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Microflow LC-Nanospray MS for Targeted Proteomics

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Key Words

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Q Exactive Plus,
TSQ Quantiva Triple Quadrupole

Goal

To develop a Microflow LC-Nanospray ESI-MS (MnESI-LC-MS) platform for targeted proteomics that achieves high throughput, high sensitivity, and robustness, by using Newomics® M3 Emitters and Thermo Fisher PepMap™ C18 columns on a Quantiva™ mass spectrometer for SRM analysis and a Q Exactive™ Plus mass spectrometer for PRM analysis.

Introduction

Liquid chromatography-mass spectrometry (LC-MS) is the enabling technology for global-scale analysis of proteins (proteomics). In order to identify and quantify peptides and proteins in small volumes of biological samples, nanoflow LC-MS (flow rate < 1 μL/min) is routinely used in order to achieve high sensitivity. However, nanoflow LC-MS lacks robustness and throughput (i.e., speed). On the other hand, high-flow LC-MS (flow rate > 300 μL/min) typically achieves high throughput and robustness with a CV < 10%, but lacks sensitivity. Microflow LC-MS (flow rate 1-50 μL/min) developed in 1970s could potentially bridge this gap. In fact, there is a recent renaissance of microflow LC-MS because of the limited sample amounts for precision medicine applications. The Newomics award-winning silicon multinozzle emitters (M3 emitters) splits the incoming microflow evenly into multiple nanoflows, thereby dramatically enhancing the ionization efficiency to achieve high throughput, high sensitivity, and robustness for LC-MS.
In this Application Note, we demonstrate the application of our microflow LC-nanospray ESI-MS platform (MnESI-LC-MS) for targeted proteomics studies of peptide mixtures spiked in human plasma digest matrix.

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Methods

Sample Preparation

Human plasma digest sample was prepared using Thermo Scientific EasyPep™ Mini MS Sample Prep Kit (Cat.#: A40006). Dried peptides were stored in -80°C. During the experiments, plasma digest sample was resuspended in Solvent A (3% acetonitrile in 97% water with 0.1% formic acid), and then the Thermo Scientific Pierce Peptide Retention Time Calibration (PRTC, Cat. #: 88320) mixture was added.

Data Processing

Raw data was imported into Skyline software (University of Washington) to obtain peak area intensities and retention times. Microsoft Excel was used to perform statistical analysis including relative standard deviation (RSD).

Instrumentation

The chromatographic separation was performed on UltiMate 3000 nanoUPLC system (Thermo Fisher Scientific) with the following conditions (Table 1). The scheduled, selected reaction monitoring (SRM) analysis of peptides was performed on a TSQ Quantiva Triple Quadrupole Mass Spectrometer and the scheduled parallel reaction monitoring (PRM) assay was achieved on a Thermo Scientific Q Exactive™ Plus MS. The position of M3 emitters relative to the ion transfer tube was tuned based on TIC signal and was about 3~4 mm.

Table 1: LC-MS conditions for evaluating sensitivity and robustness comparisons

	High-flow LC-MS	Microflow LC-MS		
Flow Rate (µL/min)	250	5	3	1.5
Column	ACE Excel C18-AR, 2.1mm ID x 100mm L, 2µm	Thermo Acclaim PepMap 100, C18, 300µm ID x 150mm L, 2µm	Thermo Acclaim PepMap 100, C18, 150µm ID x 150mm L, 2µm	
Column Temperature	RT	RT	40°C	
Mobile Phase	A: 3% acetonitrile in water, 0.1% FA B: 3% water in acetonitrile, 0.1% FA			
LC pluming	Direct injection without trap column			
Injection Mode and Volume	Microliter Pickup, 5µL out of 20µL loop	Full loop, 1 µL loop		
Sample	25fmol/µL PRTC in 120ng/µL plasma digest	25fmol/µL PRTC in 120ng/µL plasma digest	50fmol/µL PRTC in 350ng/µL plasma digest	25fmol/µL PRTC in 120ng/µL plasma digest
Run Time (min)	14	14	15	15
LC Gradient	Time %B 0 5 0.5 10 7.5 30 7.6 85 10.4 85 10.5 5 14 5	Time %B 0 5 0.5 10 7.5 30 7.6 85 10.4 85 10.5 5 14 5	Time %B 0 5 0.5 10 9.5 30 10 85 11.9 85 12 5 15 5	Time Flow %B 0 1.5 5 0.5 1.5 10 9 1.5 30 9.4 1.5 85 9.5 3 85 11.4 3 85 11.5 3 5 14.9 3 5 15 1.5 5
Mass Spec	Quantiva	Quantiva	Quantiva	QE-Plus
Acquisition Method	SRM	SRM	SRM	PRM
Emitter/Sprayer	HESI-II (110µm ID)	M3 emitter (10µm ID, 8-nozzle), stainless steel emitter (ES542)		
Spray Voltage (V)	3700	3500 for M3 emitter, 2500 for stainless steel emitter		
Spray Angle	60°	30° for M3 emitter, 10° for stainless steel emitter		
Gas Flow	40 unit for Sheath Gas 5 unit for Aux Gas	1L/min for M3 emitter 0 for stainless steel emitter		

Results and Discussion

Sensitivity Improvement

To demonstrate the performance of M3 emitters, we performed a head-to-head comparison between M3 emitters and stainless-steel emitters at microflow rates (5 µL/min) for targeted LC-MS analysis of PRTC peptides in plasma digest matrix. In addition, we compared the performance of microflow LC-MS by M3 emitters to high-flow LC-MS (2.1 mm column format). Figure 1 shows a representative extraction ion chromatogram of 15 PRTC peptides using M3 emitters at 5 µL/min. Excellent separation and detection of the pesticide compounds were achieved at microflow rates with the scheduled-SRM method. We determined the sensitivity improvement by comparing the peak area ratio for all 15 PRTC peptides, shown in Figure 2. Using the same column of 300 µm I.D. and the identical conditions, M3 emitters achieved the sensitivity gain of 2.3 on average over nanospray emitters (ES542). In addition, the average enhancement of microflow LC-MS by M3 emitter over high-flow LC-MS with HESI-II probe was about 48.9.

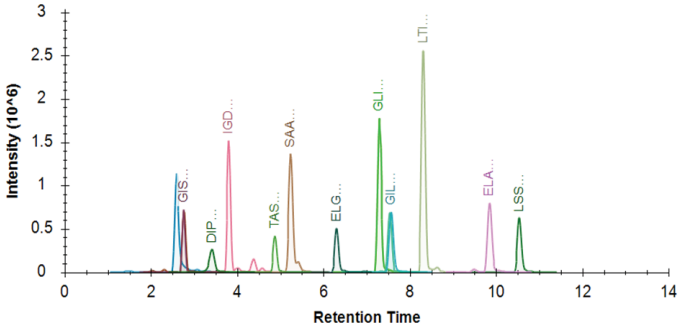

Figure 1: Extracted ion chromatogram for SRM-MS analysis of PRTC peptides in Skyline by MnESI-LC-MS using M3 emitter at 5 µL/min.

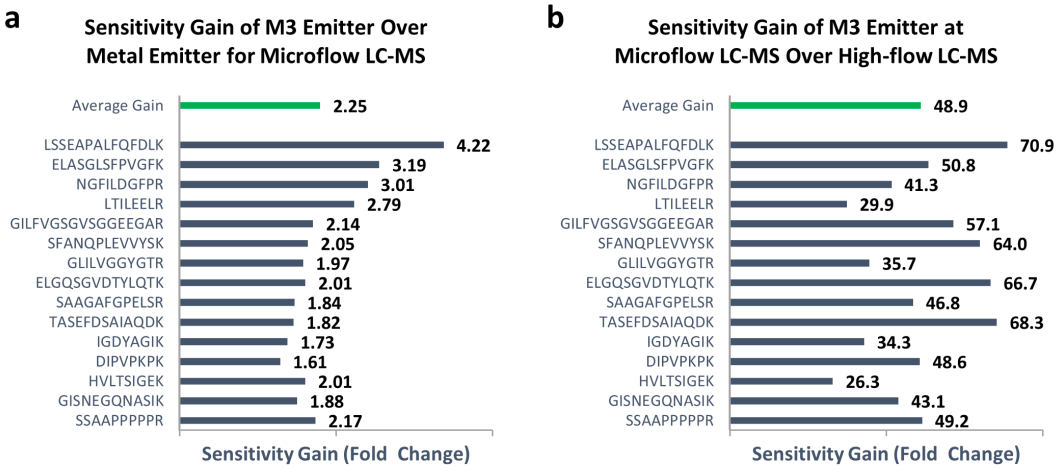

Figure 2: The sensitivity improvement of M3 emitter over conventional stainless-steel emitter (ES542) at 5 µL/min (a) and high-flow LC-MS with HESI at 250 µL/min. Sensitivity gain is determined as the ratio of peak area intensity. The PRTC peptides are arranged by their hydrophobicity. The average gain of M3 emitter over ES542 is 2.25. The average gain of MnESI-LC-MS with M3 emitter in comparison to high-flow LC-MS is 48.9.

Robustness and Reproducibility

To determine the robustness of M3 emitters for microflow LC-MS analysis, we performed over 300 injections of plasma digests spiked with PRTC standards. Figure 3 shows the peak area stability and retention time stability for both microflow LC-MS and high-flow LC-MS analyses of PRTC peptides. For all 15 peptides, we achieved the average peak area intensity RSD of 5% for microflow LC-MS with M3 emitters even without using an internal standard to correct for MS signal variability. In addition, the peak area intensity RSD for the high-flow LC-MS was about 6% on average. The slightly better RSD from M3 emitter might be due to the much stronger signals than high-flow HESI because of over 40-fold higher sensitivity. The average retention time RSD for M3 emitter was <1% over 300 injections, indicating excellent run-to-run repeatability, and was comparable to ~0.33% for high-flow LC-MS.

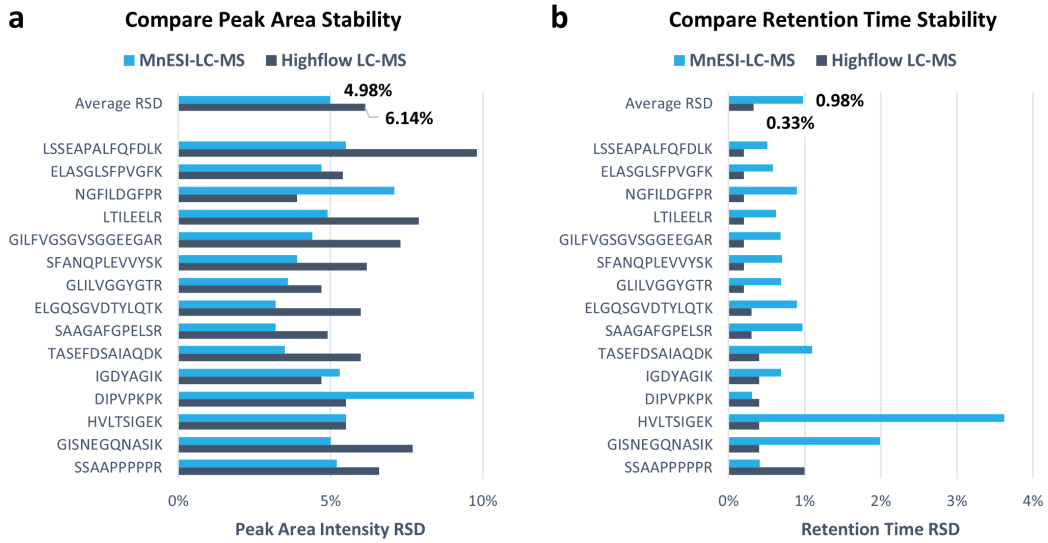

Figure 3: Peak area stability (a) and retention time stability (b) with targeted analysis of PRTC peptides in human plasma digest matrix by MnESI-LC-MS with M3 Emitter and high-flow LC-MS with HESI. The relative standard deviations (RSD) for peak area intensity and retention time were determined by performing 345 injections consecutively for MnESI-LC-MS and 300 injections for high-flow LC-MS, respectively. The PRTC peptides are arranged by their hydrophobicity from high to low.

Very consistent back pressure was obtained after over 300 injections and we did not observe any pressure change from both the column and M3 emitter. Figure 4 shows the pressure trace from the first and last injection (#345). The M3 emitter still worked well after 345 injections and there was no sign of physical damage, clogging, or decreased performance. We have not carried out the emitter lifetime test yet but will investigate it in the future. These results demonstrated the rugged performance and excellent reproducibility of M3 emitters for microflow LC-MS, delivering the same level of high throughput and robustness as high-flow LC-MS.

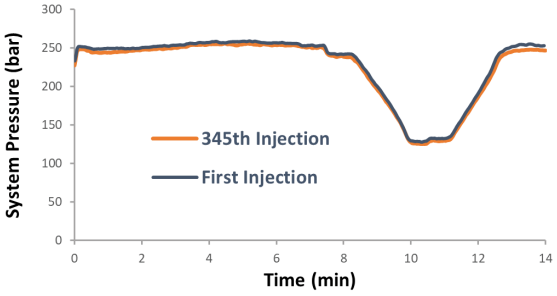

Figure 4: Consistent pressure trace recorded during the first injection and last injection (#345) of PRTC standards spiked in human plasma digest matrix by MnESI-LC-MS with a M3 Emitter. For simplicity, only the first and last injection traces were plotted, but the other injections showed almost the identical trend.

Applications for High-Sensitivity and High-Throughput Proteomics

To achieve higher sensitivity for proteomics, it is straightforward to employ smaller diameter columns, such as 150 µm ID instead of 300 µm ID ones. In this study, we evaluated the robustness of microflow LC-MS at lower flow rates, by integrating M3 emitters with 150 µm ID columns. Figures 5 and 6 show peak area stability of 15 PRTC peptides for over 300 injections at flow rates of 3 µL/min and 1.5 µL/min, respectively. We achieved an excellent peak area precision of on average 5.2% RSD at 3 µL/min and 8.5% RSD at 1.5 µL/min, indicating the robustness of coupling M3 emitters with 150 µm ID columns for lower microflow LC-MS applications.

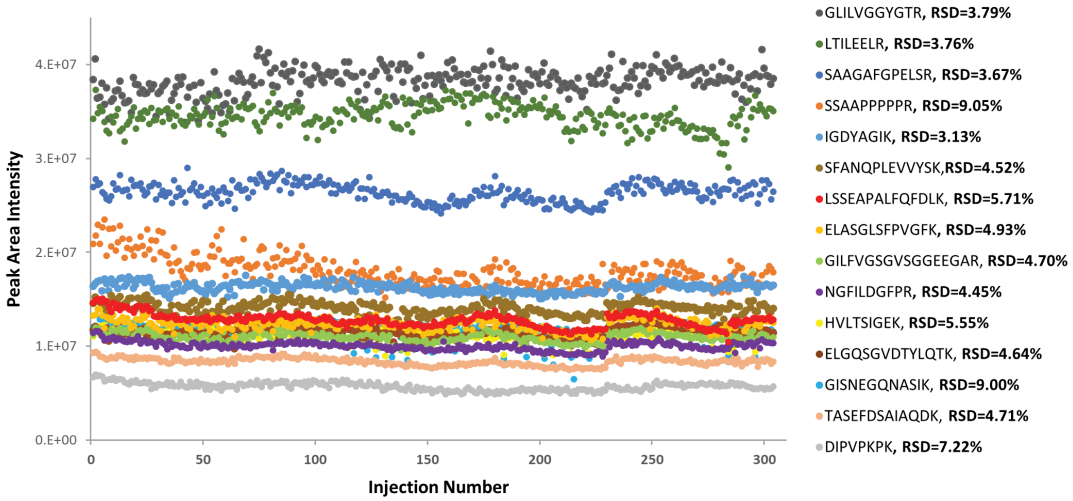

Figure 5: Peak area stability with targeted analysis of PRTC peptides at 3 µL/min using M3 emitter with a 150 µm I.D. separation column. 305 injections of 50 fmole PRTC peptides spiked in 350 ng human plasma digest were performed to obtain peak area intensity RSD. The average peak area intensity RSD was 5.2%.

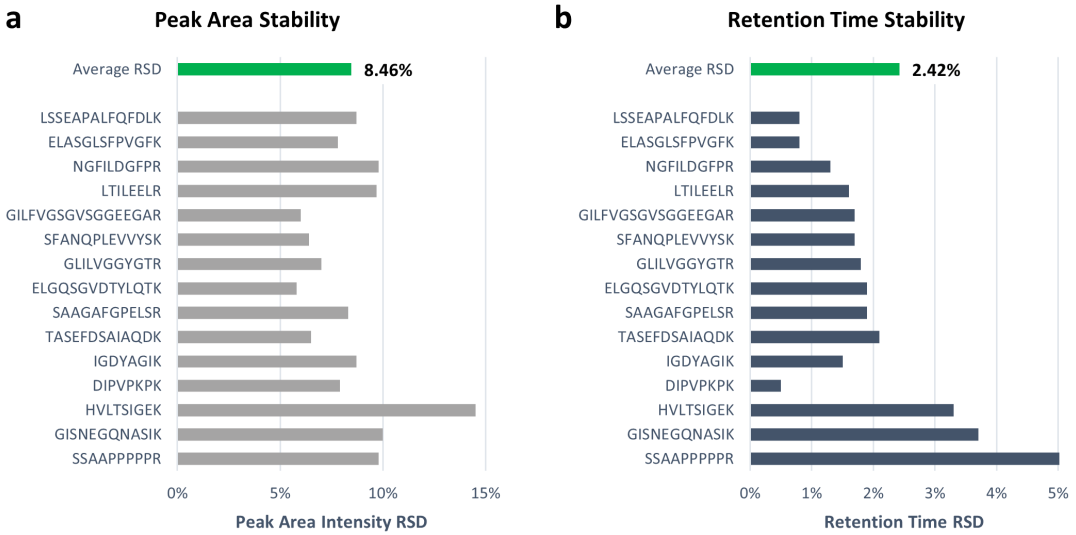

Figure 6: Peak area stability (a) and retention time stability (b) with targeted analysis of PRTC peptides in human plasma digest matrix at 1.5 µL/min using M3 emitter with a 150 µm I.D. separation column. 320 injections of 25 fmole PRTC peptides spiked in 120 ng human plasma digest were performed to obtain RSDs.

Conclusions

In summary, we have demonstrated that MnESI-LC-MS is a significant improvement over conventional microflow LC-MS. The robustness is on par with the gold standard of high-flow LC-MS and the sensitivity is approximately 50-fold higher than high-flow and 2.3-fold higher than conventional microflow LC-MS for proteomics. The combination of robustness and increased sensitivity has the potential to facilitate wide adoption of microflow LC-MS for high-throughput applications in proteomics, metabolomics, lipidomics, and glycomics.

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4. ASMS 2018 poster: po-65308-lc-ms-capillary-flow-sensitivity-proteomics-asms2018-po65308-en

Ordering Information

Catalog #	Product
SKIT-T01	Newomics® Starter Kit for Thermo Fisher Nanospray Flex Ion Sources
E8N10MU01	Newomics® M3 Emitters, 10 µm I.D. – 8 nozzles

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