

Depletion of high-abundance proteins from human plasma using a combination of an affinity and pseudo-affinity column.

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Human serum albumin (HSA) and immunoglobulin G (IgG) represent over 75% of all proteins present in human plasma. These high-abundance proteins prevent the detection of low-abundance proteins which are potential markers for various diseases. The depletion of HSA and IgG is therefore essential for further proteome analysis. In this paper we describe the optimization of conditions for selective depletion of HSA and IgG using affinity and pseudo-affinity chromatography. A BIA Separations CIM (convective interaction media) Protein G disk was applied for the removal of IgG and the Mimetic Blue SA A6XL stationary phase for the removal of HSA. The binding and the elution buffer for CIM Protein G disk were chosen on the basis of the peak shape. The dynamic binding capacity was determined. It was shown to be dependent on the buffer system used and independent of the flow rate and of the concentration of IgG. Beside the binding capacity for the IgG standard, the binding capacity was also determined for IgG in human plasma. The Mimetic Blue SA A6XL column was characterized using human plasma. The selectivity of the depletion was dependent on the amount of human plasma that was loaded on the column. After the conditions on both supports had been optimized, the Mimetic Blue SA A6XL stationary phase was combined with the CIM Protein G disk in order to simultaneously deplete samples of human plasma. A centrifuge spin column that enables the removal of IgG and HSA from 20 microl of human plasma was designed. The results of the depletion were examined using sodium dodecyl sulfate polyacrylamide gel electrophoresis and two-dimensional gel electrophoresis.

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