

Vivaspin® Turbo 4

Ultrafiltration – An Economic and Ergonomic Approach to Separate Proteins & Metabolites for Disease Detection



Application
Note

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Summary

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 the success of this study depends on establishing the link between the expression studies of the metabolites (proteins with MW <3 kDa) with proteins of size bigger than 3 kDa and their efficient "separation" from each other in the human serum. Multiple affinity removal system is generally employed to separate the high-abundant proteins (Albumin, IgG, Antitrypsin, IgA, Transferrin and Haptoglobin) based on the principle of affinity chromatography from human serum, plasma and other biological fluids. The removal of these abundant proteins improves LC/MS and the electrophoretic analysis of the serum sample by effectively expanding the dynamic range of the analysis. Apparently, using this system causes in a lot of cost (4× w.r.t. ultrafiltration) to the user. At the same time, ultrafiltration proves to be economic, equally efficient and ergonomic to accomplish this procedure. Ultrafiltration separates protein | biomolecules based on their size and configuration by passing

their solution through a membrane under a driving force either, centrifugal or pressure-driven.

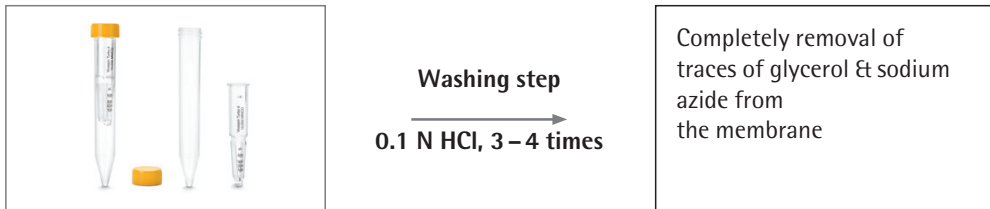
The membrane retains most of the particles above its retention rating and allows smaller molecules to pass through them. The membranes are characterized by a molecular weight cut-off (MWCO) expressed in daltons. This acts as a barrier to the protein exceeding the MWCO. In this study, Vivaspin® Turbo 4 incorporates a dual-membrane with extraordinary flow rates and a better recovery using the patented angular dead-stop pocket were used.



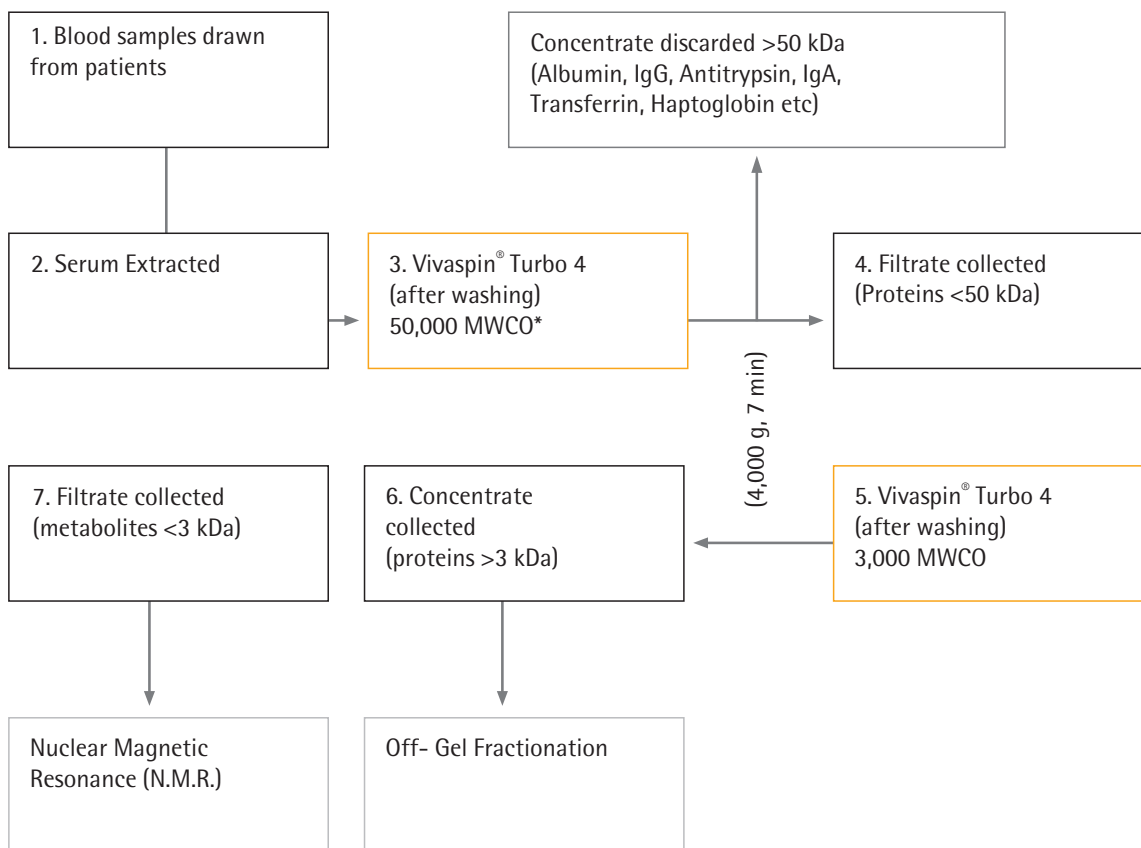
Suggested Method

The following method was suggested to obtain the desired results:

Step 1: Spin Column Preparation



Step 2: Ultrafiltration



Equipment & Plastic Ware

1. Vivaspin® Turbo 4, 3,000 MWCO, Cata No: VS04T91
2. Vivaspin® Turbo 4, 50,000 MWCO, Cata No: VS04T31
3. Centrifuge – Sigma Aldrich (15 ml rotor capacity, Fixed angle)
4. Agilent 3100 OFFGEL Fractionator
5. Agilent 2100 Bioanalyser
6. Pipette – Eppendorf
7. Bruker Avance 800 MHz NMR

Test Sample

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

The pre-rinsing was first carried out by filling the concentrator with deionised water. This resulted in observance of undesired peaks in NMR spectrum. (See Figure 1).

This caused an interference with the analysis. Hence, the pre-rinsing was once carried out with but with 0.1 N HCl solution (12 – 14 mL for one concentrator which is 3 – 4 times with Vivaspin® Turbo 4). This eliminated the unwanted peaks observed earlier in the NMR spectrum. (See Figure 2).

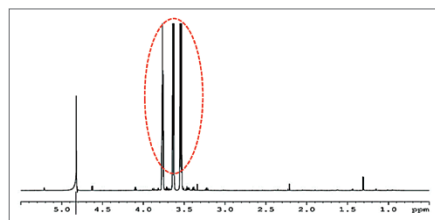


Figure 1

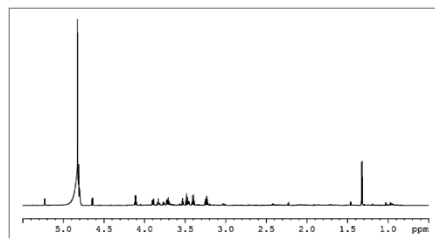


Figure 2

* The multiple Affinity Removal System – Human 6 LC Columns are applied in step 3 to remove the abundant proteins from the blood serum falling in the range of 55 to 200 kDa. This opens the door for the Vivaspin® Turbo 4, 50 kDa cut-off to replicate the same result.

Results and Discussion

The filtrate obtained after spinning the serum samples with Vivaspin® Turbo 4. The 50 kDa MWCO eliminated all abundant high molecular weight proteins like the multiple affinity removal system from Agilent. Afterwards the filtrate is concentrate with the 3 kDa MWCO concentrator to separate the proteins from metabolites. The concentrate were analyzed by the

off-gel Fractionation (Figure 3) and the filtrate containing the small metabolites were analysed by using NMR (Figure 4). There was no need to use such costly affinity chromatography based system when an easier technology still existed. The cost of analysis for 1 sample from MARS system was estimated 4 times more expensive than the cost of using Vivaspin® Turbo 4.



Figure 3: Healthy Individual

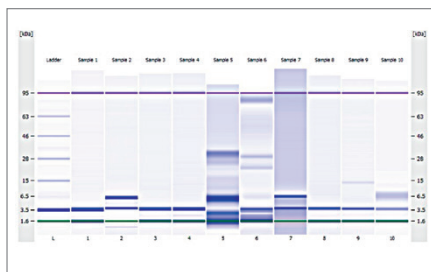


Figure 3: Diseased Individual

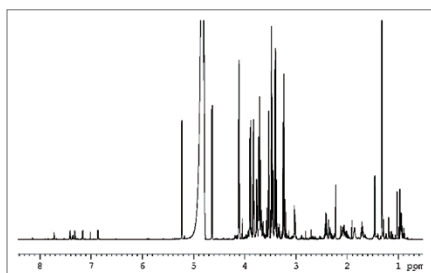


Figure 4: Healthy Individual

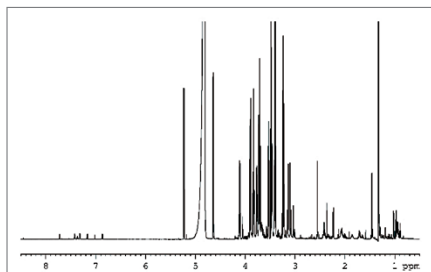


Figure 4: Diseased Individual

The result obtained from the off-gel fractionation and NMR, showed over-expression 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 concentration with Vivaspin® Turbo 4 as was expected and demanded by the aim of this research. This completely eliminated the demand of using MARS system in any of these steps.

Testimonial

"Ultrafiltration based protein separation is a highly useful approach to obtain the desired proteins without any chemical treatment. It consumes very less time, economical, and a 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."

Sartorius Lab Instruments
GmbH & Co. KG
Weender Landstrasse 94–108
37075 Goettingen, Germany
Phone +49.551.308.0
Fax +49.551.308.3289
www.sartorius.com

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