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Liquid Chromatography Mass Spectrometry

Highly Polar Pesticide Multi-Residue Analysis in Food Safety by LC-MS/MS

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■ Abstract

The analysis of highly polar pesticides by a single LC-MS/MS method is extremely challenging as a consequence of diverse separation and detection behaviour. Conventional approaches in highly polar pesticide analysis often use single residue methods or small group specific methods which are time consuming and limit throughput. In this study, the panel of target analytes selected for analysis included a series of compounds that are typically addressed by multiple methods and workflows; glufosinate, glyphosate, ethephon, fosethyl aluminium, maleic hydrazide, perchlorate, ETU, PTU, nicotine, amitrole, chlormequat, daminozide, diquat, kasugamycine, mepiquat, paraquat and trimesium.

To accelerate turnaround times and increase sample sizes for more complete testing programs two LC-MS/MS methods were developed for the measurement of a range of highly polar pesticides in their underivitised state using the LCMS-8050 triple quadrupole mass spectrometer. All target compounds were quantified at 0.01 mg/kg which is below the European Union maximum residue limit for all studied compounds delivering a measurable impact on sample cycle time and productivity.

Keywords: Highly polar pesticides, LCMS-8050, food safety, glyphosate, diquat, paraquat, perchlorate

■ Introduction

The use of pesticides in the environment is constantly under review and in recent years regulatory bodies have adopted a hazards-based approach to pesticide regulation leading to an increased use of highly polar pesticides which present lower persistence and toxicity. Enforcing pesticide limits within regulatory limits defined as the maximum residue levels (MRL's; the maximum concentration of pesticide residues permitted in food and feed) requires methods that provide results quickly and accurately for a broad spectrum of chemical structures in a diverse range of food samples.

Pesticide residue monitoring laboratories utilise multi-residue LC-MS/MS methods for the quantification of an ever increasing list of target pesticides. However, the measurement of highly polar pesticides by a single LC-MS/MS method is extremely challenging as a consequence of diverse separation and detection behaviour. For this reason, single residue methods or small group specific methods are often utilised to analyse these compounds, in some cases including the use of pre- or post-column derivatisation. Therefore, there is a clear need to reduce the number of separation methods applied to the analysis of highly polar pesticides to help accelerate sample throughput, reduce the cost platform, simplify analytical workflows and enhance data quality for regulatory reporting limits.

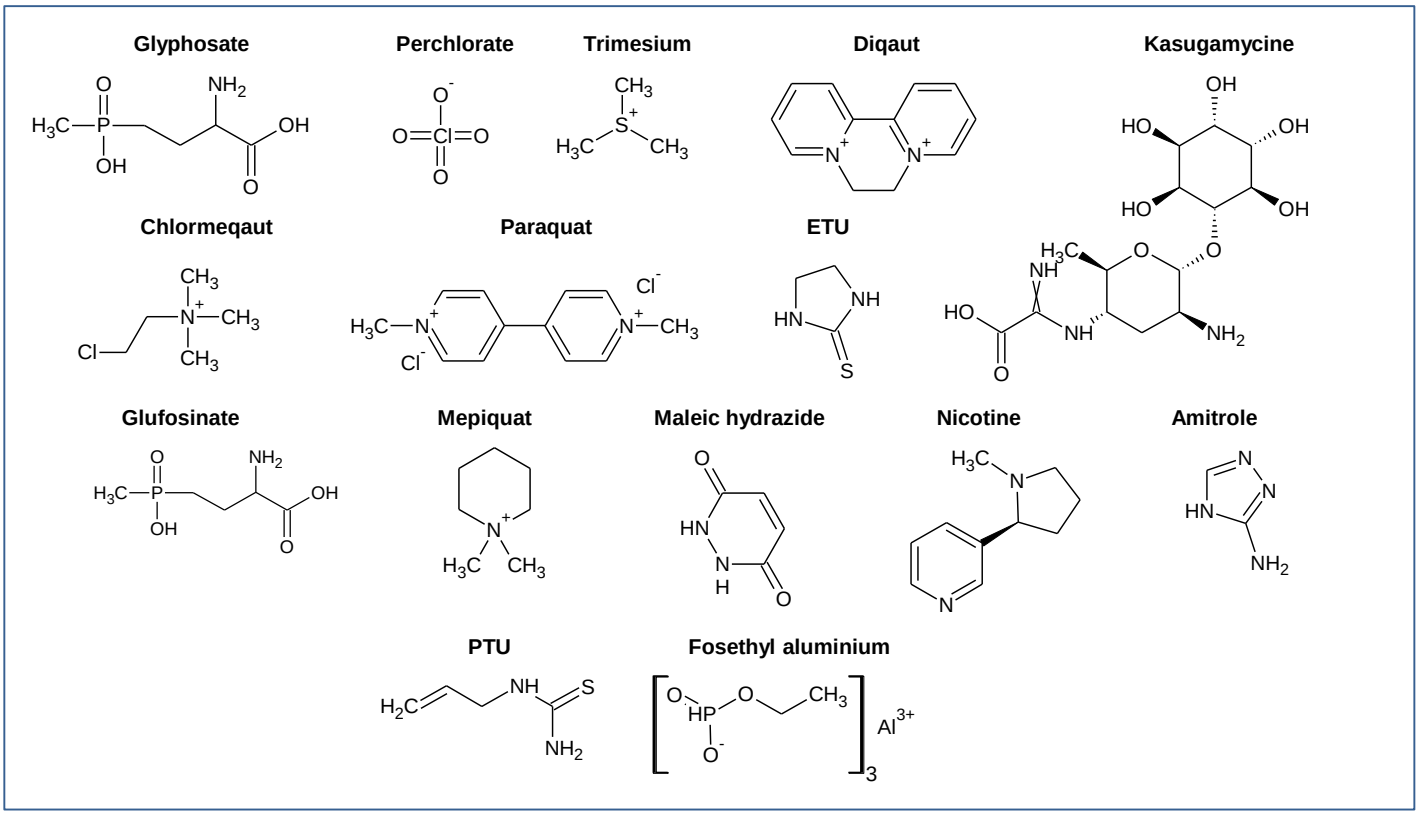

Figure 1. Target analyte structures

The highly polar pesticides targeted in this study included glufosinate, glyphosate, ethephon, fosethyl aluminium, maleic hydrazide, perchlorate, ETU, PTU, nicotine, amitrole, chlormequat, daminozide, diquat, kasugamycine, mepiquat, paraquat and trimesium. Structures for these compounds are displayed in Figure 1. All of the compounds included in this study were polar, characterised with LogKow < 1. The most polar compounds being the cationic quaternary ammonium herbicides diquat (LogKow -4.6) and paraquat (LogKow -4.5). Several of the compounds also have a low molecular mass, for example trimesium (77 g/mol), amitrole (84 g/mol) and ETU (102 g/mol).

The analysis of highly polar pesticides is extensively reported in literature but the methods have been limited to a small number of specific target compounds and not to a group with such a diverse chemical space. For example, a common approach for the analysis of one of the world's biggest selling herbicides glyphosate is typically achieved by FMOC derivatization. This derivatization step is specific for glyphosate, glufosinate and AMPA residues in water and food samples but it is relatively complex, limits throughput and repeatability and reproducibility can suffer due to the derivatisation step.

The aim of this study was to develop a fast, sensitive and simple methodology for a range of challenging highly polar pesticides that require single-residue methods, by as few multi-residue LC-MS/MS runs as possible and without the need for derivatisation. Several different analytical columns and mobile phases were evaluated in this study, in addition to assessing the MS/MS parameters. Isotopically labelled standards were used to compensate for matrix effects. Initial data was collected in food matrix using a triple quadrupole mass spectrometer in MRM mode.

■ Experimental

Individual reference standards for each compound were provided by Phytocontrol in methanol at a concentration of 10 ng/µL. Mobile phase solvents and additives were all LC–MS quality and purchased from Sigma–Aldrich. Apple extracts were provided by Phytocontrol and extracted according to the EURL-SRM QuPPe methodology.[1] Briefly, apple samples (10 g) were prepared by chopping up the sample, freezing, homogenizing with dry ice, adding 1% formic acid in methanol solution (10 mL) and centrifuging (4000 RPM). Linearity was evaluated by spiking sample extracts at the following levels:0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 mg/kg. Deuterated internal standards were used for calibration. All calibration points were analysed in duplicate. Plastic vials were used for analysis to prevent interaction of certain pesticides (e.g. paraquat, diquat and glyphosate) with glass surfaces.[1]

SRM transitions and analyte specific MS parameters (Q1 pre-bias (V), Q3 pre-bias (V) and collision energy) were optimised automatically using the SRM optimisation feature available in LabSolutions software. SRM transitions are listed in Table 2 and Table 3.

Preliminary investigations involved the testing of several different analytical columns: SIELC Obselisc R (150 x 2.1mm, 5µm); Hypercarb PGC (100 x 2.1mm, 5µm); SeQuant ZiC-HILIC (100 x 2.1mm, 3.5µm), SeQuant ZIC-cHILIC (100 x 2.1mm, 3.5µm), Scherzo SM-C18 (50 x 2, 3 µm), Scherzo SW-C18 (50 x 2, 3 µm), Fortis Phenyl (100 x 2.1mm, 5µm), Luna Phenyl-Hexyl (100 x 2.1mm, 3µm), and Restek IBD (150 x 2.1mm, 3µm). These columns were tested with several different mobile phase additives including acetic acid, formic acid, ammonium formate, ammonium acetate and ammonium hydroxide (depending on appropriate conditions for each column and the progression of results). Reversed phase, HILIC, and mixed mode chromatography were tested depending on the column suitability for each mode. The final LCMS/MS method conditions are listed in Table 1.

Table 1. LC/MS/MS parameters for Method 1 and Method 2

Liquid chromatography				
	Method 1		**Method 2**	
UHPLC	Nexera UHPLC system		Nexera UHPLC system	
Analytical column	ZIC-HILIC (100 x 2.1mm, 3.5µm)		Hypercarb PGC (100mm x 2.1mm, 5µm)	
Mobile phase	A = Water 20mM ammonium formate and 0.3% formic acid		A = Water 1% acetic acid	
	B = Acetonitrile		B = Methanol 1% acetic acid	
Gradient	**Time (mins)**	**%B**	**Time (mins)**	**%B**
	0	97	0	0
	5.8	68	10	30
	9	15	15	35
	10	15	17.5	68
	10	97	18	100
	16	Stop	22	100
			22.1	0
			33	Stop
Column temp.	35°C		35°C	
Injection volume	6µL (40µL acetonitrile co-injected)		5µL	
Flow rate	0.4mL/min		0.3mL/min	
Mass spectrometry				
LC/MS/MS	LCMS-8050			
Ionisation mode	Heated electrospray			
Polarity switching time	5 ms			
Pause time	1 ms			
Dwell times	5-50ms			
Interface temperature	350°C			
Heating block	300°C			
Desolvation line	200°C			
Gas	Heating gas 10 L/min; drying gas 10 L/min; Nebulising gas 3 L/min			

Table 2. Method 1 MS acquisition parameters, retention time and internal standard

Compound	Ret. time (min)	Polarity	SRM transitions	Q1 (V)	CE	Q3 (V)	ISTD	MS1 Res.	MS2 Res.
Amitrole	3.1	Positive	85 > 43	-14	-25	-14	Paraquat d8	Unit	Unit
			85 > 57	-14	-20	-20		Unit	Unit
			85 > 58	-14	-23	-22		Unit	Unit
Chlormequat	4.1	Positive	122 > 58	-28	-27	-21	Chlormequat d4	Unit	Unit
			122 > 59	-28	-23	-21		Unit	Unit
			122 > 63	-28	-22	-23		Unit	Unit
Daminozide	2.2	Positive	161 > 143	-16	-14	-25	Chlormequat d4	Unit	Unit
			161 > 44	-16	-22	-16		Unit	Unit
			161 > 45	-16	-23	-16		Unit	Unit
Diquat	4.0	Positive	183 > 157	-12	-21	-27	Paraquat d8	Unit	Unit
			183 > 78	-12	-39	-12		Unit	Unit
			183 > 130	-12	-34	-22		Unit	Unit
Kasugamycine	7.8	Positive	380 > 112	-18	-20	-18	Chlormequat d4	Unit	Unit
			380 > 200	-18	-13	-20		Unit	Unit
Mepiquat	4.5	Positive	114 > 98	-22	-29	-15	Mepiquat d3	Unit	Unit
			114 > 58	-22	-26	-21		Unit	Unit
			114 > 42	-22	-45	-14		Unit	Unit
Paraquat	9.0	Positive	186 > 171	-12	-20	-30	Paraquat d8	Unit	Unit
			186 > 77	-12	-45	-27		Unit	Unit
			186 > 169	-12	-35	-29		Unit	Unit
Trimesium	5.1	Positive	77 > 62	-13	-21	-22	Paraquat d8	Unit	Unit
			77 > 47	-13	-27	-17		Unit	Unit
			77 > 45	-13	-45	-16		Unit	Unit
Chlormequat d4	4.1	Positive	126 > 58	-21	-29	-21		Unit	Unit
Mepiquat d3	4.5	Positive	117 > 101	-20	-29	-18		Unit	Unit
Paraquat d8	9.0	Positive	193 > 178	-13	-21	-30		Unit	Unit

Table 3. Method 2 MS acquisition parameters, retention time and internal standard

Compound	Ret. time (min)	Polarity	SRM transitions	Q1 (V)	CE	Q3 (V)	ISTD	MS1 Res.	MS2 Res.
Glyphosate	3.7	Positive	170 > 88	-17	-9	-18	Glyphosate C13	Unit	Unit
			170 > 42	-17	-26	-17		Unit	Unit
			170 > 60	-17	-16	-24		Unit	Unit
Gluphosinate	2.9	Positive	182 > 136	-12	-11	-26	Maleic hydrazide d2	Unit	Unit
			182 > 56	-12	-24	-23		Unit	Unit
			182 > 119	-12	-19	-23		Unit	Unit
ETU	3.1	Positive	103 > 44	-19	-18	-15	ETU d4	Unit	Unit
			103 > 60	-19	-28	-23		Unit	Unit
			103 > 86	-19	-21	-28		Unit	Unit
Fosethyl	9.9	Negative	109 > 81	23	13	29	Fosethyl d15	Unit	Unit
			109 > 63	23	25	23		Unit	Unit
			109 > 79	23	24	28		Unit	Unit
Maleic hydrazide	13.7	Positive	113 > 40	-11	-27	-16	Maleic hydrazide d2	Unit	Unit
			113 > 67	-11	-19	-27		Unit	Unit
			113 > 85	-11	-17	-17		Unit	Unit
Nicotine	2.0	Positive	163 > 130	-16	-21	-22	Nicotine d3	Unit	Unit
			163 > 117	-16	-25	-20		Unit	Unit
			163 > 132	-16	-17	-23		Unit	Unit
Perchlorate	30.1	Negative	99 > 83	22	26	30	Perchlorate 18O4	Unit	Unit
			99 > 67	22	37	23		Unit	Unit
			101 > 85	22	26	30		Unit	Unit
PTU	3.1	Positive	117 > 58	-20	-16	-19	ETU d4	Unit	Unit
			117 > 60	-20	-29	-20		Unit	Unit
			117 > 72	-12	-22	-26		Unit	Unit
ETU d4	3.0	Positive	107 > 48	-18	-19	-16		Unit	Unit
Fosethyl d5	9.6	Negative	114 > 82	24	15	30		Unit	Unit
Maleic hydrazide d2	13.6	Positive	115 > 42	-11	-20	-17		Unit	Unit
Glyphosate 13C2 15N	3.6	Positive	173 > 91	-11	-8	-19		Unit	Unit
Nicotine d3	1.7	Positive	166 > 130	-30	-22	-21		Unit	Unit
Perchlorate 18O4	30.1	Negative	107 > 89	23	27	30		Unit	Unit

■ Results and Discussion

Following evaluation of several different analytical columns, mobile phases and mass spectrometer settings, two methods were developed for a range of highly polar pesticides that typically require single residue methods to analyse. A ZIC-HILIC column, a zwitterionic stationary phase covalently attached to porous silica, was used in method 1 to analyse the following; amitrole, chlormequat, daminozide, diquat, kasugamycine, mepiquat, paraquat and trimesium. While a Hypercarb PGC (porous graphitic carbon), which behaves as a strongly retentive alkyl-bonded silica gel, was used in method 2 to analyse the following; glufosinate, glyphosate, ethephon, fosethyl aluminium, maleic hydrazide, perchlorate, ETU, PTU, and nicotine.

Three MRM transitions were acquired for each analyte, with exception of two transitions for kasugamycine. Linearity was evaluated for all compounds in the range 0.005 mg/kg – 0.2 mg/kg (5 – 200 ppb) in apple matrix. The concentration of each calibration level is listed in the experimental section. All seven target compounds achieved excellent correlation coefficients greater than R^2>0.9975, using internal standards for quantitation, linear fit and 1/C weighting. Calibration curves for several compounds are displayed in Figure 3 (using LC method 1) and Figure 5 (using LC method 2). The linearity results for all target compounds is listed in Table 4.

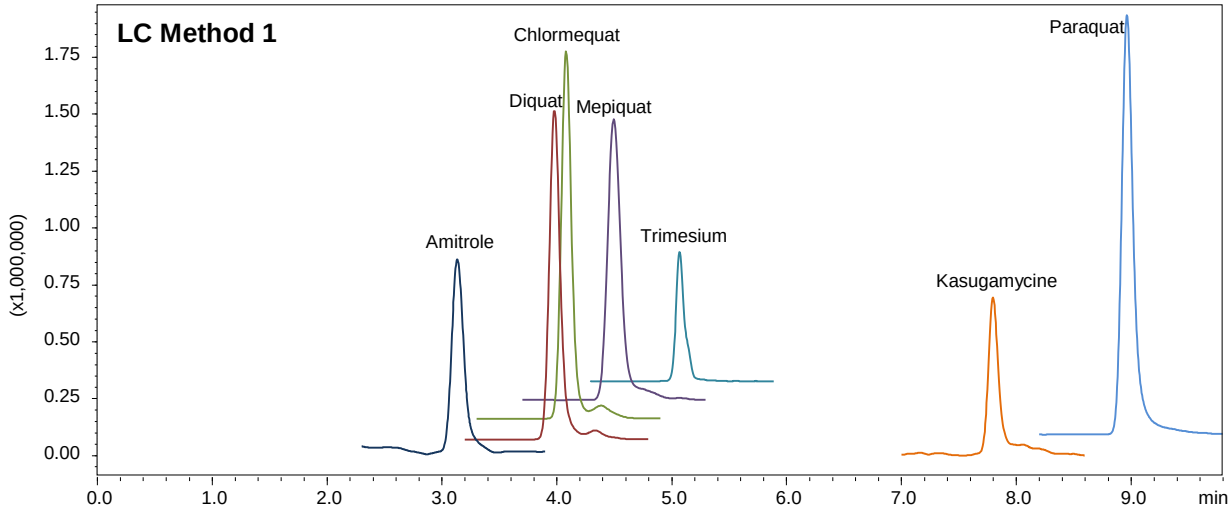

Figure 2 . Target analytes at 0.05mg/kg in apple matrix using a ZIC-HILIC based separation (LC Method 1)

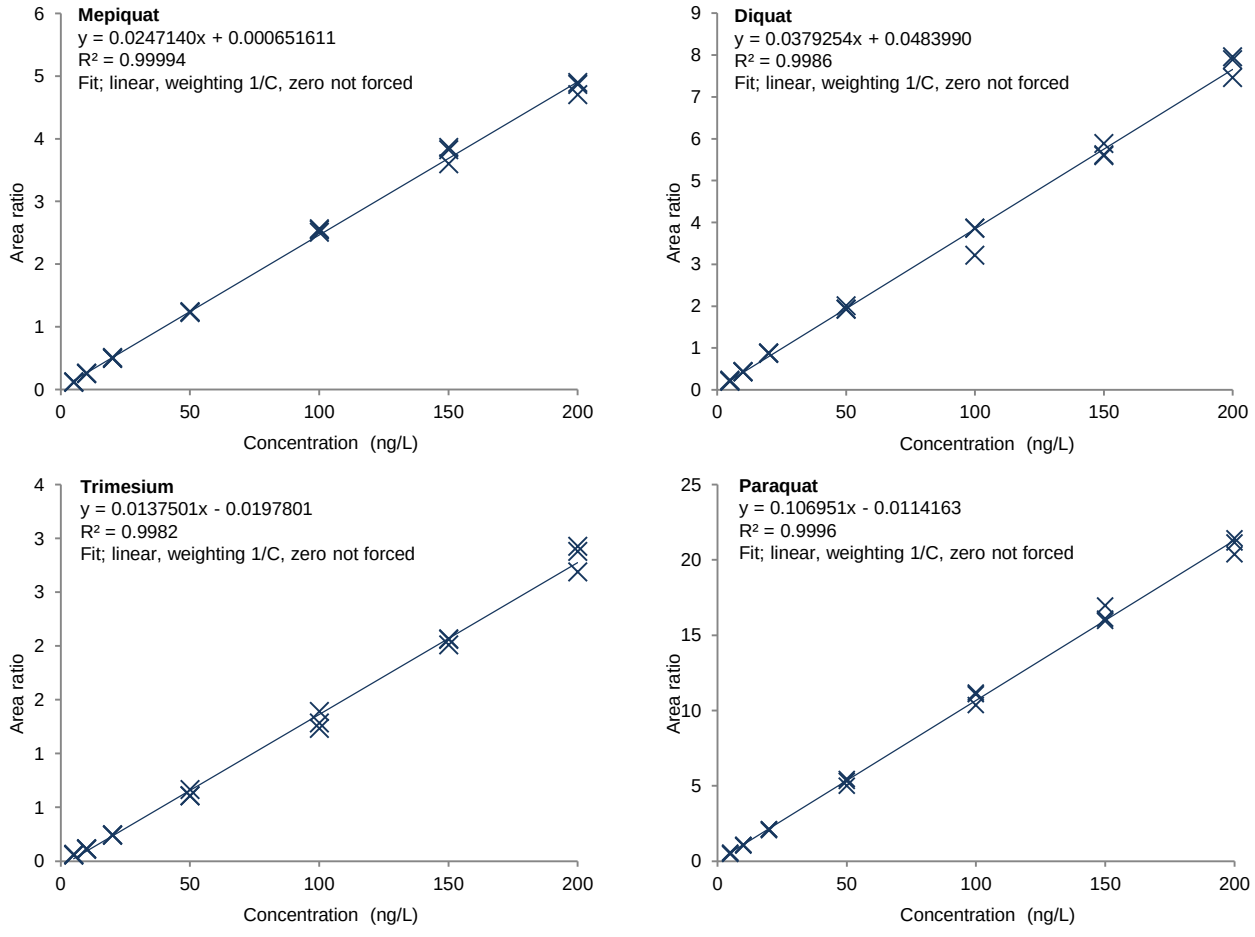

Figure 3. Calibration curves for paraquat, mepiquat, trimesium and diquat using a ZIC-HILIC based separation (LC Method 1)

Figure 2 displays a chromatogram of each compound at 0.05 mg/kg using a ZIC-HILIC based separation (LC method 1) and Figure 4 display a chromatogram using a Hypercarb PGC based separation (LC method 2). All target analytes were identified at 0.01 mg/kg. This concentration is below the European Union (EU) maximum residue limit (MRL) for all of the target analytes in this study. For example, the EU MRL for the following compounds in the majority of commodities is; glyphosate 0.1 mg/kg, glufosinate 0.1 mg/kg, chlormequat 0.05 mg/kg, paraquat 0.02, mepiquat 0.05 mg/kg, daminozide 0.02 mg/kg and ethephon 0.05 mg/kg.[2] Consequently, the sensitivity achieved in these methods is far below what is required and therefore dilution of sample extracts is possible in order to reduce matrix effects.

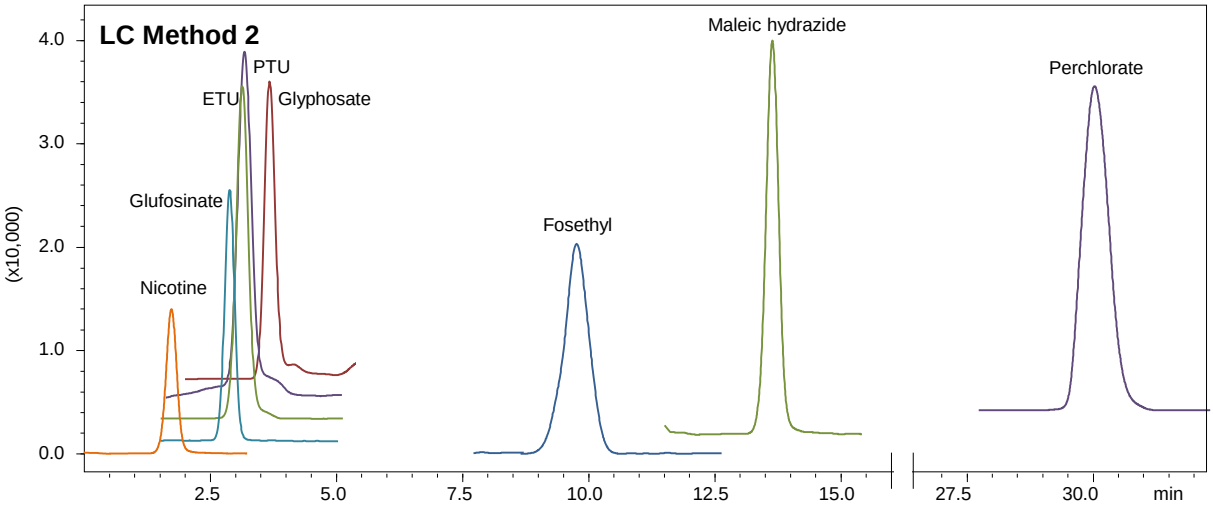

Figure 4. Target analytes at 0.05mg/kg in apple matrix using a Hypercarb PGC based separation (LC Method 2).

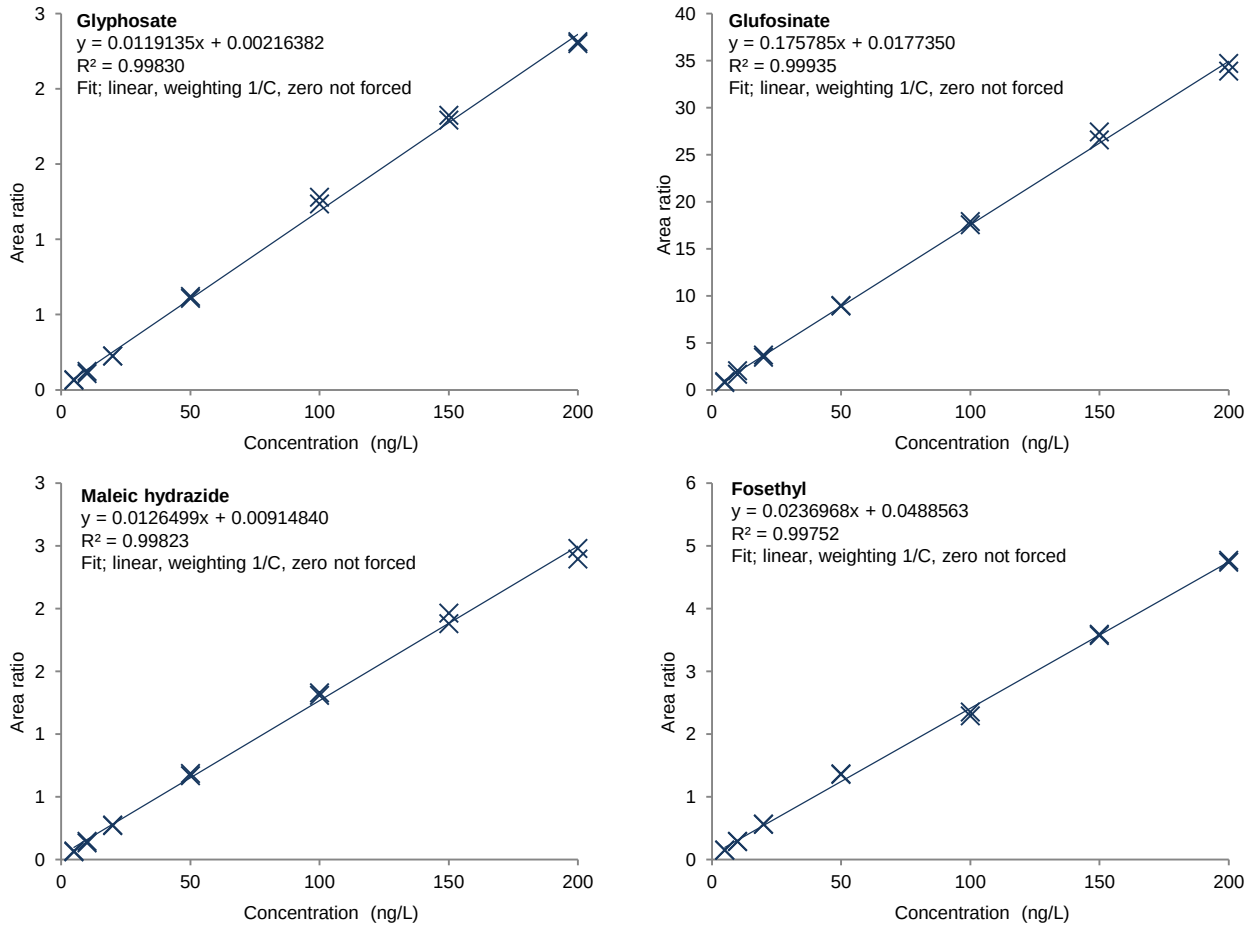

Figure 5. Calibration curves for glyphosate, gluphosinate, maleic hydrazide and fosethyl using Hypercarb PGC based separation (LC Method 2)

■ Conclusion

Two LC-MS/MS methods were developed for the measurement of a range of highly polar pesticides in their underivitised state using the LCMS-8050 triple quadrupole mass spectrometer. The developed multi-residue methods offer significant time savings in comparison to single residue methods typically used for analyse of these analytes. All compounds were quantified in the range 0.005 – 0.2 mg/kg with correlation coefficients greater than 0.997. The excellent sensitivity achieved, which is most cases is far below the EU MRL, offers the opportunity to dilute sample extracts prior to LC-MS/MS injection in order to reduce matrix effects.

■ References

1. Reference Laboratory for pesticides requiring Single Residue Methods (EURL-SRM). Quick Method for the Analysis of Residues of numerous Highly Polar Pesticides in Foods of Plant Origin involving Simultaneous Extraction with Methanol and LC-MS/MS Determination (QuPPe-Method). 2012. Version 7

2. Commission Regulation (EC). 2005. No 396/2005 of the European Parliament and of the Council, maximum residue levels of pesticides in or on food and feed of plant and animal origin. Official Journal of the European Union, L 70: 1-16. http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=homepage&language=EN

Table 4. Target analytes linearity results using LC method 1 and LC method 2

Compound	R^2	Fit type	Weight	Method
Diquat	0.9986	Linear	1/C	Method 1
Chlormequat	0.9988	Linear	1/C	Method 1
Amitrole	0.9981	Quadratic	1/C	Method 1
Kasugamycine	0.9992	Linear	1/C	Method 1
Daminozide	0.9995	Quadratic	1/C	Method 1
Mepiquat	0.9993	Linear	1/C	Method 1
Paraquat	0.9995	Linear	1/C	Method 1
Trimesium	0.9981	Linear	1/C	Method 1
ETU	0.9998	Linear	1/C	Method 2
Fosethyl	0.9975	Linear	1/C	Method 2
Gluphosinate	0.9993	Linear	1/C	Method 2
Glyphosate	0.9983	Linear	1/C	Method 2
Maleic hydrazide	0.9982	Linear	1/C	Method 2
Nicotine	0.9984	Linear	1/C	Method 2
Perchlorate	0.9998	Linear	1/C	Method 2
PTU	0.9991	Linear	1/C	Method 2

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