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High-sensitivity, high-throughput pesticide analysis by micro-flow LC-MS

Runsheng Zheng[1], Christopher Pynn[1], Alec Valenta[2], Katherine Lovejoy[1], Ece Aydin[1], Kenneth Matuszak[2], Wim Decrop[1], Martin Samonig[1]

[1]Thermo Fisher Scientific, Germany; [2]Thermo Fisher Scientific, USA

Introduction

Purpose: Demonstrate the benefits of using micro-flow (50 µL/min, 1.0 mm i.d.) for pesticide analysis vs analytical flow (300 µL/min, 2.1 mm) without impacting sample throughput.

Methods: A novel LC-MS configuration comprised of a Thermo Scientific™ Vanquish™ Neo UHPLC system coupled to an Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer affording a 15-min micro-flow separation of 50 pesticide standards at 50 µL/min using a 1.0 mm i.d. x 100 mm Thermo Scientific™ Hypersil GOLD™ HPLC column. Results were compared to an analytical flow configuration using a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system at 300 µL/min using a 2.1 mm i.d. column with identical chemistry.

Results: This study highlights the advantages of micro-flow LC-MS which affords a 2 to 4-fold increase in peak area across fifty pesticides compared with analytical flow LC-MS (2.1 mm i.d., 300 µL/min). The sensitivity gain using micro-flow results from the combined benefits of reduced column i.d. and increased ionization efficiency, yielding higher peak heights and larger peak areas without impacting in peak widths (FWHM). The micro-flow method also provided low retention time variation without sacrificing sample throughput. More, the reduced flow rate resulted in a 6-fold reduction in solvent usage. Taken together, these data suggest that microflow LC-MS provides a more sensitive, cost efficient, and environmentally friendly alternative to analytical flow for pesticide analysis.

Materials and methods

Sample Preparation

A pesticide standard containing 50 compounds (Cat. No. 31975, RESTEK GmbH, Germany) was diluted to 100 pg/µL using a solvent mixture of 12.5% acetonitrile, 0.1% formic acid (v/v). The dilution was performed in a Thermo Scientific™ SureSTART™ high recovery vial (P/N: 6PSV9-V1) which was then sealed with a talcum-free screw cap (P/N: 6PSC9STB1).

LC-MS Method

Table 1 presents the LC parameters for both methods. The required hardware components of the Vanquish Neo UHPLC system are listed in **Table 2**. The Vanquish Neo UHPLC system was configured in the micro-flow Direct Injection workflow using 50 µm I.D. capillaries. The hardware components of the Vanquish Flex Binary UHPLC system are listed in **Table 3**.

The Vanquish Flex binary pump was equipped with a 35 µL mixer set for low gradient delay volume (GDV) applications. Data were acquired on an Orbitrap Exploris 240 MS in full scan mode (MS1). More detail can be found in **Table 4**.

Data Analysis

Data analysis was conducted using Skyline[1] software and R script[2].

Table 1. LC Methods and consumables

	Analytical flow	Micro-flow
LC	Vanquish Flex UHPLC system	Vanquish Neo UHPLC system
Column	Hypersil GOLD 100 x 2.1 mm, 1.9 µm (25002-102130)	Hypersil GOLD 100 x 1.0 mm, 1.9 µm (25302-101030)
Mobile Phases	A) Water/MeOH (95/5, v/v, %), 5 mM ammonium formate, 0.1% FA B) Water/MeOH (5:95, v/v, %), 5 mM ammonium formate, 0.1% FA	
Washes	Rear seal wash: water/methanol (90/10, v/v, %) Needle wash: water/methanol (80/20, v/v, %)	Rear seal wash: water/isopropanol (25/75, v/v, %) with 0.1% FA Strong needle wash: 100% acetonitrile with 0.1% FA Weak needle wash : 100% water with 0.1% FA
Temperature	50 °C, Still Air Mode	
Injection Amount	1 µL (100 pg/pesticide)	
Gradient	0.0 -1.0 min: 2%B; 1.0 - 2.0 min: 2–50% B; 2.0 - 9.0 min: 50 –99% B; 9.0 - 11.0 min: 99% B; 11.0 - 11.1min: 2% B; 11.1 - 14.0 min: 2%B	
Flow Rate	300 µL/min	50 µL/min
Spray needle	100 µm I.D.	50 µm I.D.

Table 2. Micro-flow UHPLC system

Module	Part number
Vanquish Neo UHPLC system	VN-S10-A-01
Vanquish Display, Column Compartment N	6036.1180, VN-C10-A-01

Table 3. Analytical Flow UHPLC system

Module	Part number
Binary Pump F with 35 µL mixer set	VF-P10-A and 6044.3870
Split Sampler F, Column Compartment, and System Base	VF-A10-A, VH-C10-A, VH-S01-A

Table 4. Orbitrap Exploris 240 parameters

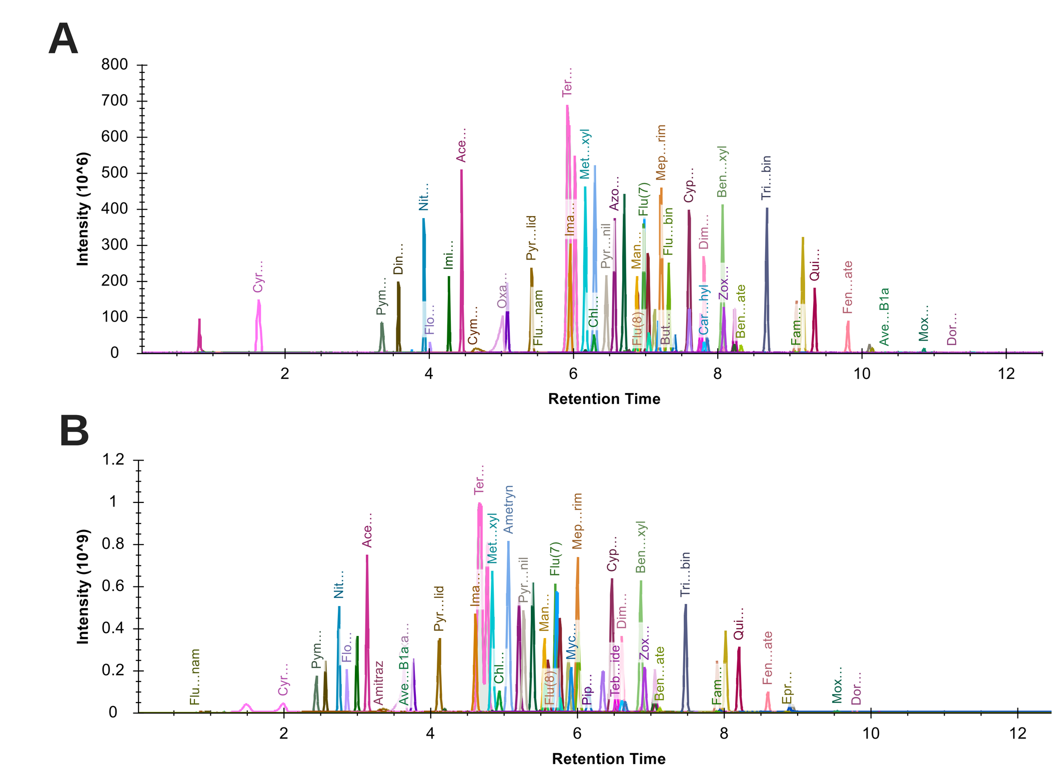
Parameter	Analytical Flow	Micro Flow
Spray Voltage (static, positive)	3300V	
Sheath Gas	30	
Auxiliary Gas	10	
Sweep Gas	0	
Ion Transfer Tube Temperature	325	
Vaporizer Temperature	350	
Acquisition Mode	Full Scan (MS1)	
Polarity	Positive	
RF Lens (%)	60	
Orbitrap Resolution	60,000	
Scan Range (m/z)	110-1100	
AGC target	Custom (300%)	
Maximum Injection Time	Auto	
Data Type	Profile	
Sweep cone	On	Off
Sweep gas	1	0

Results

Micro-flow chromatographic performance

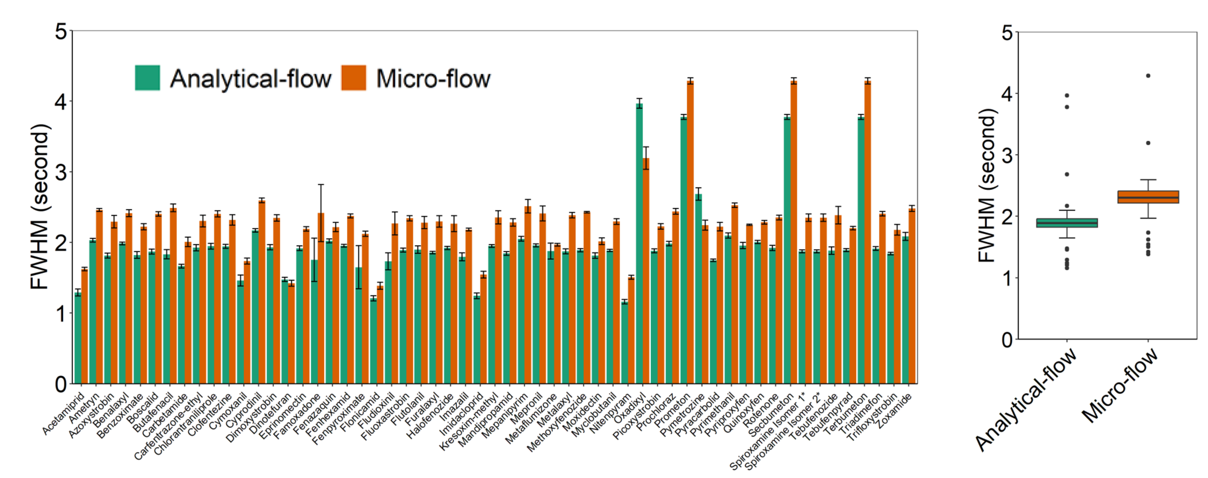
One concern of running low-flow applications is a reduction in sample throughput. Due to a low GDV (<2 µL) in the micro-flow configuration, the Vanquish Neo UHPLC system using a 1.0 mm x 100 mm column at 50 µL/min delivers a comparable chromatogram to analytical flow with a 2.1 mm x 10 cm column at 300 µL/min (**Figure 1**).

Figure 1. Extracted ion chromatograms of analytical (A) and micro-flow (B) methods.



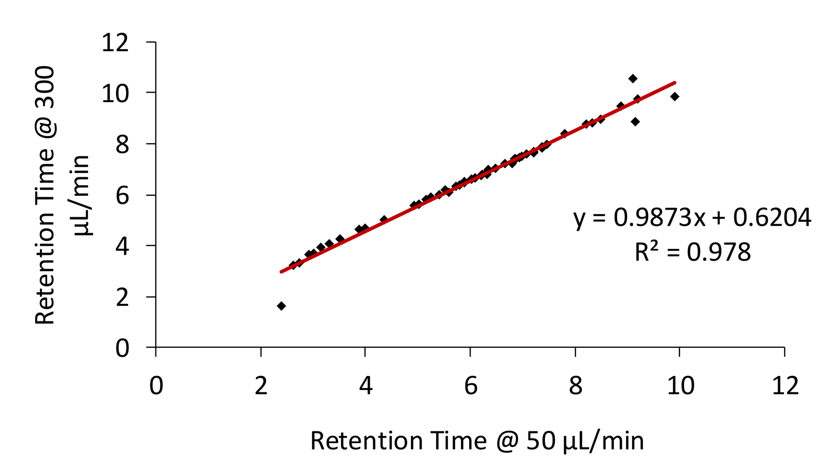
In addition, micro-flow yields similar peak FWHM, illustrating no significant loss in separation efficiency downscaling (**Figure 2**).

Figure 2. Pesticide peak width evaluation



Benefitting from the proportional downscaling of the chromatography conditions (flow rate and column i.d.), the micro-flow separation maintains the elution order of most of the analytes, simplifying method transfer (**Figure 3**).

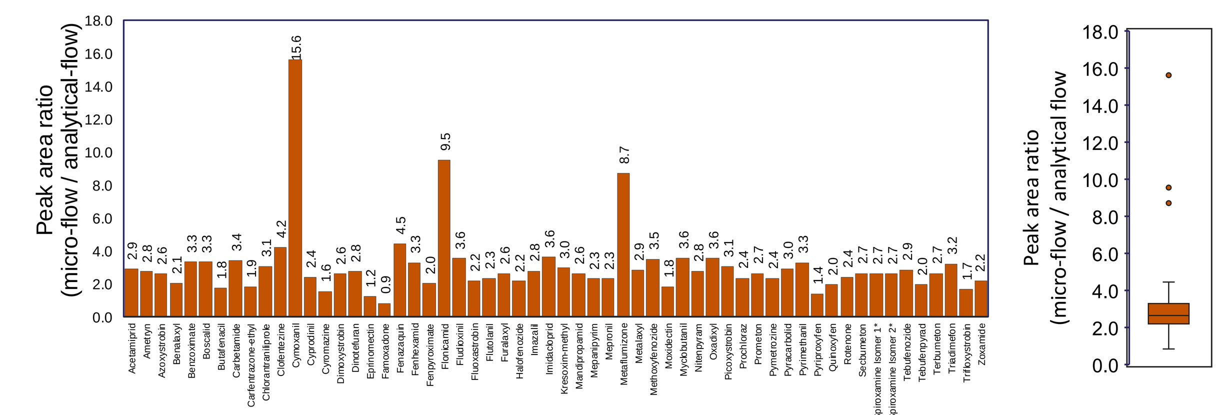
Figure 3. Retention time comparison



Boosted LC-MS micro-flow sensitivity

Using optimized LC-MS conditions micro-flow increases peak intensities and areas up to 16-fold with a median increase of 2.5-fold (**Figure 4**).

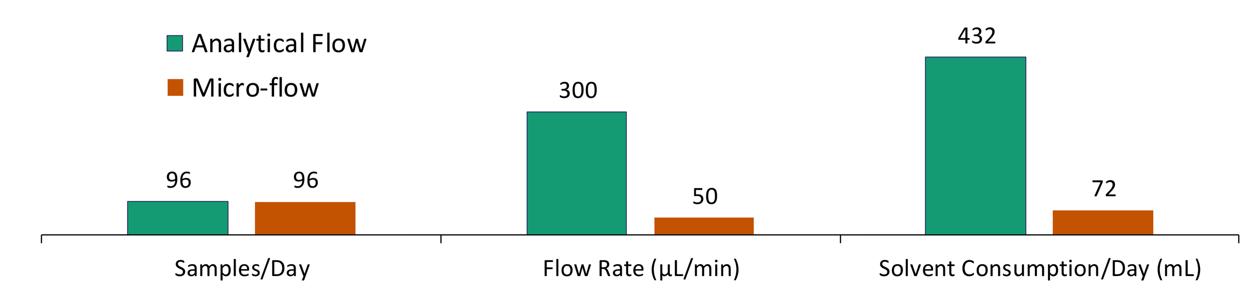
Figure 4. Pesticide peak area evaluation



Economic and environmental impact of downscaling

For a 15-min gradient, micro-flow reduced mobile phase consumption by 6-fold, from 4.5 mL to 0.75 mL per run. Solvent volume savings were ~360 mL/day or 10.8 L/month per instrument, yielding a decrease in cost and environmental footprint (**Figure 5**).

Figure 5. Method comparison



Conclusions

This work demonstrates the feasibility and benefits of analyzing pesticides using micro-flow LC-MS with a Vanquish Neo UHPLC system including:

- Comparable RTs and FWHMs to analytical flow
- Up to 16-fold (median at 2.5-fold) increased peak areas
- A 6-fold reduction in solvent consumption

References

1. Birgit Schilling, etc. Mol Cell Proteomics. 2012. 11 (5), page 202-214.

2. R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL https://www.R-project.org/.

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