A simplified multi-residue method for the rapid screening and confirmation of pesticides present in fruit and vegetable crude extracts using isocratic HPLC separation combined with electrospray tandem mass spectrometry.

Scottish Agricultural Science Agency

Michael Taylor, Ken Hunter, Kirsty Hunter, David Lindsay, Claire Blackie and Soazig Le Bouhellec
Scottish Agricultural Science Agency, East Craigs, Edinburgh

AIM: *To develop a generic LCMSMS method that improves the analytical efficiency of pesticide multi-residue analysis.*

Introduction

The application of atmospheric pressure ionisation LCMSMS techniques has facilitated the multi-residue analysis of crude extracts from fruit and vegetables. The enhanced selectivity of MSMS can significantly reduce potential interference from non-target substances and discriminate between co-eluting and isobaric analytes. The utility of electrospray MSMS detection in combination with isocratic HPLC separation was investigated to see if further efficiency gains could be made compared to gradient separations used previously in our laboratory

Experimental

HPLC Isocratic Method – Agilent 1100 HPLC system

Column:	Hypersil C_{18} 3µm BDS (4.6 x 100mm I.D.)
Mobile Phase:	Methanol:10mM aqueous ammonium acetate (70:30v/v)
Flow Rate:	0.5mlmin^{-1} (post-column split → approx. 20µlmin^{-1} into ion source)
Temperature:	35^{o}C
Injection volume:	5µl or 10µl

Mass Spectrometry Method – Micromass Quattro Ultima

Acquisition:	Electrospray ± ive ion mode Multiple Reaction Monitoring (MRM) Single Ion Recording (SIR) for 2-phenylphenol
Collision Gas:	Argon approx. 1.4x10^{-3} mbar
Data System:	MassLynx 3.4
Desolvation Gas:	Nitrogen approx. 500 lhr^{-1}
Nebuliser Gas:	Nitrogen
Cone Gas:	Nitrogen @ 80lhr^{-1}
Desolvation Temp:	350^{o}C
Capillary voltage:	3kV
Source Temp:	150^{o}C

Analytical Procedure

Extraction:	Homogenisation with ethyl acetate/sodium sulfate/sodium hydrogen carbonate followed by methanol solvent exchange (≡ 0.4g sample per ml)
Clean-up:	None required – Crude extract filtered (0.45µm PTFE Acrodisc syringe filter)

Results and Discussion:

The various pesticide/commodity combinations that have been analysed using this method are shown in Table 1. The target reporting levels (RL) used for each pesticide/commodity combination, were consistent with the levels set for the relevant part of the 2001 UK pesticide residue surveillance programme. All of the pesticides, and any important metabolites, yielded ions characteristic of the molecular weight of the neutral molecule (M) i.e. $[M+H]^+$, $[M+Na]^+$ or $[M+NH_4]^+$ in positive ion mode or intense $[M-H]^-$ ions in negative ion mode. Structurally diagnostic product-ions were generated for each compound following collision-induced dissociation (CID) of the selected precursor ion with the exception of 2-phenylphenol $[M-H]^-$ precursor ion which remained intact. MSMS parameters determined following optimisation experiments are listed in table 2.

Table 1. Mixtures of pesticides sought in each commodity and target reporting level (RL)

Pesticide	RL (mgkg^{-1})	Commodity: Apple	Grape	Kiwi	Lemon	Peach	Spinach	Strawberry
2,4-D (free acid)	0.05				•			
Aldicarb	0.05				•			
Aldicarb sulfone	0.05				•			
Aldicarb sulfoxide	0.05				•			
Azoxystrobin	0.05	•	•	•	•	•	•	•
Bendiocarb	0.50			•		•		
Butocarboxim	0.20			•	•			
Butocarboxim sulfone	0.20			•	•			
Butocarboxim sulfoxide	0.20			•	•			
Carbaryl	0.01	•		•	•	•	•	•
Carbendazim	0.01	•		•	•		•	•
Carbofuran	0.05			•	•			
Carbofuran 3-hydroxy	0.05			•	•			
Dichlofluanid	0.05		•			•		
Diethofencarb	0.05			•				
Ethiofencarb	0.20			•				
Fenhexamid	0.05					•		
Furathiocarb	0.05			•				
Imazalil	0.02	•	•		•	•	•	•
Kresoxim-methyl	0.05	•		•		•	•	•
Methiocarb	0.20			•		•		
Methiocarb sulfone	0.20			•		•		
Methiocarb sulfoxide	0.20			•		•		
Methomyl	0.05			•				
Metolcarb	0.05			•				
Myclobutanil	0.05	•				•	•	•
Oxamyl	0.05			•				
2-phenylphenol	0.10	•			•		•	•
Penconazole	0.05	•				•	•	•
Propiconazole	0.05					•		
Pymetrozine	0.05				•	•		
Pyrimethanil	0.05	•				•	•	•
Tebuconazole	0.05					•		
Thiabendazole	0.05	•	•	•	•	•	•	•
Thiodicarb	0.05				•			
Thiophanate-methyl	0.10				•	•		•
Trifloxystrobin	0.05	•			•	•		•

Table 2. Optimum experimental parameters used for screening purposes.

PESTICIDE	RMM[a]	Precursor ion assignment	MSMS transition	Cone Voltage (V) & Collision Energy (eV)
2,4-D (free acid)	220	[M-H]	219 → 161	30,10
Aldicarb	190	[M+Na]	213 → 89	25,25
Aldicarb sulfone	222	[M+NH$_4$]	240 → 86	25,20
Aldicarb sulfoxide	206	[M+H]	207 → 89	20,15
Azoxystrobin	403	[M+H]	404 → 372	34,10
Bendiocarb	223	[M+H]	224 → 167	30,15
Butocarboxim	213	[M+H]	213 → 75	34,10
Butocarboxim sulfone	222	[M+Na]	245 → 130	25,20
Butocarboxim sulfoxide	206	[M+H]	207 → 75	30,15
Carbaryl	201	[M+H]	202 → 145	20,15
Carbendazim	191	[M+H]	192 → 160	35,15
Carbofuran	221	[M+H]	222 → 165	27,15
Carbofuran 3-hydroxy	237	[M+H]	238 → 181	10,15
Dichlofluanid	332/334[b]	[M+H]	333/335 → 224/226	20,15
Diethofencarb	267	[M+H]	268 → 226	19,7
Ethiofencarb	225	[M+H]	226 → 107	26,10
Fenhexamid	301	[M+H]	302 → 97	25,25
Furathiocarb	382	[M+H]	383 → 195	26,15
Imazalil	296	[M+H]	297 → 159	42,15
Isoprocarb	193	[M+H]	194 → 95	26,11
Kresoxim-methyl	313[b]	[M+H]	314 → 206/222	23,10
Methiocarb	225	[M+H]	226 → 109	25,10
Methiocarb sulfone	257	[M+H]	258 → 226	25,10
Methiocarb sulfoxide	241	[M+H]	242 → 185	25,10
Methomyl	184	[M+H]	185 → 128	27,10
Metolcarb	165	[M+H]	166 → 109	34,10
Myclobutanil	288	[M+H]	289 → 70	25,20
Oxamyl	241	[M+H]	242 → 72	21,20
2-phenylphenol	170	[M-H]	169 (SIR)	21,n/a
Penconazole	283	[M+H]	284 → 159	30,30
Propiconazole	341	[M+H]	342 → 159	25,30
Pymetrozine	217	[M+H]	218 → 105	24,28
Pyrimethanil	199	[M+H]	200 → 107	30,28
Tebuconazole	307	[M+H]	308 → 70	30,50
Thiabendazole	201	[M+H]	202 → 175	30,20
Thiodicarb	354	[M+H]	355 → 88	25,20
Thiophanate-methyl	342	[M+H]	343 → 151	28,24
Trifloxystrobin	408	[M+H]	409 → 186	20,25

[a] **Relative Molecular Mass**
[b] **Sum of two characteristic transitions**

The selectivity of MSMS and use of Multiple Reaction Monitoring (MRM) data acquisition procedures is demonstrated in figures 1a, and 1b, which show ion chromatograms obtained following analysis of nine pesticides sought in spinach and twelve of the twenty pesticides sought in kiwi-fruit.

Quantification was carried out from interpolation against calibration data generated using matrix-matched standards that covered the analyte concentration range of interest. The use of matrix-matched standards was necessary to compensate for signal suppression observed in matrix compared to the response in pure solvent. The concentration range of matrix-matched standards spanned 0.005-0.8 µgml^{-1}. Correlation coefficients (r) greater than 0.99 were achieved routinely, following calibration of each pesticide.

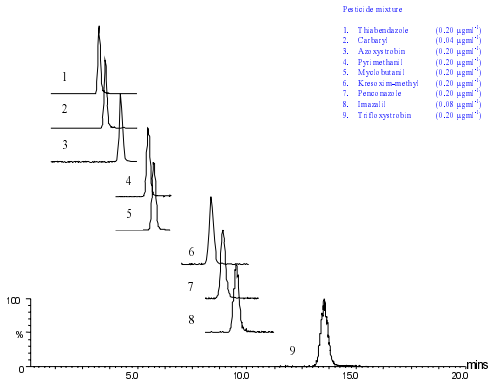


Figure 1a. Ion chromatograms of nine pesticides sought in spinach. Time-scheduled data acquisition sequence required 2 sets of three, 1 set of 2 and 1 single MRM channels.

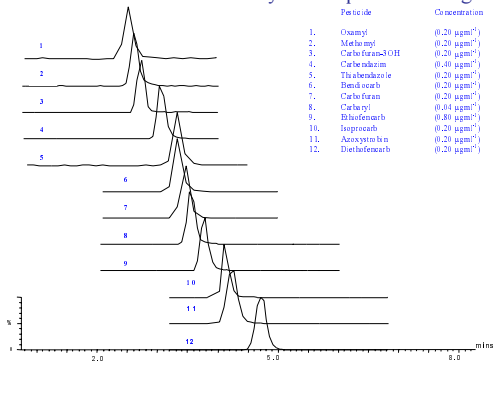
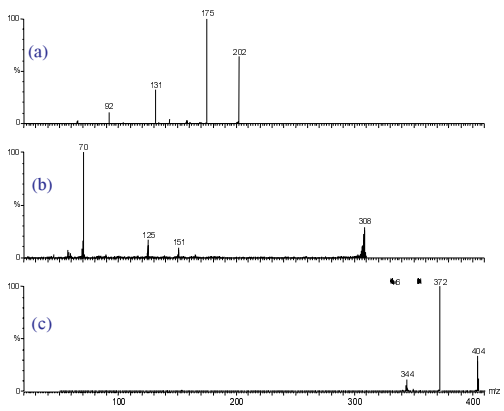


Figure 1b. Ion chromatograms of twelve pesticides (from 20) sought in kiwi fruit. 1 set of 5, 1 set of 4 and 1 set of 2 and a single MRM channels used.

Table 3. Example recovery and precision data obtained for pesticides sought in peach following multiresidue analysis of fortified organic produce.

Pesticide	Fortification Levels (mgkg^{-1})	% Mean Recovery[1]	RSD	n	Range
Azoxystrobin	0.20 & 0.05	78	9.7	6	70-86
Bendiocarb	0.20 & 0.05	79	8.7	6	70-86
Carbaryl	0.04 & 0.01	78	7.7	6	68-87
Dichlofluanid	0.20 & 0.05	84	9.7	5	60-93
Fenhexamid	0.20 & 0.05	79	5.9	6	72-84
Imazalil	0.08 & 0.02	77	7.5	6	67-87
Methiocarb	0.80 & 0.20	71	10.1	5	59-78
Methiocarb sulfone	0.80 & 0.20	64	17.5	5	55-76
Methiocarb sulfoxide	0.80 & 0.20	96	43.2	5	41-147
Myclobutanil	0.20 & 0.05	77	11.4	6	67-86
Penconazole	0.20 & 0.05	77	12.1	6	66-88
Propiconazole	0.20 & 0.05	80	9.9	6	71-91
Pymetrozine	0.20 & 0.05	73	6.1	6	66-79
Pyrimethanil	0.20 & 0.05	84	8.4	6	77-88
Tebuconazole	0.20 & 0.05	78	11.3	6	68-89
Thiabendazole	0.20 & 0.05	78	9.5	6	68-86

Figure 2. Examples of ESIMSMS mass spectra containing precursor → production-ion transitions used for screening and confirmation purposes. (a) thiabendazole, (b) tebuconazole and (c) azoxystrobin.



Retail samples of each commodity were extracted and screened for the presence of residues using the isocratic LCMSMS procedure. Confirmation of residues detected at or above the reporting level following initial screening experiments was achieved using an alternative precursor → product-ion transition and the same isocratic method. This was not possible for carbaryl or carbendazim, since alternative transitions were not of sufficient intensity under prevailing conditions, or for 2-phenylphenol. In the case of carbendazim and 2-phenylphenol, alternative HPLC methods were used to achieve confirmation of any residues, whereas confirmation of carbaryl residues was achieved using SIR and the original isocratic method.

Examples of product-ion mass spectra containing transitions used for screening and confirmation experiments are shown in figure 2.

Table 4 contains example LCMS/MS parameters used for confirmation purposes. Typical screening and confirmation results obtained for pesticide residues detected in various samples are detailed in table 5. Maximum Residue Levels (MRL) specified by the (i) CODEX Alimentarius Commission or (ii) in the UK Statutory Instrument for Pesticide Maximum Residue levels in Crops, Food and Feeding Stuffs applicable to the pesticide/commodity combinations are also shown in table 5.

Table 4. LCMS/MS methods used for confirmation of residues detected in samples.

Pesticide	Screen method	Confirmation Method
Azoxystrobin	m/z 404 → m/z 372	m/z 404 → m/z 344
Carbaryl	m/z 202 → m/z 145	SIR m/z 202
Imazalil	m/z 297 → m/z 159	m/z 297 → m/z 69
Myclobutanil	m/z 289 → m/z 70	m/z 291 → m/z 70
2-phenylphenol	SIR m/z 169	SIR m/z 169[b]
Tebuconazole	m/z 308 → m/z 70	m/z 308 → m/z 125
Thiabendazole	m/z 202 → m/z 175	m/z 202→ m/z 131

[a] **Isocratic acetonitrile:water 50:50 v/v, C_{18} Elite column 100 x 4.6mm x 5µm**
[b] **Isocratic acetonitrile:water 70:30 v/v, C_{18} Elite column 100 x 4.6mm x 5µm**

Table 5. Example of correlation between screening and confirmation measurements of various pesticide residues detected in samples.

Analyte	Fortification (mgkg^{-1})	Commodity (MRL mgkg^{-1})	Residue Level[a] (mgkg^{-1})		% Recovery[b]	
			Screen	Confirmation	Screen	Confirmation
Azoxystrobin	0.20	Grape (2.0)	0.20	0.20	72	74
Carbaryl	0.04	Kiwi Fruit (10.0)	0.10	0.10	84	68
Imazalil	0.20	Lemon (5.0)	1.40	1.50	78	83
Myclobutanil	0.20	Strawberry (1.0)	0.13	0.13	73	74
2-phenylphenol	0.40	Lemon (10.0)	2.10	2.10	88	78
Tebuconazole	0.20	Peach (1.0)	0.13	0.13	81	86
Thiabendazole	0.20	Lemon (5.0)	2.00	2.20	73	83

[a] **Not corrected for recovery**
[b] **Single spike corresponding to 4 times the reporting level included in analysis batch to monitor analytical performance**

Once the utility of isocratic LCMSMS in this application area had been established, it was then possible to assess the impact of this experimental approach upon the efficiency of analytical procedures carried out in our laboratory. This was achieved by comparing overall analysis times of the isocratic LCMSMS method with gradient LCMSMS methods used in our laboratory. It could take up to 20 minutes for gradient equilibration between each run, which compromised the benefits achieved from the direct analysis of crude extract. This was eliminated by the use of isocratic separation. In addition, the frequency of adaptation of gradient methods or the need for development of 'customised' gradients was significantly reduced with regard to the pesticides involved in this study.

CONCLUSIONS:

- A method has been developed that combines isocratic HPLC separation and tandem mass spectrometry for the quantitative and qualitative determination of pesticide multi-residues in crude extracts of a variety of fruit and vegetables.
- This experimental approach has provided significant efficiency gains of at least 25% against gradient LCMSMS methods used previously.