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Food and beverage

Practical application of microflow LC-MS to multiresidue pesticides analysis in garlic extracts

Authors

Kenneth P. Matuszak, Fernando Vargas, Ed George, Maciej Bromirski

Thermo Fisher Scientific

Keywords

Orbitrap Exploris 120 mass spectrometer, Vanquish Neo UHPLC system, pesticides, garlic extract, microflow, QuEChERS, LC-MS

Application benefits

- The Thermo Scientific™ Vanquish Neo™ UHPLC system delivers maximum performance 24/7 for reproducible nano-, capillary-, and microflow LC-MS applications and is designed for both novice and expert users.
- Microflow LC-MS for pesticide residues analysis in food offers greatly reduced solvent consumption compared to standard flow systems that are considered the “gold standard” in food control laboratories.
- Microflow with the Vanquish Neo UHPLC system provides enhanced sensitivity when using narrow bore columns (0.3–1.0 mm inner diameter (i.d.)) without sacrificing throughput, and at the same time, maintaining robust operation with food extracts prepared using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) sample preparation method.

Goal

To demonstrate the benefits of using microflow LC-MS for quantitative assays—greatly reduced solvent consumption, little to no solvent waste production, and fast (~12 min) sample-to-sample cycle times

Introduction

High-performance liquid chromatography (HPLC) coupled with high-sensitivity mass spectrometry (MS) is a commonly accepted approach for pesticide quantification in complex matrices. Column flow rates for conventional LC-MS analyses have typically been in the range of 200–1,000 µL/min with columns having internal diameters ranging from 2.0 to 4.6 mm. In recent years, the interest in greener, more environmentally sustainable LC-MS configurations offering increased sensitivity and reduced solvent consumption has grown. Nanoflow chromatography (generally in applications with flows less than 500 nL/min) has been applied to LC-MS with columns less than 100 µm in diameter to both reduce solvent consumption and increase sensitivity, as the concentration at the end of the column increases as the square of the column diameter decreases. However, these methods:

- Have much longer run times than standard or microflow LC methods
- Suffer from a higher relative dead volume
- Require the use of a specialized ionization source

Microflow rates (1–100 µL/min) with microbore LC columns in the range of 0.3–1.0 mm i.d. present the following advantages:

- Microflow LC-MS offers higher sensitivity when compared to analytical flow LC-MS.
- It decreases mobile phase consumption and waste, lowering cost and environmental impact.
- It can be optimized to obtain similar cycle times to standard flow HPLC thus avoiding a sacrifice in productivity.

In the past, microflow chromatography (1–100 µL/min) has been a less utilized flow regime, mostly because there have been no reliable HLPC pumping systems that could operate efficiently at these flow rates. These pumps lacked the ability to operate at higher backpressures, which is required for time-efficient optimized chromatography. Also, the pump must be capable of creating reproducible mobile phase gradients and have a low dead volume throughout the flow path to avoid retention time variability and band broadening at microflow rates. Finally, a lack of high-quality analytical columns with small diameters in a variety of stationary phases and particle sizes made it difficult for labs to convert from standard flow to microflow LC.

In this application note, we describe a microflow method developed using a Vanquish Neo UHPLC system coupled to a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer applied to the analysis of multiresidue pesticides in a garlic extract. The data demonstrate that the problems have been overcome with the superior performance of the novel microflow configuration in the Vanquish Neo UHPLC and suggest that microflow LC-MS based pesticide analysis can provide a robust, more sensitive, cost-efficient, and environmentally friendly alternative to analytical flow for the analysis of complex food extracts.

Experimental

Materials and methods

The analytical column chosen for the experiments was a Fortis™ Technologies Evosphere™ AQUA 0.5 × 100 mm, 5 micron particle size (P/N EVOAQ-550503) HPLC column. This was chosen over several other phases and column diameters studied as it had the best overall peak shapes, backpressure profile, and retention for the pesticides. In addition, the flow rate was set at 50 µL/min with sample extracts directly injected into the system, allowing easy and rapid method optimization.

A 3 micron version of the same packing material was also evaluated. The maximum backpressure during the gradient for the 5 micron particle size was approximately 280 bar, and for the 3 micron it was approximately 500 bar. The 3 micron did not significantly improve the chromatography, so the 5 micron particle size was chosen for the subsequent experiments.

At this flow rate, the standard Thermo Scientific™ Ion Max™ ion source could be used. A 50 µm i.d. capillary low-flow HESI needle (P/N 30139) was substituted for the standard needle. To reduce post-column band-broadening, a 50 µm × 55 cm piece of Thermo Scientific™ nanoViper™ tubing (P/N 6041.5560) was used to connect the column to the ion source grounding union, and the tubing connection from the ion source to the HESI needle was replaced with a 50 µm × 15 cm piece of nanoViper tubing (P/N 6041.5124). A 12-minute injection-to-injection cycle time was developed with chromatography suitable for quantitative analyses.

Mobile phase solvents, pesticide standards, and sample extraction materials are described in Table 1.

Table 1. Reagents, standards, and solvents used for development of the micro-flow multi-residue pesticide method

Materials and parts	P/N
Formic acid, Optima™ LC-MS grade, Fisher Chemical™	A117-50
Acetic acid (ACN), Optima™ LC-MS grade, Fisher Chemical™	A11350
Ammonium formate, Optima™ LC-MS grade, Fisher Chemical™	A11550
Methanol, Optima™ LC-MS grade, Fisher Chemical™	A456-4
Acetonitrile, Optima™ LC-MS grade, Fisher Chemical™	A955-4
Water, Optima™ LC-MS grade, Fisher Chemical™	W64
LC Multiresidue Pesticide Standards Kit, Restek	31971
Thermo Scientific™ HyperSep™ Dispersive SPE Mylar™ pouches, 6 g of $MgSO_4$ and 1.5 g of $NaCH_3CHOO$	60105-341
Thermo Scientific™ HyperSep™ Dispersive SPE Clean-Up, 150 mg $MgSO_4$, 50 mg PSA, and 50 mg GCB	60105-202

Preparation of garlic extract dilution matrix (using graphitized carbon black-GCB)

Into a 50 mL polypropylene centrifuge tube, 2 g of garlic powder and 15 mL of H_2O (+1% ACN, HPLC grade) were added and left to incubate for 10 min at room temperature. After incubation, 15 mL of ACN (HPLC grade), 6 g of $MgSO_4$, and 1.5 g of $NaCH_3CHOO$ were added to the tube, which was vortexed and centrifuged (10 min) at room temperature. After centrifugation, a 1 mL aliquot of the ACN supernatant layer was dispensed into a microcentrifuge tube containing 150 mg $MgSO_4$, 50 mg PSA, and 50 mg GCB and centrifuged (10 min) at room temperature. After centrifugation, 250 µL of supernatant was diluted with 750 µL H_2O in an autosampler vial and mixed.

Preparation of pesticide mix stocks

Pesticide substock solutions of 1,000, 100, 10, and 1 ppb were generated using Restek's LC Multiresidue Pesticide Standards Kit (P/N 31971). 100 µL was taken from each of the ten ampules and pooled into an HPLC autosampler vial creating a pesticide stock mix at a concentration of 10 µg/mL (or 10 ppm). The stock mix was then serially diluted (100 µL into 1,000 µL) using MeOH to generate the substock solutions for use in the creation of the pesticide calibration curve standards.

Preparation of calibration curve standards

The standard curve was prepared by spiking the pesticide substock mixes in blank garlic extract to obtain the 50, 25, 10, 5, 1, 0.5, 0.1, 0.05, and 0.01 ppb calibration standards. This calibration range in the final extract represents approximately 0.3 µg/kg to 1,500 µg/kg based on the extraction procedure for 2 g of the garlic powder.

HPLC method

Parameter	Value			
Column	Fortis Technologies Evosphere AQUA 0.5 × 100 mm, 5 micron (P/N EVOAQ-550503)			
Mobile phase A	H_20 with 0.1% formic acid and 5 mM ammonium formate			
Mobile phase B	90:10 MeOH:H_20 with 0.1% formic acid and 5 mM ammonium formate			
Gradient	Time (min)	%A	%B	Flow (µL/min)
	0.00	100	0	50.00
	0.50	100	0	50.00
	1.50	50	50	50.00
	6.50	100	100	50.00
	8.50	100	100	50.00
	*Equilibration controlled by the Vanquish Neo system			

MS method

The Orbitrap Exploris 120 mass spectrometer was operated in targeted Selected Ion Monitoring (tSIM) mode, with the quadrupole set to pass a 1 amu wide window centered around each of the 43 pesticides (the selected pesticides had a diversity of structures and polarities to demonstrate the method application to a broad range of pesticide classes), and the Orbitrap analyzer was set to a resolution of 60,000 FWHM for mass analysis.

Parameter	Value
ESI spray voltage (V)	3,500
Sheath gas (Arb)	25
Aux gas (Arb)	5
Sweep gas (arb)	0
Ion transfer tube temp (°C)	300
Vaporizer temp (°C)	65
RF lens (%)	60
Orbitrap resolution	60,000
Data type	Centroid

Results and discussion

An example overlay of an extracted ion chromatogram (XIC) is shown in Figure 1. The extraction window was set at ±5 ppm mass accuracy and centered on the exact mass for the pesticide compounds quantitated. All 43 compounds elute between a retention time (RT) of 1.2 and 6.65 min. The majority of the peak widths at 10% of base were approximately 0.1 min.

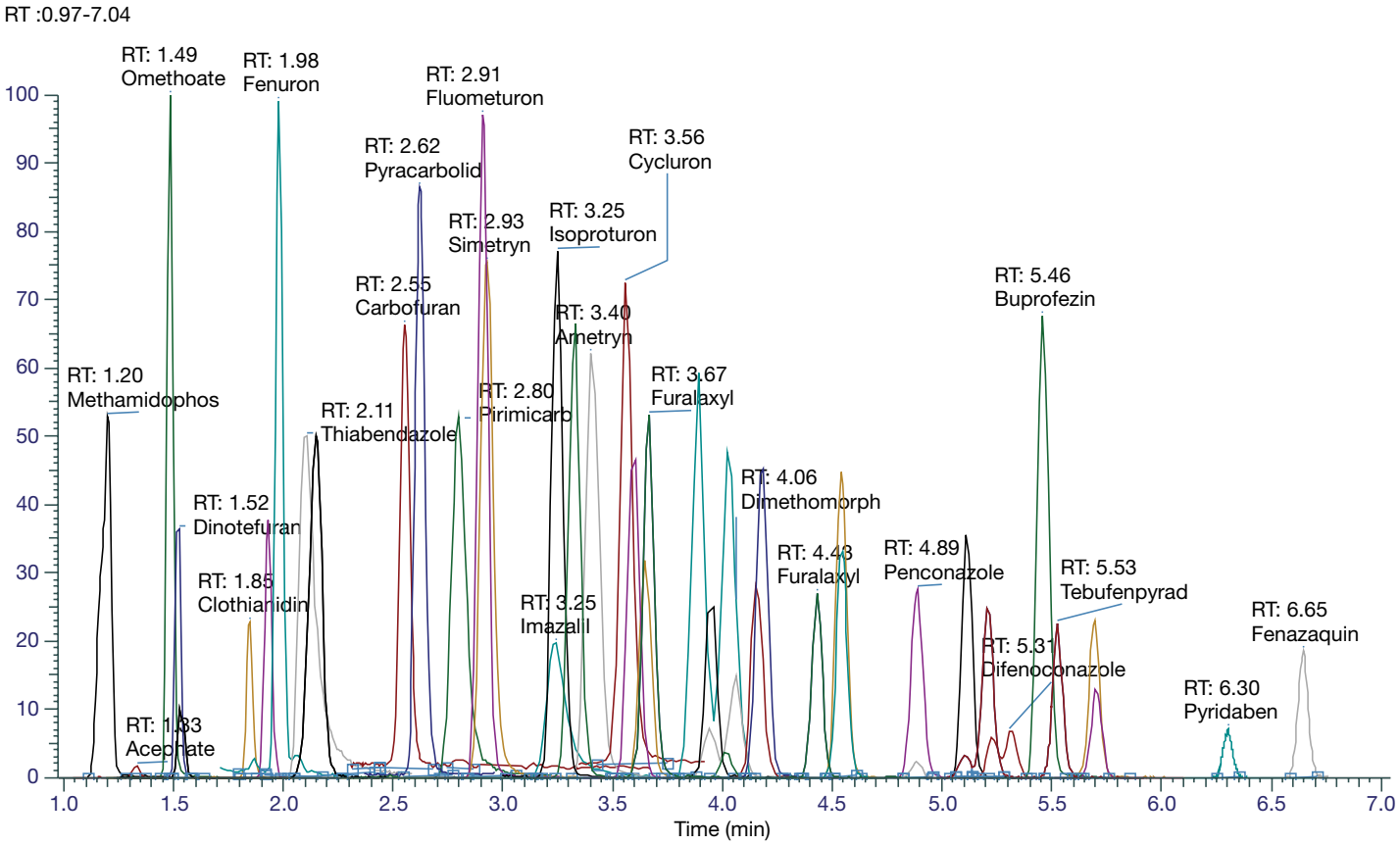

Figure 1. XIC overlay of 43 select pesticides (at 5 ppb in garlic extract) representing a wide range of chemical classes and retention times studied in the method

Calibration

Pesticides standards in matrix were injected in triplicate with 0.5 µL injection volume. There were no internal standards used in the method. The limit of quantitation (LOQ) was determined by analyzing three replicate injections at a concertation that resulted in an RSD of less than 25% and also showed a calculated amount within 25% of the theoretical amount.

Calibration curves showed little deviation of the 0.5 µL replicates. All replicate data points are plotted individually (Figure 2).

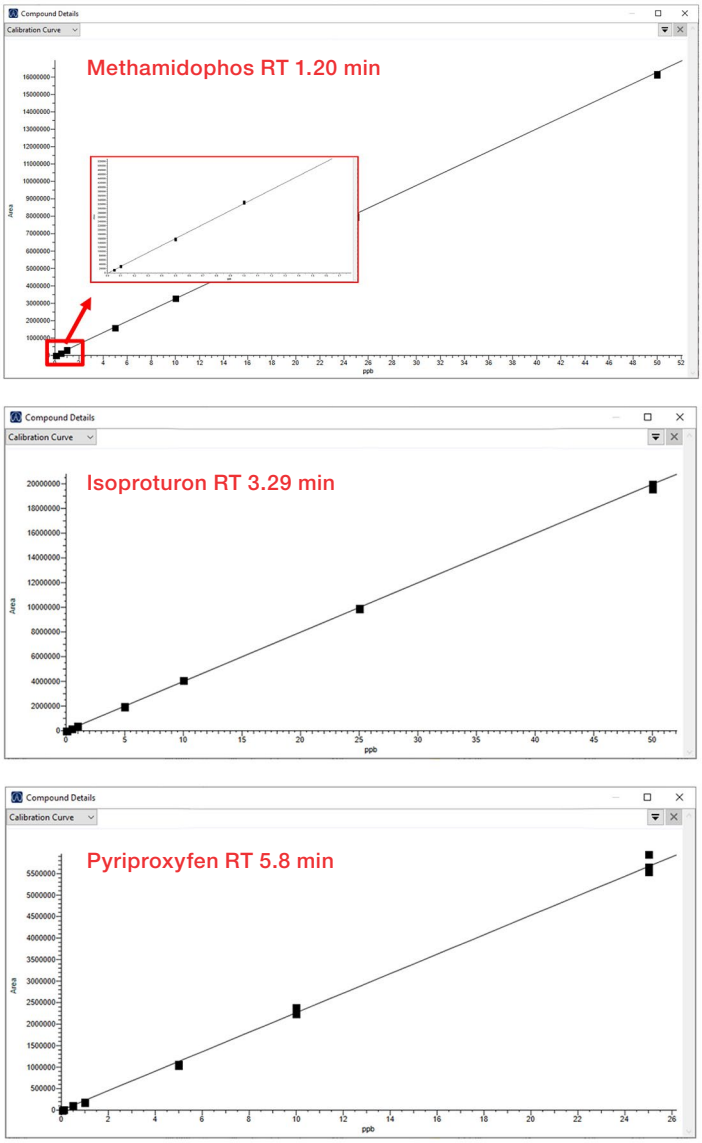

Figure 2. Calibration curves prepared in the garlic extract, with spiking concentrations of 0.01 to 50 ppb

Table 2 lists the linear ranges of the 43 pesticides in the garlic extract.

Table 2. Linear range (in ppb) for the pesticides studied in the garlic extract. The lowest point in the calibration table also represents the LOQ for each of the pesticides

Compound	RT (min)	Linear range (ppb)
Methamidophos	1.20	0.05–50
Acephate	1.33	1.00–50
Omethoate	1.49	0.01–50
Dinotefuran	1.52	0.05–50
Butoxycarboxim	1.53	0.10–50
Clothianidin	1.86	0.05–50
Imidacloprid	1.94	0.05–50
Fenuron	1.99	0.50–50
Thiabendazole	2.14	0.01–50
Acetamiprid	2.17	0.05–50
Carbofuran	2.59	0.50–50
Pyracarbolid	2.66	0.50–50
Pirimicarb	2.83	0.10–50
Fluometuron	2.95	0.01–50
Simetryn	2.96	0.01–50
Imazalil	3.30	0.05–50
Isoproturon	3.30	0.05–50
Methabenzthiazuron	3.38	0.05–50
Ametryn	3.44	0.05–50
Cycluron	3.62	0.50–50
Fenamidone	3.66	0.50–50
Azoxystrobin	3.71	0.05–50
Furalaxyl	3.73	0.05–50
Triadimefon	4.02	0.05–50
Dimethomorph	4.03	0.05–50
Terbutryn	4.03	0.05–50
Bifenazate	4.08	1.00–50
Spirotetramat	4.23	0.05–50
Mefenacet	4.26	0.05–50
Fenoxycarb	4.50	0.05–50
Flusilazole	4.62	0.05–50
Zoxamide	4.63	0.05–50
Penconazole	4.96	0.05–50
Bitertanol	4.97	0.50–50
Trifloxystrobin	5.20	0.05–50
Ipconazole	5.29	0.05–50
Difenoconazole	5.33	0.10–50
Buprofezin	5.56	0.01–50
Tebufenpyrad	5.61	0.05–50
Hexythiazox	5.80	0.50–50
Pyriproxyfen	5.80	0.05–25
Pyridaben	6.35	0.50–25
Fenazaquin	6.74	0.05–50

Robustness study

Robust operation at microflow rates is essential for laboratories operating in a high production environment. The aim of this method development was to demonstrate that a microflow method for pesticides could be practically applied without loss in productivity (sample-to-sample cycle time) and maintain good retention time and peak area response stability over time. For this study, two hundred injections of a spiked garlic extract at 5 ppb were injected to test the signal robustness using no internal standard correction and to also investigate retention time stability. An inherent advantage in microflow methods with narrow diameter columns is improved sensitivity, thus allowing smaller volumes of sample (0.5 µL) for direct injection into the system. As a result, very little matrix was being injected into the ion source, resulting in minimal contamination. Figure 3A is a composite plot of the signal stabilities (peak area) of seven representative compounds and Figure 3B is a plot showing a composite of the RTs of the same compounds.

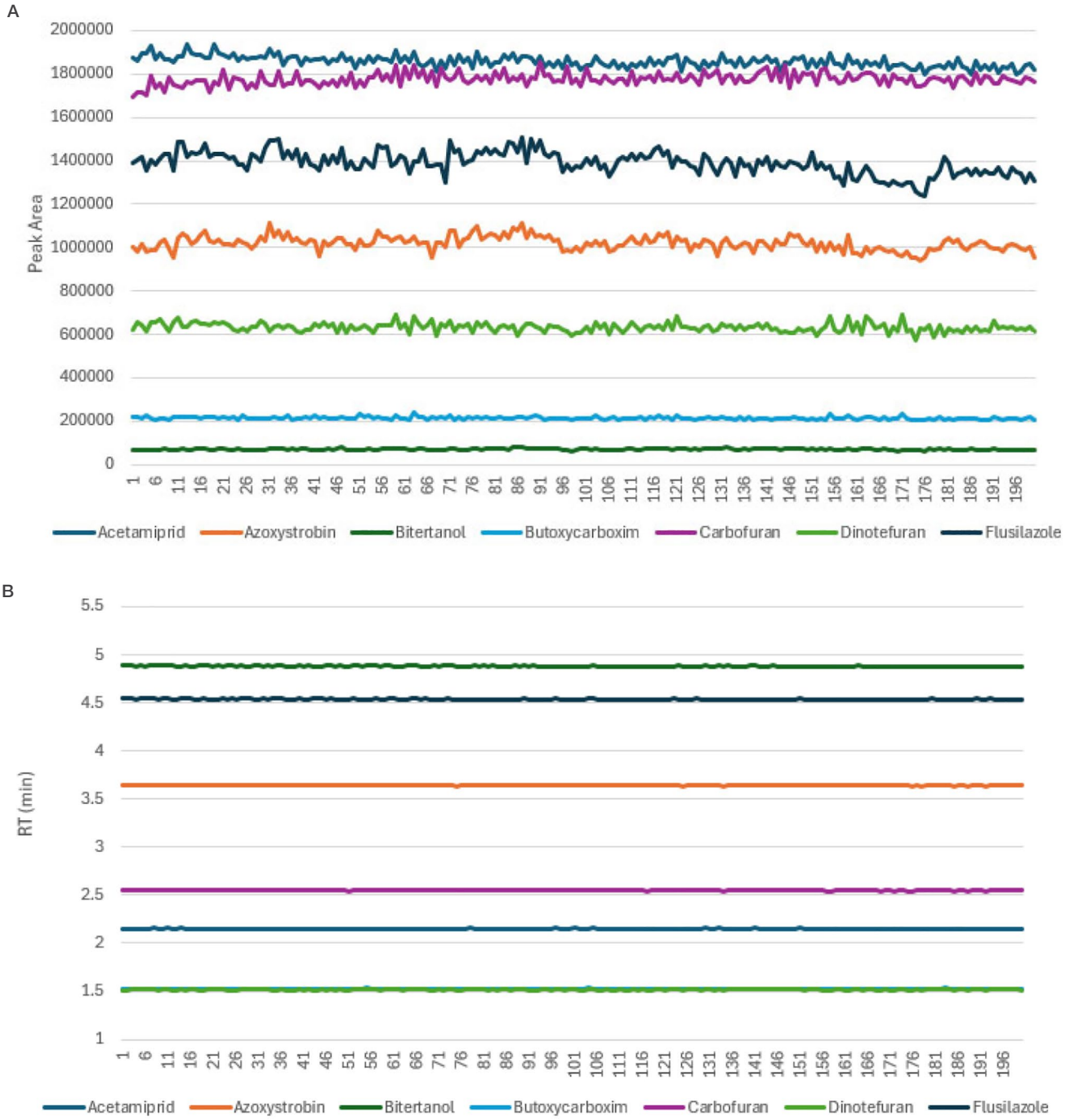

Figure 3. (A) Peak area stability for 200 injections of garlic extract spiked at 5 ppb for seven select pesticides chosen to cover a broad retention time in the method; (B) retention time stability for the same seven pesticides over 200 injections in the garlic extract

Table 3 shows %RSD of signal and RT for all 43 compounds spiked at 5 ppb in the garlic extract. These data show excellent reproducibility for the 43 pesticides in this study..

Table 3. %RSD of peak area and RT for the pesticides studied in the garlic extract

Compound	%RSD of peak area	%RSD of RT
Methamidophos	2.04	0.42
Acephate	5.76	0.47
Omethoate	2.77	0.48
Dinotefuran	3.21	0.32
Butoxycarboxim	2.88	0.29
Clothianidin	2.13	0.23
Imidacloprid	2.21	0.25
Fenuron	2.34	0.23
Thiabendazole	6.10	0.47
Acetamiprid	1.39	0.21
Carbofuran	1.59	0.16
Pyracarbolid	1.30	0.15
Pirimicarb	2.26	0.19
Fluometuron	1.19	0.12
Simetryn	3.76	0.16
Imazalil	9.98	0.33
Isoproturon	1.53	0.17
Methabenzthiazuron	1.65	0.15
Ametryn	4.40	0.10
Cycluron	1.79	0.16
Fenamidone	1.93	0.25
Azoxystrobin	3.15	0.13

Compound	%RSD of peak area	%RSD of RT
Furalaxyl	1.84	0.26
Triadimefon	2.61	0.19
Dimethomorph	2.63	0.00
Terbutryn	6.59	0.00
Bifenazate	7.74	0.19
Spirotetramat	3.00	0.17
Mefenacet	2.60	0.17
Fenoxycarb	3.24	0.14
Flusilazole	3.75	0.13
Zoxamide	2.29	0.14
Penconazole	2.73	0.10
Bitertanol	4.98	0.13
Trifloxystrobin	5.19	0.09
Ipconazole	4.23	0.11
Difenoconazole	6.02	0.00
Buprofezin	6.90	0.11
Tebufenpyrad	4.23	0.11
Hexythiazox	6.37	0.09
Pyriproxyfen	9.31	0.09
Pyridaben	13.75	0.08
Fenazaquin	15.24	0.08

Conclusion

- The developed microflow LC-MS method was shown to save mobile phase, thus reducing waste with significant cost savings benefit. For example, for every 100 injections, it was calculated that the method consumed only 37 mL of mobile phase A and 30 mL of mobile phase B.
- The cycle time from injection-to-injection was only 12 minutes, and therefore competitive with standard flow systems to maintain the high throughput required in high production labs.
- The observed robust area response and retention times demonstrate that the Vanquish Neo UHPLC system has a superior pumping system with low dead volume, overcoming the traditional roadblocks associated with transitioning from standard flow to microflow.
- The Orbitrap Exploris 120 mass spectrometer offers high resolution, low ppm mass accuracy, and the ability to acquire full scan data for retrospective analysis. Sensitivity gains in microflow LC-MS enable use of HRAM (high resolution accurate mass), and the method can be easily transferred to Themo Scientific triple quadrupole MS platforms.
- The Vanquish Neo UHPLC system can also be operated in a 'trap-and-elute' mode, and it is currently being applied to pesticide residue analysis. This is ongoing research and will be explored in further studies.

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