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New Technologies for the Simultaneous Analysis of Multiple Pesticide Residues in Agricultural Produce

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Abstract

This application note highlights recent advances in the chromatographic and mass spectrometric technologies via the analysis of a multi-component mixture for surveillance monitoring of pesticides in agricultural produce.

Introduction

Pesticides are often used in the production of foodstuffs. The concentrations of individual pesticides permitted in our food are controlled by legislation. There is, therefore, a requirement for surveillance monitoring of pesticide residues in foodstuffs. Analytical methods developed for this purpose must achieve limits of detection at or below the Maximum Residue Limit (MRL).

Given the large number of pesticides in existence and the variety of agricultural produce available, multi-residue pesticide screening methods can offer efficiency advantages over single residue and class specific methods. However, these multi-residue methods are limited both by the chromatographic separation of the analytes and the speed of data acquisition.

Tandem quadrupole mass spectrometry is often used as a detection system due to the high selectivity offered in multiple reaction monitoring (MRM) mode, which compensates for generic sample preparation methods involving minimal sample cleanup. Due to the number of potential analytes, the mass spectrometer chosen should be able to rapidly switch both between MRM channels and between positive and negative ionization modes, thereby offering the potential to achieve greater efficiency in the analysis of multi-component mixtures. Complementing these +/– ionization mode switching capabilities in the Waters Micromass Quattro Premier Mass Spectrometer is the revolutionary Waters ACQUITY UPLC System, offering improved chromatographic resolution and shorter analysis times resulting from the use of columns packed with novel 1.7 µm stationary phase particles.[1]

In this work, we highlight recent advances in these chromatographic and mass spectrometric technologies via the analysis of a multi-component mixture for surveillance monitoring of pesticides in agricultural produce.

Experimental

Method

Sample Preparation, Extraction and Cleanup Procedure

The raisin sample, Californian sun-dried seedless raisins (Thompson variety) was prepared using a procedure described below involving methanolic extraction and ChemElut cleanup, evaporation and reconstitution.[2]

The raisin sample was chopped to avoid loss of juice. A 5 g aliquot of the homogenized sample was transferred to a blender cup, to which 9 mL of water was added. After 10 minutes, 20 mL of methanol was added and the sample was blended for 2 minutes. 6 mL of the resultant extract was mixed with 2 mL of a solution of sodium chloride (20 g in 100 mL water). A 5 mL aliquot was then transferred to a ChemElut column containing 5 mL of diatomaceous earth. After 5 minutes, the ChemElut column was eluted with 16 mL of dichloromethane. The eluate was evaporated to dryness and the dry residue was reconstituted in 250 µL of methanol and further diluted with 1 mL of water. The final extract contained the residues of 0.5 g dry sample per mL. The extract was filtered through a 0.45 µm filter into a glass sample vial.

Blank matrix was prepared from organically grown sun-dried seedless raisins (Thompson variety) using the same extraction and cleanup procedure described above. Matrix-matched standards were prepared by spiking all analytes at 0.5, 1, 2.5, 5, 10 pg/L (equivalent to 1, 2, 5, 10, 20 µg/kg, respectively).

LC Conditions

LC system:	ACQUITY UPLC System
Mobile phase A:	MeOH/H_2O (1:4 v/v) + 5 mM $CH_3CO_2NH_4$
Mobile phase B:	MeOH/H_2O (9:1 v/v) + 5 mM $CH_3CO_2NH_4$
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 100 mm, 1.7 µm
Flow rate:	0.45 mL/min
Injection volume:	20 µL

Column temp.:	40 °C

Gradient elution

Time	%B
0 min	0%
8.5 min	100%
11.0 min	100%
11.1 min	0%
13.5 min	0%

MS Conditions

MS system:	Quattro Premier
Ionization mode:	ES+/ES-
Capillary voltage:	0.8 kV (+/- ionization)
Gas flow:	800 L/hr
Source temp.:	120 °C
Desolvation temp.:	400 °C
Cone voltage:	See Table 1
MS/MS:	Operated in MRM mode
Collision voltage:	See Table 1

Pesticide Residue	Retention Time (min)	Precursor Ion m/z	Product Ion m/z	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (ppb)
Daminozid	0.50	161.1	143.1	18	12	200	0.01
Methamidophos	0.79	141.8	93.8	22	14	80	0.02
			124.9	22	13	80	
Acephate	0.89	184.1	143.0	16	8	40	0.04
Butoxycarboxim-sulfoxide	1.00	207.1	132.1	17	6	30	0.05
Omethoate	1.01	214.0	183.0	20	12	30	0.01
			154.9	20	15	30	
Aldicarb-sulfoxide	1.11	207.1	132.0	16	10	30	0.1
			89.0	16	14	30	
Butoxycarboxim	1.20	240.1	106.1	10	14	30	0.04
Aldoxycarb	1.26	240.1	86.0	15	20	30	0.005
Oxamyl	1.32	237.1	71.9	12	10	30	0.003
Propamocarb	1.36	189.1	102.0	25	17	30	0.01
			144.0	25	12	30	
Oxydemeton-methyl	1.49	247.0	169.0	20	13	10	0.002
Pymetrozin	1.57	218.0	105.0	25	17	10	0.02
6-chloro-4-hydroxy-3-phenyl-pyridazin	1.60	207.1	77.0	35	30	10	0.04
			104.0	35	21	10	
Methomyl	1.60	162.9	87.8	15	8	10	0.01
			105.9	15	10	10	
Demeton-S-methyl-sulfon	1.61	263.1	169.1	28	16	10	0.02
			121.2	28	16	10	

Table 1. MRM method parameters, UPLC retention times and LODs achievable from solvent standards.

Table 1. (continued)

Pesticide Residue	Retention Time (min)	Precursor Ion m/z	Product Ion m/z	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (ppb)
Quinmerac	1.69	222.0	141.0	22	33	10	0.008
Monocrotophos	1.78	224.0	126.9	20	15	10	0.005
Bendiocarb	1.78	224.1	109.0	18	18	10	0.01
			167.1	18	9	10	
Nicosulfuron	1.80	411.0	182.1	22	18	10	0.05
Amidosulfuron	1.84	370.0	261.2	18	14	10	0.02
Metsulfuron - methyl	2.00	382.0	167.0	22	15	10	0.02
Thifensulfuron - methyl	2.00	388.0	167.1	22	15	10	0.02
Ethiofencarbsulfon	2.04	275.1	107.1	10	20	10	0.006
Rimsulfuron	2.05	431.9	182.1	30	22	10	0.02
Ethiofencarbsulfoxide	2.13	242.1	107.0	18	18	10	0.003
Thiofanox-sulfoxide	2.14	252.1	104.0	10	12	10	0.3
Imidacloprid	2.14	256.1	209.2	22	16	10	0.02
			175.1	22	20	10	
Florasulam	2.28	360.1	129.0	30	20	10	0.09
5 Hydroxy-clethodim-sulfon	2.29	408.2	204.2	22	16	10	0.1
Thiofanox-sulfon	2.32	268.1	76.0	10	10	10	0.02
Clethodim-imin-sulfon	2.35	302.2	98.1	35	30	10	0.04
Metamitron	2.37	203.0	175.1	28	16	10	0.02
Cinosulfuron	2.42	414.1	183.1	25	18	10	0.05
Chlorsulfuron	2.43	358.1	141.1	25	16	10	0.08
			167.1	25	16	10	
Bromoxynil*	2.45	273.9	78.9	40	25	30	0.2
Dimethoate	2.48	230.1	125.1	17	20	10	0.03
			199.1	17	10	10	
Clethodim-imin-sulfoxide	2.49	286.2	208.2	25	17	10	0.03
Vamidothion	2.51	288.1	146.1	17	12	10	0.005
Carbofuran-3-hydroxy	2.56	220.1	163.1	25	10	10	0.007
Flazasulfuron	2.66	408.1	182.1	25	22	10	0.5
Triasulfuron	2.85	402.0	167.1	25	17	10	0.2
			141.0	25	20	10	
Clethodim-sulfon	2.90	392.1	300.2	20	12	10	0.04
Clethodim-sulfoxide	2.95	376.1	206.2	22	15	10	0.05
Carbendazim	2.98	192.1	160.1	25	18	10	0.05
			132.1	25	30	10	
Thiacloprid	3.05	253.0	126.0	28	22	10	0.01
Difenzoquat methylsulfate	3.12	249.2	193.1	45	28	10	0.03
Butocarboxim	3.32	213.1	75.0	20	14	10	0.005
Aldicarb	3.39	208.1	116.0	7	7	10	0.3
Ioxynil*	3.40	369.8	126.9	40	30	20	0.1
Carbofuran	3.41	222.3	165.2	25	15	10	0.1
Iodosulfuron	3.63	508.2	167.2	25	18	30	1
Thiabendazol	3.78	202.0	175.1	40	25	20	0.07
			131.0	40	32	20	
Propoxur	4.17	210.1	111.0	14	15	10	0.01

Table 1. (continued) MRM method parameters, UPLC retention times and LODs achievable from solvent standards.

Table 1. (continued)

Pesticide Residue	Retention Time (min)	Precursor Ion m/z	Product Ion m/z	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (ppb)
Formetanate	4.23	222.1	165.2	20	12	10	0.005
Prosulfuron	4.46	420.0	141.1	25	20	10	0.2
			167.0	25	18	10	
Carbaryl	4.60	202.1	145.0	18	10	10	0.005
Bensulfuron-methyl	4.67	411.1	149.1	25	22	10	0.05
Ethiofencarb	4.76	226.1	107.1	15	15	10	0.01
			164.1	15	8	10	
Primisulfuron-methyl*	4.84	466.9	226.2	20	15	10	1
Triflusulfuron-methyl	4.86	493.0	264.2	28	20	10	0.8
Thiodicarb	4.88	355.1	87.9	15	16	10	0.02
Thiofanox	4.92	219.0	56.9	15	18	10	0.01
Pirimicarb	4.97	239.1	72.0	28	18	10	0.005
			182.1	28	15	10	
Atrazin	5.08	216.1	174.1	30	17	10	0.01
Isoproturon	5.26	207.1	72.1	25	18	10	0.008
Isoxaflutole	5.31	377.1	251.2	15	20	10	0.3
Metalaxyl	5.34	280.1	220.2	20	13	10	0.01
			192.2	20	17	10	
Diuron	5.35	233.1	72.1	25	18	10	0.02
3,4,5-Trimethacarb	5.41	194.1	137.1	18	10	10	0.01
Clethodim	5.52	360.2	164.1	20	19	10	0.05
Desmedipham	5.56	318.2	182.2	17	12	10	0.01
Phenmedipham	5.69	301.1	168.0	25	10	10	2
Linuron	5.92	249.1	160.0	28	16	10	0.02
			182.1	28	15	10	
Pyrimethanil	5.93	200.1	107.0	42	22	10	0.1
			82.0	42	25	10	
Azoxystrobin	5.97	404.1	372.2	22	15	10	0.02
			329.2	22	30	10	
Methiocarb	6.06	243.1	121.0	10	22	10	0.3
Fludioxonil*	6.20	247.0	180.1	45	28	20	0.1
			126.1	45	35	20	
Promecarb	6.23	208.1	151.0	20	9	10	0.03
			109.0	20	15	10	
Iprovalicarb	6.55	321.2	119.1	15	18	10	0.1
Fenhexamid	6.61	302.1	97.0	35	25	10	0.05
			55.1	35	35	10	
Metolachlor	6.81	284.1	176.1	20	25	10	0.01
			252.1	20	15	10	
Tebufenozide	7.01	353.2	133.0	13	20	10	0.4
			297.2	13	8	10	
Fenoxycarb	7.04	302.1	88.0	20	20	10	0.05
Cyprodinil	7.19	226.2	93.1	45	33	10	0.08
			108.1	45	25	10	
Tebuconazol	7.23	308.1	70.0	30	20	10	0.02

Table 1. (continued) MRM method parameters, UPLC retention times and LODs achievable from solvent standards.

Table 1. (continued)

Pesticide Residue	Retention Time (min)	Precursor Ion m/z	Product Ion m/z	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (ppb)
Imazalil	7.24	297.1	159.0	30	20	10	0.3
			69.1	30	20	10	
Triflumuron	7.49	359.1	156.0	25	18	10	0.2
			139.0	25	37	10	
Haloxyfop-methyl	7.73	376.1	316.2	30	18	10	0.03
Indoxacarb	7.80	527.9	218.1	28	20	10	0.5
Hexaflumuron*	7.85	459.1	276.1	22	22	30	5
Quizalofop-ethyl	8.00	373.1	299.2	30	19	10	0.03
Fluazifop-P-butyl	8.07	384.1	282.2	32	22	10	0.02
			328.2	32	16	10	
Haloxyfop-ethoxyethyl	8.07	434.0	316.2	25	20	10	0.05
Spiroxamine	8.11	298.3	144.1	30	20	10	0.03
Furathiocarb	8.12	383.1	195.1	20	16	10	0.04
Diflubenzuron	8.14	311.0	158.1	30	14	10	0.1
Teflubenzuron*	8.31	379.0	196.0	18	25	10	0.8
			339.1	18	15	10	
Flufenoxuron	8.68	488.9	158.1	25	18	10	0.05
Pyridate		379.1	207.1	25	16	120	0.05
Fenpropimorph	9.38	304.2	147.2	45	30	120	0.02

Table 1. MRM method parameters, UPLC retention times and LODs achievable from solvent standards.

Results and Discussion

Method Development and Performance

The work details the development of a multi-residue method for the analysis of 100 pesticide residues by UPLC-MS/MS. The work is based upon a previously developed HPLC-MS/MS method using a Waters Alliance HT/Quattro Premier System, which had an overall cycle time of 25 minutes (HPLC Conditions: XTerra MS C_{18} Column, 2.1 x 100 mm, 3.5 µm, linear gradient from 0 to 100% B in 17 min).

Comparison of UPLC and HPLC chromatograms is shown below (Figure 1). Peak widths observed for the majority of pesticide residues analyzed under UPLC conditions are approximately 0.1 min (cf 0.3 min under HPLC conditions). The narrower peak widths often resulted in an increase in signal response over that achieved under HPLC-MS/MS conditions (Figure 1).

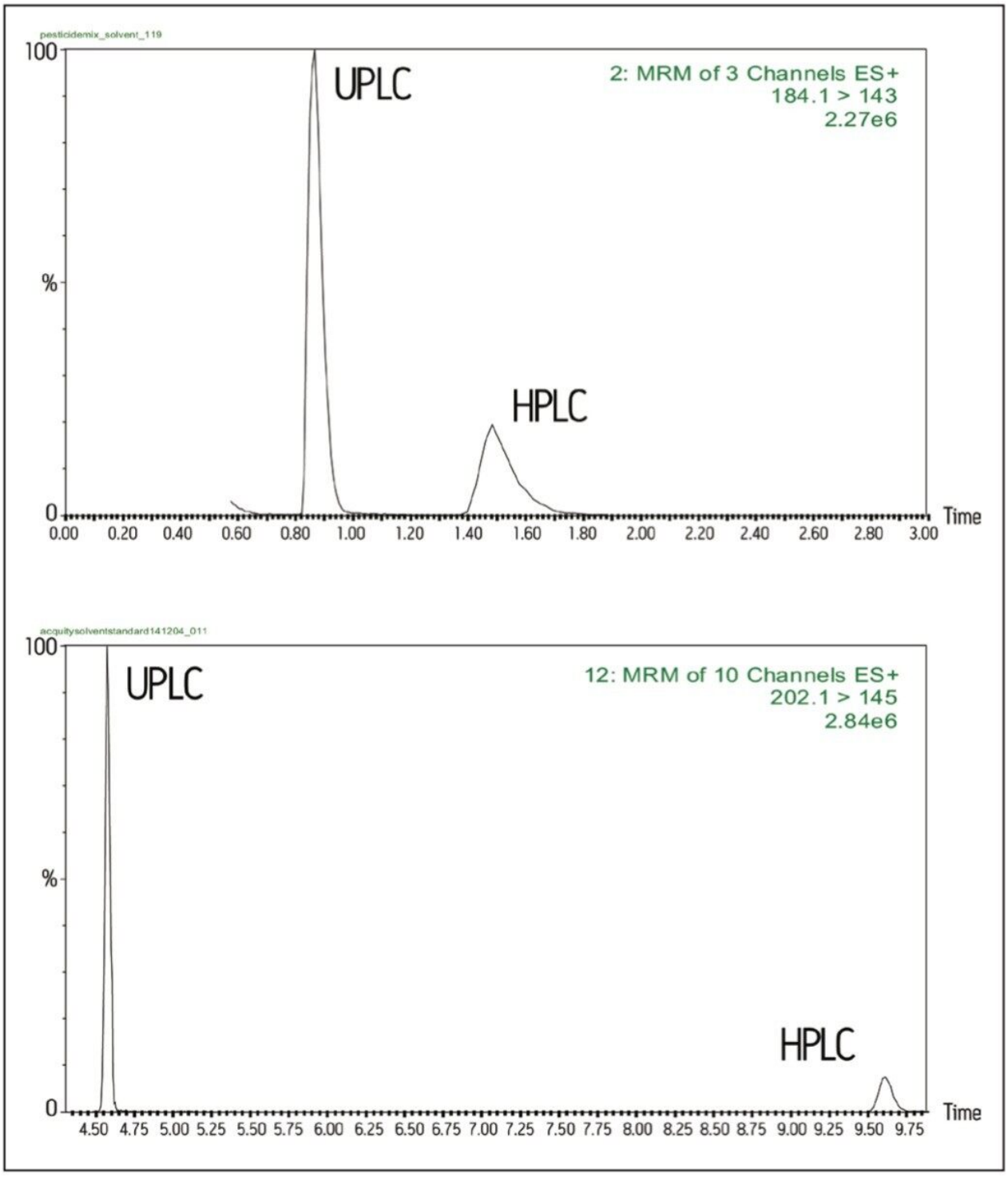

Figure 1. Comparison of UPLC (0.1 min) and HPLC (0.3 min) chromatograms. Data obtained for a) acephate and b) carbaryl (solvent standard) at 10 pg/µl.

Greater chromatographic resolution is achievable under UPLC conditions (cf. HPLC) and is illustrated in Figure 2. Butoxycarboxim sulfoxide and aldicarb sulfoxide have similar retention properties, with

butoxycarboxim sulfoxide eluting first, and the same MRM transition (*m/z* 207.1>132).

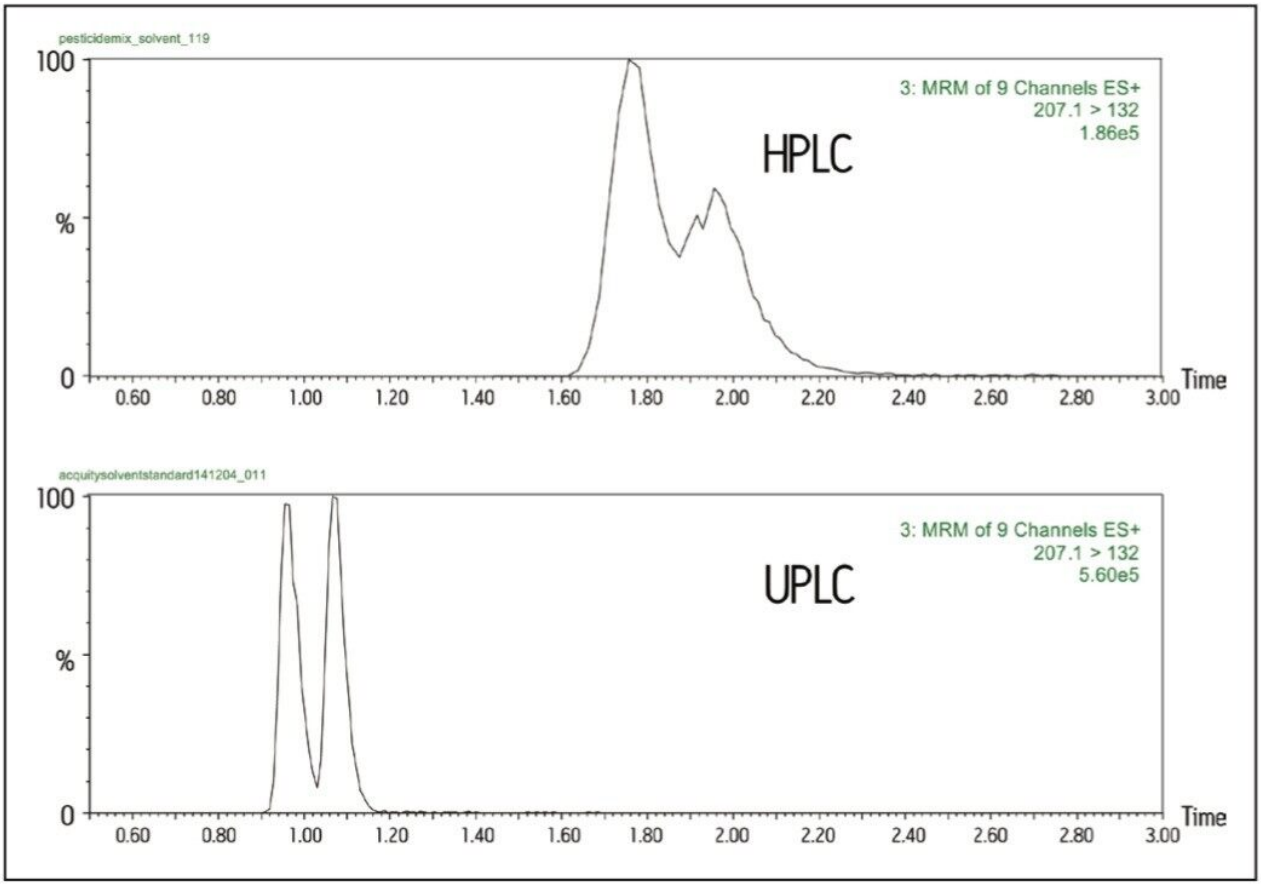

Figure 2. Increased resolution of UPLC over HPLC.

It can be seen that UPLC has the ability to separate complex mixtures. This is confirmed by considering the analysis of 100 pesticide residues in raisin matrix (Figure 3). All 100 pesticides elute within 10 minutes, and the overall cycle time is just 13.5 minutes.

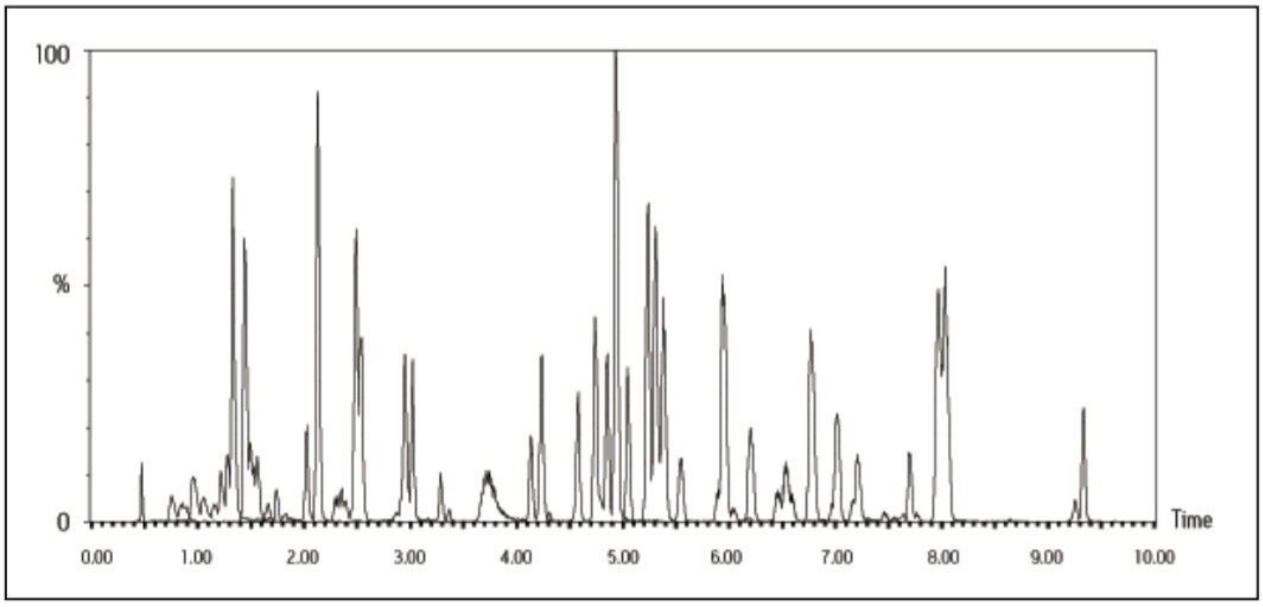

Figure 3. UPLC TIC chromatogram for the analysis of 100 pesticide residues in raisin extract (10 µg/kg).

Since the analytical method is intended for surveillance monitoring, it needs to be able to detect tens of pesticide residues; some of which are better detected under negative ES conditions (Table 1). The use of the ACQUITY UPLC System places added demands on the mass spectrometer due to the improved chromatographic resolution and short analysis times. For these reasons, the Quattro Premier Tandem Quadrupole Mass Spectrometer was selected as the detector for this application.

In order for accurate quantization to be performed, a minimum of 10 data points across each peak must be acquired. This requirement, coupled with the number of target analytes and narrow chromatographic UPLC peaks indicated that it would be advantageous if the MRM functions were arranged into time windows, based on analyte retention times (Figure 4). This system enabled the flexible use of dwell times (Table 1), such that those peaks with lower intensities can have their S/N ratios increased by employing longer dwell times, while retaining a minimal scan time.

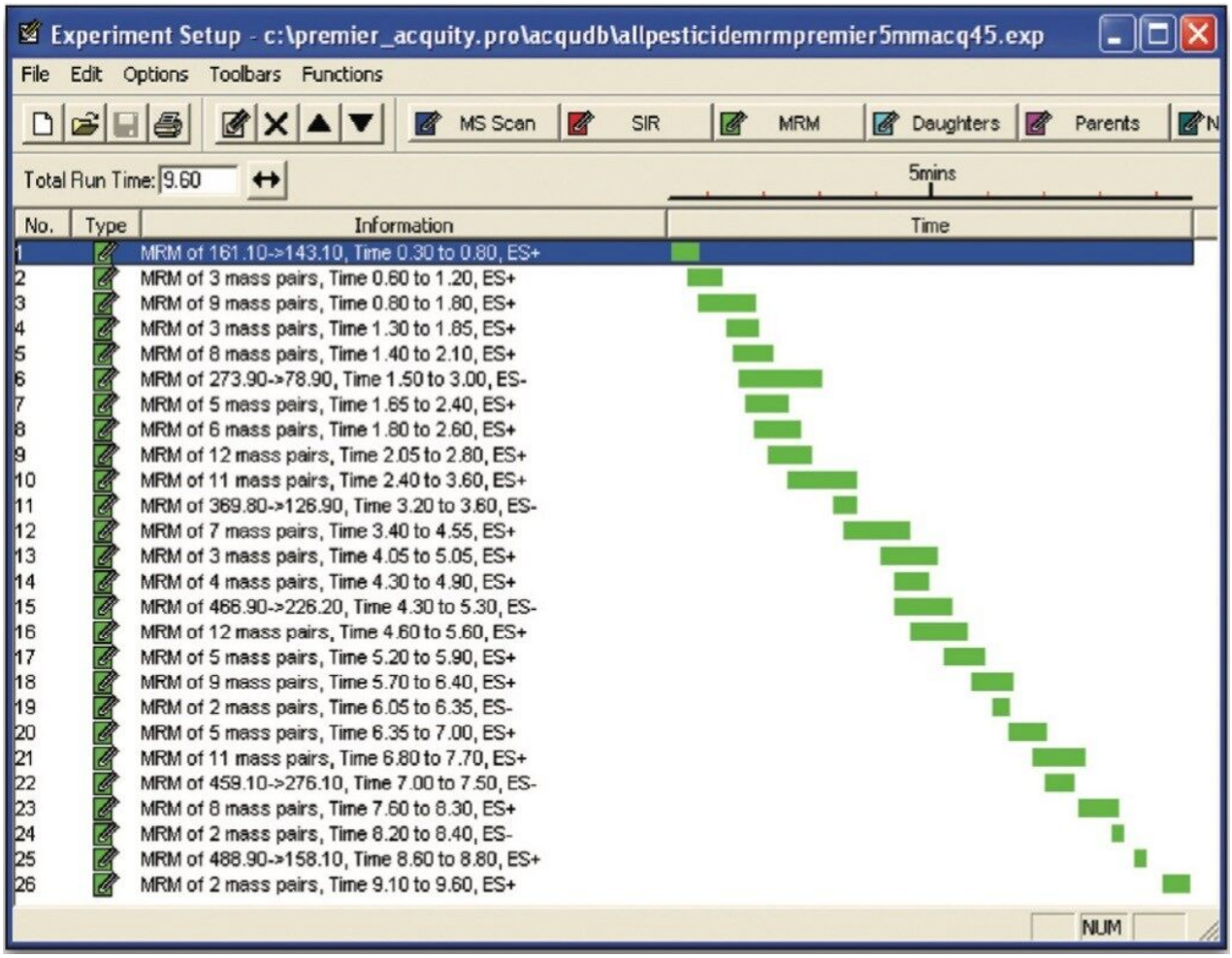

Figure 4. MRM functions arranged into time windows.

In addition to the primary MRM traces monitored for each analyte, confirmation MRM traces were incorporated into the method for the 31 most commonly found residues. In total, 131 MRM transitions were monitored in 26 time windows (Figure 4).

Six of the pesticides included within the method ionize under negative ES mode. The Quattro Premier can switch rapidly between positive and negative ionization modes, so that closely eluting analytes under both modes can be achieved within a single analytical run as illustrated above right (Figure 5), thereby minimizing the need to perform separate analyses.

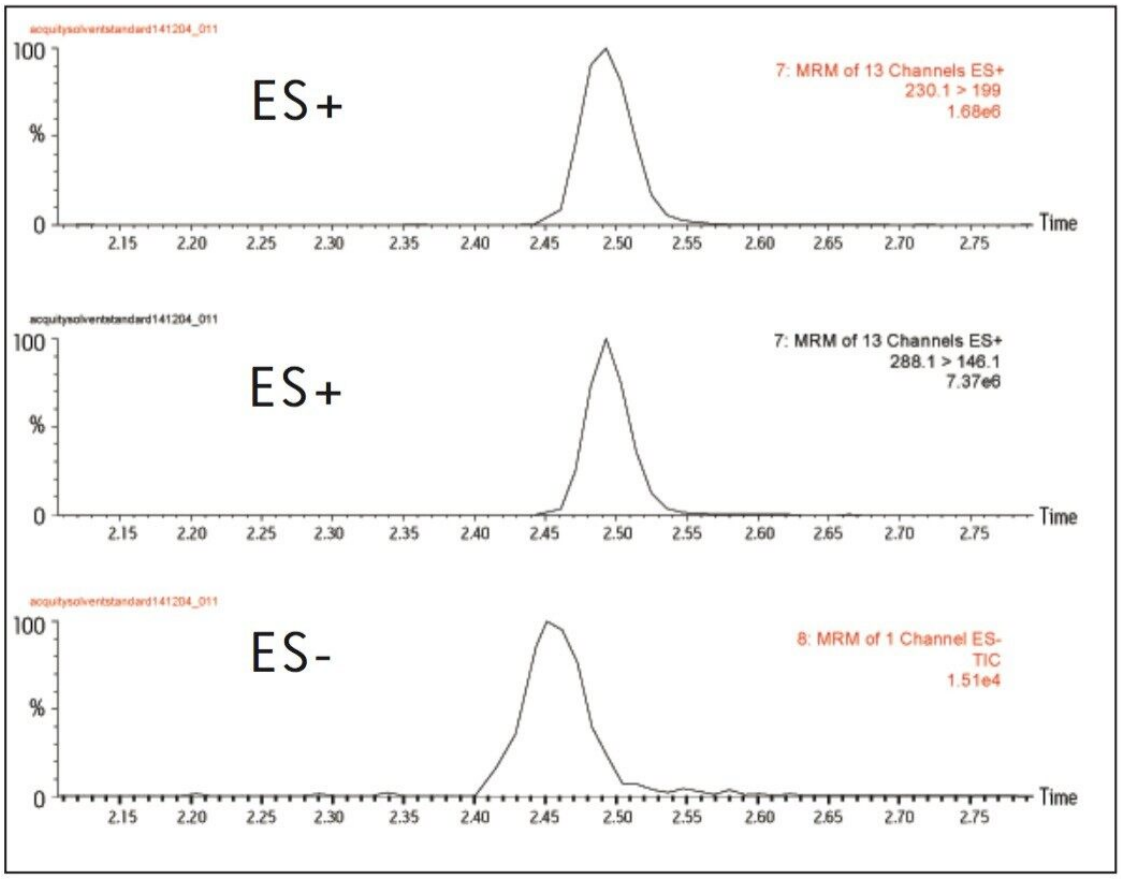

Figure 5. Chromatographic traces for dimethoate and vamidothion (ES+) and bromoxynil (ES-).

Analysis of standard solutions enabled LODs (based on 3 x S/N) to be determined (Table 1). All are well below the necessary reporting level of individual pesticides in food (10 µg/kg, 5 pg/L), indicating that this method could be applied to the analysis of pesticide residues in a variety of matrices.

Application

The analytical method was applied to the analysis of pesticide residues in raisins. The chromatogram (Figure 3) obtained for the analysis of a raisin sample containing the pesticides spiked at a level equivalent to the MRL demonstrates good signal response for all analytes at this reporting level. Since the analytical method is intended for surveillance monitoring, it needs to be able to detect tens of pesticide residues; some of which are better detected under ES- conditions (Table 1).

Good linearity in calibration was demonstrated over the range analyzed, 1-20 µg/kg (Figure 6).

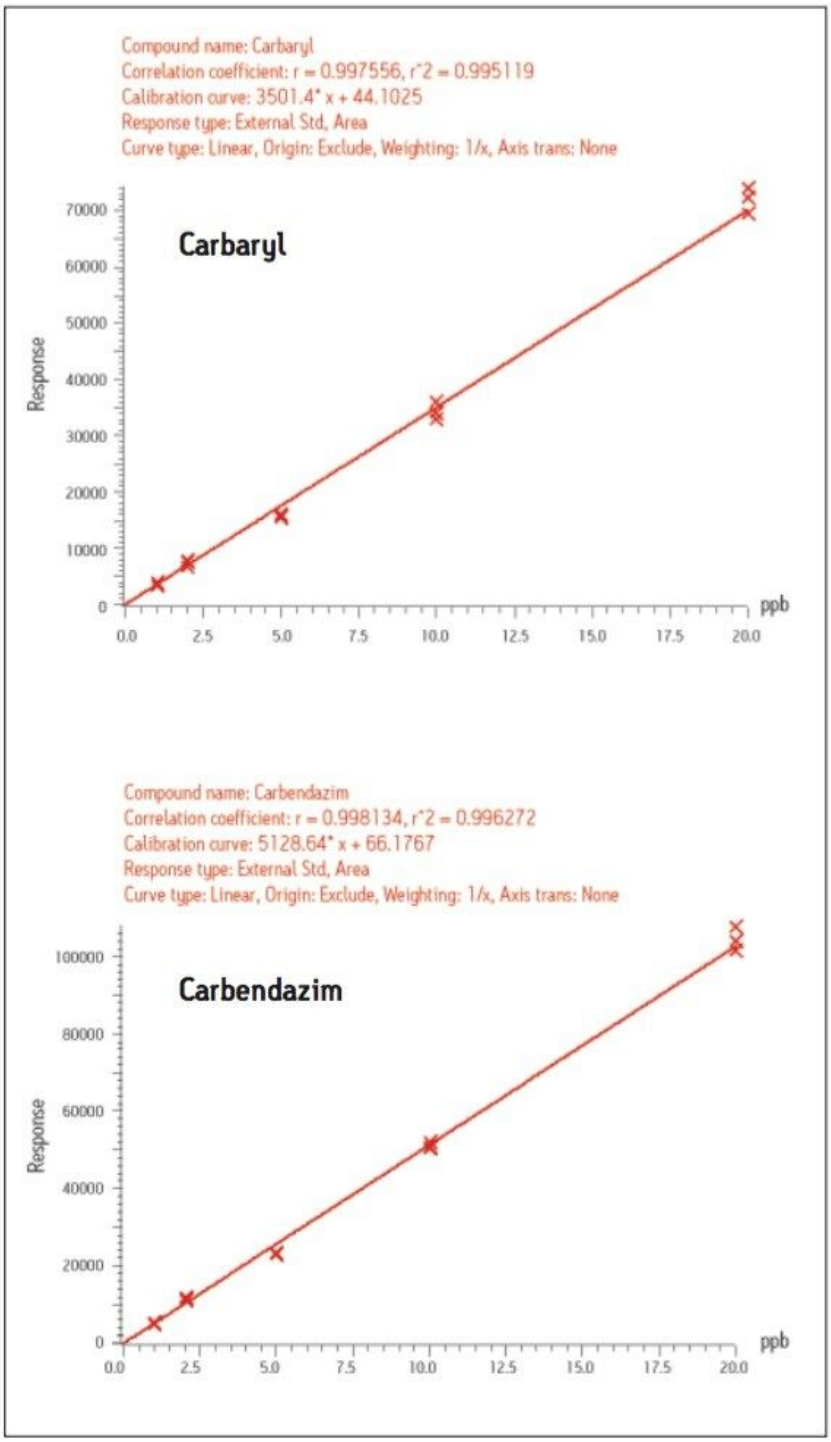

Figure 6. Representative calibration graphs for the analysis of carbaryl (top) and carbendazim (bottom) spiked into blank raisin matrix at a range of concentrations.

Inclusion of a second transition within the surveillance method enables unambiguous confirmation of the presence of a residue within the sample, without the need to perform a second confirmatory analytical run

(Figure 7) resulting in further efficiency gains. Two pesticide residues (imidacloprid and tebufenozide) were confirmed present within the raisin sample at levels below the MRL, 4.4 and 3.4 µg/kg, respectively.

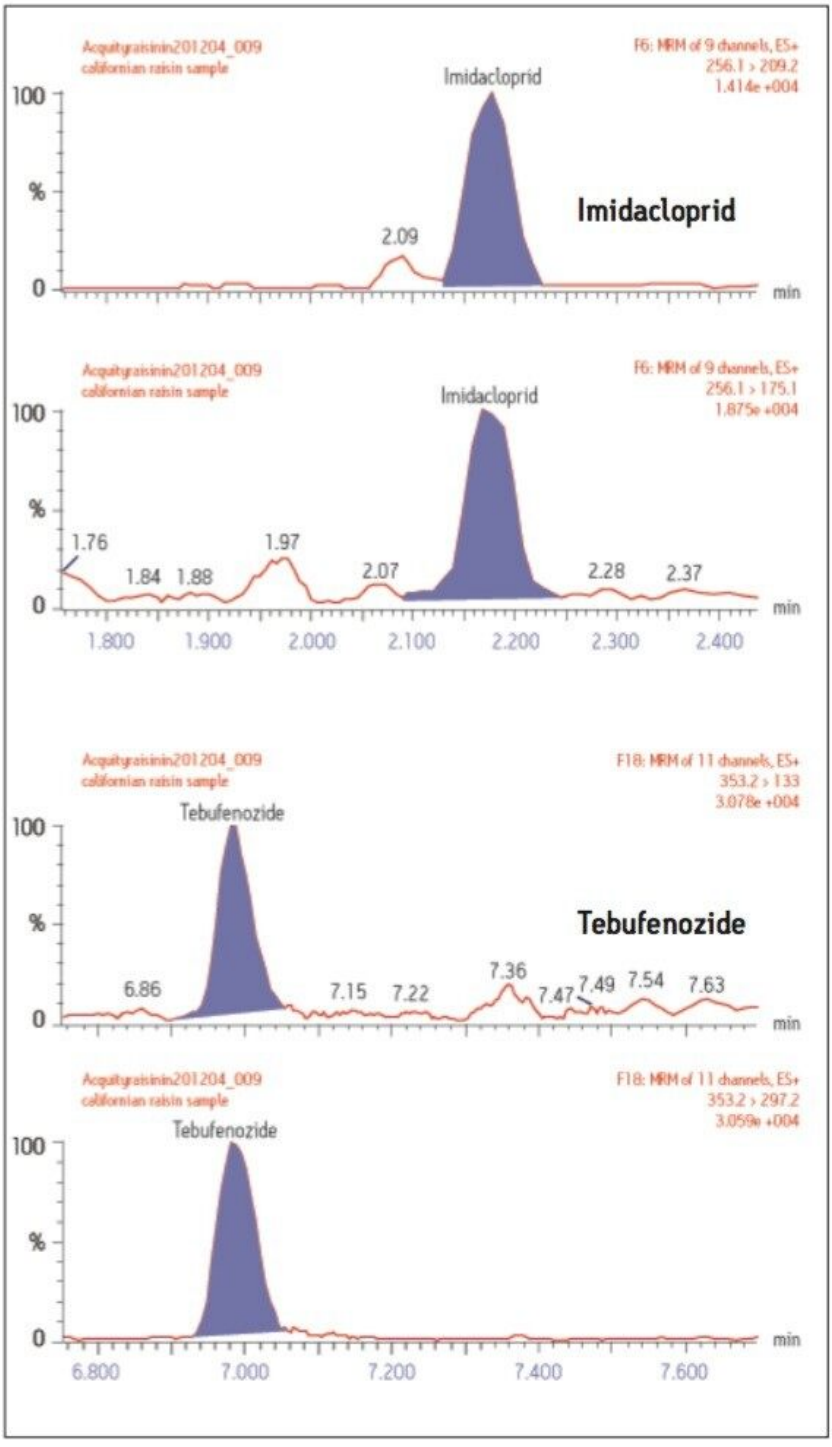

Figure 7. Confirmation that the Californian raisin sample contained imidacloprid (top) and tebufenozide (bottom).

Conclusion

- A rapid multi-residue UPLC-MS/MS method has been developed for surveillance monitoring of 100 pesticide residues and has been applied to the analysis of raisins.
- Improved efficiency and increased sample throughput has been realized through the combination of these UPLC and MS technologies which offer:
  - enhanced chromatographic resolution and short analysis times.
  - the ability to group MRM functions into time windows, enabling the incorporation of confirmatory MRM traces.
  - the capability to switch rapidly between MRM channels and between positive and negative ionization modes.
- The sensitivity achieved for the majority of pesticide residues indicates that this UPLC-MS/MS method could be applied to the analysis of pesticides in different matrices over the range analyzed.
- Given the chromatographic improvements afforded by the ACQUITY UPLC System coupled to the advances in data acquisition methods seen with the Quattro Premier Mass Spectrometer, it is feasible that this method could be extended to over three hundred compounds (provided efficient sample extraction).

References

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2. A Multi-Residue LC-MS/MS Method for the Determination of 81 Pesticide Residues in Fruit and Vegetables: Part 1. Waters Application Note 720000686EN < https://www.waters.com/nextgen/us/en/library/application-notes/2003/a-multi-residue-lc-ms-ms-method-for-the-determination-of-81-pesticide-residues-in-fruit-and-vegetables-part-1-method-overview.html> . Method Overview.

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