LC-MS/MS Analysis of Water using the Eksigent ekspert™ microLC 200 and AB SCIEX QTRAP® 4500

Maximizing performance, reducing costs

Jason Causon
AB SCIEX, Warrington, UK

Introduction

Water testing is typically performed using large volume injections combined with UHPLC-MS/MS. This utilizes high flow rates and small particle size UPLC columns. This is used to yield higher resolution and greater sensitivity, but at the sacrifice of higher column backpressures. The other drawback to UHPLC is the high consumption of solvents especially methanol and acetonitrile. This is an ever growing cost in both purchasing and disposal due to their environmental impact.

Micro flow chromatography with column diameters ≤1mm is an exciting approach for sensitive, high-throughput LC-MS/MS for environmental testing. It has been demonstrated that reducing the LC flow rate and using micro flow LC-MS/MS can result in an increase in sampling efficiency and sensitivity compared to conventional HPLC flow rates of 0.2 mL/min or greater.[1] The other benefits of micro flow chromatography include reduced solvent consumption, smaller injection volumes and reduced contamination of the mass spectrometer.

The Eksigent ekspert™ microLC 200 system is a dedicated micro flow UHPLC system that has been designed for optimal performance in the micro flow regime. It includes a new autosampler injection system with modifications for very small volume sample handling, minimal sample waste and very low carryover.

Here we present a new approach using low volume sample injections on the Eksigent ekspert™ microLC200 system on an AB SCIEX QTRAP® 4500 to quantify pesticides in environmental water samples.

Key Features of the Eksigent ekspert™ microLC 200 System

- High performance pumping system
  - Microfluidic Flow Control™ (MFC) for accurate rapid gradients with exceptional accuracy and reproducibility
  - Robust UHPLC performance with operating pressures up to 10,000 psi
- Fast reproducible sample injections
  - Small volume injections with minimal sample waste
  - Very low carry-over
  - Lowest delay volumes enable ultrafast gradient separations for LC/MS applications
- Green LC with smaller ID columns to reduce mobile phase consumption by over 95%, providing significant cost savings
- Robust integration with AB SCIEX hardware and software

Experimental

Liquid Chromatography:

All experimental data was acquired with the Eksigent ekspert™ microLC 200 system equipped with a HTC-xt Dynamic Load and Wash (DLW) autosampler. The ekspert™ microLC 200 system's solvent delivery system is based on binary gradient pumps that use patented Microfluidic Flow Control™ (MFC) pump technology

The column used was an Eksigent ChromXP™ Halo Fused-Core 2.7 µm, Phenyl Hexyl 0.5 x 150 mm. The column temperature was maintained at 30°C for all experiments. The mobile phases were ammonium formate in water and methanol. The flow rate was set at 15 µL/min with a 4 µL injection volume.

For the conventional HPLC experiments a Kinetex Fused-Core column was used with a flow rate of 600 µL/min and a 4 µL injection volume. The following gradient profile was used for both micro flow and conventional flow (Table 1).

Table 1. Analytical gradient profile used at both flow rates

Time (min)	A (%)	B (%)
0.0	95	5
0.5	95	5
5.0	25	75
7.0	5	95
8.5	5	95
8.75	95	5
15.0	95	5

Mass Spectrometry:

The AB SCIEX QTRAP® 4500 system was used for all data acquisition. Analyses were performed using multiple reaction monitoring (MRM) with simultaneous positive/negative switching electrospray ionization. The Eksigent hybrid 25 µm ID electrospray electrode was used for all micro flow analyses. This hybrid electrode is designed to minimize post column peak dispersion to maintain excellent peak shapes.[2] The source settings were setup for micro flow rates with the curtain, nebulizer and heater gas set to 20 psi with a temperature of 350°C. For the conventional flow rates the source conditions were scaled to the appropriate values.

Results and Discussion

The aim of this work was to determine the quantitative performance using low volume injections with micro flow rates. To establish the performance a number of pesticides were spiked into water and serially diluted down to the limits of detection. The dilutions were analyzed at both flow rates; Figure 1 shows a comparison for Desphenyl-chloridazon at 1ng/mL.

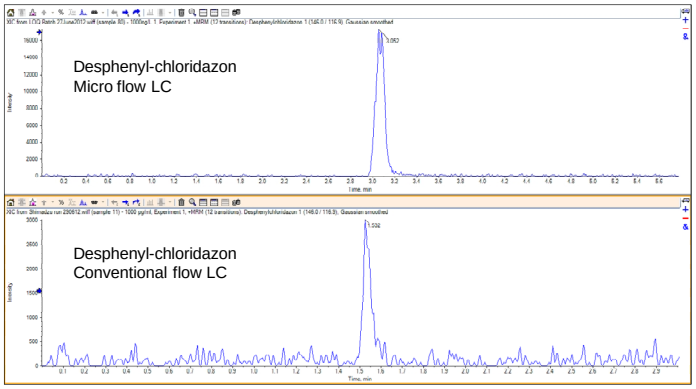

Figure 1. Desphenyl-chloridazon analyzed using micro vs. conventional flow LC-MS/MS (1 ng/mL)

At 1 ng/mL when comparing the peak areas at both flow rates the micro flow showed a 6.5 fold increase in peak area and a 10 fold increase in signal to noise. The parent Chloridazon was also analyzed and the comparison is shown in Figure 2.

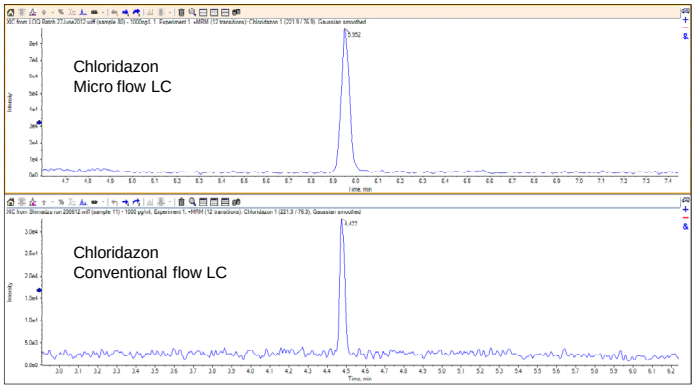

Figure 2. Chloridazon analyzed using micro vs. conventional flow LC-MS/MS (1 ng/mL)

At 1 ng/mL when comparing at both flow rates, micro flow showed a 4 fold increase in both peak area and signal to noise. Both compounds showed when using the ekspert™ microLC 200 system significant sensitivity gains. Table 2 shows the lower limits of quantitation (LLOQ, where signal to noise is 10) for both micro and conventional flow.

Table 2. Comparison of lower limits of quantitation (LLOQ)

Compound Name	LLOQ (ng/L) Micro Flow LC	LLOQ (ng/L) Conventional Flow LC
Chloridazon	50	500
Desphenyl-chloridazon	50	500
Atrazine	10	25
Atrazine-desethyl	10	50
2,4-D	50	250

Table 2 shows the ekspert™ micro LC200 system gave a 2.5 to 10 fold lower limit of quantitation compared to conventional flow HPLC. The accuracy and precision of the micro flow methodology was tested at and around the limits of quantitation. As shown in Table 3 all five pesticides gave very good precision and accuracies of less than 10% and +/- 3% respectively.

Table 3. Comparison of lower limits of quantitation (LLOQ)

Compound Name	CV (%)	Accuracy (%)
Chloridazon	6.6	102
Desphenyl-chloridazon	4.2	101
Atrazine	4.6	99
Atrazine-desethyl	8.9	103
2,4-D	3.9	103

The linearity of response for atrazine and atrazine-desethyl were analyzed from the LLOQ to 1 µg/L. shown in Figure 3. Both pesticides gave an ‘r’ value of 0.999 or better.

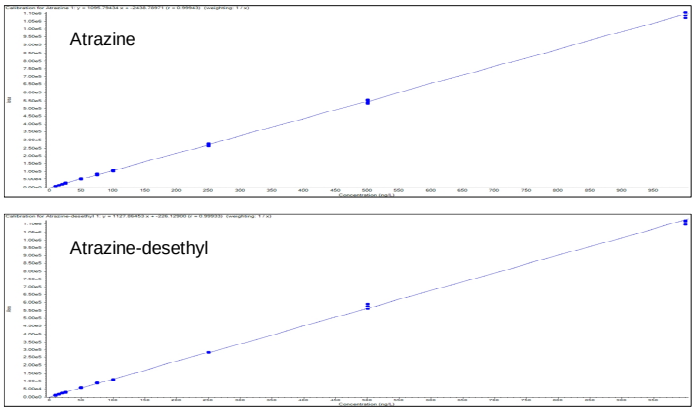

Figure 3. Calibration lines for Atrazine (top) and Atrazine-desethyl (bottom) with ‘r’ values of 0.999 or better

Conclusions

We have presented here a new approach for the analysis of pesticides in water. In order to use low volume injections, micro flow LC has been considered as a way to enhance performance and maximize sensitivity. The use of reduced diameter columns using the HALO fused-core particle allowed for significant reduction in the flow rates, injection volumes and sample consumption. This combines to give sensitivity gains reducing the lower limits of quantitation by 2.5 to 10 fold.

The other benefits of using micro flow technology is less mass spectrometer down time and reduced cost of ownership. For a typical overnight batch the conventional flow rates would use over 0.5 L of solvent. Whereas the Eksigent ekspert™ microLC 200 system ran at 15 µL/minute used just under 13 mL of solvent. Therefore over the course of a year the micro flow LC would use approximately 1/40 of the solvent.

References

1 K. Mrizig et al.: ‘Enabling Fast Separations and Smaller Sample Volumes in LC-MS/MS Using the ekspert™ microLC 200 System’ Technical Note Eksigent (2012) #6000112-01

2 K. Mrizig 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
www.absciex.com

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