Affinity Removal of the 14 Most Abundant Proteins in Human Plasma Using the Agilent 1260 Infinity Bio-inert Quaternary LC System

Application Note

Proteomics & Protein Sciences

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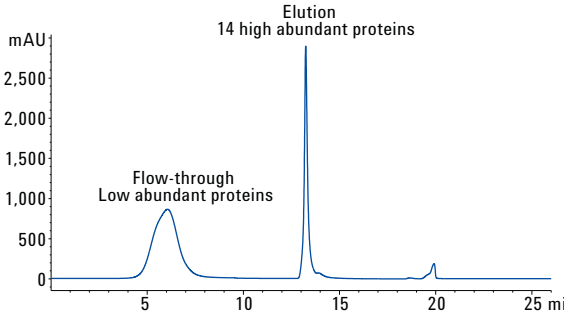
Abstract

This Application Note describes the depletion of the "Top 14" high abundant proteins from plasma using the Agilent Multiple Affinity Removal System together with the Agilent 1260 Infinity Bio-inert Quaternary LC. Using the bio-inert HPLC system ensures a high reproducibility of the depletion procedure, as metal leaching and corrosion caused by high salt/low pH buffers is avoided. Subsequent analyses using the Agilent 2200 TapeStation and Agilent 2100 Bioanalyzer Systems show efficient removal of high abundant proteins from the more clinically relevant low abundance proteins.



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Introduction

The plasma proteome is the most complex human-derived proteome, containing proteins present at concentrations which can differ by more than 10 orders of magnitude[1]. The protein with the highest abundance in plasma is serum albumin, present at concentrations up to 50 mg/mL[2], in contrast, proteins such as interleukin-6 are only present in the low pg/mL range[3].

Mass spectrometric analysis can play an essential role in proteomic studies. However, mass spectrometry based assays are particularly affected by ionization suppression. Signals corresponding to the low abundant proteins are masked by those derived from the high abundant proteins. To obtain limits of detection (LOD) and limits of quantification (LOQ) in the clinically relevant range, either an enrichment of the low abundant proteins or a depletion of the high abundant proteins must be performed.

With the Agilent Human 14 Multiple Affinity Removal System (MARS), the depletion of the "Top 14" high abundant proteins can be achieved up to an efficiency of 94 % reduction of total protein[4]. MARS is based on affinity interactions, and contains an affinity HPLC column plus optimized buffers for sample loading, washing, eluting, and regeneration[5,6]. It is specifically designed to fractionate 14 high-abundant proteins from human biological fluids such as plasma, serum, and cerebral spinal fluid (CSF). This technology enables the removal of albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3, and transthyretin in a single chromatographic step.

Due to the high salt and low pH buffers used in the depletion procedure with the MARS column, use an inert system to avoid problems arising from stainless steel systems such as metal leaching or corrosion of the system. The Agilent 1260 Infinity Bio-inert Quaternary LC system together with the Agilent 1260 Infinity Bio-inert Fraction Collector provides automated peak-based (in addition to time-based) fraction collection within a metal-free environment. Therefore, it is an ideal system to use for plasma depletion procedures with the MARS as an important sample preparation step to provide depleted plasma for subsequent proteomic studies.

Experimental

The Agilent 1260 Infinity Bio-inert Quaternary LC system consisted of the following modules:

- Agilent 1260 Infinity Bio-inert Quaternary Pump (G5611A)
- Agilent 1260 Infinity High performance Bio-inert Autosampler (G5667A)
- Agilent 1290 Infinity Thermostat (G1330B) for sample cooling
- Agilent 1290 Infinity Thermostat (G1330B) for fraction cooling
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C) with bio-inert solvent heat exchangers
- Agilent 1260 Infinity DAD VL (G1315D with a 10-mm bio-inert standard flow cell)
- Agilent 1260 Infinity Bio-inert Analytical-scale Fraction Collector (G5664A)
- Agilent 2100 Bioanalyzer Instrument (G2943CA)
- Agilent 2200 TapeStation Instrument (G2964AA)

Software

- OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.05 [35]
- Agilent 2100 Expert Software (version B.02.08 (SR2))
- Agilent 2200 TapeStation Controller Software (version A.01.04), Agilent 2200 TapeStation Analysis software (version A.01.04)

Agilent Multiple Removal System

The Agilent Multiple Affinity Removal System consisted of the following:

- Affinity column (4.6 × 50 mm dimensions were used with a capacity of 20 µL human plasma/serum (p/n 5188-6557)
- Starter Reagent Kit (5185-5986), which includes:
    - Buffer A for sample loading
    - Buffer B for eluting bound proteins
    - Spin filters 0.22 µm for sample cleanup before loading column
    - Concentrators, 5 kDa, MWCO, for concentrating flow-through fractions
- Agilent High Sensitivity Protein 250 Kit (p/n 5067-1575) for Bioanalyzer analysis
- P200 ScreenTape (p/n 5067-5371) and P200 Reagents (p/n 5067-5372) for TapeStation analysis

Human plasma and phosphate buffered saline (PBS) tablets were purchased from Sigma-Aldrich, St. Louis, USA. The plasma was diluted according to the MARS sample preparation protocol.

Chromatographic conditions

Sample processing and fractionation were performed according to the protocol provided with the Agilent Multiple Affinity Removal System. Table 1 shows the chromatographic conditions. To prevent salt crystal blockage of the capillary tubing due to the high salt concentration of the used buffers, it is highly recommended to flush the system extensively with water after the MARS procedure.

Results and Discussion

Figure 1 displays an overlay of the first, 10th, 20th, 30th, 40th, and 50th chromatogram from a 4.6 × 50 mm MARS column for the removal of the "Top 14" high abundant proteins from human plasma. For sample loading, a slow flow rate of 0.125 mL/min was used to ensure optimal antibody-antigen recognition and binding. In contrast to the binding step, the flow rate was then elevated to 1 mL/min for elution and re-equilibration. The flow-through fraction peak from minute 5 to 7 represents the low abundant proteins, which are not binding to the affinity column. After changing the loading buffer to the elution buffer at 11.51 minutes, the peak between minute 13 and 14 represents the eluted 14 high abundant proteins, which were bound to the column. Figure 1 shows that the high precision of retention time and area was achieved over 50 injections. The precision of retention time and area was < 0.052 and < 2.64 RSD, respectively, for both peaks.

Table 1. Chromatographic conditions.

Chromatographic conditions			
Gradient	Time	%B	Flow rate
	0.00	0.00	0.125
	9.50	0.00	0.125
	9.51	0.00	1.000
	11.50	0.00	1.000
	11.51	100.00	1.000
	16.00	100.00	1.000
	16.01	0.00	1.000
	25.00	0.00	1.000
Injection volume	80 µL		
Thermostat autosampler and FC	4 °C		
Temperature TCC	RT		
DAD	280 nm/4 nm, Ref.: OFF		
Peak width	> 0.05 minutes (1.0 second response time) (5 Hz)		
Fraction collection	Peak-based with a threshold at 17.5 mAU		

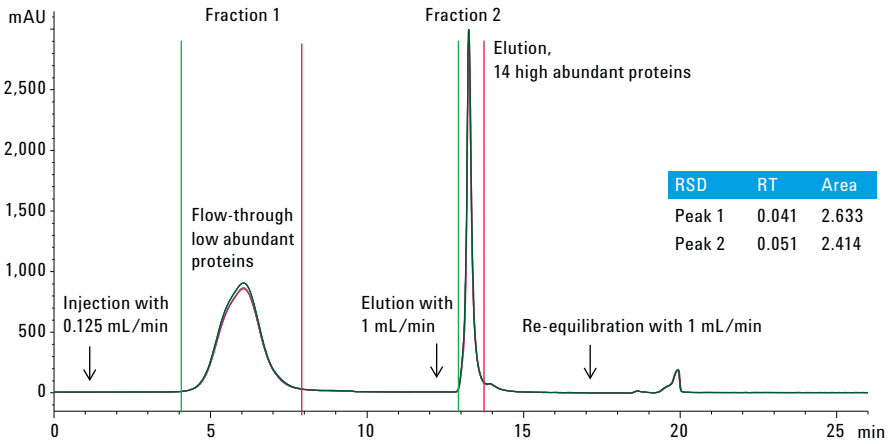

Figure 1. Overlay of the first, 10th, 20th, 30th, 40th, and 50th chromatogram of the affinity removal of the "Top 14" high abundant proteins from human plasma using a 4.6 × 50 mm MARS column.

Figure 2 A shows results obtained from the Agilent 2200 TapeStation System for the processed plasma samples using the 4.6 × 50 mm MARS column and the P200 ScreenTape Assay. Lane H2 shows unprocessed plasma, B1 represents the flow-through fraction with the low abundant proteins, and B2 represents the eluted 14 high abundant proteins. Including the sample QC of the protein samples on the 2200 TapeStation System as an automated quality control step, is a simple-to-use, rapid, and reproducible option to check the MARS purification efficiency.

Figure 2 B shows analysis of the samples on the 2100 Bioanalyzer Instrument using the High Sensitivity Protein 250 Assay. Sample 1 represents the flow-through fraction of the low abundant proteins, Sample 2 represents the eluted 14 high abundant proteins, whereas Sample 3 is unprocessed plasma.

This assay can be used to obtain quantitative information, especially for characterization of very low concentrated protein samples. As such, more protein bands are seen compared to the 2200 TapeStation Analysis. Due to the assays wider sizing range, high molecular weight proteins can also be detected although accurate sizing is only possible up to 250 kDa. Even on this high level of sensitivity, it could be shown that removal of high abundant proteins from the low abundant ones in the flow through has worked successfully.

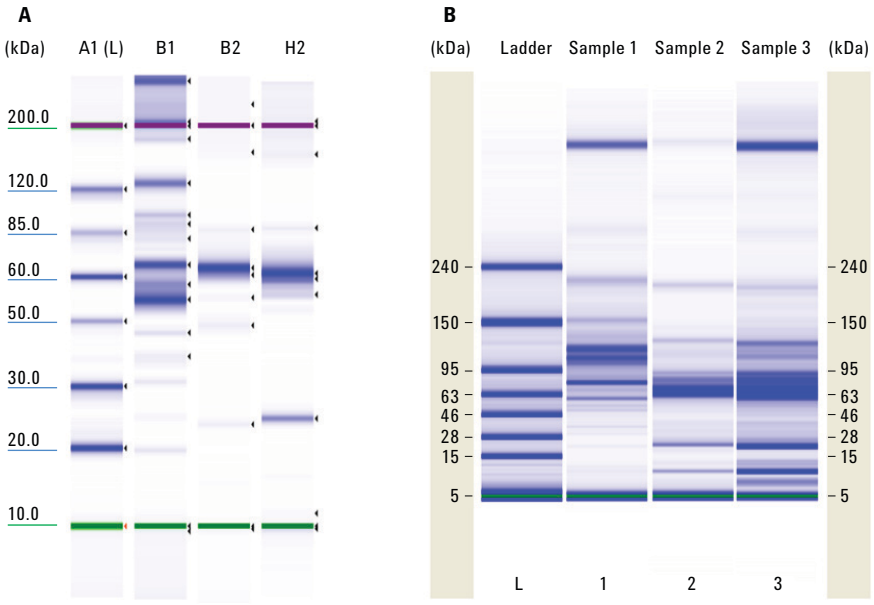

Figure 2. A) Agilent 2200 TapeStation analysis of the processed an unprocessed plasma sample. A1: Ladder, B1: Flow-through low abundant proteins, B2: Eluted high abundant proteins, H2: Unprocessed plasma sample. B) Bioanalyzer analysis using the High Sensitivity Protein 250 Assay with Sample 1: Low abundant flow-through, Sample 2: High abundant, and Sample 3: Unprocessed plasma.

Both, the 2200 TapeStation and the 2100 Bioanalyzer analyses are complimentary. The 2200 TapeStation P200 assay is a rapid and user-friendly way to qualitatively monitor effective removal of the high abundant proteins in unprocessed plasma. If a higher level of detail is required, the user can use the 2100 Bioanalyzer High Sensitivity P250 assay which shows the very low concentrated proteins.

In addition, more low abundant proteins become visible after depletion using the Agilent Multiple Affinity Removal System.

Conclusions

The Agilent Multiple Affinity Removal System together with the Agilent 1260 Infinity Bio-inert Quaternary LC system with Bio-inert Fraction Collection is an ideal solution for the removal of the "Top 14" high abundant proteins from plasma. The bio-inert system ensures high reproducibility of the Multiple Affinity Removal System avoiding corrosion and metal leaching due to the employed high salt/low pH buffers. Analysis on the TapeStation and Bioanalyzer systems after the depletion procedure show an efficient removal of high abundant proteins from the flow-through low abundant proteins. The depletion enables the detection of more protein bands representing potential low abundant proteins of clinical interest. The enriched low abundant proteins can then be subsequently used for additional proteomic studies using for example mass spectrometry.

References

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