アプリケーションノート

Comparison of SIM and MRM for the Quantitative Confirmation of Pesticide Residues in Food

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Abstract

This application note aims to compare the results from SIM and MRM using the Waters Micromass Quattro micro GC Tandem Quadrupole Mass Spectrometer for the quantification and confirmation of pesticide residues in simple through to complex food matrices.

Introduction

The inappropriate or unlawful use of pesticides on agricultural produce can result in unacceptably high levels of their residues in produce destined for human consumption. Food produce that is to be used for this purpose must contain less than the statutory maximum residue limit (MRL) of any given residue.

Worldwide, there are over 800 compounds currently in use to control pests such as insects, weeds, rodents and fungi. The legal enforcement of regulations governing pesticide use requires the regular monitoring of foodstuffs. Given the large number of residues that may be found it is often advantageous to extract and determine as many of them as possible during a single analysis. An extraction, with acetonitrile, followed by dispersive solid phase extraction (SPE) clean up was reported for the analysis of a wide range of pesticides in fruits and vegetables[1] and fatty samples.[2] As the number of target analytes is increased, the selectivity of the extraction method must be compromised, resulting in a more complex sample matrix. The potential for analytical interference from co-extracted substances is high and the analytical selectivity of such a method must be provided by the determinative step.

Mass spectrometry is a highly selective analytical technique that can be used to monitor the masses of specific ions generated from the analytes of interest.

The use of selected ion monitoring (SIM) provides a greater level of selectivity than other traditional detection methods, e.g. flame ionization detection (FID) or electron capture detection (ECD). However, when the analysis of multiresidue pesticides is required, in a variety of produce, the low selectivity of the clean-up stage means that even the use of SIM does not eliminate the potential for matrix interference.

Multiple reaction monitoring (MRM) is a tandem mass spectrometric technique that allows the monitoring of specific collision induced dissociation (CID) reactions. The nature of CID reactions depends on molecular structure as well as mass and, as a result, significant improvements in analytical selectivity may be achieved using MRM.

The aim of this work was to compare the results from SIM and MRM using the Waters Micromass Quattro micro GC Tandem Quadrupole Mass Spectrometer for the quantification and confirmation of pesticide residues in simple through to complex food matrices.

Waters Micromass Quattro micro GC Mass Spectrometer.

Experimental

Extraction Method

10 g frozen sample was weighed in a centrifuge tube. Acetonitrile (10 mL) was added along with 100 µL of internal standard solution (PCB 138 and TPP) and the tube was shaken for 1 min. $MgSO_4$ (4g) and NaCl (1g) were added and the solution buffered to pH 5.0-5.5 with citrate buffer. After shaking and centrifugation, an aliquot was transferred to a vial containing PSA sorbent and anhydrous $MgSO_4$. After undergoing further shaking and centrifugation, the extract was acidified to pH 5 to protect base-sensitive residues. The extract was analyzed by GC-MS and GC-MS/MS.

GC Method

Agilent 6890 GC with 7683 autosampler

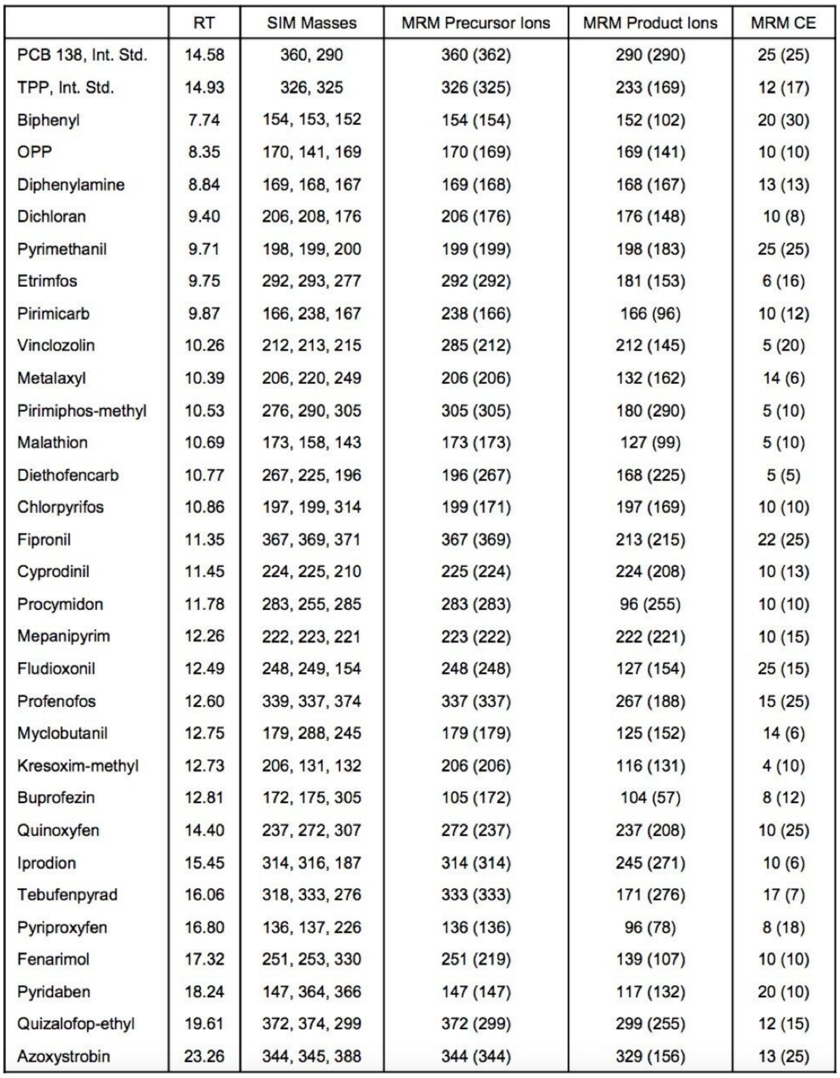
Column:	Varian FactorFour VF-5ms 30 m x 0.25 mm i.d., 0.25 µm
Flow rate	1.0 mL/min helium constant flow

Temp. ramp:	40 °C (Hold 2 min) 220 °C @ 30 °C/min 260 °C @ 5 °C/min 280 °C @ 20 °C/min (Hold 15 min)
Total run time:	32 min
Injection method:	Cyro cooled PTV in solvent vent mode, 3 µL injected
Vent method:	Vent pressure 5 kPa, Vent flow 20 mL/min for 0.5 min

GC-MS/MS Method

The Waters Micromass Quattro micro GC tandem quadrupole mass spectrometer was used in electron impact (EI+) mode. The ion source was operated at 180 °C with an electron energy of 70 eV and a trap current of 200 µA. Two modes of acquisition were employed; SIM and MRM with an argon collision gas pressure of 3.0×10^{-3} mBar.

The Quattro micro GC was tuned so that the precursor and product ions were resolved with a peak width at half height of less than 0.7 Da. The list of pesticide residues, the SIM masses and the MRM transitions, along with the collision energies for the method are listed in Table 1. The dwell, inter-channel and inter-scan times were unchanged between the methods.



	RT	SIM Masses	MRM Precursor Ions	MRM Product Ions	MRM CE
PCB 138, Int. Std.	14.58	360, 290	360 (362)	290 (290)	25 (25)
TPP, Int. Std.	14.93	326, 325	326 (325)	233 (169)	12 (17)
Biphenyl	7.74	154, 153, 152	154 (154)	152 (102)	20 (30)
OPP	8.35	170, 141, 169	170 (169)	169 (141)	10 (10)
Diphenylamine	8.84	169, 168, 167	169 (168)	168 (167)	13 (13)
Dichloran	9.40	206, 208, 176	206 (176)	176 (148)	10 (8)
Pyrimethanil	9.71	198, 199, 200	199 (199)	198 (183)	25 (25)
Etrimfos	9.75	292, 293, 277	292 (292)	181 (153)	6 (16)
Pirimicarb	9.87	166, 238, 167	238 (166)	166 (96)	10 (12)
Vinclozolin	10.26	212, 213, 215	285 (212)	212 (145)	5 (20)
Metalaxyl	10.39	206, 220, 249	206 (206)	132 (162)	14 (6)
Pirimiphos-methyl	10.53	276, 290, 305	305 (305)	180 (290)	5 (10)
Malathion	10.69	173, 158, 143	173 (173)	127 (99)	5 (10)
Diethofencarb	10.77	267, 225, 196	196 (267)	168 (225)	5 (5)
Chlorpyrifos	10.86	197, 199, 314	199 (171)	197 (169)	10 (10)
Fipronil	11.35	367, 369, 371	367 (369)	213 (215)	22 (25)
Cyprodinil	11.45	224, 225, 210	225 (224)	224 (208)	10 (13)
Procymidon	11.78	283, 255, 285	283 (283)	96 (255)	10 (10)
Mepanipyrim	12.26	222, 223, 221	223 (222)	222 (221)	10 (15)
Fludioxonil	12.49	248, 249, 154	248 (248)	127 (154)	25 (15)
Profenofos	12.60	339, 337, 374	337 (337)	267 (188)	15 (25)
Myclobutanil	12.75	179, 288, 245	179 (179)	125 (152)	14 (6)
Kresoxim-methyl	12.73	206, 131, 132	206 (206)	116 (131)	4 (10)
Buprofezin	12.81	172, 175, 305	105 (172)	104 (57)	8 (12)
Quinoxyfen	14.40	237, 272, 307	272 (237)	237 (208)	10 (25)
Iprodion	15.45	314, 316, 187	314 (314)	245 (271)	10 (6)
Tebufenpyrad	16.06	318, 333, 276	333 (333)	171 (276)	17 (7)
Pyriproxyfen	16.80	136, 137, 226	136 (136)	96 (78)	8 (18)
Fenarimol	17.32	251, 253, 330	251 (219)	139 (107)	10 (10)
Pyridaben	18.24	147, 364, 366	147 (147)	117 (132)	20 (10)
Quizalofop-ethyl	19.61	372, 374, 299	372 (299)	299 (255)	12 (15)
Azoxystrobin	23.26	344, 345, 388	344 (344)	329 (156)	13 (25)

Table 1. SIM and MRM method parameters.

Acquisition and Processing Methods

The data were acquired using Waters MassLynx Software and processed using the Waters TargetLynx Application Manager.

In SIM mode, three masses were acquired for each residue while in MRM mode it was two transitions. These were used so that quantification and confirmation could be performed with a single injection assuming that the ion ratio between the masses or transitions was consistent for standards and samples. The confirmation criteria chosen was in accordance with the European Union (EU) guidance document Quality Control Procedures for Pesticide Residue Analysis (SANCO/10476/2003).[3]

Results and Discussion

Five food matrices were compared using SIM and MRM ranging from simple through to complex; cucumber, sweet pepper, grapefruit, wheat flour and curry powder. 0.005 mg/kg was chosen to be the reporting level as this is half of the target MRL, for active substances in products for which no specific MRL is set, as specified in EU regulation (EC) NO 396/2005[4] of 23 February 2005.

For both methods matrix-matched calibration curves were linear over the range 0.005 – 0.500 mg/kg with correlation coefficients greater than 0.990 for all analytes in all matrices.

The difference in matrix interference from simple and complex foodstuffs is illustrated in Figure 1. In the case of pirimiphos-methyl from cucumber, there are few peaks from matrix interference whereas from curry powder there are many more.

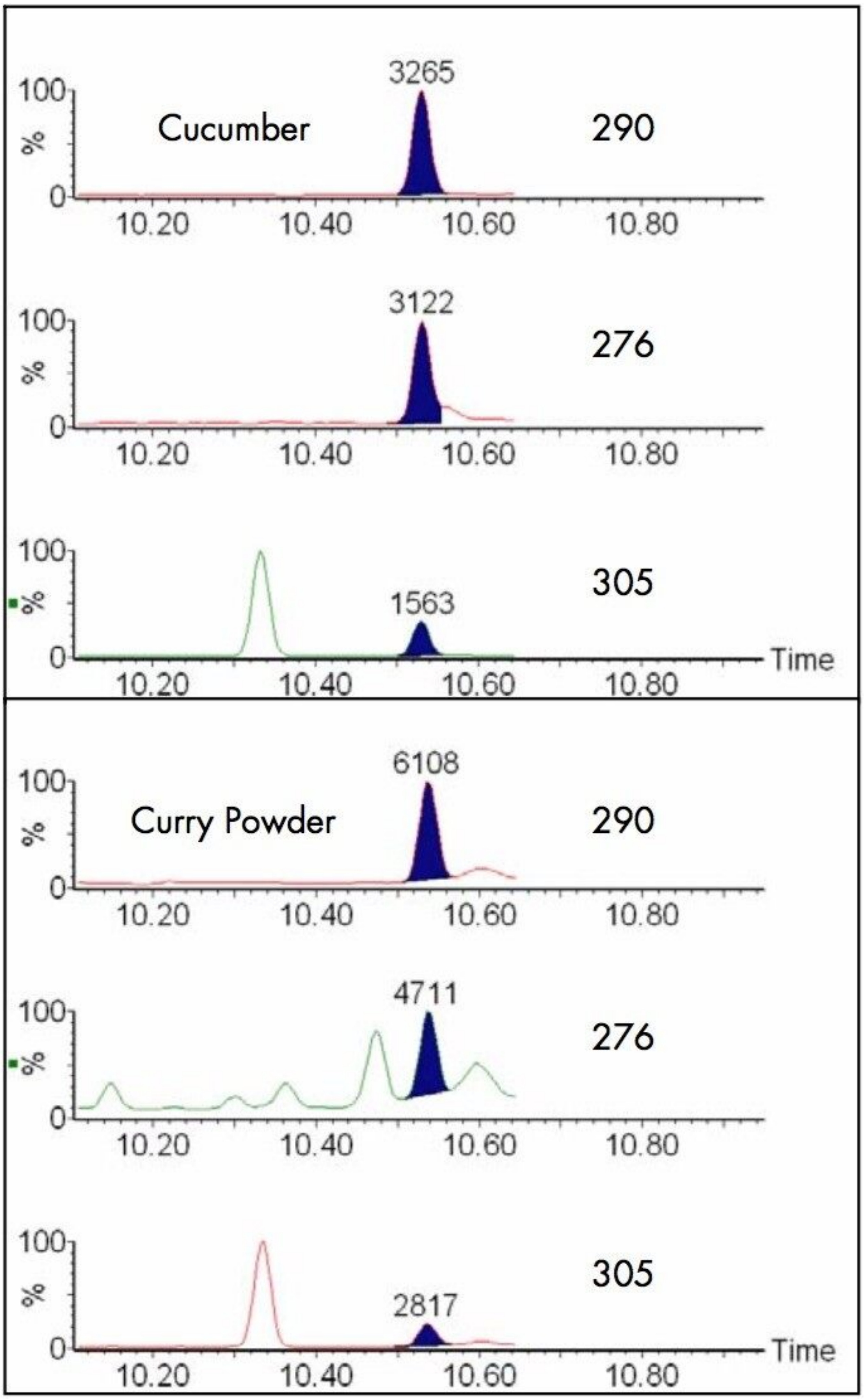


Figure 1. 0.005 mg/kg pirimiphos-methyl in cucumber and 0.025 mg/kg in curry powder using SIM.

In SIM mode, for our confirmation criteria to be passed, any concentrations in the extracts must have ion ratios of 0.934 (± 30%) and 0.497 (± 30%). In this example, the ion ratios are 0.956 and 0.479 in cucumber, and 0.771 and 0.461 in curry powder which pass our confirmation criteria. For pirimiphosmethyl, quantification and confirmation by SIM is possible in both matrices.

Changing the mode of acquisition from SIM to MRM significantly increases the selectivity of the determinative step. Pirimiphos-methyl in curry powder with no matrix interference is illustrated in Figure 2. The lack of matrix interference peaks allows routine, automatic integration to be performed.

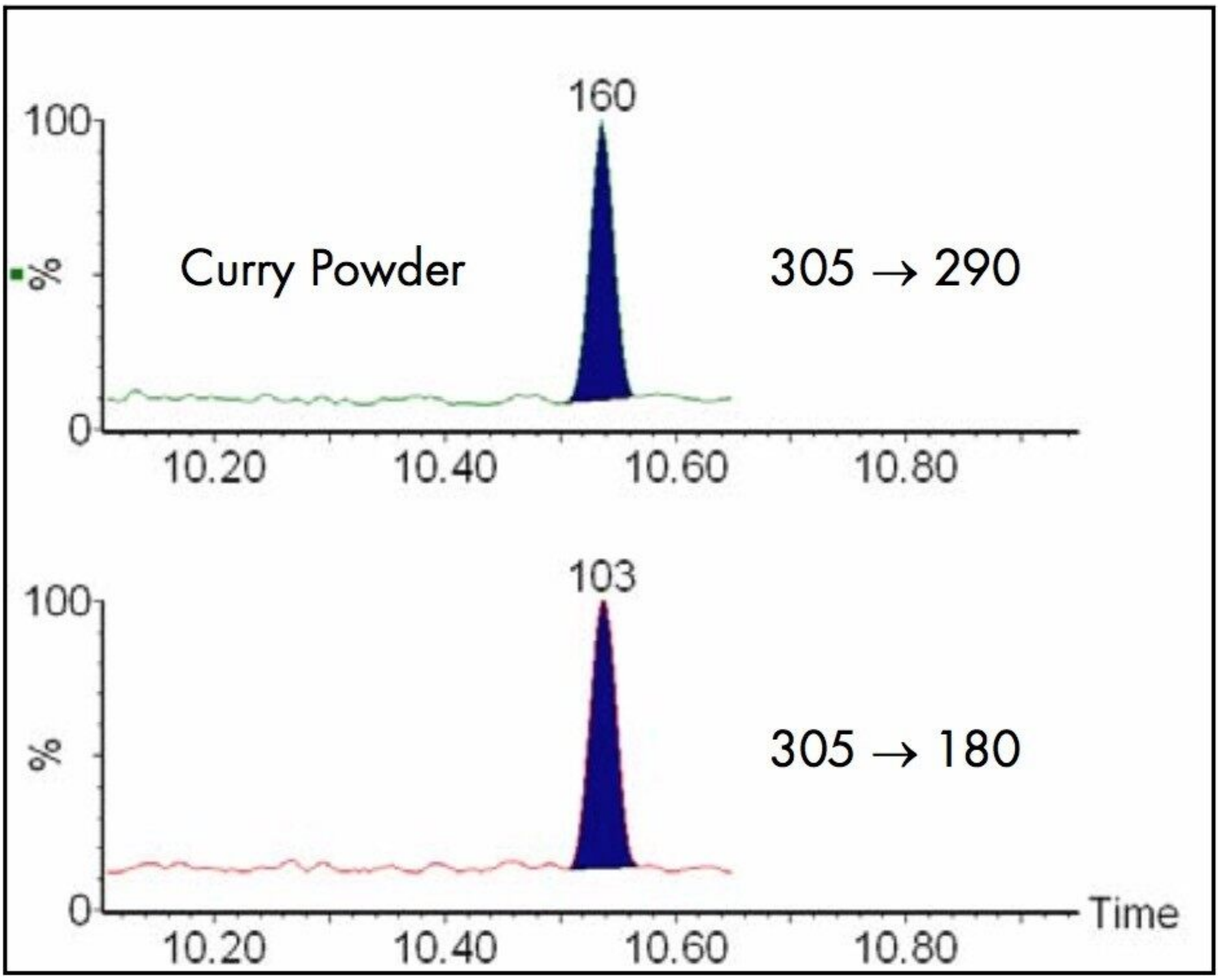


Figure 2. 0.025 mg/kg pirimiphos-methyl in curry powder using MRM.

For our confirmation criteria to be passed in MRM mode, any concentrations in the extracts must have an ion ratio of 0.747 (± 30%). In this example, the ion ratio is 0.644 in curry powder, which passes our confirmation criteria. For pirimiphos-methyl, quantification and confirmation by MRM is possible in curry powder.

Other pesticide residues yield similar results. Biphenyl, vinclozolin and fludioxonil are illustrated in Figures

3, 4 and 5, respectively. Screening would be possible using SIM while confirmation is likely to be problematic if not impossible. However, moving to MRM significantly improves the selectivity in all cases, increasing the probability of being able to perform routine, automatic integration.

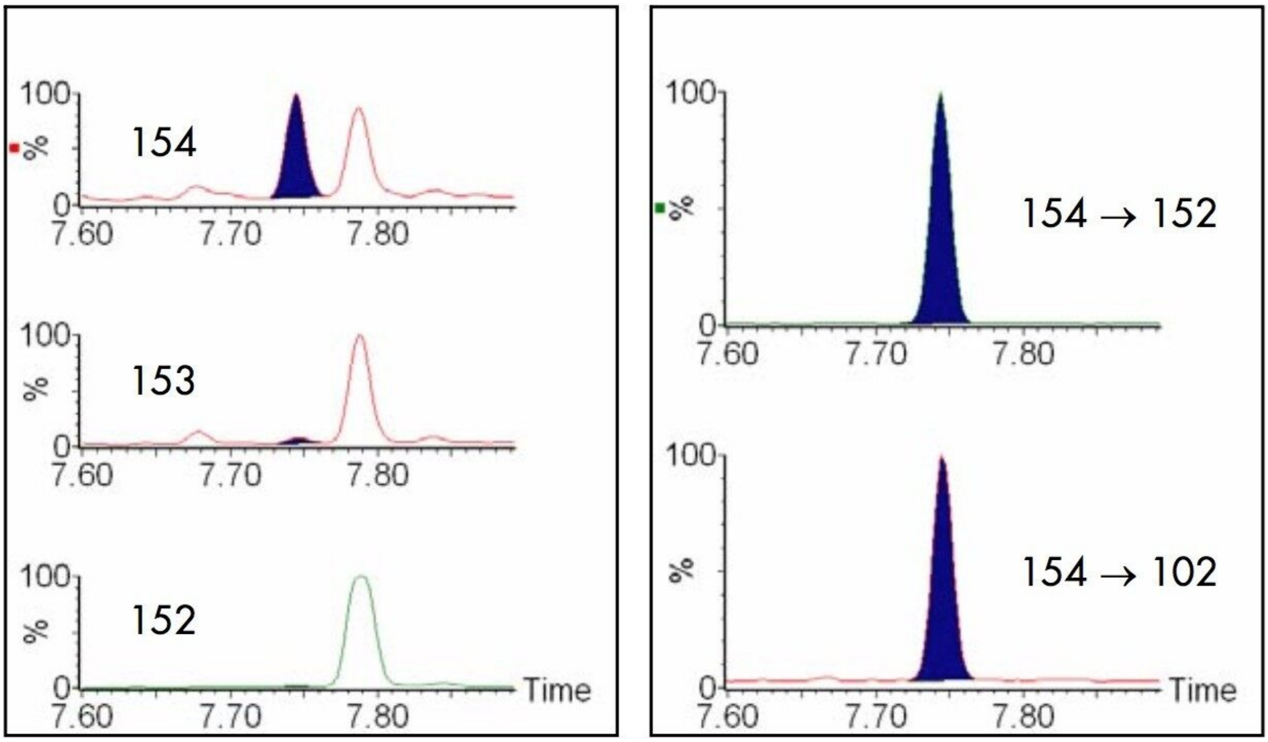


Figure 3. 0.025 mg/kg biphenyl in curry powder using SIM and MRM.

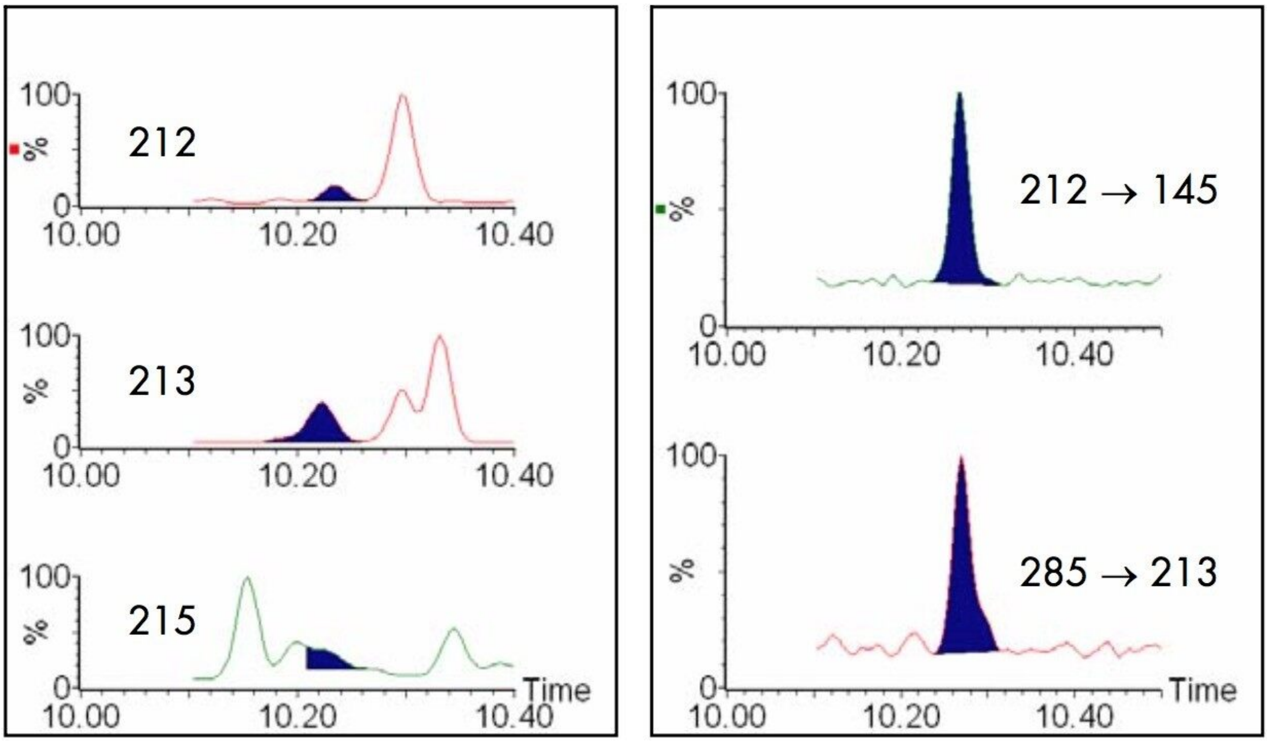


Figure 4. 0.025 mg/kg vinclozolin in curry powder using SIM and MRM.

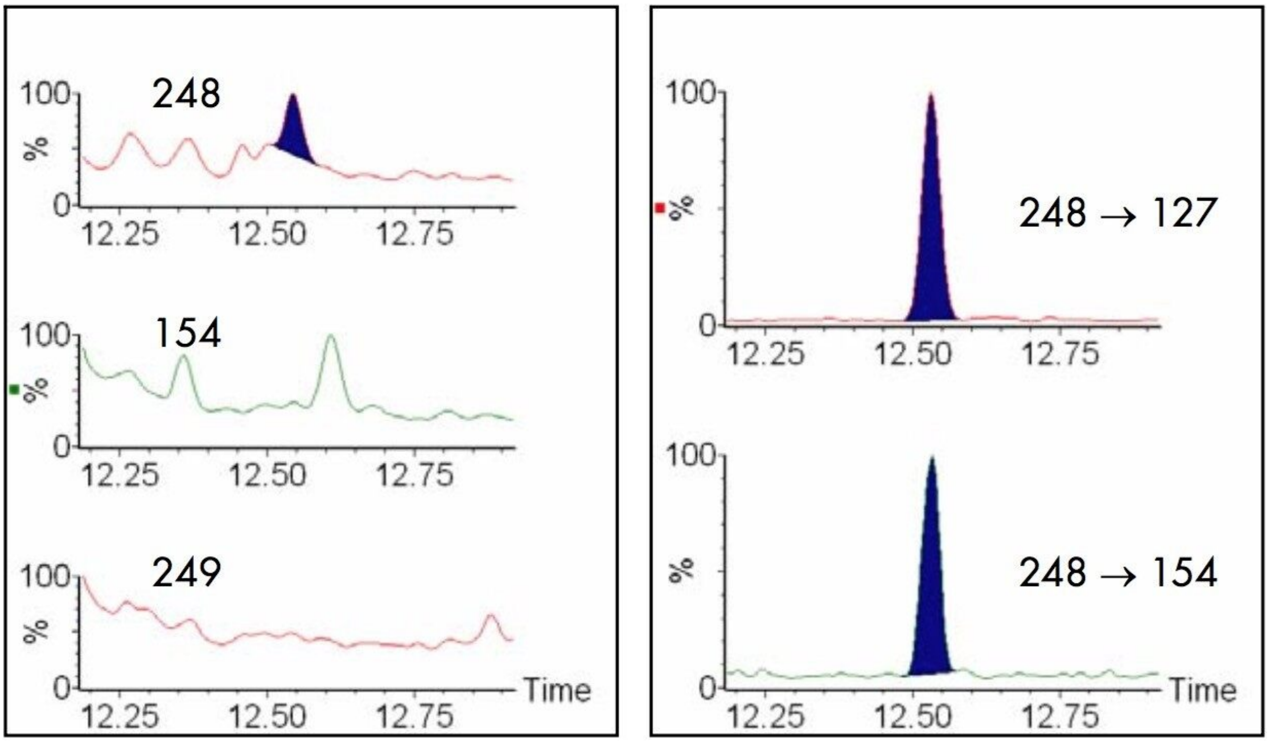
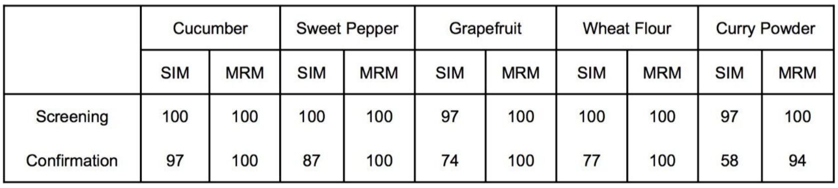


Figure 5. 0.025 mg/kg fludioxonil in curry powder using SIM and MRM.

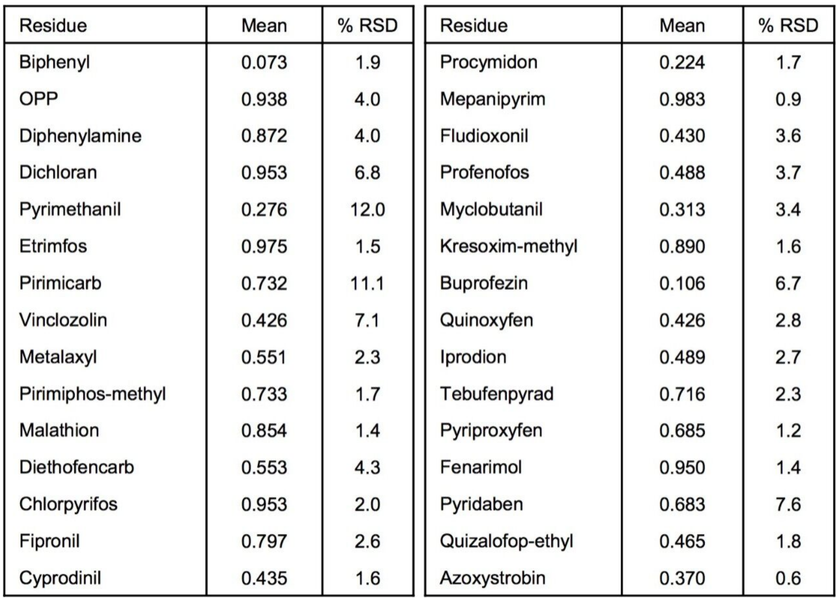
The results for all of the pesticide residues in all of the matrices are summarized in Table 2. They show that both SIM and MRM can be used successfully for the screening of the majority residues. However, there are significant differences between the percentage of residues confirmed by MRM compared to SIM, particularly in the more complex matrices.



	Cucumber		Sweet Pepper		Grapefruit		Wheat Flour		Curry Powder	
	SIM	MRM	SIM	MRM	SIM	MRM	SIM	MRM	SIM	MRM
Screening	100	100	100	100	97	100	100	100	97	100
Confirmation	97	100	87	100	74	100	77	100	58	94

Table 2. Percentage of residues successfully screened or confirmed in all five matrices using SIM or MRM.

The ion ratios are important as they provide the basis of confirmation. The ion ratio statistics presented in Table 3 are calculated for the 25 matrix injections across all five matrices. The low % RSDs indicate the good repeatability of the MRM method.



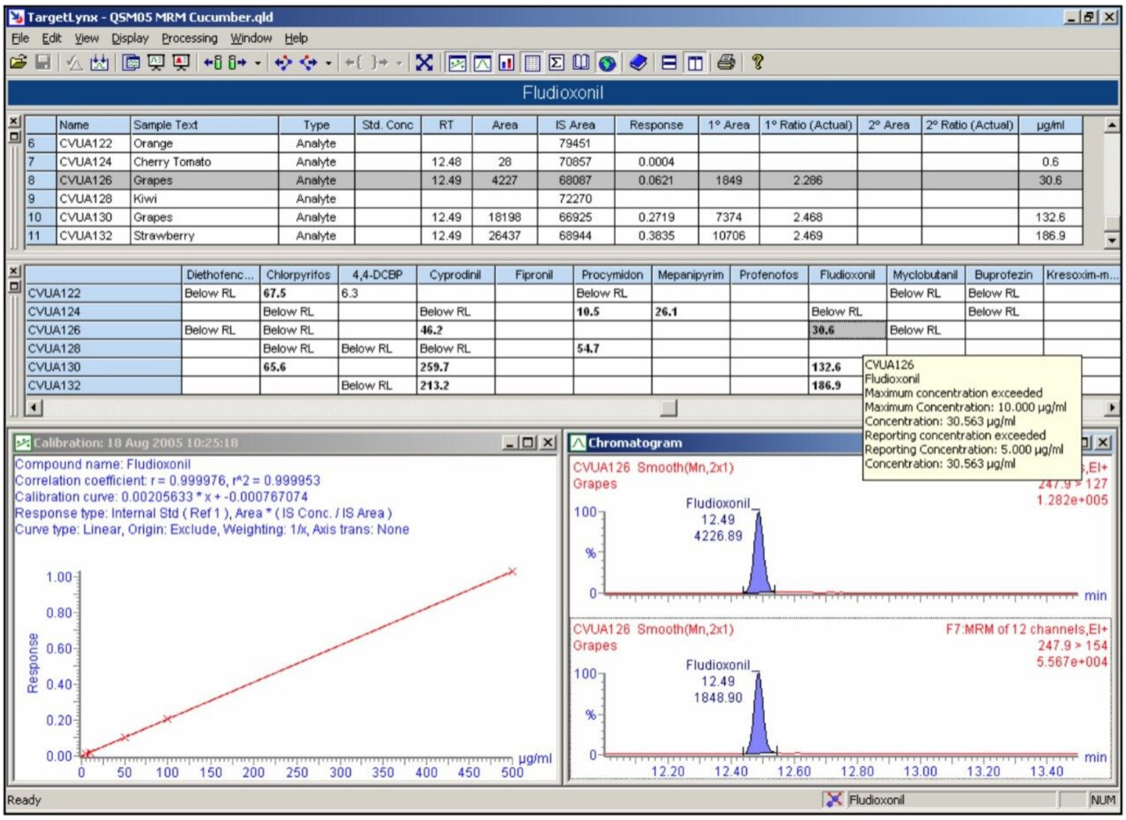
Residue	Mean	% RSD	Residue	Mean	% RSD
Biphenyl	0.073	1.9	Procymidon	0.224	1.7
OPP	0.938	4.0	Mepanipyrim	0.983	0.9
Diphenylamine	0.872	4.0	Fludioxonil	0.430	3.6
Dichloran	0.953	6.8	Profenofos	0.488	3.7
Pyrimethanil	0.276	12.0	Myclobutanil	0.313	3.4
Etrimfos	0.975	1.5	Kresoxim-methyl	0.890	1.6
Pirimicarb	0.732	11.1	Buprofezin	0.106	6.7
Vinclozolin	0.426	7.1	Quinoxyfen	0.426	2.8
Metalaxyl	0.551	2.3	Iprodion	0.489	2.7
Pirimiphos-methyl	0.733	1.7	Tebufenpyrad	0.716	2.3
Malathion	0.854	1.4	Pyriproxyfen	0.685	1.2
Diethofencarb	0.553	4.3	Fenarimol	0.950	1.4
Chlorpyrifos	0.953	2.0	Pyridaben	0.683	7.6
Fipronil	0.797	2.6	Quizalofop-ethyl	0.465	1.8
Cyprodinil	0.435	1.6	Azoxystrobin	0.370	0.6

Table 3. Ion ratio statistics for 25 matrix injections across five matrices using MRM.

The MRM method was also applied to “real” samples containing incurred pesticide residues.

Five different matrices were supplied to further test the method; orange, cherry tomato, grapes, kiwi and strawberry. The cucumber matrix-matched calibration curve was used for quantification purposes. The TargetLynx Application Manager was used to provide automatic quantification and confirmation with two MRM transitions acquired for each pesticide residue. For illustration purposes, the reporting level was chosen to be 0.005 mg/kg.

An example TargetLynx browser containing 0.031 mg/kg fludioxonil in grapes is illustrated in Figure 6. A summary of all the incurred pesticide residues detected and confirmed above the reporting level is listed in Table 4.



TargetLynx - QSM05 MRM Cucumber.qld

Fludioxonil

	Name	Sample Text	Type	Std. Conc	RT	Area	IS Area	Response	1° Area	1° Ratio (Actual)	2° Area	2° Ratio (Actual)	µg/ml
6	CVUA122	Orange	Analyte				79451						
7	CVUA124	Cherry Tomato	Analyte		12.48	28	70857	0.0004					0.6
8	CVUA126	Grapes	Analyte		12.49	4227	68087	0.0621	1849	2.286			30.6
9	CVUA128	Kiwi	Analyte				72270						
10	CVUA130	Grapes	Analyte		12.49	18198	66925	0.2719	7374	2.468			132.6
11	CVUA132	Strawberry	Analyte		12.49	26437	68944	0.3835	10706	2.469			186.9

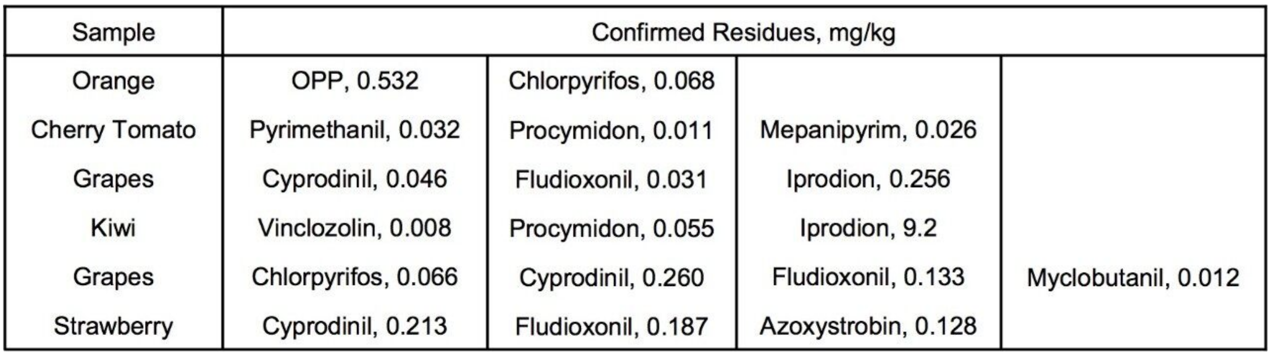
	Diethofenc...	Chlorpyrifos	4,4-DCBP	Cyprodinil	Fipronil	Procymidon	Mepanipyrim	Profenofos	Fludioxonil	Myclobutanil	Buprofezin	Kresoxim-m...
CVUA122	Below RL	**67.5**	6.3			Below RL				Below RL	Below RL	
CVUA124		Below RL		Below RL		**10.5**	**26.1**		Below RL		Below RL	
CVUA126	Below RL	Below RL		**46.2**					**30.6**	Below RL		
CVUA128		Below RL	Below RL	Below RL		**54.7**						
CVUA130		**65.6**		**259.7**					**132.6**			
CVUA132			Below RL	**213.2**					**186.9**			

CVUA126
Fludioxonil
Maximum concentration exceeded
Maximum Concentration: 10.000 µg/ml
Concentration: 30.563 µg/ml
Reporting concentration exceeded
Reporting Concentration: 5.000 µg/ml
Concentration: 30.563 µg/ml

Calibration: 18 Aug 2005 10:25:18

Compound name: Fludioxonil
Correlation coefficient: r = 0.999976, r^2 = 0.999953
Calibration curve: 0.00205633 * x + -0.000767074
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Figure 6. Example TargetLynx browser for grapes containing incurred fludioxonil.



Sample	Confirmed Residues, mg/kg			
Orange	OPP, 0.532	Chlorpyrifos, 0.068		
Cherry Tomato	Pyrimethanil, 0.032	Procymidon, 0.011	Mepanipyrim, 0.026	
Grapes	Cyprodinil, 0.046	Fludioxonil, 0.031	Iprodion, 0.256	
Kiwi	Vinclozolin, 0.008	Procymidon, 0.055	Iprodion, 9.2	
Grapes	Chlorpyrifos, 0.066	Cyprodinil, 0.260	Fludioxonil, 0.133	Myclobutanil, 0.012
Strawberry	Cyprodinil, 0.213	Fludioxonil, 0.187	Azoxystrobin, 0.128	

Table 4. Confirmed residues found in incurred sample matrices using MRM.

Conclusion

Selected ion monitoring (SIM) is suitable for the screening and confirmation of pesticide residues in relatively simple matrices.

Multiple reaction monitoring (MRM) offers significant improvements for the screening and confirmation of pesticide residues, especially in more complex matrices.

The combination of QuEChERS extraction, the PTV injector in solvent vent mode, the Quattro micro GC in MRM mode and TargetLynx allow routine pesticide monitoring to easily be achieved.

The described MRM method was tested on “real” samples where incurred pesticide residues were confirmed above the reporting level.

References

1. M. Anastassiades, S. Lehotay, D. Stajnbaher, F. Schenck, *J. AOAC Int*. 86 (2) (2003) 412.
2. S.J. Lehotay, K. Maštovska, S.J. Yun, *J. AOAC Int*. 88 (2) (2005) 630.
3. http://europa.eu.int/comm/food/plant/protection/resources/qualcontrol_en.pdf < http://europa.eu.int/comm/food/plant/protection/resources/qualcontrol_en.pdf>
4. Regulation (EC) NO 396/2005 of 23 February 2005 on Maximum Residue Levels of Pesticides in or on Food

and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC, *Off. J. of the European Communities* No. L70/1.

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