Extended lifespan of innovative column assemblies in low-flow ion sources

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Abstract

Purpose: To evaluate reproducibility of proteomics data across large HeLa and plasma sample sets using a novel column and emitter assembly.

Methods: 200 ng plasma digest or 200 ng HeLa Protein Digest with 100 or 50 fmol PRTC was analyzed with Thermo Scientific™ OptiSpray™ PepMap™ Neo and Thermo Scientific™ µPAC™ Neo cartridges.

Results: Reproducibility of chromatography and protein and peptide IDs (<0.6 %CV) over 200 plasma injections is demonstrated on the µPAC Neo HT cartridge. Reproducibility of chromatography and protein and peptide IDs (<10 %CV) over 2000 HeLa injections is demonstrated on the µPAC Neo HT cartridge and 500 injections on the PepMap Neo 75 µm x 15 cm with a single emitter on each cartridge.

Introduction

Driven by the aim of obtaining sufficient statistical significance, nano LC-MS study cohorts typically comprise several hundreds to thousands of biological samples and technical replicates. A suitable proteomics workflow must accommodate the need to process, measure, and analyze 100s of samples with high performance and reproducibility over the duration of the entire study. One of the key challenges for low-flow LC-MS workflows is increasing column and emitter backpressure and subsequent clogging or degradation of spray quality. A low-flow ion source and a cartridge that contains a novel column assembly, integrated replaceable emitter, and automated spray position optimization have been developed to address these longevity challenges and provide a platform for reliable, long-term LC-MS data acquisition.

Materials and methods

Figure 1. Experimental setup for three studies of OptiSpray cartridge lifetime A) µPAC Neo HT HeLa digest workflow up to 2000 injections B) PepMap Neo 75 µm x 15 cm with FAIMS HeLa digest workflow up to 500 injections and C) µPAC Neo HT plasma digest workflow up to 211 injections

Sample preparation

µPAC HT HeLa digest workflow: To analyze the performance of the cartridges, 200 ng HeLa Protein Digest Standard was reconstituted in 0.1% FA with 50 fmol Thermo Scientific™ Pierce™ LC-MS/MS System Suitability Standard (7x5 mix) at a concentration of 200 ng/µL. For “aging injections” to bring cartridge to 2000 injections, 300 ng/uL HeLa was used.

PepMap Neo 75 µm x 15 cm with FAIMS HeLa digest workflow: 200 ng Thermo Scientific™ Pierce™ HeLa Digest/PRTC Standard was reconstituted in 0.1% FA to generate a 200 ng/µL HeLa and 100 fmol/µL PRTC sample.

µPAC HT plasma digest workflow: Pooled human blood plasma was purchased from Sigma Aldrich and underwent S-Trap™ mini spin column (ProtiFi) digestion. The protocol is scaled to yield approximately 300 µg of digested serum or plasma. The steps include determining protein concentration, diluting the sample in SDS lysis buffer, reducing with TCEP, alkylating with MMTS, acidifying the sample, binding proteins to the S-Trap column, washing, and digesting with trypsin. Peptides are then eluted with TEAB and formic acid, followed by hydrophobic peptide elution with acetonitrile/formic acid. Finally, peptides are dried down and resuspended for analysis in 0.1% TFA. For analysis, 30 µg aliquots were resuspended in 150 µL to yield an approximate sample concentration of 200 ng/µL. 200 ng Pierce HeLa Protein Digest Standard was reconstituted in 0.1% TFA at a 200 ng/µL.

Test methods

µPAC HT HeLa digest workflow: HeLa was loaded onto an OptiSpray µPAC Neo HT cartridge and separated in direct inject mode using a Thermo Scientific™ Vanquish™ Neo UHPLC system. DDA data was acquired with the Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer.

PepMap Neo 75 µm x 15 cm with FAIMS HeLa digest workflow: HeLa was analyzed using an Orbitrap Exploris 480 mass spectrometer with FAIMS Pro Duo connected to a Vanquish Neo UHPLC system, utilizing DDA. Separation was achieved with an OptiSpray PepMap Neo 75 µm x 15 cm cartridge and a PepMap Neo trap cartridge (P/N 174500) operating in backflush trap-and-elute mode. FAIMS gas was set to 3.5 L/min and FAIMS CV was -45 V.

µPAC HT plasma digest workflow Neat plasma digests were analyzed using a Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer connected to a Vanquish Neo UHPLC system, utilizing a data DIA method. Separation was achieved with an OptiSpray µPAC Neo HT cartridge and a µPAC trap column operating in backflush trap-and-elute mode. The isolation window was configured at 10 Th, with MS1 and MS2 resolutions set at 60K and 15K, respectively. Precursor mass ranges were established between 525-825 m/z, resulting in 30 scan events for the 170 SPD method.

For each workflow, the position of the emitter was determined by running the OptiSpray capillary flow quick optimization routine.

Table 1. Selected LC-MS and OptiSpray ion source parameters for each workflow and cartridge type

Cartridge Type	Emitter Type	LC Gradient (min)	LC Flow Rate (µL/min)	Inj Workflow	Pos Ion Voltage (V)	Sheath Gas (arb)	Sample Type
µPAC Neo HT	Tapered tip (15 µm ID)	5	1.5	Direct Injection	1900	5	HeLa digest
PepMap Neo 75µm x 15cm	Tapered tip (15 µm ID)	11.8	1	Trap-Elute	1900	5	HeLa digest
µPAC Neo HT	Tapered tip (15 µm ID)	5.5	1.25 - 2.5	Trap-Elute	2000	5	Plasma digest

Data analysis

LC-MS data were analyzed either using Thermo Scientific™ Proteome Discoverer™ 3.1 with CHIMERYS™ using prediction model INFERYS™ 3.0.0 by MSAID or with Spectronaut® 19. Results shown have been filtered to a 1% FDR. Neat plasma and intermittent HeLa QC data have been analyzed in directDIA™ mode with matching between runs between triplicates. PRTC ions were analyzed in Skyline.

Results

Longevity of OptiSpray µPAC HT cartridge: HeLa digest

To isolate the impact of cartridge aging across 2000 injections from MS cleanliness, data was only acquired on the Exploris 480 MS every 500 injections. A second “aging” setup with a Thermo Scientific™ TSQ Quantis™ or Thermo Scientific™ TSQ Altis™ mass spectrometer was used to inject HeLa onto the cartridge. Moving the cartridge between two systems also demonstrates robustness during storage and reinstallation. Furthermore, the OptiSpray source automatically returns the cartridge to the same position on the Exploris 480 MS each time, eliminating the variability from manual emitter positioning.

A total of 604 µg of HeLa and ~40 mL of solvent was injected on each µPAC Neo HT column across 2000 injections (151 µg Hela and 9.75 mL per 500 injections). Each cartridge had reproducible peptide and protein IDs that decreased less than 10% from its initial IDs over the tested lifetime. On one µPAC Neo HT cartridge (Figure 2, dark blue trace), the emitter was replaced due to damage at 1000 injections. The peptide and protein IDs obtained after 1000, 1500, and 2000 injections were within 9% of those obtained with the original emitter.

Retention times for one PRTC ion from 0 to 2000 injections for were relatively stable, with a maximum RT CV of 2.8%. The variation is due to the LC not being kept constant throughout the 2-month experiment. For the five replicates at 0, 500, 1000, 1500, and 2000 for each cartridge, the median RT CV is 0.1%. Peak area reproducibility was excellent with %CVs less than 11% for each cartridge from 0 to 2000 injections.

Figure 2. Protein and Peptide IDs of 200 ng HeLa digest on 5 µPAC Neo HT cartridges up to 2000 injections. Each trace represents an individual cartridge.

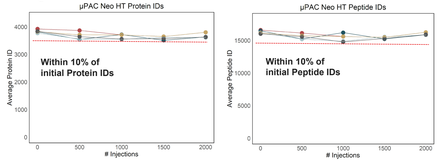
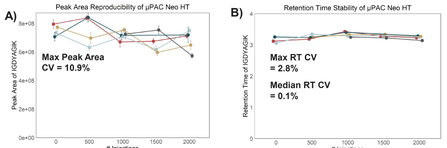


Figure 3. A) Peak area and B) Retention time precision of one PRTC peptide for digest on 5 µPAC Neo HT cartridges up to 2000 injections. Each trace represents an individual cartridge.



Longevity of OptiSpray PepMap Neo 75 µm x 15cm cartridge with FAIMS: HeLa digest

An additional longevity experiment with HeLa digest was performed on the PepMap Neo 75µm x 15cm cartridge. FAIMS Pro Duo was installed to reduce background noise and to prevent the MS source region from being fouled. A total of 500 injections were made on each cartridge, using a 100 SPD method. To test the robustness of cartridge reinstallation, every 100 injections the cartridge was removed and stored for ≥24 hours. Upon reinstallation, the OptiSpray source automatically returned to the previously optimized position.

Protein and peptide IDs were highly reproducible run-to-run and cartridge-to-cartridge, with CVs of 2.1% and 2.0%, respectively, across all injections on all cartridges. Three PRTC ions were monitored across the 500 injections – the peak area CV was ≤12%. Retention time stability was excellent, with a median rolling CV of 0.5%, calculated across all injections on all cartridges.

Figure 4. Protein and Peptide IDs of 200 ng HeLa digest on 3 PepMap Neo 75 µm x 15 cm up to 500 injections. Each color represents an individual column.

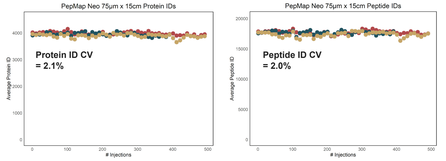
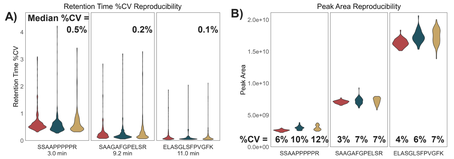


Figure 5. A) Retention time rolling %CVs over the past 20 injections and B) Peak areas of three PRTC peptide for three PepMap Neo 75 µm x 15 cm up to 500 injections. Each color represents an individual column.



Longevity of OptiSpray µPAC HT cartridge: Neat human plasma digest

To evaluate OptiSpray compatibility with complex matrices, a longevity experiment was conducted where neat human plasma digest was injected over 36 hours, with intermittent blank and HeLa digest QC runs to monitor column performance.

HeLa QC sample identifications remained stable, with CVs for protein group and peptide identification at 0.1% and 0.3%, respectively, based on 33 QC runs. Chromatographic metrics showed a CV of 1.2% for median peak width and a max retention time deviation of 1.5 seconds for five endogenous HeLa peptides, resulting in retention time CV below 0.7% for all peptides. Metrics from plasma raw files also showed excellent stability, with CVs for protein group and peptide identification at 0.6% and 0.5%, respectively, based on 60 neat plasma runs.

Figure 6. Base peak chromatograms (BPC) acquired from a series of intermittent A) neat human plasma digest analyses and B) HeLa digest QC. For each sample, 200 ng of tryptic digested protein material was injected. Injection number within the sequence is indicated to the left of the respective BPC.

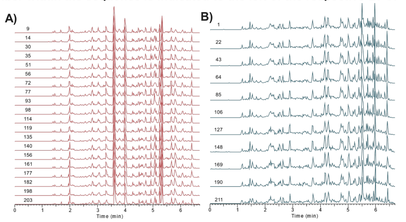


Figure 7. Chromatographic metrics from intermittent HeLa QC and neat human plasma digest analyses. A) Retention times for 5 endogenous HeLa peptides. B) Column pressure recorded at the beginning of each run at 4% B and 2.5 µL/min during HeLa QC (blue) and neat human plasma digest analyses (red).

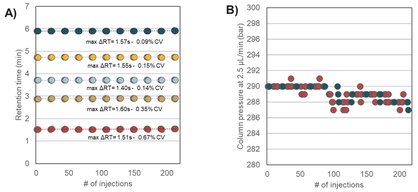
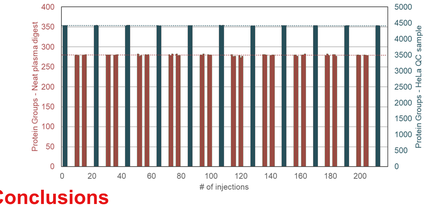


Figure 8. Protein groups identified from intermittent HeLa digest QC (right y-axis - blue bars) and neat human plasma digest analyses. (Left y-axis – red bars). For each sample, 200 ng of tryptic digested protein material was injected.



Conclusions

OptiSpray showed robust and reproducible results over hundreds to thousands of injections:

- The OptiSpray cartridge performed well for high-throughput neat plasma proteome profiling, offering excellent stability with a 0.5% CV for protein group identifications and no impact on cartridge performance, indicated by consistent chromatographic metrics (FWHM, retention time and pressure).
- Reproducible peptide and protein IDs (within 10% of the initial value) and reproducible PRTC areas (max 10.8% CV) and retention times (median 0.1% CV) were achieved over 2000 injections of HeLa on a µPAC HT cartridge. Reproducible peptide and protein IDs (max 2.1%) and reproducible PRTC areas (max 12% CV) and retention times (median 0.5% CV) were achieved over 500 injections of HeLa on a PepMap Neo 75 µm x 15 cm cartridge operated with FAIMS.
- Both µPAC and PepMap cartridges have robust performance when removed from a system and reinstalled, in part due to the automated positioning of the cartridge with the OptiSpray source. With an exception of one µPAC Neo HT cartridge, emitters were not exchanged, but the emitters can easily be replaced to further extend lifetime.

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