Comparing microflow vs analytical flow high-performance liquid chromatography for the analysis of pesticides

Using an OptiFlow® Turbo V Ion Source on a SCIEX QTRAP® 6500+ LC-MS/MS System

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Liquid chromatography (LC) has been applied to a wide range of environmental samples and combining this with tandem mass spectrometry (MS/MS) allows for highly sensitive and accurate measurements.[1] A large majority of LC-MS/MS methods rely on electron spray ionization (ESI), which is susceptible to matrix effects, including ion suppression.[1] However, the versatility of ESI and the wide range of compounds of environmental concern that are able to be analyzed using this method have made LC-MS/MS an important tool for environmental research and monitoring. As LC-MS/MS becomes a more important tool for the analysis of nonvolatile and polar contaminants, research has focused on creating more sensitive methods. Microflow LC has been shown to achieve sensitivity gains but until recently has primarily been used for peptide LC-MS/MS quantification.

Microflow LC operates at significantly lower flow rates (up to 100x lower) compared to traditional analytical high-performance LC systems.[1,2] These systems operate at flow rates in the range 1–200 µL/min and the droplets created have a diameter of only a few microns. These smaller droplets allow for more ions to be generated by ESI. Additionally, by decreasing the size of these droplets, ion suppression effects can be minimized, as there are fewer molecules to compete for charge.[1] This could be extremely valuable for environmental matrices, since these matrices are notoriously complex, even after extensive sample extraction techniques.[2,3]

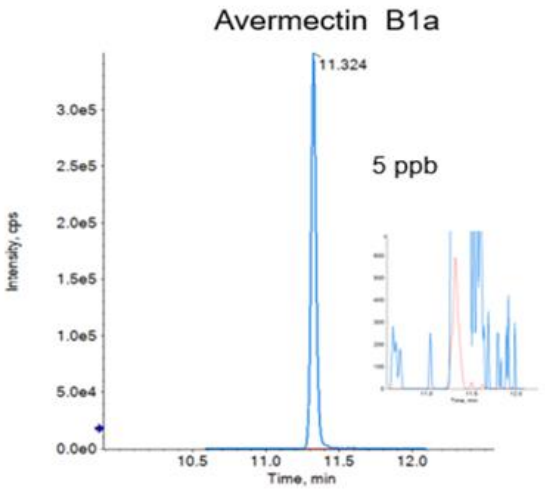

Figure 1. Significant gains in sensitivity. Large signal gains were observed when using microflow as compared to analytical flow chromatography, example shown here for avermectin B1a.

Here, a comparison of microflow LC and analytical flow LC for the analysis of 69 frequently analyzed pesticides was performed (Figure 1). Both methods use the same SCIEX QTRAP 6500+ LC-MS/MS System. The microflow method utilizes an OptiFlow Turbo V Ion Source in tandem with an M5 MicroLC System, while the analytical flow method utilizes an IonDrive™ Turbo V Ion Source coupled with an ExionLC™ AD System.

Key advantages of microflow liquid chromatography over high-performance liquid chromatography

- Sensitivity gains of up to 240x for the select pesticides
- No manual manipulation of optimal probe position needed for microflow analysis using the OptiFlow Source
- Solvent cost reduction compared to analytical methods

Methods

Shared method conditions: One of the key objectives of this study was to compare microflow and analytical flow HPLC methods. To accomplish this, parameters were kept as consistent as possible between the two methods. The MS/MS conditions that are compound specific were kept consistent between the two methods. Only source and gas conditions were adjusted (Table 1). The data were processed using SCIEX OS Software 1.7.

HPLC-specific method conditions: The HPLC separation was carried out using a SCIEX ExionLC AD System equipped with two LC-30AD binary pumps and a CTO-30A Column Oven. The chromatography column used was a Luna Omega 3 µm Polar C18 100 Å 100 × 4.6 mm (Phenomenex), and the temperature was maintained at 45 °C. The gradient conditions are outlined in Table 2. The injected sample volume was 1 µL. The extracted sample was analyzed using a QTRAP 6500+ System equipped with an IonDrive Turbo V Ion Source. The optimized source conditions can be found in Table 1.

Microflow-specific method conditions: The microflow analysis was performed using an M5 MicroLC System at a flowrate of 15 µL/min. An identical stationary phase was used but in a smaller diameter column (Luna Omega 3 µm Polar C18 100 Å 100 x 0.5mm (Phenomenex). The gradient conditions are outlined in Table 2. A 1 µL sample volume was directly injected. The same mobile phases were used, however, the gradient was optimized for the low flow rate. The sample was analyzed using the same QTRAP 6500+ System equipped with a OptiFlow Turbo V Ion Source that was designed specifically for lower flow rates. The optimized source conditions for this method can be found in Table 1.

Table 1. Comparing the source conditions used for analytical flow using the IonDrive Turbo V™ Ion Source vs microflow using OptiFlow Turbo V Ion Source settings for pesticide analysis.

Parameter	High flow source	Microflow source
Curtain Gas (CUR):	*45 psi*	*20 psi*
Ionspray Voltage (IS):	*4500 V*	*4000 V*
Heater Temperature (TEM):	*300 ºC*	*200 ºC*
Gas 1	*60 psi*	*15 psi*
Gas 2	*50 psi*	*60 psi*

Table 2. Comparing gradients used for microflow and analytical flow LC.

Analytical Flow (800 µL/min)		Microflow (15 µL/min)	
Time	% B	Time (min)	%B
0	*55*	*0*	*0*
0.5	*55*	*1*	*10*
2.5	*80*	*5*	*40*
8.5	*90*	*7*	*80*
12.5	*100*	*9*	*100*
16.5	*100*	*16*	*100*
16.6	*55*	*16.1*	*0*

Mobile phase A: 100% Optima grade water with 0.1% formic acid and 5mM ammonium formate
Mobile phase B: 100% acetonitrile with 0.1% formic acid and 5mM ammonium formate

Sensitivity comparison between microflow and analytical flow LC

The sensitivity between microflow and traditional analytical flow LC was compared by dividing the signal to noise (S/N) for the compound using the microflow method by the signal to noise of the compound using the analytical flow method. This ratio was measured at two points (1) the lowest point of the calibration curve in the analytical flow data, and (2) the highest point of the microflow calibration curve (Figure 2). The lowest point of the analytical flow data was used because microflow method

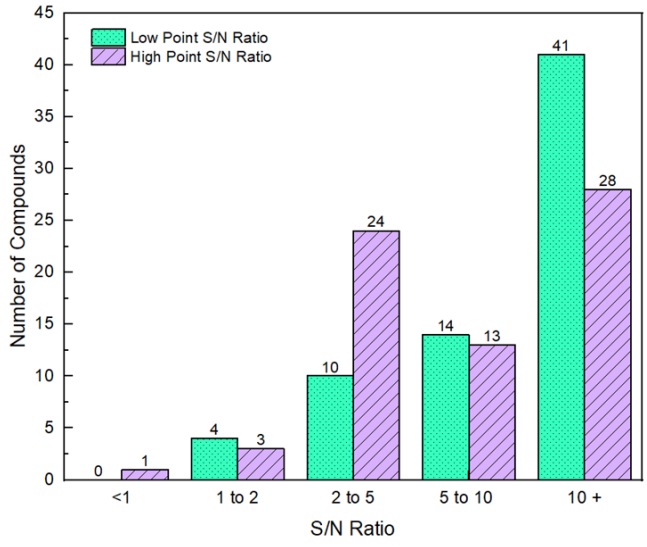

Figure 2. Signal to noise (S/N) gains between microflow and analytical flow LC. Here the S/N was compared at the LLOQ of the analytical flow data (green) and the highest point of the microflow data (purple). All 69 compounds analyzed saw an increase in S/N over analytical flow at its LLOQ. Many compounds had large S/N gains of 5 fold or higher.

provided calibration curve points below the LOQ of the analytical flow method. The highest point of the microflow calibration curve was used because detector and source saturation occurred first in the microflow LC data. Both the low point and high point calibration points were compound dependent.

All 69 of the compounds saw an increase in S/N using microflow LC at the low concentration point (Table 1). Three compounds were observed in the microflow data method that were not observed in the analytical flow method: methyl parathion, chlorfenapyr, avermectin B1b.

Of the remaining 69 compounds, 4 had a ratio between 1 and 2, 10 had a ratio between 2 and 5, 14 had a ratio between 5 and 10, and 41 (over 59%) have a ratio greater than 10 (Figure 2, green). The average S/N ratio at the low point was 29, with a standard deviation of 39 and a median value of 12 (Figure 3, green).

Table 1. Signal to noise gains using microflow chromatography. Signal to noise (S/N) ratio of the microflow peak area over the analytical flow peak area at lowest point of the calibration curve of the analytical flow data.

Compound	Low STD S/N Ratio	Compound	Low STD S/N Ratio	Compound	Low STD S/N Ratio
Acephate	*2*	*Dimethomorph I*	*31*	*Oxamyl*	*2*
Acequinocyl	*62*	*Dimethomorph II*	*31*	*Paclobutrazol*	*13*
Acetamiprid	*4*	*Ethoprophos*	*15*	*Permethrin, trans-*	*69*
Aldicarb	*29*	*Etofenprox*	*5*	*Permethrins cis*	*11*
Avermectin B1a	*89*	*Etoxazole*	*3*	*Phosmet*	*6*
Avermectin B1b	*>200x**	*Fenhexamid*	*4*	*Piperonyl butoxide*	*3*
Azoxystrobin	*39*	*Fenoxycarb*	*52*	*Prallethrin*	*3*
Bifenazate	*1*	*Fenpyroximate*	*59*	*Propiconazole*	*4*
Bifenthrin	*95*	*Flonicamid*	*49*	*Propoxure*	*8*
Boscalid	*12*	*Fludioxonil*	*26*	*Pyrethrins Pyrethrin I*	*6*
Carbaryl	*68*	*Hexythiazox*	*8*	*Pyridaben*	*3*
Carbofuran	*2*	*Imazalil*	*4*	*Spinetoram J*	*7*
Chlorantraniliprole	*24*	*Imidacloprid*	*6*	*Spinetoram L*	*6*
Chlorfenapyr	*>200x**	*Kresoxim-methyl*	*24*	*Spinosyn A*	*73*
Chlorpyrifos	*21*	*Malathion A*	*7*	*Spinosyn D*	*76*
Clofentezine	*62*	*Metalaxyl*	*25*	*Spiromesifen*	*7*
Coumaphos	*40*	*Methiocarb*	*7*	*Spirotetramat*	*15*
Cyfluthrin	*12*	*Methomyl*	*4*	*Spiroxamine*	*1*
Cypermethrin	*6*	*Methyl parathion*	*>200x**	*Tebuconazole*	*10*
Daminozide	*5*	*Mevinphos I*	*10*	*Thiacloprid*	*62*
Diazinon	*58*	*Mevinphos II*	*6*	*Thiamethoxam*	*20*
Dichlorvos	*239*	*Myclobutanil*	*27*	*Trifloxystrobin*	*143*
Dimethoate	*11*	*Naled*	*46*	*Fipronil*	*38*

*Compounds were not present under analytical flow conditions.

The three outliers that were observed in Figure 3 were dichlorvos, trifloxystrobin, and bifenthrin, with S/N gains in microflow of 239, 143, and 95 respectively.

The S/N ratios between microflow and analytical flow were also compared at the high concentration point of the microflow curve. Three had a ratio between 1 and 2, 24 had a ratio between 2 and 5, 13 had a ratio between 5 to 10, and 28 had a ratio larger than 10 (Figure 2). The average high standard S/N ratio was 14, with a standard deviation of 23 and a median value of 7 (Figure 3, purple). The 7 outliers observed were fipronil, cypermethrin, dichlorvos, spinosyn D, acequinocyl, phosmet, and Imidacloprid, with ratios of 110, 110, 85, 55, 52, 47 and 37 respectively.

Examples of the MRM chromatograms highlight the signal gains and the S/N gains observed in the microflow LC experiments (Figure 4).

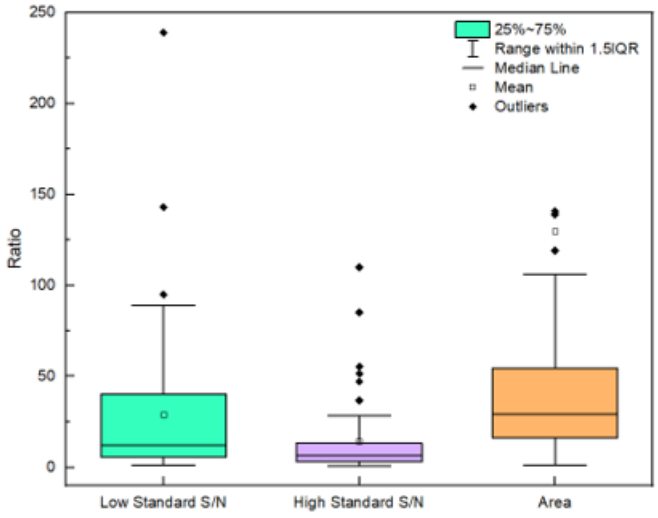

Figure 3. Comparison of S/N gains in microflow vs analytical flow HPLC. Again the S/N ratios for each compound between the flow regimes were compared at the low (green) and high (purple) concentration points. A range in S/N gains were observed at each but most compounds showed S/N gains., The peak areas between microflow and analytical was also compared (orange) at the low concentration point.

Cause of sensitivity gains

To determine the cause of the increase in sensitivity, several factors were investigated, including pKa, Log P, polar surface area, and temperature sensitivity. A Kendall's rank correlation was performed to identify significant correlations. Temperature was found to have the largest impact on sensitivity. To determine the compounds' temperature sensitivity, a standard containing all the pesticides was injected at a series of source temperatures, ranging from 350ºC to 550°C in 50ºC increments. The area was then plotted against the source temperature and a linear regression model was used to calculate the slope. The slope of this line was used to represent the compounds' temperature sensitivity. There was a significant correlation between the slope of this line and the increase in sensitivity.

To further assess this, two groups were made: (1) compounds with a negative slope and an r^2 above 0.7, and (2) compounds with a positive slope and a r^2 above 0.7. The compounds with a negative slope saw an increase in area with lower source temperatures, while the compounds with a positive slope saw an increase in area under higher source temperatures. It was found that the compounds that preferred a lower temperature had a median value of ~4x higher than the compounds that preferring higher temperatures (Figure 5).

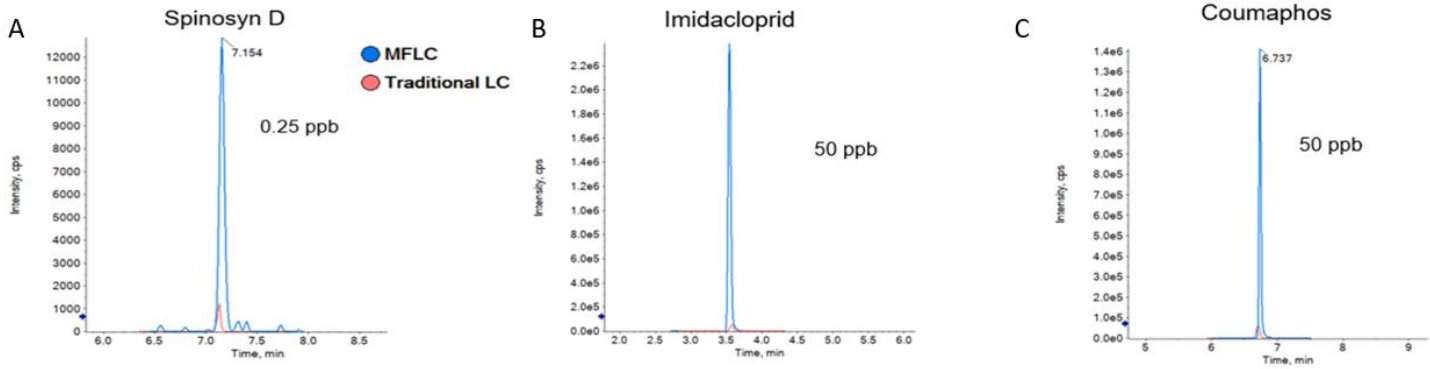

Figure 4. Example chromatograms comparing the two different flow regimes. MRM chromatograms comparing microflow liquid chromatography (blue) and traditional analytical flow liquid chromatography (orange) for four selected pesticides are shown; A) spinosyn D, B) imidacloprid, C) coumaphos.

Conclusions

Microflow LC was compared to analytical flow LC for the analysis of 69 commonly analyzed pesticides. All 69 pesticides showed an increase in sensitivity at the low point of the concentration curve. A major factor for the observed increase in S/N was related to temperature sensitivity. Compounds that were temperature sensitive experienced a larger increase in the S/N ratio. The M5 Microflow LC System coupled with an OptiFlow Turbo V Ion Source allows for more sensitive environmental methods and may play a key role in environmental monitoring efforts as lower limits of detection are required to protect public health.

References

1. Uclés Moreno A, et al. (2015) Microflow Liquid Chromatography Coupled to Mass Spectrometry-an Approach to Significantly Increase Sensitivity, Decrease Matrix Effects, and Reduce Organic Solvent Usage in Pesticide Residue Analysis. *Anal. Chem*. **87 (2),** 1018–1025.
2. Herrera López S, *et al.* (2016) Screening of Pesticide Residues in Honeybee Wax Comb by LC-ESI-MS/MS. A Pilot Study. *Chemosphere* **163**, 44–53.
3. Gil García MD, *et al.* (2017) MCM-41 as Novel Solid Phase Sorbent for the Pre-Concentration of Pesticides in Environmental Waters and Determination by Microflow Liquid Chromatography-Quadrupole Linear Ion Trap Mass Spectrometry. *Microchem. J.* **134**, 181–190.

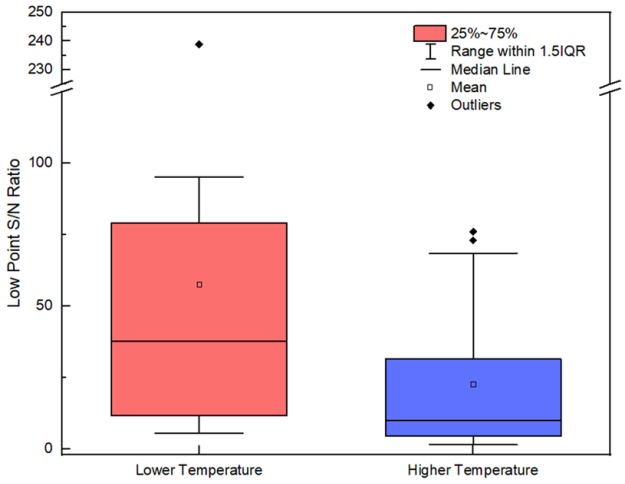

Figure 5. Impact of source temperature on pesticide signals. Signal to noise (S/N) ratios at the low concentration point were compared between microflow and analytical flow for the two observed classes of compounds; those that saw an increase in intensity with lower source temperatures (red) and compounds that saw an increase with higher source temperatures (blue).

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