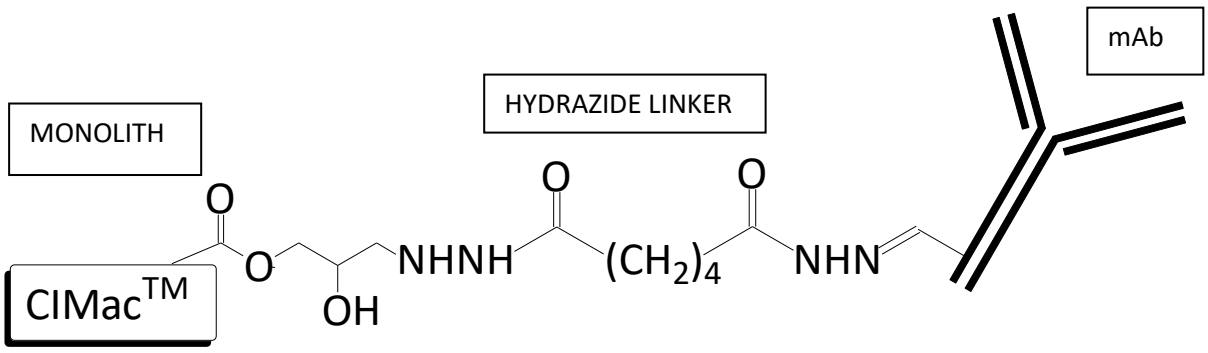
A042

The power of immunoaffinity chromatography on CIMac™ platform– studying glycosylation profile of fibrinogen in human plasma

The **CIMac** antibody immobilisation platform enables an elegant immobilisation of antibodies, which can be used as immunoaffinity adsorbents in specific diagnostic applications. Immobilization of antibodies to solid chromatographic supports is avery elegant solution for preparation of reusable immunoaffinity assays that can lower the price but also increase the accuracy and lower the limit of detection of biological assays for diagnostic purposes. Therefore, it is important that reusing a column for multiple sample analysis does not affect chromatographic/analysis performance. In this application note, an example of reusability of the hydrazide-based CIMac column with immobilised monoclonal antifibrinogen (Scheme 1) for consecutive purification of fibrinogen from human plasma is shown (Figure 1).

> Scheme 1

Schematic presentation of immobilisation of monoclonal antibody onto the CIMac column bearing the hydrazide linker



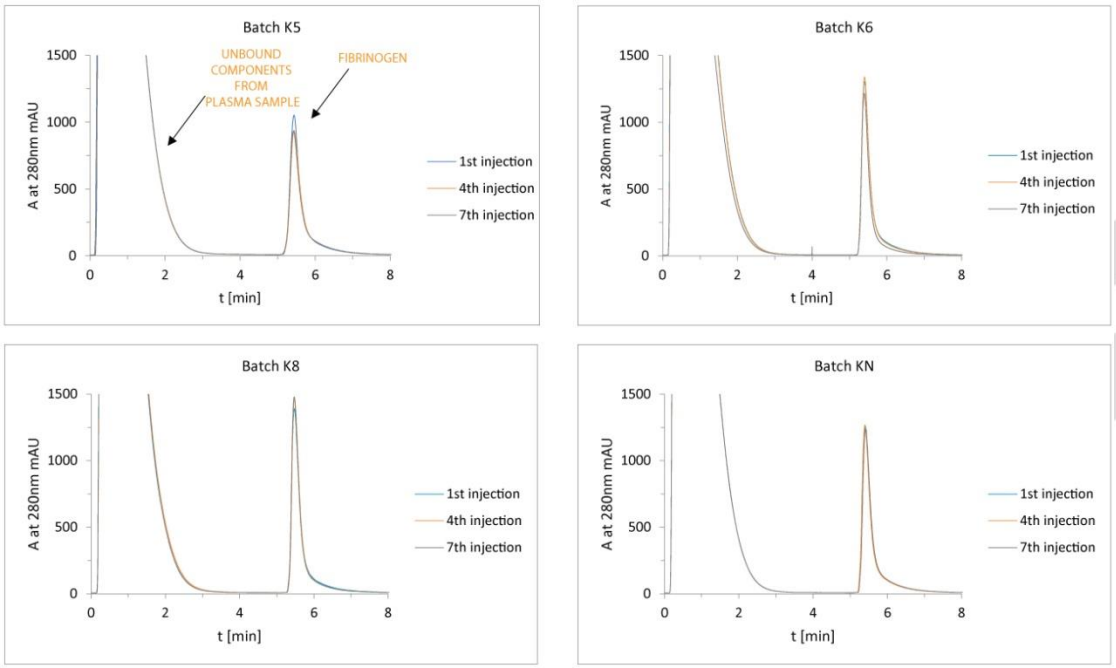
METHOD

Column:	hydrazide-based CIMac™ column with immobilised monoclonal antifibrinogen
Load:	100µL of plasma sample, 10x diluted with PBS
Flow rate:	1.0 mL/min
Mobile phases:	Solvent A: phosphate-buffered saline (PBS) Solvent B: 0.1M formic acid, pH 2.4
Gradient elution method:	Solvent A (4min), solvent B (4min)
Wash:	Solvent A (2min)
Regeneration:	Not additionally regenerated between the runs

Publication #: A042-Fibrinogen-1509

> Figure 1

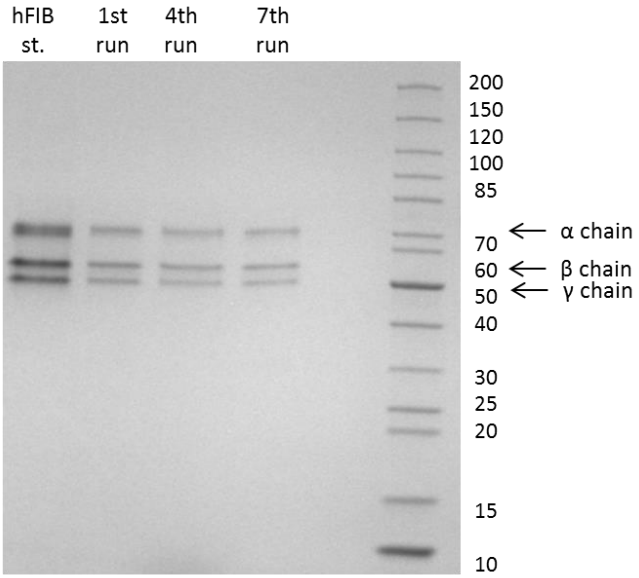
Elution peaks of fibrinogen from CIMac HDZ-antifibrinogen columns on four different column batches. Elution in the first, fourth and seventh application of human plasma on each column is shown.



Human fibrinogen is a fibrous plasma protein that is synthesized in the liver and has a crucial role in blood clotting after tissue injury. It is composed of three polypeptide chains (α, β, γ), each in duplicate and linked together by disulphide bonds. The purity of eluted fibrinogen from human plasma samples was confirmed by SDS-PAGE analysis from the elution fractions and a comparison with the fibrinogen standard (Figure 2).

> Figure 2

SDS-PAGE analysis of elution fractions from first, fourth and seventh run on a CIMac HDZ-antifibrinogen column.



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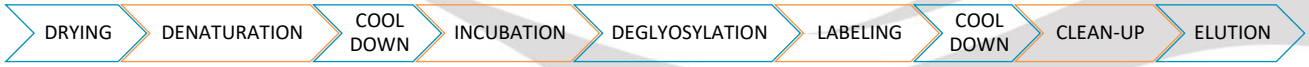
There are two biantennary N-glycans on each of β and γ chain, while α chain is considered not to be N-glycosylated. In addition to N-glycosylation, fibrinogen is also O-glycosylated at its α and β chains.[1] Fibrinogen's N-glycosylation pattern differs in patients with liver cirrhosis and was shown to be important for the structural and biophysical characteristics of a fibrin clot. [2]

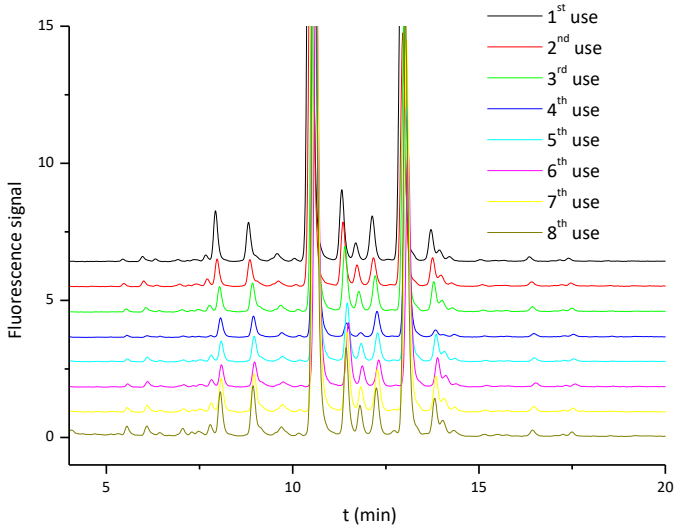
The protein content from eluates of the chromatographic runs was deglycosylated, treated according to Scheme 2 and its glycan profile was anaylsed with hydrophilic intraction chromatography using a Waters BEH Glycan chromatography column. As shown in Figure 3, nearly identical profile from the same plasma sample was obtained for 8 consecutive chromatographic runs from one column, confirming the stability and selectivity of the CIMac immunoaffinity column.

> Scheme 2



> Figure 3

UPLC analysis of a glycan profile (human plasma) from the 8 consecutive elution fractions of CIMac HDZ-antifibrinogen column.



1. G. Zauner, M. Hoffmann et al, Journal of proteome research 11 (2012) 5804-5814
2. J. Weisel, Advances in protein chemistry 70 (2005) 247-299

HIGH GLYCAN
Methods for high-throughput (HTP) analysis of protein glycosylation

GENOS

Publication #: A042-Fibrinogen-1509

ORDERING INFROMATION

Used product

Catalogue No.	Product description
110.8002-1.3	CIMac™ HDZ-0.1 Analytical Column (Hydrazide) (Pores 1.3µm)

Related Products

Catalogue No.	Product description
311.8002-2	CIMmultus™ HDZ-1 Advanced Composite Column (Hydrazide) (Pores 2 µm)
102.8002-2	CIMmic HDZ-0.2 (Hydrazide) (Pores 2 µm)

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BIA Separations has a commitment to cater for customer's needs in the field of chromatography and CIM® monolithic columns. Beside column production, BIA offers immobilization service. Immobilization of antibodies (Abs) is a challenging task. Let us do the hard work for you. For more information please contact our technical support at help@biaseparations.com.

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