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Abstract



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P09 Extraction of human serum albumin for functional studies using novel immunoaffinity-based CIMacαHSA monolithic columns

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The depletion of abundant proteins from biological samples by affinity extraction is an important stage in routine sample preparation for tandem mass spectrometry (MS/MS) analysis, where the identification of low-abundance proteins is hampered by charge competition effects and by the limited dynamic range of MS/MS. Novel immunoaffinity-based Convective Interaction Media analytical columns with specificity to human serum albumin (CIMac^M- α HSA) were developed to address the need for fast and automated protein depletion systems for plasma and serum samples [1]. This communication reports the optimization of an immunoaffinity method on monolithic supports for the extraction of HSA from plasma samples. The study aims at the recovery of HSA while preserving its native state, thus enabling functional investigations on the glycation of HSA in type-II diabetes mellitus [2]. For this purpose, a set of four CIMac- α HSA columns characterized by different pore sizes $(1.3-2.1 \mu m)$ and HSA binding capacities (0.7-1.4 mg HSA/mL support)were prepared by covalent coupling of oxidized polyclonal aHSA onto hydrazide-activated CIMac monoliths. The feasibility of recovering HSA in a reversibly denatured state will be evaluated by varying the pH and composition of the mobile phase used for the elution step; preliminary data of long-term stability tests for the employed immunoaffinity surfaces will also be reported.

References:

[1] Tarasova I.A., Lobas A.A., Cernigoj U., et al., Electrophoresis 2016, 37, 2322-2327.

[2] GA-RAGE research project funded by the Alma Idea program (University of Bologna, Italy).