PRODUCT MANUAL

for

ProSwift® ConA-1S

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PRODUCT MANUAL

FOR

ProSwift ConA-1S column

5 x 50mm, PEEK P/N 074148

DIONEX® Corporation
Document No. 065346
Revision 02
August 2010

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1. INTRODUCTION

1.1. Principles of affinity chromatography

Affinity chromatography is used for sample purification based on the reversible interactions between the target molecules and the specific ligands immobilized on a chromatographic matrix. The process usually consists of three steps: (1) bind, (2) wash and (3) elute. In the binding step, samples containing the target molecules are loaded onto the column and allowed to bind. In the washing step, components in the sample that do not bind to the ligand are washed off the column, while the target molecules are retained. In the eluting step, specific components in an eluting solution are used to break the target-ligand interactions and then elute the target molecules. The column can them be equilibrated to remove the competing elution components.

1.2. Lectins and Con A

Lectins are a group of proteins of non-immune origin that can recognize and bind to specific sugars. They have been important tools in purification and analysis of glycoconjugates due to their recognition towards specific carbohydrate structures. Most of the lectin-carbohydrate interactions are reversible and can be inhibited by the inhibiting sugar of the lectin. This makes lectins ideal candidates as affinity ligands in affinity purification of glycoconjugates, such as glycoproteins, glycopeptides and glycans.

Concanavalin A (Con A) is a lectin derived from *Canavalia ensiformis* (Jack bean) seeds. At neutral and alkaline pH, Con A exists as a tetramer of four identical subunits with a total molecular weight of approximately 104 kDa. Below pH 5.6, Con A dissociates into active dimers of 52 kDa. Con A is one of the most well characterized and widely used lectins. It binds to α-mannose, and to α-glucose with weaker affinity. Divalent metal ions such as calcium (Ca^{2+}) or magnesium (Mg^{2+}) need to be present to keep Con A active for its binding to carbohydrates. Figure 1 shows the four monomer units. Each of which binds a calcium and a transition metal, typically manganese.

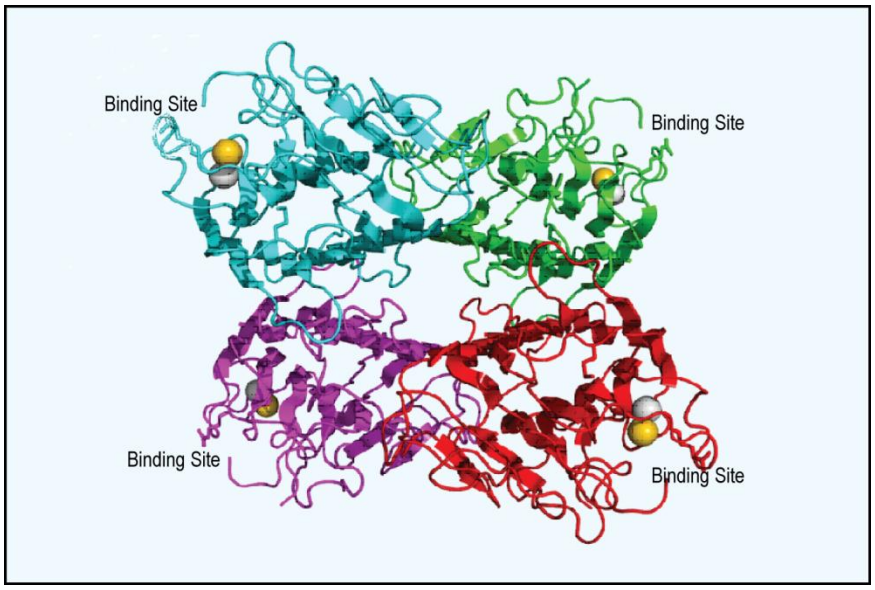


Figure 1

Crystallographic structure of Con A based on the PDB 3CNA crystallographic structure. Graphic rendered with PyMol

As α-mannose is commonly expressed on most glycoproteins, affinity Con A column is a useful tool for purification and enrichment for glycans, glycopeptides, and glycoproteins. Usually high-mannose type glycans bind to Con A strongly, and some hybrid type glycans can bind to Con A with good affinity as well, while complex type glycans usually have very weak affinity towards Con A.

Figure 2 demonstrates the specificity of the ProSwift ConA-1S column. Horseradish peroxidase (HRP), which is a glycoprotein with rich high-mannose type glycans, binds to the Con A column and is only eluted with the inhibiting sugar, α-methyl-mannopyranoside (bottom trace). When HRP is loaded and the column is flushed with galactose the HRP is not released. This is because galactose has a very poor affinity to Con A and therefore does not compete with the Con A-HRP interaction (middle trace). When the galactose is switched to mannose, which will compete for the Con A binding, the HRP is eluted.

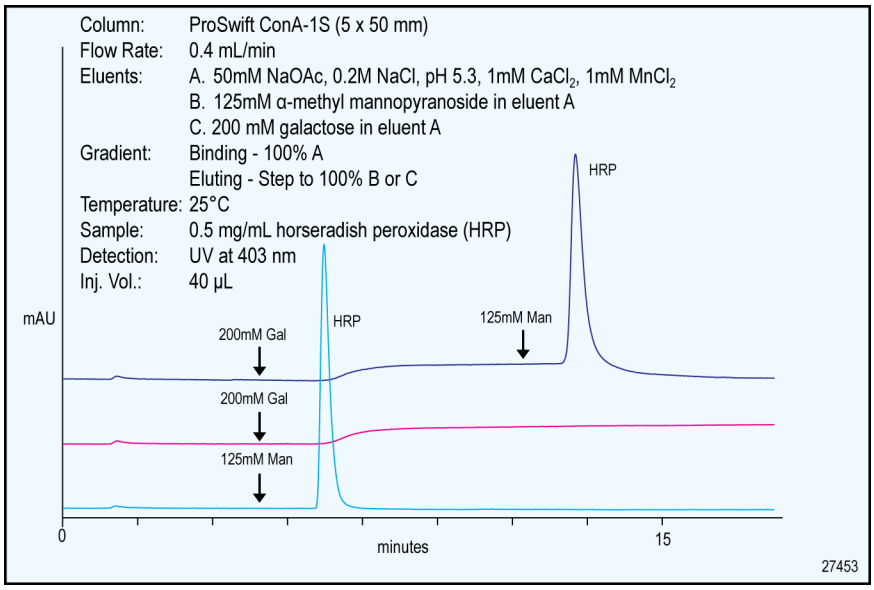


Figure 2

Elution of HRP on the ProSwift ConA-1S (5x50mm) Column with Different Sugars

1.3. Monolith Technology

The ProSwift ConA-1S column is specifically designed to provide fast enrichment or laboratory-scale purification for glycans, glycopeptides, and glycoproteins. The HPLC compatibility of this column allows automation, provides higher throughput and more accurate analysis. ProSwift Con A columns are polymeric monoliths prepared by in-column polymerization, followed by functionalization with Con A. The monolith is a cylindrical polymer rod containing uninterrupted, interconnected, through pores, with surface area intermediate between porous and non-porous bead-based columns. The monolith structure contains pore sizes in two ranges (shown in Figure 3), 0.62 µm pores contribute high surface area enabling high ligand density and 3.25 µm pores that allow reduced operational backpressure. This approach results in small mass-transfer distances that produce improved efficiency, even at elevated flow-rates. High quality Con A is covalently attached to the monolith column through their amine groups. The sugar binding sites are protected during the conjugation process so the Con A activity is well maintained.

The ProSwift affinity monolith offers increased loading capacity as well as improved efficiency. The format allows rapid automation of loading, binding, elution and collection using Dionex systems.

1.3.1. Column Parameters:

Column Dimension:	5×50mm
Protein Coated On Monolith:	Concanavalin A
Bi-Modal Monolith Pore Diameter:	3.25 and 0.62 µm
Accessible Surface Area	5.5 m^2/g
Ligand Density	~14 mg/mL Con A
Bed Height	39 mm
Bed Volume	0.98 mL
Binding capacity	~2mg HRP/column

1.3.2. Typical Operating Parameters:

Operating Flow range:	0 to 1.0 mL/min
Maximum flow:	2.0 mL/min
Eluent pH range:	pH 5-8
Operating Temperature:	≤ 30°C
Pressure:	≤ 2000 psi
Organic Solvent Limit:	10% Methanol

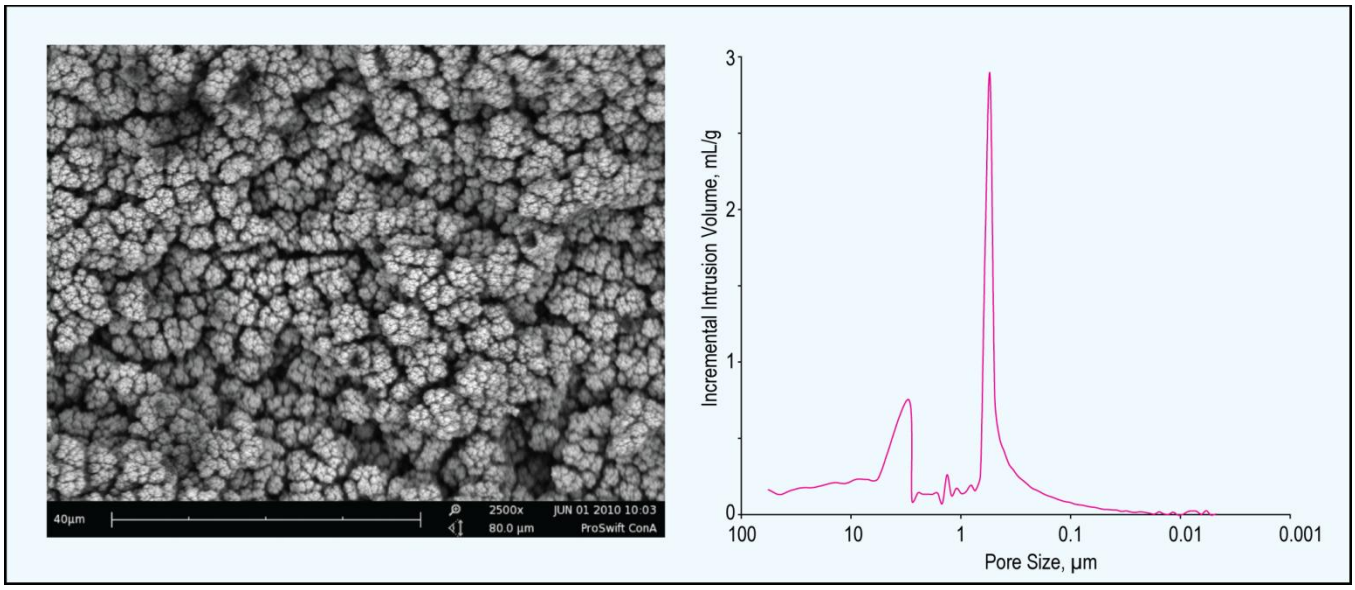


Figure 3

SEM image and pore size distribution of ProSwift ConA-1S 5x50mm

2. CHROMATOGRAPHY SYSTEM SET-UP

The ProSwift ConA-1S column can be used on any HPLC system that is compatible with high salt aqueous buffers, although a Dionex HPLC system is highly recommended. The common set-up of a chromatography system for this column should contain a gradient pump, an autosampler, a thermal compartment, and a detector. A fraction collector is beneficial when collection of eluted samples is needed.

2.1. Detection

For glycoproteins and glycopeptides, UV absorbance at 280nm or 214nm is commonly used for detection in chromatography analysis. The UV detector should be connected immediately after the ProSwift Con A column. UV detectors from various manufacturers can be used. (However, the UVD 170/340U, VWD-300x or PDA100 from Dionex are highly recommended.) Various cell volumes and path lengths are available to suit analytical or semi-preparative applications.
Glycan samples are usually labeled with fluoresphores and detected with a fluorescence detector. Fluorescence detectors from various manufacturers can be used. Dionex fluorescence detectors (FLD-3100, FLD-3400) are recommended.

2.2. Pumps

Gradient LC pumps from any manufacturer can be used. The pump should have at least two channels to allow step or linear gradient of sugar elution of glycoconjugate samples from the ProSwift ConA-1S column. Due to the use of chloride salts Dionex recommends the use of inert systems to prevent column damage due to metal leaching.

2.3. Injectors

An autosampler is used to inject samples into the system. A manual injector can be used if an autosampler is not available.

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 is highly recommended. This can also be beneficial for temperature sensitive samples.

3. OPERATION

3.1. Eluent

The ProSwift ConA-1S column can be used with most of the common buffers and eluents used in biological analysis, provided that they are compatible with Con A and the samples. Acetate and Tris buffers are two commonly used buffers for Con A affinity columns. Phosphate buffer should be avoided, as calcium is needed to maintain Con A activity while calcium phosphate is essentially insoluble in water. High concentration salt such as 0.2M NaCl in the eluents can be used to help prohibit non-specific binding of protein samples to the column. Although the following eluents are recommended for applications in this manual, the optimal buffer can vary for different applications. Method development is needed for different applications to find the optimal conditions.

Eluent A: 50mM NaOAc, 0.2M NaCl, pH 5.3, with 1mM $CaCl_2$, 1mM $MgCl_2$
Eluent B: 100mM α-methyl-mannopyranoside in eluent A

All chemicals should be at least ACS reagent grade. Deionized water with resistance of 18.2 mΩ-cm should be used. All eluents need to be filtered through 0.2µm filters before use.

CAUTION	***Although some literature states that Ca^{2+}, Mg^{2+}, and Mn^{2+} are all required to maintain binding activity of Con A, our results have shown that Con A is still active with only Ca^{2+} present. At low pH, such as pH 5.3, all these three metals can be added if preferred. However, when alkaline pH is used, only Ca^{2+} can be added as Mg^{2+} and Mn^{2+} will form insoluble hydroxides and precipitate resulting in serious column and system damage.***

3.2. Column Installation

Install the column on the LC instrument in the correct flow direction as indicated on the column label.

3.3. Column Start-up

The column is shipped in the recommended eluent A (50mM NaOAc, 0.2M NaCl, pH 5.3, 1mM $CaCl_2$ 1mM $MgCl_2$) with 0.1% NaN_3. After the column is installed, gradually increase the flow rate to 1 mL/min over 2 minutes. Pump at least 20 mL of eluent A through the column to remove the sodium azide and equilibrate the column.

3.4. Common Gradient and Equilibration

A step gradient with mannose solution is usually used to purify glycoconjugate samples on the Con A column. As alpha-methyl-mannopyranoside has much higher affinity to Con A than mannose, alpha-methyl-mannopyranoside is recommended as a more efficient eluting reagent. After sample elution, wash the column with eluent A to remove the alpha-methyl mannospyranoside and the column can be recovered. Equilibration time is flow rate dependant. The column should be equilibrated with **at least 10 column volumes (10 mL at 1 mL/min)** eluent A prior to next sample loading.

3.5. Sample Preparation

As the Con A bound to the column a protein, samples containing proteases may cause column deterioration and decreased capacity. Any protease should either be removed from the samples or protease inhibitors added to the sample to avoid any unnecessary reduction in performance.

3.6. Flow Rate

The ProSwift ConA-1S column can be used at a flow rate up to 2 mL/min. The recommended flow rate is 0.5-1 mL/min., higher flow rates will generate higher column pressure (Figure 4), and may also decrease the binding efficiency of the samples to the column.
Figure 5 and Table 1 show that as you increase the flow rate there is little effect on the amount of HRP that binds to the column. The decrease in total area is due to the use of the same data collection rate of the detector.

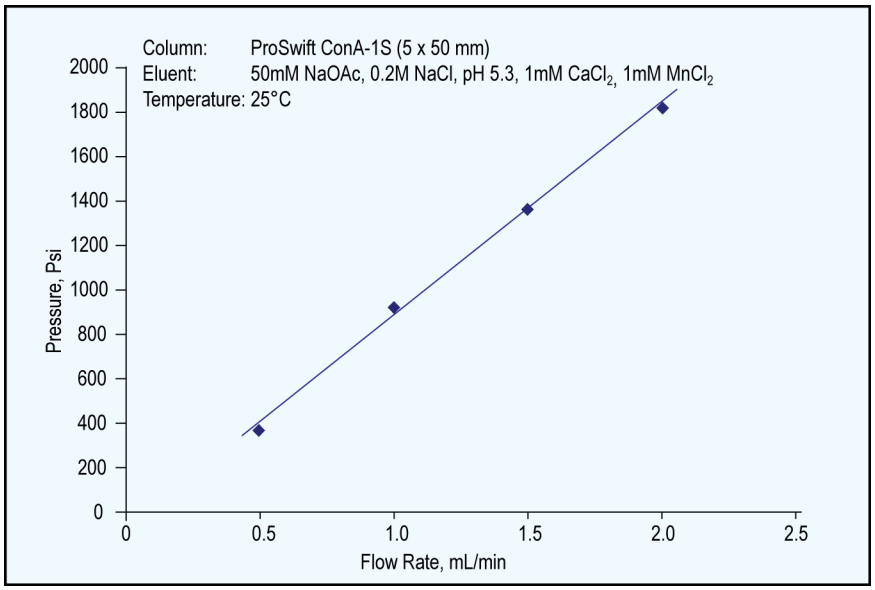


Figure 4

Example of Effect of Flow Rate on Column Backpressure.
(Actual back pressure may vary slightly from column to column)

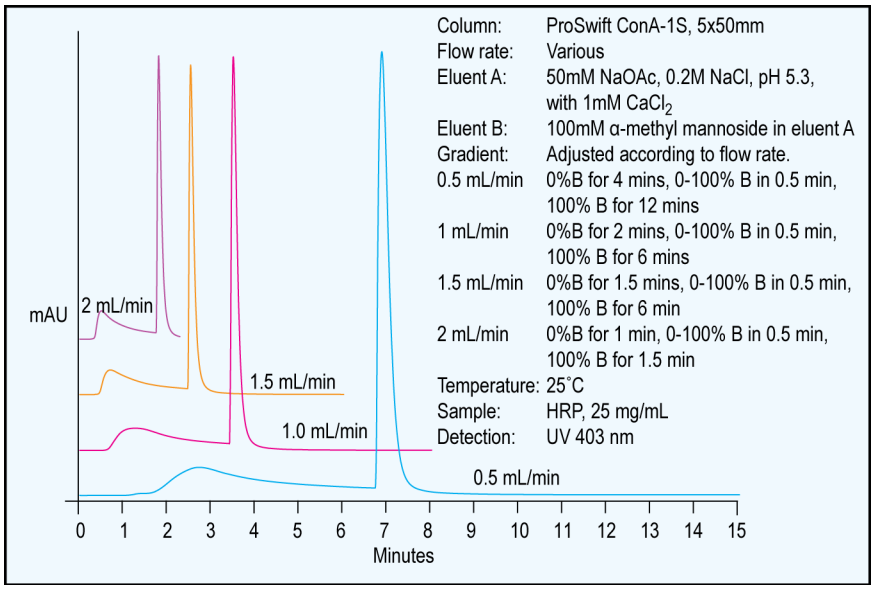


Figure 5

Effect of Flow Rate on Binding

Flow Rate, mL/min	Total Area, mAU.min	Unbound area, mAU.min	Unbound relative area, %	HRP area, mAU.min	HRP relative area, %
0.5	256.69	90.94	35.43	165.75	64.57
1.0	113.17	32.95	29.11	80.22	70.89
1.5	86.87	31.66	36.43	55.24	63.57
2.0	62.83	22.67	36.09	40.16	63.91

Table 1

Effect of Flow Rate on HRP Peak Area

3.7. Loading Capacity

The loading capacity of horseradish peroxidase (HRP), a glycoprotein with rich high-mannose type glycans, is no less than 2.0 mg on the ProSwift ConA-1S column at 1 mL/min flow rate. Figure 6 shows the linearity of area to sample load for HRP when loaded onto the ProSwift ConA-1S column. This correlation allows the ProSwift ConA-1S to be used for quantitation of enriched species.

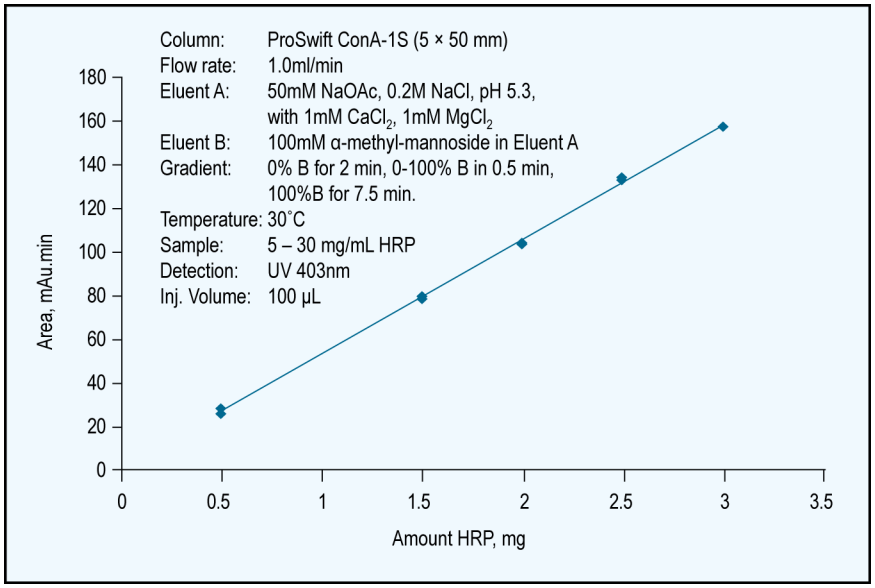


Figure 6

Total area dependence on HRP Loading

3.8. Column Temperature

It is recommended to use the ProSwift ConA-1S at temperatures no higher than 30˚C. To ensure data consistency and to elongate the column life time, it is recommended to use a column oven to control the temperature at 25˚C or lower. Figure 7 shows how HRP is minimally affected by temperature. Proteins with different binding association may be affected differently.

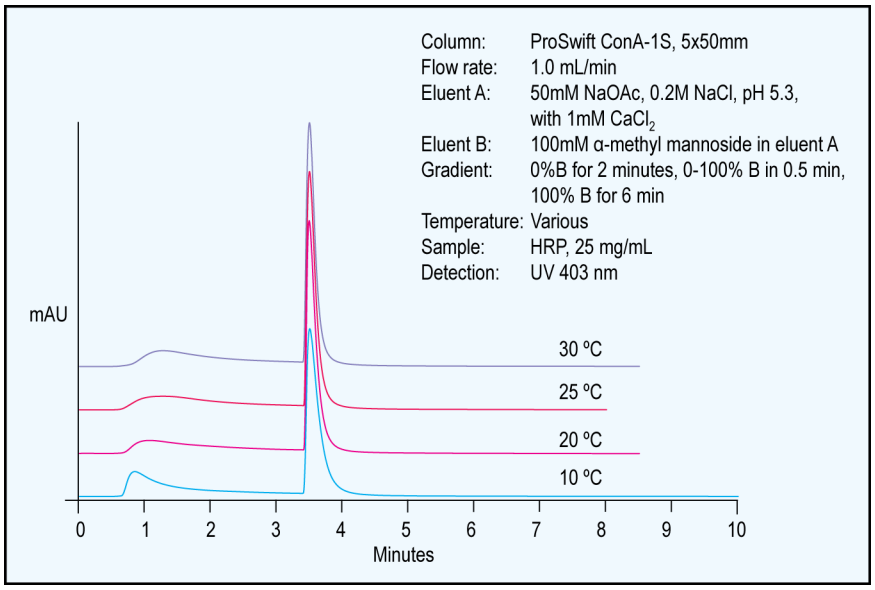


Figure 7

Temperature effect on binding efficiency

3.9. Column Ruggedness

Loading capacity loss over time is commonly observed for affinity columns with protein ligands, as the ligand will gradually lose activity after repeated binding and elution cycles. About 10-20% loading capacity loss of the ProSwift ConA-1S can be expected after one hundred continuous injections at maximum loading. This is shown in Figure 8. The rise in the baseline prior to the target peak is due to column overloading. When lower loading amounts of protein is applied to the column the observable activity loss is negligible. (Figure 9.)

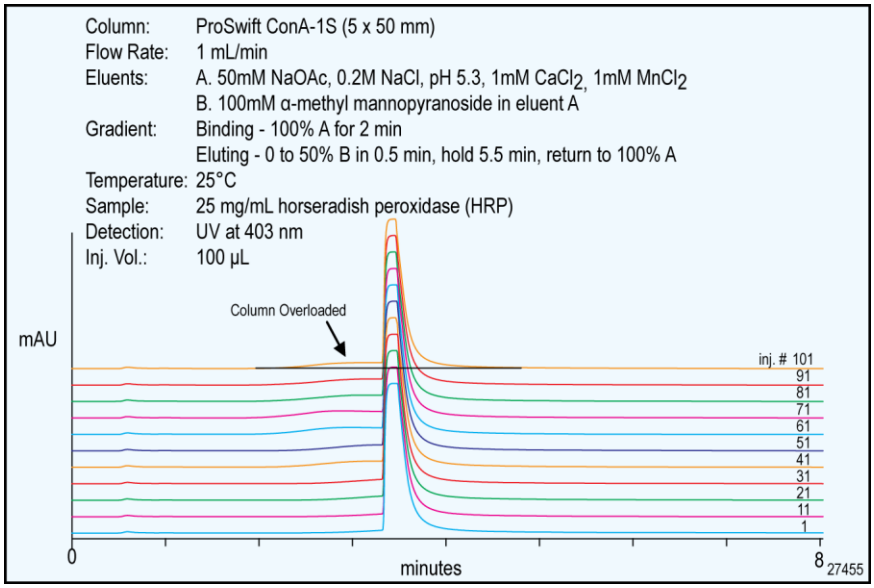


Figure 8

2.5 mg HRP Loaded onto ProSwift ConA-1S (5x50mm) for 100 injections at 1 mL/min.

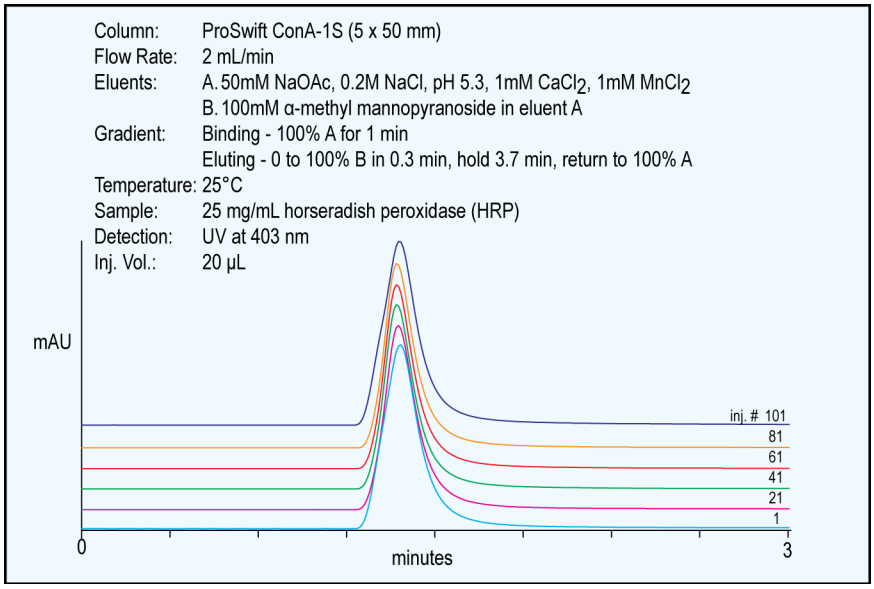


Figure 9

Loading of 0.5 mg HRP for 100 injections at 2 mL/min on the ProSwift ConA-1S (5x50mm).

3.10. pH range

It is recommended to use the ProSwift ConA-1S column at pH 5 – pH 8.

CAUTION	***Note: Although the ProSwift ConA-1S column can be used at slightly higher or lower pH, such as pH 8.5 and pH 4.5, the column degrades faster outside the recommended range. For best lifetime of the column, it is recommended to avoid extreme pH and use it within the recommended pH range.***

3.11. Column Storage

To maintain column lifetime, the ProSwift ConA-1S column should to be stored at 2-8°C when not in use. For short term storage (<2 days), the column may be left in eluent A at room temperature. For long term storage (>2 days), the column should to be stored in the recommended eluent A with 0.1% NaN_3 at 2-8˚C.

4. EXAMPLE APPLICATIONS

4.1. Purification of Horseradish Peroxidase

Con A is used to purify horseradish peroxidase from extraction of horseradish roots. In the chromatograms below pure HRP is applied to the ProSwift Con A column and released with sugar solution. High capacity (>2mg) and good peak efficiency is demonstrated. In this application an analytical UV cell is used. The maximum absorbance of the detector is reached with the high loading. A preparative cell should be used if quantification for high loading sample is needed.

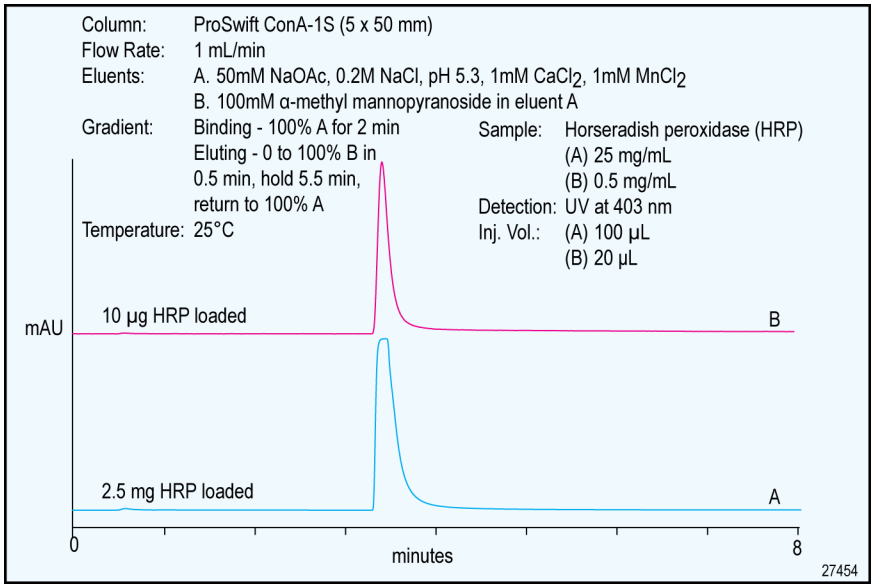


Figure 10

Binding and Elution of HRP on the ProSwift ConA-1S (5x50mm) column.

4.2. Separation of pNP-labeled sugars

The ProSwift ConA-1S column can separate different sugars. As the sugars themselves do not absorb UV, UV absorbing p-nitrophenol (pNP) labeled sugars were used to demonstrate this. The mannose, which specifically binds to the Con A elutes much later than the glucose. This application indicates that different protein glycosylations may be separated using the ProSwift ConA-1S in combination with a gradient of releasing buffer.

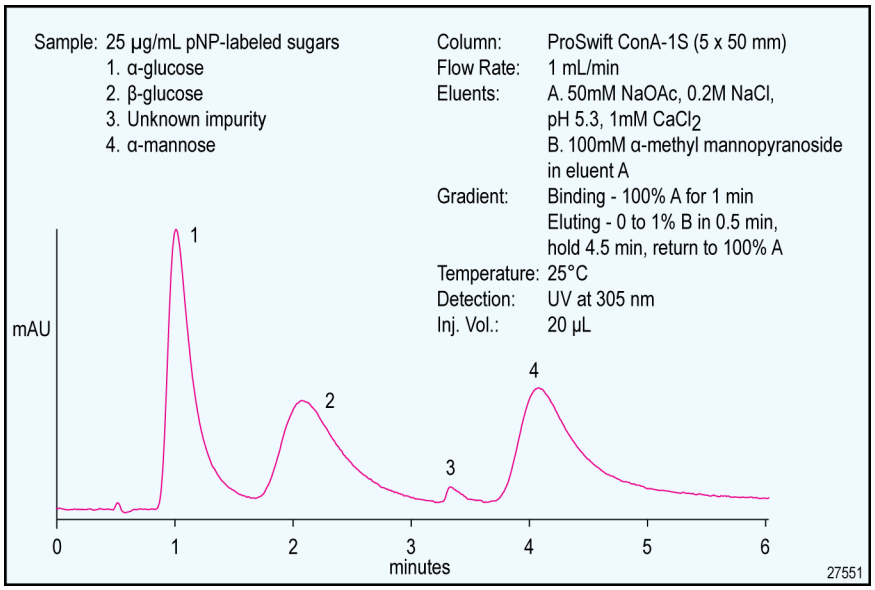


Figure 11

Three pNP-labeled Sugars are Separated on the ProSwift ConA-1S (5x50mm) Column.

4.3. Separation of glycoprotein glycoforms

Protein glycoforms with different affinities to Con A can be separated on the ProSwift ConA-1S column. Chicken ovalbumin was fractionated into an unbound and a bound fraction on this column. The unbound fraction was collected and re-loaded onto the column. The presence of an unbound fraction indicates there are different glycoforms or non-glycosylated species present in the sample.

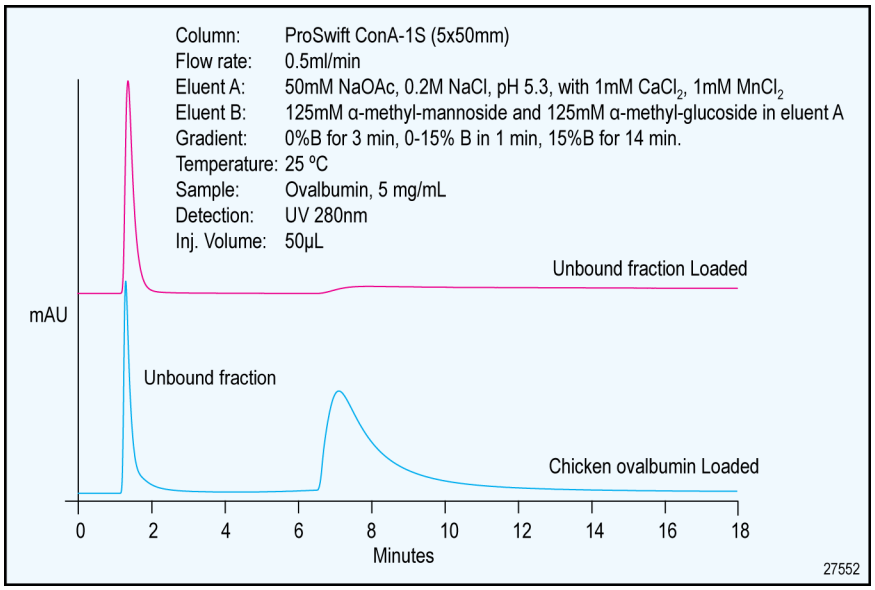


Figure 12

Binding of Ovalbumin glycoproteins on the ProSwift ConA-1S (5x50mm) column.

4.4. Fractionation of glycans

Glycan samples can be fractionated on the Con A column based on their different affinities. Fluorescent labeled serum *N*-glycans were nicely fractionated on the ProSwift ConA-S column (shown in Figure 13).

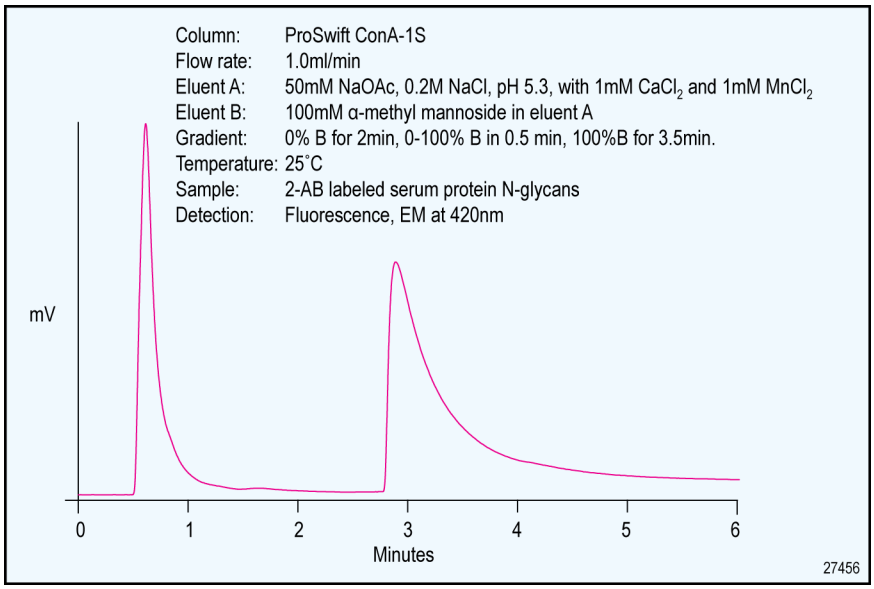


Figure 13

Separation of Glycans of the ProSwift ConA-1S (5x50mm) column.

4.5. Depleted human serum proteins

Depleted human serum proteins were fractionated on the ProSwift ConA-1S column. Enriched bound fractions can be further analyzed with mass spectrometry downstream analysis for identification. Figure 14 show the unbound and bound fraction which can be collected and subjected to further analysis.

Glycation of HSA can cause abnormal biological effects as a result of the formation of Advanced Glycosylation End Products (AGE). This can lead to tissue damage as well as causing mutations and DNA transposition. Elevated glycoalbumin is observed in diabetes mellitus[1] through non-enzymatic glycation via formation of a Schiff base between ε-amino groups of lysine (or arginine) residues and glucose molecules in blood. This reaction can be inhibited in the presence of antioxidant agents[2].

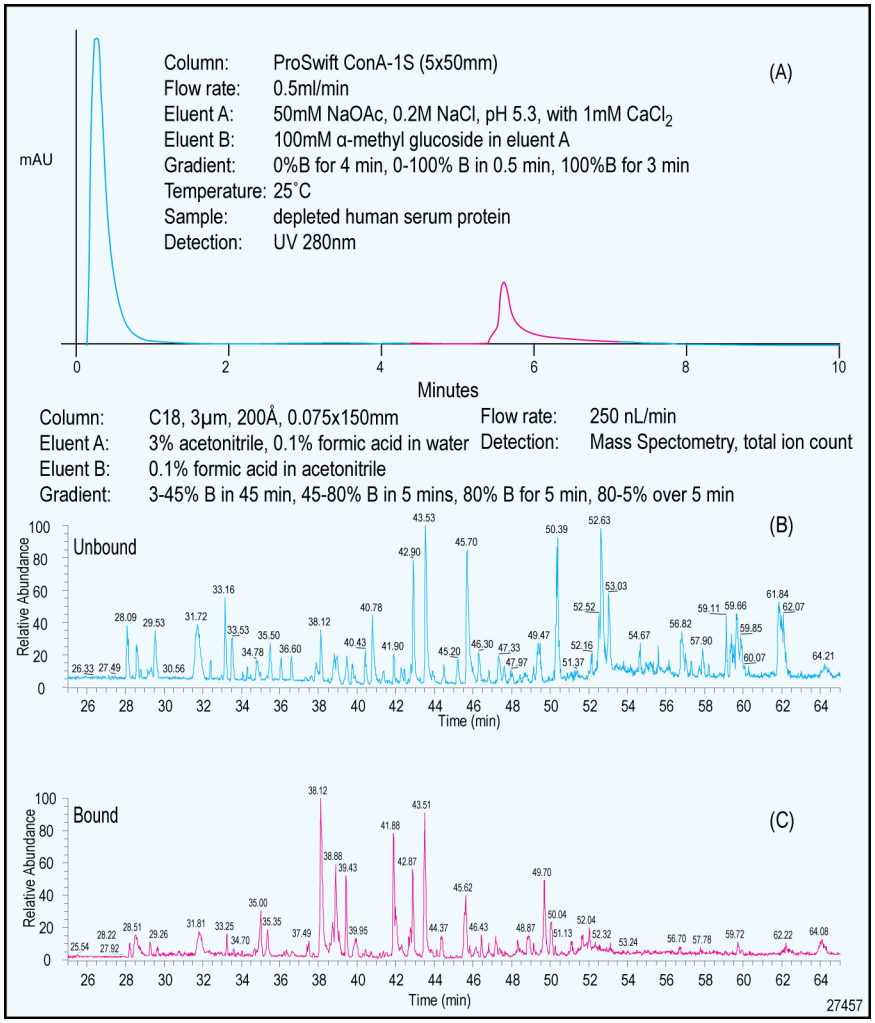


Figure 14

Depleted Human Serum Protein Samples Fractionated on the ProSwift ConA-1S (5x50mm) column with MS analysis.

(Data courtesy of B. Mann)

[1] Iberg N, Flückiger R (1986). "Nonenzymatic glycosylation of albumin in vivo. Identification of multiple glycosylated sites". *J Biol Chem* **261** (29): 13542–5

[2] Jakus V, Hrnciarová M, Cársky J, Krahulec B, Rietbrock N (1999). "Inhibition of nonenzymatic protein glycation and lipid peroxidation by drugs with antioxidant activity". *Life Sci* **65** (18-19): 1991–3

4.6. Enrichment of glycopeptides

Horseradish peroxidase (HRP) was digested using trypsin. The tryptic digest was fractionated on the ProSwift ConA-1S column (Figure 15). The bound and unbound fractions were collected and analyzed on a reversed-phase column (Figure 16). The glycopeptides can clearly be identified.

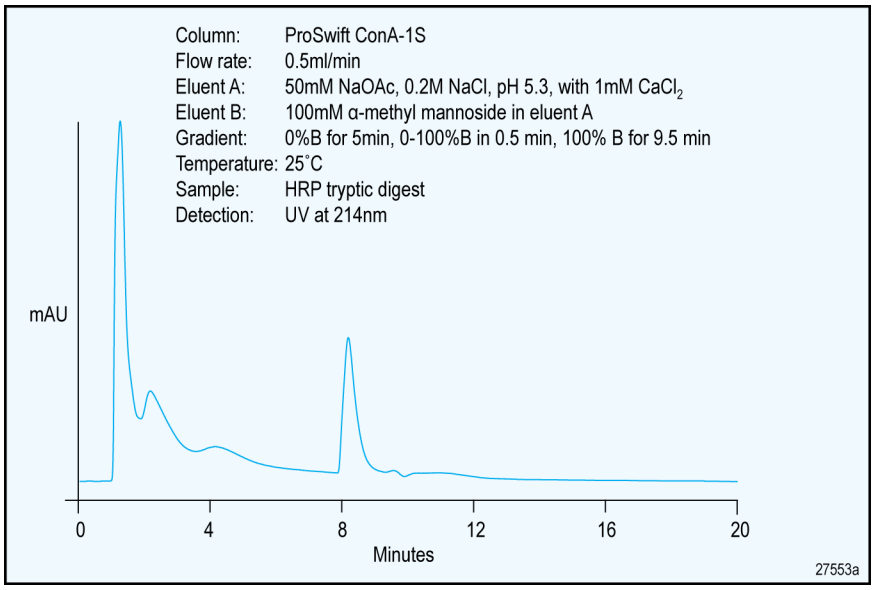


Figure 15

Separation of Tryptic Peptides on the ProSwift ConA-1S (5x50mm) column.

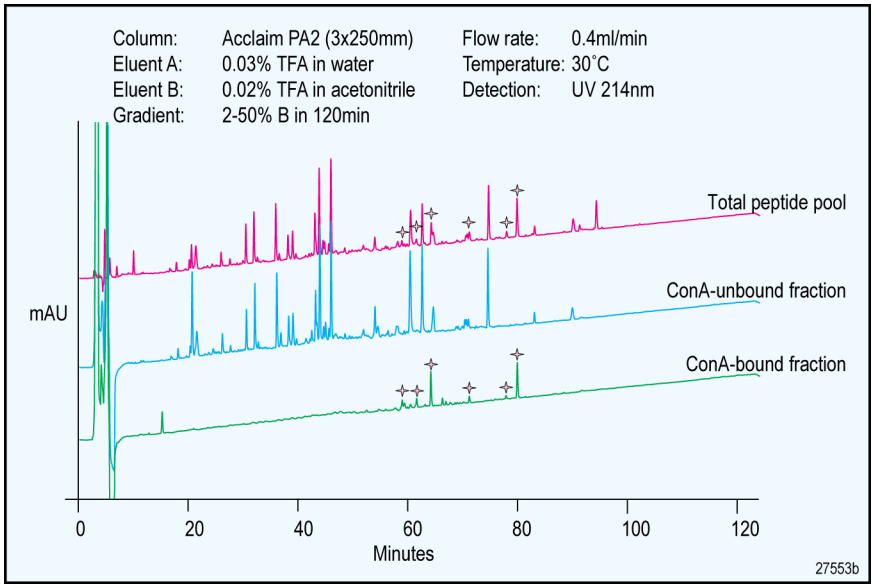


Figure 16

Analysis of Tryptic Digest Fractions from Figure 15

5. TROUBLE SHOOTING

The purpose of the Troubleshooting Guide is to help solve operating problems that may arise while using ProSwift ConA-1S column.

5.1. High Backpressure

If the system pressure is very high, it is advisable to find out what is causing the high system pressure.
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 ~1200 psi (8.3 MPa) at a flow rate of 1.0 mL/min when the 5-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.

5.1.1. System Flow Path:

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.

5.1.2. 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 Section 7.

5.1.3. Flow Rate:

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.

5.2. High Background or Noise:

5.2.1. Contamination of Eluents:

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

5.2.2. 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 Section 7- Column Cleanup.

5.2.3. Contaminated Hardware:

To eliminate the hardware as the source of the high background signal, remove 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 mAu at UV_{280}. If it is not, check the detector cell by injecting deionized water directly into it. See the appropriate detector manual for further details.

5.2.4. Air Bubbles in Detector:

If the pump loses prime, or a large injector loop filled with air is activated, air can enter the system. The air can dissolve at high pressure, but comes out of solution when it elutes from the column (low back pressure), and become trapped in the detector cell. Ensure that the pump is not pumping air, and that the injection system is working properly, and then clear the bubble by applying increased back pressure to the detector cell.

5.3. Low Capacity

If no binding or sudden loading capacity loss is observed, check the following:

5.3.1. Eluents:

Improper eluent concentration or pH may be the problem. Remake the eluent as required for your application. Ensure that the water and chemicals used are of the required purity. In order to activate the binding sites of Con A, Ca^{2+} must be present in both eluent A (loading) and eluent B (eluting).

5.3.2. Samples:

Column overloading may be the problem. If the sample contains more than one species that will bind to the column they will be in competition for binding sites. Reduce the amount of sample injected onto the column by either diluting the sample or injecting a smaller volume onto the column.

Check the glycosylation pattern of the target protein. If the target does not contain mannose residues that are accessible to the Con A the protein will not bind to the column. Some glycoproteins may have different glycoforms, which have different affinities towards the con A column. Therefore, it can be expected that a non-bound fraction and a bound fraction will be observed even if the column is not overloaded.

5.3.3. Column Equilibration:

The column may not be equilibrated well. Equilibrate the column by running through eluent A at 1ml/min for at least 15min.

5.3.4. Flow Rate:

Flow rate can affect binding efficiency and release. During loading, at high flow rates the target protein may pass through the column before having the chance to bind to the column. Reduce the flow rate. During elution, if flow rate used is too low then the released protein may not have time to elute from the column within the specified analysis time. Increase analysis time or increase the flow rate.

5.3.5. Temperature:

High temperatures can be detrimental to the column. Use the column at temperatures under 30˚C with a thermostatted column oven.

5.4. Low Sample Recovery

The sample may bind to the column too strongly. Try washing the column with 0.25M methyl-mannopyranoside and 0.25M alpha-methyl glucopyranoside in the eluting eluent. For more washing options, see Section 7 - Column Cleanup.

5.5. Decreased Detection Sensitivity:

Detection sensitivity may be caused by sample degradation, column degradation leading to increased peak width (lower peak height), or limitations to light throughput in the absorbance detector (e.g., dirty or improperly installed flow cell, near failure of D_2 lamp). Refer to detector manual for clean up procedures. It could also be due to inappropriate detection wavelength. Check wavelength in use.

5.6. 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. At increased flow rates the data collection rate may need to be increased.

5.7. Poor Peak Shape:

When using the ProSwift ConA-1S column to purify concentrated samples containing particulate matter the column inlet frit may become contaminated. You may see a split elution peak as shown in Figure 17. As the monolithic ProSwift columns can be run in either direction without damaging the bed this can often be solved by reversing the column flow direction.

Disconnect the column from the detector and attach the column out let to the pump. Allow at least 10 column volumes of eluent to flow through the column before attaching the inlet of the column to the detector. Direct connection may flush debris into the cell and can damage the cell. This procedure can be carried out many times to extend the lifetime of the column.

It should be noted that the frits used in the ProSwift family of products is not serviceable.

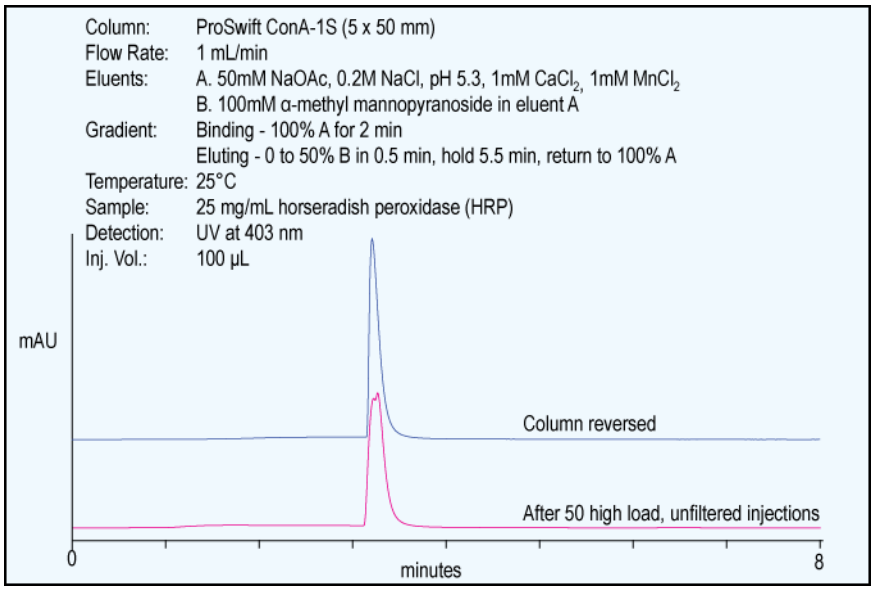


Figure 17

Column contamination on the ProSwift ConA-1S (5x50mm) column

5.8. System Problems:

5.8.1. High Detection Background Caused by the System:

a) Verify the problem is not related to the detector or column.
b) Prepare new eluents with freshly filtered deionized water.
c) Rinse all eluent lines with the new eluents (at least 40mL using the priming syringe)
d) If new eluent introduces high background without the column set installed, your deionized water source, or eluent components have become contaminated.

5.8.2. No Peaks, Poor Peak Area Reproducibility or Unexpectedly Small Peak Area:

a) Check the position and filling levels of sample vials in the autosampler.
b) Check injector needle height setting.
c) Check each line of the schedule for proper injector parameters. Employ full loop methods if other injection modes (partial loop fill) do not provide acceptable reproducibility.
d) Service the injection valve (check for leaks, rotor fragments, or sediments inside the valve)
e) Check sampling needle for bits of vial septa clogging the flow path.

5.8.3. Incorrect or Variable Retention Times:

a) Check your eluent preparation procedure for possible errors.
b) Prime the pump if necessary.
c) Measure the flow rate by weighing out the eluent collected during exactly five minutes of flow. Recalibrate the pump if necessary.
d) Set the eluent composition for 100% for each eluent and draw out at least 40mL of eluent from each of the lines to verify pump is primed.
e) Check and/or service the pump's proportioning valve. With the pumping turned off, the flow through the pump outlet tubing (disconnected from the injector) should be zero in all eluent positions. Check this separately for each eluent line.

For further information on system troubleshooting please refer to the appropriate system component manual.

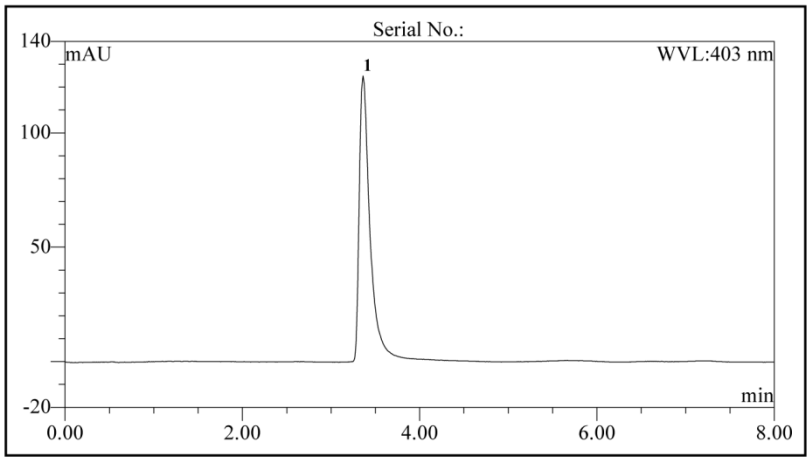
6. QUALITY ASSURANCE REPORTS

6.1. ProSwift ConA-1S (5x50mm)

ProSwift® ConA-1S
5 x 50 mm
Product No. 074148

Date: 22-Dec-09 08:39
Serial No. :
Lot No. :

Eluent A:	50 mM Sodium Acetate, pH 5.3, 0.2M Sodium Chloride, 1mM CaCl2, 1mM MgCl2
Eluent B:	100mM alpha-methyl-mannopyranoside in Eluent A
Equilibration:	100% A for 8 minutes.
Gradient:	100% A for 2 minutes, 0% to 100% B in 0.5 minute, Hold 5.5 minutes.
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Detection:	UV 403nm
Injection Volume:	100 µL of 5mg/mL HRP
Storage Solution:	Eluent A with 0.1% Sodium Azide



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Peak Width (10%) (min)	Peak Width (50%) (min)	Amount (mg)
1	HRP	3.36	2.0	0.26	0.119	0.5

QA Results:

Component	Parameter	Specification	Results
	Pressure	<=1320	735
HRP	Asymmetry (EP)	1.0-2.6	Passed
HRP	Ret. Time	3.2-3.5	Passed
HRP	PW (10%)	<=0.33	Passed
HRP	PW (50%)	<=0.16	Passed

QA Results from Capacity Test:

HRP	Capacity (mg/column)	=>2	Passed

Production Reference:

Datasource: MonoBio
Directory: Production\ConA-1S
Sequence: 074148_PS_CONA-1S_5X50MM_EFFICIENCY
Sample No.: 41

Chromeleon® Dionex® Corporation 1994-2010

Doc.No.: 074150-01 QAR (HRP Efficiency Test) 6.80 SR9a Build 2680 (163077)

7. COLUMN CLEANUP:

The column can be cleaned using the following washing options to remove proteins that bind strongly to Con A either through affinity binding or non-specific interactions.
For flow rates higher or lower than 1 mL/min the equivalent number of column volumes should be used based of a column volume of 1 mL.

7.1. Sugar Wash

1. Add 0.25M alpha-methyl mannopyranoside and 0.25M alpha-methyl glucopyranoside in the recommended eluent A
2. Flush the column at 1 mL/min for 30min.
3. Equilibrate the column with eluent A at 1 mL/min for at least 30min before use.

7.2. Salt Wash

1. Prepare eluent containing 2M NaCl, 50mM NaOAc, pH 5.3, with 1mM $CaCl_2$
2. Flush the column at 1 mL/min for 40-60min.
3. Equilibrate the column with eluent A at 1ml/min for at least 30min before use.

7.3. Solvent Wash

1. Add 10% Methanol to eluent A.
2. Flush the column at 1 mL/min for 40-60min.
3. Equilibrate the column with eluent A at 1 mL/min for at least 30min before use.

CAUTION	***Always use high purity grade chemicals and water to prepare the washing solutions. Always filter the washing solution through 0.2µm filters before use.***
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.***

NOTES

For further information call 1-800-DIONEX-0 (1-800-346-6390)

