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Applying Micro Flow LC and High Speed Data Acquisition MS/MS to the Analysis of Pesticides Residues in Complex Spice Matrix

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1. Introduction

In the UK, the Committee on Pesticide Residues in Food (PRiF) monitors levels of residues in food. The 2012 survey included the analysis of spices, which are recognised as a highly challenging matrix. Spices are known as "difficult" matrices, since they contain a bulk of extractable compounds (essential oils, pigments, etc.) causing significant problems during LC–MS/MS analysis including loss of chromatographic performance, system robustness, matrix effects and isobaric interference. Clean-up usually limits the scope of target analytes, thus, an extensive clean-up is not practical when faced with multi-residue analysis.

Here, we present the application of micro flow LC in comparison to conventional flow LC to minimise the challenges associated with the analysis of spice extracts. Lower flow rates can lead to increased sensitivity for concentration-dependent detection techniques such as electrospray ionization (ESI) mass spectrometry; which consequently permits the injection of smaller volumes of complex matrix into the LCMS system. Matrix effects were compared between conventional flow with the standard injection volume and micro flow employing a lower injection volume.

2. Methods

2-1. Sample preparation

Samples of spices were extracted using QuEChERS (quick, easy, cheap. effective, rugged and safe) methods developed by the Food and Environment Research Agency, UK. Sample extracts in acetonitrile were spiked with 210 pesticides. Sample extracts were directly injected or diluted 10 times with water; refer to main text.

2-2. LC-MS/MS analysis

Table 1 LCMS analysis conditions for the conventional flow and micro flow experiments

	Conventional flow	Micro flow
UHPLC	Nexera UHPLC system	
Flow rate	400 µL/min	90 µL/min
Mobile phase	A= Water with 5 mM ammonium formate B= Methanol with 5 mM ammonium formate	
Gradient	10% B - 100% B (12 min), 100% B (14 min), 10% B (17 min)	
Analytical column	ACQUITY UPLC HSS T3; 2.1 mm × 100 mm, 1.8 µm	ACQUITY UPLC HSS T3 1 mm × 100 mm, 1.8 µm
Column temperature	35°C	
Injection volume:	5 µL	
MS	LCMS-8040 triple quadrupole mass spectrometer	
Ionisation	Electrospray, positive and negative mode	
SRM	210 pesticides (469 SRMs) Dwell 3 msec. / Pause 1 msec.	
Desolvation line	250°C	200°C
Drying/Nebulising gas	15 L/min, 2 L/min	
Heating block	400°C	

3. Results and discussion

3-1. Conventional flow compared to micro flow

An established pesticide method using a 2.1mm ID × 100mm column and a 400 µL/min flow rate was compared to a micro flow method. Linear velocity was maintained during the micro flow analysis on a 1.0 mm ID × 100 mm column by using a 90 µL/min flow rate. A chromatogram of the micro flow LC is displayed in Fig. 1.

Both methods were utilised for the analysis of 210 pesticides in spice extracts. A comparison of the conventional flow method and the micro flow method resulted, on average for all 210 pesticides, in an increase of 1.9 times peak height. Peak area was found to increase on average 2.5 times. Signal to noise ratios were not calculated as noise was typically extremely low or not visible in the scheduled MRM window. A comparison of selected compounds is displayed in Fig. 2.

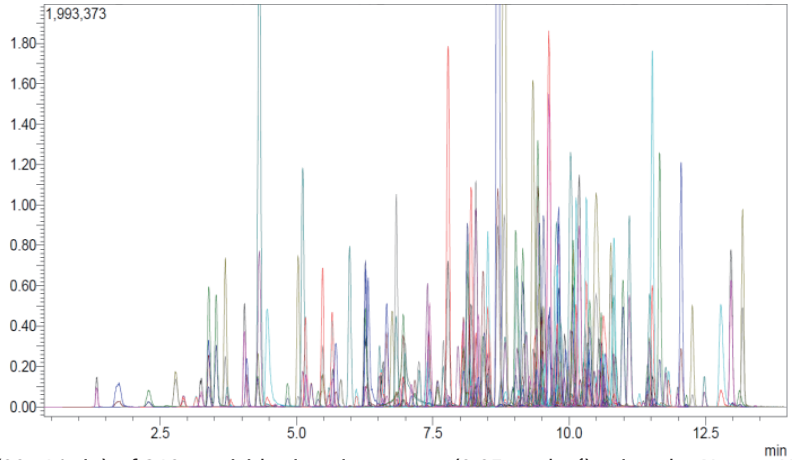

Fig. 1 Micro flow analysis (90 µL/min) of 210 pesticides in spice extract (0.05 mg kg^{-1}) using the Nexera UHPLC and the LCMS-8040

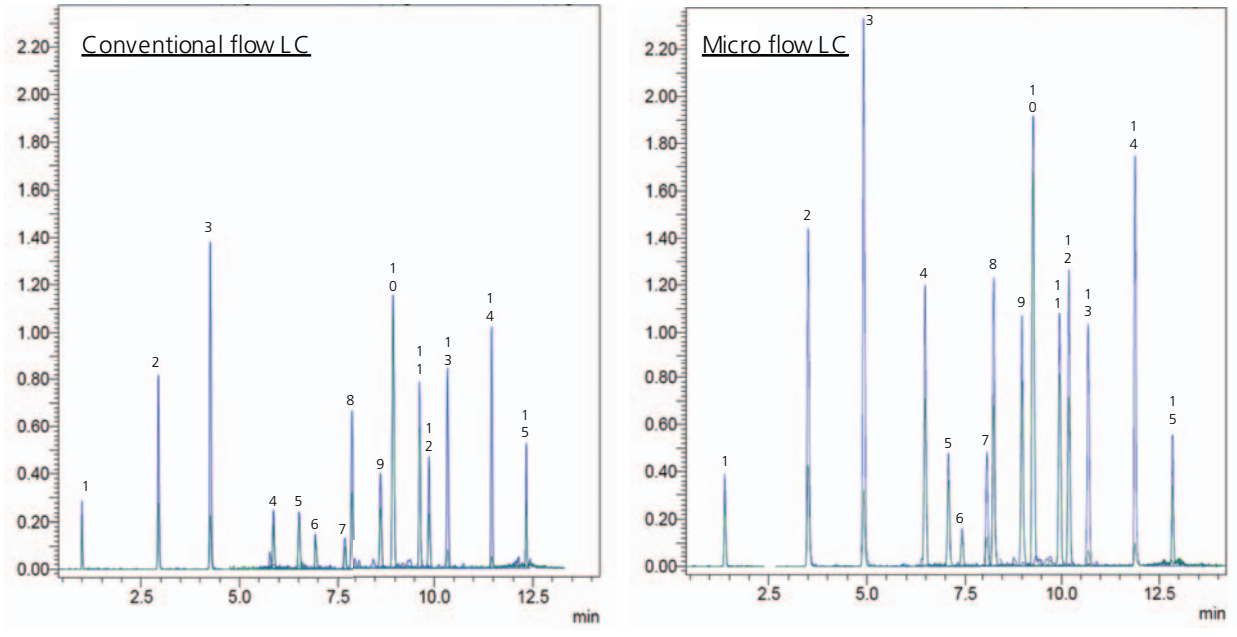

Fig. 2 Conventional flow analysis (left) at 400 µL/min and micro flow analysis (right) at 90 µL/min for selected pesticides over the run time. Injection volume 5 µL of spice extract (0.005 mg kg^{-1}) diluted 10 times with water.

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3-2. Linearity comparison

Linearity was investigated over a seven-point calibration with samples ranging from 0.005 mg kg^{-1} - 0.2 mg kg^{-1}. Fig. 3 compares the calibration curves for nine pesticides analysed using conventional flow LC and micro flow LC. The same set of calibration samples was used in both experiments. Linearity was excellent for both conventional flow and micro flow; typically $R^2 > 0.998$ (No weighting). Fig. 3 also highlights the increased peak area achieved by micro flow LC.

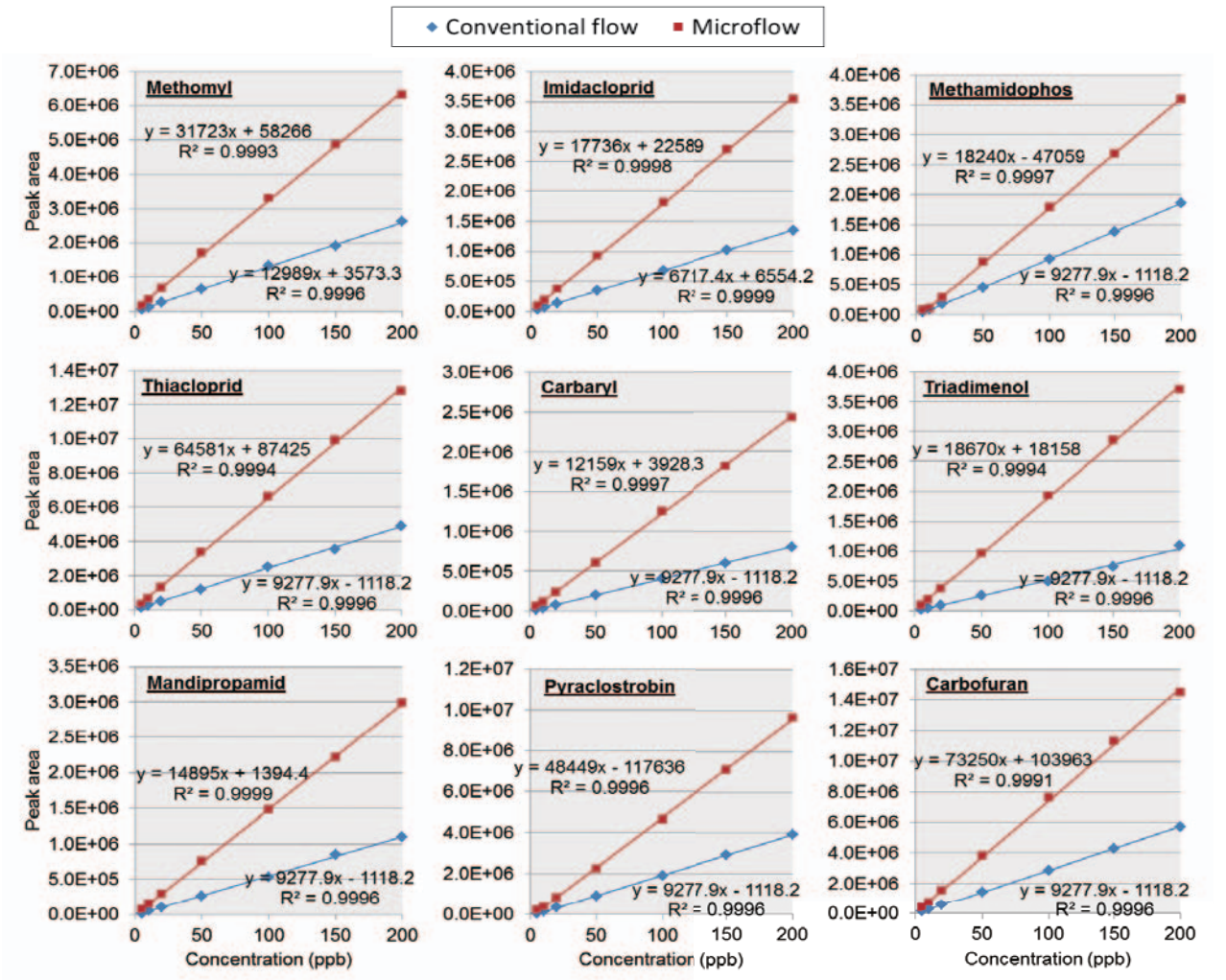

Fig. 3 Comparison of calibration curves using conventional flow LC and micro flow LC. Calibration samples prepared at 0.005 mg kg^{-1} - 0.2 mg kg^{-1} in acetonitrile extracts; samples subsequently diluted 10 times with water and injected

3-3. Matrix effects comparison

As a consequence of the superior sensitivity demonstrated by micro flow LC, injection volumes were reduced. Injection volumes were initially reduced by 40% from 5 µL to 3 µL (acetonitrile extract diluted 10 times with water), which achieved similar peak height responses for most pesticides to conventional flow with a 5 µL injection. Given the reduced injection volume, samples were analysed by micro flow LC without dilution; consequently a 0.3 µL (not diluted) injection was performed. Fig. 4 displays a comparison of matrix effects using conventional flow with a 5 µL injection (10x diluted sample) and micro flow with a 0.3 µL injection (not diluted; acetonitrile extract).

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Matrix effects were reduced for a large number of compounds with the smaller injection volume afforded by the micro flow method, typically by approximately 10-20%.

Furthermore, the ability to inject acetonitrile extracts directly saves on the time needed to dilute samples and improves compound stability.

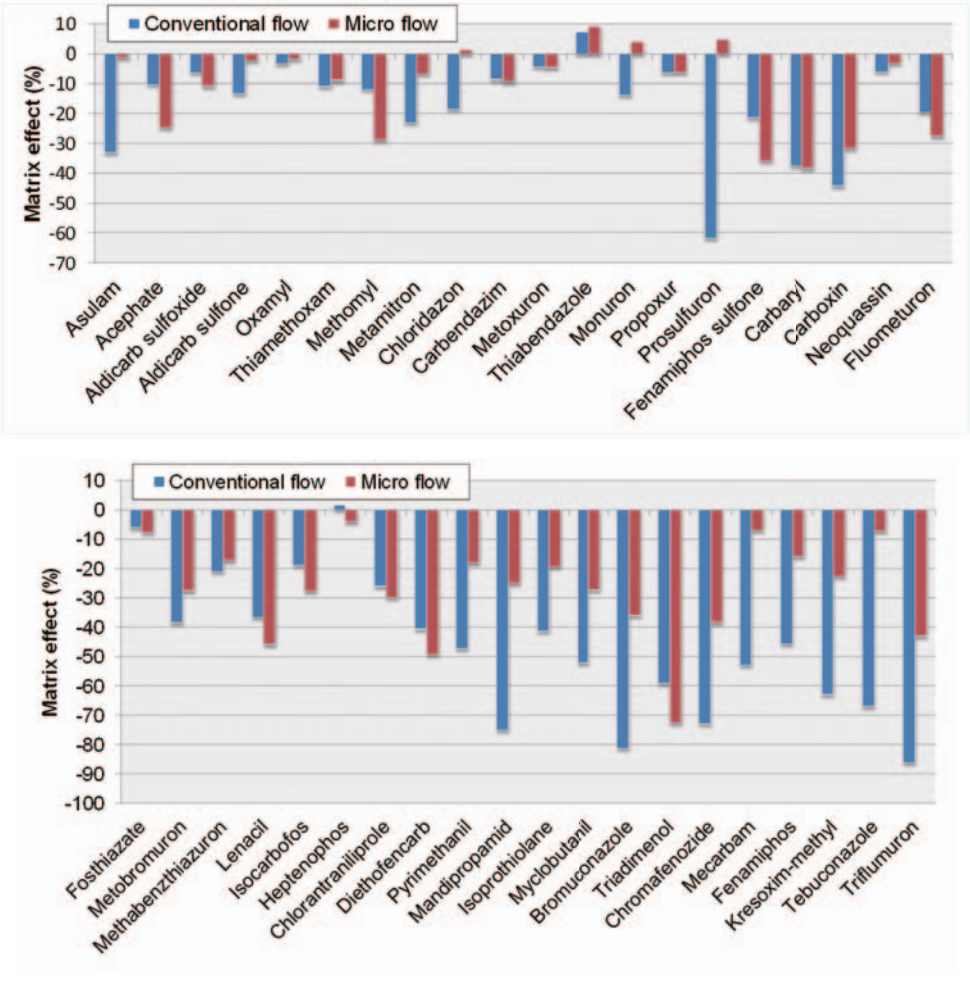

Fig. 4 Comparison of matrix effects in conventional flow LC (5 µL injection of an acetonitrile extract diluted 10 times with water) and micro flow LC (0.3 µL injection of an undliuted acetonitrile extract). Pesticides added at 0.005 mg kg^{-1} before dilution. Errors were typically less than 10%RSD.

4. Conclusion

- Application of a micro flow LC method, in comparison to a conventional flow LC method, provided on average an increase in peak height of 1.9 times for 210 pesticides.
- The increased sensitivity afforded by micro flow LC was used to reduce injection volumes by 40%.
- Due to the reduced injection volume the direct injection of solvent extracts was possible without the need for dilution
- A combination of the reduced sample injection volume and the injection of solvent extracts reduced matrix effects by 10-20% for many pesticides in complex spice matrix.
- Micro flow LC is a possible alternative to conventional flow LC if improved sensitivity is required.

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