Optimized plasma proteomics pipeline from sample to insight for large-scale biomarker discovery

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INTRODUCTION

Blood plasma is a highly valuable biofluid for protein biomarker discovery, but meaningful analysis is often hindered by its wide dynamic range, variability in instrument performance, and the complexity of the resulting datasets.

To overcome these challenges, we present a workflow that integrates high-throughput, automated enrichment of low-abundance plasma proteins (P2-iST Plasma) with a high-performance chromatography solution (PepSep Advanced) and a state-of-the-art DIA analysis platform (Spectronaut) that delivers high identification depth, robust quantification, and excellent data completeness across large cohorts.

Together, these advanced solutions enable more reliable and comprehensive plasma proteomics.

METHODS

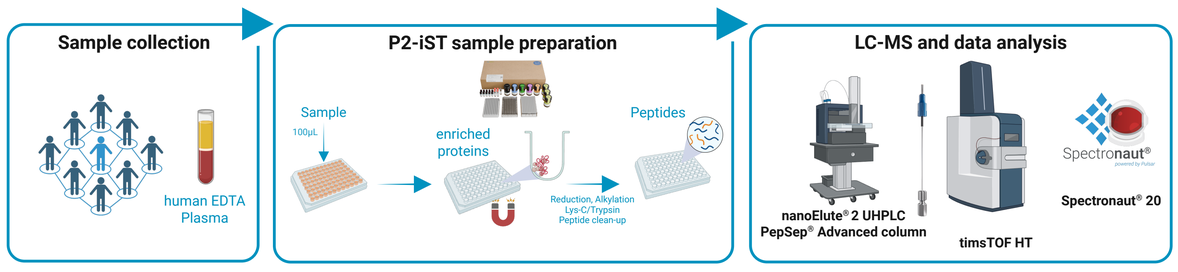
Input:
Human EDTA blood plasma, collected and pooled from 2 healthy donors by DiaServe Laboratories GmbH (centrifugation at 2000 × *g*).

Sample preparation:
Multiple replicates of 100 µL plasma were processed using the P2–iST Plasma Kit (Biognosys Group) according procedure.

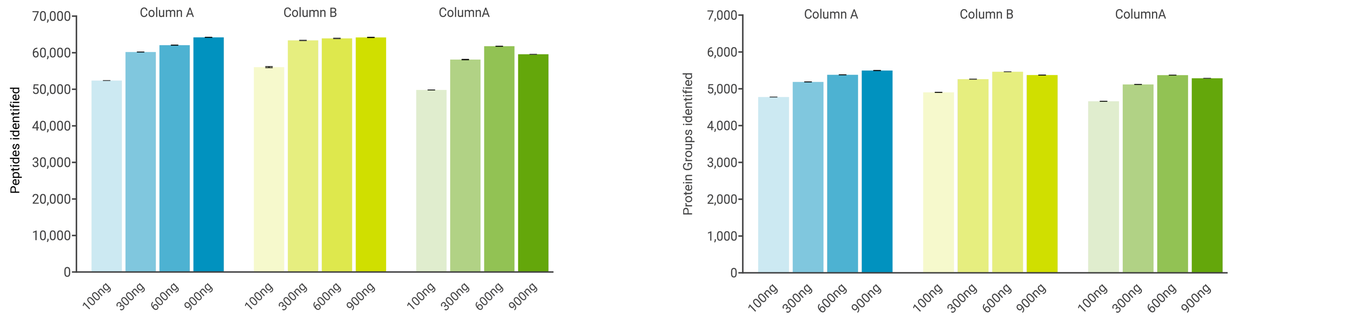
LC-MS analysis:
A dilution from 900 to 100 ng of pooled peptides loaded on a nanoElute® 2 (Bruker) equipped with either a 25 cm PepSep® Advanced column (Bruker; Column A) combined with a PepSep® CaptiveSpray 2 emitter (10 µm) or a 25 cm integrated-emitter column (competitor; Column B). Data were acquired on a timsTOF HT (Bruker) operated in DIA-PASEF® mode. Injection triplicates were analyzed for each column.

Data processing:
Data was processed using Spectronaut® 20 software with directDIA+™ (Biognosys). For visualization, the search-engine-agnostic visualization tool Proteo-Vision™ and GraphPad Prism were used.

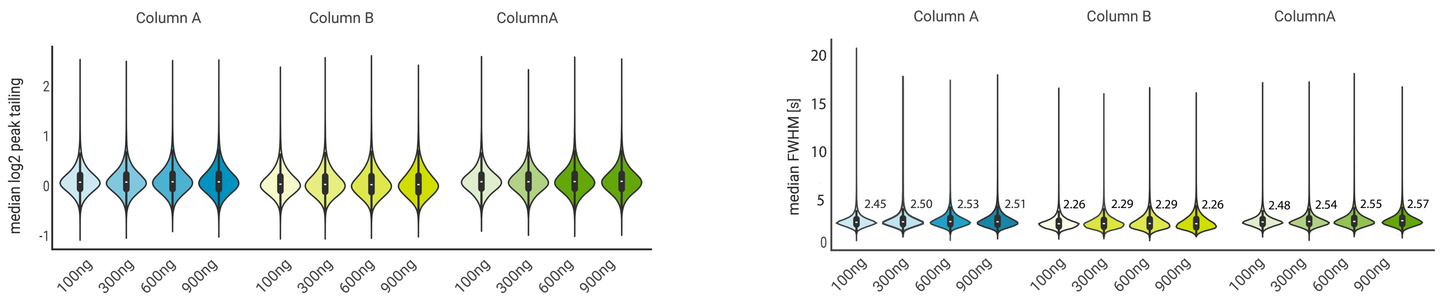
RESULTS



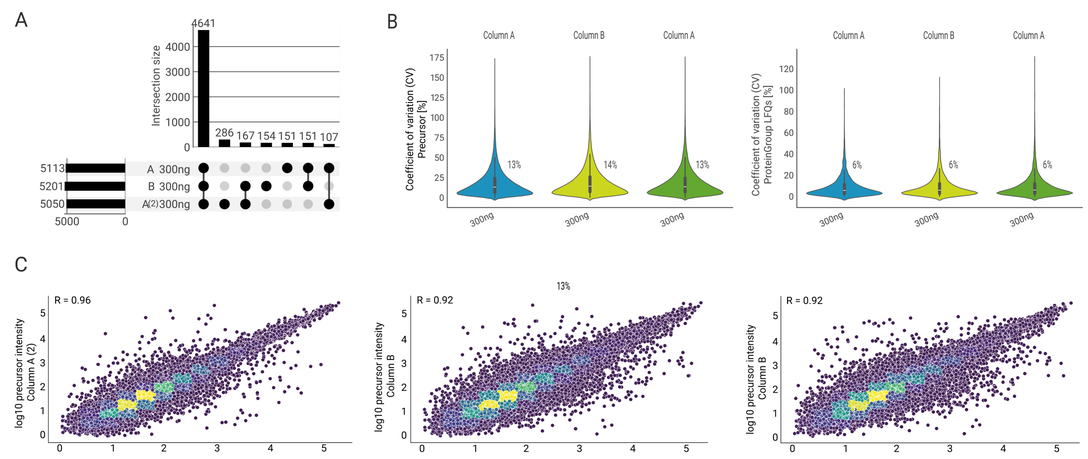
P2-iST Plasma workflow overview for a complete deep proteomic analysis of blood plasma, from sample collection to data visualization.



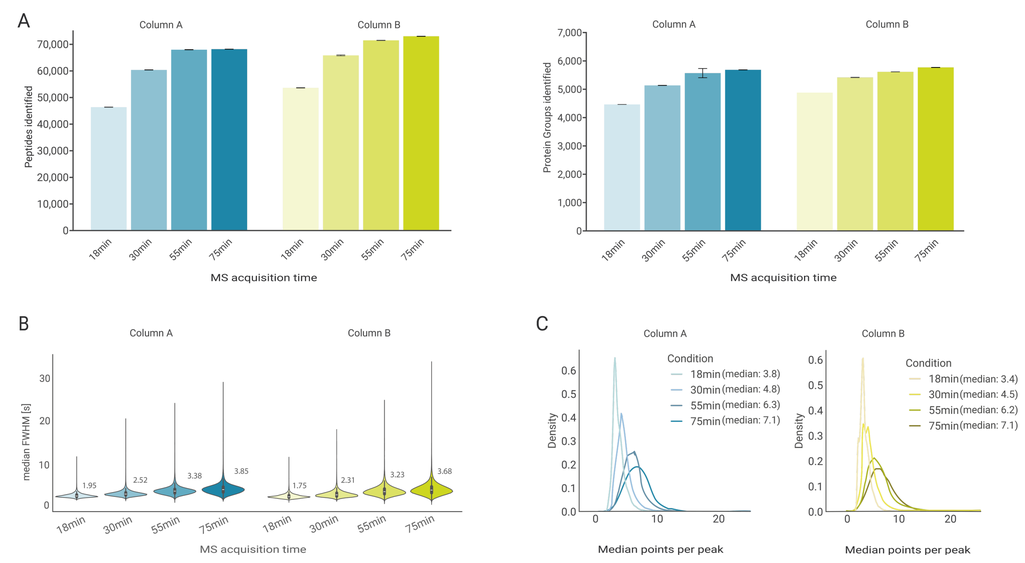
Comparison of peptide and protein identifications at different injection amounts. Enriched plasma peptides were injected at increasing amounts across three independent LC–MS sessions: first on a 25 cm PepSep Advanced column (Column A), followed by analysis on a 25 cm integrated-emitter column (Column B), and finally again on Column A. The PepSep Advanced column showed consistent performance across runs and achieved identification numbers comparable to the integrated-emitter column.



Comparison of peak width and peak shape. Peak sharpness on the PepSep Advanced column (Column A) was compared with the integrated-emitter column (Column B) across all injection amounts and showed very similar performance. Both column types exhibited symmetrical peak shapes (tailing factors), supporting good chromatographic resolution, accurate peak area determination, and reliable quantification.



Protein overlap and quantitative comparison at 300 ng injection. The PepSep Advanced column (Column A) showed strong overlap in protein identifications with the integrated-emitter column (Column B). Quantification precision, assessed by the coefficient of variation (CV), was equally low for both columns. Precursor intensities showed high concordance between the two column types, comparable to replicate injections on the same column.



Versatility of the PepSep Advanced column across different gradient lengths. The novel PepSep Advanced column enabled deep plasma proteome coverage while maintaining excellent performance across a wide range of gradient lengths, from 75 min down to just 18 min of MS acquisition time.

KEY TAKEAWAYS

P2-iST Plasma enables deep plasma proteome coverage
The P2-iST Plasma kit provides access to thousands of low-abundance plasma proteins from just 100 µL sample input using a streamlined workflow that can be performed manually or automated within a single day.

PepSep® Advanced UHPLC columns deliver high-performance separations
The separate column and emitter configuration boosts identifications and improves peak shape while maintaining strong quantitative performance and signal concordance. Broad gradient compatibility supports both high-throughput and deep-profiling workflows.

This integrated toolkit delivers a streamlined, end-to-end plasma proteomics workflow
By leveraging advanced separation columns, nanoLC systems, mass spectrometry, and data analysis software, a robust pipeline is established from sample preparation to actionable biological insights.

CONTACT & MORE

PepSep®

P2-iST Plasma

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Conflict of Interest Disclosure
Grey, M. is employed by PreOmics Inc.
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