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

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Traditionally in pesticide screening of food, samples are prepared using generic extraction procedures, like QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) [1] and then analysed 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 utilising the Scheduled MRM™ algorithm to screen for over 100 pesticides in QuEChERS food extracts. The method was applied to an extract from chilli powder, a matrix notorious for producing dirty extracts, revealing that microflow LC is robust enough to handle the challenging samples faced in a routine food testing lab.

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). Chilli 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 (10mL) and acetonitrile (10mL containing 0.05% acetic acid) in a 50mL 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 (6mL) 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

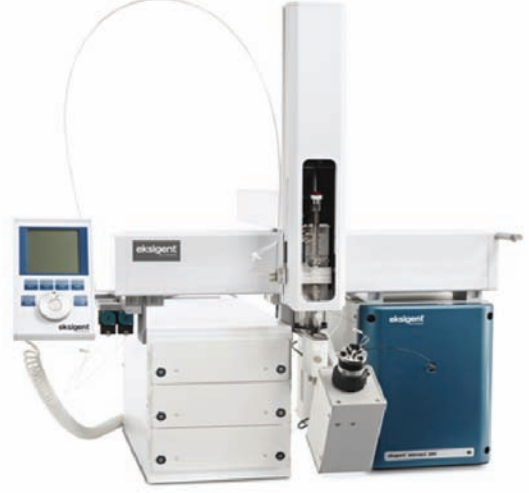
The LC system used for these tests was the Eksigent ekspert™ microLC 200. The system was run at 40 µL/min, which is at least 10 times lower than conventional LC separations using a 4.6mm ID column. The separation of the 2 µL injection was done using a 0.5 x 50 mm Halo C18 column held at 50ºC and with the gradient profile shown in *Table 1* where A = water and B = methanol, with both phases containing 2mM ammonium acetate and 0.1% formic acid.

LC Conditions for UHPLC

The LC system used for comparative tests was a Shimadzu UFLCXR 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 5.0mm 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 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 550ºC, gas 1 (nebuliser 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) [2]. 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, utilising 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 optimised for speed, although the micro flow LC methods could be optimised 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*.

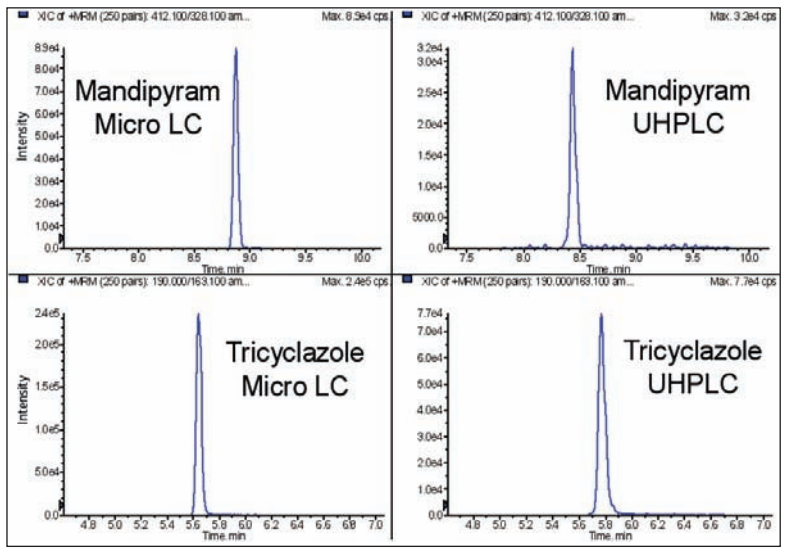


Figure 1. A comparison of micro flow LC and high flow LC

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 2µL 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%.

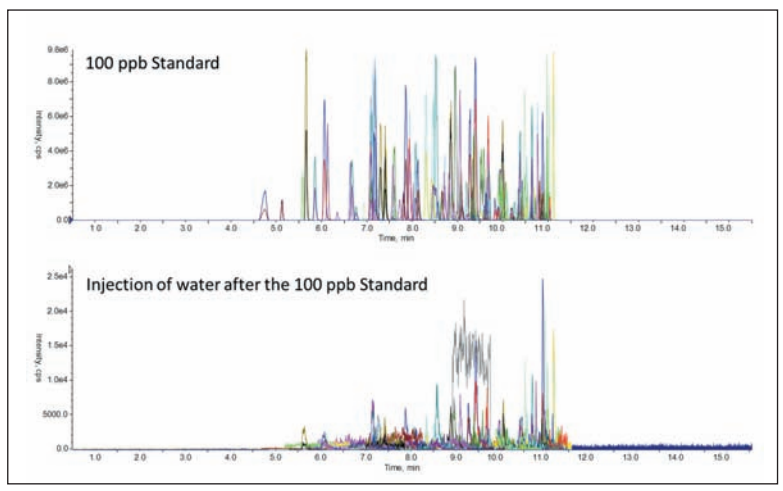


Figure 2. The top pane shows a 100 ppb calibration standard injected using the micro flow LC MS/MS set-up. The bottom pane shows water injected directly after this standard showing very low carryover.

The linearity of response for Flutolanil, analysed 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

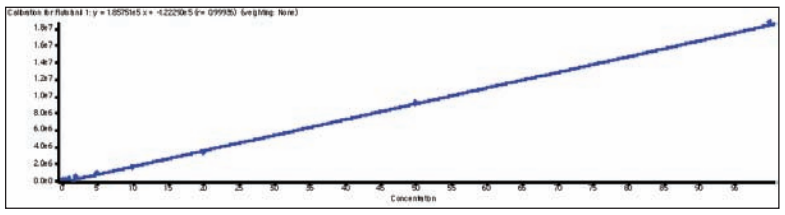


Figure 3. Example of a calibration line for one of the pesticides, Flutolanil, from 0.2 to 100 ppb. The fit used was Linear and the 'r' value obtained was greater than 0.999.

other pesticides in this analysis.

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 chilli powdered (totalling 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.

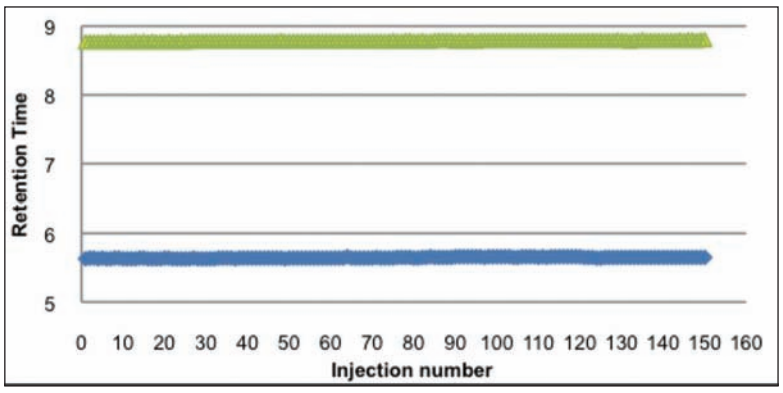


Figure 4. In this graph, retention time of two pesticides, Flutolanil (top) and Tricyclazole (bottom) were plotted against the injection number. The graph shows that the retention times obtained are rock solid with little or no variation between injections, confirming the low dead volume of the system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

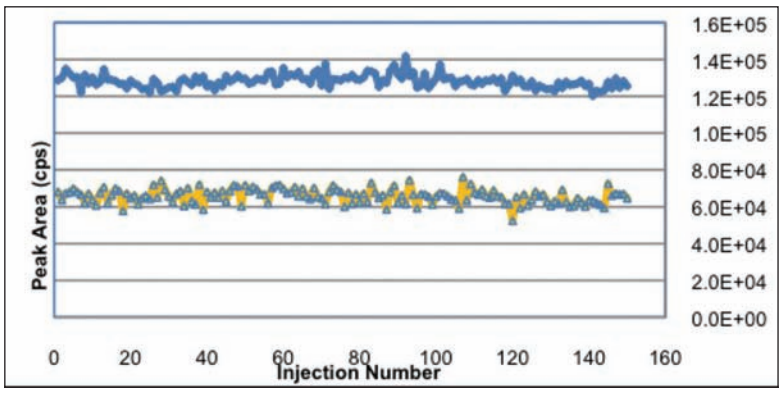


Figure 5. This graph shows the peak areas of two pesticides, Flutolanil (bottom) and Tricyclazole (top), which elute at different times during the run. It shows that the robustness is excellent with no deterioration in response even after 150 injections of a crude spice extract.

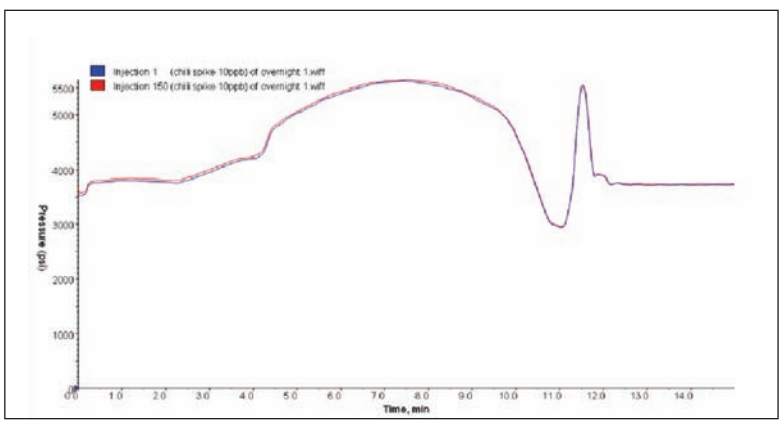


Figure 6. This figure compares the pressure profiles obtained from two injections of chilli extract, 150 injections apart.

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.

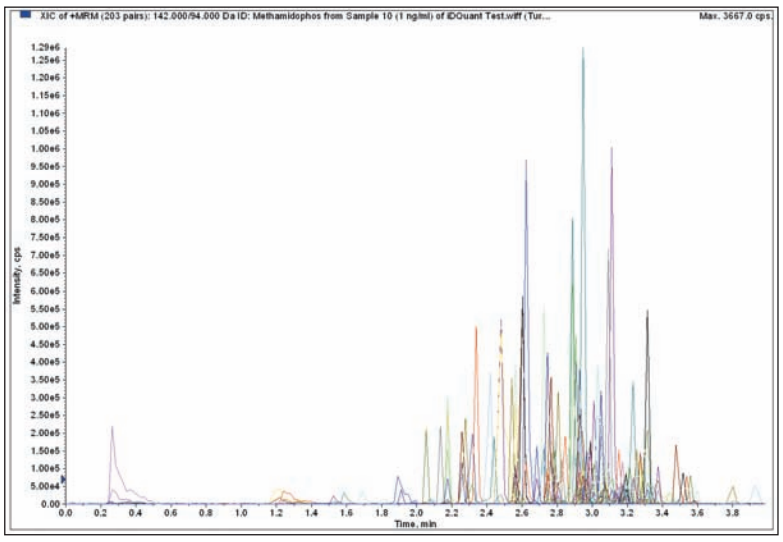


Figure 7. An example of the rapid gradient conditions that can be achieved using micro flow LC for pesticide residue analysis.

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 (0.6mL/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

Headspace Sampler for GC and GCMS Serves a Wide Range of High Sensitivity Applications

Shimadzu has launched the HS-20 gas chromatography headspace sampler for accurate analysis of an even wider range of volatile compounds with boiling points ranging from low to high. The HS-20 heats liquid or solid samples sealed in a container to a specific temperature, and injects the volatile compounds diffusing into the gaseous phase into a GC or a GCMS. These systems are widely used in the fields of environmental and pharmaceutical applications as well as in materials and food products analysis and forensics. The HS-20 will support these analyses particularly in testing and inspection organisations.

The unique configuration of flow lines and the oven enables the analysis of high boiling point compounds while minimising carryover. Using the electronic cooling trap, it is also possible to concentrate the headspace gas for analysis of compounds with extremely high sensitivity.

Headspace samplers enable easy analysis of volatile compounds. They are used in various fields requiring higher reliability, such as analysis of VOCs (volatile organic compounds) in the environmental and quality control applications of pharmaceuticals whereas in food products and materials control a wide range of volatile compounds with low to high boiling points has to be detected with high sensitivity in order to provide accurate qualitative and quantitative results for numerous measurement targets.

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Release of a New Control Driver for ELSD System

DataApex is pleased to announce the release of a new control driver for the Evaporative Light-Scattering Detector 90LT, manufactured by Sedere Company. This is already the 3rd Sedere detector its control and digital data acquisition has been incorporated into Clarity Chromatography SW. The driver for ELSD 90LT has been officially released with Clarity Chromatography Software version 4.0.3.

Sedere is a leading manufacturer of Low-Temperature Evaporative Light-Scattering Detectors. Sedere has more than 25 years of experience in the development of versatile detection systems for all chromatography applications and their detectors are distributed worldwide.

Clarity Chromatography Software has a strong position in the chromatography data systems market. Clarity, the third generation of DataApex products, allows controlling more than 400 different instruments from the single environment and offers its users very high flexibility. Clarity is highly regarded for its intuitive approach, excellent performance, cost-effectiveness and proficient technical support.

DataApex is solely focused on chromatography software development. A strong emphasis is placed on technological innovation, visionary adoption of new laboratory standards, best practices and extensive customer support. DataApex products are sold in over 80 countries around the world. Ten chromatography instrument manufacturers privately resell labelled versions of DataApex's software.

MORE INFO. 314

Interview on Technologies and Services Available for Download

In a recent interview by American Laboratory editors with Collin D'Silva and Rohan Thakur, President and Vice President of the **Bruker** CAM division were probed on their business strategies, technology, services and markets and the article is available for download http://bit.ly/BCA450. Find out how Bruker CAM has developed its product lines, what is next on the agenda, and how this affects you as either a soda drinker or airplane traveller. Over the past two years Bruker has made a strategic move to extend their capabilities into the chemical and applied markets. Through innovation and a higher R&D investment than average, Bruker has designed new instrumentation and software platforms that directly address the needs of the user. With gas chromatographers (GC) suitable for almost any application, Bruker has become a major player in the GC field. By eliminating barriers to the use of GC – for example with a 14 language user interface – their global presence is increasing. Double award winning system such as the SCION GC-MS TQ, and state-of-the-art electronic architecture has allowed Bruker to support companies worldwide in markets and applications such as food testing (pesticide residues and beverage grade CO_2), renewable energy and biofuels, such as creating biojet and using beverage grade CO_2 analysers. Turnkey solutions requiring minimal user input and training mean all of these benefits are available for any laboratory undertaking routine chemical analysis in the food testing, environmental monitoring, water testing, toxicology, forensics and sports medicine markets.

"With a significant presence in over 30 countries, Bruker CAM sets the standard not only for analytical instrumentation, but software and methodology too. We continue to invest our R&D resources in the redevelopment and advancement not only of existing product lines but also new ones such as the SCION" explained Collin D'Silva. " The introduction of our new websites such as globalfoodtesting.com and globalenergytesting.com means our customers can match our capabilities in relation to their needs. With the pressure on analysts to 'do more, with less, more quickly' we have had proven success with applications such as pesticide screening."

MORE INFO. 315