AURORA® SERIES 5

TECHNICAL NOTE

Aurora® Elite™ XT 15 × 150 C18

More from every gradient. From first injection, to last.

Peak properties, identification rates, quantitative precision and column lifetime for the Aurora® Elite™ 15 cm × 150 µm column on a Thermo Scientific Orbitrap Astral Zoom, from 50 to 170 samples per day and across more than 1,350 injections.

Aurora Series® 5 chromatography, benchmarked at the throughputs modern proteomics actually runs at.

> 9,400	1.19 s	< 1%	1,350+
PROTEIN GROUPS PER 5.25-MINUTE RUN	MEDIAN PEAK WIDTH AT 170 SPD	CHANGE IN BACK-PRESSURE ACROSS ENTIRE SERIES	HIGH-PRESSURE INJECTION CYCLES

Short gradients only pay off if the chromatography keeps up.

Every peptide that co-elutes, broadens or loses intensity is a peptide the mass spectrometer has to work harder to see, and at 170 samples per day there is no time to spare. This note reports what the IonOpticks Aurora® Elite™ 15 cm × 150 µm column delivers under exactly those conditions.

We separated 20, 50 and 200 ng of HeLa digest standard at 50, 90 and 170 SPD and evaluated peak parameters, identification rates and quantitative precision. A like-for-like Thermo Scientific EASY-Spray ES906 (15 cm × 150 µm) column was run alongside on the same system, with the gradient optimised for each column to ensure a fair comparison.

Column lifetime was then followed over more than 1,350 high-pressure injection cycles, using a 50 ng HeLa standard at 170 SPD (5.25-minute gradient). Across every gradient and every sample load the IonOpticks column gave narrower peaks, higher signal, more identifications and better quantitative precision, holding that performance from the first injection to the last.

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INSTRUMENTATION USED



MASS SPECTROMETER

Thermo Scientific Orbitrap Astral Zoom with EASY-Spray source



UHPLC

Thermo Scientific Vanquish Neo



DATA PROCESSING

DIA-NN Enterprise 2.6.1
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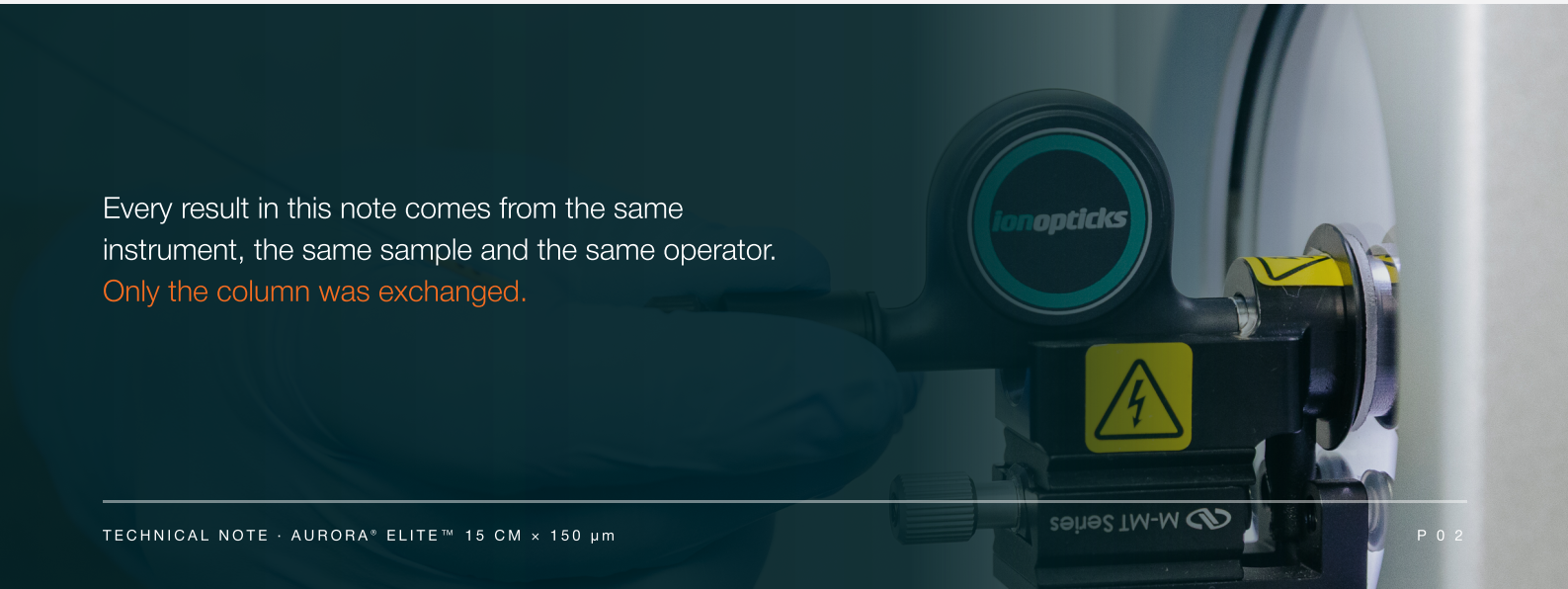
COLUMN

IonOpticks Aurora® Elite™ XT 15 × 150 C18 UHPLC column



COLUMN HEATING

IonOpticks HeatSync™ system set at 50 °C



Every result in this note comes from the same instrument, the same sample and the same operator. Only the column was exchanged.

Deeper coverage, with precision to match.

+44%

QUANTIFIED UNIQUE PEPTIDES*

+15%

QUANTIFIED UNIQUE PROTEIN GROUPS*

Coverage only counts if it quantifies reproducibly. At equivalent sample input, the Aurora® Elite™ 15 cm × 150 µm column quantifies up to 44% more unique peptides and 15% more unique protein groups than the ES906 column across gradients from 50 to 170 SPD. Peptides and protein groups were considered as quantified if their CV across five replicates was <20%.

*CV < 20% across n = 5 replicates, relative to the ES906 column at matched input.

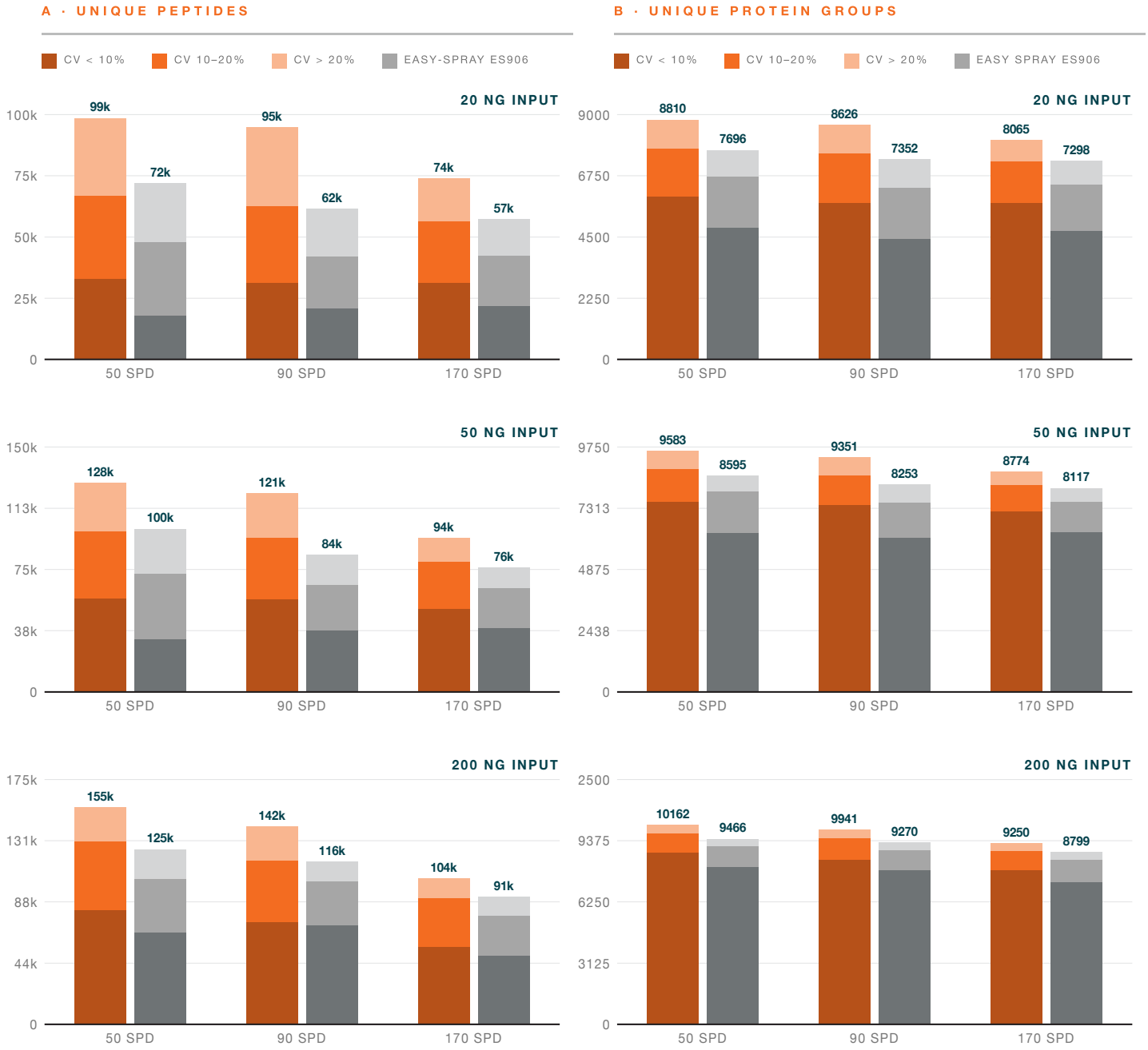


Figure 1: Unique peptides (A) and unique protein groups (B) identified from injections of HeLa digest standard, separated using Aurora® Elite™ 15 cm × 150 µm and ES906 columns (n = 5). Rows are 20, 50 and 200 ng sample input; bars are grouped by gradient throughput. Identifications are stacked by CV: < 10%, 10–20% and > 20%.

MORE UNIQUE PEPTIDES

Up to 44% more unique peptides quantified from the same sample.

GAINS AT EVERY SAMPLE LOAD

Ahead of ES906 at 20, 50 and 200 ng, not just at high input.

HOLDS AT 170 SPD

The advantage is maintained at the shortest gradient tested.

Narrow peaks across the elution window.

30%
NARROWER MEDIAN FWHM

< 3 s
FWHM ACROSS A 50 SPD RUN

Peak width sets the ceiling on everything downstream. The Aurora® Elite™ 15 cm × 150 µm column produces consistently sharp peaks throughout the entire peptide elution window across a range of throughputs, with a median full width at half maximum (FWHM) up to 30% narrower than the ES906 column.

PRECURSOR PEAK WIDTH DISTRIBUTION

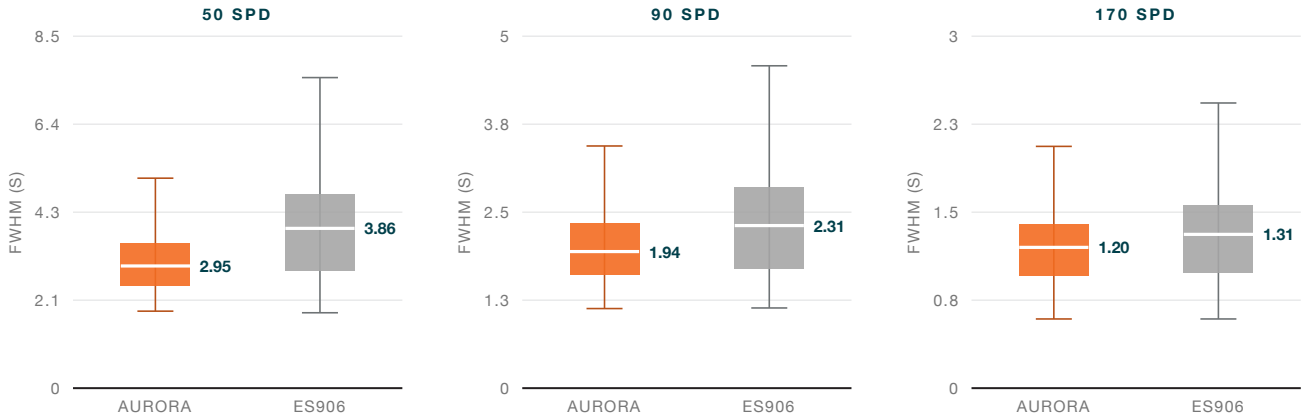


Figure 2: 50 ng of HeLa digest standard separated on either the Aurora® Elite™ 15 cm × 150 µm or the ES906 column using a 50, 90 or 170 SPD gradient (n = 5). Box plot of precursor peak width distributions.

MEDIAN PEAK WIDTH ACROSS THE ELUTION WINDOW

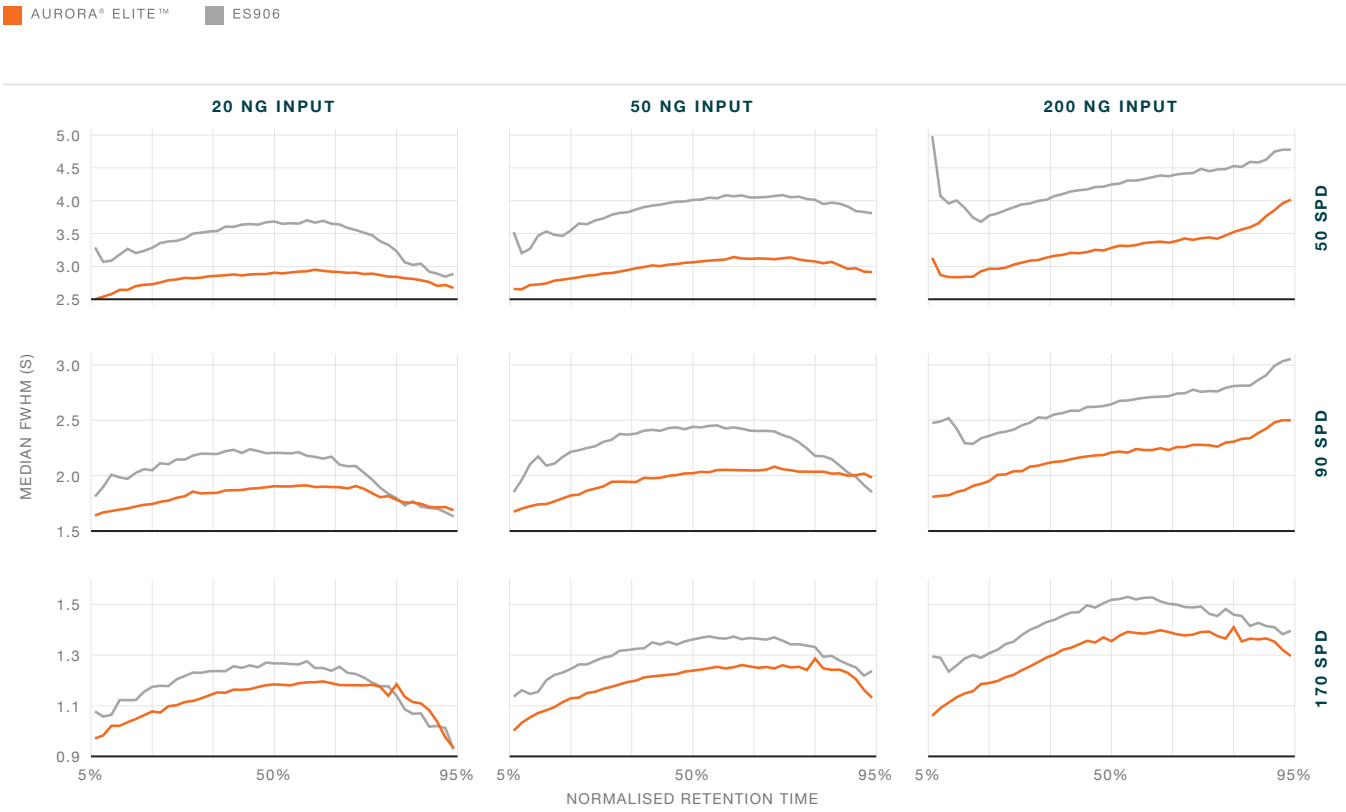


Figure 3: Trendline of median peak width as a function of retention time. HeLa digest standard was separated on either the Aurora® Elite™ 15 cm × 150 µm or ES906 column using 50, 90 and 170 SPD gradients at 20, 50 and 200 ng input. Median peak width was calculated for every 1.2 s retention time bin per sample, then averaged across replicates and plotted against the normalised retention time bin (n = 5).

Sharper peaks put more signal into every scan.

22.8 min

MATCHED GRADIENT
BOTH COLUMNS

Narrow chromatographic peaks obtained with the Aurora® Elite™ 15 cm × 150 µm column raise base peak intensity and increase peak capacity for any given gradient length. The same sample, concentrated into less time, is simply easier for the instrument to see.

AVERAGED BASE PEAK CHROMATOGRAM · 50 SPD · 50 NG

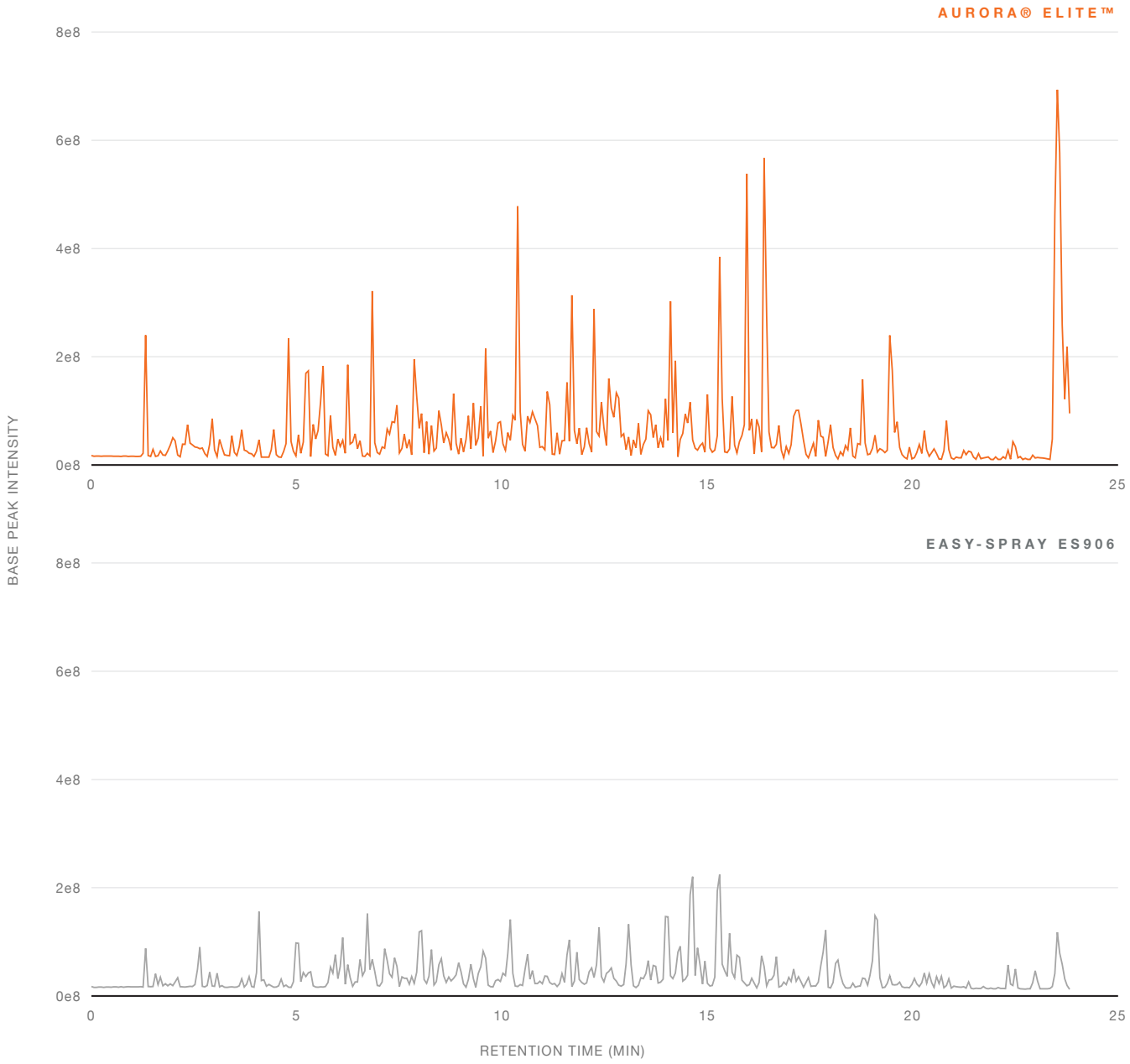


Figure 4: Averaged base peak chromatograms (n = 5) of a 50 ng HeLa digest standard separated at 50 SPD throughput (22-minute gradient) using the IonOpticks Aurora® Elite™ 15 cm × 150 µm column (top) and the ES906 column (bottom).

MORE INTENSITY

Taller, better resolved peaks from the same 50 ng load at 50 SPD.

MORE PEAK CAPACITY

More resolved features fit inside the same gradient window.

CLEANER PROFILE

Peaks resolve to baseline across the whole gradient.

Half the run time. Or half the sample.

5.25 min
MATCHES A 22.8-MIN ES906 RUN

50 ng
MATCHES A 200 NG ES906 INJECTION

Better separation can be spent in one of two ways. Spend it on time: from 20 ng, the Aurora® Elite™ 15 cm × 150 µm column quantifies 7,282 proteins in a 5.25-minute run at 170 SPD, more than the 6,729 the ES906 column reaches in a 22-minute run at 50 SPD. That is 3.4 times the samples per day from the same instrument. Spend it on sample instead: hold the gradient at 90 SPD and 50 ng on the Aurora® Elite™ 15 cm × 150 µm column quantifies 8,646 proteins, within 3% of the 8,873 the ES906 column needs 200 ng to reach.

More IDs, less time

QUANTIFIED PROTEINS BY RUN TIME

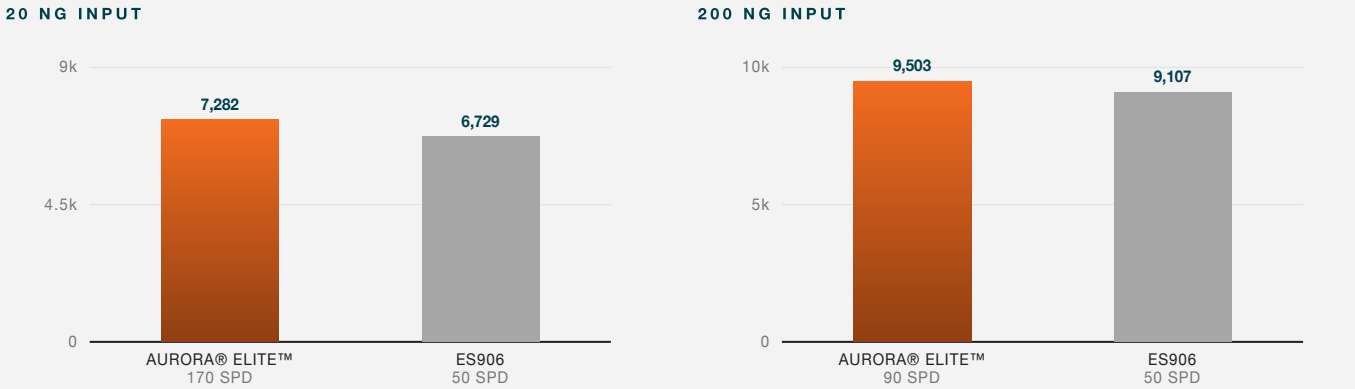


Figure 5: Number of quantified proteins (CV < 20%, n = 5) from 20 ng (left) and 200 ng (right) HeLa digest standards, separated using 50–170 SPD gradients on the Aurora® Elite™ 15 cm × 150 µm and ES906 columns.

More IDs, less input

QUANTIFIED PROTEINS BY SAMPLE INPUT

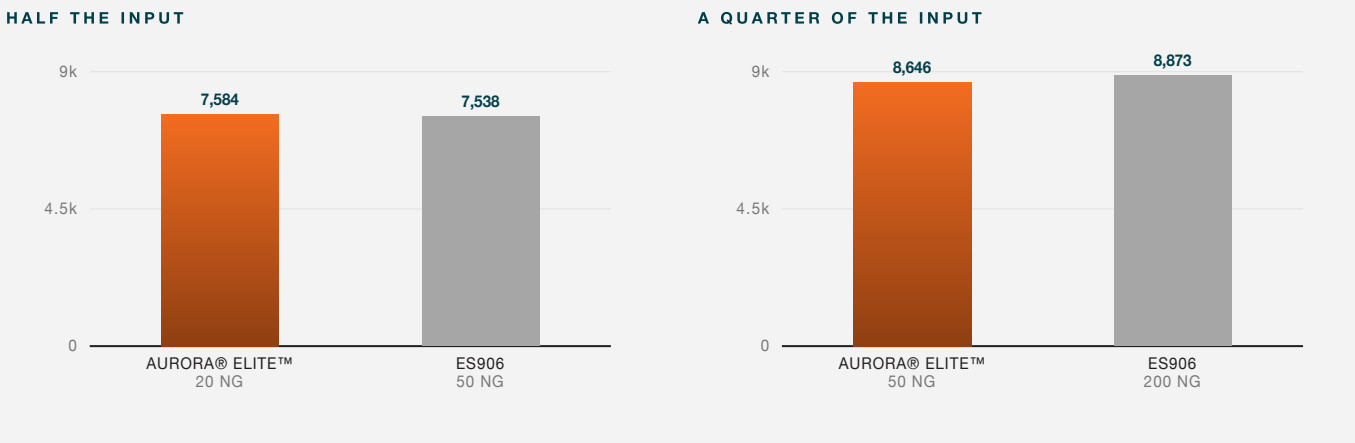


Figure 6: Number of quantified proteins (CV < 20%, n = 5) from 20 ng, 50 ng and 200 ng HeLa digest standards when separated using a 90 SPD gradient on the Aurora® Elite™ 15 cm × 150 µm and ES906 columns.

MORE SAMPLES PER DAY

From 20 ng, 7,282 proteins at 170 SPD beats ES906 at 50 SPD.

LESS SAMPLE CONSUMED

At 90 SPD, 50 ng matches what the ES906 column needs 200 ng to reach.

IDEAL FOR LIMITED SAMPLES

Headroom where material is scarce and cannot be repeated.

The last injection looks like the first.

The Aurora® Elite™ 15 cm × 150 µm columns maintain exceptional performance even after > 1,350 injection cycles. Back-pressure, peak properties, identification rate and quantitative precision were monitored using 50 ng injections of HeLa protein digest standard separated with high-throughput 170 SPD runs throughout the entire injection series.

Rising back-pressure, broadening peaks and drifting retention times are the usual signs that a column is nearing the end of its life, and they rarely arrive at a convenient point in a cohort. None of them appeared here.

QC CHECKPOINTS

50 ng HeLa digest at 170 SPD, on a 5.25-minute gradient, sampled periodically across the whole series.

> 9,400
UNIQUE PROTEIN GROUPS PER RUN

91.7%
UNIQUE PROTEIN GROUPS SHARED ACROSS ALL 1,350 RUNS

r > 0.99
LOG_2 PROTEIN INTENSITY CORRELATION

680 ± 4
BAR MAXIMUM BACK-PRESSURE

Coverage that does not drift across a cohort.

8,627

UNIQUE PROTEIN GROUPS IN EVERY SINGLE RUN

91.7%

OF UNIQUE PROTEIN GROUPS SHARED THROUGHOUT

The protein identification rate remained high across the dataset, consistently exceeding 9,400 unique protein groups per run. Of the unique protein groups identified, 8,627 (91.7%) were shared across all runs, with a Log_2-intensity Pearson correlation coefficient of > 0.99. Retention times held to the same window throughout, so a method validated at the start of a cohort still applies at the end of it.

UNIQUE PROTEIN GROUPS IDENTIFIED PER RUN



Figure 7: Number of unique protein groups identified from runs throughout a > 1,350 injection series. Data points represent 50 ng HeLa digest standard injections separated at 170 SPD throughput (5.25-minute gradient).

RETENTION TIME OF 15 SELECTED PRECURSORS

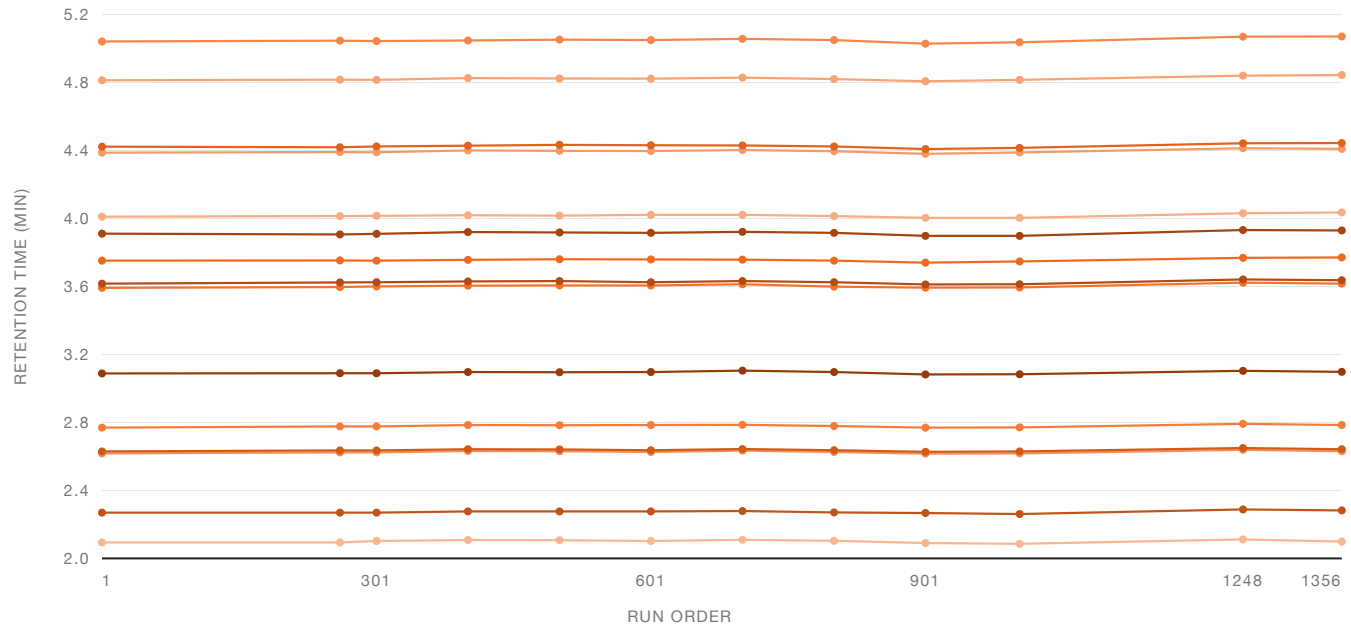


Figure 8: Retention time stability across the > 1,350 injection series. Data points represent 15 selected precursors identified across the dataset in 50 ng HeLa digest standard injections separated at 170 SPD throughput (5.25-minute gradient).

No creep in pressure, no change in peak shape.

The maximum back-pressure recorded during the gradient throughout the entire series remained stable at 680 ± 4 bar. The peak capacity of the column also remained stable: eluting peaks stayed symmetrical (Figure 10A) and the peak width at half maximum height held at around 1.19 ± 0.01 s (Figure 10B). Quantitative agreement between runs held just as firmly, with Log_2 protein intensities correlating above r = 0.99 across every pair of QC runs (Figure 11).

± 4 bar
MAXIMUM PRESSURE STANDARD DEVIATION

1.19 s
MEDIAN FWHM, ± 0.01 S

r > 0.99
LOG_2 INTENSITY CORRELATION

MAXIMUM BACK-PRESSURE PER GRADIENT

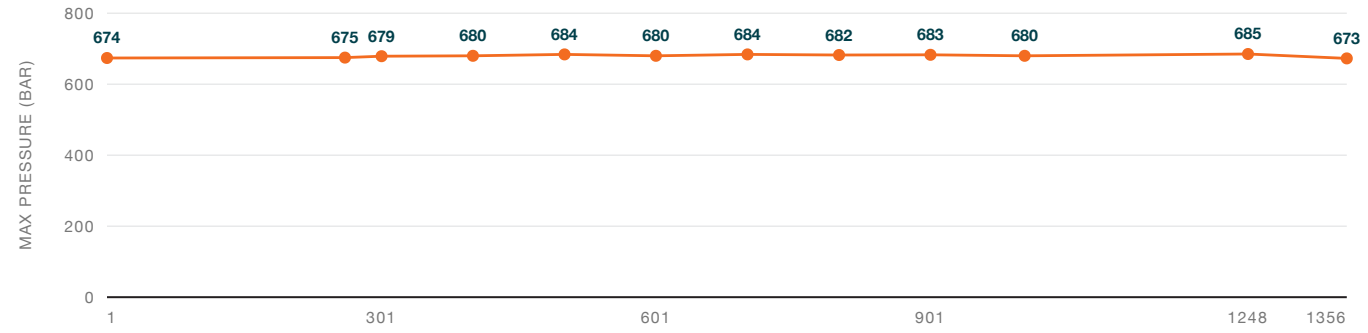
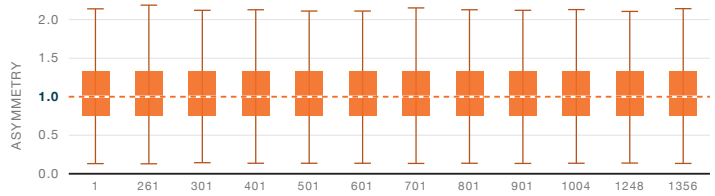


Figure 9: Maximum back-pressure recorded during each of the 170 SPD gradients throughout a > 1,350 injection series.

A · PEAK ASYMMETRY FACTOR



B · FULL WIDTH AT HALF MAXIMUM

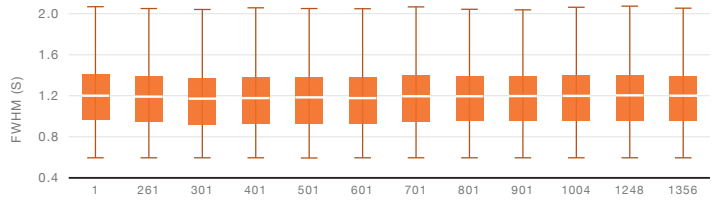


Figure 10: Peak shape parameter distributions across injections of a 50 ng HeLa digest standard separated with the Aurora® Elite™ 15 cm × 150 µm column at 170 SPD throughput. (A) Peak asymmetry factor and (B) full width at half maximum for each monitored run throughout the > 1,350 injection series.

PEARSON r, LOG_2 INTENSITY

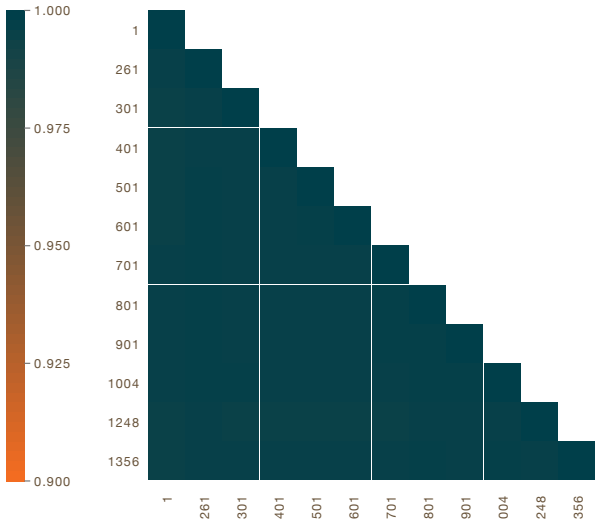


Figure 11: Pearson correlation coefficient of Log_2 protein intensities for the protein groups identified across all runs of the > 1,350 injection series (n = 8,627 common protein groups).

NO BLOCKAGE BUILDING

Back-pressure sits within ± 4 bar of 680 across the whole series.

PEAKS STAY SYMMETRICAL

Asymmetry and FWHM distributions overlay run to run, so no tailing or broadening creeps in.

QUANTITATION COMPARABLE

At r > 0.99 between every run pair, samples acquired at any point in the series can be compared directly.

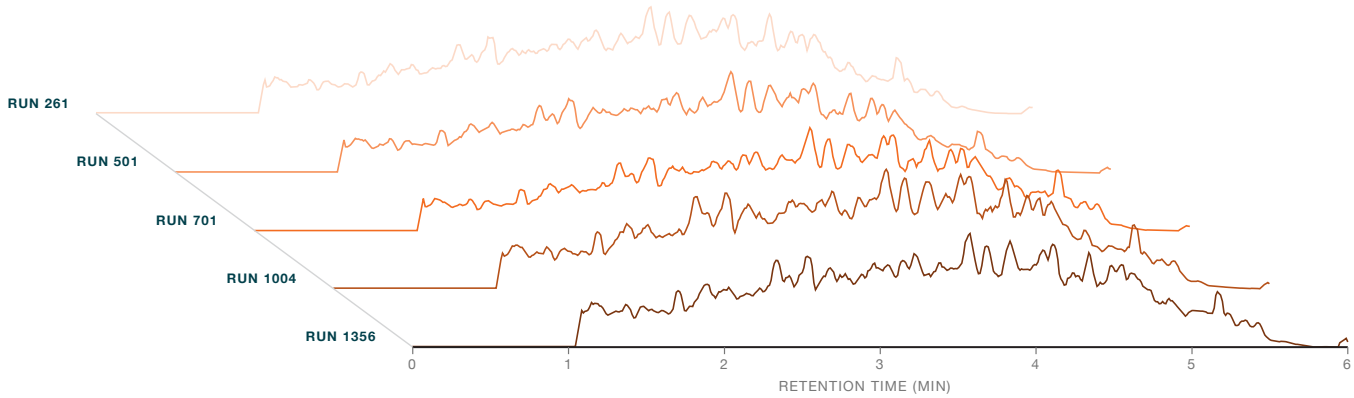
Chromatograms that overlay, run after run.

1,350+

INJECTIONS WITH UNIFORM PEAK PROFILE

Chromatographic profile stability was maintained uniformly across the entire gradient window, with stable signal intensities and retention times throughout the > 1,350 injection series.

A · TIC CHROMATOGRAM CASCADE



B · EXTRACTED ION CHROMATOGRAMS

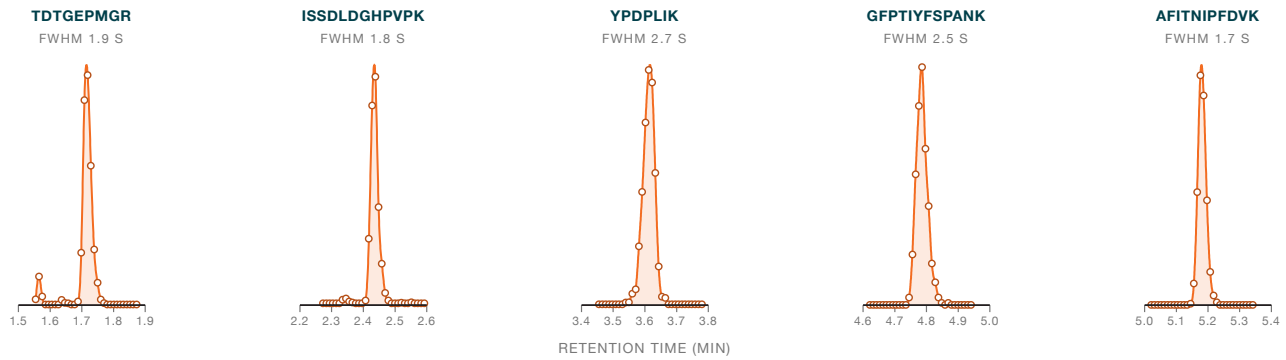


Figure 12: Robustness of chromatographic profiles and elution dynamics across the entire elution window. (A) Cascade of total ion current (TIC) chromatogram across representative runs throughout the >1,350 injection series, offset for clarity. (B) Extracted ion chromatograms of representative precursors across the elution window.

STABLE SPRAY

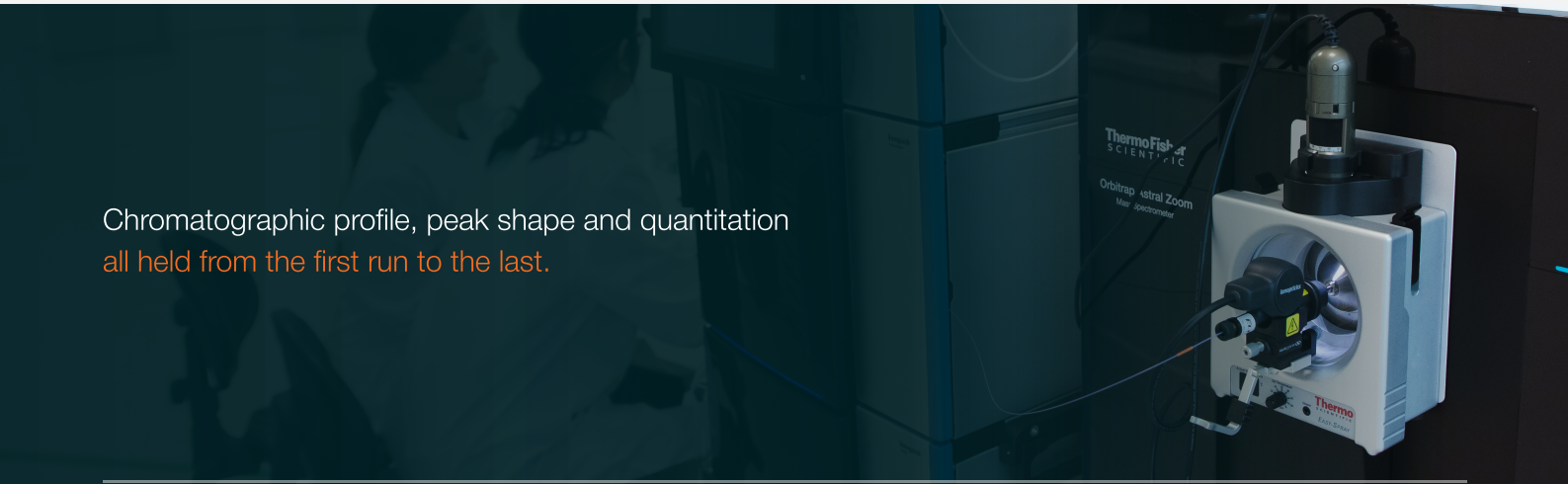
Continuous TIC traces from the first run to the last.

UNIFORM TRACES

The same profile shape and intensity from one run to the next.

PEAK SHAPE PRESERVED

Each precursor keeps its width and retention time across the series.



LC conditions.



Peptides were separated on an Aurora® Elite™ 15 cm × 150 µm column connected to the Thermo Scientific Vanquish Neo UHPLC system using a 20 µm ID nanoViper transfer line. The UHPLC was configured for a direct injection workflow. General instrument parameters, including mobile phase solvents, are described in Table 1. Recommended column settings for the Vanquish Neo A03 script are described in Table 2; specific gradient details are given in Tables 3–5.

TABLE 1 · UHPLC SETUP

Mobile phase A	0.1% formic acid (FA) in water
Mobile phase B	0.1% FA in 99.99% acetonitrile (ACN)
Gradient flow rate	2.5 µL/min
Column	Aurora® Elite™ XT 15 cm × 150 µm
Column temperature	50 °C
Autosampler temperature	7 °C
Injection wash solvents	Strong: 0.1% FA in 80% ACN Weak: 0.1% FA in water
Needle wash	Enabled, after-draw
Loading volume	Automatic
Fast loading and equilibration	Pressure control

TABLE 2 · COLUMN SETTINGS

Inner diameter	150 µm
Length	15.0 cm
Void volume	1.779 µL
Maximum pressure	1000 bar
Maximum flow	6.0 µL/min
Maximum temperature	60 °C
Max. pressure change up	1000 bar/min
Max. pressure change down	1000 bar/min

CONSUMABLES

Fisher Scientific LC-MS Optima formic acid (A117-50)
Optima LC-MS grade water (10505904)
Optima LC-MS acetonitrile (A955-1)
Optima LC-MS isopropanol (A461-212)
Thermo Scientific Pierce HeLa protein digest standard (88328)

TABLES 3–5 · GRADIENT PARAMETERS

Table 3 · 50 SPD

TIME	DUR.	FLOW	%B
0.000	0.000	2.500	1.0
0.500	0.500	2.500	3.0
22.500	22.000	2.500	29.0
22.800	0.300	2.500	32.0
23.000	0.200	2.500	85.0
24.000	1.000	2.500	85.0

Table 4 · 90 SPD

TIME	DUR.	FLOW	%B
0.000	0.000	2.500	1.0
0.300	0.300	2.500	4.0
12.900	12.600	2.500	31.0
13.000	0.100	2.500	85.0
13.650	0.650	2.500	85.0
13.700	0.050	2.500	1.0

Table 5 · 170 SPD

TIME	DUR.	FLOW	%B
0.000	0.000	2.500	1.0
0.100	0.100	2.500	5.0
5.250	5.150	2.500	33.0
5.300	0.050	2.500	85.0
5.950	0.650	2.500	85.0
6.000	0.050	2.500	1.0

Time and duration in minutes; flow rate in µL/min. Gradients were optimised for each stationary phase to ensure a fair comparison between columns.

MS parameters and data processing.



The Thermo Scientific Orbitrap Astral Zoom MS was operated in “Peptide” application mode with data acquisition parameters optimised to maximise the number of identified precursors for each gradient length. Table 6 gives the full acquisition method.

TABLE 6 · MS DATA ACQUISITION PARAMETERS

Source and method

Source voltage	1.8 kV
Inner transfer tube temperature	280 °C
Polarity	Positive
Expected LC peak width	3 s
Advanced peak determination	On
Default charge state	2
Lock mass correction	Off

Orbitrap MS1

Resolution	240,000
Enhanced dynamic range	Off
Scan range (m/z)	380–980 (50, 90 SPD) 400–800 (170 SPD)
RF lens	45%
Normalised AGC target	500%
Maximum injection time	3 ms
Data type	Profile

Astral MS2

Acquisition mode	Data-independent
Precursor mass range (m/z)	380–980 (50, 90 SPD) 400–800 (170 SPD)
Isolation window	4 m/z
Window overlap	0
Window placement optimisation	On
Collision energy	25%
Scan range (m/z)	150–2000
RF lens	45%
Pre-accumulation	On
Normalised AGC target	500%
Maximum injection time	6 / 3 / 2 ms (50 / 90 / 170 SPD)
Loop control	Time: 0.6 s

Raw data files were processed in DIA-NN Enterprise © Aptila Biotech GmbH (version 2.6.1) using a custom spectral library, generated from a library-free search of 2 and 3 hour DIA HeLa runs acquired on an IonOpticks Aurora® Frontier™ column. Raw files were processed in batches, grouped by column and gradient length, with match-between-runs disabled. Mass tolerances were 5 ppm for MS1 and 10 ppm for MS2. Quantification used Legacy (direct) mode with global normalisation enabled. Protein inference was performed at the empirical library generation step and library protein groups were used for analysis. Knowledge base functionality was not used.

SUMMARY

The Aurora® Elite™ 15 cm × 150 µm column delivers superior separation efficiency, durability and quantitative performance when compared to the EASY-Spray ES906 column. Narrower chromatographic peaks give up to a 50% increase in peak intensity and increased peak capacity for any given gradient length, yielding up to 45% more peptide and 13.3% more protein identifications with greater quantitative precision at equivalent sample inputs.

At constant run times, the same results are achieved from down to a quarter of the sample material. Backed by an extended lifetime study at 170 SPD, the column maintains that performance over more than 1,350 high-pressure injection cycles, consistently exceeding 9,400 protein groups per run. The Aurora® Elite™ architecture successfully eliminates the trade-off between throughput, sensitivity, and column lifespan.

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