LFQ HR-DIA WORKFLOW FOR PLASMA PROTEOMICS ON AN AUTOMATED PLATFORM

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Introduction

Plasma proteome continues to be a good source of biological information to diagnose health and monitor health[1,2]. Recently, it has been indicated to provide a source and means of providing therapy for age related diseases as well[3]. One of the best tool to quantify the plasma proteome is mass spectrometry-based proteomics as it enables discovery of new proteins in the plasma as well as characterization of post-translationally modified plasma proteome. Additionally, it enables protein-protein interaction analysis within the relevant matrix. However, a major challenge in plasma proteomics is the protein dynamic range where close to 60% of the plasma consists of albumin. This challenge exists all the way from plasma preparation, MS analysis, and data analysis (Table 1). Here, we highlight a label-free quantitation high-resolution data-independent acquisition (LFQ-HR-DIA) workflow for plasma sample processing using the new Thermo Scientific™ AccelerOme™ automated sample preparation platform comprised of standardized and optimized software, liquid handler, and reagent kit in combination with Thermo Scientific™ Vanquish™ Neo UHPLC system coupled to Thermo Scientific™ Orbitrap Exploris™ 480 MS system.

Table 1. Challenges in Plasma Proteomics

Sample Preparation	Sample Analysis	Data Quantification
• High missed cleavage • Processing variation between sample • *in vitro* modification	• Protein dynamic range • Speed & sensitivity • Robustness	• Data processing • Confident & high-quality quantification • Missing quantification values

Materials and methods

Control human plasma samples were aliquoted in an input plate by four different individuals with five aliquots per individual (Table 2) and were processed for mass spectrometry analysis using the AccelerOme platform (Figure 1). Briefly, proteins were lysed, reduced, alkylated, and digested to peptides using AccelerOme LFQ kit. All 20 sample digests were then analyzed by LC-MS/MS. Briefly, ~1µg of peptides were separated using a Thermo Scientific™ EASY-Spray™ 50 cm column on a Vanquish Neo UHPLC system coupled to an Orbitrap Exploris 480 MS (Figure 2).

Instrument	Setup Kit	Method
Input sample	Pooled plasma from same source	2 µl (~100µg)/well 20 samples 5 aliquots per person (4)
Sample preparation: AccelerOme	LFQ Kit	LFQ
HPLC: Vanquish Neo UHPLC	Analytical column: EASY-Spray™ PepMap™ C_{18} 2 µm 75 µm X 50 cm (Direct injection)	Column Temperature: 60ºC Gradient: 60 min Flow rate: 350 nl/min
Mass Spectrometer	Orbitrap Exploris™ 480	Data-independent acquisition
Data analysis	Spectronaut™ 17 (Biognosys)	DirectDIA and Library search

Table 2. HR-LFQ-DIA set up with automated sample preparation

Figure 2. Hardware used for the LC-MS/MS analysis

LC Method

- Total Run time: 60 min
- Analytical column: EASY-Spray Neo PepMap™ C_{18} 2µm 75µm X 50cm
- Mobile phases: [A] 0.1% FA in H_2O, [B] 0.1% FA in 80% ACN
- Flow rate: 300 nl/min
- Workflow: Direct injection
- Column temperature: 60˚C
- 1 µL injections (1 µg protein digest on the column)
- Gradient: 7% to 30% B in 55 minutes (Figure 3).

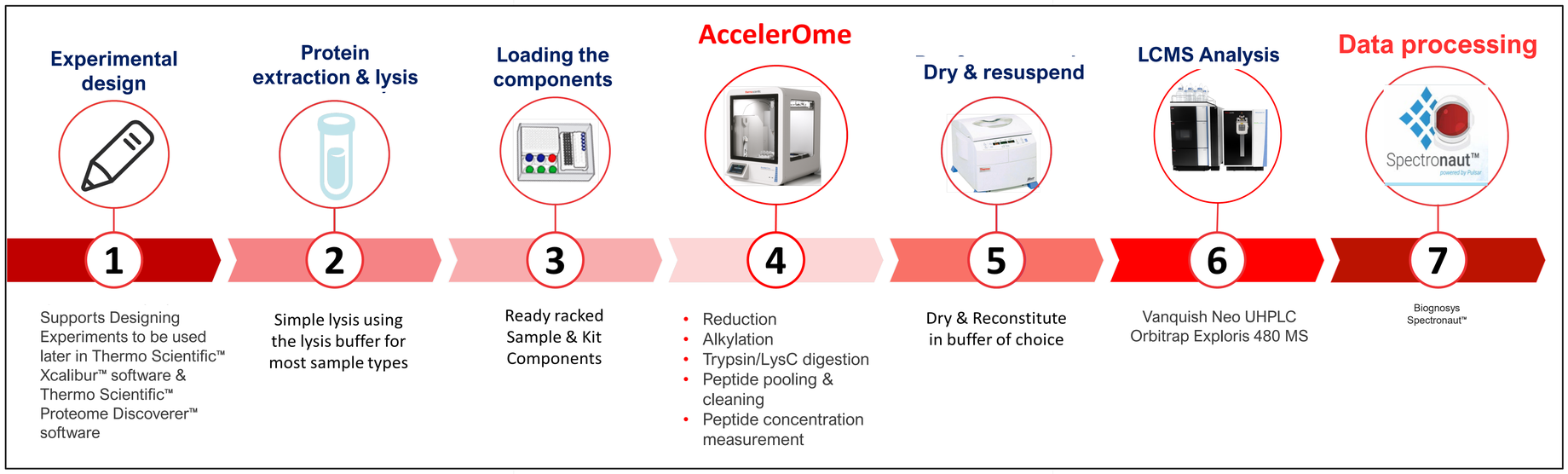


Figure 1. Complete "end to end" proteomics workflow. The experimental design and other metrics such peptide amount information are carried out along the process to be consumed for improved results and reduce operator errors.

No	Time	Duration [min]	Flow [µl/min]	%B	Volume [µl]	No. of Column Volumes
1	0.000	Run				
2	0.000	0.000	0.700	7.0	0.00	0.00
3	1.900	1.900	0.700	7.0	1.33	0.90
4	2.000	0.100	0.350		0.05	0.04
5	57.000	55.000	0.350	30.0	19.25	13.01
6	57.000	Column Wash				
7	57.100	0.100	0.700	99.0	0.05	0.04
8	60.000	2.900	0.700	99.0	2.03	1.37
9	60.000	Stop Run				
10	60.000	Column Equilibration				

Figure 3. Liquid chromatography gradient details

MS method

The samples were analyzed using high resolution data-independent acquisition method. Briefly, the survey scan was acquired using m/z 400-900 at a resolution of 60K @200mz. The DIA scans were performed at a resolution of 15K @200 mz. DIA scans were acquired using isolation window of 12 mz and isolated precursor ions were fragmented using higher-collisional induced dissociation (HCD) with 30% normalized collision energy (NCE). Figure 4 shows details about the MS parameters.

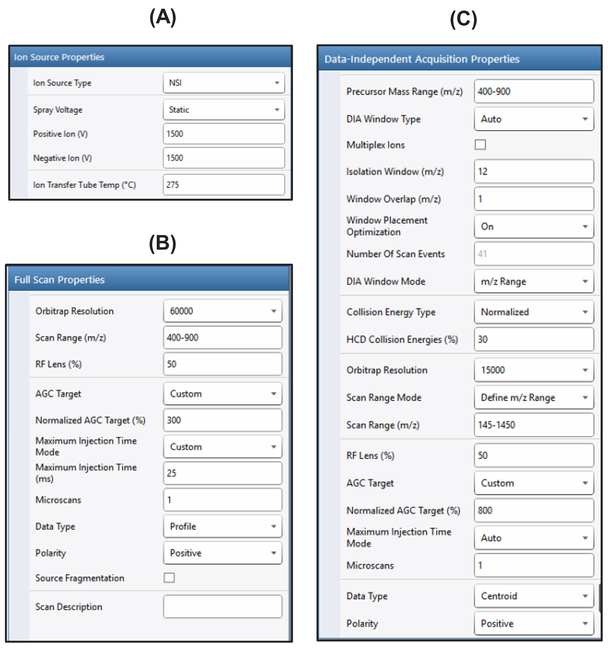


Figure 4. Data-independent acquisition parameters

Data processing

DIA raw data files were processed using Spectronaut™ 17 (v17.3.230224.55965) with DirectDIA approach against Human UniProt protein database (20,607 sequences). Quantification was based only on unique peptides with a false-discovery rate (FDR) of 1%. Protein groups were filtered 1% FDR on experiment level. Protein quantities are reported from such filtered protein groups exclusively.

Results

HR-LFQ-DIA workflow for plasma sample processing using AccelerOme platform resulted in consistent sample to sample peptide recovery, protein identifications and quantitation with minimal person to person variation.

Figure 5. Peptide recovery (A), Base peak chromatogram overlap (B) and protein identifications (C) across all samples

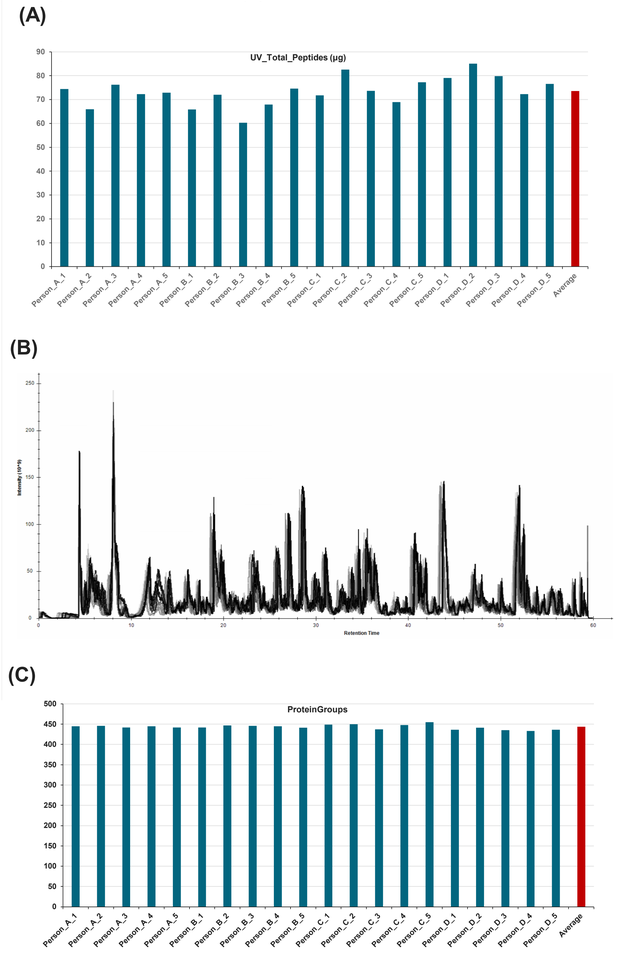
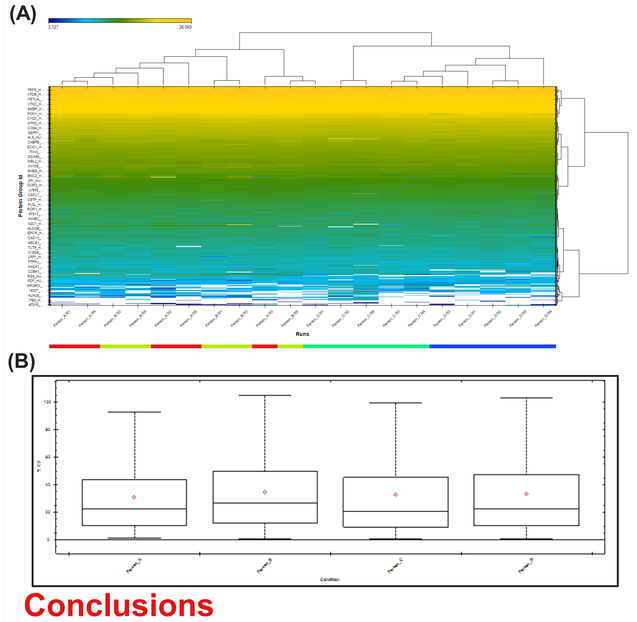


Figure 6. Heatmap showing protein abundances across all samples (A) Group-specific % CV (B)



Conclusions

- AccelerOme platform provides consistently high protein alkylation, digestion efficiency and low *in vitro* artefacts.
- AccelerOme system provides a hands-free solution to overcome this challenge by combining standardized, optimized, and verified hardware, software, and reagents to produce quality samples for plasma proteome quantification.
- AccelerOme platform also enables future development into additional applications for samples preparation for mass spectrometry analysis including high abundant proteins depletion.

References

1. Enroth et.al. Protein profiling reveals consequences of lifestyle choices on predicted biological aging. Scientific Reports. 2015 Dec 1;5:17282. PMID: 26619799
2. Williams et.al. Plasma protein patterns as comprehensive indicators of health. Nature Medicine. 2019 Dec;25(12):1851-1857. PMID: 31792462
3. Villeda et.al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nature Medicine. 2014 Jun;20(6):659-63. PMID: 24793238

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