High flow LC-MS analysis of plasma digest for protein identification

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INTRODUCTION

Proteome analysis can play an important role in the discovery of target biomarkers in matrices such as plasma, serum or urine. One way to examine the human proteome is through liquid chromatography mass spectrometry (LC-MS). LC-MS analysis of digested human plasma allows the detection of 1000s of peptides within one run. Bioinformatic analysis then allows the identification of signature peptides that may be used to identify proteins. Data from high flow rate LC-MS has been shown to be able to simultaneously identify 100s of proteins that may then be used as targets for future studies.

MS2 scans may be data-dependent (DDA), data-independent (DIA) or a targeted scan, such as parallel reaction monitoring (PRM). Each of these scan types is accessible through high resolution accurate mass (HRAM) mass spectrometry (MS) or Tribrid mass spectrometers, both of which could efficiently screen for proteins in plasma.

MATERIALS AND METHODS

Sample Preparation

100 µL of 8 M GuHCl, 275 mM Tris-Hcl, 2% n-propanol, 10 mM DTT, pH 8.6, 30 µL of plasma and 4.5 µL of 1M iodoacetic acid sodium salt was vortexed (1200 rpm, 5 minutes) and centrifuged (2500 RCF, 2 minutes). Samples were transferred to 2.2 mL deepwell plates filled with 1.7 mL of 50 mM Tris-Hcl, 5 mM $CaCl_2$, before 120 µL of Pierce TPCK trypsin, 1mg/mL was added. The plate was then incubated (37 °C, 5 hours).

Liquid chromatography and mass spectrometry

From each sample 45 µg of digested plasma was injected into a Vanquish™ Horizon UHPLC system fitted with a 2.1 x 50 mm PS-DVB trap column, and an Acclaim ™ C18 120 Bonded phase 2.1 x 250 mm C18 2.2 µm 120Å pore analytical column with a 52-minute gradient and a flow rate of 250 µL/minute. The Orbitrap Exploris™ 240 was set to a 50% AGC target value for the full scan with EASYIC. DDA, DIA and PRM data were collected in triplicate from various optimized MS2 scans. PRM precursor transitions were generated from a library created from DIA gas phase fractionation data. The Tribrid system used only the quadrupole and linear ion trap for data collection.

Data analysis

Data was processed with Thermo Scientific Proteome Discoverer™ 3.0 (using MSPep Search and INFERYS nodes) and Skyline software.

More selective databases were generated by including only proteins ID'd by DDA and DIA GPF scans. PRM transitions were selected based on relative and absolute area counts of peaks.

RESULTS

Protein and peptide IDs were determined from DDA and DIA scans (Figure 1). Selectively reducing the database size was demonstrated to increase the resolving power of the identification method, significantly increasing the number of protein and peptide IDs for DDA and DIA scans (Figure 2/3).

Figure 1: DIA MS scan for 45 µg of plasma

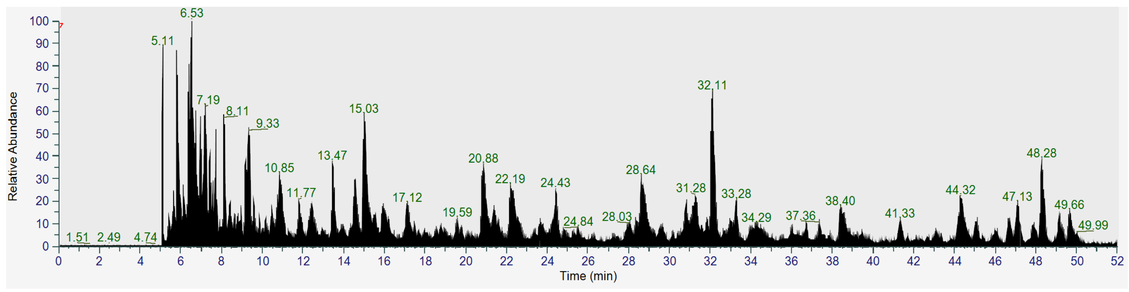
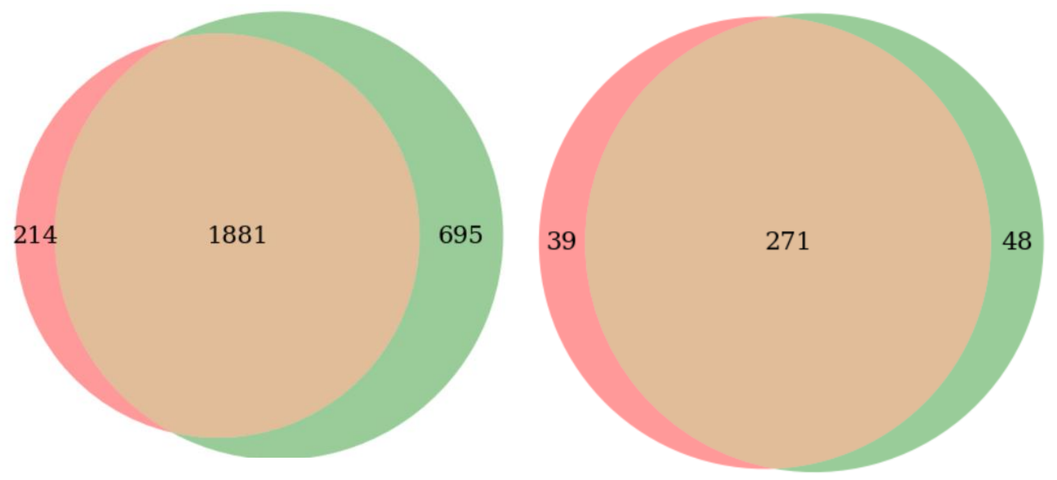
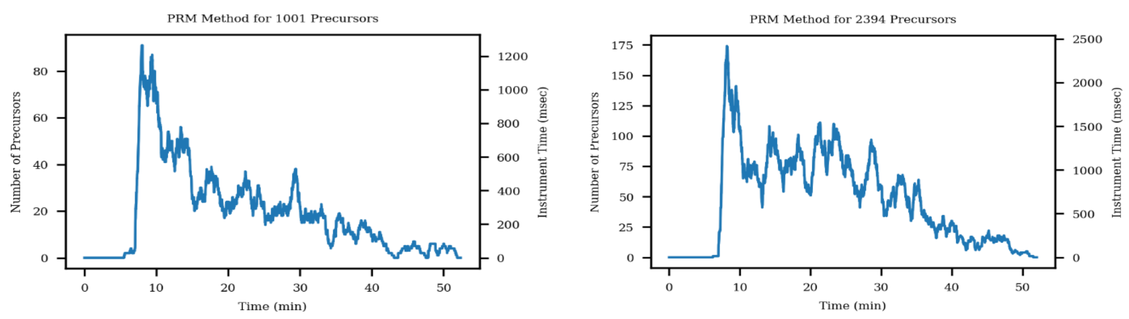


Figure 2: Venn diagrams of unique peptides (left) and proteins (right) from the DIA method on the *Orbitrap Exploris 240* and *Tribrid* mass spectrometers (quadrupole/linear ion trap)



Precursor transitions for the PRM scans were chosen using seven small window gas phase fractionation (GPF) DIA scans (Figure 4). Criteria for selecting transitions included meeting specific intensities and relative intensities. The Tribrid instrument was able to generate 2394 suitable precursors, as opposed to the Orbitrap Exploris' 1001.

Figure 4: Distribution of PRM precursors generated from DIA data on the Orbitrap Exploris 240 (left) and a Tribrid system (right)



Optimized PRM methods on both instruments demonstrated the identification of proteins and peptides at varying concentrations (Figure 5).

Figure 5: PRM peptide and protein IDs (left, N ≥ 3) and Venn diagrams of unique peptides (center) and proteins (right) for various injection loads

Injection load (µg)	Tribrid	Orbitrap Exploris 240
45	237 (1114)	166 (610)
10	213 (933)	125 (379)
2.5	109 (395)	74 (182)

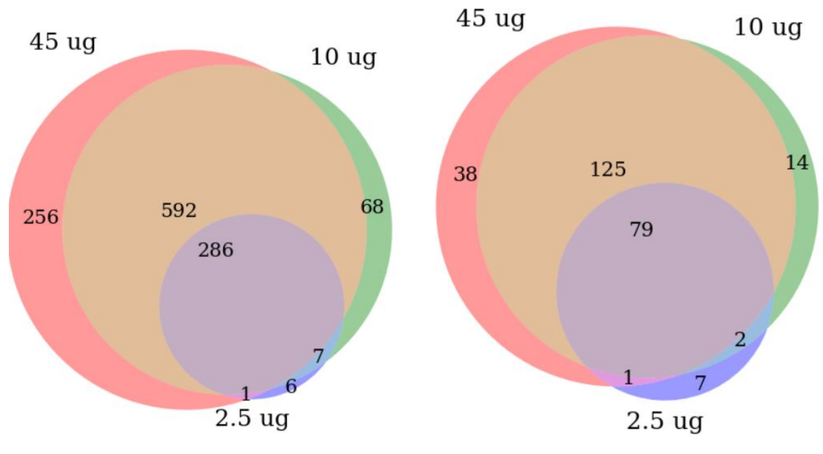
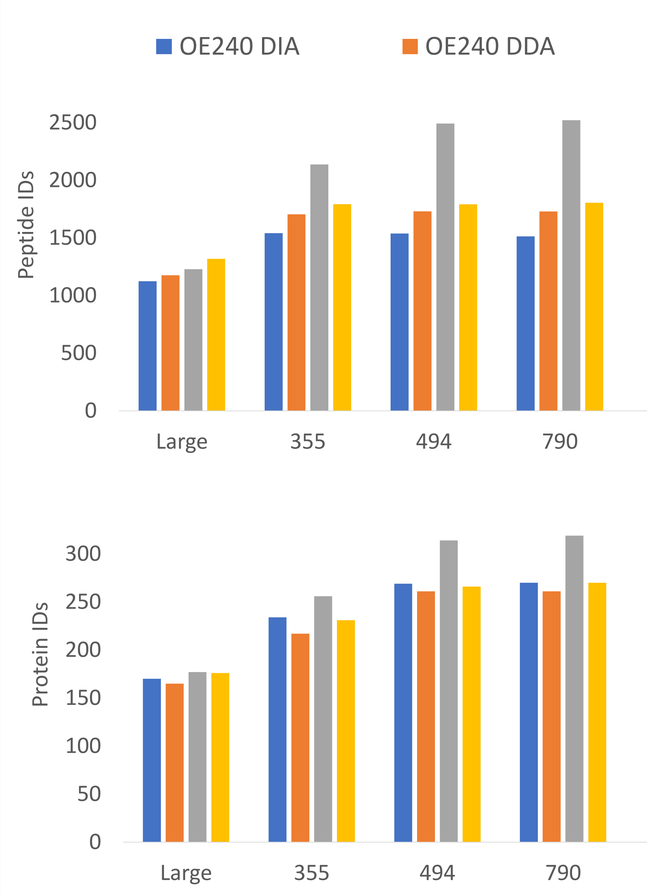


Figure 3: Results of database optimization on peptide (top) and protein (bottom) IDs from DDA and DIA scans



CONCLUSIONS

Protein and peptide IDs were calculated for DDA, DIA and PRM data, highlighting the versatility of HRAM and the quadrupole/linear ion trap on a Tribrid mass spectrometer. Successful collection of DDA, DIA and PRM data highlights orbitrap technology or the quadrupole/linear ion trap combination both being suitable for quickly acquiring discovery data and translating it to a targeted method on the same instrument.

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TRADEMARKS/LICENSING

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