Comparison of the Quantitative Measurement of Albumin in Human Plasma Using ELISA and uHPLC-High Resolution Mass Spectrometry

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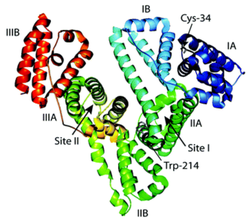
Introduction

Introduction: Human serum albumin (HSA) is the most abundant protein in human blood plasma. It is comprised of 585 residues with 17 disulfide bonds. Cys34 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 polystyrene plate and incubation for 5 minutes at ambient temperature. Following incubation, the plate is read on a plate reader (e.g. SpectraMax 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.



HSA Structure

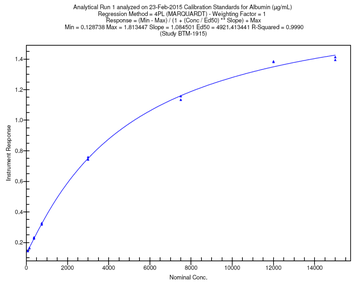
HPLC & HR-MS Conditions

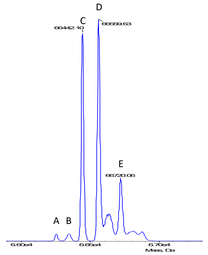
HPLC Conditions	Description
HPLC	Shimadzu LC20AD
Mobile Phase A	0.1% Formic acid in H_2O
Mobile Phase B	0.1% Formic acid in ACN
Autosampler Temp	Ambient
Separation Type	Gradient
Column	Waters C4 Column, 2.1 x 50 mm, 3.5 µm
Flow Rate	0.50 mL/min
Injection Volume	10 µL
Sciex TT 6600	ESI
Ionization Mode	Positive
Assay Range	1 – 200 µg/mL
Sample Treatment	PBS Dilution
Anticoagulant	Na Heparin

MS Conditions	Description
Mass Spectrometer	Sciex TT 6600
Ion Source	ESI
CUR	50
GS1	50
GS2	50
IS	4500
TEM	500 °C
DP	200

Time (Minutes)	Flow Rate (mL/min)	%B
0	0.5	20
0.5	0.5	25
2.0	0.5	80
2.5	0.5	95
3.5	0.5	95
3.6	0.5	20
5.6	0.5	20

Typical BCG Albumin Assay Curve



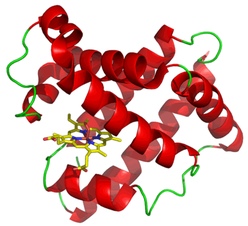


Description of Albumin Peaks Observed by LC-HRMS

Peak ID	MW (Da)	Isoforms	Functional Consequence [4]
A	66253 (-187)	HSA – DA	Loss of chelating function, loss of free radical scavenging activity
B	66343 (-97)	HSA + Cysteine - DA	Impaired chelating function, loss of free radical scavenging activity
C	66439	Native Form of HSA	Native Form of HSA with fully preserved activity
D	66560 (+120)	HSA + CYS (+O_2)	Loss of antioxidant activity
E	66720 (+280)	HSA + CYS + GLY	Impaired antioxidant and binding activity, conformational alteration

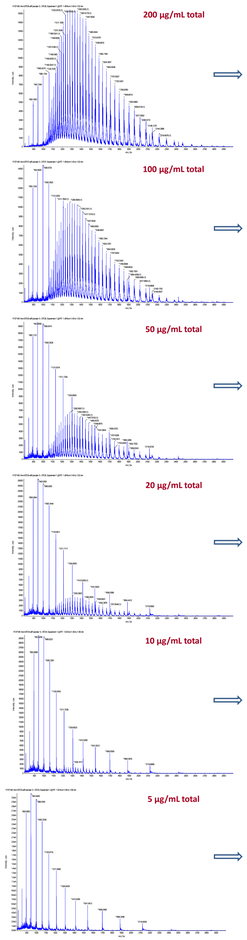
Sample Preparation for BCG Assay Kit

- ❑ Starting from a 250 mg/mL solution of HAS in DI water, dilute using PBS buffer into a curve range of 150 – 15,000 µg/mL Albumin concentration
- ❑ Prepare QCs at the LLOQ (150 µg/mL), LQC (450 µg/mL), MQC (7,500 µg/mL), HQC (12,000 µg/mL), and ULOQ (15,000 µg/mL) for run acceptance.
- ❑ Pre-dilute samples 1:10 by adding PBS for example 20 µL sample + 180 µL of PBS.
- ❑ Transfer 50 µL of standards, controls, blank, and pre-diluted samples from tubes to 96-well polystyrene plate in duplicate as per plate map.
- ❑ Add 200 µL of reagent and tap lightly to mix, avoiding bubbles.
- ❑ Incubate 5 minutes at RT and measure absorbance at 620 nm on plate reader.
  - ❑ SpectraMax M2e ROM V3.022

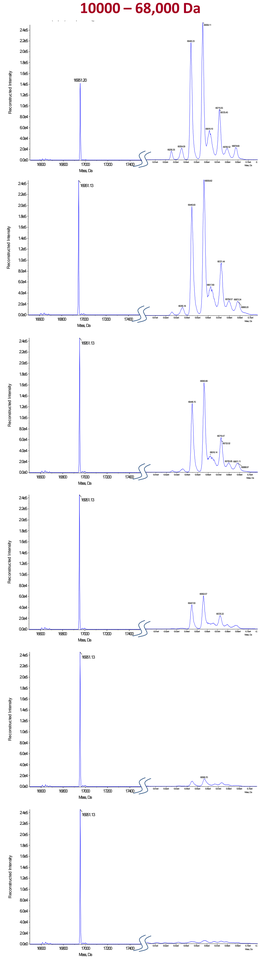


Myoglobin Structure

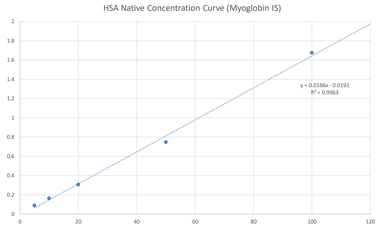
Mass Spectrum m/z 600-4000



Deconvoluted Mass Spectrum 10000 – 68,000 Da



Typical LC-HRMS Albumin Curve



Conclusions

Results: We compared the difference between the colorimetric assay of total HSA vis-à-vis the quantitative analysis of HSA using uHPLC coupled to HRMS of the intact proteins.[3] Differences between sample analysis using the colorimetric assay versus the uHPLC-HRMS assay were observed and are attributed to the speciation of the different albumins chromatographically. Generally, the native form of HSA attributed to approximately 1/3 of the total albumin in the plasma.

Conclusions: With this method a complete picture of the albumins present in human plasma can be determined, i.e., total albumin as compared to different albumin types (oxidation states, glycation forms, etc.). Differences between the colorimetric assay of total HSA and the quantitative analysis of whole HSA protein types, using uHPLC-HRMS, were observed providing a more complete picture of potential health issues.

References

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[2] S. Era, K. Kuwata, H. Imai, K. Nakamura, T. Hayashi, M. Sogami, Biochim. Biophys. Acta 1247 (1995) 12-16.
[3] L. Turell, H. Botti, L. Bonilla, M. Torres, F. Schopfer, B. Freeman, L. Armas, A. Ricciardi, B. Alvarez, R. Radi. J. Chrom. B. 944 (2014) 144-151.
[4] M. Domenicali, M. Baldassarre, F. Giannone, M. Naldi, M. Mastroroberto, M. Biselli, M. Laggetta, D. Patrono, C. Bertucci, M. Bernardi, and P. Caraceni, Hepatology. 60:6 (2014) 1851-1860.
[5] http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/2/mak124bul.pdf

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