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Application Note

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Vivaspin® Turbo 4 PES: An Economic Approach to Separate Disease Metabolites and Proteins

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Abstract

The presence of biomolecules, such as disease metabolites and protein markers, in patient samples provide a key measurement for disease diagnostics. The efficient separation of these biomolecules is required both for diagnostic analysis and for disease marker research, such as co-expression profiles. Available separation methods commonly employing precipitation chemicals, are time consuming or come with high cost and set-up optimization. Here we demonstrate ultrafiltration with Vivaspin® Turbo 4 PES centrifugal devices as a highly effective method for the rapid and precise separation of biomolecules from blood serum, using NMR and off-gel fractionation to evaluate.

Find out more: www.sartorius.com/en/products/lab-filtration-purification/ultrafiltration-devices

Introduction

The currently perceived wisdom is that the application of the new "omics" sciences to biological systems will result in new biomarkers for disease diagnostics, patient stratification and the monitoring of drug efficacy.

One area to benefit from such an application is cancer biology. The key to success in this research area depends on establishing the link between the expression of metabolites (proteins with MW <3 kDa) with proteins larger than 3 kDa and their efficient separation from each other in the human serum. Multiple Affinity Removal System (MARS) is generally employed to separate the high-abundant proteins (Albumin, IgG, Antitrypsin, IgA, Transferrin and Haptoglobin) from human serum, plasma and other biological fluids, based on the principle of affinity chromatography. The removal of these abundant proteins improves LC/MS and electrophoretic analysis of the serum sample by effectively expanding the dynamic range. Apparently, using this system results in high cost (4× w.r.t. ultrafiltration) to the user. In contrast, ultrafiltration proves to be economic, equally efficient and ergonomic to accomplish this procedure.

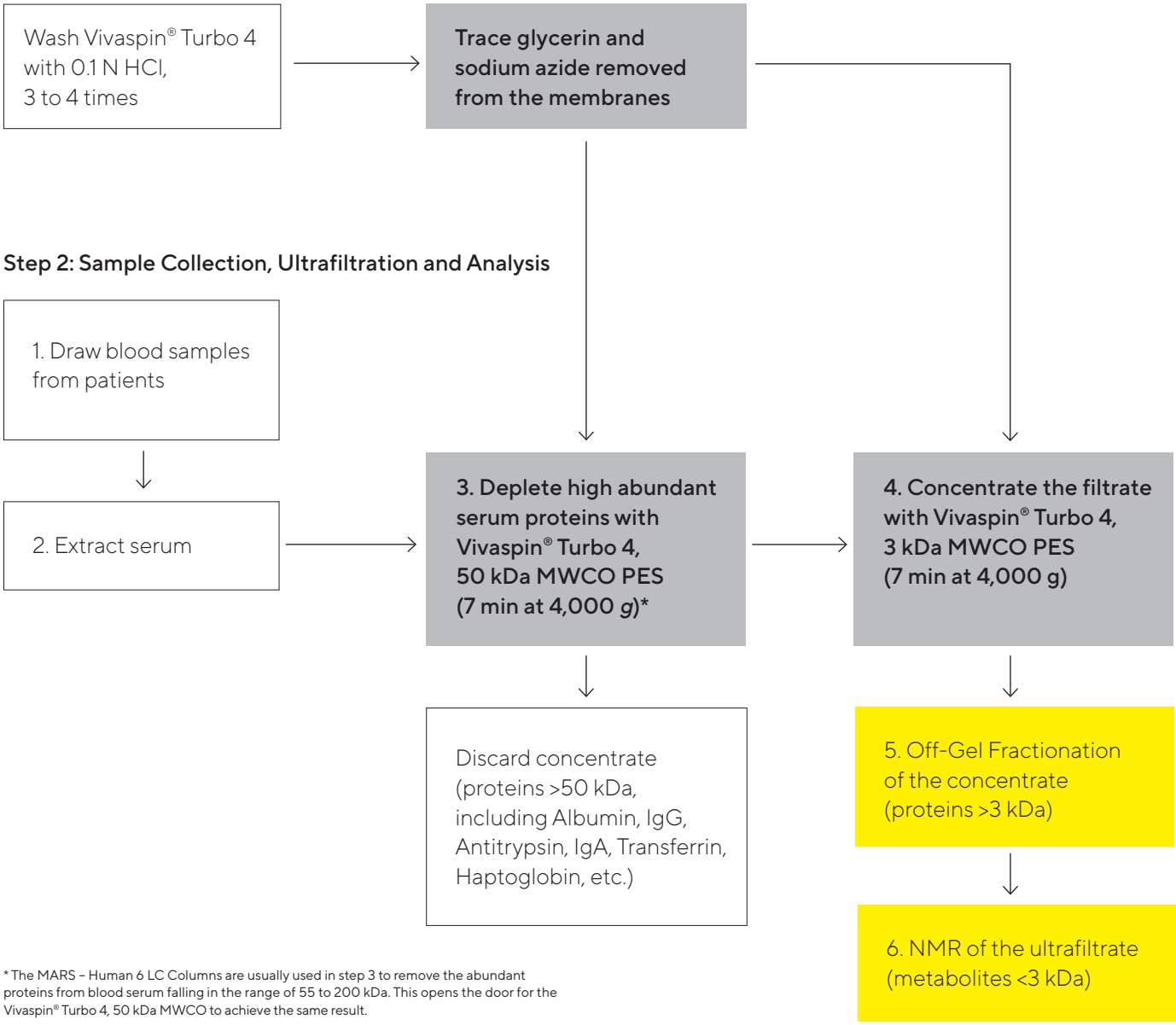
Ultrafiltration separates proteins | biomolecules based on their size and configuration by passing a sample through a membrane under a driving force - usually generated by centrifugation or pressure.

Ultrafiltration membranes are characterizd by a molecular weight cut-off (MWCO) expressed in kilodaltons (kDa). This acts as a barrier to larger proteins, which are retained, whilst smaller molecules and can pass through. In this study, Vivaspin® Turbo 4 PES, incorporating a dual membrane with extraordinary flow rates and convenient recovery from the patented angular dead-stop pocket, were used.

Suggested Method

The following method was suggested to obtain the desired results:

Step 1: Concentrator Preparation



* The MARS – Human 6 LC Columns are usually used in step 3 to remove the abundant proteins from blood serum falling in the range of 55 to 200 kDa. This opens the door for the Vivaspin® Turbo 4, 50 kDa MWCO to achieve the same result.

Equipment & Consumables

1. Vivaspin® Turbo 4 PES, 3 kDa MWCO (Order No. VS04T91)
2. Vivaspin® Turbo 4 PES, 50 kDa MWCO (Order No. VS04T31)
3. Centrifuge – Sigma Aldrich (fixed angle with cavities for 15 mL tubes)
4. Agilent 3100 OFFGEL Fractionator
5. Agilent 2100 Bioanalyser
6. Pipette – Eppendorf
7. Brucker Avance 800 MHz NMR

Test Samples

Intravenous Blood drawn from unhealthy individuals | patients admitted at S.G.P.G.I Hospital, Lucknow, India were used as test samples and analyzed in comparison with the healthy individual.

Notes

Pre-rinsing was first carried out by filling the concentrator with deionized water. This resulted in observance of undesired peaks in NMR spectra (Figure 1).

Since this could cause interference with the analysis, the pre-rinsing was performed with 0.1 N HCl solution (12 – 14 mL for one concentrator, which is 3 – 4 times with Vivaspin® Turbo 4 PES). This eliminated the unwanted peaks (Figure 2).

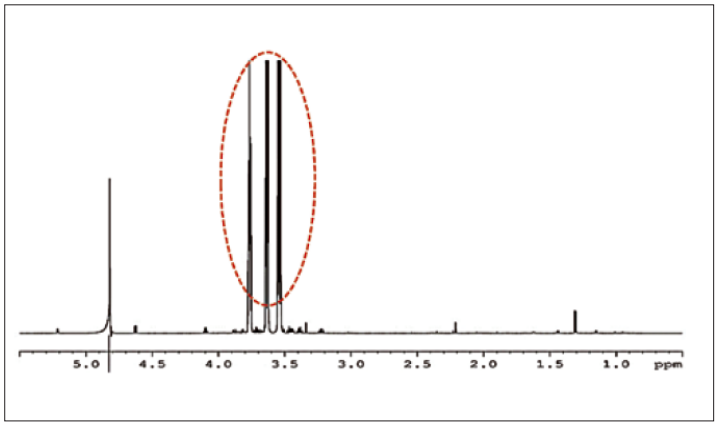


Figure 1

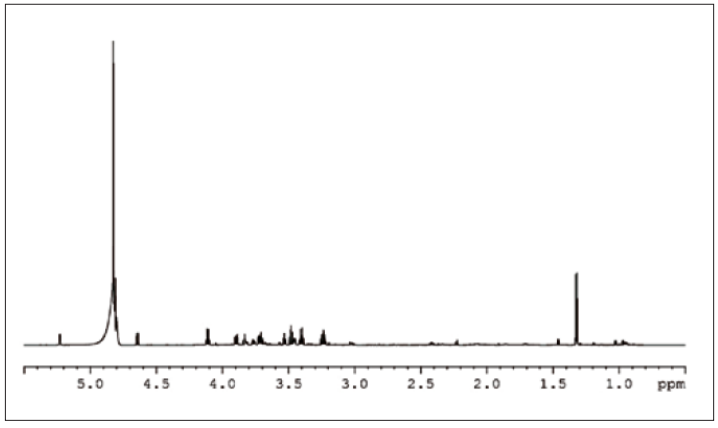


Figure 2

Results and Discussion

The filtrate obtained after ultrafiltration of serum samples with Vivaspin® Turbo 4 PES (50 kDa MWCO) eliminated all abundant high molecular weight proteins, comparably to the MARS (Agilent). Afterwards the filtrate was concentrated using a 3 kDa MWCO to separate the proteins from metabolites. The concentrate was analyzed by Off-Gel Fractionation (Figure 3) and the filtrate containing small metabolites was analyzed by NMR (Figure 4). There was no need to use such costly affinity chromatography based system when a simpler technology existed. The cost of analysis for 1 sample from MARS was estimated 4 times more expensive than the cost of using Vivaspin® Turbo 4 PES.

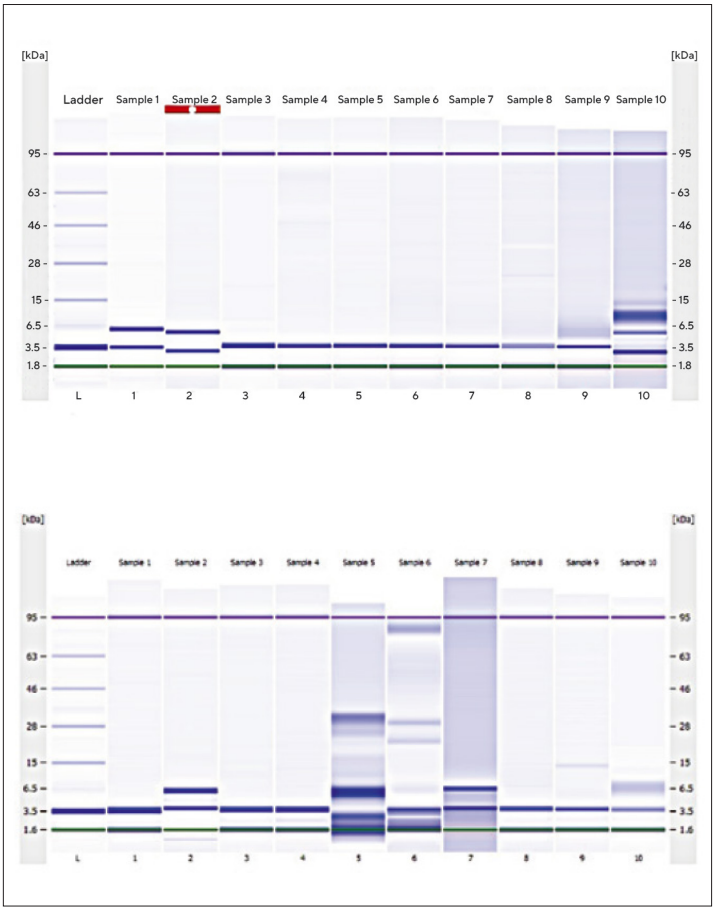


Figure 3: Off-Gel Fractionation analysis of proteins from serum of healthy (top) and diseased (bottom) individuals.

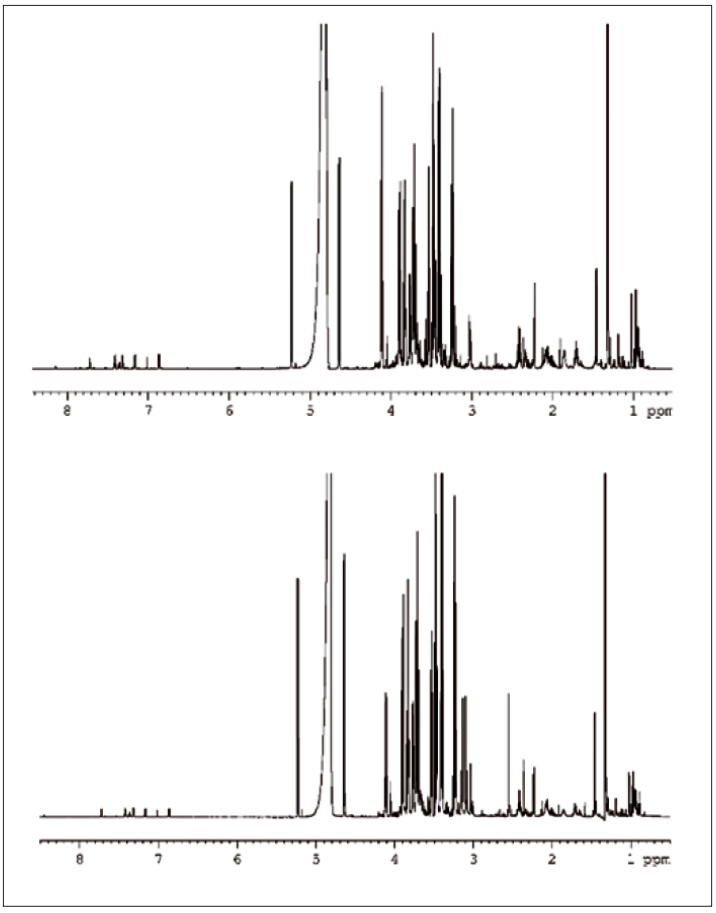


Figure 4: NMR analysis of metabolites from serum of healthy (top) and diseased (bottom) individuals.

The result obtained from Off-Gel Fractionation and NMR, showed over- and under-expression of certain protein and metabolites respectively when compared between the healthy and diseased individual's serum samples. This was made possible with the two step ultrafiltration using Vivaspin® Turbo 4 PES as expected and demanded by this research. This completely eliminated the need to use MARS.

Testimonial

"Ultrafiltration based protein separation is a highly useful approach to obtain the desired proteins without any chemical treatment. It consumes less time, and is an economical and robust technology. The results are reproducible. They can be used for targeted or non-targeted approach to remove the desired proteins and small molecular weight molecules. I saved a lot of cost which might've incurred using the MARS system just for one step. I thank the team of Sartorius for enlightening me about the efficiency of using ultrafiltration spin columns for my application at the right time."

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