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Quantitative Targeted Proteomics of Mouse Plasma Protein Biomarkers by using Nano LC coupled to Triple Quadrupole Mass Spectrometer

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Introduction

Biomarkers are a measurable indicator of a specific biological state, especially the states of diseases or therapeutic response to medicine. Because the goal of biomarker analysis is to develop blood tests for diagnosis, targeted therapy, and monitoring therapeutic response across many diseases, plasma/serum biomarkers are urgently needed. The challenge for blood protein biomarker discovery is the complexity of plasma protein, which has tens of thousands of proteins spanning ten orders of magnitude in abundance, such as from albumin to cytokines. Here we present a targeted proteomics LC-MS method that can efficiently quantify 375 mouse plasma proteins with the Evosep nano-flow LC coupled to an Agilent triple quadrupole mass spectrometer in a single analysis with 2250 MRM transitions. The goal of this study is to demonstrate the capability to acquire large biologically relevant targeted panels of protein biomarkers highlighting fast acquisition rate-short dwell time, without compromising precision.

Experimental

All materials (Acetonitrile, formic acid, Evotips, column, and PeptiQuant Plus kit) were purchased from commercial sources.

Sample preparation was performed according to MRM Proteomics PeptiQuant Plus proteomics kit protocol with modification. Briefly, bovine serum albumin (BSA) was reduced by DTT at 60°C for 1 hour in 9 M urea solution, then alkylated by iodoacetamide at room temperature in dark for 30 min, finally digested by trypsin at 37°C overnight with shaking. The BSA digest was used as surrogate matrix to prepare the calibration curve. Peptide standards and stable isotope labeled internal standards (SIS) were rehydrated to desired concentration. The calibration curve was prepared by spiking 10 µL of peptide standards to 1 µg BSA digest and 2 µL of SIS to all samples.

Final solution was loaded onto Evotip pure following the manufactures protocol including rinsing, conditioning, equilibrating, loading, washing and wetting steps. Evotips with trapped peptides were injected into an Evosep One Agilent 6495 QQQ LC/MS system as shown in Figure 1.

Experimental

Figure 1. Evosep One and 6495C triple quadrupole LC/MS system.

All data was collected with an Evosep One LC coupled to an Agilent 6495 triple quadrupole LC/MS (G6495C) system using the condition shown in Table 1. Data was analyzed with Skyline and Agilent Quantitative Analysis software.

Table 1.LC/MS conditions

LC Conditions	
Column	EV1106 (Endurance)
Emitter	EV1117
Source	Agilent Nano ESI source
Method	30 SPD/44 min
MS Conditions	
Gas temperature	225 ºC
Drying gas flow	11 L/min
Capillary voltage	2500 V
High pressure RF	150 V
Low pressure RF	60 V

LC/MS Analysis of PeptiQuant Plus Mouse kit.

750 peptides including 375 light peptides and 375 stable isotope labeled peptides were analyzed with a 44-minute method (30 SPD method) using Evosep One coupled to an Agilent 6495 triple quadrupole. Dynamic MRM was used to acquire all 2250 MRM transitions, which is 3 transition per peptide as shown in Figure 2, with shortest dwell time 1.2 ms. A representative extracted ion chromatogram of all 2250 MRM was shown in Figure 3.

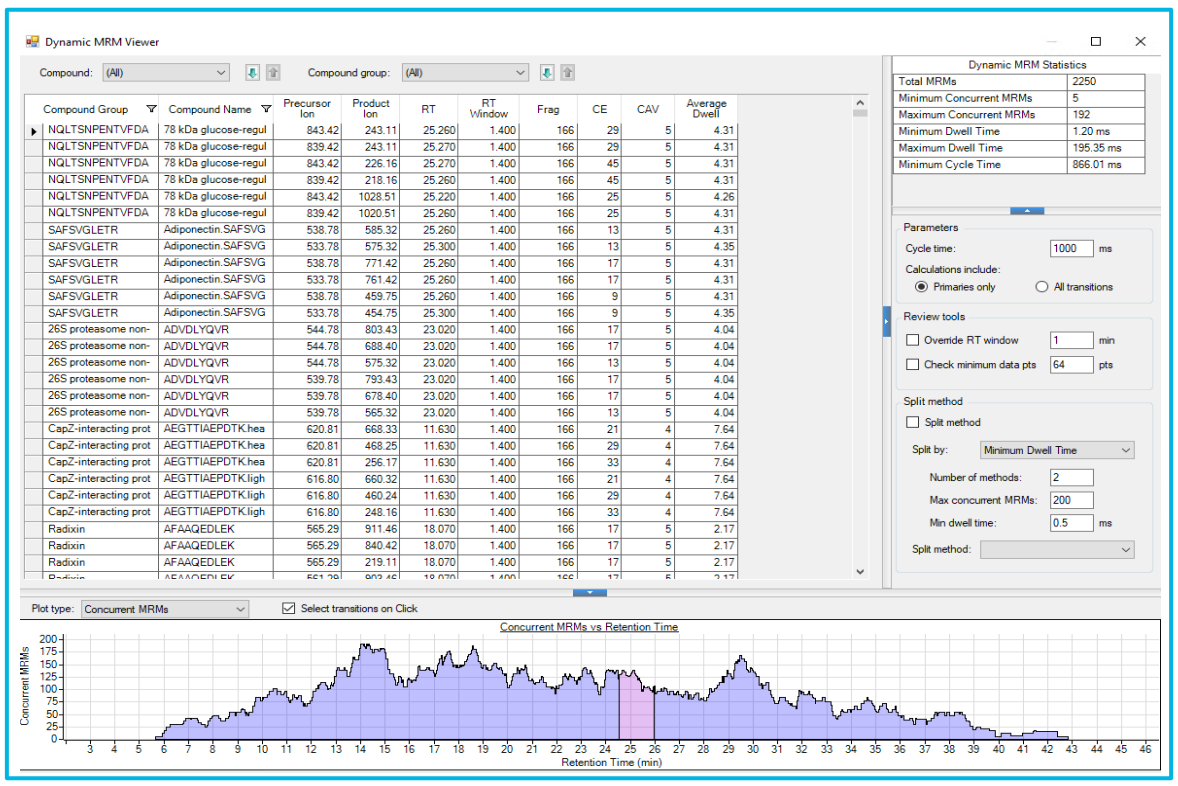

Figure 2. Dynamic MRM viewer showing total MRMs of 2250 in one nano LCMS method.

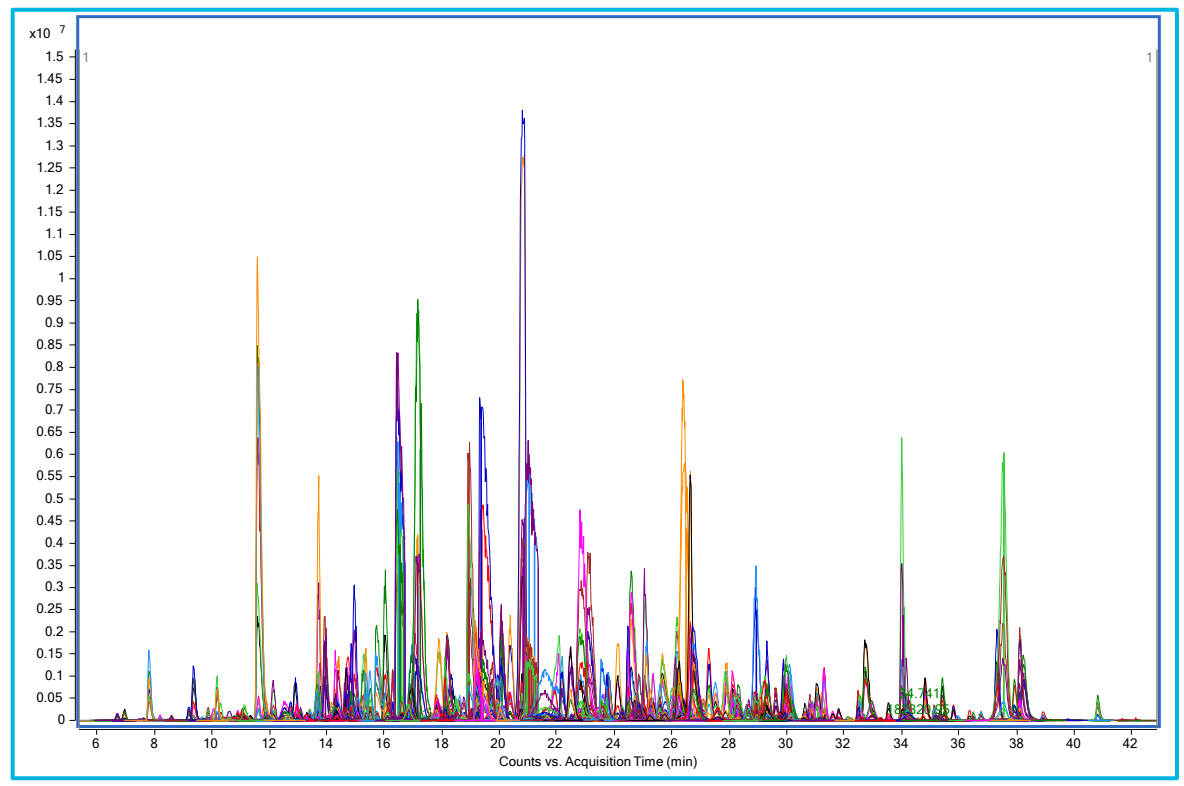

Figure 3. Extracted ion chromatograms of 2250 MRM transitions for all 750 peptides.

The peptide detection limits are shown in Figure 4. The lower limit of quantification (LLOQ) was below 100 amol/µL for more than 85% of the peptides, which is at least 10 times lower compared to a standard protocol using UHPLC. Figure 5 shows an MRM chromatogram overlay of peptides SLEDLNR (n=2) at low level demonstrating excellent retention time and MS response reproducibility.

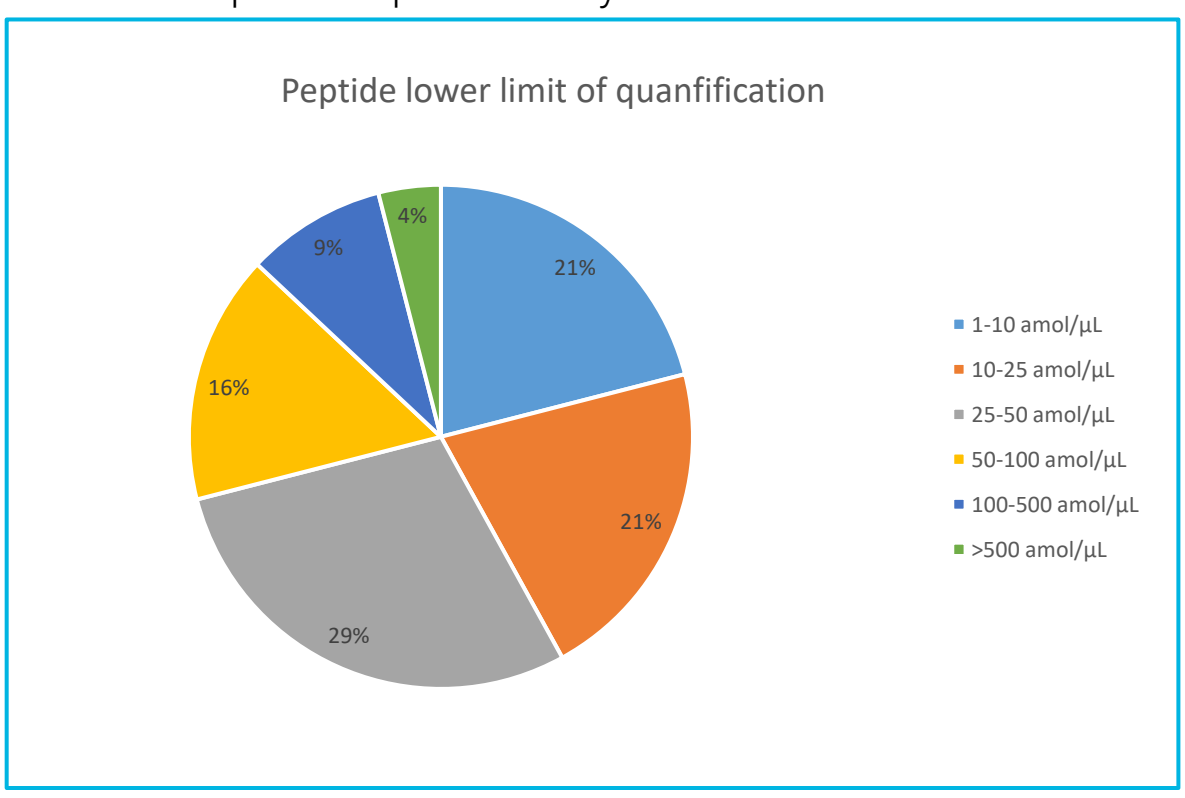

Figure 4. Summary of the LLOQ of 375 peptides with concentration factors accounted for.

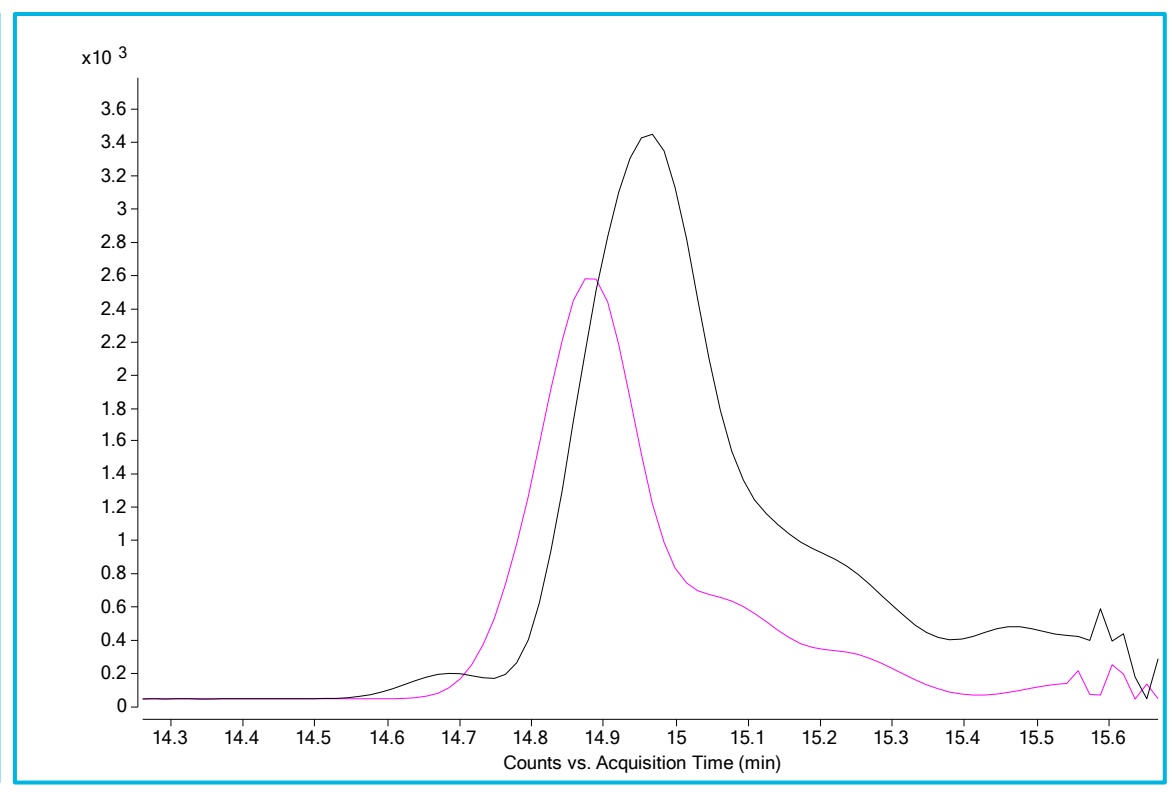

Figure 5. MRM chromatogram overlay of peptides (n=2) of at the low end of calibration curve .

Results and Discussion

An example of a peptide (SLEDLNR from Apolipoprotein A-IV) calibration curve from 38.6 amol/µL to 965 fmol/µL is shown in Figure 6, which is over 4 orders of magnitude. Calibration curve performance is shown in Table 2 demonstrating excellent precision and accuracy. The intra-day and inter-day precision and accuracy of quality control samples were determined from two dependent runs performed over two days. The precision and accuracy of quality control samples is shown in Table 3.

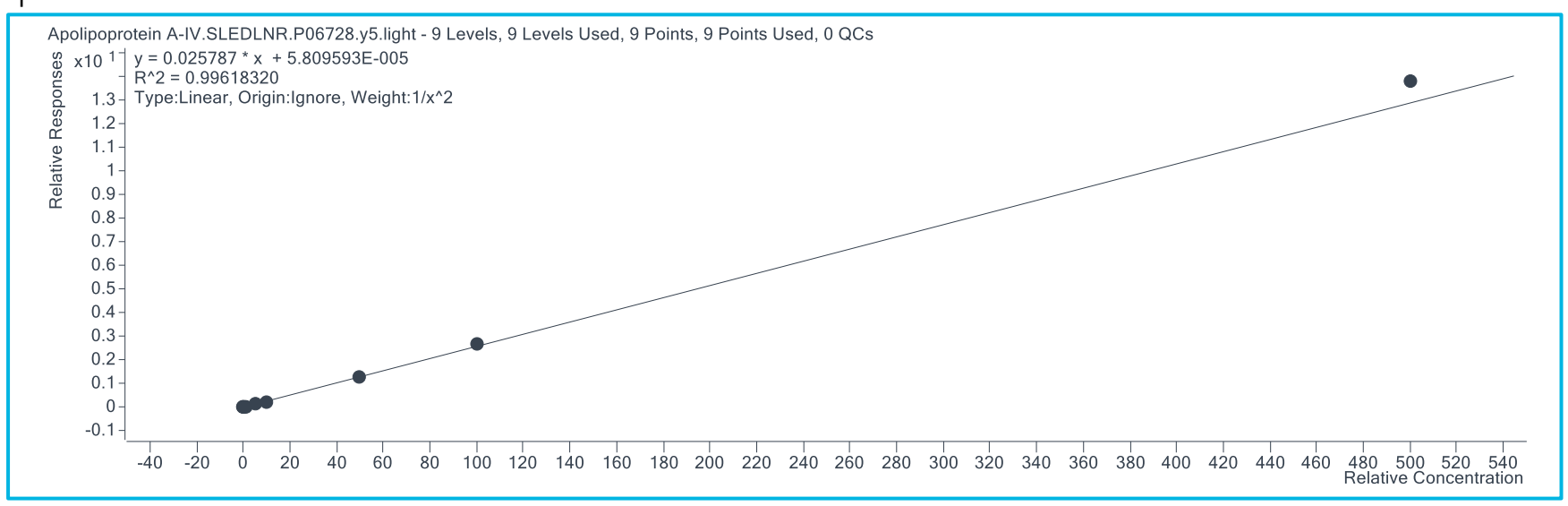

Figure 6. Peptide (SLEDLNR from Apolipoprotein A-IV) calibration curve from 38.6 amol/µL to 965 fmol/µL.

Table 2. Precision and accuracy of peptide SLEDLNR calibration curve

Concentration (amol/uL)	38.6	193	965	1930	9650	19300	96500	193000	965000
Run 1	38.8	186	1029	1722	9829	18038	97674	199241	1035764
Run 2	39.4	174	1034	1720	9024	18475	110398	212673	946083
Mean	39.1	180	1031	1721	9426	18256	104036	205957	990924
% Bias	1.3	-6.9	6.9	-10.8	-2.3	-5.4	7.8	6.7	2.7
% CV	1.0	4.9	0.3	0.1	6.0	1.7	8.6	4.6	6.4

Table 3. Precision and accuracy of peptide SLEDLNR quality control samples

	Concentration (amol/uL)	579	2895	38600	386000
Run 1	Mean	496	2404	34785	357156
	% Bias	-14.4	-17.0	-9.9	-7.5
	% CV	4.9	2.4	2.9	4.3
Run 2	Mean	486	2462	35966	393706
	% Bias	-16.1	-15.0	-6.8	2.0
	% CV	4.2	3.0	3.5	1.4
Inter-day	Mean	491	2433	35376	375431
	% Bias	-15.2	-16.0	-8.4	-2.7
	% CV	4.3	2.8	3.4	6.0

Conclusions

- We were able to quantify 375 peptides from PeptiQuant Plus mouse kits by coupling the Evosep One to the 6495C LC/TQ using Evosep 30 SPD method.
- This targeted proteomics workflow provides high analytical sensitivity, precision and accuracy, as well as linearity, throughput, robustness, and ease of use with more than 85% of target peptides having an LLOQ below 100 amol/µL.
- The 6495C LC/TQ dMRM feature was able to monitor 2250 MRMs in one analytical run with short dwell time as low as 1.2 ms.

References

[1]Bache, N et al. A Novel LC System Embeds Analytes in Pre-formed Gradients for Rapid, Ultra-robust Proteomics. Mol. Cell Proteomics 2018, 17(11), 2284–2296

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