The Use of Micro Flow UHPLC in Pesticide Screening of Food Samples by LC-MS/MS

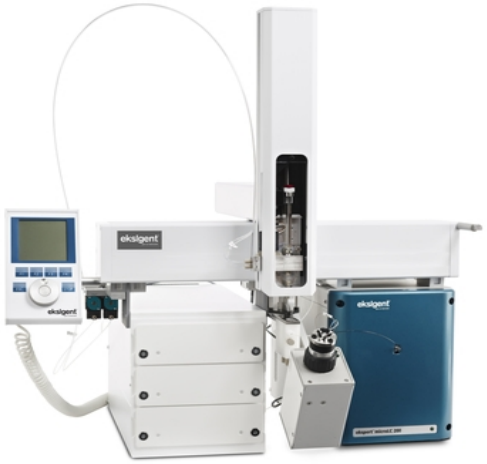
Reduce costs without sacrificing analytical performance by the use of micro LC

Stephen Lock
AB SCIEX Warrington (UK)

Introduction

Traditionally in pesticide screening of food, samples are prepared using generic extraction procedures, like QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe)[1, 2] and then analyzed by LC-MS/MS or GC-MS/MS. Usually in LC-MS/MS analysis, LC flow rates exceed 400 µL/min and are used in combination with small particle size HPLC columns with high pressures to maintain sharp peaks and fast chromatography. These flow rates produce excellent peak shapes and results, but have a draw back in that they require higher volumes of organic solvents. The consumption of HPLC organic solvents, such as acetonitrile and methanol, is a growing cost of analysis, and their disposal can have an adverse environmental impact. Therefore, new approaches to reduce solvent consumption in pesticide residue testing will be beneficial to the environment while also reducing the running costs of a testing lab.

Here we present new data using Eksigent ekspert™ microLC 200 System in combination with a LC-MS/MS method developed on an AB SCIEX QTRAP® 4500 system and utilizing the *Scheduled* MRM™ algorithm to maximize the number of data points across each peak. This approach was applied to a screen of over 100 pesticides in QuEChERS food extracts, and for the majority of these tests, the method was applied to an extract from chili powder, a matrix notorious for producing dirty extracts.



Materials and Methods

Sample Preparation

For linearity and sensitivity tests, calibration standards were prepared in water from concentrations 0.2 – 100 parts-per-billion (ppb). Chili powder and fresh basil were extracted using a QuEChERS method supplied with a kit from Supelco. Herb or spice (5 g) was mixed with water (10 mL) and acetonitrile (10 mL containing 0.05% acetic acid) in a 50 mL PTFE tube. Dispersive SPE (dSPE) $MgSO_4$ QuEChERS salts were added and the tube shaken (1 min) and centrifuged (5 min, 3500 rpm). The top layer (6 mL) was mixed with a dSPE PSA/C18 clean-up mixture and shaken (1 min) and centrifuged (5 minutes, 3500 rpm). The supernatant (100 µL) was diluted with water (900 µL) and injected (2 µL).

LC Conditions for Eksigent ekspert™ microLC 200 System

The LC system used for these tests was the Eksigent ekspert™ microLC 200. The system was run at 40 µL/min, which is at least 10 times lower than conventional LC separations using a 4.6 mm ID column. The separation of the 2 µL injection was done using a 0.5 x 50 mm Halo C18 column held at 50 ºC and with the

gradient profile shown in Table 1 where A = water and B = methanol, with both phases containing 2 mM ammonium acetate and 0.1% formic acid.

LC Conditions for UHPLC

The LC system used for comparative tests was a Shimadzu UFLC$_{XR}$ system consisting of two Shimadzu LC20AD pumps, SIL 20AC autosampler and a CTO20A column oven. The system was run at 400 µL/min with a conventional 4.6 x 5.0 mm Kinetex 2.6 µm core shell HPLC column held at 50ºC for a direct comparison. The same injection volume of 2 µL and gradient separation (Table 1) was used with the same mobile phases as with the micro flow LC analysis.

Table 1. Gradient conditions used for separation

Eksigent ekspert™ microLC 200			UHPLC		
Time (min)	A %	B %	Time (min)	A %	B %
0.0	98	2	0.0	98	2
2.0	98	2	2.0	98	2
9.5	30	70	9.0	30	70
10.5	5	95	10.5	5	95
11.0	5	95	11.5	5	95
11.5	98	2	11.5	98	2
15.0	98	2	15.0	98	2

M/MS Conditions

In this work, the AB SCIEX QTRAP® 4500 LC/MS/MS system (Figure 1) was used in positive mode with an IonSpray voltage (IS) of 5500 V. The method was set-up to detect 125 pesticides (250 MRM transitions), in a single injection, taken from the list contained in the SCIEX iDQuant™ Standards kit. Data was acquired using the *Scheduled* MRM™ algorithm.

For the high flow injection using the Shimadzu UHPLC, a standard electrospray electrode and Turbo V™ probe was used with a source temperature of 550 ºC, gas 1 (nebulizer gas) setting of 50 psi and a gas 2 (heater gas) settings of 60 psi. When the micro LC was used, the electrode was changed to a micro LC hybrid electrode (50 µm ID).[3] The installation of the micro LC electrode was fast and simple, requiring only the replacing of the standard electrode, taking approximately one minute for the exchange. The micro LC electrode is a hybrid PEEKSIL/stainless steel tip electrode, designed for low dead volume to eliminate peak dispersion and improve peak shape. The source settings were set-up for low flows, utilizing a lower source temperature and lower gas flow settings; however, the MRM settings were the same as used in the high flow method. This enables easy transfer of methods from a traditional high flow HPLC to the new Eksigent ekspert™ microLC 200 system.

Results and Discussion

In this work, all data was acquired and processed using Analyst® software version 1.6 and MultiQuant™ software version 2.1. The aim of this work was to test the micro flow LC applicability for routine food testing and compare the sensitivity and performance with a traditional, higher flow method already established for pesticide analysis. In this study, the chromatography was not optimized for speed, although the micro flow LC methods could be optimized to reduced run times, if desired (described briefly at the end of this application note). To compare the micro flow LC method with a higher flow analysis, a 2 ppb standard was injected. Extracted ion chromatograms comparing 2 pesticides eluting at different regions of the chromatograms are shown in Figure 1.

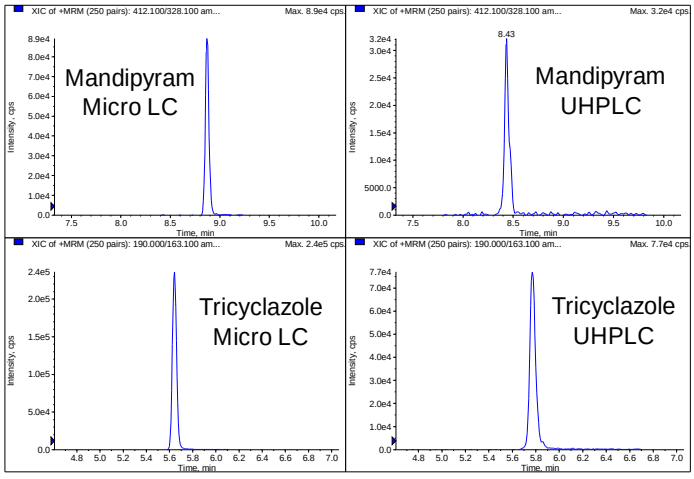


Figure 1. A comparison of micro flow LC and high flow LC

This result shows that the micro flow LC produces similar peak shapes when compared to normal flow rates due to the very low dead volume of the system. The comparative sensitivities are shown in Table 2, where a list of 10 pesticides spanning the run was compared. The results clearly demonstrate the increases in response, which ranged from a 3 fold to > 10 fold increase across the chromatographic separation (signal / noise values were taken directly from the MultiQuant™ software).

Table 2. Comparison of the signal / noise observed from a 2 µL injection of a 2 ppb standard using micro flow LC versus high flow LC

Pesticide	Retention time (min)	Signal / Noise micro LC	Signal / Noise UHPLC
Monocrotophos	4.05	1083.5	229
Tricyclazole	5.62	758.4	56.8
Simetryn	6.18	414.8	126.3
Monolinuron	6.89	432.6	40.2
Isoproturon	7.57	613.5	65.7
Terbutryn	8.03	883.7	92.5
Flutolanil	8.77	416.9	80.7
Fenoxycarb	9.44	99.8	16.7
Pyridaben	10.62	903.7	22.9

To confirm that the carryover between injections was very low, a 100 ppb standard was injected (producing a saturated response for most of the pesticides) followed by a water blank (Figure 2). For the majority of the pesticides, no carryover was observed in the water blank, with overall carryover estimated at < 0.1%.

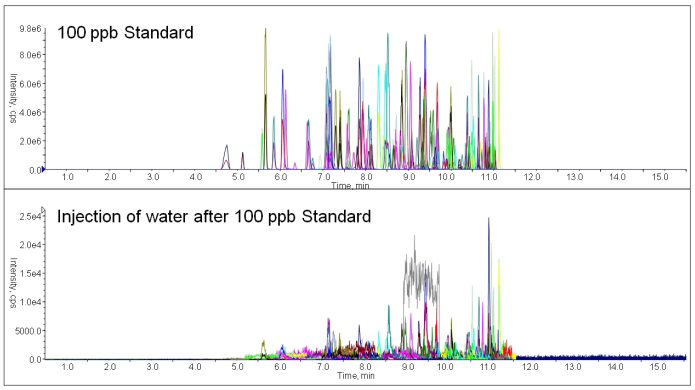


Figure 2. The top pane shows a 100 ppb calibration standard injected using the micro flow LC MS/MS set-up. The bottom pane shows water injected directly after this standard showing very low carryover.

The linearity of response for Flutolanil, analyzed using micro flow LC, is shown in Figure 3. This curve clearly demonstrates that the linearity of the method is preserved using micro flow LC, and this result is typical of what was observed for other pesticides in this analysis.

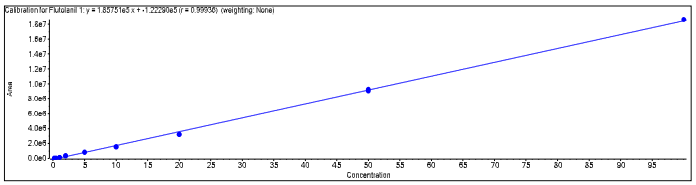


Figure 3. Example of a calibration line for one of the pesticides, Flutolanil, from 0.2 to 100 ppb. The fit used was Linear and the 'r' value obtained was greater than 0.999.

The robustness of the micro flow LC was also evaluated. In these tests, the system was stressed by repeatedly injecting unfiltered diluted QuEChERS extract of chili powdered (totaling over 150 injections). The retention time stability (Figure 4), response (Figure 5), and pressure curves (Figure 6) were then compared to see if the system had been affected by the large number of crude samples injected. The results showed outstanding reproducibility for the duration of the 150 injections, showing that micro flow LC is very robust and capable of withstanding long analytical runs that include 'dirty matrix' samples.

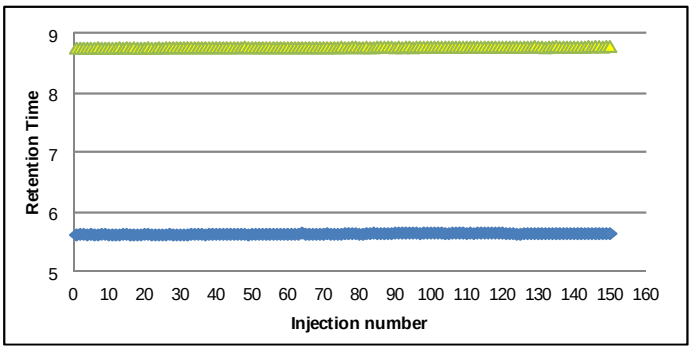


Figure 4. In this graph, retention time of two pesticides, Flutolanil (top) and Tricyclazole (bottom) were plotted against the injection number. The graph shows that the retention times obtained are rock solid with little or no variation between injections, confirming the low dead volume of the system and that fast equilibration times are possible.

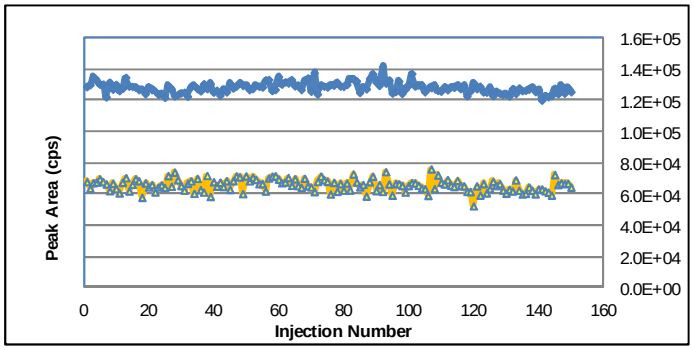


Figure 5. This graph shows the peak areas of two pesticides, Flutolanil (bottom) and Tricyclazole (top), which elute at different times during the run. It shows that the robustness is excellent with no deterioration in response even after 150 injections of a crude spice extract.

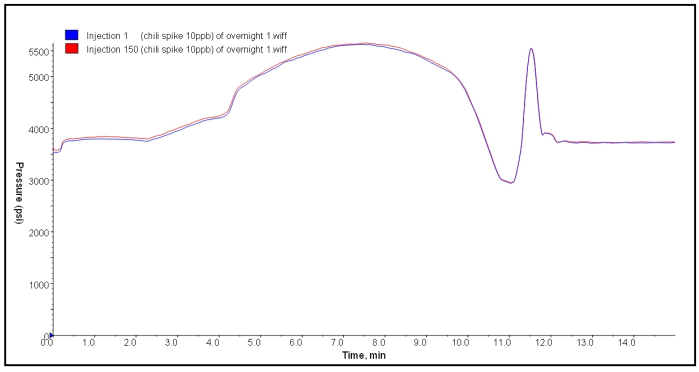


Figure 6. This figure compares the pressure profiles obtained from two injections of chili extract, 150 injections apart.

Finally, an additional advantage of micro flow LC is the ability to shorten the run times due to the low dead volume of the system. An example of this is shown in Figure 7 where the run time has been shortened from 15 minutes to less than 5 minutes. In this example, 6 µL of a 1 ppb pesticide standard containing over 200 pesticides was injected at 30 µL / min onto the same type HALO C18 column used in the above chilli extract analysis. The sensitivity was excellent, and the peak heights for some of the pesticides exceeded 1 million cps.

Conclusions

This study has clearly demonstrated that using micro flow LC is a valid approach in residue analysis in food samples.

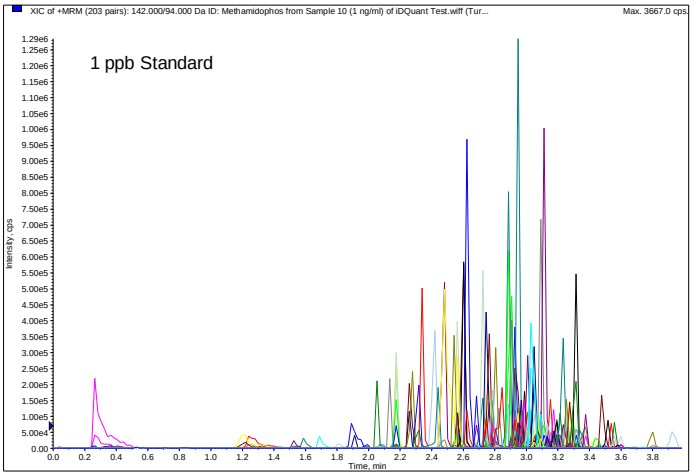


Figure 7. An example of the rapid gradient conditions that can be achieved using micro flow LC for pesticide residue analysis.

The method using the Eksigent ekspert™ microLC 200 system was quick, sensitive, robust and reproducible but also provides a huge cost saving to labs. With LC grade acetonitrile running at a cost of £100/L, this 3 day study could have cost about £ 100 with convention chromatography (0.6 mL/min running for 24 hours per day) and less than £10 with micro flow LC. Over one year, this corresponds to a savings of over £4000 (£90 x 50 weeks) in solvent consumption alone.

In addition, due to the very low dead volume of the micro flow LC, run times can easily be reduced by speeding up the gradient, greatly improving throughput for high volume testing laboratories. Finally, a great added benefit of micro flow LC analysis is the improvement in sensitivity, allowing greater dilution of sample extracts and the use of lower injection volumes to reduce matrix effects and improve robustness of the whole analysis.

References

1 M. Anastassiades et al.: 'Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce' J. AOAC Int. 86 (2003) 412-431

2 K. Mriziq et al.: 'Higher Sensitivity and Improved Resolution Microflow UHPLC with Small Diameter Turbo V™ Source Electrodes and Hardware for use with the ExpressHT™-Ultra System' Technical Note Eksigent (2011) # 4590211-01

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Headquarters
500 Old Connecticut Path, Framingham, MA 01701 USA
Phone 508-383-7700
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