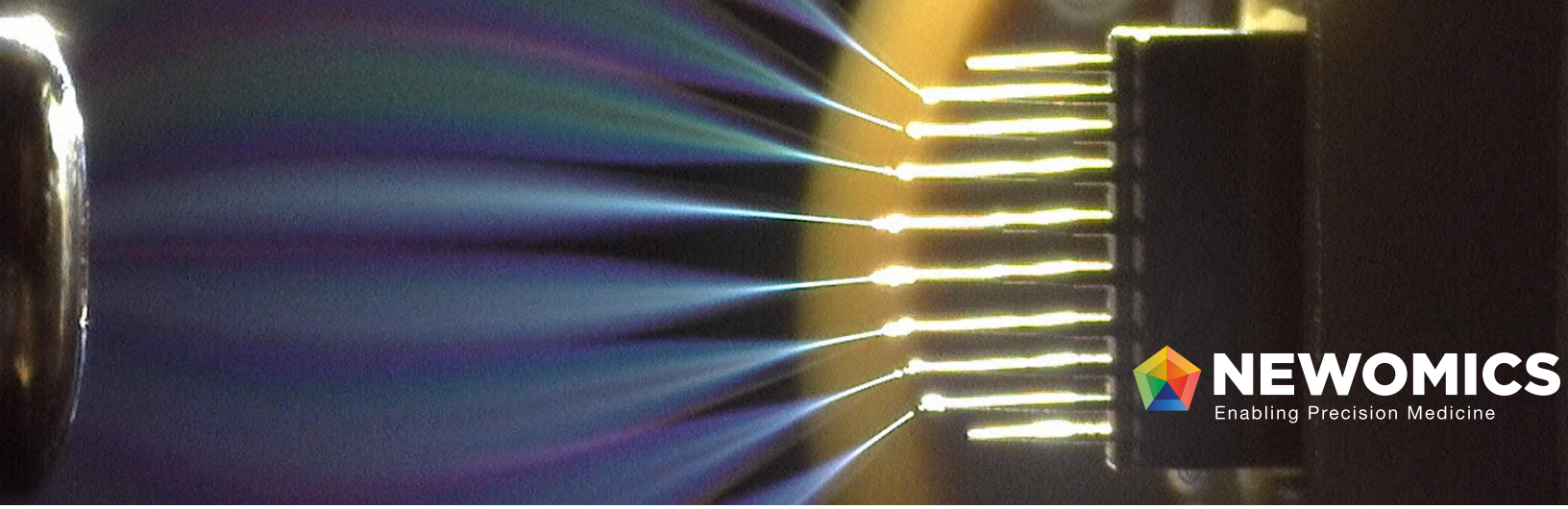

Enhanced Sensitivity and Robustness Using a Microflow LC and Nanospray MS Platform for Small Molecules Analysis

Authors

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Keywords

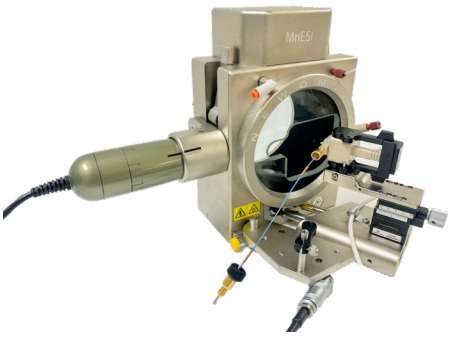
Microflow-nanospray ESI-MS (MnESI-MS), Ion source, M3 emitter, Multinozzle, Small Molecules, Pesticides, Limit of Detection, Microflow LC-MS, High-flow LC-MS, Sensitivity, Reproducibility

Goal

To demonstrate significant sensitivity improvement and rugged performance for analyzing small molecules (with pesticides as an example in this note), using the Newomics microflow-nanospray ESI-MS (MnESI-MS) platform.

Introduction

The sensitivity of liquid chromatography coupled to mass spectrometry (LC-MS) increases significantly with a decreasing column diameter and reduced flow rates. The relationship has been shown to follow a power law function empirically [1]. As a result, microflow LC-MS has benefits with increased sensitivity, reduced sample and solvent consumption, and decreased matrix effects, compared to conventional high-flow LC-MS. However, it still lacks sensitivity compared to nanoflow electrospray because of its lower ionization efficiency at the microflow. Newomics unique multinozzle emitters divide the microflow LC eluent into eight nanoflow electrospray nozzles. The 10 micron ID electrospray nozzles achieve nanoflow desolvation efficiency. As a result, the theoretical spray current increases proportionally to the square root of the number of nozzles [2]. Here, using pesticides in the fruit matrix as a case study, we performed three-way comparison between MnESI-MS with M3 emitters, high-flow LC-MS with HESI (heated ESI) needles, and microflow LC-MS with stainless steel nanospray tips (SStip). We have demonstrated the sensitivity gain and robust and reproducible performance of our MnESI-MS platform.



Methods

1. Sample Preparation

Organic grapefruit sample was extracted with acetonitrile (ACN) using a QuEChERS method, which is a sample preparation procedure to extract pesticides from food. The grapefruit extract was diluted twice with water and filtered through a 0.2 µm syringe filter. After centrifugation at 10,000 x g for 5 min, the supernatant was taken out and mixed with 241 pesticide standards. The final ACN concentration in the sample was about 35%. For calibration experiments, ten concentrations of pesticide standards were spiked in grapefruit matrix at the concentration of 0, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 ppb, respectively, and in triplicates. For robustness experiments, 300 injections of 1 ppb pesticide standards spiked in grapefruit matrix (1 µL injection volume) were performed.

2. Instrumentation

The chromatographic separation was performed on UltiMate 3000 RSLCnano UHPLC system (Thermo Fisher Scientific) with the following conditions.

	High-flow LC	Microflow LC
Column	ACE Excel C18, 2.1 mm ID x 100 mm L, 2 µm	ACE C18, 300 µm ID x 100 mm L, 3 µm
Mobile phase	A) Water with 5 mM ammonium formate and formic acid 0.1% (v/v, %) B) Methanol with 5 mM ammonium formate and formic acid 0.1% (v/v, %)	
Temperature	25 °C	
Injection volume	1 µL, full loop injection	
Flow rate	250 µL/min	5 µL/min
Run time	14.5 min	15 min
Gradient	Time (min) %B 0 2 0.25 30 10 100 12 100 12.1 2 14.5 2	Time (min) %B 0 2 0.25 40 9.5 100 11.5 100 11.6 2 15 2

The scheduled, selected reaction monitoring (SRM) analysis of pesticides was performed on a TSQ Quantiva Triple Quadrupole Mass Spectrometer with a Thermo Scientific Ion Max NG source, a Thermo Scientific Nanospray Flex NG ion source, or a Newomics MnESI platform.

	High-flow LC-MS	MnESI-MS	Microflow LC-MS
Ionization Source	Ion Max NG	MnESI	Nanospray Flex NG
ESI Emitter	HESI needle (110µm ID)	M3 emitter (10µm ID, 8 nozzle)	SStip (30µm ID, ES542)
Polarity	Positive		
Gas flow rate	Sheath gas: 35 units Aux gas: 5 units	1.2 L/min	No gas
Spray voltage	3,500 V	3,500 V	2,500 V
Spray angle	60 °	30 °	10 °
Ion transfer tube temperature	350 °C	300 °C	300 °C
Vaporizer temperature	300 °C	N/A	N/A
CID gas	2 mTorr		

3. Data Analysis

Tracefinder™ software (Thermo Fisher Scientific) was employed to automatically process raw data after acquisition.

Results and Discussion

1. MnESI-MS platform significantly improves sensitivity for pesticide analysis

We first developed and optimized the scheduled LC-SRM/MS assay to quantify the 241 pesticides. **Figure 1** shows a representative extracted ion chromatogram of grapefruit extract spiked with 1 ppb of 241 pesticide standards, using MnESI-MS platform with M3 emitters at a flow rate of 5 µL/min. With nearly the same chromatographic run time (15 min) as high-flow LC (14.5 min), excellent separation and detection of the pesticide compounds were achieved at micro flow rates. We then performed a three-way comparison between MnESI-MS, conventional microflow LC-MS, and high-flow LC-MS. We determined the sensitivity gain for all 241 pesticide compounds by injecting the equal amount of sample and comparing the signal enhancement between the three different platforms.

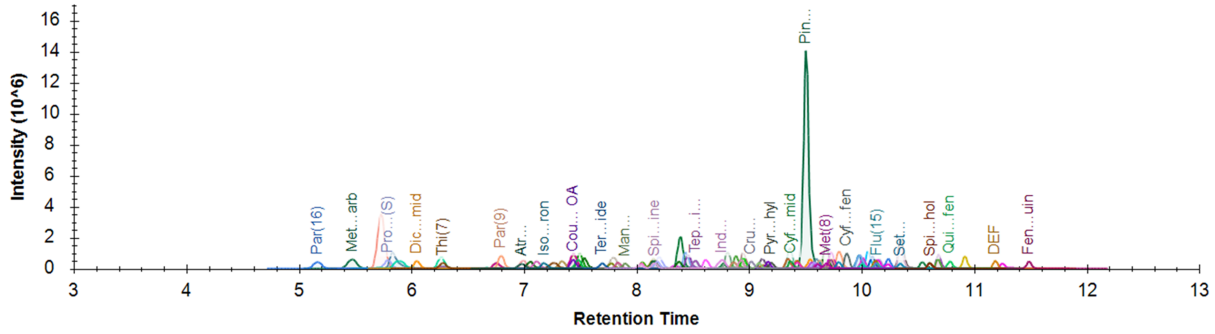


Figure 1. A representative extracted ion chromatogram (EIC) of 241 pesticides in grapefruit matrix extract by MnESI-MS using a M3 emitter at 5 µL/min.

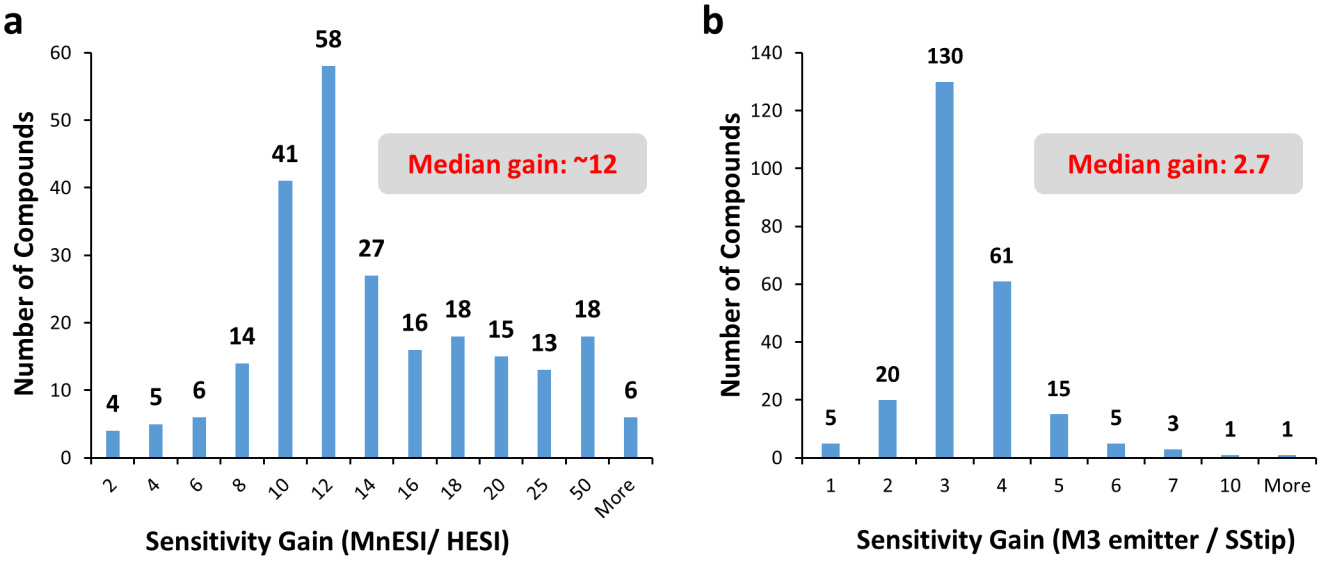


Figure 2. The signal enhancement with M3 emitter in comparison to stainless steel tip (SStip) and HESI. a) Histogram of the sensitivity gain of 241 pesticide compounds measured by microflow LC-MS with M3 emitter in comparison to high-flow LC-MS with HESI probe. b) Histogram of the sensitivity gain of 241 pesticide compounds measured by microflow LC-MS with a M3 emitter in comparison to a SStip. The number of compounds out of the total 241 for each closest RSD value was shown on the bar graph.

The results are summarized in **Figure 2**. The enhancement from MnESI-MS with the M3 emitter over high-flow LC-MS with HESI ranged from 2 to 150-folds with the median gain of ~12-fold (**Figure 2a**). In addition, using the same column and identical conditions, MnESI-MS achieved the median sensitivity gain of ~2.7 over conventional microflow LC-MS with a nanospray emitter (SStip).

We also performed calibration experiments for all pesticide compounds and quantified their limit of detection (LOD) individually. As shown in **Figure 3** for a representative compound (parathion methyl OA), excellent linearity was obtained and the LOD was determined to be 0.01 ppb by MnESI-MS in comparison to 1 ppb from high-flow LC-MS with HESI. The LOD values are consistent with the sensitivity improvement of ~110-fold between M3 emitter and HESI for this pesticide.

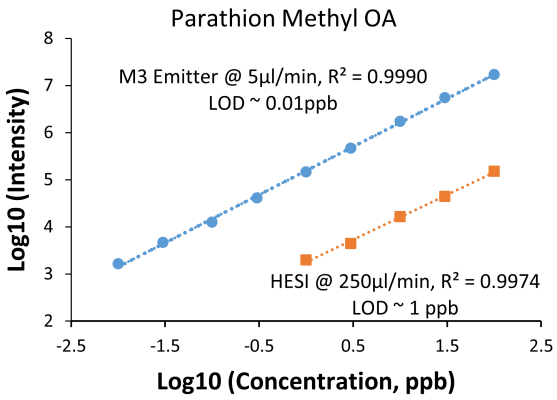


Figure 3. Calibration curve for parathion methyl OA using the MnESI-MS platform with a M3 emitter (LOD of 0.01 ppb) and high-flow LC-MS with HESI probe (LOD of 1 ppb).

2. MnESI-MS platform delivers excellent reproducibility and robustness

To assess the robustness and reproducibility of the MnESI-MS platform, we performed over 300 injections of grapefruit extracts spiked with 241 pesticides. **Figure 4** shows the peak area reproducibility with relative standard deviation (RSD) for six representative compounds. The RSD is less than 3.5 % for all of them. For all 241 compounds (**Figure 5**), we achieved the median RSD of 4.7% using the MnESI-MS platform in comparison to 6.4% using the high-flow LC-MS. The slightly better RSDs from MnESI-MS might be due to their much stronger MS signals than high flow HESI because of the over 10-fold sensitivity gain. In addition, we didn't observe any clogging or decreased performance after 300 injections on our M3 emitters. These results demonstrated the rugged performance and excellent reproducibility over time for MnESI-MS with our M3 emitters.

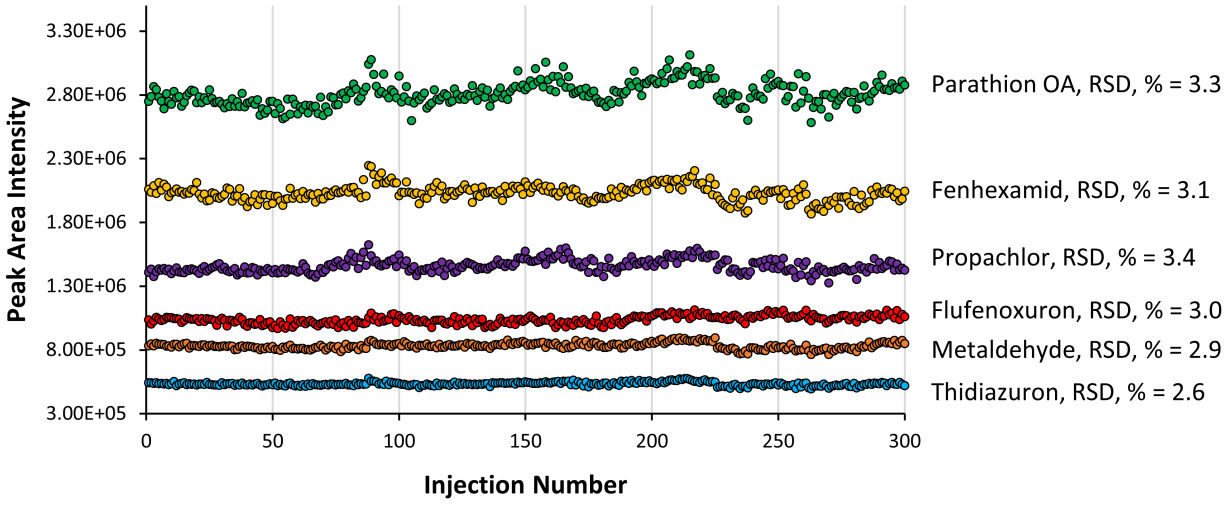


Figure 4. Peak area reproducibility of 300 injections of pesticides spiked in grapefruit by MnESI-MS with M3 emitters. The RSDs for six representative compounds are shown.

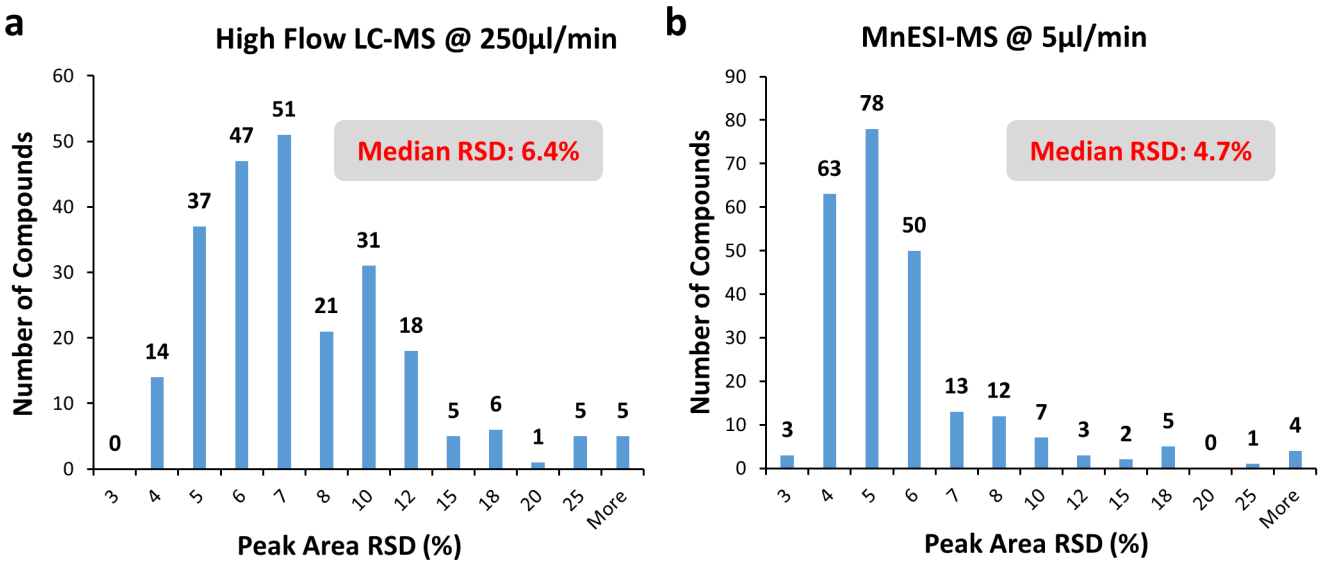


Figure 5. Peak area reproducibility by MnESI-MS with a M3 emitter in comparison to high-flow LC-MS by HESI over 300 injections. a) Histogram of the peak area RSD of 241 pesticide compounds measured by high-flow LC-MS with HESI probe. b) Histogram of the peak area RSD of 241 pesticide compounds measured by microflow LC-MS with a M3 emitter. The number of compounds out of the total 241 for each closest RSD value was shown on the bar graph.

Conclusion

In summary, we have demonstrated a MnESI-MS platform with M3 emitters as a unique solution that integrates micro-flow LC with nanospray MS, with the benefit of significantly enhanced mass spectrometry sensitivity over conventional high-flow needles and nanospray emitters. In addition, we have achieved the same level of reproducibility and rugged performance as high flow LC-MS, for LC-MS analysis of small molecules such as pesticides.

Reference

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[2] P. Mao, H-T. Wang, P. Yang, and D. Wang, Multinozzle Emitter Arrays for Nanoelectrospray Mass Spectrometry, Anal. Chem. 83, 6082 (2011)
[3] M. Anastassiades, S.J. Lehotay, D. Stajnbaher and F.J. Schenck, Journal of AOAC International, 86(2), 412-431 (2003)

Ordering Information

Product	Catalog #
MnESI Source for Thermo Scientific New Generation Mass Spectrometers	IS-T01
MnESI Source for Thermo Scientific Legacy Mass Spectrometers	IS-T02
MnESI Source for Bruker Mass Spectrometers	IS-B01
M3 Emitter, 10 µm ID, 8-nozzle	E8N10MU01
M3 Emitter, 20 µm ID, 8-nozzle	E8N20MU01
M3 Emitter, 20 µm ID, 5-nozzle	E5N20MU01
Flow Splitting Kit for Microflow LC-MS, 1-5 µl/min	FSK-01
Flow Splitting Kit for Microflow LC-MS, 5-15 µl/min	FSK-02
Flow Splitting Kit for Microflow LC-MS, 10-25 µl/min	FSK-03
Flow Splitting Kit for Microflow LC-MS, custom	FSK-10

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