

# A New Monolithic ConA Affinity Column for Purification and Analysis of Glycans, Glycopeptides, and Glycoproteins



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## INTRODUCTION

Lectin-affinity chromatography has been widely used for the purification and analysis of oligosaccharides, glycopeptides, and glycoproteins. However, most of the lectin-affinity columns currently available are agarose bead-based spin columns, which have to be operated manually and can only be used for a limited number of purification cycles. With the growing interest in glycoproteomic studies, such as biomarker identification, there is an increasing need for a robust HPLC lectin column. Presented here is the development and applications of a new monolithic Concanavalin A (Con A) affinity column. Concanavalin 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  $\alpha$ -mannose, and to  $\alpha$ -glucose with weaker affinity. Divalent metal ions such as calcium ( $\text{Ca}^{2+}$ ) or magnesium ( $\text{Mg}^{2+}$ ) 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. The high-capacity ProSwift® ConA-1S column can provide fast and efficient purification and analysis for various Con A-binding glycoconjugate samples. The HPLC compatibility of this column allows automatic sample injection, high throughput, and excellent reproducibility.

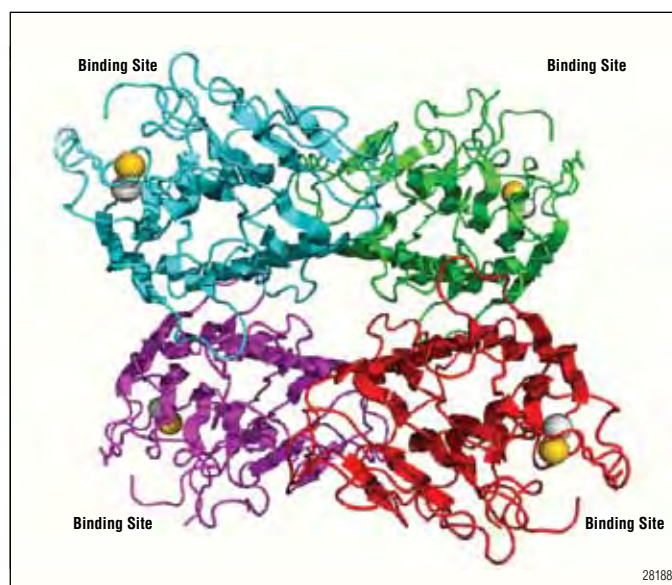


Figure 1. Structure of Concanavalin A at 2.4 Å resolution; Hardmad, K.D.; Ainsworth, C.F. *Biochemistry*, **1972**, 11, 4910–4919.

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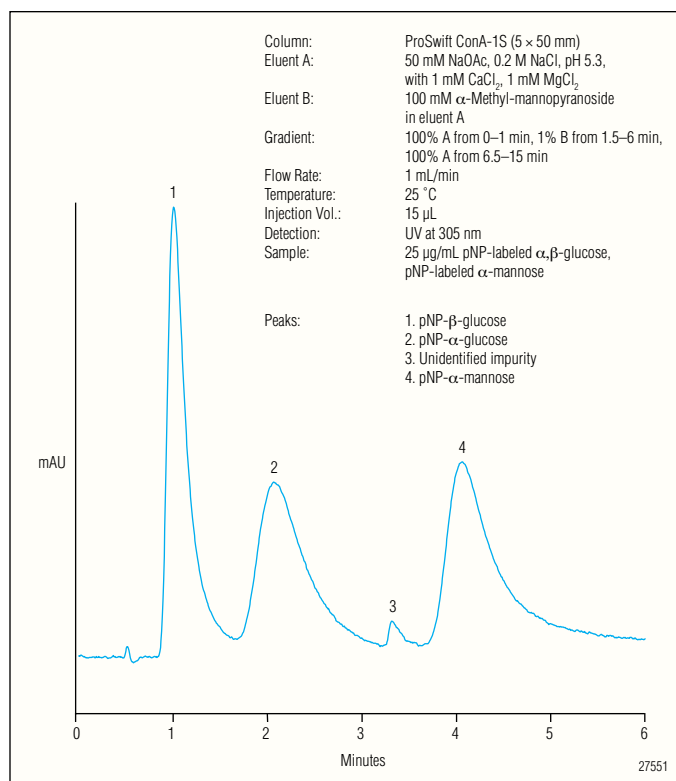


Figure 2. Specificity of the ProSwift ConA-1S column. Separation of three pNP-sugars.

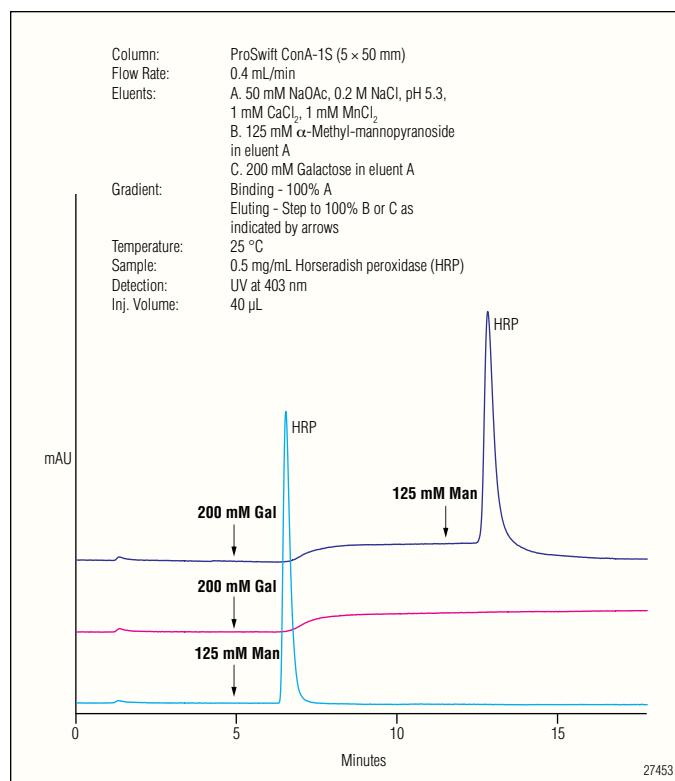


Figure 3. Specificity of the ProSwift ConA-1S column. Specific elution of horseradish peroxidase.

## SYSTEM REQUIREMENTS

The ProSwift ConA-1S column can be used on any compatible HPLC system, which usually consists of a gradient pump; an autosampler; a thermal compartment; and a UV, fluorescence, or other type of detector, depending on the sample type.

## COLUMN SPECIFICATIONS

Column Dimension:	5 × 50 mm
Protein Coated on Monolith:	Concanavalin A
Bimodal Monolith Pore Diameter:	3.25 and 0.62 µm
Binding Capacity:	~2 mg HRP/column
Operating Flow Rates:	Up to 2 mL/min
pH Range:	pH 5–8
Operating Temperature:	≤30 °C
Maximum Pressure:	2300 psi
Organic Solvent Limit:	10% Methanol

## COLUMN CHEMISTRY AND SPECIFICITY

The ProSwift ConA-1S column is a polymeric monolith prepared by in-column polymerization, followed by functionalization with Con A. The monolith is a cylindrical polymer rod containing uninterrupted, interconnected, flow-through pores, with surface area greater than nonporous bead-based columns. The structure consists of small pores that contribute surface area, and larger pores that allow reduced backpressure at elevated flow rates. This approach results in short mass-transfer distances that produce improved efficiency, even at elevated flow rates. High quality Con A is covalently attached to the monolith column through the amine groups of Con A (see Figure 1). The sugar binding sites are protected during the conjugation process so the Con A activity is well maintained. Figures 2 and 3 show the specificity of the ProSwift ConA-1S column. Three pNP-labeled sugars were separated based on their different affinities towards Con A. Horseradish peroxidase (HRP), which is a glycoprotein with rich high-mannose type glycans, was bound to the Con A column and was eluted with α-methyl-mannopyranoside, which has high affinity for Con A.

## LOADING CAPACITY OF ConA-1S COLUMN

The loading capacity for HRP is no less than 2.0 mg on the ProSwift ConA-1S column at a flow rate of 1 mL/min. Figure 4 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.

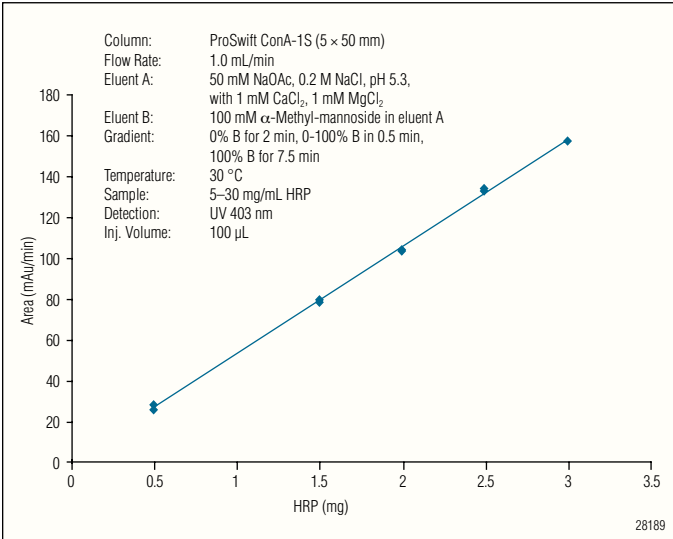


Figure 4. Linearity of area to sample load for HRP on ConA-1S column.

## COLUMN RUGGEDNESS

The ProSwift ConA-1S column can be regenerated easily by washing with conditioning buffer after sample binding and elution. The rugged column chemistry allows hundreds of run cycles with minimal capacity loss. Figure 5 shows the ProSwift ConA-1S column maintains good capacity after 100 injections and elutions of HRP.

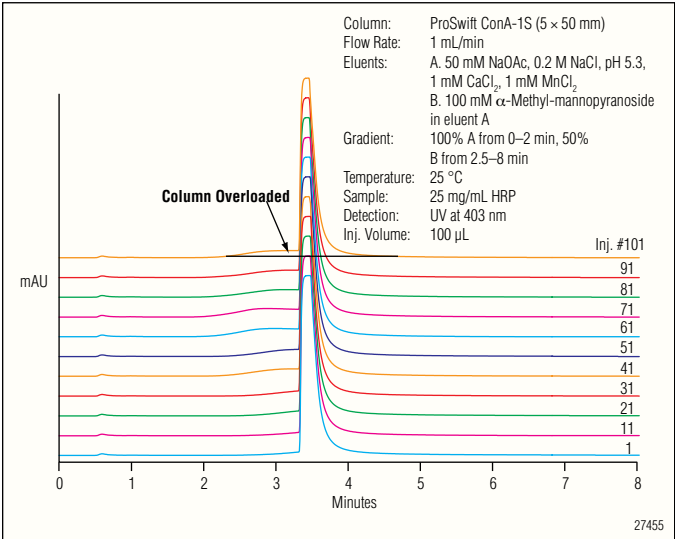


Figure 5. One hundred binding-elution cycles of HRP.

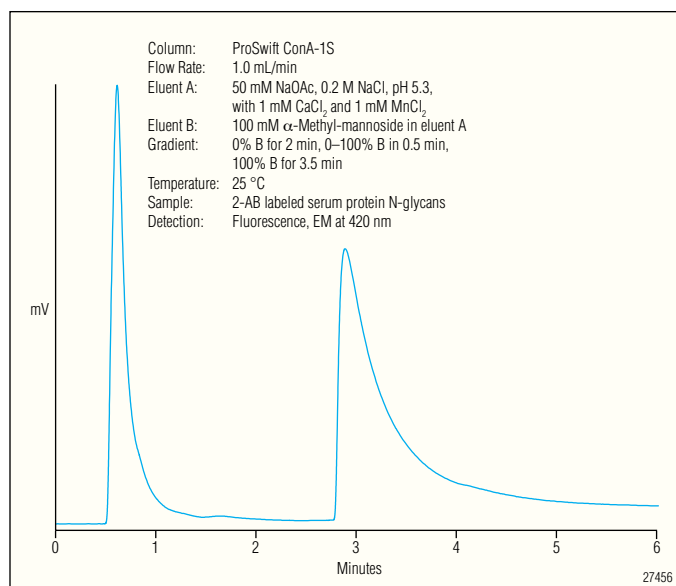


Figure 6. Purification of fluorescent-labeled glycans.

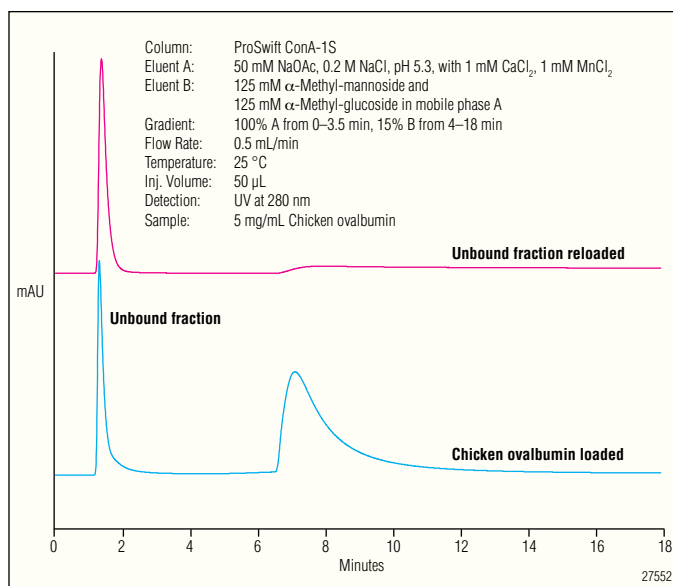


Figure 7. Separation of chicken ovalbumin glycoforms.

## APPLICATIONS

Con A is one of the most well characterized and widely used lectins. It binds to  $\beta$ -mannose, and to  $\beta$ -glucose with weaker affinity. Usually high-mannose type glycans bind to Con A strongly, and some hybrid type glycans can also bind to Con A with good affinity, while complex type glycans usually have very weak affinity towards Con A. As  $\beta$ -mannose is commonly expressed on most glycoproteins, a Con A-affinity column is a useful tool for purification and enrichment for glycans, glycopeptides, and glycoproteins.

### Fractionation of Glycans

Glycan samples can be fractionated on the Con A column based on their different affinities to Con A. Figure 6 shows fluorescence-labeled serum N-glycans were fractionated into two fractions on the ProSwift ConA-1S column.

### Separation of Glycoprotein Glycoforms

Protein glycoforms with different affinities to Con A can be separated on the ProSwift ConA-1S column. In Figure 7, chicken ovalbumin was fractionated into an unbound and a bound fraction on this column. The unbound fraction was collected and reloaded onto the column. All of the previously unbound fraction again eluted in the flow-through. Elution of the unbound fraction indicates that it has different glycosylation patterns than the bound fraction.

# Enrichment of Glycopeptides

HRP was digested with trypsin. The tryptic digest was fractionated on the ProSwift ConA-1S column. The bound and unbound fractions were collected and analyzed on a reversed-phase column (Figure 8).

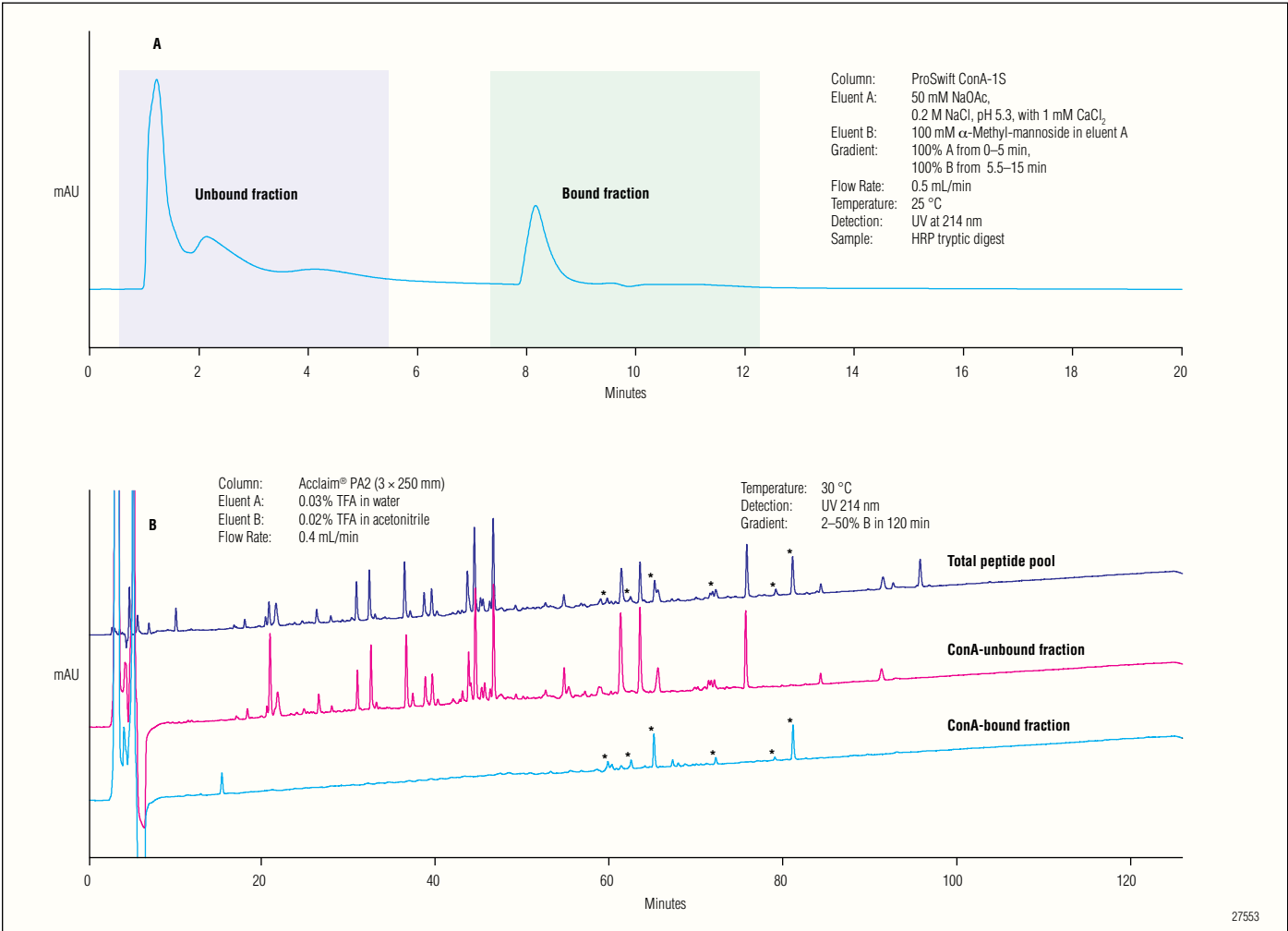


Figure 8. Enrichment of HRP glycopeptides. A) Enrichment of glycopeptides on ProSwift ConA column. B) Profiling of HRP peptide pools on a reversed-phase column.

## Enrichment of Glycoproteins

Depleted human plasma proteins were fractionated on the ProSwift ConA-1S column (Figure 9).

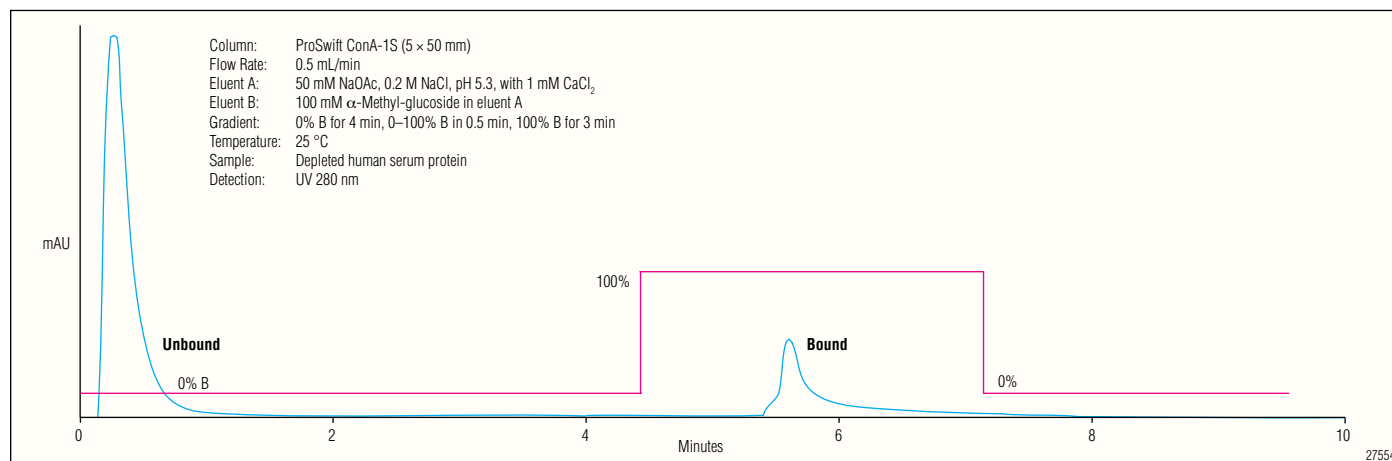


Figure 9. Enrichment of glycoproteins.

## CONCLUSION

- A novel monolithic HPLC Con A-affinity column with high capacity and specificity was developed.
- The ProSwift ConA-1S column provides highly efficient enrichment, purification, and analysis on glycan, glycopeptide, and glycoprotein samples.
- The HPLC-compatibility of the ProSwift ConA-1S column allows automation, high throughput, and excellent reproducibility.

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LPN 2751-01 3/11  
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