INTRODUCTION

Introduction: Human serum albumin (HSA) is the most abundant protein in human blood plasma. It is comprised of 585 residues with 17 disulfide bonds. Cy3 is its single free thiol group which is thought to act as an oxidant scavenger. Previous chromatographic studies have shown that the HSA-SH fraction of HSA decreases with age; 76% vs. 48%, young vs elderly healthy male subjects, respectively. 1-2 The lower percent of SH present is a measure of the frailty of the person. Since ELISA can only measure total albumin, it does not provide a complete assessment of the person’s overall health. Although typical serum albumin levels range from 35-50 mg/mL of serum, lower or higher levels of certain serum albumins indicate disease states (e.g., Cirrhosis of the liver, severe dehydration-kidney disease) which requires treatment. Thus test kits that simply measure the total amount of HSA present in blood plasma may not completely describe the patient condition regarding a measure of frailty of the patient. This poster presents the ability of using LC coupled to high resolution mass spectrometry (HRMS) for the analysis of the different intact albumin proteins.

METHOD

Method: A sensitive colorimetric assay kit from Sigma-Aldrich, the Bromocresol Green Assay kit (catalog MAK124), was used to quantitate albumin levels in human sodium heparin plasma samples. The method involves addition of single working reagent to standards, controls and samples in a 96-well polyethylene plate at ambient temperature. Following incubation, the plate is read on a plate reader (e.g. SpectraxMax M2e) at 620 nm. The kit utilizes bromocresol green, which forms a colored complex specifically when bound to albumin. The intensity of color (measured at 620 nm) is directly proportional to the albumin concentration in sample. By comparison, a uHPLC-HRMS method which determines the concentration and intact molecular weight of the various whole albumin proteins was used. The advantage of the LC-MS system is the ability to separate different Albumin species present in the plasma.