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Micro Flow UHPLC-MS/MS in Pesticide Analysis of Infant Foods

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David R. Baker[1], Neil Loftus[1], Simon Hird[2]
[1]Shimadzu, Manchester, UK
[2]Food and Environment Research Agency, York, UK

1. Introduction

Food safety with regards to infant food is of the upmost importance; however, it is also recognised as a challenging matrix to analyse due to the low maximum residue limit (MRL) of 0.01 mg/kg required by European Directive 2006/141/EC for all pesticides. Furthermore, the European Directive prohibits the use of certain very toxic pesticides in the production of infant foods and establishes even lower MRLs for a few other very toxic pesticides. Additionally, the analysis of infant food is complicated by their wide range of fat content.

LC-MS/MS has been widely used for the quantitation of pesticides in infant food. The analytical methods typically use conventional LC flow rates (approximately 0.5 mL/min). Micro flow LC uses significantly lower flow rates (10 to 100 µL/min). With the same sample amount and identical LC peak width, the reduction in LC flow rate can result in an improved detection limit for concentration-dependent detection techniques such as electrospray ionization (ESI) mass spectrometry. Here, we utilise the improved response from micro flow LC to achieve the required low limits of detection for over one hundred pesticides in infant food. Initial validation results are presented for the micro flow LC method, in addition to robustness data.

2. Materials and Methods

2-1. Sample preparation

Samples 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 130 pesticides. Sample extracts were diluted five times with water before LCMS injection.

2-2. LC-MS/MS analysis

UHPLC	Nexera UHPLC system
Flow rate	90 µL/min
Mobile phase	A= Water (95%) and methanol (5%) with 5 mM ammonium formate
	B= Methanol with 5 mM ammonium formate
Gradient	0% B - 100% B (12 min), 100% B (14 min), 10% B (17 min)
Analytical column	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	130 pesticides (260 SRMs) Pause 1 msec./Dwell 3 msec.
Desolvation line	200°C
Drying/Nebulising gas	15 L/min, 2 L/min
Heating block	400°C

3. Results and discussion

3-1. Micro flow LC method

Micro flow LC was utilised for the analysis of 130 pesticides using a 90 µL/min flow rate and a 1.0 mm I.D. analytical column. The flow rate was reduced from conventional flow rates (approximately 500 µL/min) in order to increase analyte response and reduce costly solvent consumption. All 130 pesticides were eluted within 12.7 minutes with a typical peak width of 7 seconds. An example chromatogram is displayed in Fig. 1. A high speed data acquisition mass spectrometer was utilised to acquire reliable data with a 1 msec. pause and 3 msec. dwell time, which allowed the acquisition of a large number of overlapping MRM transitions.

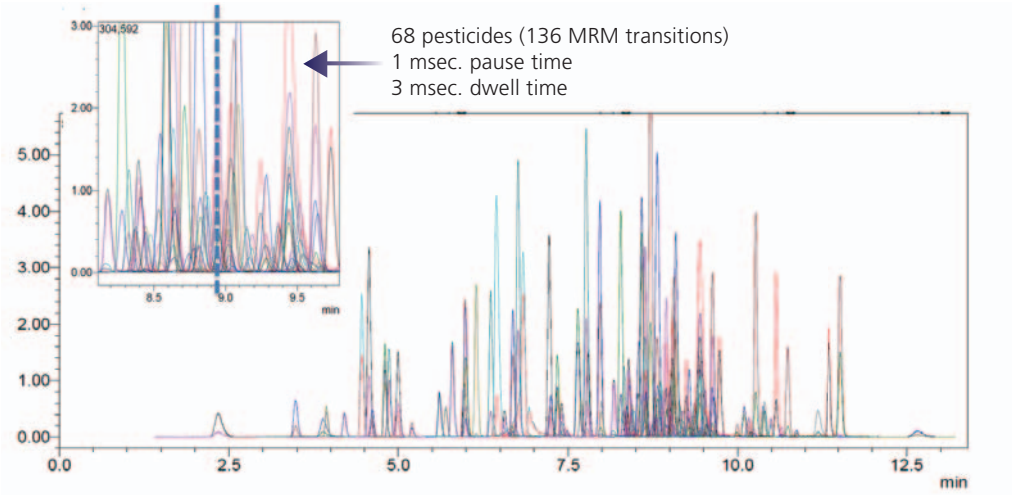

Fig. 1 Micro flow LC analysis of 130 pesticides in infant food (0.05 mg /kg)

3-2. Linearity

Calibration curves were prepared in the range 0.5xRL (reporting limit) to 20xRL. For the vast majority of pesticides this was in the range 0.005 - 0.2 mg/kg (0.5 – 200 ppb). The developed method achieved the necessary sensitivity to detect all 130 pesticides at the lowest required level. Fig. 2 displays calibration curves for nine example pesticides including the first eluting analyte (methamidophos) and latest eluting analyte (fenbutatin). All analytes displayed linearity with $R^2 > 0.996$, with typical R^2 of 0.9990.

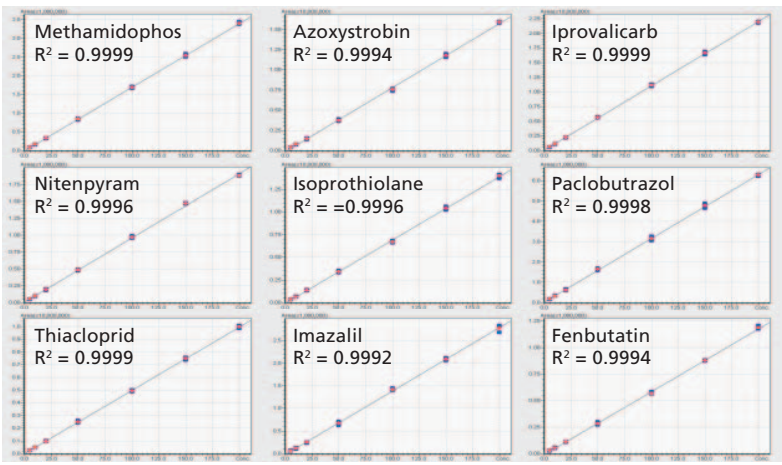

Fig. 2 Calibration curves (weighting 1/x), 0.005 0.2 mg/kg (0.5 200 ppb) of nine pesticides in infant food

3-3. Intra- and inter-day precision

To assess the intra-day and inter-day variability of the LC-MS/MS method, QC samples were prepared in infant food at low (0.01 mg/kg) and high (0.2 mg/kg) concentrations. Intra- (n=6) and inter-day (n=3 over 3 days) precision were determined as %RSD of peaks areas. Table 2 lists precision data for compounds over the run time, including the first eluting analyte (methamidophos) and latest eluting analyte (fenbutatin). Intra-day precision was typically less than 4%, while inter-day precision was typically less than 7%.

Table 2 Intra- and inter-day precision of selected pesticides over the run time

Compound	Retention time	Intra-day (%RSD)		Inter-day (%RSD)	
		Low	High	Low	High
Methamidophos	2.3	2.14	3.53	2.80	4.50
Acephate	3.4	1.56	2.55	3.54	3.85
Aldicarb sulfoxide	4.2	1.31	0.70	4.84	3.91
Methomyl	4.8	2.02	2.03	3.61	4.86
Imidacloprid	5.6	2.05	0.64	3.46	4.59
Carbendazim	6.5	1.70	0.72	2.69	3.80
Carbaryl	7.4	3.41	0.93	5.38	4.77
Fosthiazate	7.6	1.21	1.19	2.55	4.50
Azoxystrobin	8.2	1.90	1.78	2.78	4.30
Dimethomorph	8.5	3.05	2.28	5.03	4.95
Triazophos	8.7	1.45	1.19	3.47	4.78
Iprovalicarb	8.8	2.40	1.71	3.08	4.47
Cyazofamid	8.9	2.60	2.16	2.89	5.74
Penconazole	9.3	1.58	1.61	4.55	4.15
Hexaconazole	9.4	2.37	1.77	4.16	4.35
Pencycuron	9.6	2.59	2.27	2.69	4.43
Pyriproxyfen	10.2	1.02	1.02	1.32	5.39
Fenpyroximate	10.7	2.96	1.29	12.8	2.97
Etofenprox	11.4	2.55	1.00	2.03	4.72
Fenbutatin	12.6	2.40	2.90	2.72	4.51

3-4. Carryover assessment

Carryover was assessed after the injection of six injections of the highest concentration calibration sample. No analytes were detected above the LOQ (Fig. 3).

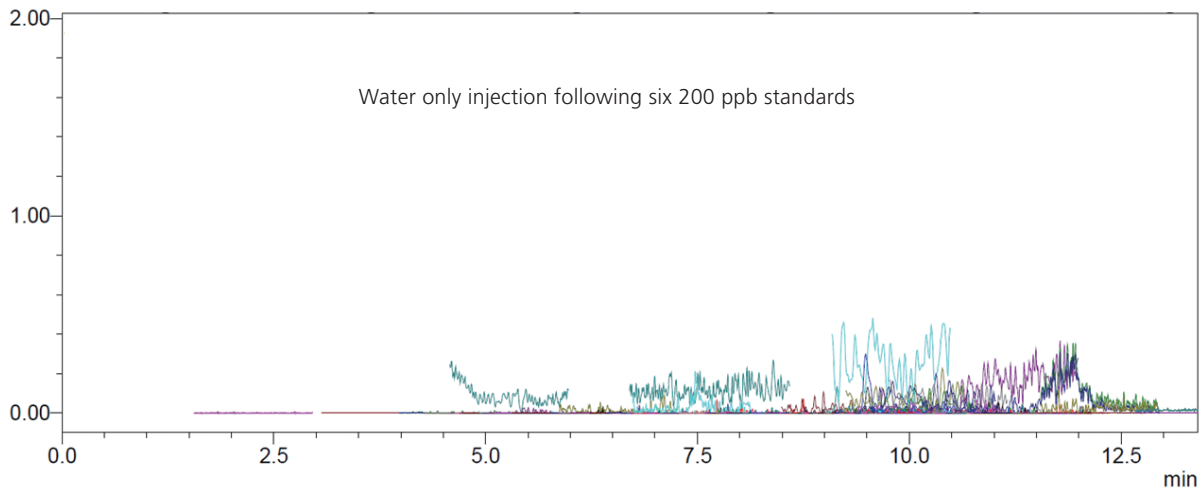

Fig. 3 Water only injection following the injection of six 200 ppb standards

3-5. System robustness

To the investigate the robustness of the micro flow system infant food extract was repeatedly injected over 48 hours. In total, 162 injections were performed with infant food extract at 0.02 mg/kg. The stability of the system was assessed in terms of peak area stability (Fig. 4) and retention time stability. The data showed excellent stability with peak area deviation typically 3-5% and retention time stability typically less than 0.12%RSD.

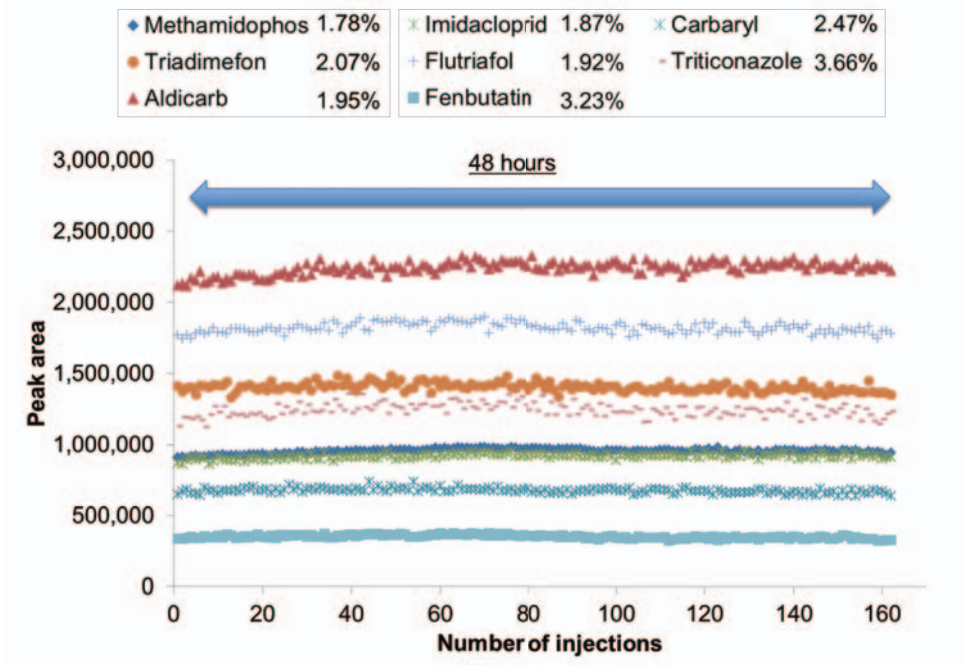

Fig. 4 Peak area stability (%RSD) of pesticides in infant food extract over 48 hours of repeat injection

4. Conclusion

- The developed micro flow LC methodology achieved the required MRL of 0.01 mg/kg for all 130 pesticides with all compounds eluted within 12.7 minutes.
- Initial validation data displayed excellent linearity for all compounds, low intra- and inter-day precision, no observed carryover, and good peak area and retention time stability over 48 hours.
- Precise data was acquired with the use of low pause and dwell times (1 msec. pause and 3msec. dwell times)
- Micro flow analysis was successfully carried out a UHPLC system capable of both conventional higher flow rates and lower micro flow rates.
- Micro flow LC is a possible alternative to conventional flow LC if extra sensitivity is needed or reduction in solvent consumption is required.

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