BECKMAN COULTER
Life Sciences

Quality Control of High-Throughput Automated Proteomic Sample Preparation on a Biomek i7 Hybrid Automated Workstation

Reproducible high-quality proteomic sample preparation ensures consistency and is important in biomarker discovery and to identify novel molecular targets for drug discovery. Previously we have demonstrated a proteomic sample preparation method on the Beckman Coulter Biomek i7 hybrid automated workstation[1]. Here, we demonstrate data from four Quality Control (QC) samples run in a 96 well plate in multiple batches with cerebrospinal fluid (CSF) and plasma, which were prepped for proteomics analysis or protein and peptide analysis on the Biomek i7 Dual hybrid liquid handler.

The automated method on the Biomek i7 Hybrid Automated Workstation provides:

1. Reduced hands-on time
2. Reduction in system setup and potential pipetting errors
3. Device-integrations for complete walkaway sample preps
4. Standardized workflow for improved results
5. Quick install with ready-to-implement methods

Figure 1. Biomek i7 Hybrid Automated Workstation.

Timings:

The estimated time for processing 96 samples for proteomics on a Biomek i7 automated workstation is 4.5 hours.

Experimental Design:

The tryptic digestion for both biofluids was done on the Biomek i7 hybrid workstation using a 96 well plate. For digestion quality control (QC), each plate contained 4 QC reference samples along with patient samples. These QC reference samples were placed in 4 wells of a 96 well plate (designated well in each quadrant (Q1, Q2, Q3 & Q4) of the plate) (Fig 1). CSF and plasma protein were denatured, reduced and alkalized followed by a 4 hour trypsin digestion using the Biomek i7 hybrid workstation (Beckman Coulter) programmed for uniform pipetting and mixing temperature of 37°C. The CSF samples were quenched and proceeded for LC-MS analysis performed on Evosep- Exploris-480 system with 45 min gradient method. Whereas desalting was done on plasma using a positive pressure apparatus (Amplius Positive Pressure ALP, Beckman Coulter) mounted on the Biomek i7 hybrid workstation deck. After desalting, samples were dried and reconstituted for LC-MS analysis performed on Eksigent–Sciex Triple TOF 6600 system with 60 min gradient method.

	1	2	3	4	5	6	7	8	9	10	11	12
A	H20	QC digestion sample- Q1	8	15	22	29	35	44	QC digestion sample- Q3	59	66	H20
B	H20	1	9	16	23	30	37	45	52	60	67	H20
C	H20	2	10	17	24	31	38	46	53	61	68	H20
D	H20	3	11	18	25	32	39	47	54	62	69	H20
E	H20	4	12	19	25	33	40	48	55	63	70	H20
F	H20	5	12	20	26	34	41	49	56	64	71	H20
G	H20	6	13	21	27	35	42	50	57	65	72	H20
H	H20	7	14	QC digestion sample- Q2	28	36	43	51	58	65	QC digestion sample- Q4	H20

Figure 1. Schematic representation of the sample plate setup.

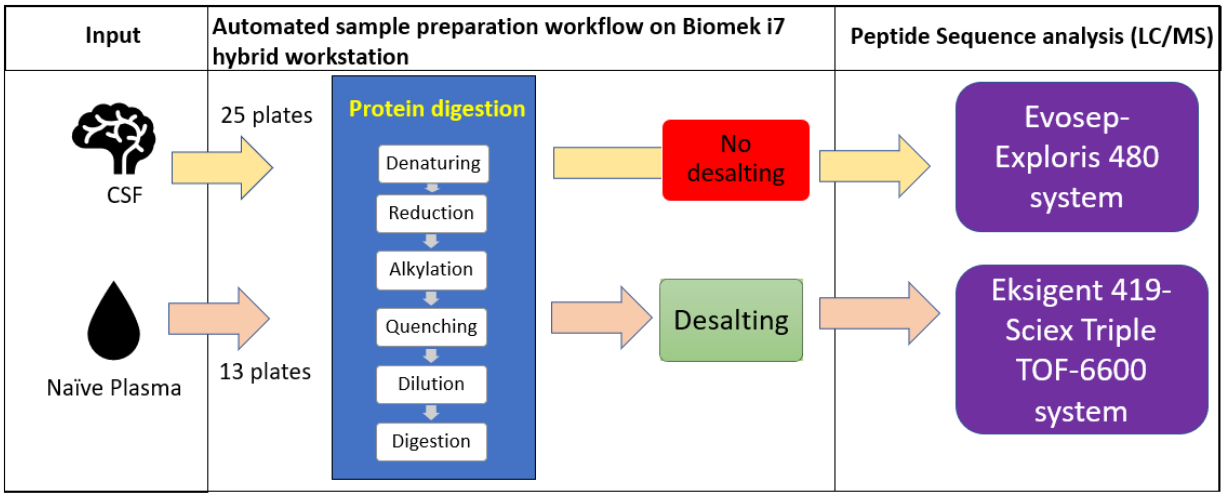

Figure 2. Workflow for automated sample preparation and peptide analysis of biofluid samples.

Results:

Analysis of the proteins and peptides quantified for each sample type was performed to determine inter-day versus intra-day workflow suitability. The cumulative frequency of peptides identified through intra-day and inter-day analysis had a %CV of <20% (Table1). The distribution of peptides and proteins for each plate and quadrant are shown in Figure 3.

Input sample	Proteomic Output	Inter-day		Intra-day	
		Mean	% CV	Mean	% CV
CSF QC digestion sample	protein	540.39	8.02	540.39	8.93
	protein	1602.08	2	1602.08	10.52
Plasma QC digestion sample	protein	430.15	9.77	429.86	15.21
	protein	1759.45	9.03	1758.706	15.36

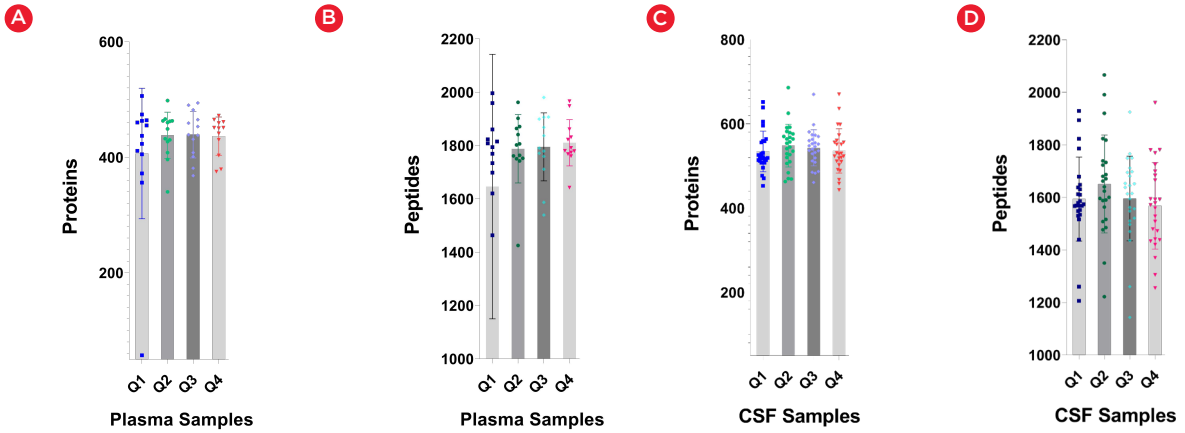

Fig 3: Quantified protein and peptide for the QC digested samples on all plates for plasma (A&B) and CSF (C&D).

Conclusions:

The inter-day and intra-day data from the QC digested samples demonstrate the robustness of method and automated sample preparation is reproducible across both the sample types. This in turn helps in efficient and reliable data generation for biomarker discovery and therapeutic development by overcoming potential user-introduced errors during sample preparation.

References: 1. Fu Q, Johnson CW, Wijayawardena BK, Kowalski MP, Kheradmand M, Van Eyk JE. A Plasma Sample Preparation for Mass Spectrometry using an Automated Workstation. J Vis Exp. 2020 Apr 24;(158). doi: 10.3791/59842. PMID: 32391810.

Acknowledgement: We would like to thank Rakhi Pandey, Alejandro Rivas, Kirstin Washington, Matthew Ayres, Qin Fu and Jennifer Van Eyk at the Smidt Heart Institute, Advanced Clinical Biosystems Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA, United States for their collaborative work on the workflow implementation and data generation and analysis. Funding in part from FNIH and NIDDK for the AMP-PD program.

2022-GBL-EN-100266-V2