SUB 1µG/KG DETECTION OF GLYPHOSATE AND OTHER ANIONIC POLAR PESTICIDES USING A GENERIC EXTRACTION AND DETECTION BY LC-MS/MS

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INTRODUCTION

Routine analysis of anionic polar pesticides has become a requirement for many laboratories. In the past 20 years these would often be analysed by time consuming single residue methods. With the introduction of the Quick Polar Pesticides (QuPPe) method this has allowed a generic extraction method to be employed and introduced the possibility of analysing several anionic polar pesticides by one method.[1] Waters™ have published several applications in the area of anionic polar pesticide analysis focusing on how the Anionic Polar Pesticide Column solves several of the critical challenges with this approach as well as expected extraction method performance.[2,3,4,5] With the introduction of the Xevo™ TQ Absolute MS system, low and even sub µg/kg limits of detection can be combined with such generic extraction methods as the QuPPe method to bring a multi-residue approach to this analysis.

METHODS

Sample preparation

Blank matrix extracts were generated following the QuPPe version 12 protocol.[1] Cucumber matrix standards were prepared over the 0.5 to 200 µg/kg range (0.25 to 100 ng/mL in vial concentration) and wheat flour matrix standards were prepared over the 2 to 200 µg/kg range (0.25 to 25 ng/mL in vial concentration).

Instrument methods

LC System:	ACQUITY™ UPLC I-Class PLUS (FL-SM)
Column:	Waters Anionic Polar Pesticide Column (5µm, 2.1 x 100mm) (186009287)
Mobile phase:	A: 0.9% formic acid in water B: 0.9% formic acid in acetonitrile
Injection volume:	10 µL
Column temp:	50 ºC
LC Separation:	Available in our APP method start-up guide (link)
MS System:	Xevo TQ Absolute
Ionisation:	Electrospray
Acquisition:	MRM in negative ionisation mode
MS Parameters:	Transitions listed in Table 1

	Glyphosate		N-Acetyl-Glyphosate		AMPA		N-Acetyl-AMPA		Glufosinate			N-Acetyl-Glufosinate		MPPA			Ethephon			HEPA		Fosetyl-Al	
Precursor (m/z)	168		210		110		152		180			222		151			143		145	125		109	
Fragment (m/z)	63	150	150	192	63	79	63	110	85	63	95	136	69	63	107	133	107	79	107	79	95	63	81
Cone voltage (V)	15	15	25	25	15	15	30	20	15	15	15	20	20	20	20	15	15	15	15	15	15	15	15
Collision energy (eV)	15	10	13	9	15	15	15	17	17	25	15	20	14	25	16	12	8	13	8	14	12	16	10

Table 1. Transition information for anionic polar pesticides

RESULTS

Limit of quantification (LOQ) for the Xevo TQ-Absolute system was tested for the anionic polar pesticides by analyzing matrix standards over the range of 0.5–200 µg/kg (0.25–100 ng/mL in vial concentration) for cucumber matrix and 2–200 µg/kg (0.25–25 ng/mL in vial concentration) for wheat flour matrix. The limit of quantification was defined as the lowest calibration standard in these sequences. Table 2 lists the achieved LOQs for the anionic polar pesticides. The difference in LOQs observed between the two different sample types is attributable to the different dilution factors within the QuPPe v12 extraction procedure for "wet" commodities such as cucumber versus "dry" commodities such as wheat flour. Example chromatograms for the anionic polar pesticides in cucumber matrix at 1 µg/kg are displayed in Figure 1.

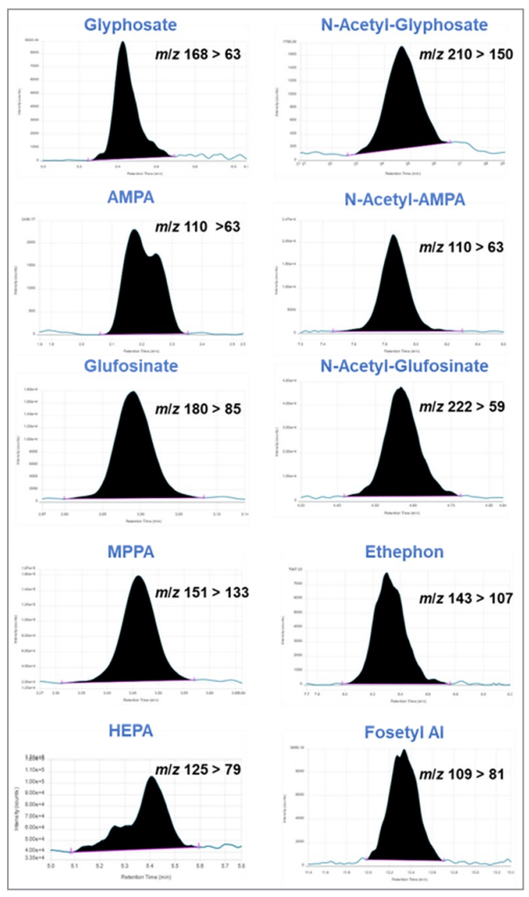

Figure 1. Chromatograms of the anionic polar pesticides and metabolites from the analysis of a cucumber matrix standard at 1 µg/kg

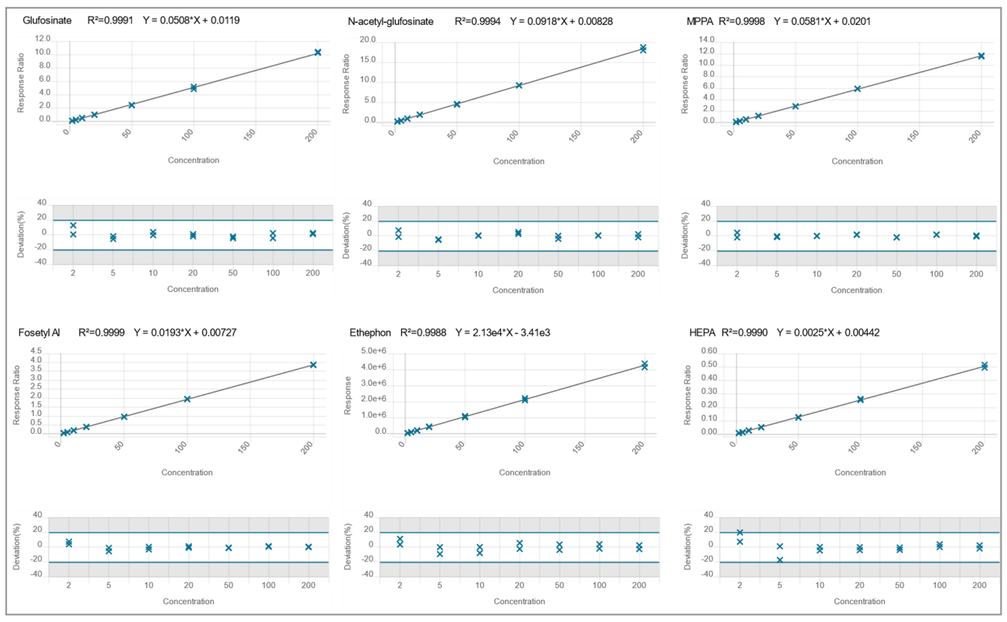

Figure 2. Calibration and residual plots for anionic polar pesticides in wheat flour 2–200 µg/kg (0.25 to 25 ng/mL in vial concentration) for Glufosinate, N-Acetyl-Glufosinate, MPPA, Fosetyl Al, Ethephon and HEPA.

For all compounds except ethephon, internal standards were used in the calibration assessment. In all cases the residuals for calibration were <20% and correlation of determination (R^2) values were all 0.99 or greater. Example calibrations from matrix standards are demonstrated in Figure 2.

	LOQ (µg/kg)									
	Glyphosate	N-Acetyl-Glyphosate	AMPA	N-Acetyl-AMPA	Glufosinate	N-Acetyl-Glufosinate	MPPA	Ethephon	HEPA	Fosetyl Al
Cucumber (wet commodity	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.45
Wheat Flour (dry commodity)	2	2	5	2	2	2	2	2	2	2

Table 2. Method limit of quantification for ten anionic polar pesticides.

The accuracy of the Xevo TQ Absolute system was assessed by quantifying residues of anionic polar pesticides matrix standards at concentrations of 1 and 10 µg/kg in cucumber (a representative vegetable matrix) and at 10 and 50 µg/kg in wheat flour (a representative cereal matrix) with the results displayed in Table 3.

DISCUSSION

Compound	Cucumber			Wheat Flour		
	Matrix Standard Conc. (µg/kg)	Trueness (%)	RSD (%)	Matrix Standard Conc. (µg/kg)	Trueness (%)	RSD (%)
Glyphosate	1	100	8.1	10	102	5.3
	10	109	3.6	50	104	6.0
N-Acetyl-Glyphosate	1	94	2.1	10	95	1.1
	10	109	0.3	50	98	0.5
AMPA	1	89	8.3	10	99	9.2
	10	108	3.5	50	100	6.5
N-Acetyl-AMPA	1	90	2.6	10	99	1.9
	10	109	1.6	50	99	1.6
Glufosinate	1	92	2.6	10	99	3.7
	10	108	1.3	50	97	4.3
N-Acetyl-Glufosinate	1	91	1.9	10	101	1.8
	10	108	0.8	50	99	2.4
MPPA	1	91	4.8	10	101	1.7
	10	109	0.6	50	99	0.6
Ethephon	1	117	2.9	10	98	3.4
	10	115	2.7	50	101	2.5
HEPA	1	97	8.7	10	98	4.1
	10	113	1.8	50	96	2.7
Fosetyl Al	1	96	3.4	10	100	1.9
	10	105	1.1	50	96	1.0

Table 3. Summary of measured concentrations from a matrix standard and the repeatability of the measurement (n=10 at each concentration level).

Extraction method performance for the QuPPe extraction is well documented and Waters has an established method for the anionic polar pesticide analysis.[5] By using a high sensitivity MS such as the Xevo TQ Absolute lower limits can be achieved for this challenging analysis as demonstrated by this work. This extra sensitivity of the method can also be used to lower the injection volume, whilst maintaining current performance limits. With this approach there would be an expected increase in method system robustness as there would be less matrix introduced into the LC-MS/MS system.

CONCLUSION

- **Using the QuPPE extraction with no clean-up limits of quantification as low as 0.5 ug/kg in cucumber and 2 ug/kg in wheat flour (expect AMPA where 5 ug/kg was the limit) can be achieved using the Xevo TQ Absolute system.**
- **The additional sensitivity of the Xevo TQ Absolute system can be used to achieve lower limits of quantification or to reduce the amount of sample injected into the system and maintain current method performance limits.**

References

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