Application of SFC-MS/MS for the Quantification of Highly Polar Pesticides in a Range of Food Samples

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1. Introduction

The analysis of highly polar pesticides by LC-MS/MS is typically achieved by several specific methods with limited chemical space. The EU Reference Laboratories for Residues of Pesticides Single Residue Methods (EURL-SRM) have published QuPPe (Quick Polar Pesticides) methods for the simultaneous analysis of a number of highly polar pesticides which cannot be easily analysed using routine QuEChERS based extraction methods and multi-residue methods (M. Anastassiades et al; QuPPe of EURL-SRM (Version 9.1; 2016). To meet the needs of analysing highly polar pesticides by LC-MS/MS several chromatographic conditions have been used including hydrophilic interaction liquid chromatography (HILIC), zwitterionic-type mixed-mode, normal-phase columns operated in HILIC-mode (bare silica and silica-based chemically bonded columns (cyano and amino)), reversed-phase C18 and carbon based phases. In this work SFC-MS/MS was applied to the analysis of a panel of highly polar pesticides extracted from several food matrices by QuPPe.

2. Methods and Materials

Food extracts of pepper, flaxseed and lemon provided by Concept Life Sciences were extracted using QuPPe based methods. These extracts were then spiked with pesticides and directly injected into the SFC-MS/MS.

Supercritical fluid chromatography		Mass spectrometry	
SFC	Nexera UC system	LC-MS/MS	LCMS-8060
Analytical column	Restek Ultra Silica (150 x 2.1mm 3um)	Ionisation mode	Heated ESI
Column temperature	50°C	Scan speed	15,000 u/sec
Flow rate	0.8mL/min (0.6mL/min 13-22min)	MRM Dwell time	3 msec
Pump A	CO2	Pause time	1 msec
Pump B (modifier solvent)	Acetonitrile + 0.5% formic acid	Interface temp.	300°C
Pump C (modifier solvent)	Water + 0.5% formic acid + 10mM ammonium formate	Heating block	350°C
Pump D (make up solvent)	Methanol	Desolvation line	250°C
Makeup solvent flow rate	0.2mL/min		

Table 1. LC-MS/MS data acquisition conditions.

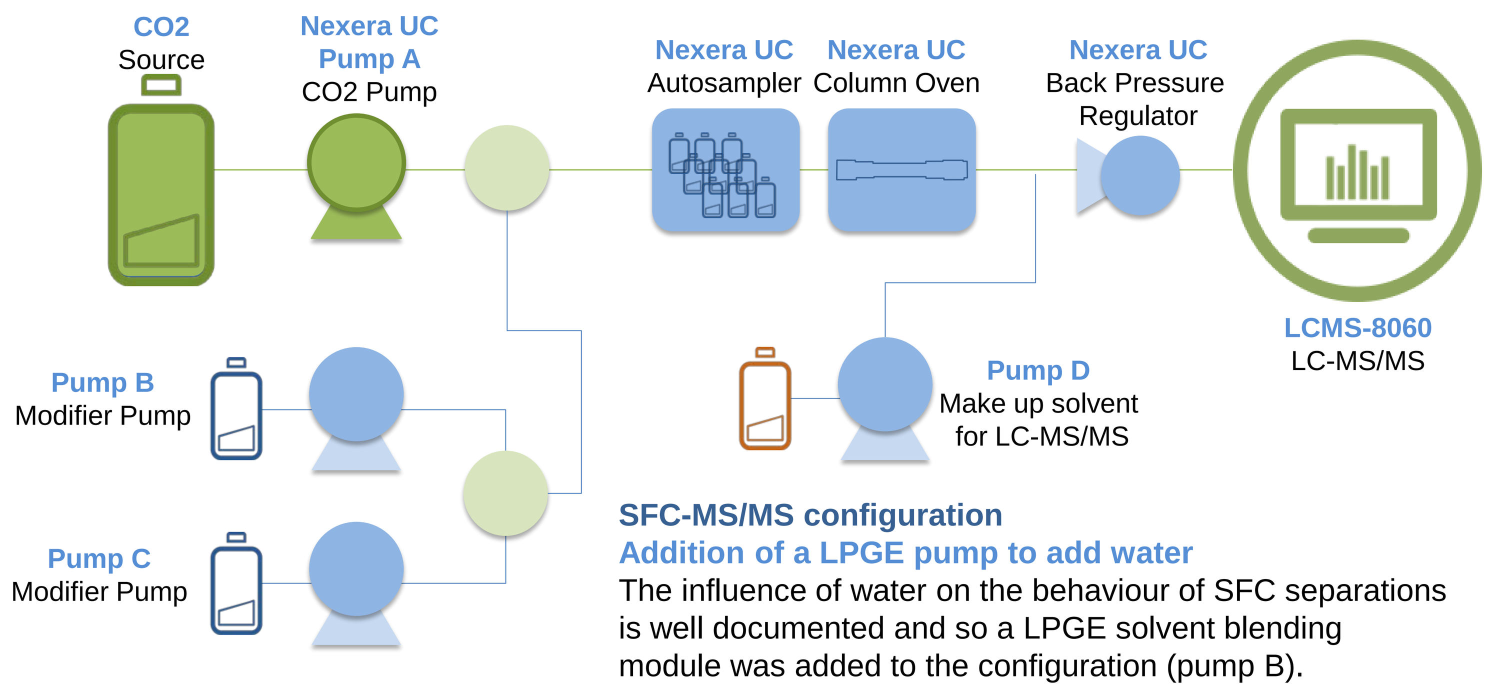

Figure 1. System configuration for the SFC-MS/MS system.

2-1. Method development

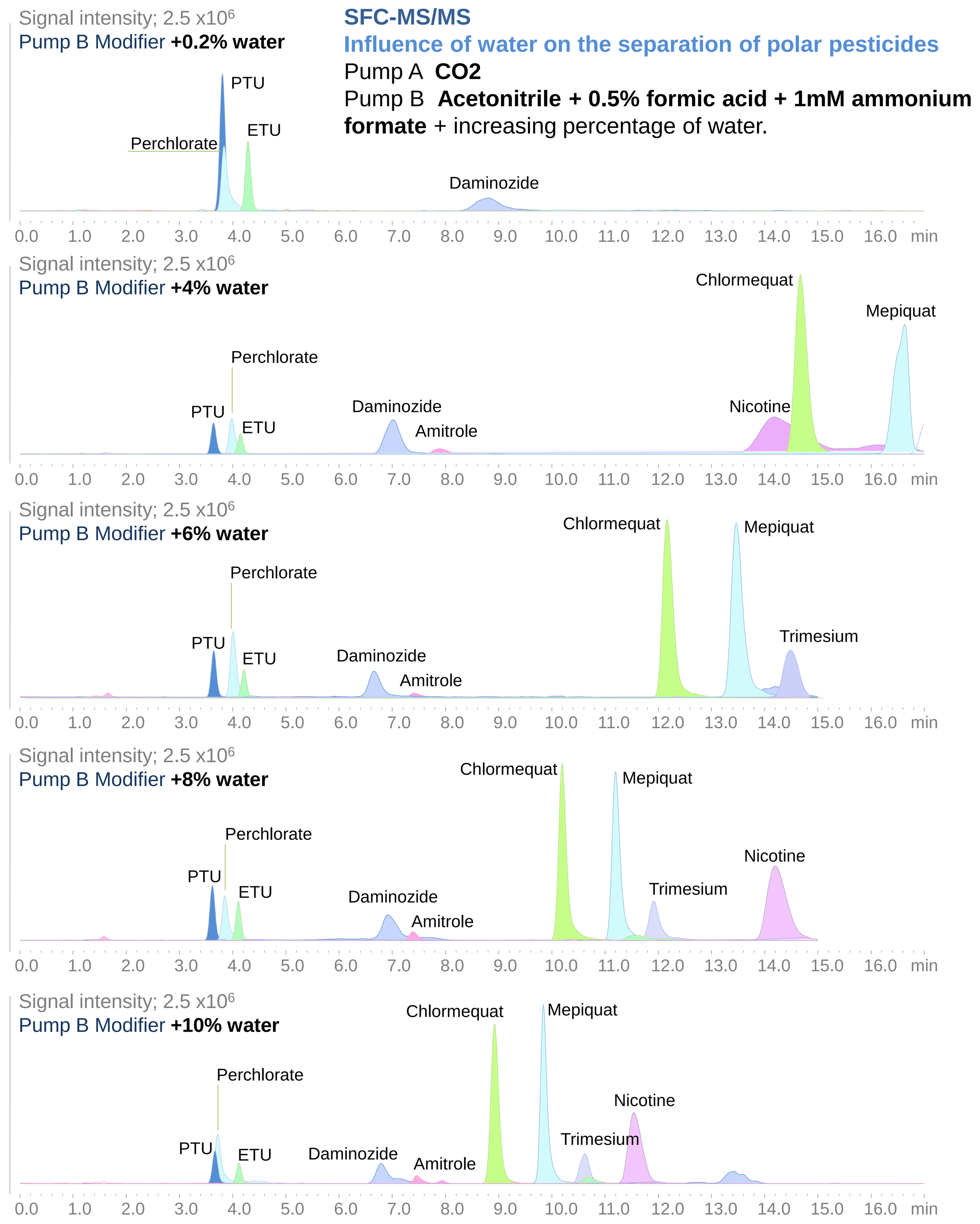

Figure 2. Influence of water on the chromatographic behaviour of highly polar pesticides in SFC-MS/MS. All conditions remained constant with the exception of changing the water content. (Changing water content was achieved by using a LPGE solvent blending module on Pump B with acetonitrile and water, both containing 0.5% formic acid and 1mM ammonium formate. Changing the concentration of ammonium formate had a positive effect on peak shape and a concentration of 10mM was used in all subsequent analyses).

From a historical perspective, the influence of water in early SFC work has been recognised for some time. Although the miscibility of water in carbon dioxide is very limited it has been usually added in the range of 0.1-10% (although recent papers have several applications with water added up to 30%). In the final method used in this study, the water content used in pump B was set to 6% water.

3. Results

3-1 Optimized separation

The SFC-MS/MS method used for the analysis of a panel of highly polar pesticides extracted from food matrices using QuPPe required alternating gradient conditions to optimize signal response and peak shape.

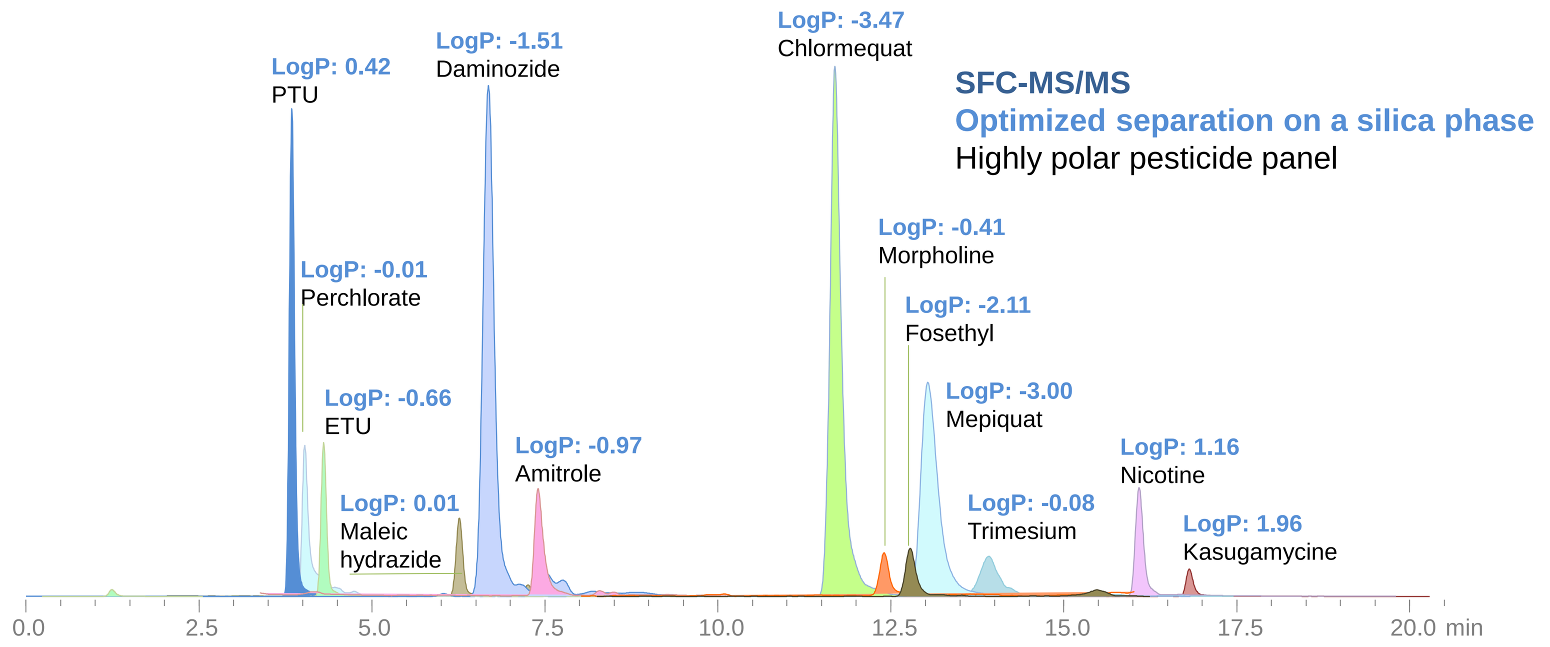

Figure 3. MRM chromatograms of a panel of highly polar pesticides (log *P* ranging from -3.47 to 1.96) analysed by SFC-MS/MS. The MRM chromatogram is from a QuPPe extract of flaxseed spiked at 200ppb.
Several compounds have been rescaled in the chromatogram; perchlorate 5x (negative ion), maleic hydrazide 50x, amitrole 5x, morpholine 50x, fosethyl 100x (negative ion) and kasugamycine 10x.
To optimize the separation and response to a panel of highly polar pesticides by SFC-MS/MS a Restek Ultra Silica (150 x 2.1mm 3um) column was used. The LC time program was modified to change CO2, water and acetonitrile gradients dynamically during the run.

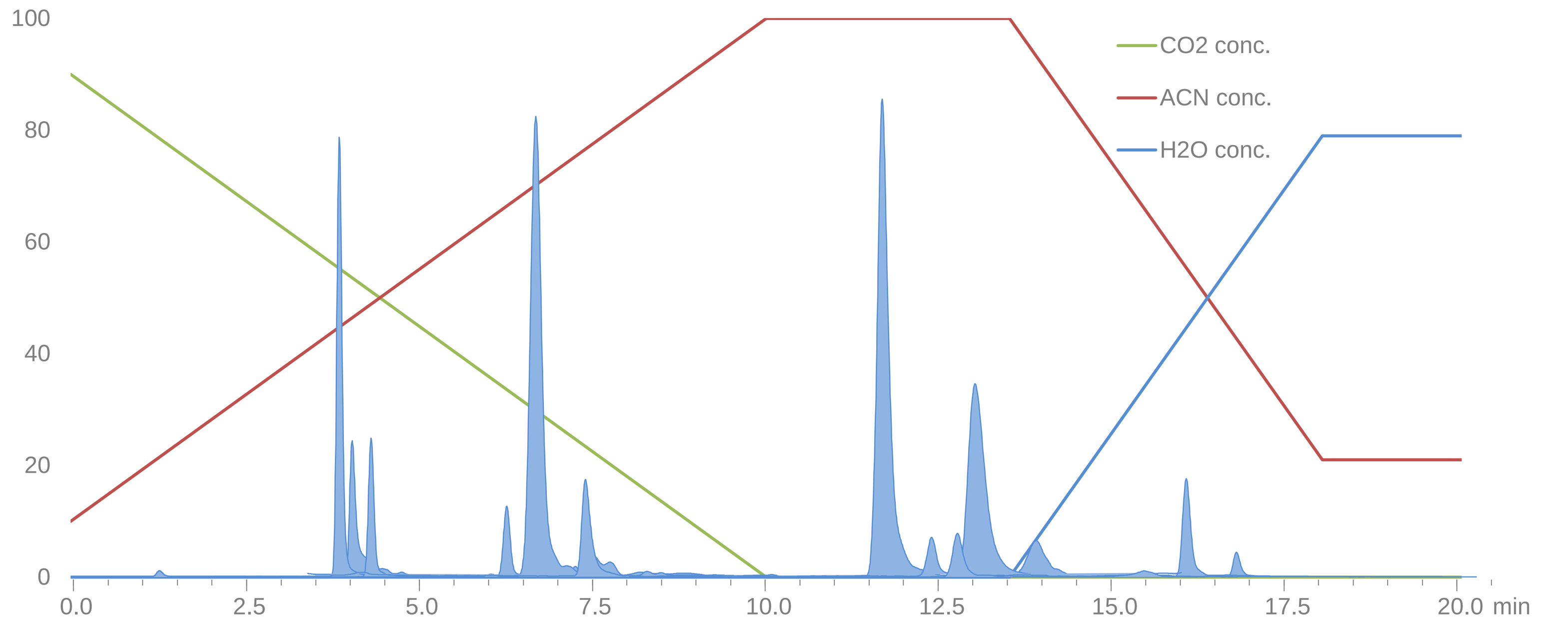

Figure 4. Mixed mode ternary gradient separation of highly polar pesticides.
The initial SFC-MS/MS conditions;
Pump A 90% Carbon Dioxide
Pump B 10% Acetonitrile + 0.5% formic acid containing 6% water
Pump C 0% Water + 0.5% formic acid + 10mM ammonium formate

3-2. Quantitative performance

To assess the impact of SFC-MS/MS on routine sample analysis several food matrices (pepper, flaxseed, lemon) were extracted using QuPPe and spiked with a panel of highly polar pesticides. The calibration range was between 10-200ppb and where possible stable isotope labelled internal standards were used.

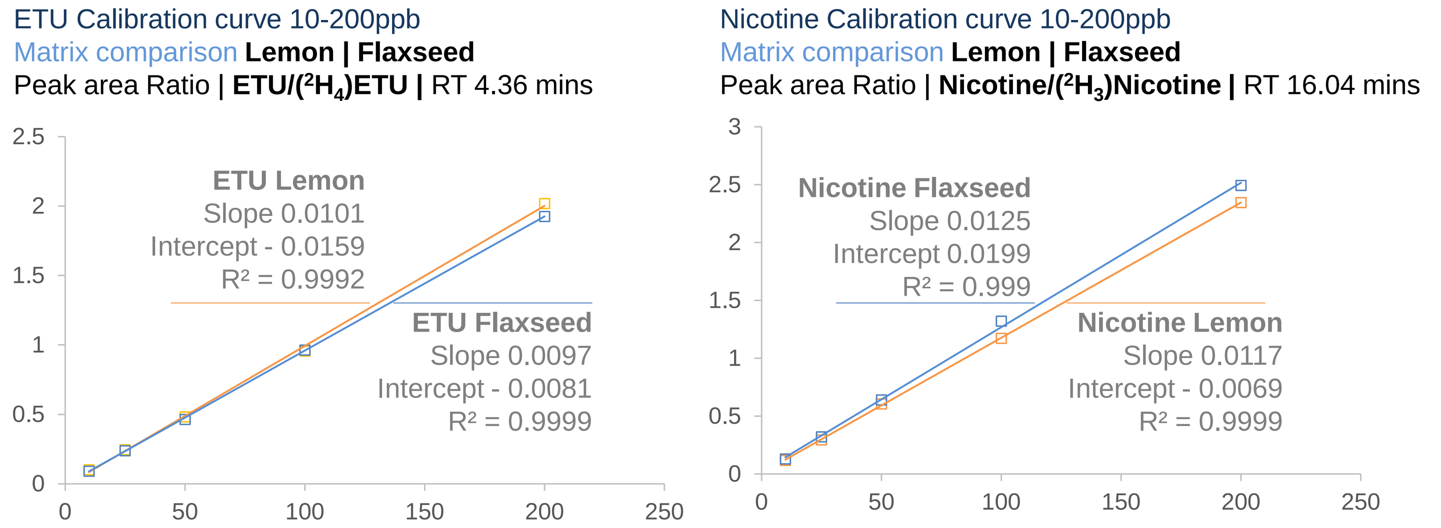

Figure 5. Calibration curve data for an early eluting compound (ETU) and a late eluting compound (nicotine) from lemon and flaxseed matrices extracted using a QuPPe method. Regardless of the matrix, peak area ratio's are in good agreement between each matrix.

3-3. Linearity and Reproducibility

A panel of highly polar pesticides was repeatedly injected (n=10) at concentration of 100ppb with stable isotope labelled internal standards.

Compound	RT (min)	Internal Standard	IS RT (min)	Quan MRM	%RSD 100ppb	R^2
Perchlorate	3.95	$^{18}O_4$ Perchlorate	3.91	99.00 > 82.90	4.98	0.968
ETU	4.36	$^{2}H_4$ ETU	4.26	103.10 > 44.05	4.84	0.999
Maleic hydrazide	6.28	$^{2}H_2$ Maleic hydrazide	6.28	113.00 > 67.10	6.81	0.997
Chlormequat	11.58	$^{2}H_4$ Chlormequat	11.54	121.90 > 58.10	1.75	1.000
Fosethyl	12.50	$^{2}H_{15}$ Fosethyl	12.50	109.00 > 80.95	6.78	0.999
Morpholine	12.19	$^{2}H_8$ Morpholine	12.23	87.90 > 70.05	10.74	0.996
Mepiquat	12.72	$^{2}H_3$ Mepiquat	12.69	114.30 > 98.10	7.66	0.998
Nicotine	16.06	$^{2}H_3$ Nicotine	16.03	163.00 > 130.00	2.31	0.999

Table 2. Linearity and %RSD of 8 highly polar pesticides measured by SFC-MS/MS (100ppb lemon matrix sample; n=10).

4. Conclusions

- SFC-MS/MS provides an alternative approach to measure highly polar pesticides in food safety. In this work, stable isotope labelled internal standards resulted in robust quantitation regardless of the matrix.
- Optimisation of the method considered the chemical space of the target analytes and required a combined ternary gradient approach. Including water into the gradient system had a positive effect on peak shape and chromatographic behaviour of several compounds.
- Column selection highlighted the need to evaluate phases empirically in SFC separations and in this work a silica based column resulted in selective retention and acceptable peak shape for highly polar pesticides.