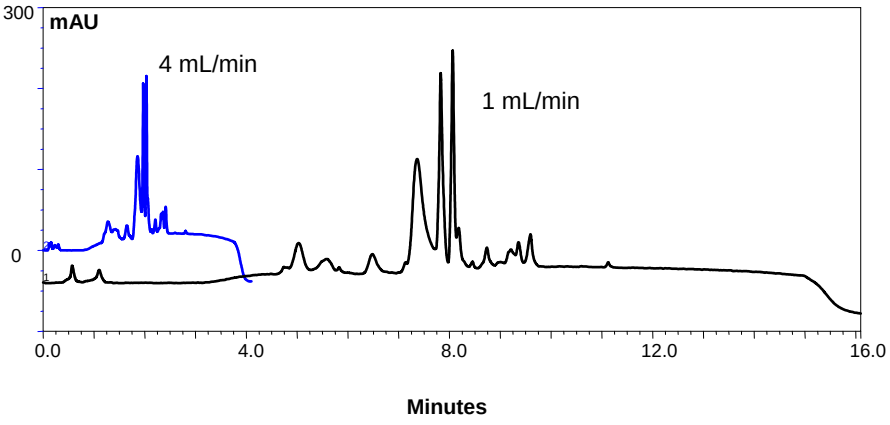


Figure 19
Separation of Snake Venom Proteins on RP-2H Column

5.5.3. Separation of Pancreatin

The separation of Pancreatin on ProSwift RP-2H is shown in Figure 20. Pancreatin contains a variety of protease (protein digesting enzymes like trypsin and pepsin), amylase (carbohydrate digesting enzyme), and lipase (fat digesting enzyme). The separation shows an early eluting unbound peak and a bound fraction. Bound fraction is made up of several resolved peaks. The identities of individual peaks are not assigned. Productivity is improved using the higher flow rate supported by the ProSwift monoliths (4 mL/min).

Column:	RP-2H (4.6 x 50mm)
Flow rate:	1or 4 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient:	1 mL/min: 1-75% B in 12 min 4 mL/min: 1-75% B in 3 min
Detection:	UV at 214 nm
Sample:	Pancreatin (21mg crude protein suspended in 1mL water, centrifuged and the clear supernatant used.)
Injection volume:	5 µL

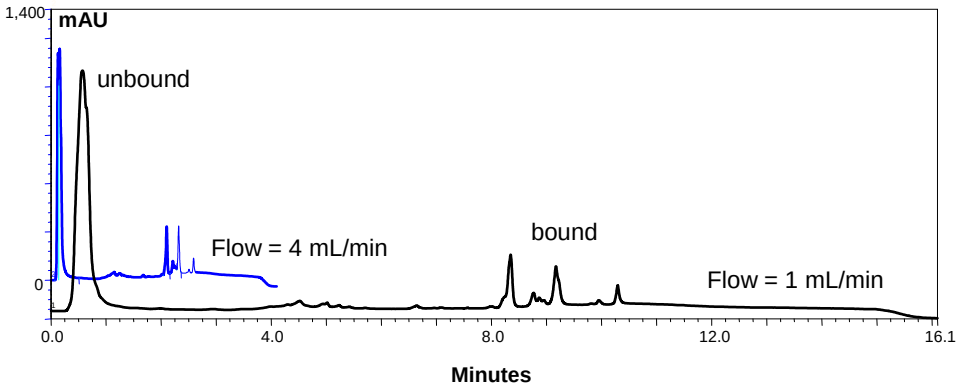


Figure 20
Separation of Pancreatin on RP-2H Column

5.5.4. Separation of Thrombin

Figure 21 shows separation of Thrombin on a ProSwift RP-2H column. Thrombin is a serine protease that converts fibrinogen to fibrin monomers in the blood coagulation process. It activates Factor XIII and interacts with other coagulation factors to initiate platelet secretion and aggregation. Thrombin exists in multiple isoforms. Alpha (α)-thrombin is biologically active, but β and γ are not. The identities of individual peaks are not established. ProSwift columns allow high flow rates with low back pressures thus increasing the productivity.

Column:	RP-2H (4.6 x 50mm)
Flow rate:	1or 4 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient	1 mL/min: 1-75% B in 12 min 4 mL/min: 1-75% B in 3 min
Detection:	UV at 214nm
Sample:	Thrombin (200 µg/mL)
Injection volume:	5 µL

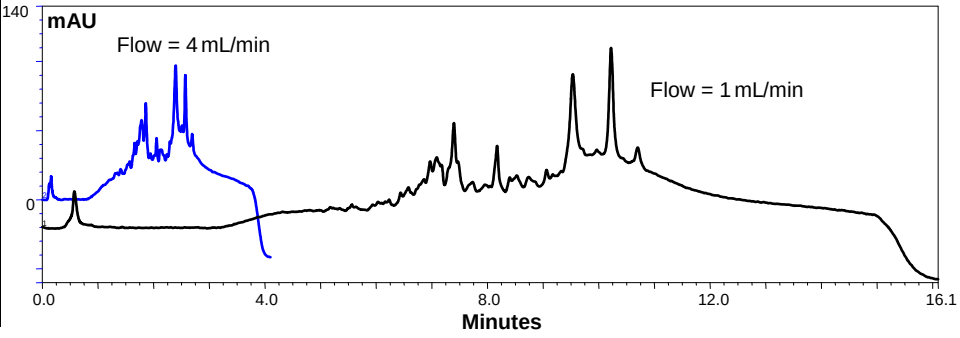


Figure 21
Separation of Thrombin on RP-2H Column

5.5.5. Separation of Pegylated Proteins

Pegylation technology is widely used for the modification of proteins, peptides, antibody fragments, and other biomolecules. Pegylated species provide advantages over the underivatized molecules by increasing bio-availability with extended half-life in blood circulation of the drug and an optimized pharmacokinetics profile. Different PEG derivatized (MW range from 2,000 to 40,000 Da) proteins / peptides are used for various applications.

Figure 22 shows the separation of pegylated chymotripsin (PEG: 5,000 MW) and chymotripsin on a ProSwift RP-3U column.

Column:	RP-3U (4.6 x 50mm)
Flow rate:	1 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Gradient	40-65% B in 6 min
Detection:	UV at 214 nm
Sample:	Chymotrypsin/ Pegylated Chymotrypsin (1mg / mL)
Injection volume:	10 µL

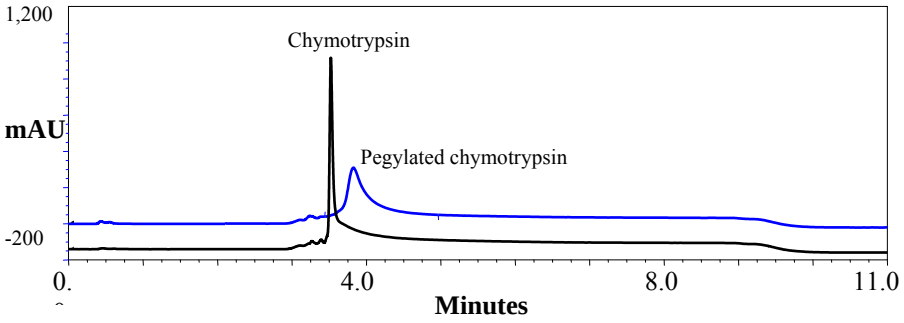


Figure 22
Separation of Pegylated Chymotrypsin on RP-3U Column

5.6. Comparison of ProSwift RP-2H with a Competitor

ProSwift monoliths are designed to minimize the problems associated with low diffusivity and steric hindrance that limit resolution of large molecules in conventional porous HPLC packing materials. Mass transfer of large biomolecules in and out of pores is limited by their low diffusivity and steric hindrance. This results in peak broadening, especially at higher flow rates. Figure 23 compares conventional porous packing material with ProSwift Monolith columns. The ProSwift columns' large channels and pores overcome these limitations, resulting in greater resolution when increasing flow rates.

Columns:	RP-2H (4.6 x 50mm) Competitor (15µ) (4.6 x 100mm)
Flow rate:	2 or 8 mL/min 2 or 8 mL/min
Eluents:	A: DI H_2O / CH_3CN (95:5: v/v) + 0.1% TFA B: DI H_2O / CH_3CN (5:95: v/v) + 0.1% TFA
Detection:	UV at 214 nm
Sample:	Mixture of five proteins.
Injection volume:	5 µL
Order of Elution:	1. Ribonuclease A (1.5 mg/mL) 2. Cytochrome C (0.5 mg/mL) 3. BSA (1.5 mg/mL) 4. Carbonic anhydrase (0.9 mg/mL) 5. Ovalbumin (1.5 mg/ mL)

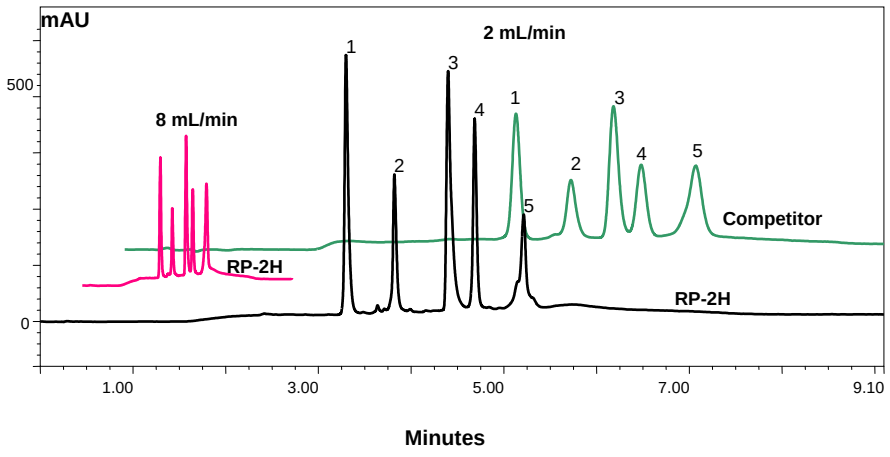


Figure 23
Separation of Protein Standard Mixture
Comparison of Competitor Column with RP-2H Column

6. TROUBLESHOOTING

The purpose of the Troubleshooting Guide is to help solve operating problems that may arise while using ProSwift (RP-1S, RP-2H, and RP-3U) 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 4
RP-1S, RP-2H and RP-3U Troubleshooting Summary

Observation	Cause	Action	Reference Section
High back pressure	Unknown	Isolate blocked component	6.1.1
	Plugged column bed supports	Clean, regenerate or replace column	6.1.2
	Other system modules	Disconnect, replace	System module manual
High background noise	Bad eluents	Remake eluents	6.2
	Contaminated columns	Clean column	6.2.2
	Contaminated hardware	Clean component	6.2.3
Poor resolution	Poor efficiency due to large system void volumes	Re-plumb System	6.3.1
	Column headspace	Reverse column orientation. If that does not work, replace column	6.3.1.A
	Contamination of the column or frit	Clean up and regenerate	6.3.1.B
Short retention times	Un-equilibrated System	Lengthen first eluent time before injecting	6.3.3.C
	Flow rate too fast	Recalibrate pump or reduce flow	6.3.2.A
	Bad eluents	Remake eluents	6.3.2.B
	Column contamination	Clean column	6.3.2.C
Poor front end resolution	Bad eluents	Remake eluents	6.3.3.A
	Column overloading	Reduce sample size or concentration	6.3.3.B
	Insufficient column equilibration	Increase pre-equilibration time	6.3.3.C
	Sluggish injection valve	Service valve	6.3.3.D
	Large system void volumes	Re-plumb system	6.3.1.A 6.3.3.E
Spurious peaks	Sample contamination	Pre-treat samples	6.4.A
	Sluggish injection valve	Service valve	6.4.B
	Contaminated eluents	Test contamination, and remake eluents	6.4.C

6.1. High Back Pressure

6.1.1. 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 Eluent Filter. The filter should be positioned between the pump and the injection valve. Make sure you have a 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. If necessary, measure the pump output at various flow rates by collecting the flow of deionized water into a pre-weighed graduated cylinder. Calculate the flow rate based on the collected volume of eluent as a function of time.

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, 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's components (injection valve, column(s), and detector) one by one, while watching the system pressure. The pressure should increase up to a maximum of 1,200 psi (8.3 MPa) at a flow rate of 2.0 mL/min when the 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.

6.1.2. Clogged Column Bed Support Assemblies

If the column inlet frit or the media is determined to be the cause of the high back pressure, clean the column by pumping eluent in the reversed direction, or regenerate the columns using the methods described in Appendix B 4, "Column Cleanup and Regeneration". Replace the column if the wash and regeneration method does not help.

6.2. High Background or Noise

6.2.1. 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.

6.2.2. 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."

6.2.3. 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 mA_{260}. If it is not, check the detector cell by injecting deionized water directly into it. See the appropriate manual for details.

6.3. Poor Peak Resolution

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

6.3.1. 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 tubing with an i.d. < 0.010" to make all eluent liquid line connections between the injection valve and the detector cell inlet on 4-mm systems. Make all tubing lengths as short as possible. Check for leaks. Similarly, column headspace can cause a loss of efficiency. If you suspect column headspace, perform the procedure in the column QuickStart (Section 4.1) in the reverse direction. If this does not help, replace the column.

B. Contamination of media or frit due to irreversible binding of sample can be responsible for the loss of column efficiency. Use stronger solvent to clean up the media with the column clean-up or regeneration method. Refer to Appendix B, “Column Care” for recommended column cleanup procedures.

6.3.2. Poor Resolution Due to Shortened Retention Times

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

A. Check the eluent flow rate. Measure the eluent flow rate after the column using a stopwatch and graduated cylinder. If it is different than the flow rate specified by the analytical protocol, recalibrate the pump.

B. Check to see if the eluent compositions and concentrations are correct.

   For isocratic analysis, an eluent that is too strong will cause the peaks to elute faster. 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. This may be a problem when one of the proportioned eluents is less than 5%. Use one reservoir containing the correct eluent composition to see if this is the problem.

   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 - Column Care, for recommended column cleanup procedures.

   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.

6.3.3. 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 to the first eluent. Increase the amount of time that 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.

6.4. Spurious Peaks

A. The column may be contaminated. If the samples contain an appreciable level of non-polar components and the column is used with a weak eluent system, these components may contaminate the analytical column. The retention times for the analytes will then decrease and spurious, inefficient (broad) peaks can show up at unexpected times. Clean the column as indicated in Appendix B, “Column Care.”

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 torqued (see valve manual). Check to see that there are no restrictions in the tubing connected to the valve. Also check the valve port faces for blockage and leaks. 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 re-torqueing 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, re-make the eluents. 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 RP columns, refer to Appendix B.5, “Column Cleanup Procedure”. If this does not help, please contact Dionex Technical Support.

6.5. 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.

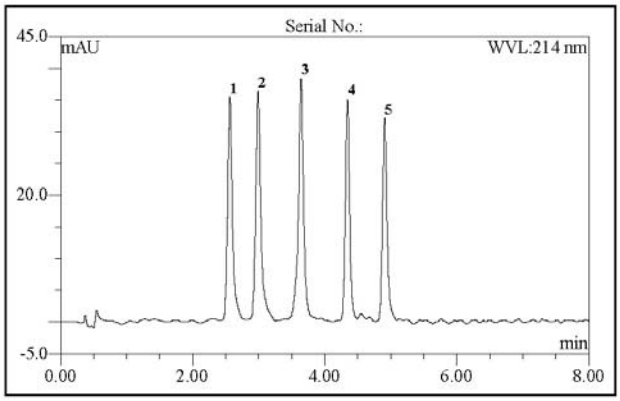
APPENDIX A - QUALITY ASSURANCE REPORT

A.1 ProSwift RP-1S

ProSwift™ RP-1S
4.6 x 50 mm
Product No. 064297

Date: 02-Jun-06 23:25
Serial No. :
Lot No. :

Mobile Phase A:	0.10% TFA in 95/5 Water/Acetonitrile (v/v)
Mobile Phase B:	0.10% TFA in 95/5 Acetonitrile/Water (v/v)
Gradient:	1% to 30% B in 8 minutes
Flow Rate:	1.5 mL/min
Temperature:	Ambient
Detection:	UV 214 nm
Injection Volume:	10 µL
Storage Solution:	100% Acetonitrile



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Resolution (EP)	Peak Width (50%) (min)	Concentration (µg/mL)
1	Met-Enkephalin	2.56	1.4	3.38	0.07	30
2	Leu-Enkephalin	2.99	1.4	5.08	0.08	30
3	Angiotensin II	3.64	0.9	5.89	0.07	30
4	Physalaemin	4.34	1.1	4.85	0.07	30
5	Substance P	4.91	1.2	n.a.	0.07	30

QA Results:

Component	Parameter	Specification	Results
	Pressure	<=990	801
Physalaemin	Asymmetry	0.95-1.7	Passed
Substance P	PW (50%)	0.05-0.11	Passed
Substance P	Ret. Time	4.71-5.10	Passed
T1/T2	Ret.Time Ratio	1.27-1.41	Passed

T1 = Ret.Time of Substance P minus Physalaemin
T2 = Ret.Time of [Leu]-enkephalin minus [Met]-enkephalin

Production Reference:
Datasource: MonoBio 6.70 Build 1820 (Demo-Installation)

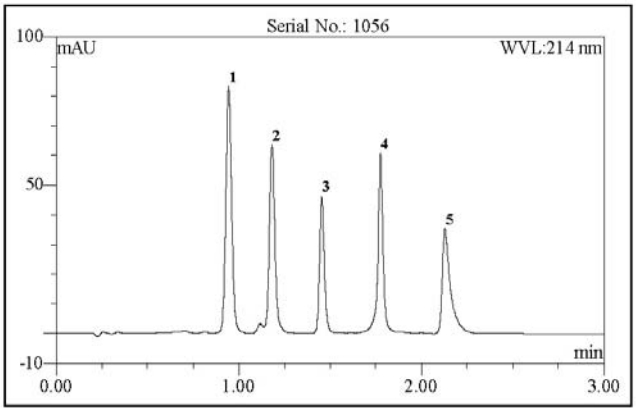
Chromeleon® Dionex Corp. 1994-2006

A.2 ProSwift RP-2H

ProSwift™ RP-2H
4.6 x 50 mm
Product No. 064296

Date: 09-May-06 09:44
Serial No. : 001056
Lot No. : 006-06-057

Mobile Phase A: 0.10% TFA in 95/5 Water/Acetonitrile (v/v)
Mobile Phase B: 0.10% TFA in 95/5 Acetonitrile/Water (v/v)
Gradient: 1% to 60% B in 3 minutes
Flow Rate: 2.5 mL/min
Temperature: Ambient
Detection: UV 214 nm
Injection Volume: 10 µL
Storage Solution: 100% Acetonitrile



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Resolution (EP)	Peak Width (50%) (min)	Concentration (µg/mL)
1	Angiotensin II	0.94	1.1	4.35	0.03	30
2	Substance P	1.18	1.1	5.52	0.03	30
3	Ribonuclease A	1.45	1.2	7.23	0.03	30
4	Cytochrome C	1.78	0.9	6.17	0.03	30
5	BSA	2.13	2.1	n.a.	0.04	30

QA Results:

Component	Parameter	Specification	Results
	Pressure	<=528	358
Ribonuclease A	Asymmetry	0.95-1.5	Passed
BSA	PW (50%)	0.03-0.05	Passed
Cytochrome C	Ret. Time	1.75-1.80	Passed
T1/T2	Ret.Time Ratio	1.40-1.50	Passed

T1 = Ret.Time of BSA minus Cytochrome C
T2 = Ret.Time of Substance P minus Angiotensin II

Production Reference:
Datasource: MonoBio 6.70 Build 1820 (Demo-Installation)

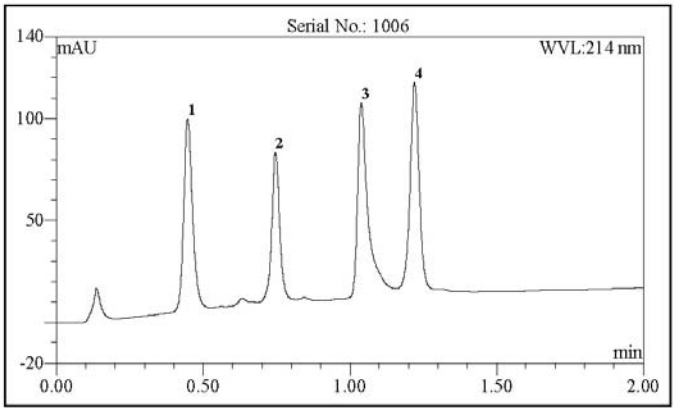
Chromeleon® Dionex Corp. 1994-2006

A.3 ProSwift RP-3U

ProSwift™ RP-3U
4.6 x 50 mm
Product No. 064298

Date: 20-Mar-06 19:15
Serial No. : 001006
Lot No. : 006-06-002

Mobile Phase A: 0.10% TFA in 95/5 Water/Acetonitrile (v/v)
Mobile Phase B: 0.10% TFA in 95/5 Acetonitrile/Water (v/v)
Gradient: 15% to 80% B in 3 minutes
Flow Rate: 4.0 mL/min
Temperature: Ambient
Detection: UV 214 nm
Injection Volume: 10 µL
Storage Solution: 100% Acetonitrile



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Resolution (EP)	Peak Width (50%) (min)	Concentration (mg/mL)
1	Ribonuclease A	0.45	1.2	6.01	0.03	0.10
2	Cytochrome C	0.75	1.1	5.82	0.03	0.05
3	BSA	1.04	2.2	3.32	0.03	0.10
4	Carbonic Anhydrase	1.22	1.0	0.82	0.03	0.10

QA Results:

Component	Parameter	Specification	Results
	Pressure	<=495	203
Cytochrome C	Asymmetry	0.95-1.4	Passed
BSA	PW (50%)	0.02-0.04	Passed
BSA	Ret. Time	1.01-1.06	Passed
T1/T2	Ret.Time Ratio	0.55-0.69	Passed

T1 = Ret.Time of Carbonic Anhydrase minus BSA
T2 = Ret.Time of Cytochrome C minus Ribonuclease A

Production Reference:
Datasource: MonoBio 6.70 Build 1820 (Demo-Installation)

Chromeleon® Dionex Corp. 1994-2006

APPENDIX B - COLUMN CARE

B.1 Recommended Operating Pressure

The maximum recommended operating pressure for ProSwift RP-1S, RP-2H, and RP-3U columns is < 2800 psi (<190 Bar <19.30 Mpa). Operating a column above its recommended pressure limit can cause irreversible loss of column performance.

B.2 Column Start-Up

The ProSwift RP-1S, RP-2H, and RP-3U are shipped in 100% acetonitrile. The columns should be washed and conditioned using the QuickStart method.

B.3 Column Storage

For storage, use 100% acetonitrile. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

B.4 Column Cleanup and Regeneration

If the column inlet frit or the media is fouled by sample or eluent contaminants, these may be removed by treatment with solvent, acid, base, or denaturant. Appropriate solubilizing agents (6 M Guanidine HCl) and detergents (1-2% SDS) and other clean-up solution should be chosen to remove organic, acid-soluble, or base-soluble contaminants with the following general cleanup procedures.

CAUTION ***Always ensure that the cleanup protocol used does not switch between eluents that may create high-pressure eluent interface zones in the column. High-pressure zones can disrupt the uniformity of the monolith and irreversibly damage its performance.***

B.5 Column Cleanup Procedure

1. Prepare the following eluents:
Eluent A: HPLC grade water
Eluent B: 80:20 THF: Water (v/v)

2. Connect the column to a LC system in the *reversed flow* direction and install a 1 mL loop

3. Set the pump flow rate to 0.50 mL/min.

4. Run the following eluents in sequence:
   a. 10 CV (ca. 7.5 ml for 4.6 x 50 mm column) 5% eluent B.
   b. 20 CV gradient from 5% to 100% eluent B.
   c. 5 CV gradient from 100% to 5% eluent B.
   d. 10 CV of 5% eluent B.

5. Load 1 mL denaturant of choice (Formic acid or 1M HCl or 1M NaOH or 2% SDS or 6M guanidine HCl)
Switch to 100% eluent A, inject the denaturant and run 10 CV of eluent A.

6. Repeat steps 4 and 5 several times with the same or different denaturants until a constant baseline is achieved during the gradient run in step 4.

7. Reconnect the ProSwift column in its proper orientation.

8. Equilibrate the column with eluent before resuming normal operation.

APPENDIX C - REFERENCES

1. Rapid Reversed-Phase Separation of Proteins and Peptides using Optimized 'Moulded' Monolithic Poly(Styrene-co-Divinylbenzene) Columns. S. Xie, R. W. Allington, F. Svec, J. M. J. Fréchet, *J. Chromatogr. A*, 865, 169, 1999.
2. Fast/High Resolution Monolithic Capillary Columns for High Throughput Biomolecular Separation, B. Kunnummal, S. Xie, T. Jiang, J. Kraska and R. W. Allington, *LC/GC*, 20, June 2004.
3. Solid Phase Extraction, S. Xie, T. Jiang, F. Svec, Monolithic Materials: Preparation, Properties, and Applications, Chapter 4.3, Journal of Chromatography Library, Vol. 67: Monolithic Materials - Preparation, Properties and Applications, 2003.
4. 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.