

A Novel Nanoflow LC-MS Platform for the Robust Identification of HLA Peptides from High Value Clinical Samples

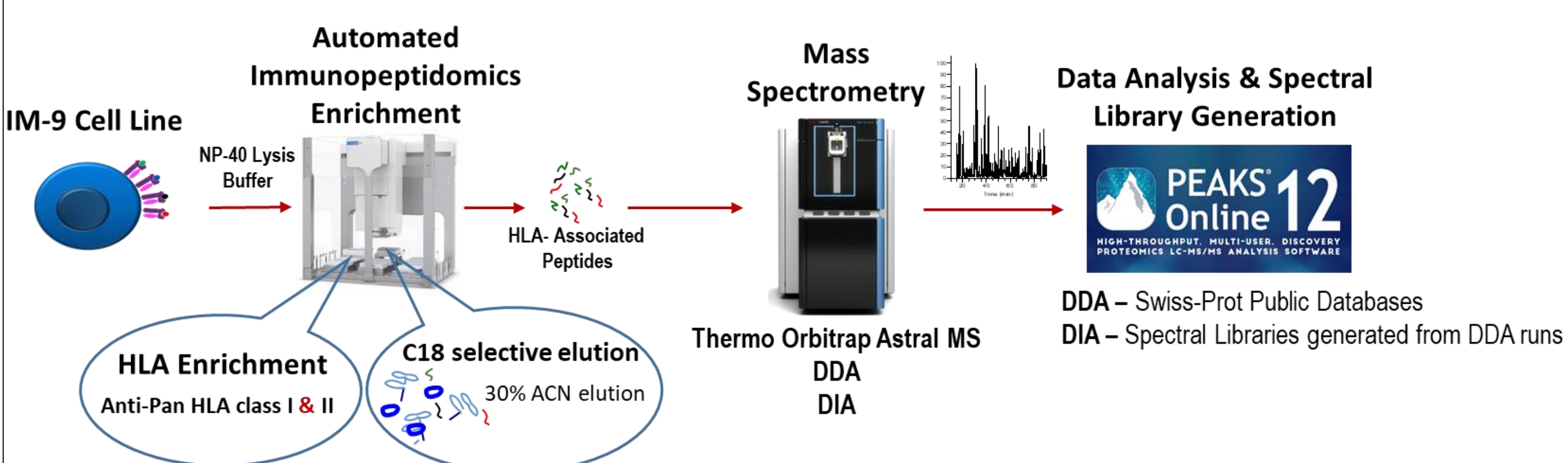
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Abstract

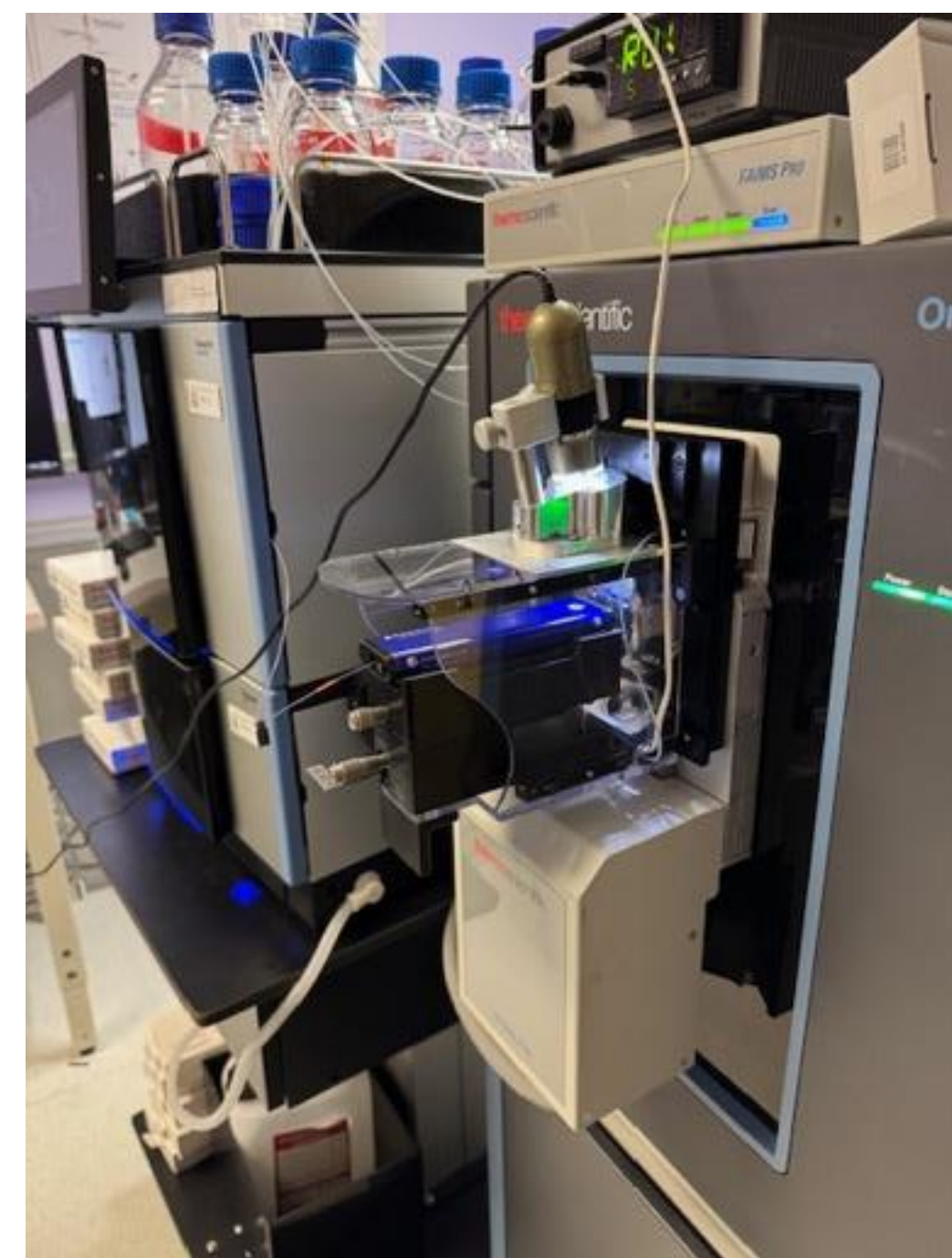
Cancer immunotherapy is a significant and effective treatment option for various tumor types. High sensitivity nanobore LC-MS plays a fundamental role in the process of recognizing human leukocyte antigen (HLA) peptides used in the development of immunotherapy treatment. Application to high-value, clinically relevant samples, has been limited by the robustness of sensitive nanobore LC methodology. Here we present the development and optimization of novel nanobore LC methodology for analyzing HLA Class I and HLA Class II targets using a comprehensive integrated platform: The PicoChip® enabled Universal Flow Station (UFS™) from New Objective on the Thermo Astral™ mass spectrometer. The newly developed methods are capable of screening small biopsy sample sizes (10-30 mg) while providing a high level of reproducibility and accuracy.

Methods



Methods

Samples were prepared using an optimized workflow involving gentle lysis, enrichment by antibody affinity purification, and clean-up by C18 SPE. An LC-MS framework using a microscale trap and elute UHPLC strategy (Vanquish Neo, Thermo Scientific) combined with a novel integrated and heated (50 C), UHPLC (1.9 μm C18) chip based column-emitter (15 μm emitter x 75 μm x 25 cm; PicoChip New Objective, Inc.) platform (Universal Flow Station, New Objective, Inc) in tandem with FAIMS, on the Orbitrap Astral Mass Spectrometer (Thermo Scientific). Sample separation was achieved by 60 min., 2-35% ACN gradient at 300 nl/min. Resulting proteomics data was processed using PEAKS Online Proteomics analysis software (Bioinformatics Solutions Inc).



PicoChip/UFS™ on FAIMS equipped Astral

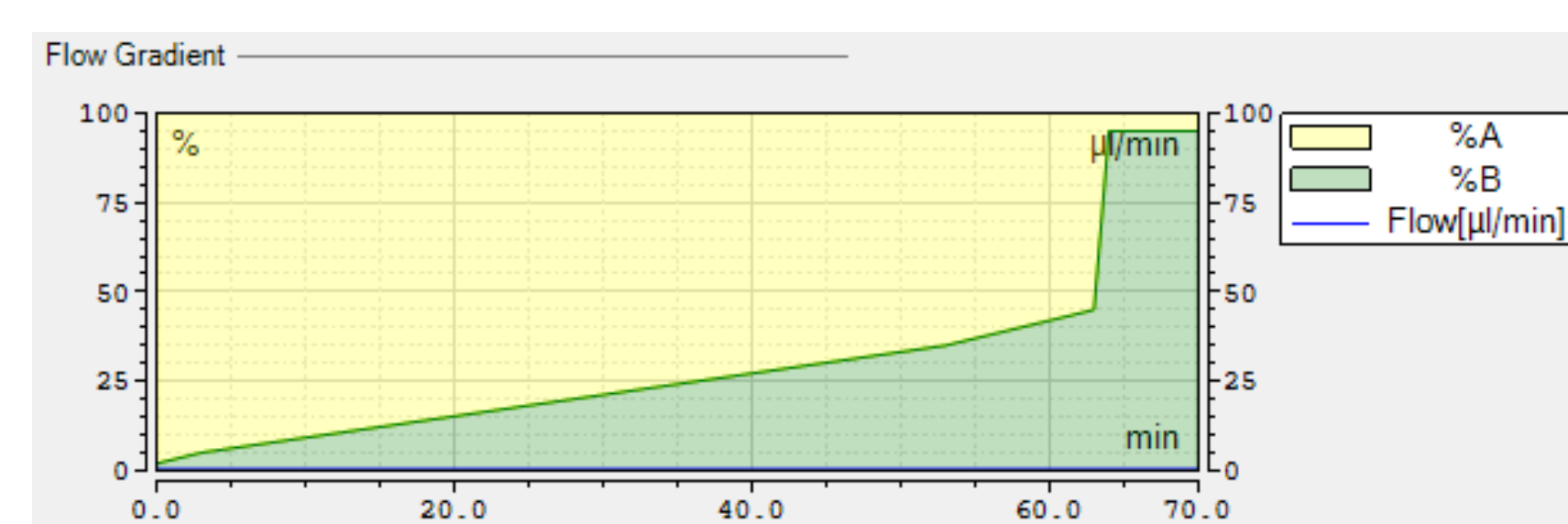
LC: Vanquish Neo UHPLC System

Trap Column: Thermo Scientific Acclaim PepMap 100 15cm x 75 μm – C18 Trap

Column: New Objective Picochip® 25cm x 75 μm x 1.9 μm – C18 column

Column Temp: Heated to 50°C

Mobile Phase: A: 0.1% Formic Acid in Water,
B: 0.1% Formic Acid in 80% Acetonitrile
Gradient Flow Rate: 300 nl/min
Gradient Profile: 2% to 35% B in 60 min



Detail of Gradient Profile

Workflow Information

Trap-and-Elute Injection
Nanol/Cap (20 μm ID, < 5 μl/min)

Separation Column(s) Specifications

Property	Value
Inner Diameter:	75 [μm]
Length:	25.0 [cm]
Void Volume:	0.740 [μl]
Maximum Pressure:	600 [bar]
Maximum Flow:	0.5 [μl/min]
Maximum Temperature:	65.0 [°C]
Maximum Pressure Change Up:	600 [bar/min]
Maximum Pressure Change Down:	600 [bar/min]
Supports Backward Flush:	No

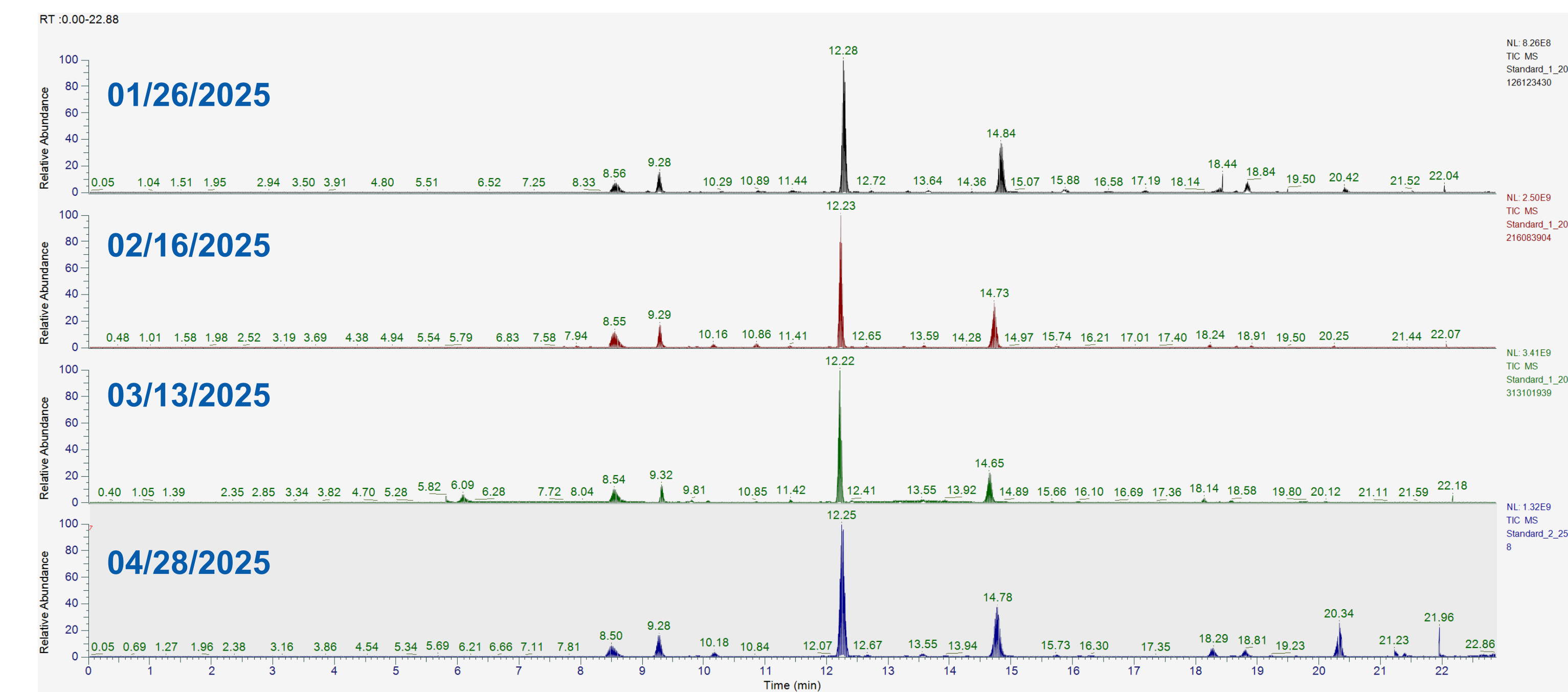
Trap Column(s) Specifications

Property	Value
Inner Diameter:	75 [μm]
Length:	15.0 [cm]
Void Volume:	0.444 [μl]
Maximum Pressure:	600 [bar]
Maximum Flow:	200.0 [μl/min]
Maximum Temperature:	60.0 [°C]
Maximum Pressure Change Up:	1000 [bar/min]
Maximum Pressure Change Down:	1000 [bar/min]
Supports Backward Flush:	No

Detail of Trap-Elute Injection

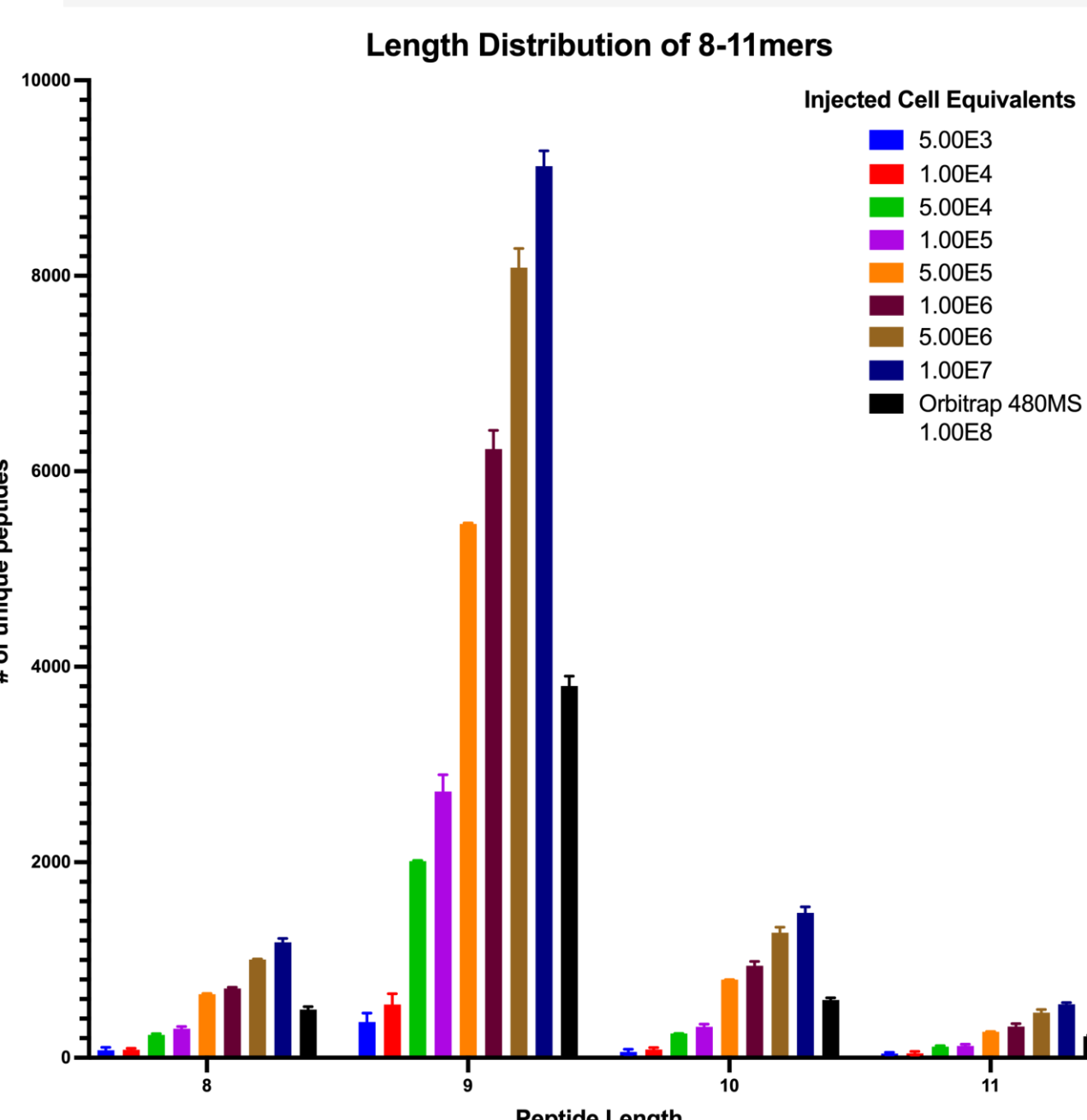
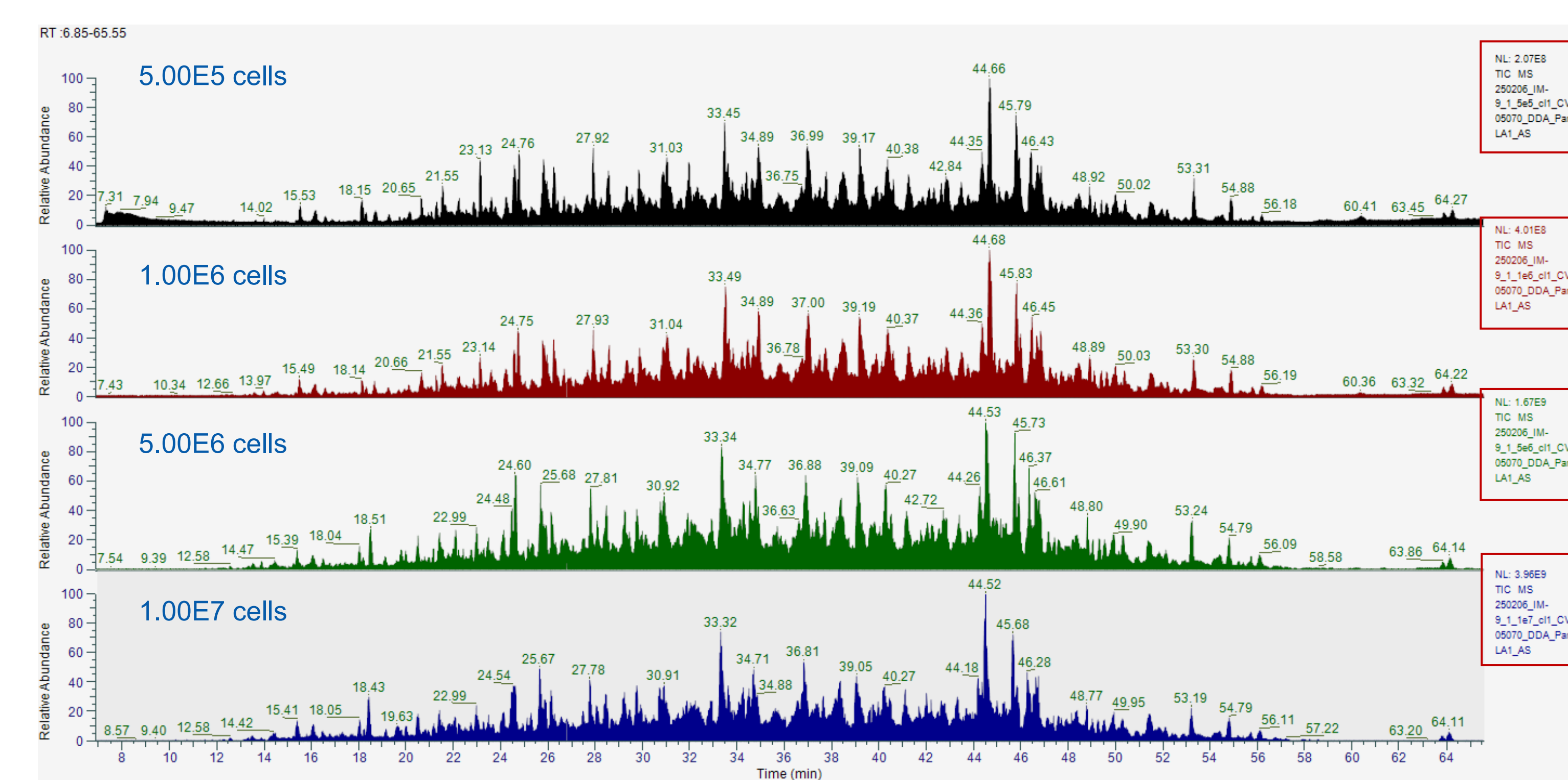
Consistent Chromatography for Quality Assurance

Peptide Standard mix shows consistency across several months and different columns



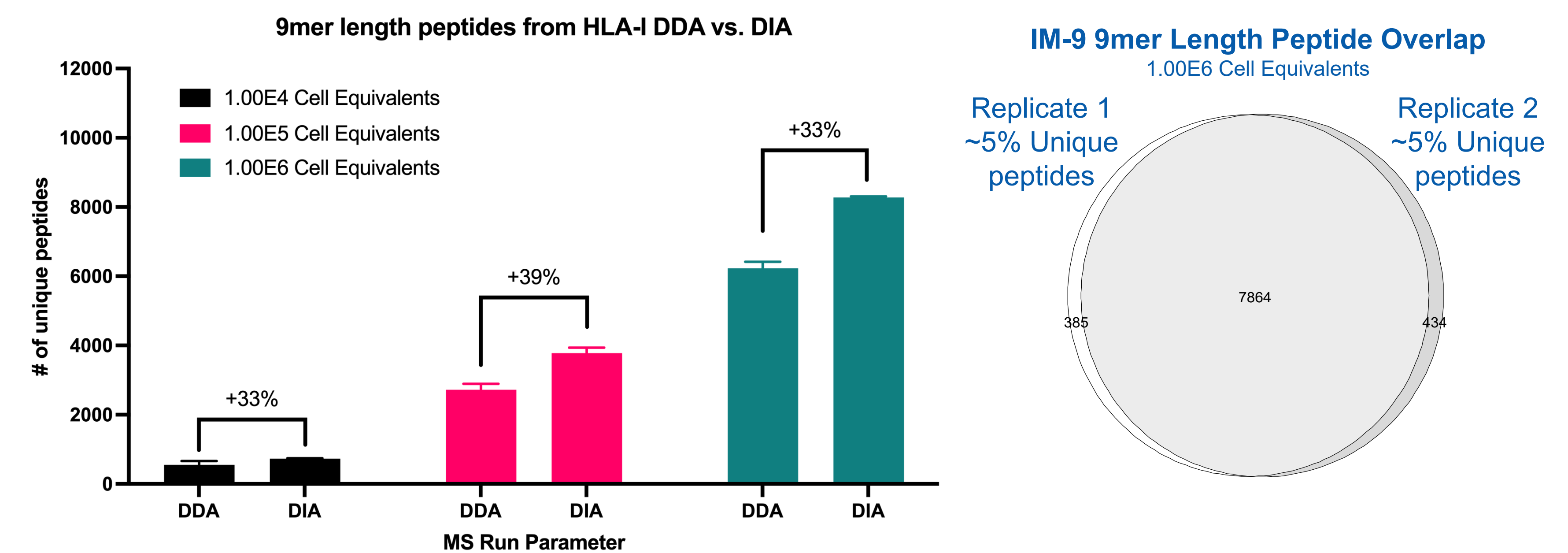
Peptide standard validation runs across 3-month time span. Highly reproducible peak shape and retention time from different PicoChip™ columns.

Integrated System Yields Robust Sensitivity

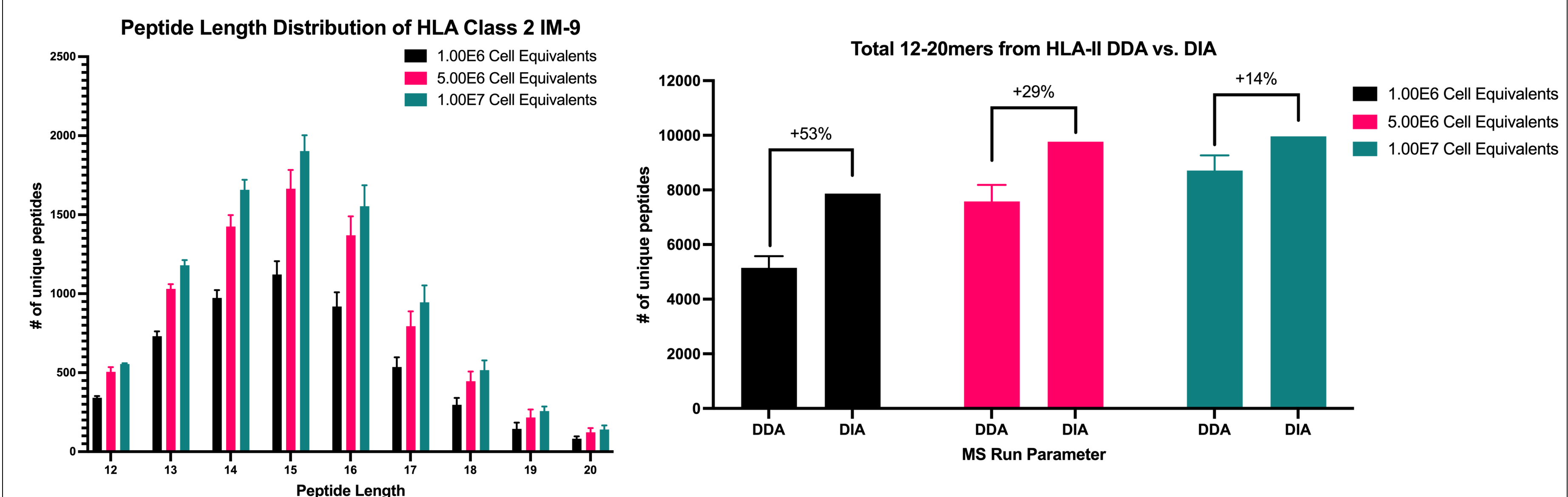


Technical replicate injections of IM-9 enriched immunopeptides from as low as 5.00E3 cell equivalents still showed an optimal peptide length distribution. Increasing injection amounts shows a steady increase in identified 9mer length peptides from 5.00E3 – 1.00E7 cell equivalents.

Consistent Performance Across DDA and DIA Workflows



There are more peptides identified from the DIA mass spec runs compared to the DDA runs of the sample samples. ~30-40% increase in peptide IDs. Additionally, we are observing a much greater similarity in unique peptide IDs from low sample amounts with ~95% peptide similarity between DIA mass spec runs of 1.00E6 Cell equivalent injections



HLA class II immunopeptidomics show very consistent results from both DDA and DIA immunopeptidomics. Additionally, we are identifying more peptides from DIA runs as expected with high reproducibility between replicate runs (~85-90% similarity, data not shown)

Summary

- Integrated nano-LC/MS platform enhances performance of latest generation mass spectrometry
- Highly reproducible chromatographic performance demonstrated across multiple columns
- Long term column-to-column consistency enables ready transition from established MS platforms to new MS platforms
- Existing foundational data, using the identical column and source framework, facilitated rapid system qualification and deployment to the Astral.
- Relevant peptide ID's increased by more than 2-fold, while reducing sample size by an order of magnitude
- Gradient times were reduced to 70 min (Astral) vs 137 min (Exploris 480) due to increased MS acquisition speed
- An increase of peptide ID's of between 30 to 40% was observed for DIA vs DDA acquisition
- Sample size could be reduced an order of magnitude (1E7 vs 1E6 cells) with a concomitant decrease in peptide IDs of only 30%
- Demonstrated high reproducibility of HLA Class II analytes across DIA replicate runs (85-90% similarity)