Leveraging a higher duty cycle DIA acquisition on a novel QTOF for enhanced proteomics analysis

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ABSTRACT

We used a research version of ZenoTOF 7600 system coupled to EvoSep One LC system to evaluate gains in protein identifications at various HeLa peptide loads when using variable window Zeno SWATH relative to SWATH acquisition. EvoSep was operated at throughputs of 200 SPD (samples per day, 5.6 min gradient), 100 SPD (11.5 min gradient), 60 SPD (21 min gradient) and nano-flow 30 SPD (44 min gradient). When we utilize the Zeno trap to increase the duty cycle at the MS/MS level to over 90% in Zeno SWATH mode (Zeno trap turned ON), the quality of the MS/MS spectra increases greatly relative to that acquired in SWATH acquisition alone. Analyzing the same sample loads with Zeno SWATH rather than SWATH acquisition, we obtain 1.5-2.4x more protein group identifications at 20% CV threshold,1.8-3.2x more at 10% CV threshold and 1.2-1.5x increase in overall identifications at low (25-50 ng) protein loads at all SPD throughputs. At higher protein loads (200-500 ng), the increase in protein group identifications is 1.3-1.5x at 20% CV threshold and 1.4-1.8x at 10% CV threshold. With Zeno SWATH we are able to identify over 8400 protein groups for 200 ng and 500 ng HeLa load for the 30 SPD throughput with 80-90% of identifications at 20% CV, or 7600-7700 proteins at 60 SPD with comparable CVs. The 'library-free' search approach for 500 ng load at 30 SPD identified 7700 protein groups, comparable number of IDs at CV thresholds relative to spectra-library approach and demonstrates the ease-of-use of the Zeno SWATH methodology in high-throughput proteomics.

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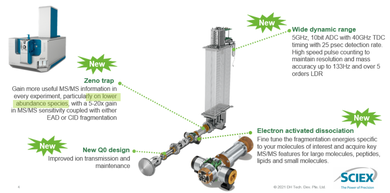
INTRODUCTION

The ability to identify and quantify large number of proteins and peptides is of great importance in translational medicine and life science research. Data independent acquisition (DIA) approaches have been shown to surpass data dependent acquisition (DDA) methodologies in terms of protein identifications in complex matrices especially at shorter acquisition speeds. Our new QTOF system equipped with a novel Zeno trap[1] is able to deliver sensitivity gains in variable window SWATH acquisition. The built-in Zeno trap increases duty cycle at MS/MS level to over 90%, allowing for unprecedented gains in sensitivity (5-20x) at MS/MS, resulting in more identifications using Zeno SWATH acquisition. We evaluated increases in protein and peptide identifications using SWATH acquisition vs. Zeno SWATH at various EvoSep sample throughputs.



What is duty-cycle?
- % of ions injected into TOF
  - Typically 5-25% on TOF

How does Zeno trap help?
- Increases duty cycle to 90% at MSMS level
  - Rich MSMS spectra

MATERIALS AND METHODS

Sample Preparation:
Lyophilized HeLa digest was purchased from Thermo Fisher Scientific and reconstituted with 95% buffer A (water with 0.1% formic acid) and 5% buffer B (acetonitrile with 0.1% formic acid) to a working concentration of 0.1 µg/µL. Working stock was further diluted to 1.25 ng/µL for 25 ng load; 2.5 ng/µL for 50 ng load; 10 ng/µL for 200 ng load; 25 ng/µL for 500 ng load. 20 µL of each concentration was loaded onto EvoTip.

HPLC:
EvoSep One (EvoSep, Denmark) had buffer A and buffer B as running solvents and operated in 200 SPD (samples per day, 5.6 min gradient), 100 SPD (11.5 min gradient), 60 SPD (21.0 min gradient) and 30 SPD (44.0 min gradient) throughputs. 200 SPD method used a 4 cm x 150 µm ID, 1.9 µm particle column (EV1107); 100 SPD and 60 SPD method used a 8 cm x 150 µm ID, 1.5 µm particle column (EV1109), 30 SPD method used a 15 cm x 150 µm ID, 1.9 µm column (EV1106).

MS Conditions:
Research version of ZenoTOF 7600 system was operated in SWATH and Zeno SWATH acquisition mode using the OptiFlow source. 200 SPD, 100 SPD and 60 SPD was run in micro-flow configuration whereas 30 SPD was in the nano-flow configuration.

200 SPD (5.6 min) and 100 SPD (11.5 min): Accumulation time: TOF MS = 50 ms (SWATH acquisition), 25 ms (Zeno SWATH), TOF MS/MS = 12 ms (SWATH), 11 ms Zeno SWATH; variable window SWATH: 56 windows 400-750 m/z; Voltage = 4500, Temp = 150˚C, CUR = 60, GS1 = 12, GS2 = 25

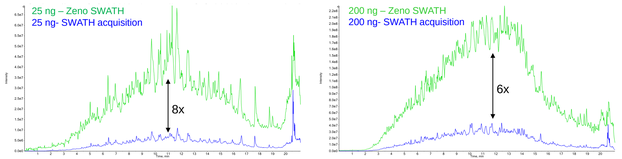
60 SPD (21.0 min): Same as for 200 SPD and 100 SPD except for: TOF MS accumulation time = 50 ms (SWATH acquisition and Zeno SWATH), variable window SWATH: 60 windows 400-900 m/z

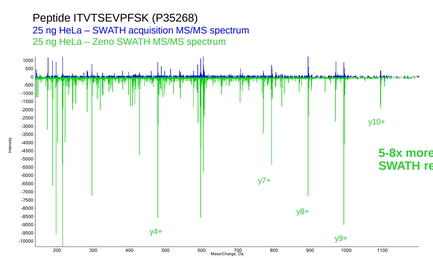
30 SPD (44.0 min): Accumulation time: TOF MS = 50 ms (SWATH and Zeno SWATH), TOF MS/MS = 20 ms (SWATH acquisition), 18 ms (Zeno SWATH); variable window SWATH: 85 windows 400-900 m/z; Voltage = 3400, Temp = 300˚C, CUR = 25, GS1 = 10

Data processing:
SWATH acquisition and Zeno SWATH data was processed using DIA-NN (v. 1.8) software[2] using (a) in-house generated pH fractionated spectral library of HeLa and K562 cell lines (11269 protein groups and 169395 precursors) or (b) SwissProt-Human canonical+isoform (Jan. 2021) FASTA database for library-free searches. pg.matrix.tsv and pr.matrix.tsv reports were used for reporting protein groups and precursors and for calculating IDs at %CV thresholds

RESULTS

MS/MS TIC (60 SPD, 21 min gradient)





5-8x more intense MS/MS with Zeno SWATH relative to SWATH acquisition

Figure 1: 200 SPD

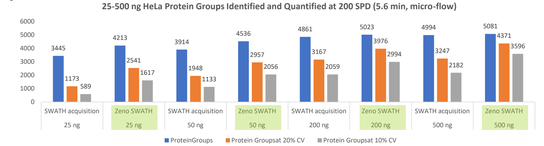


Figure 2: 100 SPD

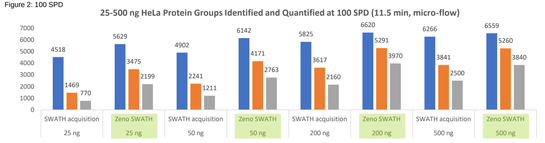


Figure 3a: 60 SPD

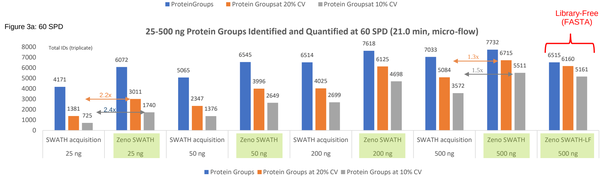


Figure 3b: 60 SPD

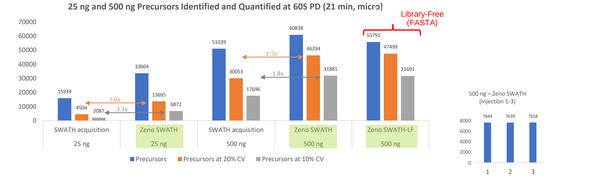
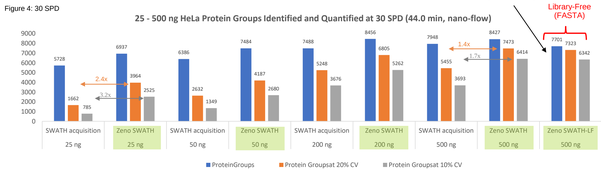


Figure 4: 30 SPD



Protein groups
SWATH acquisition vs Zeno SWATH at all (200, 100, 60, 30) SPD throughputs (Figure 1-4)
- At lower protein loads:
  - 25 ng: we see 2.2-2.4x (120-140%) and 2.4-3.2 (140-220%) more protein groups quantified at 20% and 10% CV, respectively
  - 50 ng: we see 1.5-1.9x (50-90%) and 1.8-2.3x (80-130%) more protein groups quantified at 20% and 10% CV, respectively
- At higher protein loads:
  - 200 ng: we see 1.3-1.5x (30-50%) and 1.4-1.8x (40-80%) more protein groups quantified at 20% and 10% CV, respectively
  - 500 ng: we see 1.3-1.4x (30-40%) and 1.5-1.7x (50-70%) more protein groups quantified at 20% and 10% CV, respectively
- With Zeno SWATH at:
  - 60 SPD: 7600-7700 protein groups for 200 ng and 500 ng with 80% and 87% of IDs at 20% CV (Figure 3)
  - 30 SPD: 8400 protein groups for 200 ng and 500 ng loads with 80% and 89% of IDs at 20% CV (Figure 4)
  - 30 SPD: 7700 protein groups for 500ng with library-free search with 95% of IDs at 20% CV (Figure 4)
    - Absolute number of protein groups at 20% and 10% CV is very comparable to those with a spectral-library

Precursors (Figure 3b)
SWATH acquisition vs Zeno SWATH at all (200, 100, 60, 30) SPD throughputs (Figure 3b)
- At lower protein loads (25 ng) we see (3x) 200% and (3.3x) 230% more precursors quantified at 20% and 10% CV, respectively
- At higher protein loads (500 ng), we see 1.5x (50%) and 1.8x (80%) more precursors quantified at 20% and 10% CV, respectively

Protein detection vs SPD (Figure 1-4)
- For 25-50 ng protein load, 100 SPD provides similar IDs at 20% and 10% CVs compared to 60 SPD and 30 SPD
- For 200-500 ng protein load, 60 SPD and 30 SPD provides more IDs at 20% and 10% CVs compared to 100 SPD and 200 SPD

CONCLUSIONS

- Zeno SWATH enables a higher number of proteins identified and quantified (2-3x) especially a lower protein loads relative to SWATH acquisition
  - Important for low-load samples such as single-cell, laser-capture microdissection (LCM), formalin-fixed paraffine embedded (FFPE) workflows
- Speed and selectivity of variable window SWATH acquisition and Zeno SWATH addresses the need in a high-throughput environment
- Zeno SWATH and library-free approach demonstrates the ease-of-use of DIA methodology for high-throughput proteomic workflows

REFERENCES

1. Chernushevich I., Loboda A. *J Am Soc Mass Spectrom*., 20 (7), 2009.
2. Demichev, V., et al. *Nature Methods*, 17 (1), 2020.

TRADEMARKS/LICENSING

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