Multi-site assessment of precision and reproducibility of high-throughput capillary-flow LC-MS proteome profiling with novel ultra-high-pressure LC coupled to HRAM MS

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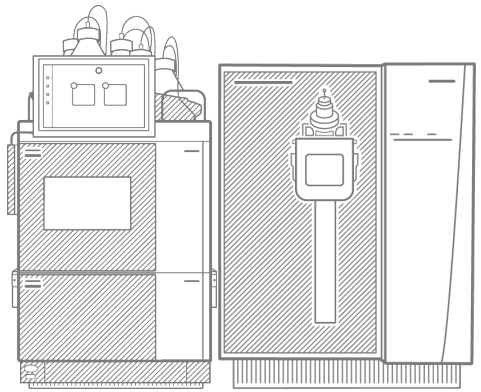
Abstract

- In this work we demonstrated the performance of the Thermo Scientific™ Vanquish™ Neo UHPLC System, the next−generation nano-, capillary- and micro-flow LC, coupled to a Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer for high-throughput bottom−up proteome profiling using a 75 µm I.D. x 15 cm Thermo Scientific™ EASY-Spray™ PepMap™ Neo Column. The developed high-throughput methods allowed to analyze up to 180 samples per 24 hours. The methods enabled up to 95% MS utilization with trap-and-elute workflow. The excellent reproducibility was achieved both intra- and inter-laboratories, regardless of the sample, column, instrument, and operator.



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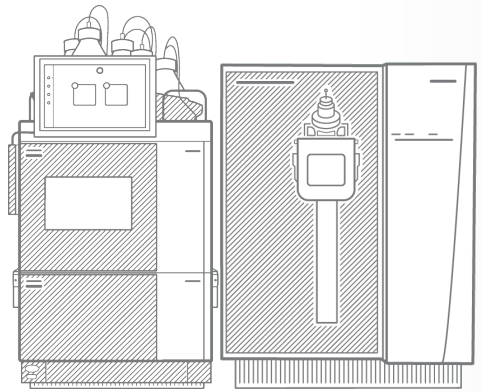

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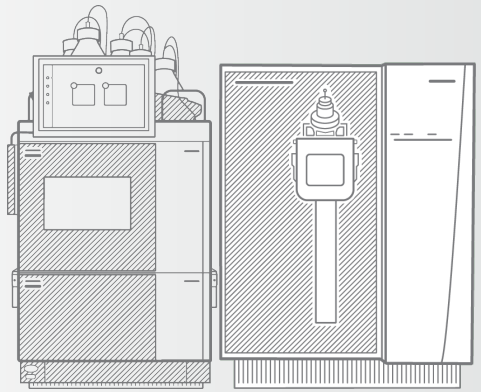

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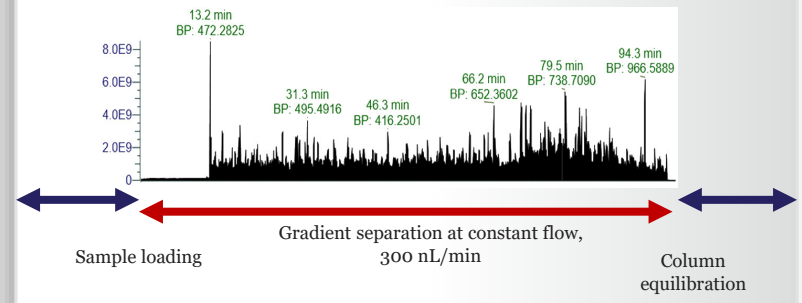
Introduction

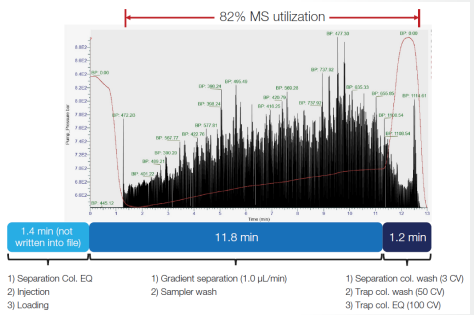
Primary Challenge

- Throughput limitations often preclude the adoption of nano-LC methods because overhead times can account for up to 50% of the total analysis time



Novel approach

- High-throughput methods using the Vanquish Neo UHPLC system and a PepMap Neo column for fast bottom-up proteome profiling to achieve 24, 30, 60, 100, and 180 samples per 24 hours.



Overview content

- High-throughput LC-MS methods overview
- Reproducible long-term performance
- Column-to-column reproducibility
- Inter-laboratory reproducibility

Summary

- excellent reproducibility both intra- and inter-laboratories, regardless of the sample, column, instrument, and operator

Materials and Methods

Sample prep

- Thermo Scientific™ Pierce™ HeLa Digest/PRTC Standard (A47996, 10 µg/vial) was reconstituted by adding 50 µL of 0.1% formic acid (FA) in water

LC-MS parameters

- 5 fast methods

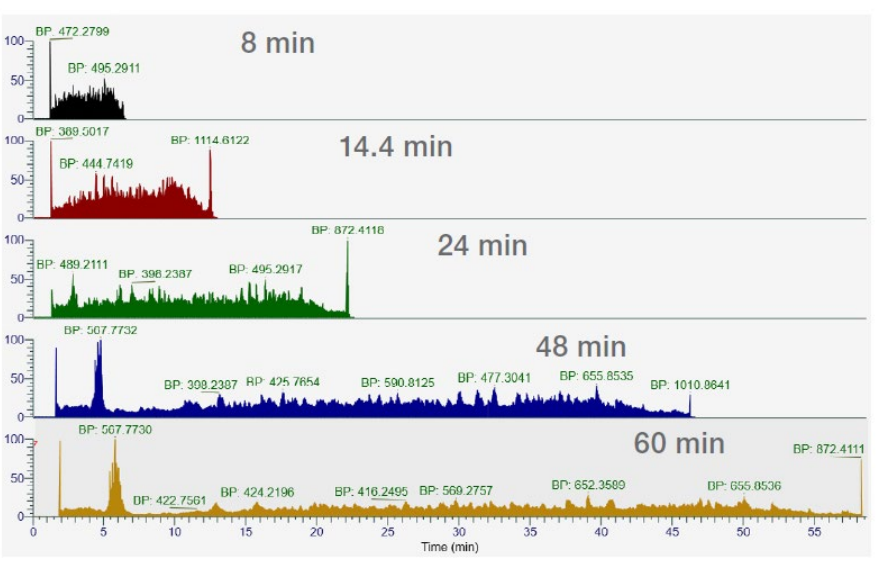
Flow rate (µ/min)	Sample throughput/day	Cycle time (min)
1.3	180	8
1.0	100	14.4
0.8	60	24
0.4	30	48
0.3	24	60

Data Analysis methods

- Thermo Scientific™ Proteome Discoverer™ Software (version 2.5) using a 2-step Sequest™ HT search algorithm and INFERYS rescoring node.

Results: standardization and productivity

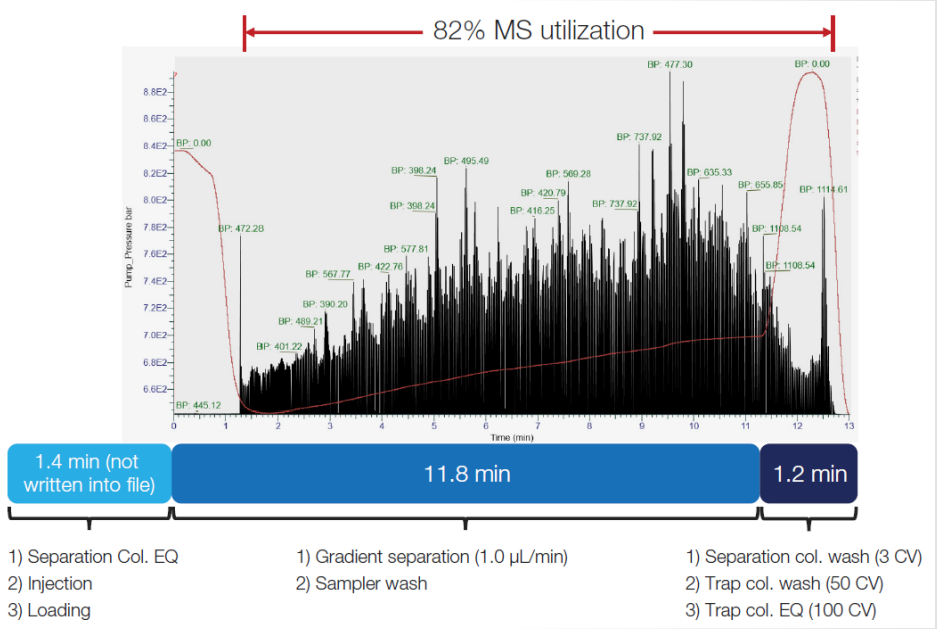
Standardized LC-MS methods



- Five standard high-throughput LCMS methods provide median value for FWHMs ranging from 2 to 11 sec (calculated for 15 PRTC peptides in 200 ng HeLa/PRTC sample) and deliver good separation profiles for methods with 8 to 60 min total cycle time.

Maximizing productivity

- The typical chromatogram of the 14.4 min method overlaid with the pressure profile (red). A breakdown of the steps demonstrates 82% MS utilization through parallel gradient separation and sampler washing procedures along with fast sample loading and column equilibration.



Results: precision and reproducibility

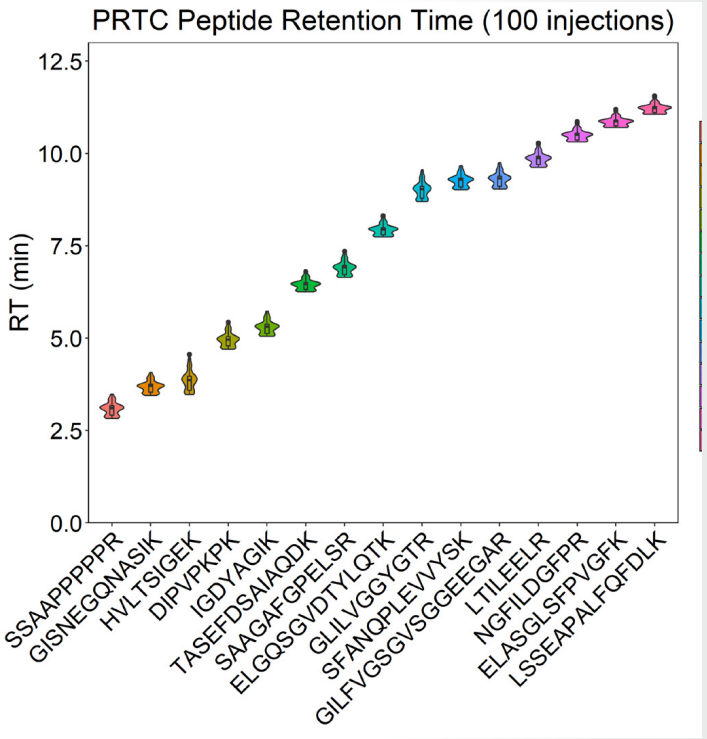
Column-to-column reproducibility

- Below 2% RSD for the number of peptide and protein identifications within each of the five methods using the same set of trap and separation columns. We also observed low inter-column variability that resulted in below 6% RSD in the number of proteins and peptides identified while using three different sets of PepMap Neo trap and separation columns on the same LC and MS hardware

	Method	Average ID (n=15)	RSD, %, Intra-column			RSD, % Inter-columns (n=15)
			Column #1 (n=5)	Column #2 (n=5)	Column #3 (n=5)	
Peptide group	8 min	5670	0.8	0.7	1.2	5.8
	14.4 min	12056	0.9	0.9	0.4	2.3
	24 min	18469	0.4	1	0.7	2.4
	48 min	27110	0.3	0.4	0.4	4.3
	60 min	29081	0.5	0.3	0.4	5.5
Protein	8 min	1218	0.9	1.2	1.1	3.4
	14.4 min	2063	0.7	0.8	0.7	1.6
	24 min	2881	0.4	1.1	0.6	1.9
	48 min	3810	0.4	0.5	0.6	3.2
	60 min	4051	0.5	0.2	0.2	4.1

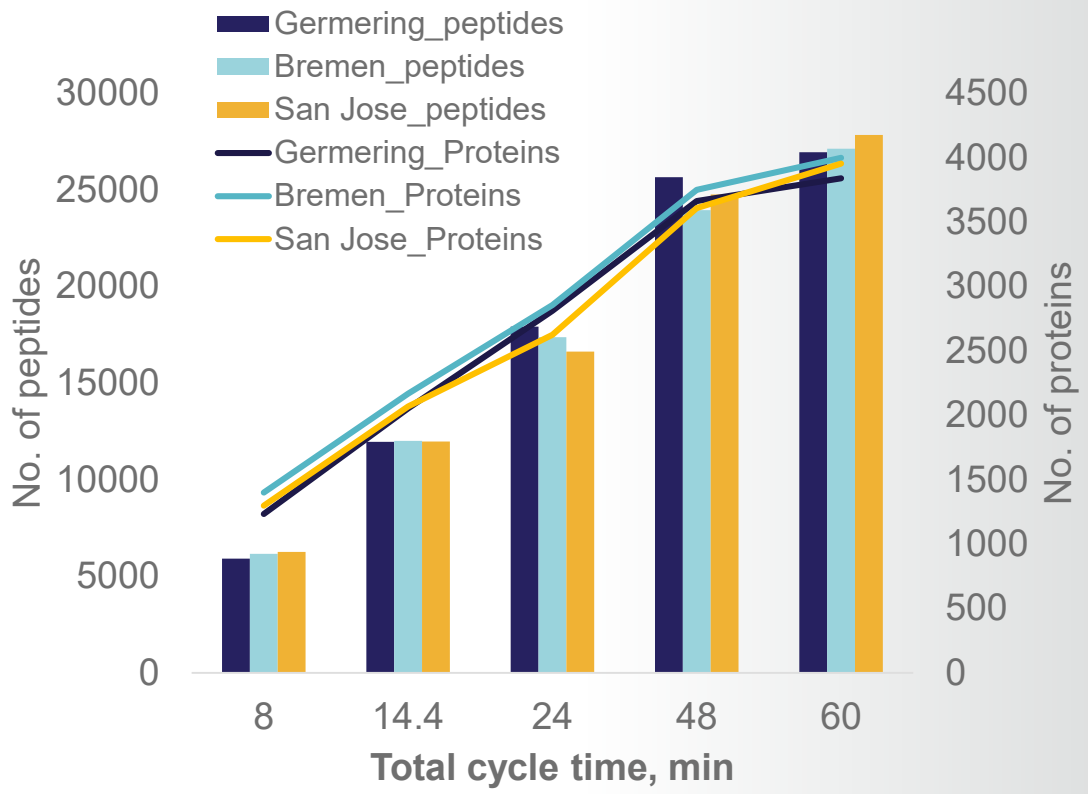
Retention time precision

- Stable retention times for PRTC peptides over 100 injections in 200 ng HeLa protein digest matrix with the 14.4-min method



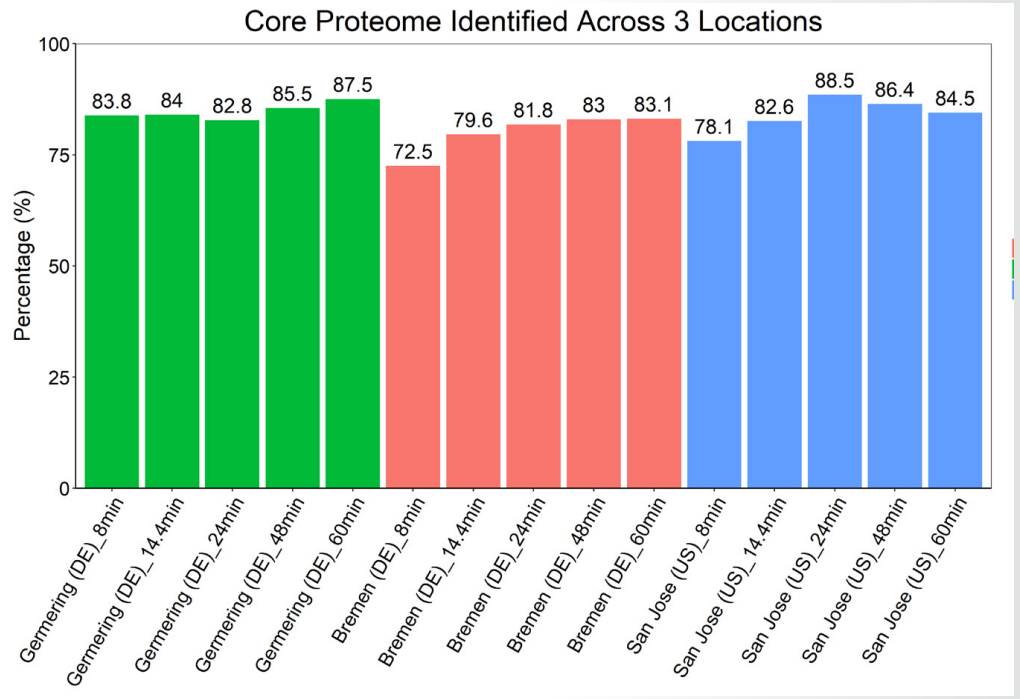
Results: site-to-site reproducibility

Total identifications



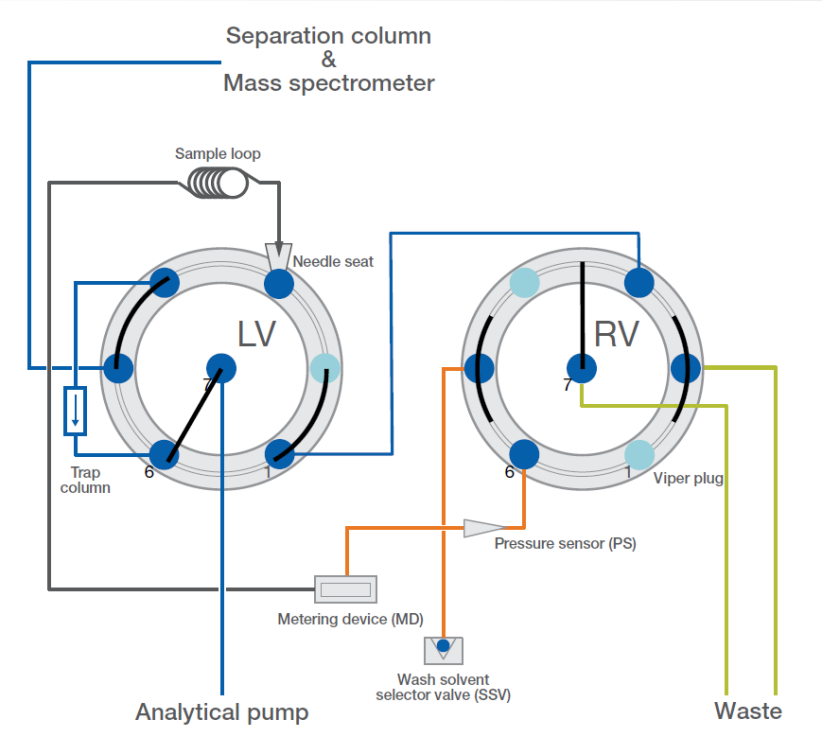
Common identifications

- The proportion of proteins that were commonly identified across three laboratories for each method length is above 72% for all laboratories and methods



Conclusions

We developed five high-throughput methods using the Vanquish Neo UHPLC system and a PepMap Neo column coupled with an Orbitrap Exploris 480 mass spectrometer for fast bottom-up proteome profiling to achieve 24, 30, 60, 100, and 180 samples per 24 hours. The Vanquish Neo UHPLC system enabled up to 95% MS utilization for enhanced peptide and protein identification and quantification when using a 75 µm I.D. x 150 mm column (2 µm particle) in the trap-and-elute workflow. The methods showed excellent reproducibility both intra- and inter-laboratories, regardless of the sample, column, instrument, and operator. Taken together, the Vanquish Neo UHPLC system combined with the latest PepMap Neo columns and Orbitrap HRAM MS is well-suited for sensitive, fast and robust LC-MS analysis of large sample cohorts.



Vanquish Neo UHPLC system trap-and-elute workflow in the backward flush mode

TRADEMARKS/LICENSING

PO66100 EN0921S