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Microflow-Nanospray ESI-MS (MnESI-MS) Platform for Human Plasma Proteomics

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OVERVIEW

- Newomics unique multi-nozzle M3 emitter splits the LC eluent evenly to multiple flows, thereby significantly enhancing the ionization efficiency to achieve improved sensitivity, throughput and robustness for LC/MS.
- A new MnESI (**M**icroflow **n**anospray **ESI**) source has been designed specifically for Thermo mass spectrometers, allowing minimal adjustment for using M3 emitter, in a truly Plug-and-Play manner.

INTRODUCTION

Compared to nanoflow LC-MS for proteomics, microflow LC-MS achieves higher throughput and robustness, but lacks sensitivity. The Newomics award-winning multinozzle emitters (M3 emitters) enable optimization of sensitivity, throughput, and robustness by splitting microflow eluent evenly into multiple nanoflows, thereby dramatically enhancing ionization efficiency. We demonstrated the applications of our MnESI platform (microflow LC-nanospray ESI-MS) for both bottom-up and targeted proteomics studies of human plasma samples.

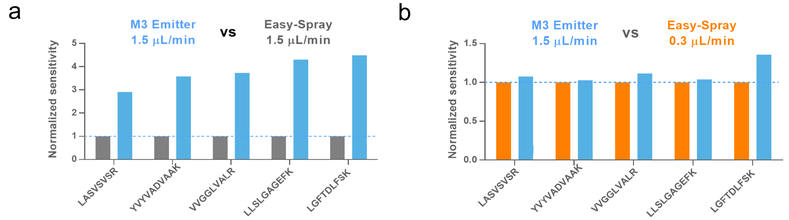
METHODS

A 300 µm ID or 150 µm ID C18 column was paired with the Newomics MnESI ion source and M3 emitters to perform LC-MS analysis of human plasma digest on a QE-Plus and TSQ Quantiva MS (Thermo Fisher), respectively. Both the sensitivity and robustness performance of MnESI platform at 1.5 µL/min were compared to that of nanoflow LC-MS at 0.3 µL/min using a Nanospray Flex ion source. For targeted proteomics analysis, the performance of MnESI platform at 5 µL/min was compared to that of high-flow LC-MS using a column of 2.1 mm ID at 250 µL/min using a HESI source.

RESULTS

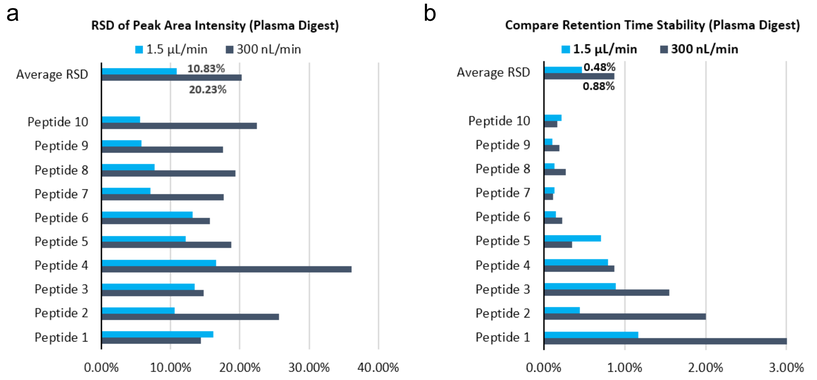
1. MnESI source with M3 emitter achieved on an average a gain of 3.8 in sensitivity, using the peak area intensity, relative to a single nozzle emitter at 1.5 µL/min. In addition, MnESI platform achieved the same sensitivity as nanoflow LC-MS at 0.3 µL/min.

Figure 1: Comparison of sensitivity between MnESI-LC-MS (M3 Emitter), microflow LC-MS (ES791) and nanoflow LC-MS (ES803).



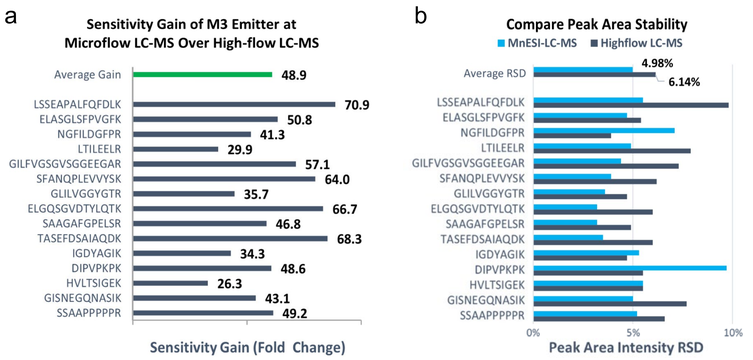
2. As shown in Figure 2, the CVs of peak area intensity and retention times from over 25 consecutive injections from MnESI platform were about 50% smaller than the values obtained by nanoflow LC-MS.

Figure 2: Comparison between nanoflow LC-MS (300 nL/min) and MnESI-LC-MS (1.5 µL/min) in terms of RSD of peptide peak area intensity and peptide retention time for analyzing human plasma digest.



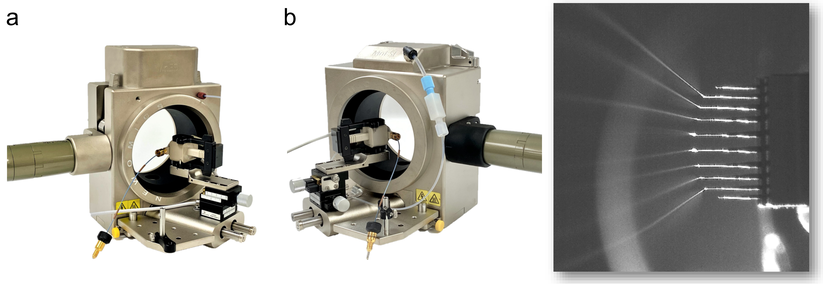
3. For targeted proteomics application, the average sensitivity enhancement from MnESI platform at 5 µL/min was about 50-fold higher than high-flow LC-MS. By performing 300 consecutive injections of plasma digests, we achieved the average peak area intensity CV of 4.98% for MnESI platform, compared to 6.14% on average obtained from high-flow LC-MS.

Figure 3: (a) The sensitivity improvement of M3 emitter over high-flow LC-MS with HESI at 250 µL/min. Sensitivity gain is determined as the ratio of peak area intensity. (**b**)Peak area stability 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.



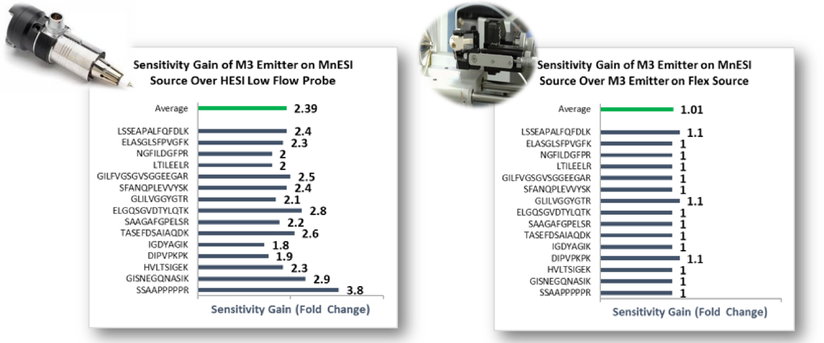
4. New MnESI (Microflow nanospray ESI) sources are designed specifically for Thermo mass spectrometers, allowing minimal adjustment for using M3 emitter, enabling truly Plug-and-Play operation.

Figure 4: New MnESI sources designed specifically for Thermo mass spectrometers. (**a**) NG interface; (**b**) Legacy interface.



5. M3 emitter on Newomics MnESI sources delivers comparable results with that on Thermo Nanospray Flex (NG) ion sources.

Figure 5: Comparison between HESI source, M3 emitter on MnESI source, and M3 emitter on Nanospray Flex NG source.



CONCLUSION

Newomics MnESI platform (MnESI source plus M3 Emitter) enables microflow LC-nanospray ESI-MS to deliver the same sensitivity as nanoflow LC-MS for bottom-up proteomics, while achieving better stability of retention time and peak area intensity. In addition, it achieves the same level of high throughput and robustness as high-flow LC-MS for targeted proteomics, while dramatically increasing the sensitivity. MnESI platform opens up new opportunities for clinical applications of human plasma proteomics.

ACKNOWLEDGEMENT

We acknowledge Junhwan Kim, Manda Van La, and Weimin Ni on their work on the design and testing of the MnESI sources.