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DUAL-COLUMN ZenoTOF CONFIGURATION TO ACHIEVE ROBUST AND HIGH-QUALITY PLASMA PROTEOMICS

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INTRODUCTION

- Liquid chromatography/mass spectrometry (LC/MS)-based plasma proteomics is recognized as a promising tool for clinical and biomarker discovery research
- To enable large-scale plasma biomarker discovery studies, it is critical to have a robust, reproducible, and high-throughput LC/MS assay
- Increased throughput enables larger-scale studies in less time, resulting in increased statistical power and the identification of robust biomarkers
- To enable higher-throughput studies, we have created a dual-column LC configuration coupled with a ZenoTOF that provided a 20-25% increase in throughput

OBJECTIVE

- Test and validate the design of dual-column LC configuration as compared with the default single-column LC configuration
- Examine the influence of increasing flowrate on the performance of untargeted/targeted MS methods
- Systematically compare the difference between single- and dual-columns with the same lot number

RESULTS

TABLE 1. Void volumes for single- and dual-column configurations.

LC configuration	Total void volume	Length of LC method	Injection per day
Single-column, 5 µl/min	22.9 µL	30 min/run	48 injections/day
Dual-column, 5 µl/min	24.1 µL	24 min/run	60 injections/day
Dual-column, 8 µl/min[a]	24.1 µL	22 min/run	65 injections/day

[a]Theoretically, the LC method for 8 µl/min can be optimized to 22 min due to the higher linear velocity resulting in earlier peptide elution times.

FIGURE 2. Dual-column configuration provided increased throughput versus a single-column configuration.

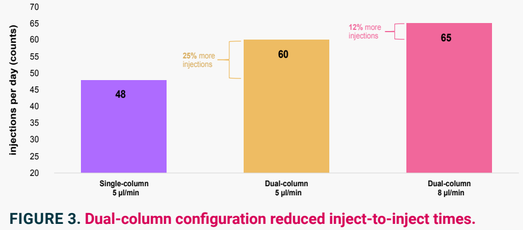
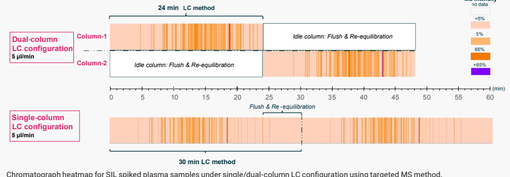
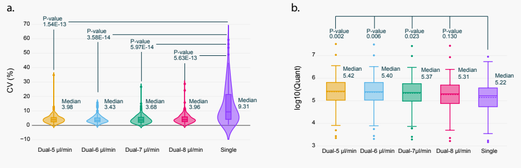


FIGURE 3. Dual-column configuration reduced inject-to-inject times.



Chromatograph heatmap for SIL spiked plasma samples under single/dual-column LC configuration using targeted MS method.
LC, liquid chromatography; MS, mass spectrometry.

FIGURE 4. Dual-column configuration improved reproducibility due to increased column re-equilibration.



*Data of dual column-1 was used.
(a) Comparison of the Peak Area CV's for 129 targeted analytes in different LC conditions; (b) The IQR for log10(Peak Area) of 129 targeted analytes was not statistically different between all LC configurations.
CV, coefficient of variance; IQR, interquartile range; LC, liquid chromatography.

- Interquartile range values for both dual- and single-column configurations were not meaningfully different; however, the coefficients of variance were significantly improved in the dual-column configuration

FIGURE 5. Dual-column configuration increased sensitivity at multiple flow rates.

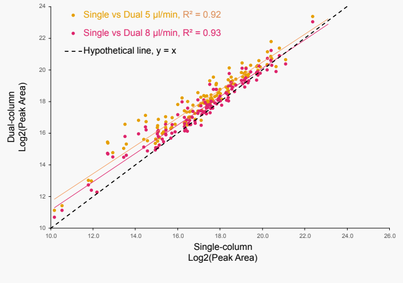
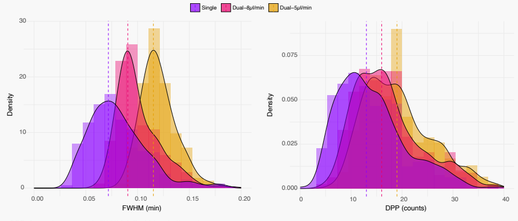


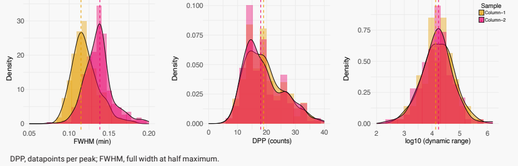
FIGURE 6. Dual-column configuration resulted in higher full width at half maximum (FWHM) and data points per peak (DPP).



DPP, datapoints per peak; FWHM, full width at half maximum.

- Increased flow rates in the dual-column configuration helped to reduce the increase in FWHM

FIGURE 7. No significant differences were observed between columns in a dual-column configuration.

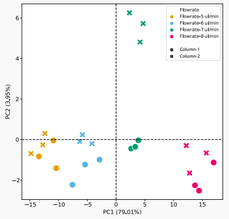


DPP, datapoints per peak; FWHM, full width at half maximum.

- Metrics impacting quantitation (DPP & Dynamic range) were not meaningfully different between 2 columns at 5 µl/min, yet there was an observed difference in FWHM between the 2 columns
- It is important to note that the difference in FWHM between columns is ~1.5 sec (6.75 vs 8.25 sec), which could result from small column manufacturing or flow path differences (1.5 sec = 125 nL at 5 µl/min)

FIGURE 8. Statistical analysis suggested minor differences between columns in a dual-column system.

a.



b.

Kendall's Tau Correlation log2(Peak Area)

	Column-1 5 µl/min	Column-1 6 µl/min	Column-1 7 µl/min	Column-1 8 µl/min	Column-2 5 µl/min	Column-2 6 µl/min	Column-2 7 µl/min	Column-2 8 µl/min
Column-1 5 µl/min	1	0.97	0.97	0.96	0.98	0.98	0.95	0.96
Column-1 6 µl/min	0.97	1	0.98	0.97	0.97	0.98	0.96	0.97
Column-1 7 µl/min	0.97	0.98	1	0.96	0.97	0.98	0.96	0.96
Column-1 8 µl/min	0.96	0.97	0.96	1	0.96	0.96	0.94	0.96
Column-2 5 µl/min	0.98	0.97	0.97	0.96	1	0.98	0.96	0.96
Column-2 6 µl/min	0.98	0.98	0.98	0.96	0.98	1	0.96	0.97
Column-2 7 µl/min	0.95	0.96	0.96	0.94	0.96	0.96	1	0.95
Column-2 8 µl/min	0.96	0.97	0.96	0.96	0.96	0.97	0.95	1

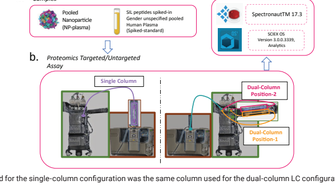
(a) The PCA analysis of all 129 targeted analytes; (b) Kendall's Tau correlation of peak area of 129 targeted analytes between 2 columns and all flow rates.

- PCA highlighted differences between different flow rates (PC1) but not between columns (PC2) (**Figure 8a**)
- Kendall Tau analysis highlighted that differences between flow rates and columns do not have a meaningful impact on quantitation (Peak Area) (**Figure 8b**)

METHODS

- Sample Preparation (**Figure 1a**):
  - Nanoparticle (NP)-plasma samples from 40 clincial subjects were processed by Seer Proteograph™ with Early Access of Proteograph XT Assay kit. NP specific samples were pooled and prepared as 100 ng/µL
  - Neat Plasma Stable Isotope Labeled (SIL) Spiked (K2EDTA filtered plasma digest samples) were spiked with 118 SIL peptides, where the concentration of SIL peptides was 13.5 fmol/µL and the final concentration of neat plasma was 192 ng/µL
- LC/MS (**Figure 1b**):
  - Waters M-Class LC + Sciex ZenoTOF 7600 instrument configuration was used to conduct the entire experiment
  - A 24 min total LC run time (22 min effective gradient) was used for the dual-column configuration while a 30 min total LC run time (22 min effective gradient) was designed for the single-column configuration
  - Both configurations had calculated void volumes shown in **Table 1** and were tested at different flowrates with the same LC method
  - Data-independent acquisition (DIA) and high-resolution multiple reaction monitoring (MRM-HR) acquisition methods were used for untargeted and targeted experiments, respectively. 129 SIL peptides were spiked into neat plasma and utilized to collect MRM-HR data
- Data analysis (**Figure 1c**):
  - MRM-HR data were analyzed in SCIEX OS 3.0.0; MS2 Quant data were presented

FIGURE 1. Overall experimental workflow.



The column used for the single-column configuration was the same column used for the dual-column LC configuration at position-1.

CONCLUSIONS

- We demonstrated a ~25% increase in sample throughput on a single MS system when the LC was in a dual-column configuration; theoretically, this increase in throughput could extend to 35%
- The increased column equilibration time in a dual-column configuration provided improved peak area coefficients of variance
- Both columns in a dual-column configuration provided similar analytical figures of merit (eg, FWHM, DPP, Quantitation, etc.)

DISCLOSURES
Study funded by PrognomiQ, Inc. All authors are current or former employees of PrognomiQ, Inc.

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