Enabling high throughput proteomics with short gradients and ZenoSWATH DIA by utilising the speed and sensitivity of the ZenoToF 7600

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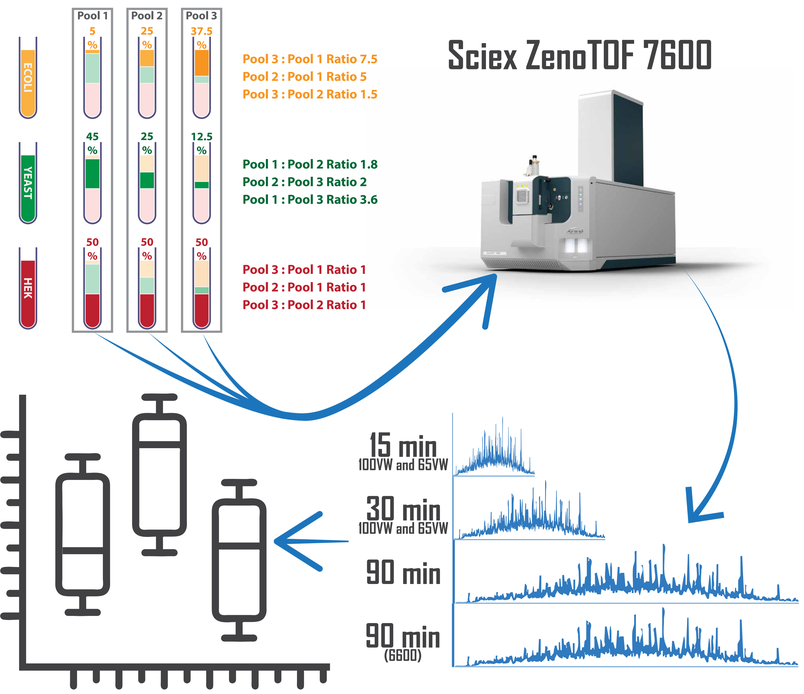
Introduction

Limited sample quantities and the need for long MS acquisition times to achieve reasonable proteome coverage remain a major bottleneck in achieving high-throughput proteomics. The ZenoToF 7600 mass spectrometer's speed and sensitivity allow for shorter MS acquisition runs while also reducing the amount of sample needed. Our objective was to test shorter MS acquisition times thereby increasing sample processing efficiency, while maintaining accurate protein identification and quantification.

Methods

To assess the impact of shorter gradients on quantitation accuracy and protein identifications, a mix of HEK293, E. coli, and yeast digests in varying proportions across 3 pools were prepared. The 3 pools were analysed in triplicates using 15, 30 and 90min gradients runs using ZenoSWATH on the Sciex ZenoTOF 7600 and compared to 90min standard SWATH run on the Sciex TripleTOF 6600. 100 variable Windows (VW) across a 400-1250 Da mass range were used for the analysis. For the shorter 15 and 30min gradient runs, additional triplicate runs were used to analyse the samples using 65VW per cycle covering a mass range of 400-750Da mass range. The reduced number of VW was used to ensure 8-10 points across a peak while the reduced mass range was used to reduce spectral complexity across the fewer windows used. 200ng was loaded per run for ZenoSWATH and 2µg was loaded per run for Standard SWATH on the TripleTOF 6600. All data was analysed using DIA-NN 1.8.



Results

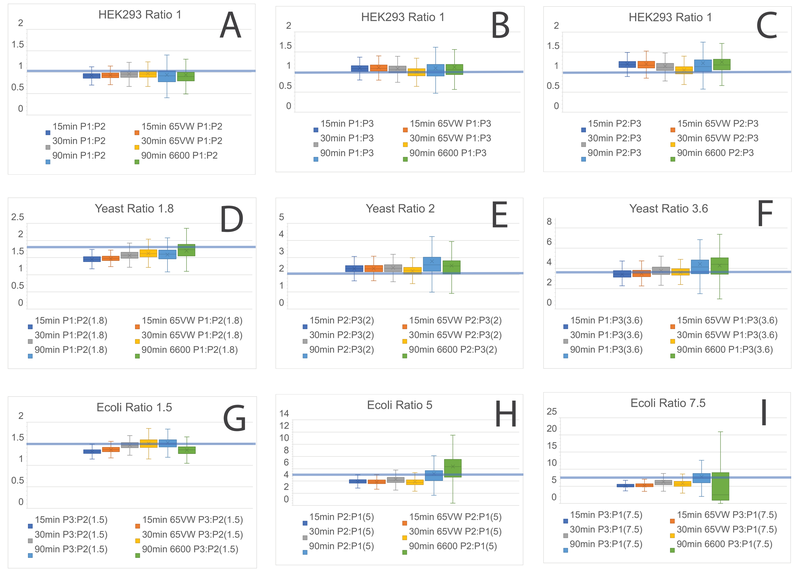


Fig1 Comparison of Pool ratios run at 15min(100VW, 65VW),30min(100VW, 65VW) and 90min run using ZenoSWATH and 90min run on the TripleTOF 6600 using standard SWATH to assess quantitation accuracy of shorter gradients. **(A,B,C)** HEK protein areas for P1:P2, P2:P3, P1:P3 Expected ratio 1, 1, 1. **(D,E,F)** Yeast protein areas for P1:P2, P2:P3, P1:P3 Expected ratio 1.8, 2, 3.6. **(G, H, I)** E.coli identified protein areas for P1:P2, P2:P3, P1:P3 Expected ratios 1.5, 5 ,7.5

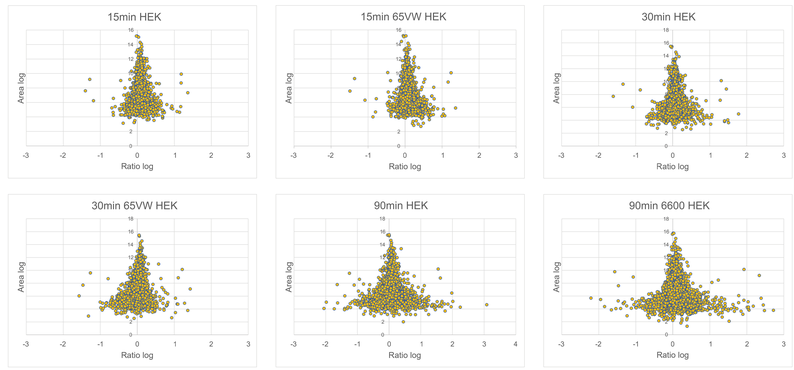


Fig2. Average HEK protein area ratios (P1:P2, P2:P3, P1:P3) log values plotted against log values of their quantified areas. **(A)** 15min MS Run, **(B)** 15min MS run with 65VW and 400-750Da mass range, **(C)** 30min MS Run, **(D)** 30min MS run with 65VW and 400-750Da mass range, **(E)** 90min MS Run, **(F)** 90min MS run on the TripleTOF 6600

Results

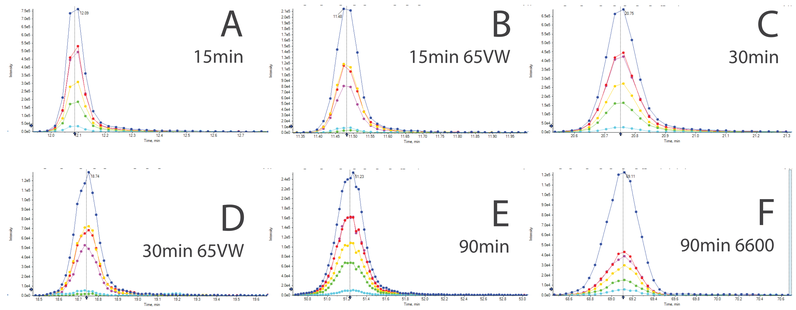


Fig3. Peak widths across different gradient lengths and the number of points acquired across each peak. **(A)** 15min MS Run, **(B)** 15min MS run with 65VW and 400-750Da mass range, **(C)** 30min MS Run, **(D)** 30min MS run with 65VW and 400-750Da mass range, **(E)** 90min MS Run, **(F)** 90min MS run on the TripleTOF 6600

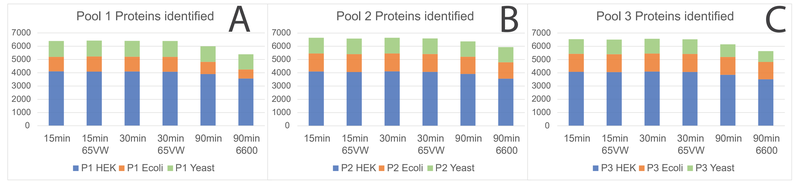


Fig4. Protein identified from the HEK, E.coli and Yeast fractions across the different run times. **(A)** Pool 1 identified proteins **(B)** Pool2 identified proteins **(C)** Pool3 identified proteins

Conclusion

1. We identified similar number of proteins on the ZenoTOF using ZenoSWATH with 15 and 30min MS runs when compared to 90min MS runs on the TripleTOF 6600 with good quantitative data with similar or better accuracy.
2. The ZenoTOF enable the use of 1/10 the sample quantity required for an analysis on the TripleTOF 6600(200ng vs 2µg)
3. Shorter MS run benefited from sharper chromatography with increased peak intensities that resulted in better quantitative data, especially for low abundant peptides and proteins.
4. The 15min MS runs with 100VW, despite suffering a reduction in the number of points across a peak were able to achieve sufficient points across a peak to achieve good quantitative results

Acknowledgements

Australian Government Department of Health
Medical Research Future Fund
Australian Government National Health and Medical Research Council NHMRC
NSW Government cancer institute NSW
THE UNIVERSITY OF SYDNEY
ICPC International Cancer Proteocenome Consortium
AUSTRALIAN CANCER RESEARCH FOUNDATION