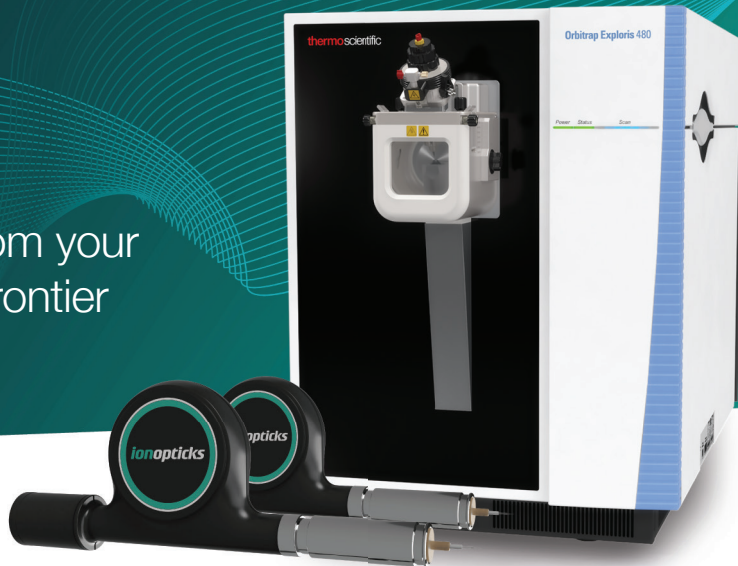


# Maximizing protein identifications from your Orbitrap Exploris 480 with Aurora Frontier UHPLC columns



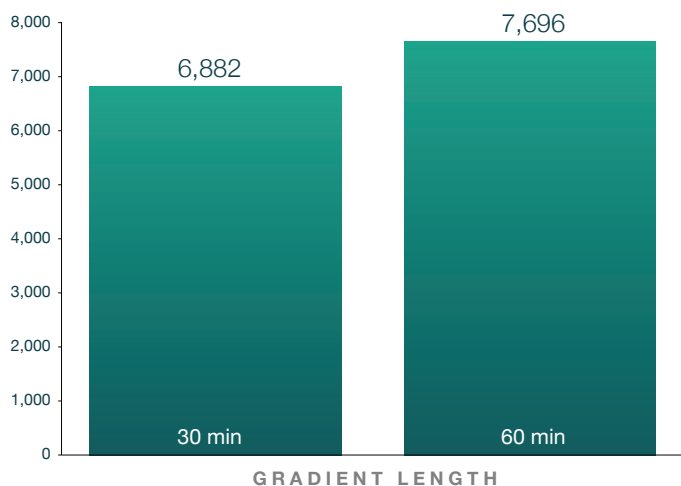
## INTRODUCTION

IonOpticks' Aurora Series packed emitter columns feature our revolutionary nanoZero® fitting, allowing for simplified plug-and-play high-throughput proteomics. When coupled with cutting-edge mass spectrometry system, Aurora Series columns demonstrate exceptional performance. In particular, the combination between IonOpticks' Aurora Frontier 60 cm x 75 µm XT columns and Thermo Fisher Scientific's Orbitrap Exploris 480 mass spectrometer creates a powerful analytical platform, enabling in-depth analysis in proteomics research. To demonstrate the capabilities of this system, experiments were conducted using 3 replicates across different gradient lengths (30 min and 60 min), allowing for an evaluation of the column's performance under various analytical conditions.

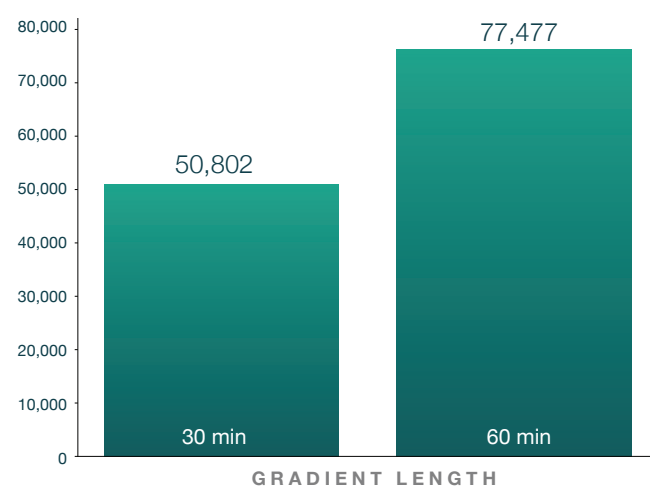
## Maximize identifications of peptides and proteins

IonOpticks' Aurora Frontier 60 cm x 75 µm XT columns offer exceptional performance in proteomics applications. These results demonstrate the advantages of using longer columns that deliver high-resolution separations, allowing researchers to gain deeper proteome coverage in their studies.

**(A) UNIQUE PROTEIN GROUPS**



**(B) UNIQUE PEPTIDES**



**Figure 1:** (A) Average protein group identifications across different gradients. (B) Average unique peptide identifications across different gradients. A HeLa tryptic digest (200 ng) was separated on Aurora Frontier 60 cm x 75 µm XT column. 3 replicates were run per condition. Samples were run on Thermo Fisher Scientific's Vanquish Neo LC and Orbitrap Exploris 480 mass spectrometer with FAIMS Pro Duo. FAIMS CVs of -45V for 30 min gradient and -45/-65V for 60 min gradient were used. Data analysis was performed using Spectronaut 18 (Biognosys AG).

More than  
**50k**  
unique peptides  
using a 30 min gradient

More than  
**77k**  
unique peptides  
using a 60 min gradient

  
**3.0**secs  
Full-width half-max  
using a 30 min gradient

  
**5.5**secs  
Full-width half-max  
using a 30 min gradient



## FEATURED PRODUCT

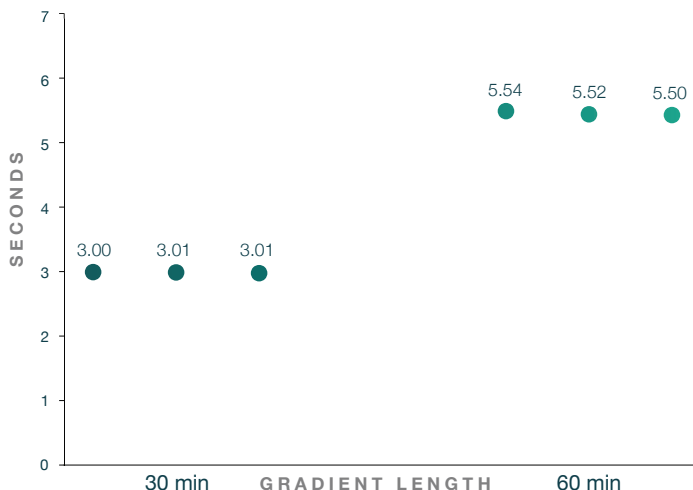
**Aurora Frontier 60x75 XT packed emitter column for EASY-Spray and Nanospray Flex**  
(60 cm x 75µm ID, 1.7µm C18) Part No. AUR3-60075C18A-XT

## Narrow peak widths

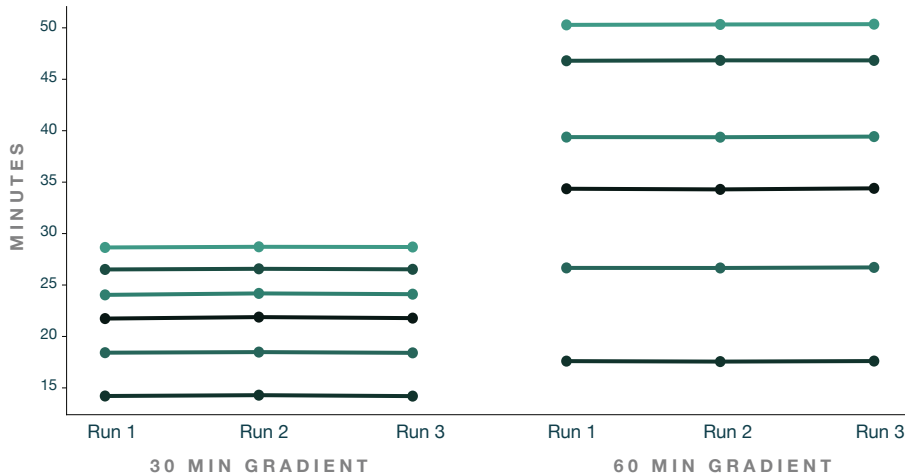
The Aurora Frontier 60 cm x 75 µm XT column shows exceptional chromatographic performance by delivering narrow peak widths across varying gradient times. Peak widths (FWHM) average 3.0 seconds for 30 minute gradients and 5.5 seconds for 60 minute gradients.

**Figure 2:** Average full width at half maximum (FWHM) for all identified peptides from HeLa Tryptic Digest injections (200 ng) on an Aurora Frontier 60 cm x 75 µm XT column. 3 replicates were run per condition. Each point represents the average FWHM for an individual replicate. Samples were run on Thermo Fisher Scientific's Vanquish Neo LC and Orbitrap Exploris 480 mass spectrometer with FAIMS Pro Duo. FAIMS CVs of -45V for 30 min gradient and -45/-65V for 60 min gradient were used. Data analysis was performed using Spectronaut 18 (Biognosys AG).

**FIG 2 AVERAGE FULL-WIDTH HALF-MAX**



**FIG 3 RETENTION TIME FOR SELECTED PEPTIDES**



## Stable retention times ensure confidence in results

The Aurora Frontier columns consistently show exceptional retention time stability over multiple runs for both 30 minute and 60 minute gradients. This high level of reproducibility is crucial in proteomics, as it ensures dependable peptide identification across various experimental conditions.

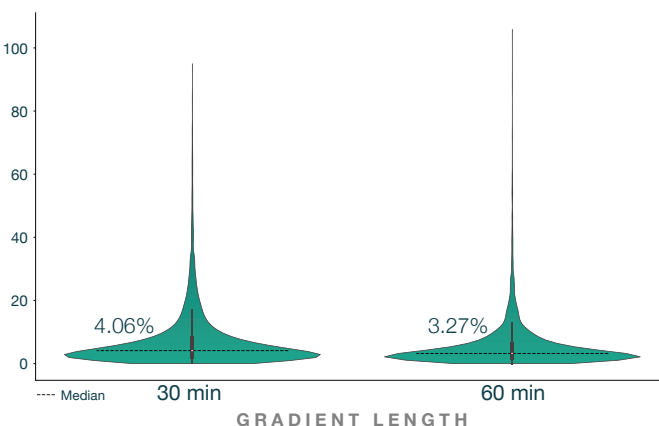
**Figure 3:** Stable peptide retention times across all runs. 6 peptides were selected and their retention time assessed across all runs from HeLa Tryptic Digest injections (200 ng) on an Aurora Frontier 60 cm x 75 µm XT column. Samples were run on Thermo Fisher Scientific's Vanquish Neo LC and Orbitrap Exploris 480 mass spectrometer with FAIMS Pro Duo. FAIMS CVs of -45V for 30 min gradient and -45/-65V for 60 min gradient were used. Data analysis was performed using DIA-NN software (Version 1.9).

## Low CVs

Analyzing protein intensity reproducibility using the coefficient of variation (CV) reveals the exceptional consistency of the Aurora Frontier columns across various gradient lengths. Both 30 minute and 60 minute gradients shows low median CV values: 4.06% and 3.27% respectively. These outcomes highlight the robustness of the Aurora Frontier columns in delivering consistent performance.

**Figure 4: Low CVs across different gradients.** Violin plot of the coefficient of variation for all protein intensities identified across all replicates from HeLa Tryptic Digest injections (200 ng) on an Aurora Frontier 60 cm x 75 µm XT column. 3 replicates were run per condition. Samples were run on Thermo Fisher Scientific's Vanquish Neo LC and Orbitrap Exploris 480 mass spectrometer with FAIMS Pro Duo. FAIMS CVs of -45V for 30 min gradient and -45/-65V for 60 min gradient were used. Data analysis was performed using Spectronaut 18 (Biognosys AG).

**FIG 4 COEFFICIENT OF VARIATION (CV %)**



## CONCLUSION

The integration of IonOpticks' Aurora Series columns with Thermo Fisher Scientific's Vanquish Neo LC and Orbitrap Exploris 480 mass spectrometer achieves maximum protein and peptide identifications. This powerful combination enhances analytical capabilities, providing robust and precise results in proteomics research. By uniting these advanced technologies, researchers gain a reliable and precise platform for conducting cutting-edge proteomics studies, unlocking deeper insights into complex samples.