International Journal of Medicine and Pharmaceutical Sciences (IJMPS)
ISSN (P): 2250-0049; ISSN (E): 2321-0095
Vol. 5, Issue 2, Apr 2015, 15-22

DEPLETION OF ALBUMIN FROM HUMAN SERUM FOR HIGH THROUGH PUT PROTEOMICS STUDIES

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ABSTRACT

Sample preparation from serum and plasma to use in proteomics, mass spectrometry (MS) and two dimensional gel (2D gel) based analytical methods, has received a greater focus to get reliable and reproducible data. Particular emphasis has been placed on the removal of the most abundant proteins such as albumin. The commercial kits based on affinity chromatography are expensive for a large sample size and have shelf life limitations. Our objective through this study was to develop an efficient and cost effective method for removal of albumins. Blue-sepharose, a 6% cross-linked agarose with a particle size range of 45-165µm, was prepared in a syringe column. Total proteins of the serum were allowed to bind to the column and five different elution buffers were used to elute proteins devoid of albumin/ immunoglobulin. The eluted proteins were subjected to gel fractionation. Our results indicated that binding capacity of the sepharose slurry to serum albumin was approximately 4mg/ml. Moreover, buffer "C" (0.1M Tris, 0.04M BME pH 7.5) showed the best retention of albumin on the column as indicated by the quality of albumin free eluted proteins from diabetic patients and healthy control group. Further, we observed that a careful selection of the elution buffer was required for a particular set of proteins to be removed from serum. A simple and cost effective micro-column, albumin purification method from serum is described. This provided good quality and quantity of albumin depleted samples for analysis of serum proteome complexity.

KEYWORDS: Diabetic Patients, Serum, Blue-Sepharose, Albumin Removal, Proteomic Study