# CHROMATOGRAPHIC MONOLITHS WITH HYDRAZIDE MODIFIED SURFACE – NEW MEMBER OF CIM® FAMILY

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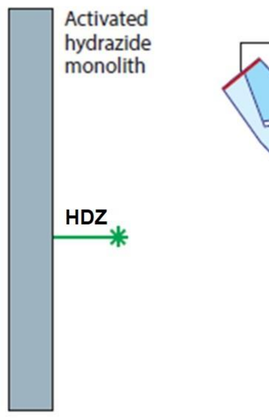
## INTRODUCTION

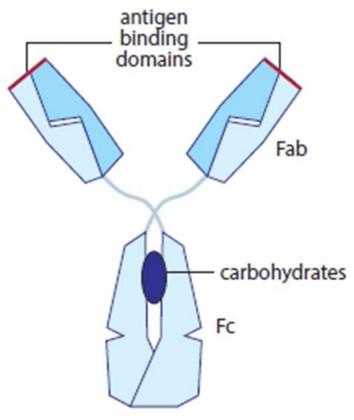
Hydrazide-activated (HDZ) columns were proven to be a product of choice for making the most effective immunoaffinity columns. They take advantage of a special hydrazide linkage that binds antibodies through the carbohydrate residues on their Fc regions. This leaves the antigen-binding domains fully accessible to enable the most effective capture of desired target (Figure bellow).

CIMac™ HDZ monoliths make HDZ-immobilized antibody columns even more effective. Because of their large channel size and the efficiency of convective mass transport, they eliminate the long loading residence times that are required for affinity chromatography on porous particle columns. Flow rates of 5–10 column volumes per minute allow complete purifications in a few minutes, even when the source material contains a low concentration of antigen. The same performance is achieved whether a small peptide or a large bio-assemblage like a virus particle or extracellular vesicle is isolated.

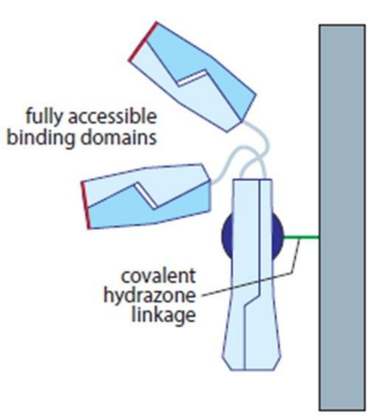
The combination of HDZ monoliths and the immobilization protocol offers a strong tool for fast antigen isolation from complex biological sample (plasma, lysate, etc.) and consequently sensitive antigen quantification. An example of CIMac™ HDZ application is a purification of fibrinogen from human plasma (Figure below).



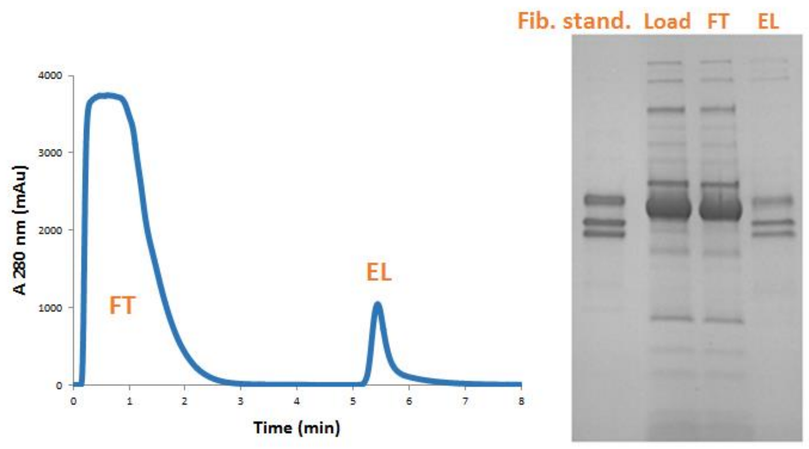

HDZ Column




Antibody




HDZ-immobilized antibody column



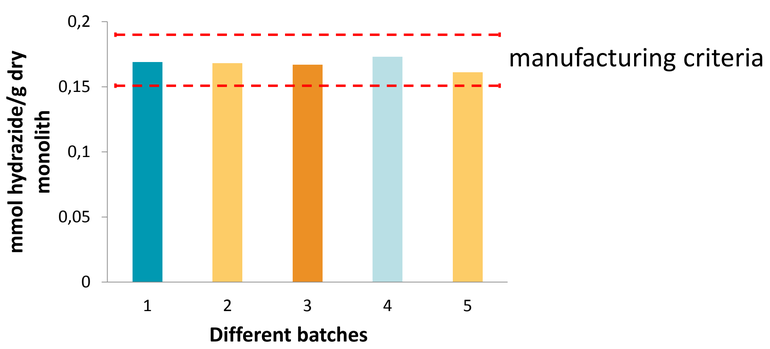

CIMac™ HDZ-0.1 Analytical column with immobilized monoclonal anti-fibrinogen. Equilibrated with phosphate buffered saline, eluted with formic acid, pH 2.0.

## RESULTS

### CHARACTERISTICS OF HDZ COLUMNS and HDZ-IMMOBILIZED ANTIBODY COLUMNS

#### 1.a REPRODUCIBLE INTRODUCTION OF HDZ GROUPS ON MONOLITHS

When transfer the product CIMac™ HDZ column into the production, the manufacturing criteria was defined to be from 0.15 to 0.19 mmol hydrazide/g dry monolith, what was successfully implemented on five different production batches (RSD was 2.6 %).



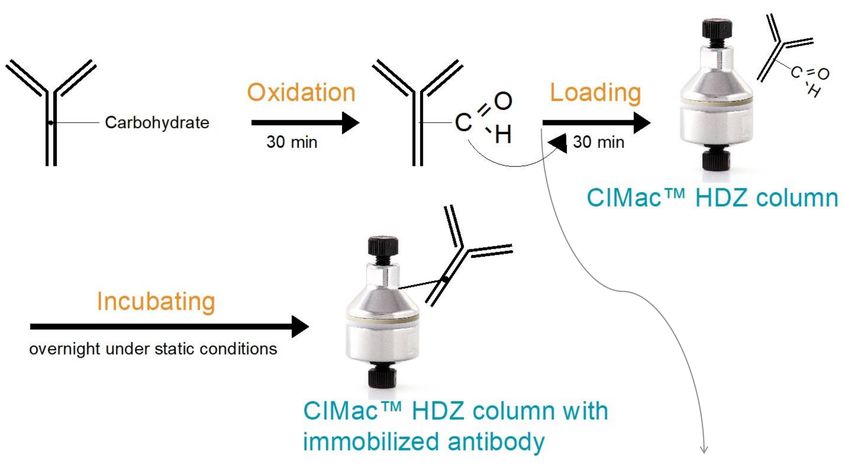

#### 1.b REPRODUCIBLE FUNCTIONALITY OF HDZ-IMMOBILIZED ANTIBODY COLUMNS

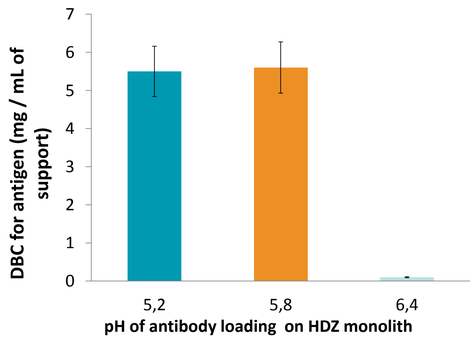
A polyclonal anti-albumin antibody was coupled to the HDZ monoliths, resulting in HDZ-immobilized @HSA columns. Then human serum albumin dynamic binding capacity (HSA-DBC) was tested on different batches as well as new and 2 years aged HDZ monoliths. The RSD was below 5 % and no changing of monolith functionality was observed even after 2 years of HDZ monolith storage at room temperature.

| Column | Batch | Age of HDZ monolith | HSA- DBC (mg HSA / mL support) |
|---|---|---|---|
| CIMac™-0.1 @HSA-col1 | 1 | 1 week | 1,40 |
| CIMac™-0.1 @HSA-col2 | 2 | 1 week | 1,42 |
| CIMac™-0.1 @HSA-col3 | 3 | 2 years | 1,35 |

#### 2. OPTIMIZATION OF IMMOBILIZATION PROCEDURE

CIMac™ HDZ differs from other hydrazide media which require IgG concentration, buffer exchange, or cycling during immobilization. This is due to a unique feature of the monolithic polymer backbone that makes it possible to simply concentrate IgG molecules on a monolith surface while loading the immobilization solution. We have developed and optimized the immobilization procedure (shematically presented below).




The amount of immobilized antibody using the proposed protocol is controlled by adjusting the pH of the loading buffer. Increasing the pH from 5.2 to 5.8 and then to 6.4 decreases the interaction between chromatographic support IgG, influencing the amount of adsorbed antibody.

## CONCLUSIONS

Preparation of HDZ columns from different batches resulted in products with reproducible amount of HDZ groups. RSD was 2.6%.

No changing of monolith functionality was observed even after 2 years of HDZ monolith storage.

CIMac™ HDZ-0.1 columns provide:

- Easy site-directed Immobilization of IgG
- Simple and convenient coupling process
- High capacity because Ab binding sites are fully accessible
- Short run times because of convective mass transport
- High sensitivity and selectivity of immunoaffinity columns