AccelerOme platform

AccelerOme automated sample preparation platform enables highly reproducible quantitative proteomics analysis in Velocity Data-Independent Acquisition (DIA) workflow

Kevin Yang[1], Amirmansoor Hakimi[1], Amarjeet Flora[2], Julian Saba[3], Julia Kraegenbring[4], Lilian Heil[1], Santosh Renuse[1], Sally Webb[1]
[1]Thermo Fisher Scientific, San Jose, CA. USA, [2]Thermo Fisher Scientific, Rockford, IL, USA, [3]Thermo Fisher Scientific, Winnipeg, MB, Canada, [4]Thermo Fisher Scientific, (Bremen) GmbH, Germany

Abstract

Purpose: To evaluate the Thermo Scientific™ AccelerOme™ automated sample preparation platform, together with the Thermo Scientific™ Velocity LFQ DIA workflow for highly efficient and reproducible end-to-end quantitative data-independent acquisition (DIA) analysis.

Methods: HeLa S3 proteome was digested into peptides using the AccelerOme platform as well as commonly used manual digestion methods. Ensuing digested samples were analyzed using the Velocity DIA workflow on Thermo Scientific™ Orbitrap™ mass spectrometers. Performance was evaluated by the protein group identification, dig efficiency, cysteine alkylation, and deamination of asparagine.

Results: The results demonstrated that Velocity DIA workflow on Orbitrap mass spectrometers together with AccelerOme platform affords excellent and reproducible protein identification and quantitation. We found that AccelerOme platform outperforms manual preparation, where it provides the lowest median coefficient of variance (CV) between injections. In addition, we were able to identify over 6000 proteins within a 30-min gradient for all sample preparation methods except for urea-C18, suggesting an exhaustive protein extraction and a minimal sample loss provided by AccelerOme platform.

Introduction

DIA coupled with label-free quantification (LFQ) has emerged as a powerful tool for quantitative proteomics. The sensitivity and reproducibility of DIA analysis for proteomics makes it attractive for large-scale biological investigations. Despite recent advances in liquid chromatography and mass spectrometry, LFQ-DIA is hampered by sample preparation uniformity, a critical step for robust protein quantitation. Manual sample preparation is time-consuming, labor-intensive, and lacks reproducibility due to its inherent error-prone processes. In this study, we coupled a previously developed Velocity DIA workflow with an AccelerOme platform, an automated sample preparation solution to ensure uniformity across different samples and minimize variation.

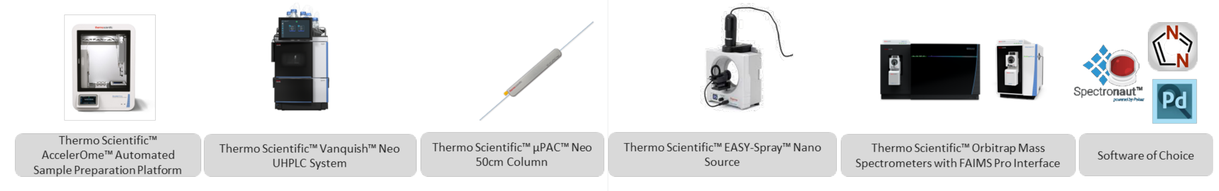
The AccelerOme platform has a liquid handling robot to process up to 36 samples for LFQ and 33 samples for Thermo Scientific™ TMT11plex™ isobaric label reagents or 32 samples for Thermo Scientific™ TMTpro™ 16plex label reagents per session. Coupling the AccelerOme sample preparation platform with the Velocity DIA workflow on Orbitrap mass spectrometers enables maximized reproducibility and productivity in discovery proteomics (Figure 1).

Materials and methods

Sample preparation

HeLa S3 cells were cultured according to the instruction from ATCC. Cell pellets were lysed, reduced, alkylated, and digested to peptides in the AccelerOme automated platform with Thermo Scientific™ AccelerOme™ Label-Free MS Sample Prep Kit (Catalog No. A50945). As a comparison for sample preparation, proteins extracted from HeLa S3 cells were also processed through commonly used manual preparation methods, including Thermo Scientific™ EasyPep™ MS Sample Prep Kit (Catalog No. A40006), S-Trap™ micro MS sample prep kit (PROTIFI), and urea denaturation followed by trypsin digestion and C18 desalting spin column clean-up (Figure 2A).

Figure 1. Complete 'end-to-end' Velocity DIA workflow. The AccelerOme automated sample preparation platform is part of an integrated Velocity DIA workflow on Orbitrap mass spectrometers.



Test method(s)

The Velocity DIA workflow was operated by directly injecting the ensuing samples into a Thermo Scientific™ µPAC™ Neo HPLC column (50 cm) and the peptides were resolved in stepped gradient operated (Figure 2B) by a Thermo Scientific™ Vanquish™ Neo UHPLC system. The eluted peptides were analyzed on an Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer or a Thermo Scientific™ Orbitrap™ Ascend Tribrid™ mass spectrometer operated in DIA mode with/without Thermo Scientific™ FAIMS Pro interface (Figure 2C). We used 200 ng HeLa digest standard (Catalog No.33329) to evaluate the performance of Velocity DIA workflow of different gradients (Figure 3). For comparing the AccelerOme platform with manual sample preparation, 30-min gradient was employed.

Data analysis

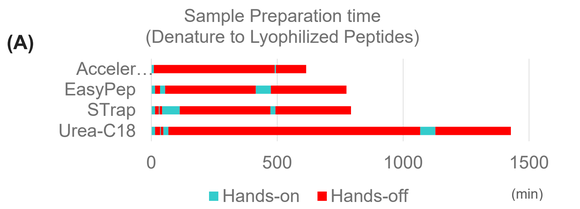
Spectronaut™ 18.1 software and Thermo Scientific™ Proteome Discoverer™ 3.1 software was used for direct DIA search. For Spectronaut software, trypsin was set as the enzyme of choice with a maximum missed cleavages set as 2. Asparagine deamidation, methionine oxidation, and cysteine alkylation, including sulphenylation (+45.988 Da) for STrap and carbamidomethylation (+57.021 Da) for the other sample preparation methods, were set as variable modifications. Spectra were searched against Uniprot Human proteome (UP000005640). Both the peptides and proteins were filtered at 1% false discovery rate (FDR). The ensuing proteins were further filtered with 5% q-value at the run level and 1% at the experimental level. For Proteome Discoverer 3.1 software, CHIMERYS™ intelligent search algorithm by MSAID™ was used for DIA search with similar parameters set up.

Results

Velocity DIA workflow on Orbitrap mass spectrometers allows for robust protein identification and quantitation.

To evaluate the Velocity DIA workflow, 200 ng of HeLa digest was analyzed with different LC gradients on Orbitrap Ascend mass spectrometer. For 80 sample per day (SPD) method that has an active gradient of 9 min, two methods were employed to maximize either protein identification (Max ID) or quantitation (Max Quan). We were able to identify >5000 proteins with our Max ID 80 SPD method and obtain ~7% CV from Max Quan 80 SPD method (Figure 3). For longer gradients, we were able to identify ~7000 proteins in 30 min gradient and ~8000 in 60 min gradient from 200 ng of HeLa digest with ~5% CV, suggesting excellent proteome coverage and quantitation precision (Figure 3).

Figure 2. LC-MS/MS analysis of Velocity DIA workflow.



(B)

Time	Duration [min]	Flow [µl/min]	%B	Volume [µl]	No. of Column Volumes
0.000	Run				
0.000	0.000	0.350	4.0	0.00	0.00
22.500	22.500	0.350	30.0	7.88	7875.00
30.000	7.500	0.350	45.0	2.63	2625.00
30.000	Column Wash				
30.100	0.100	0.350	97.5	0.04	35.00
33.000	2.900	0.350	97.5	1.02	1015.00
33.100	0.100	0.350	4.0	0.04	35.00
33.100	Stop Run				
33.100	Column Equilibration				

(C)

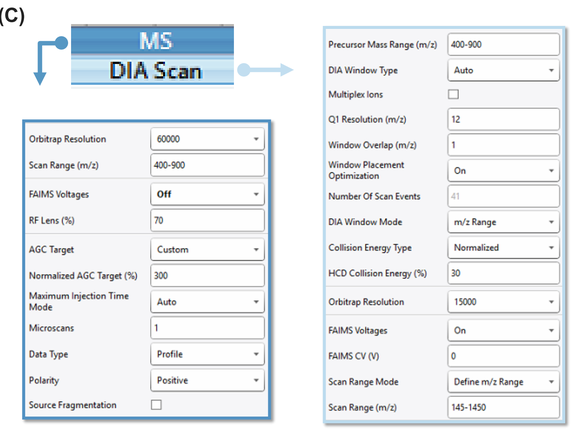


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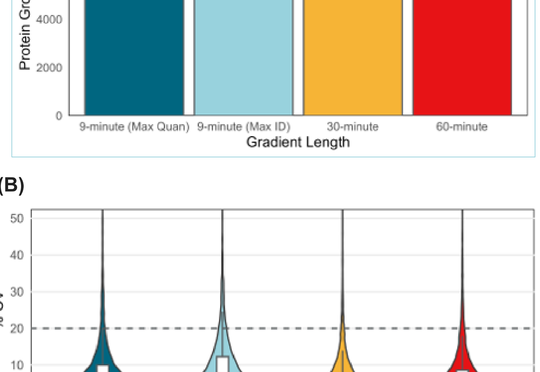
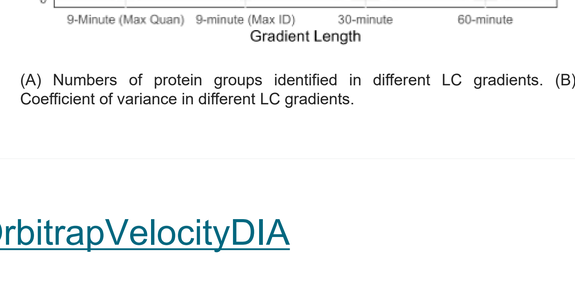
Figure 3. Velocity platform DIA for maximized protein identification and quantitation.

(A)



(B)



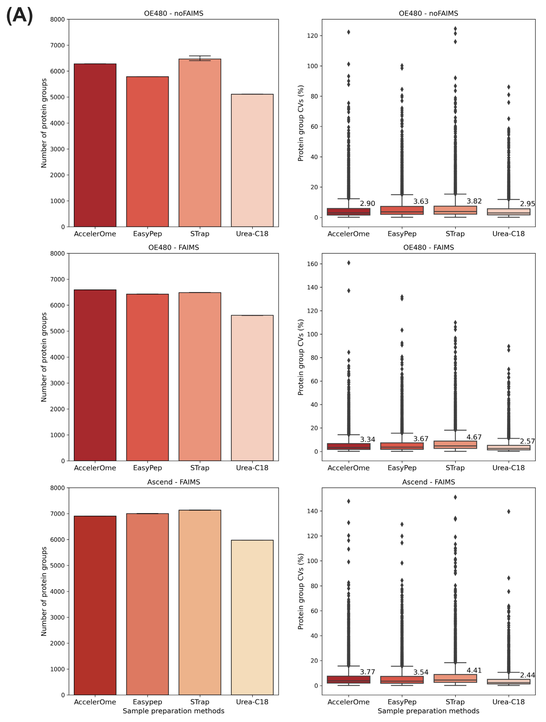
(A) Numbers of protein groups identified in different LC gradients. (B) Coefficient of variance in different LC gradients.

AccelerOme platform provides highly reproducible sample preparation and affords exhausted trypsin digestion to enable robust protein identification and quantitation.

Samples prepared by the AccelerOme platform demonstrated deep proteome coverage in Velocity platform DIA analysis along with low CVs in protein quantitation (Figure 4), suggesting excellent peptide recovery and minimal technical variations. Notably, we incorporated 3 replicates in this study for each sample preparation method for proof-of-concept evaluation. For larger cohort studies, we expect the benefits of incorporating the AccelerOme platform into quantitative proteomics to minimize technical variation to be even more pronounced. In addition, the AccelerOme platform provides the highest digestion efficiency (Figure 5) along with complete alkylation and minimum asparagine deamidation (Figure 6), which are crucial in generating optimal peptides length for LC-MS/MS analysis and the downstream data analysis.

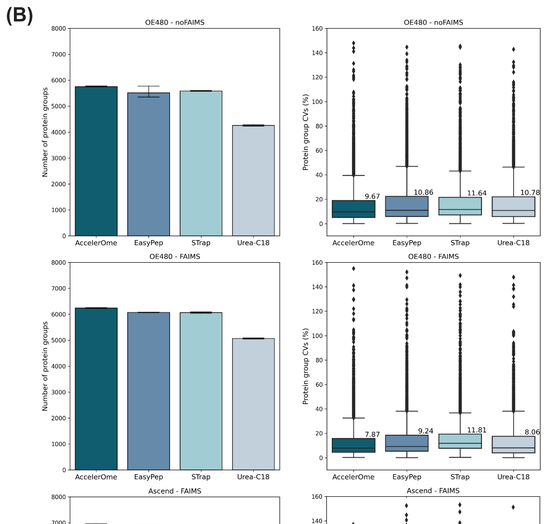
Figure 4. AccelerOme platform enables deep proteome coverage and robust quantitation in Velocity platform DIA analysis.

(A)



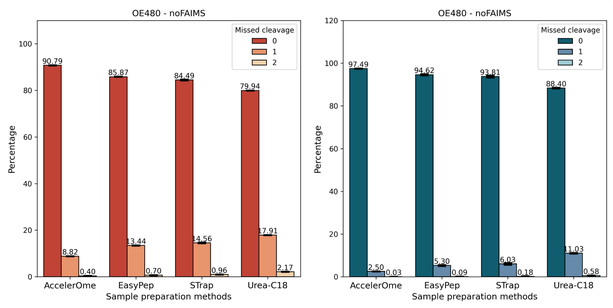
Protein group numbers (left) and the percentage of coefficient of variance (right) of samples prepared by AccelerOme platform or commonly used manual preparation methods. The data was analyzed by Spectronaut software.

(B)

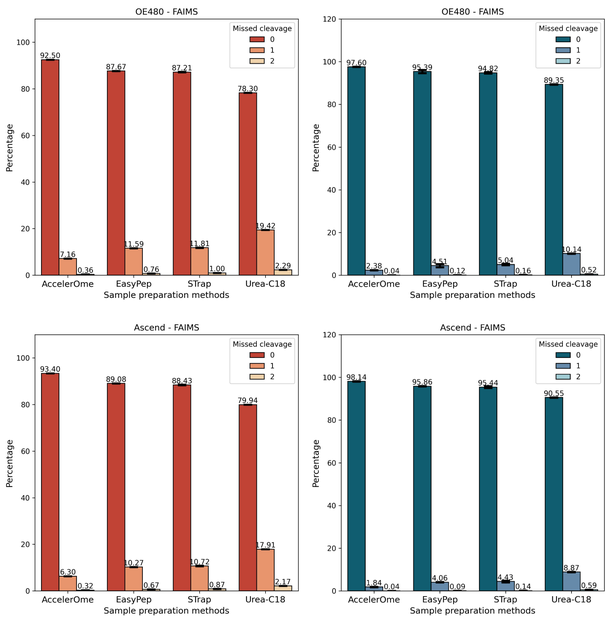


Protein group numbers (left) and the percentage of coefficient of variance (right) of samples prepared by AccelerOme platform or commonly used manual preparation methods. The data was analyzed by Proteome Discoverer 3.1 software.

Figure 5. The AccelerOme platform affords the best digestion efficiency.

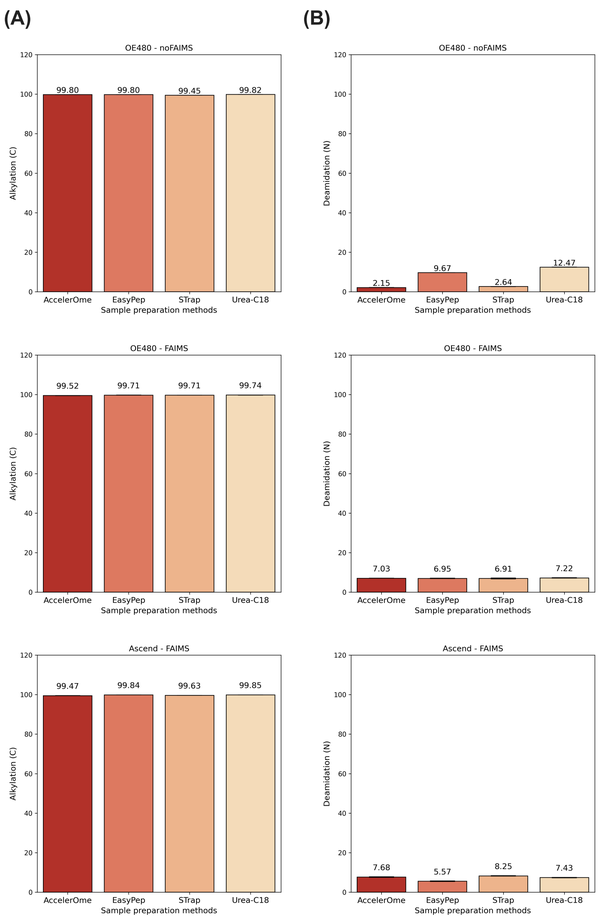


Percentage of (A) cysteine alkylation and (B) asparagine deamidation for samples analyzed on Orbitrap Exploris 480 MS without FAIMS. Data was analyzed by Spectronaut software (left) or Proteome Discoverer 3.1 software (right).



Percentage of (A) cysteine alkylation and (B) asparagine deamidation for samples analyzed on Orbitrap Exploris 480 MS or Oibitrap Ascend MS with FAIMS Pro interface. Data was analyzed by Spectronaut software (left) or Proteome Discoverer 3.1 software (right).

Figure 6. AccelerOme platform provides complete cysteine alkylation and minimal asparagine deamidation.



Percentage of (A) cysteine alkylation and (B) asparagine deamidation of samples prepared by AccelerOme platform or commonly used manual preparation methods.

Conclusions

- Velocity DIA workflow on Orbitrap mass spectrometers provides deep proteome coverage and robust quantitation.
- The AccelerOme platform can be incorporated into Velocity DIA workflow to provide an end-to-end platform for high-throughput and reproducible quantitative DIA analysis, allowing for improved productivity and minimized variations.

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