AN064 Depletion of human serum albumin from plasma samples by immunoextraction on immobilised immuno-affinity CIMmic™

Pre-activated CIMmic™ monolithic columns are tools for screening of immobilisation conditions and small scale proof-of-concept testing of custom affinity columns and enzymatic reactors. Each column is assembled from a dedicated housing and discs containing the chromatography medium. With a bed volume of 100 µL, sample requirements are minimal, while inserting multiple discs in the housing adapts the column volume to application requirements. Different surface modifications of the discs enable immobilisation of a wide variety of ligands.

Polyclonal anti-HSA antibodies were immobilised on CIMmic™ HDZ (hydrazide chemistry, 100 µL bed volume), following an optimised protocol. This CIMmic™ α-HSA column was prepared to quantitatively remove human serum albumin (HSA), the most abundant plasma protein in humans, and obtain albumin-free samples which can be further processed.

The CIMmic™ αHSA column is optimized to selectively bind and remove HSA from plasma; albumins from other sources are bound with low efficiency. HSA depletion is carried out in two steps: (i) a loading step in which the diluted and filtered plasma sample is loaded into the column, HSA is bound and HSA-depleted plasma is collected downstream of the column and (ii) a regeneration step in which the bound HSA is removed and the column is regenerated.

Depletion of HSA from plasma sample

Monolithic column:	CIMmic™ αHSA 0.1 mL (DBC at 50% breakthrough: 0.87 mg HSA/mL support)
Mobile phases:	Loading buffer: PBS 1X, pH 7.4 Elution buffer: 0.1 M glycine hydrochloride, pH 2.0
Flow rate:	0.6 mL/min
Sample and preparation:	Human plasma. Sample filtered and diluted 1:15 with PBS 1X, pH 7.4 (final HSA concentration 35 µM)
Injection volume:	20 µL
Detection:	UV absorbance at 280 nm
Method:	Equilibrate the column by flushing with at least 10 column volumes (CV) of loading buffer. Load sample with 15 CV of loading buffer, elute HSA from the column with 15 CV of elution buffer.

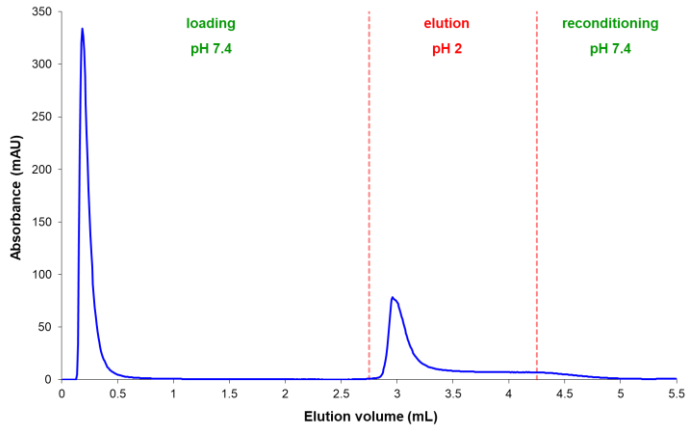

Figure 1: Chromatographic profile (UV absorbance at 280 nm) of diluted human plasma (HSA concentration: 35 µM) loaded onto the CIMmic™ αHSA column. First peak: HSA-depleted plasma; second peak: HSA.

The chromatographic fraction eluting within 1 min contains the HSA-depleted plasma (first chromatographic peak), while HSA remains fully bound to the stationary phase. The collected fraction can be concentrated by ultrafiltration or directly subjected to further analysis.

The regeneration of the column, i.e. elution of bound HSA, is obtained by flushing the column with a glycine HClbuffer (pH 2.0). No memory effect was observed.

The maximum extraction efficiency of CIMmic™ αHSA toward HSA in plasma is observed at roughly half the maximum binding capacity of the support determined by frontal analysis (DBC at 50% breakthrough). In these conditions, depletion of HSA from plasma is 100%, confirmed by ESI-Q-ToF analysis. The dynamic binding capacity of CIMmic™ αHSA at 50% breakthrough was determined as 0.87 mg HSA/mL support.

LC-MS analysis results

Biomarker discovery by tandem mass spectrometry of biological matrices is complicated due to their low concentration and the interference of high abundance proteins. The dynamic range of protein concentrations in biological matrices can span over 10 orders of magnitude. Since these impede detection of low-abundance proteins, they must be removed before analysis. The tool of choice is immunoaffinity-based extraction and it has become the routine strategy for sample preparation in proteomic analysis.

The use of CIMmic™ αHSA allowed the complete removal of HSA from plasma. The LC-MS analysis of human plasma before and after the depletion shows the disappearance of HSA (t_r = 6 min); the residual signals at 6 min, indeed, are not ascribable to HSA but to other co-eluting plasma proteins.

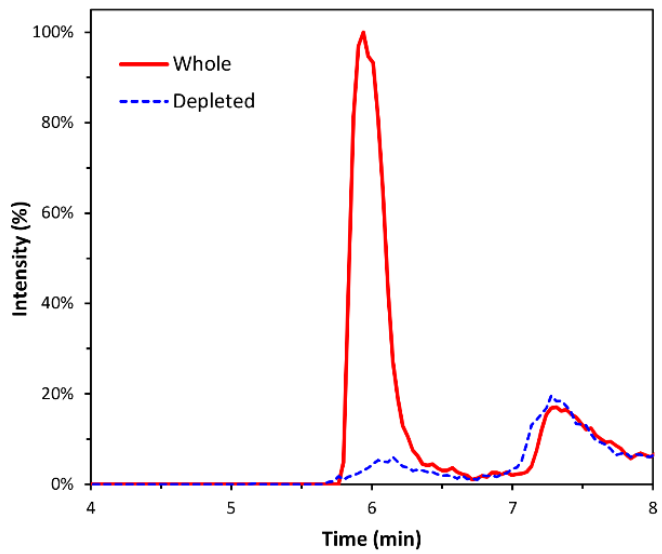

Figure 2: Efficiency of depletion by CIMmic™ αHSA columns. LC-MS analysis on whole (red) and depleted (blue) plasma sample. The residual signal at 6 min was not ascribable to HSA.

Parameters: LC-MS system: Agilent 1200 coupled with a ESI-Q-ToF mass spectrometer (Micromass Waters), reverse phase column: Phenomenex Jupiter C4 (150 x 2.0 mm; 5 µm) thermostated at 40 °C, Mobile phases A [water/acetonitrile/formic acid, 99/1/0.1 (v/v/v)] and B [water/acetonitrile/formic acid, 1/99/0.1 (v/v/v)] were used to develop a gradient as follow: from 30 to 70% B in 5 min. Column was equilibrated with the starting conditions for 5 min before further injection, flow rate 0.4 mL/min, Whole human plasma

was diluted 1:100 with PBS and 5 µL were injected onto the LC-MS system. Depleted plasma collected from CIMac-αHSA column was directly analysed by LC-MS (injection volume 18 µL), MS analysis: Total Ion Current acquisition (*m/z* range 800-3000) in positive polarity.

Conclusions

Affinity chromatography can be an excellent choice for rapid and selective depletion of HSA from plasma samples. Development of an affinity chromatography medium can be costly, principally due to the cost of the ligand. Using the smallest amount of sample during development is therefore critical. CIMmic™ monolithic columns are a suitable small-scale (100 µL bed volume) platform to develop and optimise immobilisation protocols.

Covalent immobilisation of polyclonal anti-HSA antibodies onto CIMmic™ HDZ has allowed complete immune-affinity depletion of human serum albumin from plasma. The processed sample could then be analysed by LC-MS without the interference of HSA. This immobilisation protocol can be easily transferred to a larger column volume as needed.

Monoliths make immobilised 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 let you do complete purifications in a few minutes. In this case, complete depletion requires less than 1 min. With a suitable closed chromatography system, the process can be automated, and the risk of operator contamination is minimised when handling plasma from risky donors.

References

- Irina A. Tarasova, Anna A. Lobas, Urh Černigoj, et al. “Depletion of human serum albumin in embryo culture media for in vitro fertilization using monolithic columns with immobilized antibodies”. Electrophoresis 37 (2016): 2322–2327.
- Immobilisation procedure available on BIA Separations’ website: https://www.biaseparations.com/en/library/immobilisation-procedures

Services

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.

Ordering information

Catalog No.	Product description
103.8001-2	CIMmic™ ALD-0.1 Disk (Aldehyde) (Pores 2 µm) - Pack of 3
103.8000-2	CIMmic™ CDI-0.1 Disk (Carbonyldiimidazole) (Pores 2 µm) - Pack of 3
103.8002-2	CIMmic™ HDZ-0.1 Disk (Hydrazide) (Pores 2 µm) - Pack of 3
103.8005-2	CIMmic™ Screening Pack (1x CDI 103.8000-2, ALD 103.8001-2, HDZ 103.8002-2) (Pores 2 µm)
311.8002-2	CIMmultus™ HDZ-1 Advanced Composite Column (Hydrazide) (Pores 2 µm)

Acknowledgments

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