Improved data-independent acquisition (DIA) and data-dependent acquisition (DDA) performance on low-level proteomic samples using a novel Zeno trap

Ihor Batruch[1], Stephen Tate[1], Nic Bloomfield[1], Anjali Chelur[1], Jose Castro-Perez[2]
[1]SCIEX, Canada; [2]SCIEX, USA

ABSTRACT

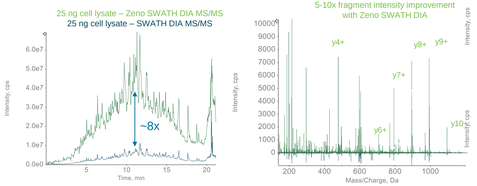
We used a ZenoTOF 7600 system in-line with a Waters M-Class LC system to determine protein identifications across varying commercial K562 tryptic digest loads in either Zeno SWATH DIA or Zeno DDA modes.
Using Zeno SWATH DIA at sample loads of 0.25, 0.5 and 1 ng loads, more than 900-1100, 1400-1500 and 2100-2300 protein groups were identified, respectively, and 45-55% of these identifications had a CV less than 20% when searched against a spectral library. At the peptide precursor level for the same loads, there were 2900-4100, 5000-5700 and 8700-12200 corresponding precursors for the 0.25, 0.5 and 1 ng loads. We tested higher loads and identified 4200, 5000 and 6100 protein groups for 5, 10 and 25 ng loads, respectively, and 64-83% of these identifications satisfied the 20% CV cutoff. For a 50 ng load, more than 6300 protein groups were identified, of which 90% had less than 20% CV, and 56000 precursors were identified. When the data were searched against a FASTA library in library-free mode, the overall number of identifications and those at 20% CV cutoffs approach those achieved when processed using the spectral library approach.
A 200 ng and 500 ng load of K562 tryptic digest was tested in Zeno DDA mode. From these experiments, we were able to identify 4600 and 5100 protein groups for the 200 and 400 ng loads, respectively, with 43000 and 56000 peptides for each load.

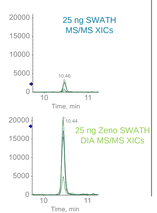
INTRODUCTION

The ability to capture biological heterogeneity at the level of an individual cell is important in translational research to gain insight into cellular composition and function. Data-independent acquisition (DIA) approaches have been shown to surpass data-dependent acquisition (DDA) methods in terms of protein identifications in complex matrices, especially at shorter LC-MS run times. The ZenoTOF 7600 system equipped with a novel Zeno trap[1] is able to deliver sensitivity gains in variable window SWATH DIA. The built-in Zeno trap increases duty cycle at the MS/MS level to over 90%, allowing for 5-20x gains in MS/MS sensitivity, resulting in more identifications using Zeno SWATH DIA. We evaluated protein and peptide identifications with Zeno SWATH DIA and Zeno DDA for a commercial digest starting at single-cell level loads up to 200 ng.

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 MS/MS level
  - Rich MS/MS spectra





MATERIALS AND METHODS

Sample preparation: Lyophilized K562 tryptic digest (SCIEX) was 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 0.5 µg/µL. The working stock was further diluted to 200, 50, 25, 10, 5, 1, 0.5 and 0.25 ng/µL. For dilutions of 5, 1, 0.5 and 0.25 ng/µL, a matrix of 5 fmol/µL bovine serum albumin (BSA) was used a diluent. A blank sample was prepared as a control with 5 fmol/µL BSA, containing 0 ng/L K562 digest.
digest
HPLC conditions: A Waters M-Class LC system running at 300 nL/min was connected to an Evosep EV-1106 analytical column (15 cm x 0.150 µm ID, 1.9 µm bead) with a 45 min gradient or to a PharmaFluidics 200 cm µPAC column with a 180 min gradient.

MS/MS conditions: ZenoTOF 7600 system was operated in Zeno SWATH DIA or Zeno DDA mode the using the OptiFlow Turbo V ion source in nano-flow configuration.
Zeno SWATH DIA: the accumulation time for TOF MS was set to 50 ms (SWATH DIA and Zeno SWATH DIA), and the TOF MS/MS accumulation time was set to 20 ms for SWATH DIA, and 18 ms for Zeno SWATH DIA. The variable Q1 windows for SWATH DIA were 85 windows covering 400-900 m/z. Source conditions were: Voltage = 3200; Temp = 225˚C; CUR = 25; GS1 = 10
Zeno DDA: the accumulation time for TOF MS was set to 100 ms, and 20 ms for TOF MS/MS. Other method parameters were charge state = 2-5; Top 45 precursors; Exclusion time = 12 sec; Exclusion mass tolerance = 15 ppm; Zeno, Voltage = 3200; Temp = 225˚C; CUR = 35; GS1 = 20

Data processing: SWATH DIA and Zeno SWATH DIA data were processed using DIA-NN software[2] (v. 1.8.1 beta 11) using (a) an in-house generated pH fractionated spectral library of HeLa and K562 cell lines created with ProteinPilot in OneOmics suite (cloud, searched against SwissProt-Human canonical+isoform FASTA file) using the 'Per-File' search strategy followed by fraction level retention time alignment and merging using the Extractor application in OneOmics suite (11269 protein groups and 169395 peptide precursors) or (b) a SwissProt-Human canonical+isoform (Jan. 2021) FASTA database for library-free searches. The pg.matrix.tsv and pr.matrix.tsv reports were used for reporting protein groups and precursors and for calculating identifications at %CV thresholds.
Zeno DDA files were processed with the ProteinPilot application search engine in OneOmics suite (cloud) against a SwissProt-Human canonical+isoform FASTA database. Iodoacetamide was selected as a fixed modification and results were reported at 1% FDR.

RESULTS

Figure 1a.

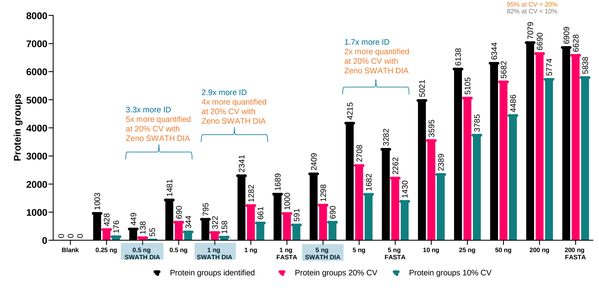


Figure 1b.

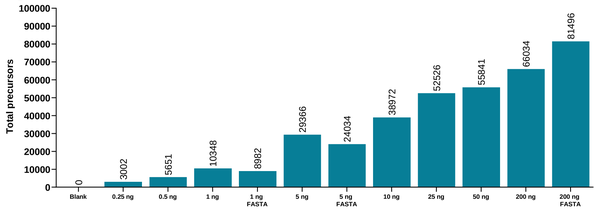


Figure 1c.

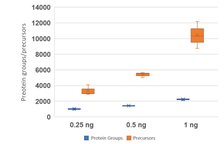


Figure 1d.

Figure 1. (a) Protein groups and (b) precursors detected with SWATH DIA and Zeno SWATH DIA for 0.25-200 ng K562 loads. (c) Number of protein groups and precursors detected with Zeno SWATH DIA for 0.25 ng, 0.5 ng and 1 ng sample loads across 30 days with different dilutions. (d) Overlap of protein groups detected with a spectral-library and a library-free (FASTA) mode for a 1 ng load of K562 digest in Zeno SWATH DIA.

Figure 2a.

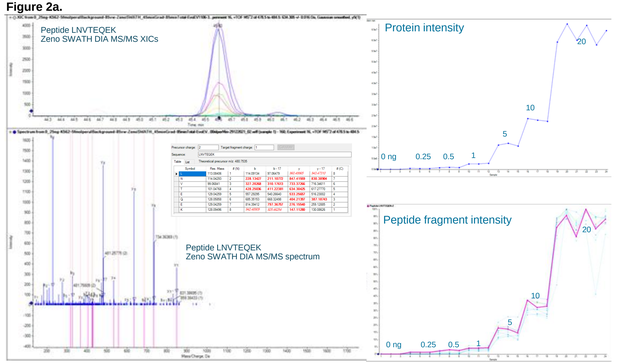
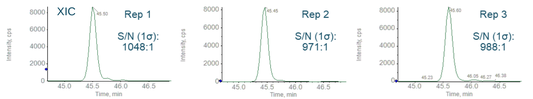


Figure 2b.



CV across triplicate injection = 0.73%

Figure 2. (a) Spectral match of peptide LNVTEQEK at 0.25 ng and XIC intensity at protein and peptide fragment level up to 20 ng load. (b) XIC of peptide LVNTEQK at 0.25 ng in triplicate with a calculated CV for summed y5, y6, y7 and y8 fragments ions.

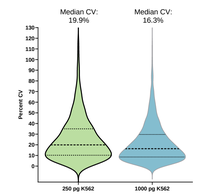


Figure 3. Median protein CV for 0.25 and 1.0 ng K562 load with Zeno SWATH DIA.

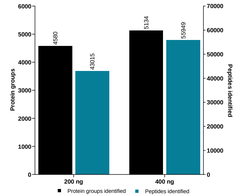


Figure 4. Protein and peptide identifications with Zeno DDA for 200 and 400 ng K562, using <1% FDR filter.

CONCLUSIONS

- Zeno trap improves duty cycle at MS/MS level to over 90%, thereby improving MS/MS sensitivity 5-10x
- At single-cell level protein loads, Zeno SWATH DIA increases protein detection over 3x and increases number of proteins quantified by 4-6x relative to SWATH DIA (Figures 1a, 1b). Consistent detection of low loads was achieved across 30 days:
  - 0.25 ng load: 900-1100 protein groups (3 sets over 1 month, Figure 1c)
  - 0.5 ng load: 1400-1500 protein groups (3 sets over 1 month, Figure 1c)
  - 1.0 ng load: 2100-2300 protein groups (3 sets over 1 month, Figure 1c)
  - 0.25 and 1 ng K562 loads had median CVs of 20% and 16%, respectively (Figure 3)
- For a 200 ng protein load, Zeno SWATH DIA enabled detection of over 7000 protein groups with 95% of detections having a CV < 20% (Figure 1a) and over 66,000 precursors (Figure 1b)
- Library-free (FASTA database) search performed well and eliminated the need to generate spectral libraries for different biological cohorts (Figures 1a, 1d)
- Excellent peptide spectral matching at 0.25 ng and corresponding fragment peptide and protein intensity up to 20 ng (Figure 2a) were observed with median CVs of 20% and 16% for 0.25 and 1.0 ng loads, respectively (Figure 3)
- In Zeno DDA, over 4500 protein groups with 43,000 peptides were identified for a 200 ng K562 load and over 5100 protein groups with 56,000 peptides were identified for a 400 ng load (Figure 4)

REFERENCES

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

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