

Speeding up Biomarker Discovery: Next Generation Technology for Reproducible and Precise Proteome Profiling

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Diseases like cancer are complex and require another level of discipline in analytical proteomics measurements. Having set the standards for discovery research with deep comprehensive proteome profiles, new advances in Orbitrap technology and workflows allow for large scale analysis across large sample numbers.

Here we present a highly robust novel capillary-flow LC-MS platform that combines a capillary-flow chromatographic system (capLC), a new 150 μm column and a new Thermo Scientific™ Q Exactive™ HF-X mass spectrometer. The Q Exactive HF-X encompasses a high capacity transfer tube (HCTT), an electrodynamic ion funnel for increased ion flux and a scan rate of 40 Hz.

The new capLC-MS platform performances were assessed for data dependent acquisition (DDA) label free quantification high-resolution accurate-mass (HRAM) proteomics experiments demonstrating high precision and reproducibility for the analysis of complex samples including bio-fluids. The DDA-based label free quantification workflow using the Proteome Discoverer™ 2.2 software leads to near complete (97%) peptide and protein quantification where 97% of the peptides quantified have a CV less than 20% displaying the high precision.

A novel high resolution MS1 based data independent acquisition (DIA) method was also developed for comprehensive proteome profiling. The capLC-MS platform identifies and quantifies more than 4800 protein groups and 46000 peptide precursors in a 1-hour total run time (1% FDR and median CV<10%) delivering the analytical reproducibility and scalability required for rigorous results that matter.

The new Thermo Scientific™ Q Exactive™ HF-X mass spectrometer allow for the identification of the same number of proteins in half the time or 40% more peptide identifications using the same time as compared to the Thermo Scientific™ Q Exactive™ HF mass spectrometer.

The use of capillary LC rather than nano LC allows more robustness, 2x shorter total run time meaning greater throughput with only a minor loss of the total number of identified peptides and proteins and with a similar level of quantification to nano LC.