Lifting the Albumin Curtain to Increase Plasma Proteome Profiling: Incorporating Differential Ion Mobility for Increased Proteome Coverage

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ABSTRACT

Purpose: Increase the plasma proteome coverage for routine profiling analysis.

Methods: Incorporation of the Thermo Scientific™ FAIMS Pro™ interface with the Thermo Scientific™ Orbitrap™ Tribrid™ mass spectrometer to expand the precursor features reproducibly measured and sequenced. The proposed FAIMS DDA method utilizes two CV settings.

Results: Introduction of the FAIMS Pro interface into the DDA method resulted in detection and quantitation of ca. 1200 additional peptides and over 100 proteins. In addition, the FAIMS DDA experiment converted 33 proteins identified as "1-hit wonders" as defined in the standard DDA experiment to proteins quantified by 2 or more peptides.

INTRODUCTION

Overcoming the dynamic range effects in non-depleted plasma profiling remains challenging. Following tryptic digestion, high abundant proteins yield many different high abundant peptides that elute through the entire chromatographic gradient. Due to the large abundance differences between the high and low intensity peptides, ion suppression effects at the source and trapping/detection efficiencies limit proteome coverage. Efforts to maximize loading and peak capacities have increased the number confident identification of over 400 proteins with a 90-minute gradient. Research, however, requires greater depth to detect and evaluate potential biomarkers. Our approach is to incorporate differential ion mobility to enhance selectivity prior to entering the mass spectrometer resulting in greater numbers of precursors reproducibly sampled.

MATERIALS AND METHODS

Sample Preparation

A stock solution of plasma was prepared from a healthy donor following standard preparation methods. The resulting plasma was digested with trypsin and used without further clean up. A total of 1 µg of plasma digest was loaded on column for all experiments.

Test Method(s)

All experiments were performed on a Orbitrap Fusion Lumos Tribrid mass spectrometer with and without a FAIMS Pro interface. Specifically, the Top Time DDA method was used that restricted the complete acquisition time consisting of a full scan MS and as many MS/MS spectra as can be acquired in either 2 seconds for the standard DDA method or two 1-second per FAIMS compensation voltage (CV) setting. The HRAM full scan MS was acquired at a resolving power of 120,000 and all MS/MS spectra acquired in the linear ion trap with an average scan time of 20 msec. For the FAIMS DDA method, two CV steps (-50V and -70V) were alternated to filter two different precursor peptide populations into the Tribrid mass spectrometer for precursor detection and subsequent MS/MS detection. The plasma digest was loaded onto a 500 x 0.75 mm Thermo Scientific™ EASY-Spray™ column and separation was performed using a Thermo Scientific™ EASY-nLC™ 1200 system. A 140 minute separation method was performed with a 0.3% per minute gradient over 100 minutes. The remainder of the time was spent loading the sample on column and re-equilibration prior to the next injection.

Data Analysis

All raw files were processed using Thermo Scientific™ Proteome Discoverer™ 2.3. The UniProt human database (April 2018) was used for performing sequence matching. Peptide and Protein Validator scoring was used for classifying the resulting matches. Unique peptides scored medium or high were exported as a function of CV setting into Excel for comparative scoring and precursor integration analysis. Peptides acceptance required a minimum of one matched MS/MS spectra across the three technical replicates, and a maximum coefficient of variance on the precursor m/z signal of 40% or better determine if the FAIMS DDA method increased detection, scoring, or reproducible measurements across the three technical replicates.

RESULTS

The goal of the experiment is to increase the plasma profiling capabilities on non-depleted plasma without significantly modifying the experimental method. Our approach is to utilize a FAIMS Pro interface directly inserts into the source housing between the emitter tip and the ion transfer tube on the mass spectrometer. The use of 2 different CV settings increased the probability of suppressing the transmission efficiency of the high abundant peptides in 1 CV setting, extending the dynamic range of peptide detection.

RESULTS

Figure 1. Base peak chromatographic plot of the plasma digest acquired using the standard DDA method. The peptide mapping represents the base peptides per abundant peak. Note that almost all peptides are attributed to HSA. The elution peak at 63.77 minutes is highlighted to demonstrate the comparative full scan MS acquired over the eluting peak width.

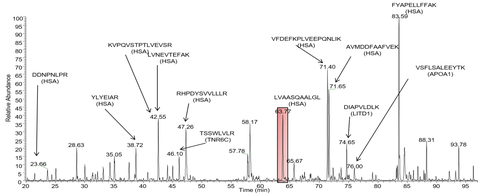

Figure 2. Comparative full scan MS averaged across the 30-second peak width for the abundant peak shown in red. The top spectrum demonstrates the affects of abundant peptide elution that suppresses the detection capabilities of low-level peptides, the base peak at m/z 507 has a measured intensity of 1.3e8 and the majority of the other precursors detected are predominantly singly charged and low abundance. The inset shows a low-level precursor measured in the 3+ charge state. The measured ion intensity for the m/z 628 ion is 4.3e3 and has a S/N ca. 4. The second full scan MS representing a low-level chromatographic peak shows an abundance of multiply charged precursors with the base peak at m/z 872 measured with an ion abundance of 8.3e6. The second inset shows the measured 3+ precursor with a measured intensity of 1.1e4 and S/N over 10.

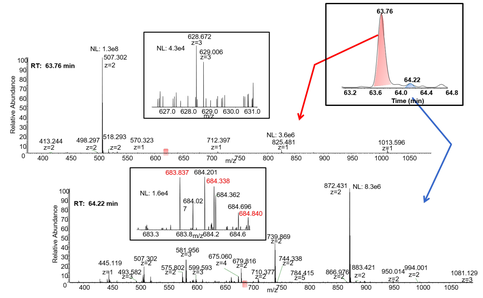

Figure 3. Comparative base peak plots acquired using the standard and FAIMS DDA methods on the same plasma sample. The FAIMS DDA method shows the two different chromatographic traces representing two different CV settings. Evaluation of the trace shows the two different CV steps enable both the transmission of the high abundant peak at 1 CV and a different peak profile at the second CV setting.

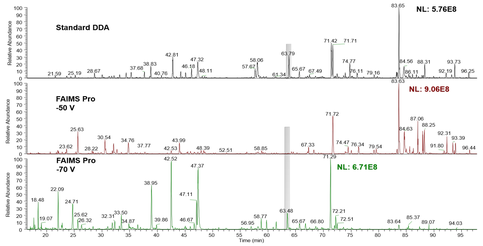

Figure 4. Evaluation of the comparative full scan MS at 63.77 minutes. The MS spectra look similar for the standard DDA and FAIMS DDA method using -70V. The full scan MS spectra acquired with a FAIMS CV setting of -50 V results in significantly more precursors that enabled the detection and sequencing of 14 peptides not originally measured in the Standard DDA method. The insets shows the mass extraction for the TTHY peptide.

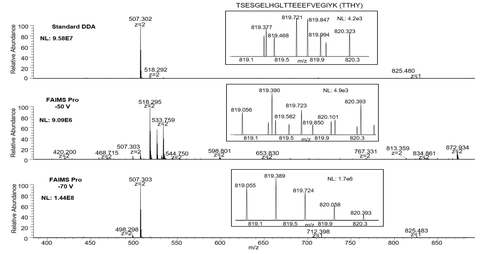

Table 1. List of high abundant HSA peptides identified in Fig.1 and the comparative AUC values and variance across three technical replicates per method. The values listed for the FAIMS DDA experiment were recorded for the optimal CV setting. In addition, the numbesr of co-eluting peptides successfully measured under the HSA peptide are listed.

HSA Peptide	RT Window (min)	HSA Peptide AUC (%CV)		# of Peptides Sequenced Under the HSA Peptide				
	0.6 minutes	Standard	FAIMS Pro	Standard	FAIMS			
					-50V	-70V	Common	Unique
DDNPNLPR	27.3	8.0e7 (31%)	3.9e9 (3%)	3 (1)	4	15	4	21
YLYEIAR	38.9	6.4e8 (25%)	7.34e9 (3%)	24 (8)	16	15	6	21
KVPQVSTPTLVEVSR	42.6	1.9e9 (28%)	1.6e10 (3%)	22 (6)	14	10	10	18
TSSWLVLR	46.1	5.7e8 (15%)	4.3e8 (2%)	16 (5)	12	6	20	31
RHPDYSVVLLLR	47.6	2.0e9 (11%)	1.91e10 (3%)	13 (8)	14	6	6	21
LVAASQAALGL	63.8	1.8E9 (5%)	2.93e9 (10%)	19 (12)	9	5	5	12
VFDEFKPLVEEPQNLIK	71.4	4.9E9 (7%)	1.81e10 (4%)	12 (5)	9	6	6	14
AVMDDFAAFVEK	72.0	3.6E9 (3%)	1.02e10 (7%)	18 (8)	8	4	8	10
VSFLSALEEYTK	75.9	6.5E8 (3%)	1.95e9 (2%)	13 (10)	15	3	5	20
FYAPELLFFAK	83.6	3.6E9 (6%)	1.53e10 (2%)	6 (2)	4	3	11	14
			Totals	168(71)	118	83	91	200

Figure 5. Venn diagram showing the overlap of peptides identified and reproducibly measured across three technical replicates. There were 1210 unique peptides identified using the FAIMS DDA method compared to 253 using the standard DDA method. The 1120 peptides measured across both methods were used to evaluate relative method performance.

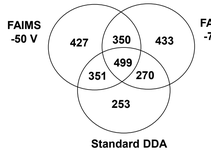

Figure 6. Evaluation of the measured AUC response for the overlapping peptides between methods. The difference in measured AUC value is binned for each method with values in red indicating greater measured AUC values resulting from the FAIMS DDA method compared to the responses shown in black.

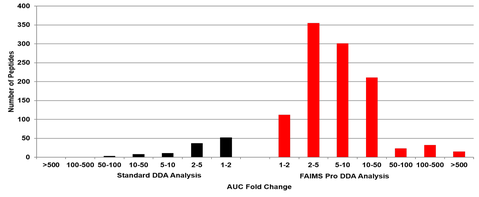

Figure 7. Comparative analysis of the measured variance between methods for the commonly identified peptides. The reported variance for the FAIMS DDA experiments have been broken down by CV setting and some peptides were measured at each CV setting and included.

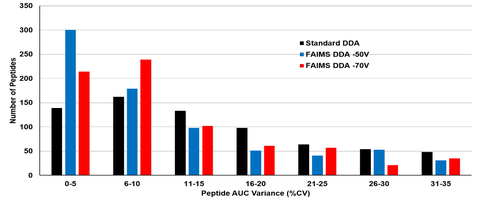

Figure 8. Evaluation of the sampling frequency for the overlapped peptides between the standard DDA and FAIMS DDA methods across the three technical replicates per experiment. For the FAIMS DDA experiment, two different analysis are presented as a portion of the peptides detected and successfully measured in each FAIMS CV setting. The values reported for each setting as well as that reported for the best response.

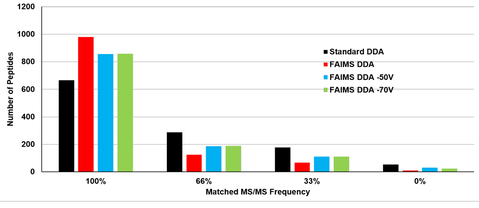

Figure 9. Evaluation of common peptide response attributed to 1-hit wonders from the standard DDA method. The inset shows the comparative variance difference between the two experimental methods for each peptide..

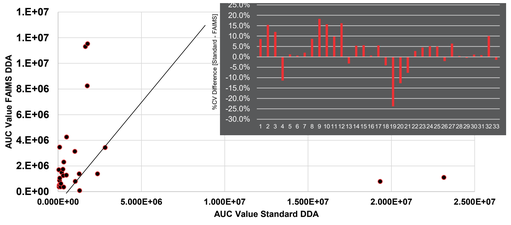

Table 2. List of key proteins that were identified using the standard DDA method as 1-hit wonders but were identified by multiple peptides using the FAIMS DDA method. Each peptide was confidently sequenced and reproducibly measured across the three technical replicates.

A2GL_HUMAN	3	CPN2_HUMAN	2	IPSP_HUMAN	5
AMBP_HUMAN	2	CXCL7_HUMAN	2	ITIH3_HUMAN	2
APOA2_HUMAN	5	ECM1_HUMAN	3	KLKB1_HUMAN	5
APOC1_HUMAN	2	F13B_HUMAN	2	LCAT_HUMAN	2
APOC2_HUMAN	3	FA10_HUMAN	2	LG3BP_HUMAN	2
APOD_HUMAN	4	FA12_HUMAN	2	RET4_HUMAN	2
APOL1_HUMAN	6	FIBG_HUMAN	12	TBA4A_HUMAN	2
APOM_HUMAN	3	GELS_HUMAN	5	TBB1_HUMAN	3
ATRN_HUMAN	3	H4_HUMAN	4	TPM4_HUMAN	2
C1QA_HUMAN	3	HBA_HUMAN	2	URP2_HUMAN	6
CD5L_HUMAN	2	HBB_HUMAN	2	ZA2G_HUMAN	6
CO2_HUMAN	3	HPTR_HUMAN	2		
CO4B_HUMAN	3	HV307_HUMAN	2		
CO8A_HUMAN	3	IGHG4_HUMAN	2		

CONCLUSIONS

Introducing the FAIMS Pro interface into the routine plasma profiling workflow provides an orthogonal degree of selectivity post-chromatographic separation and before entering the Orbitrap mass spectrometer. Performing DDA sampling at each CV setting significantly increases the precursor m/z space per retention time.

- Multiple CV settings enables increased detection of the high abundant peptides at one CV setting while suppressing its ion transmission in the second CV setting resulting in low-level peptide transmission and detection
- Incorporation of the FAIMS Pro interface significantly increases the measured AUC values for detected peptides as compared to the standard DDA method.
- The measured reproducibility for detected peptides was increased using the FAIMS Pro interface
- The frequency at which peptides were sampled across technical replicates was increased reducing the need for "match-between-runs"

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TRADEMARKS/LICENSING

PO65515-EN0519S

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