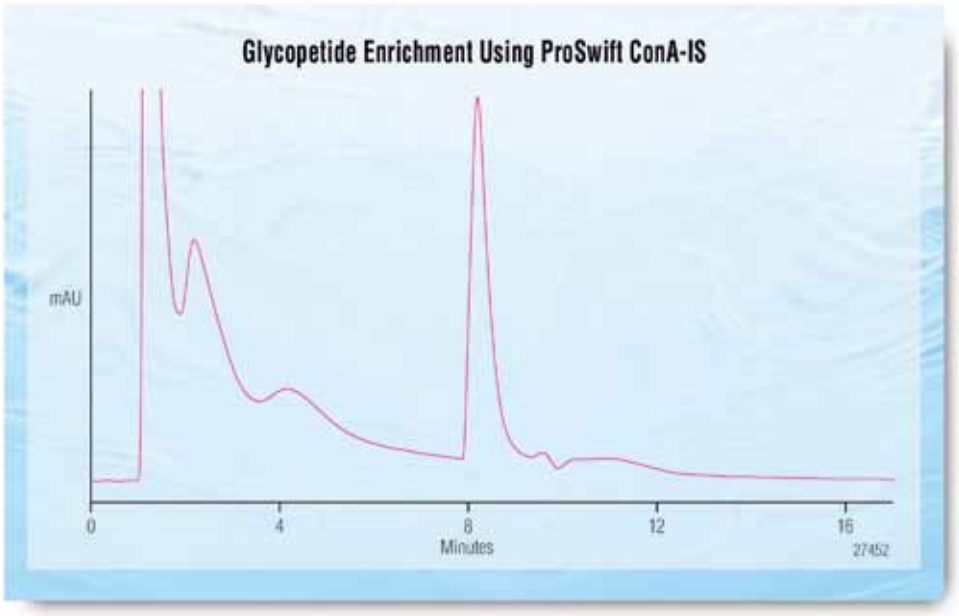
columns

ProSwift ConA-1S Affinity Column for the Enrichment of Glycans, Glycopeptides, and Glycoproteins



ProSwift® ConA-1S affinity monolith column is for the enrichment and purification of Con A binding glycans, glycopeptides, and glycoproteins by HPLC.

- *Highly efficient enrichment and purification*
- *High capacity and ligand density*
- *High sample recovery*
- *Low elution volumes*
- *Fast separations*
- *Reusable; over a hundred purifications*

Now sold under the Thermo Scientific brand

Thermo SCIENTIFIC

Optimal Enrichment and Purification of Con A Binding of Glycans and Glycoconjugates

The ProSwift ConA-1S affinity monolith column is unsurpassed for fast, highly efficient enrichment and purification of Concanavalin A (Con A) binding glycans, glycopeptides, and glycoproteins from complex samples. This HPLC compatible affinity column maintains the specificity and selectivity for Con A binding glycans and glycoconjugates. The high capacity and ligand density of the ProSwift ConA-1S column facilitates the highly efficient enrichment of samples. The high peak efficiency of the column produces sharp narrow peaks resulting in low elution volumes of highly concentrated products. The column is reusable, and over a hundred enrichments and purifications are possible with minimal loss of capacity.

The ProSwift ConA-1S column, designed for and used in HPLC systems, provides many advantages compared to standard manual methods. These advantages include faster separations, better enrichment and sample recovery, efficient washing capabilities, high peak efficiency, and small elution volumes. Other advantages of using the ProSwift ConA-1S column with HPLC systems include automation, reusability, high-throughput capability, and more accurate analysis with on-line monitoring.

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Passion. Power. Productivity.

Column Chemistry

The ProSwift ConA-1S column is a polymeric monolith functionalized with the lectin, Concanavalin A (Con A). The monolith is a cylindrical polymer containing uninterrupted interconnected flow through pores and smooth surfaces. The monolith morphology provides high ligand density and fast mass transfer. The high ligand density gives the column its high capacity and facilitates highly efficient enrichment. The fast mass transfer enables high peak efficiency resulting in highly enriched products in low elution volumes. The monolith morphology also increases the binding rate compared to bead-based media for faster separations. The ProSwift ConA-1S column is efficient with continuous-flow binding and elution which enables fast purifications with concentrated small volume fractions of highly enriched eluted products.

Specificity

The ProSwift ConA-1S column maintains the specificity and selectivity of the Con A lectin, high affinity towards alpha-mannose, and weaker affinity to glucose. High mannose and some hybrid types of glycans have high affinity to Con A. Therefore, samples containing these type of glycans can bind strongly to the ProSwift ConA-1S column. Complex biantennary *N*-glycans usually have very weak affinity to Con A, and generally samples containing only this type of glycan bind weakly to the ProSwift ConA-1S column.

Figure 1 shows how horseradish peroxidase (HRP), a glycoprotein with rich high-mannose type glycans, was strongly retained on the ProSwift ConA-1S column and was only eluted with a specific Con A inhibiting sugar, α-methyl-mannopyranoside. Washing with galactose, a non-inhibiting sugar, did not displace and elute the protein. The HRP protein was retained on the column through Con A-mannose interactions. This demonstrates the excellent mannose-binding specificity of ProSwift ConA-1S column.

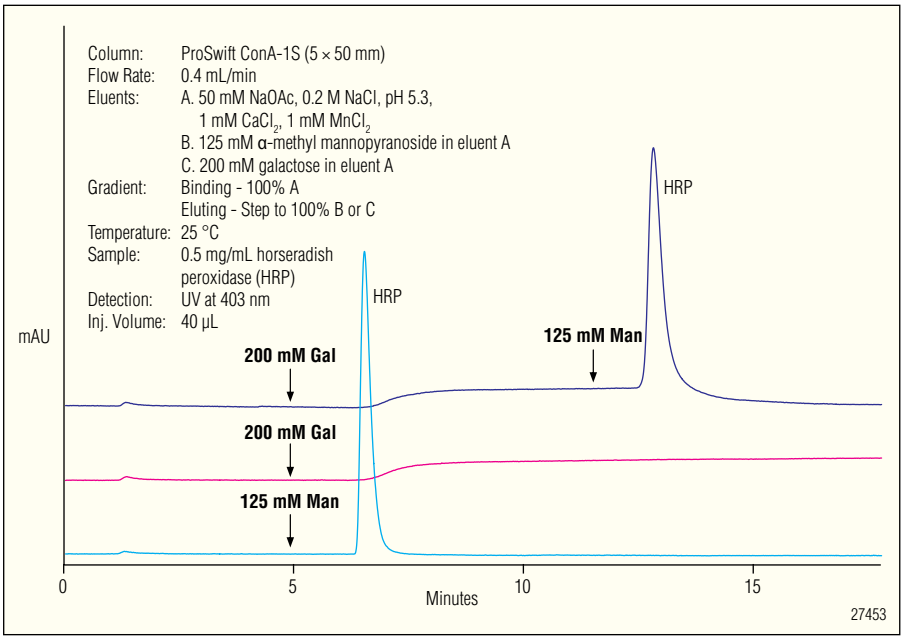

Figure 1. Mannose-binding specificity of Con A demonstrated using HRP on the ProSwift ConA-1S column.

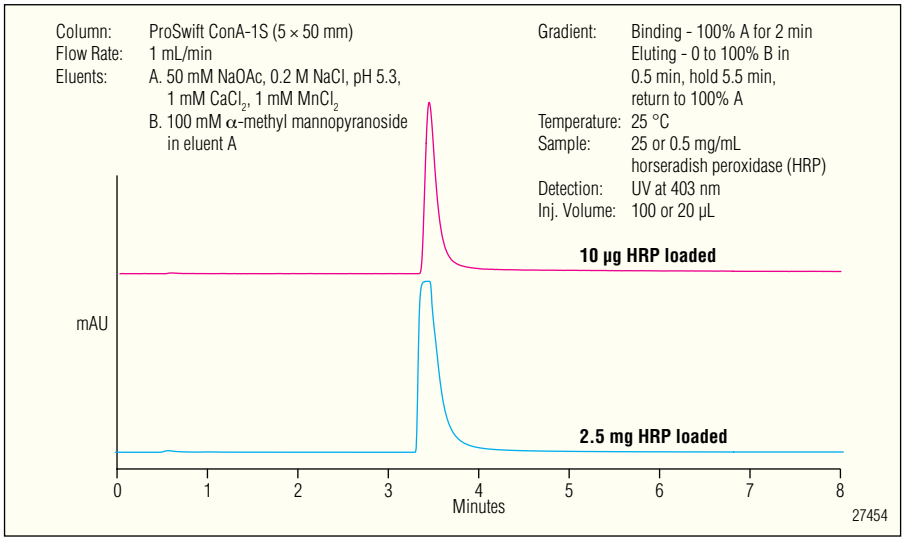

Figure 2. Horseradish peroxidase (HRP) injected at high (2 mg) and low (10 µg) loading on the ProSwift ConA-1S column.

High Capacity and Peak Efficiency

The high capacity and peak efficiency of the ProSwift ConA-1S column contribute to the highly efficient enrichment and purification of glycan and glycoconjugate products from complex samples. The high peak efficiency of the column results in sharp, narrow peak shapes, yielding low elution volumes of highly concentrated products. The high capacity and ligand density provides large quantities of efficiently enriched purified products for downstream applications and separations. The ProSwift ConA-1S column has a wide linear capacity range from 0.1 to 2 mg for glycoproteins. Figure 2 shows the high capacity of the ProSwift ConA-1S column with the 2 mg purification of HRP. Efficient peak shapes were obtained for both the high and low HRP sample loadings.

Reusable for Over a Hundred Enrichments and Purifications

The HPLC compatible ProSwift ConA-1S column can be used over a hundred times for the enrichment and purification of glycoconjugate type samples. The high capacity and stability of the affinity monolith column enables it to be used repeatedly with minor capacity loss. The ProSwift ConA-1S column can easily be recovered by conditioning with buffer after each affinity separation. The ProSwift ConA-1S column is ideal for high throughput glycan and glycoconjugate applications. Figure 3 shows the ProSwift ConA-1S column maintained good capacity after 100 injections.

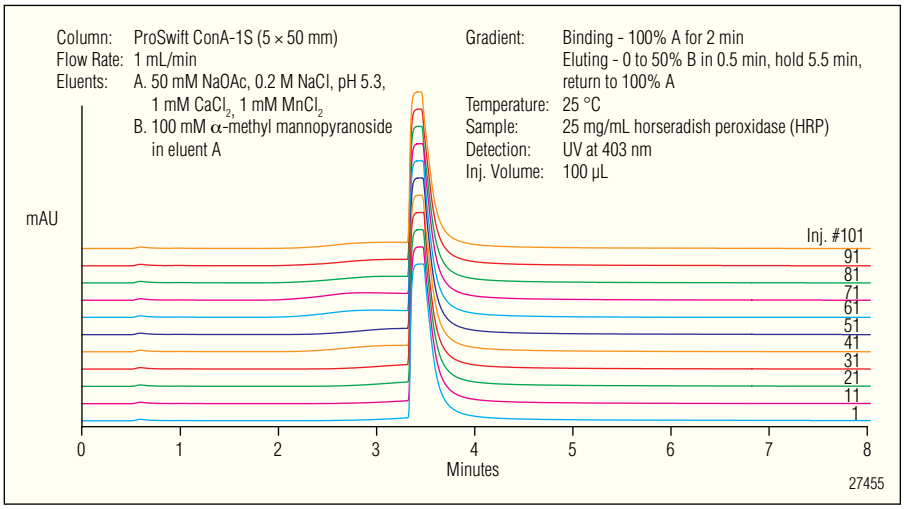

Figure 3. High reusability by maintaining good capacity after 100 injections on the ProSwift ConA-1S column.

Applications

The ProSwift ConA-1S Affinity column provides fast, highly efficient enrichment, and purification of a variety of glycan and glycoconjugate applications including glycoproteins, glycopeptides, and fluorescently derivatized glycoconjugates. Figure 4 shows the fractionation of fluorescently labeled glycans removed from human blood serum on the ProSwift ConA-1S column. The glycan pool was fractionated into unbound and bound fractions based on their different affinity to Con A. The process was completed in under 6 min. This demonstrates the fast enrichment and purification of a group of fluorescently labeled glycans on the ProSwift ConA-1S column.

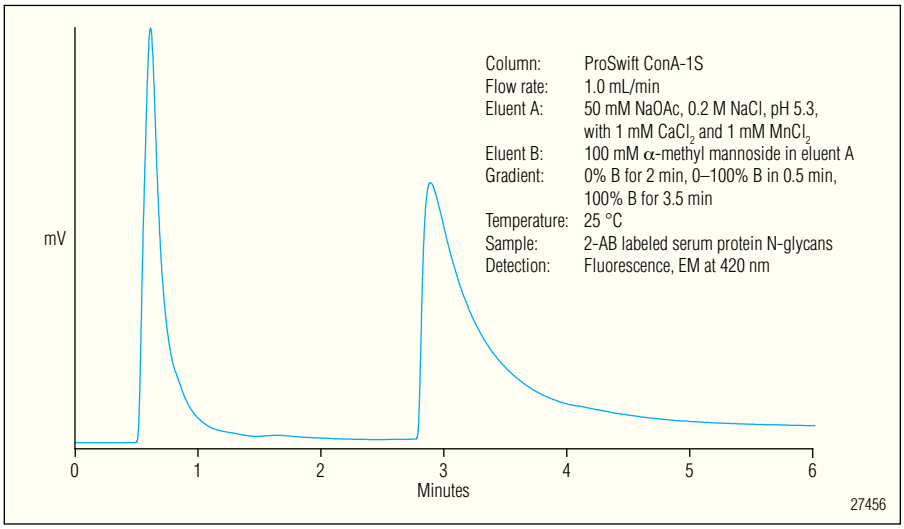

Figure 4. Fast, highly efficient enrichment and purification of a group of fluorescently labeled glycans in under 6 min using the ProSwift ConA-1S column.

Figure 5A shows the fast enrichment of glycoproteins from immunodepleted human serum proteins on the ProSwift ConA-1S column. The bound fraction was eluted with sugar solution, collected, subjected to a tryptic digest, and then analyzed by LC-MS using a reverse-phase column. Figure 5 shows the MS analysis of the bound (B) and unbound (C) fractions. This data confirms the capability of the ProSwift ConA-1S column for the highly efficient enrichment and purification of a large group of glycoproteins.

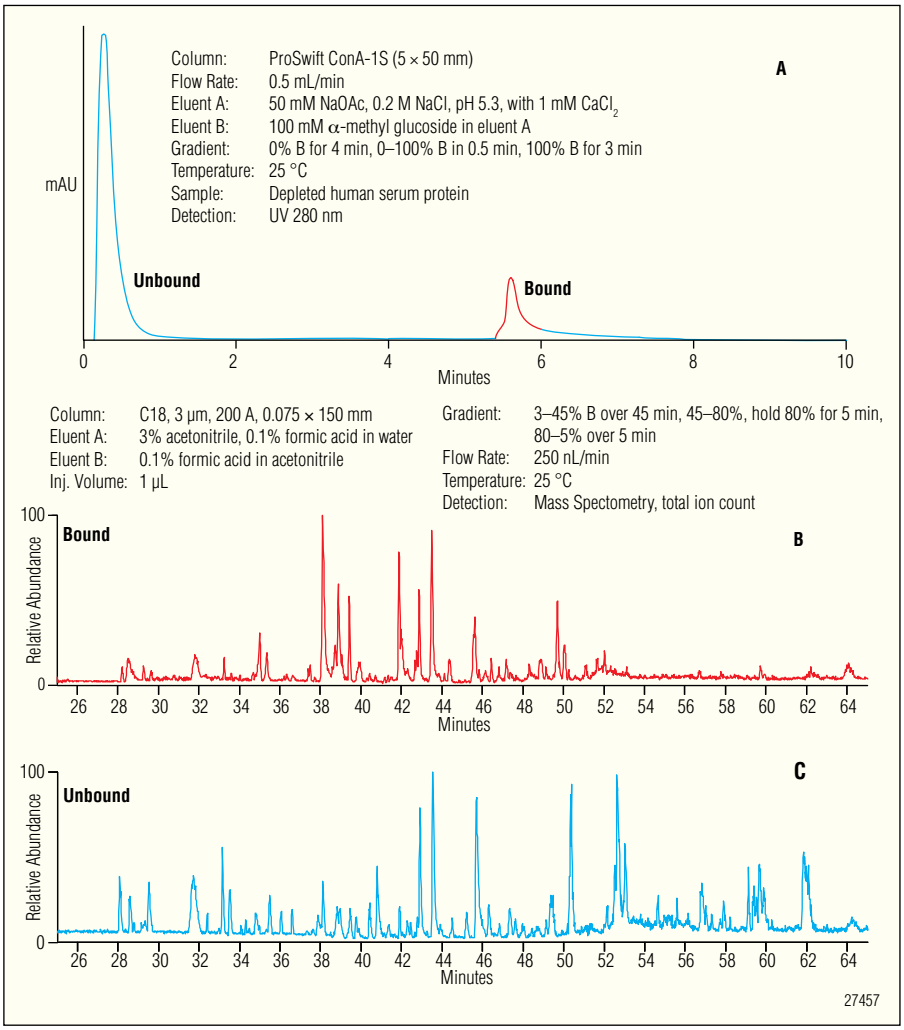

Figure 5. Enrichment and purification of a group of immunodepleated glycoproteins using the ProSwift ConA-1S column, A) followed by analysis of the digested unbound, B) and bound, and C) fractions using RP-LC-MS.

The ProSwift ConA-1S column can also be used for the successful enrichment of glycopeptides. Figure 6 shows enrichment of Con A-binding glycopeptides from an HRP tryptic digest using the ProSwift ConA-1S column. The HRP glycopeptides containing Con A-binding glycans were bound to the column and eluted with sugar solution, while other peptides having weaker or no affinity to Con A eluted earlier.

The ProSwift ConA-1S column is capable of separating different glycoforms of proteins. Figure 7 shows the separation of different glycoforms of chicken ovalbumin. The protein was fractionated into two fractions based on the different affinities of the glycoforms.

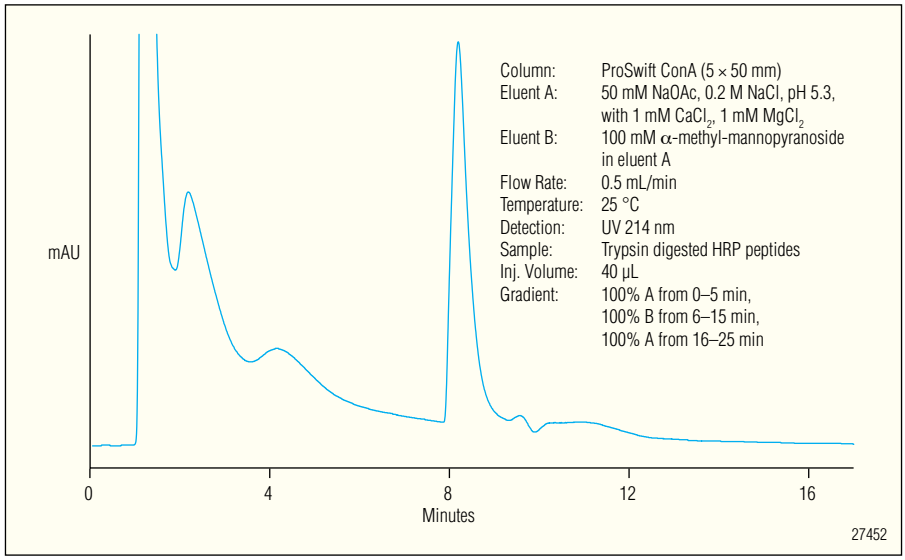

Figure 6. Glycopeptide enrichment using the ProSwift ConA-1S column.

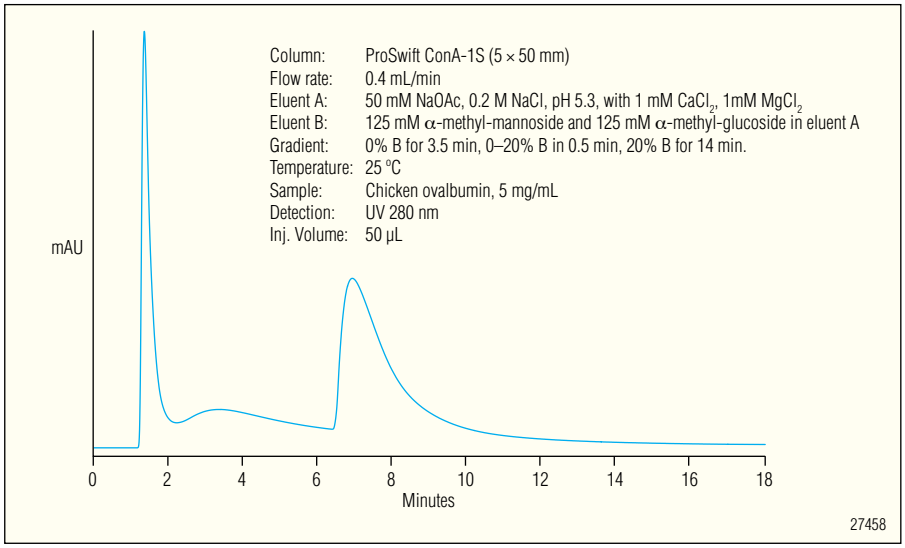

Figure 7. Different glycoforms of chicken ovalbumin separated on the ProSwift ConA-1S column.

SPECIFICATIONS

Column dimension:
5 × 50 mm

Base matrix material:
Polymethacrylate

Surface ligand:
Concanavalin A (Con A)

Column protein binding capacity:
>2 mg HRP per column

Bound protein per column:
12–16 mg Con A

Ligand density:
~14 mg/ml

Bed height:
9 mm

Bed volume:
0.77 mL

pH Range:
5–8

Maximum flow rate:
Up to 2 mL/min

Maximum pressure:
<2000 psi
8.9 MPa

Operating temperature:
<30 °C

Solvent compatibility:
Up to 10% methanol

ORDERING INFORMATION

In the U.S., call (800) 346-6390 or contact the Dionex Regional Office nearest you. Outside the U.S., order through your local Dionex office or distributor. Refer the following part numbers:

ProSwift ConA-1S Column

ProSwift ConA-1S Column (5 × 50 mm) 074148

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