

IMMUNOAFFINITY COLUMNS: REPRODUCIBLE AND RELIABLE TOOL FOR ANALYTICS AND PURIFICATION



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INTRODUCTION

There are many cases, where a single protein needs to be purified from a complex sample. Such proteins manifest themselves as impurities, which can affect further analysis, either by causing specific equipment malfunction or lower yield in the products. In other cases the specific protein is our molecule of interest, for example in glycomics analysis. In both cases high specificity for

proteins, reproducibility and reliability is necessary. We have developed a model immunoaffinity column and 96-well plate based on an anti-fibrinogen monoclonal antibody, covalently immobilized onto CIMacTM analytical chromatographic monolith.

RESULTS

Monoclonal anti-fibrinogen antibodies (mouse, IgG2a), were purified within HighGlycan project on a CIM[®] r-Protein A-8 Tube Monolithic Column and later immobilized using the hydrazide immobilization platform.

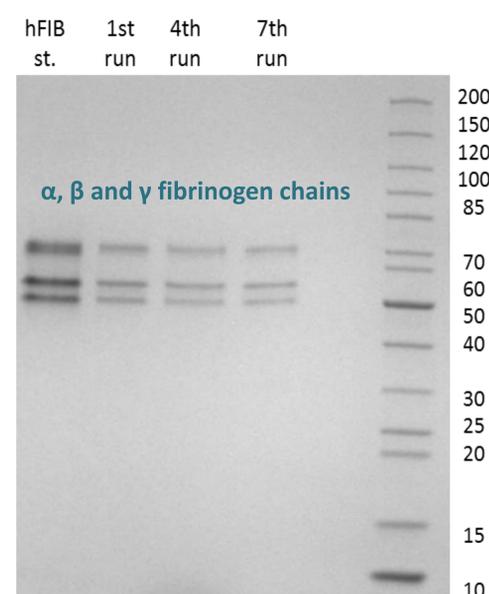
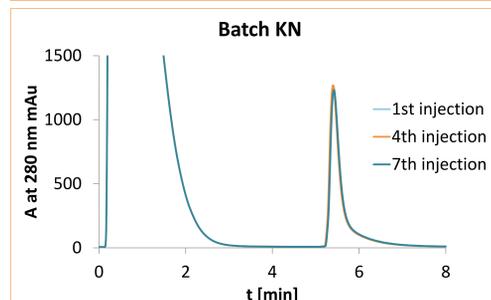
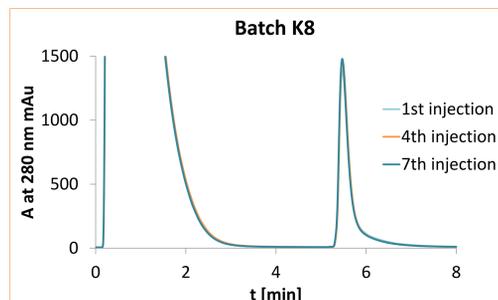
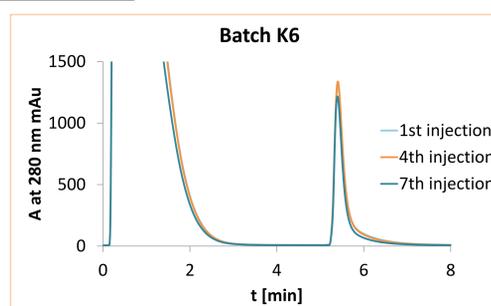
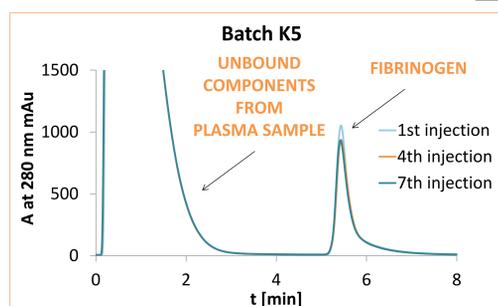
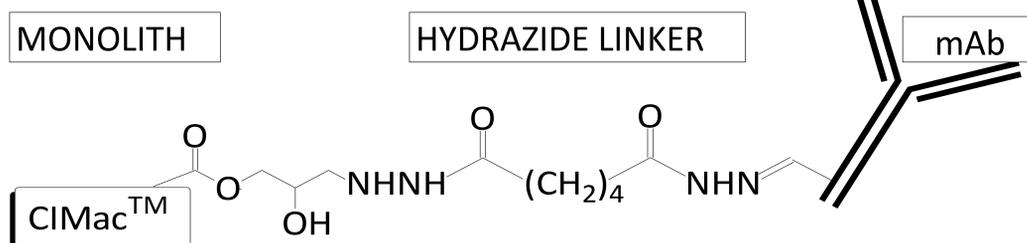
Columns with immobilized mAbs were tested several cycles with fibrinogen from a plasma sample. Run to run reproducibility was evaluated by SDS-PAGE and further glycosylation analysis at GENOS (partner in HighGlycan project).

REPRODUCIBILITY:

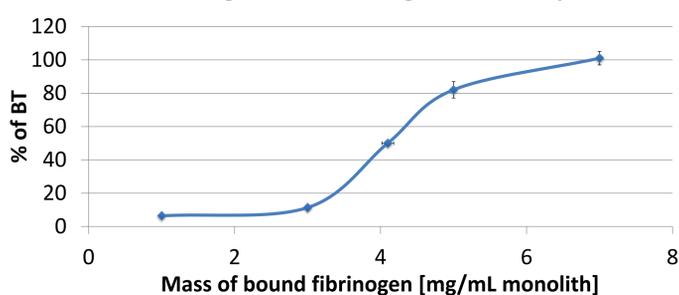
Image shows overlay of chromatograms from different column batches. A similar elution profile is observed on each column in repeated runs from 1 to 7. An additional confirmation are practically identical bands of elution peaks on SDS-PAGE, without impurities or losing band intensity.

Conditions:

- Column: CIMacTM HIDA
- Mouse anti-fibrinogen mAb (IgG2a)
- Loading buffer: PBS
- Elution buffer: 0.1 M formic acid or 0.1 M glycine pH 2.0



Breakthrough curve of fibrinogen – 8 well strip



We have prepared an 8 well strip on a 96 well plate format. Each well housed a 200 μ L monolith.

Dynamic binding capacity was measured by loading aliquots of fibrinogen and measuring unbound fibrinogen.

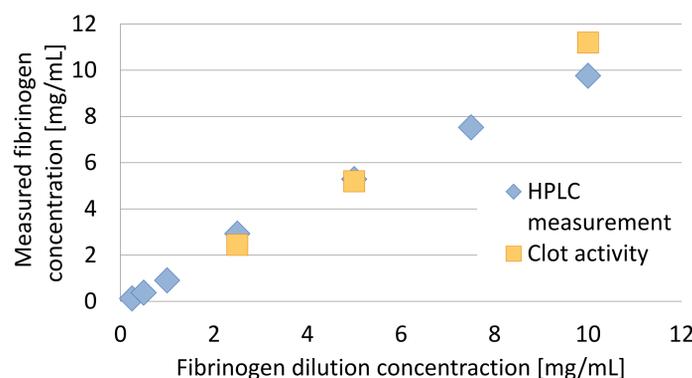
The prepared strip has been thoroughly tested and used for fibrinogen isolation from plasma samples. After 4 months of use no significant drop in fibrinogen binding capacity has been observed.

USE AS ANALYTICAL TOOLS:

An example of CIMac application is a comparison between a HPLC quantification of fibrinogen concentration and the blood processing industry standard, clot activity measurement.

We have proven higher sensitivity of HPLC-based measurements.

Additionally, immunoaffinity method is insensitive to buffer composition and presence of anticoagulants.



Measurement method/ sample	CIMac TM antifibrinogen column c_{FIB} [mg/mL]	Clot activity measurement c_{FIB} [mg/mL]
Plasma 10x diluted	2.71	Not Detected
Plasma 5x diluted	2.18	Not Detected
Plasma undiluted	2.16	2.14

CONCLUSIONS

- Platform process for immobilization of antibodies on hydrazide monoliths
- Robust method and high repeatability of elution of fibrinogen
- Combined platform for analytical or preparative use

- Improved sensitivity and range for analytical methods
- Platform suitable for parallelization and automation



GLYCOMET

