ionopticks

FEATURED PRODUCTS:

5cm Aurora Series packed emitter column with CaptiveSpray Insert
(5cm x 150μm ID, 1.6μm C18)
Part No. AUR2-50150C18A-CSI

5cm Aurora Series packed emitter column
(5cm x 150μm ID, 1.6μm C18)
Part No. AUR2-50150C18A

APPLICATION NOTE

DDA High-throughput proteomics for plasma samples using the Aurora Rapid column

INTRODUCTION

Ultra-sensitive, high-throughput proteomics

IonOpticks' Aurora Series 5cm RAPID UHPLC packed emitter columns featuring our revolutionary nanoZero® fitting, allow for simplified plug-and-play high-throughput proteomics. These columns are capable of analysing 180 samples per day with a 5 min gradient. Tryptic digested Human Plasma was injected and separated using our 180 samples per day method and data dependent acquisition using PASEF resulting in >230 protein identifications (>1,600 unique peptide identifications) [Fig1, Fig 2] with highly reproducible peptide retention times between runs [Fig 3].

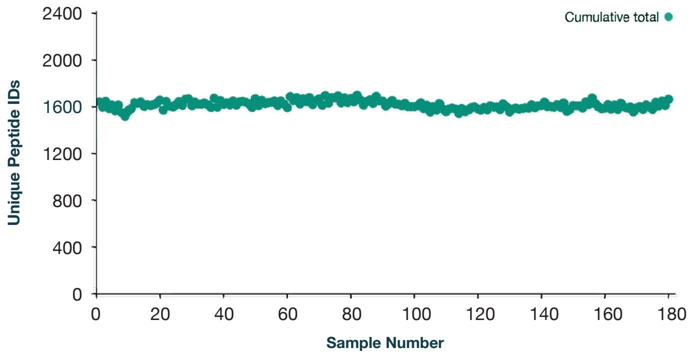


Fig 1 Number of unique peptide IDs

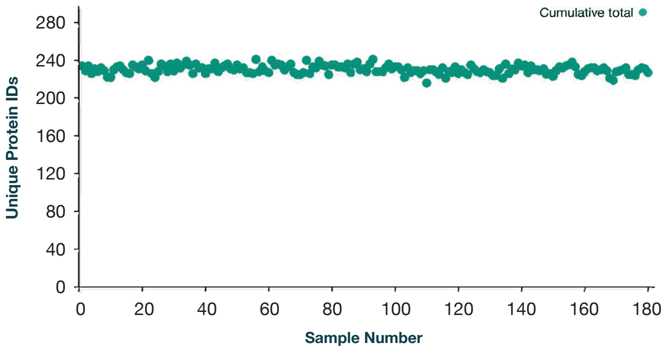


Fig 2 Number of unique protein IDs

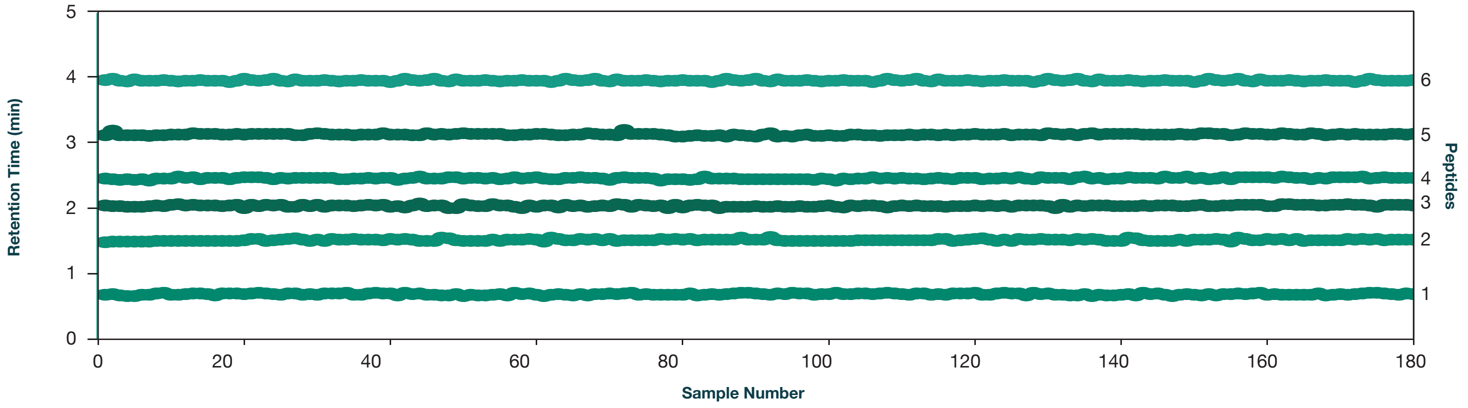


Fig 3 Retention time comparison for 6 different peptides across 180 samples

More than
230
proteins per run
DDA-PASEF

180
times per day

Integrated
nanoZero
high-pressure
fitting

1.3 secs
Full-width
half-maximum

Fig 4 Median and standard deviation of FWHM for peptides

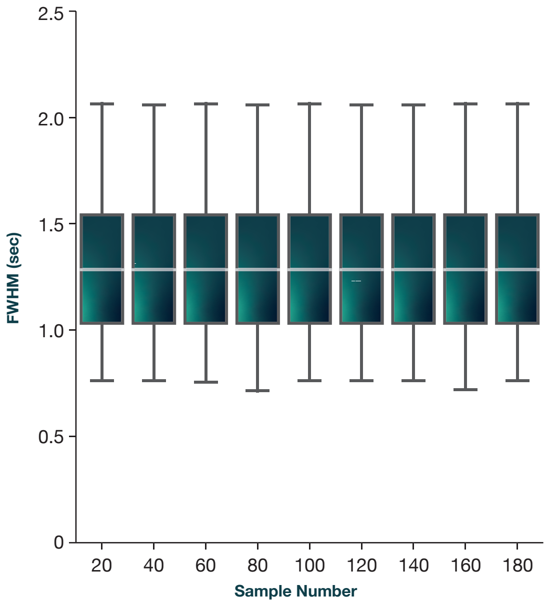
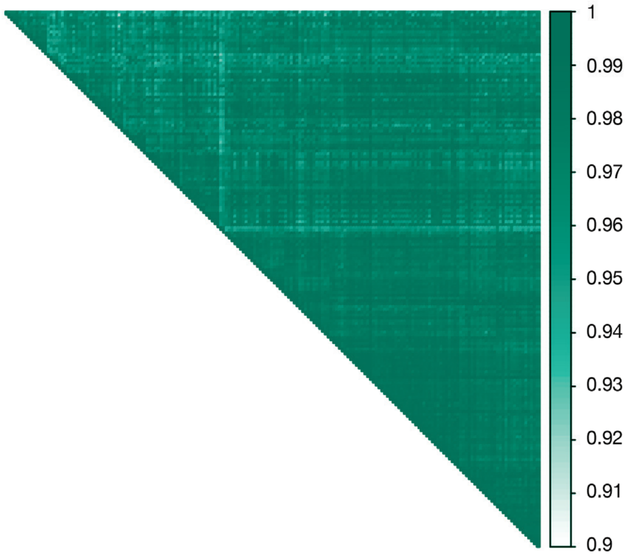


Fig 5 Pearson correlation plot of peptide intensities



>0.97
mean Pearson correlation

across
180
samples

METHODS

Human Plasma tryptic digest was reconstituted in 2% acetonitrile/1% formic acid in MilliQ water to a concentration of 50 ng/µL. Samples were analysed on a M-class (Waters, USA) coupled to a timsTOF Pro (Bruker) equipped with a CaptiveSpray source. 50ng (1µl) of peptides were separated on an Aurora Series 5cm X 150µm RAPID UHPLC packed emitter column, using a constant flow rate of 2µl/min. The column was maintained at room temperature. Sample was injected into a sample loop which takes approximately 0.5min. Mobile phase at 100% buffer A continues to flow over the analytical column during this period facilitating column equilibration. The sample loop was switched on-line for 1min at 100% buffer A. A recommended sample gradient is shown here.

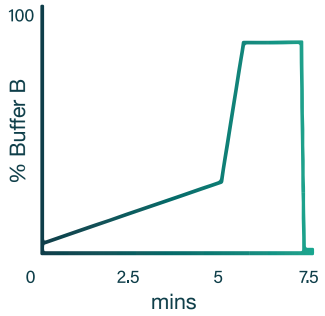
Separately, 20µg of the Human Plasma tryptic digest was resuspended in 10mM Ammonium Formate pH 10. Peptides were separated into 12 fractions using a stage-tip containing 4 X C18 plugs. Fractions were lyophilised to dryness using a CentriVap (Labconco) before reconstitution in 2% ACN, 1% FA prior to analysis. Fractions were analysed using the same LC-MS method as the single shot samples.

Data was analyzed by MaxQuant software using the integrated Andromeda search engine and searched against the human Uniprot Reference Proteome and matched to a high pH fractionation library using the Match-Between-Runs feature.

Aurora Rapid 5x150,
5 minute gradient

(5cm x 150µm ID, 1.6µm C18) Part No. AUR2-50150C18A

Example gradient



Time (mins)	Comp (% Buff. B)	Flow rate (µl/min)
0	5	2
5	34	2
5.5	85	2
6.5	85	2
6.8	0	2
7.5	0	2

FURTHER READING

For futher resources and technical support, visit our Help Centre at helpcentre.ionopticks.com.

To view other application notes, read the latest publications featuring Aurora Series columns, or view the full range of IonOpticks products, visit our website at www.ionopticks.com

Find these additional documents in our help centre:

PDF Aurora Series UHPLC packed emitter column user guide

Robust reproducibility

- High-throughput analysis
- Maximum protein IDs
- Minimum peak widths
- Consistence performance over large sample cohorts

ABOUT IONOPTICKS

IonOpticks produces high-performance chromatography solutions for the global research community. We specialise in the development and manufacture of columns for analytical applications in liquid chromatography with mass spectrometry (LC-MS) and high-end proteomics. Our highly reproducible methods provide a unique ability to enhance the sensitivity of mass spectrometry sample analysis, enabling scientists and clinicians to discover more from their samples. Our team are experts in a broad array of LC-MS platform technologies and are driven by the need to improve chromatographic performance in order to achieve data quality and deep proteome coverage on a whole new scale.