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Routine and Robust Plasma Protein Profiling for Clinical Research Applications on the Vanquish Horizon/Exploris platform

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ABSTRACT

Purpose: Profiling the plasma proteome using liquid chromatography-mass spectrometry has been a long sought after application that holds promise for monitoring health. The execution has been limited in the past by the high analytical dynamic range needed. Ultra High Performance Liquid Chromatography coupled with Orbitrap detection has revolutionized the depth at which this proteome can be quantified. Here we show using modified Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer with S-lens interface with Easy Internal calibration allows for a compact platform that can deliver the routine robust high performance necessary for profiling proteins in plasma samples.

Methods: Plasma was centrifuged out from whole blood at either 800 RCF for 10min or 2000 RCF for 30 min. These plasma samples differ in platelet, white blood cell and red blood cell numbers, and associated proteins from these contaminants are well characterized. Data dependent MS2 acquisition was used to collect full scan for quantification and identifications, with accurate mas/retention time/spectral matching being done against a comprehensive spectral library from the samples. Replicate LC-MS-MS data dependent runs were performed both sets of plasma samples. A spectral library was created by offline fractioning of a pool of both digested plasma samples into 24 fractions using a neutral pH, negative ion paring method.

Results: The combined library contain 320 high confidence protein groups and 2200 high confidence (1% FDR) peptides. The combined searched library was then used to enhance the depth of the data dependent individual runs. Quantification of peptides from individual runs was done using the Pinnacle software package from Optys with imported searched data. 105 proteins in the runs had a combined CV in both sample types of 10% or less.

INTRODUCTION

Whole blood is the most commonly collected and available bio-specimen collected from clinical trials and other studies – so donor panels tend to be larger. Medium- to high-level proteins in plasma have been associated as potential disease markers so there is a desire to develop highly multiplexed workflows to profile the known markers, but also incorporate comprehensive methods to increase the profiling capabilities of the plasma proteome in hopes of increasing the number of putative markers. While plasma is easily obtained, contains numerous FDA approved protein targets, there are serious challenges to increasing the plasma proteome coverage for routine quantitation.

1. Dynamic range – there are around 20,000 (or more) proteins reported to be in plasma and they cover a wide dynamic range (12 orders of magnitude).
2. Within the protein numbers, Ig's, by nature, have many different forms and sequence variants. Many of the variants have very small sequence differences or the PTMs (glycosylations) differ, causing many issues in performing database searching. Does the selected database used for searching contain the correct sequences for each individual?
3. Composition of the plasma proteome. There are many proteins covering a wide dynamic range, but there are very few proteins that are highly abundant such as albumin, transferrin, and the Igs that can "mask" detection of low-level proteins and hormones. With the stated goal of increasing the plasma proteome coverage, ways of handling the high abundant proteins is mandatory.

The best way to overcome these limitations and sample derived challenges is to combine several critical technology pieces, leveraging each one to yield a superior workflow. The Thermo Scientific™ Vanquish™ Horizon UHPLC provides exquisitely accurate gradients at up to 1500bar to provide highly efficient separations of peptides at high loading capacity. The Orbitrap Exploris 240 with Easy-IC provides accurate full scan quantification, even in the presence of high chromatographic ion peak flux achieved with the Vanquish. Combined with accurate retention time MS/MS spectral libraries, a new depth of routine plasma protein profiling is achievable.

MATERIALS AND METHODS

Sample Preparation

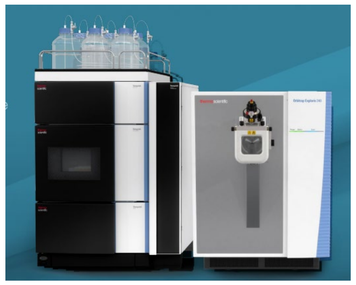
Plasma samples (heparin tubes) from a single donor obtained with IRB approval were spun out at either 800 RCF for 10minutes or at 2000 RCF for 30 minutes. Plasma was removed from the tubes, mixed and aliquoted into 1mL aliquots in a 1.5mL tube, and frozen to -80degC until use. An AB-1300 200uL PCR plate is filled with 100uL per well of 8M GuHCl 275mM Tris-HCl 2% n-propanol 10mM DTT, pH 8.6. Plasma samples, thawed on ice for 1 hour, are vortexed, and 30uL is pipetted into the GuHCl in the plate and mixed with repeated pipetting 3x. The plate is sealed using the Easypeel foil on an APLS 3000 plate sealer(155degC 1.3s) and heated to 37degC for 1 hour. The seal is then removed, and 4.5 uL of 1M iodoacetic acid sodium salt (acros organics) is added to each well. The plate is then sealed again, vortexed for 5 min at 1200rpm and centrifuged for 2 min at 2500RCF. Nunc 2.2mL deepwell plates (Nunc, 278752) are filled with 1.7mL of 50mM Tris-HCl 5mM CaCl2. Samples are transferred to the deepwell plates. To each well is added 120uL of Pierce TPCK trypsin, 1mg/mL in 25mM acetic acid. And the plates sealed with an Easypeel seal (AB-3739). The plates are then incubated for 5 hours at 37degC. after which 100uL of acetic acid is added to each well, and the plates resealed, vortexed and centrifuged for 20 min at 2500RCF.

MATERIALS AND METHODS

Sample analysis

From each sample 45ug(45uL) of plasma digest was injected into a Vanquish Horizon UHPLC system fitted with a 2.1x50mm 3um 100A pore PS-DVB trap column, and an Acclaim C18 120 Bonded phase 2.1x25cm C18 120 2.2um 120A pore analytical column with a 52 min gradient at 250uL a min was used for the separations. The Orbitrap Exploris 240 was set to a 300% target value for the full scan with EASY-IC and a top 20 data dependent MS2 acquisition was used to collect full scan for quantification and identifications.

Vanquish Horizon UHPLC and the Orbitrap Exploris 240



RESULTS

Comprehensive plasma profiling workflow

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Figure 1. Plasma fractionation and spectral library generation

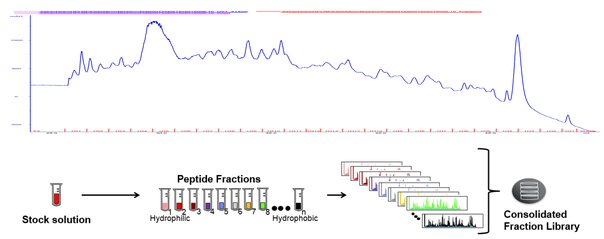

Figure 1. Generation of a sample specific library with accurate retention times provides the most reliable way of assigning both full scan and fragmentation features in individual samples.

Figure 2. Chromatographic Distribution of the spectral library components

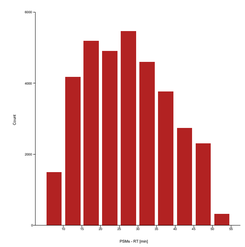

Figure 2 Gradient optimized to yield a durable method and wide distribution of PSM over the gradient

Figure 3. Mass Accuracy distribution of library

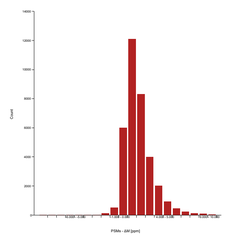

Figure 3 Distribution of PSM mass accuracy for the fractionated libraries and individual sample runs

Figure 4 Non-Differential peptide

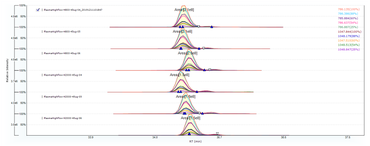

Figure 4 Transthyretin is not found in a platelet or cell compartment, so is not differentially expressed.

Figure 5 Quantification of a differential contaminating protein from platelets

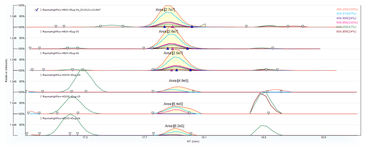

Figure 5 Beta actinin is a protein found in cell structures and is typically at 6ng/mL in normally prepared plasma

Figure 6 Unaligned peptide standards in plasma digests, 140 continuous injections

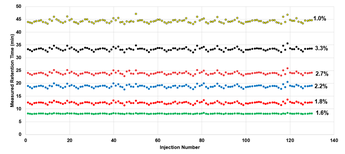

Figure 6 Retention time reproducibly over time is a key feature for a proper plasma profiling platform.

Figure 7 Pinnacle™ aligned peptide standards in plasma digests, 140 continuous injections

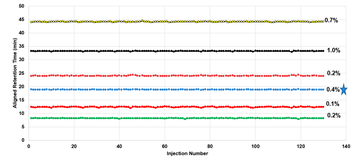

Figure 7 Small retention time shifts over time can easily be corrected. This greatly improves assignment to feature in the library.

CONCLUSIONS

Routine plasma profiling has become more routine and accessible with a combination of several key technology platforms.

- The Vanquish Horizon UHPLC platform combined with the Orbitrap Exploris 240 provides a robust and durable translational and clinical research platform for the routine profiling of plasma proteins.
- The addition of the Easy-IC provides additional functionality for long term mass accuracy for alignment with accurate mass/accurate retention time databases.
- This is critical for low level quantifications that may lack spectral assignments.
- Accurate retention time spectral libraries improve the coverage and identifications of plasma proteins at depth.

ACKNOWLEDGEMENTS

We would like to thank Dr. Mingming Ning of the Clinical Proteomics Research Center at Massachusetts General Hospital for assistance with the phlebotomy.

TRADEMARKS/LICENSING

PO65782-EN 0422S

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