SUB 1 µG/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. These challenging analytes and their metabolites are not "amenable" to common multi-residue approaches, such as QuEChERs and mini-Luke, nor to reversed-phase chromatography.[1,2] Polar pesticide approaches were typically a series of selective single residue methods which required significant effort for the analysis. The introduction of the Quick Polar Pesticides (QuPPe) method has allowed the analysis of foodstuffs for highly polar pesticides not amenable to common multi-residue methods.[3] 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.[4,5,6,7]

The demand for lower limits of quantification for the anionic polar pesticides can be addressed with the enhanced negative ion sensitivity of the Xevo™ TQ Absolute system. This now allows limits of detection in the low and even sub µg/kg region and can be combined with a generic extraction such as the QuPPe method to bring a multi-residue approach to this analysis. This application work focused on achieving a lower limit of quantification with this enhanced sensitivity. Reduced injection volume to reduce matrix load on the liquid chromatography tandem mass spectrometry (LC-MS/MS) system is also possible using this approach.

METHODS

Sample preparation

Blank matrix extracts were generated following the QuPPe version 12 protocol.[3] 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). Solvent standards were prepared corresponding to each of these ranges to assess matrix effects.

Instrument methods

LC: ACQUITY™ UPLC I-Class PLUS system with Sample manager (FL), mobile phase A: 0.9% formic acid in water, mobile phase B: 0.9% formic acid in acetonitrile, Waters Anionic Polar Pesticide Column 5µm, 2.1 x 100mm was used for separation, Flow rate 0.5 mL/min, 0 min: 90% B, 4 min 15% B, 30 min 15% B, 35 min 90% B.

MS Settings: Xevo TQ Absolute system, ESI negative mode, capillary voltage 2.4 kV, desolvation temperature: 600°C, source temperature: 150°C, desolvation gas flow: 1000 L/hr

MS transitions: See Table 1.

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

Table 1. MRM transitions of polar pesticides.

RESULTS

The linear response range for the anionic polar pesticides was tested 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. Limit of quantification was defined as the lowest calibration standard in these calibration sequences, 0.5 µg/kg for cucumber matrix and 2 µg/kg for wheat flour matrix. 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 cucumber and wheat flour matrix standards are demonstrated in Figure 1 and 2.

From the calibration experiments the method limit of quantification was calculated as the lowest calibration standard where the quantifier and qualifier transition were detected. Those limits are listed in Table 2. The difference in sample limits of quantification observed between the two different sample types is attributable to the different dilution factor within the QuPPe v12 extraction procedure for "wet" commodities such as cucumber versus "dry" commodities such as wheat flour. The in-vial concentrations that were detected were 0.25 ng/mL for all anionic polar pesticides studied for both commodities except for AMPA which had a slightly higher in vial concentration of 0.63 ng/mL in wheat flour matrix which is attributed to signal suppression from the matrix.

Compound	Cucumber		Cereals	
	Vial Concentration (ng/mL)	Sample Concentration (µg/kg)	Vial Concentration (ng/mL)	Sample Concentration (µg/kg)
Glyphosate	0.25	0.5	0.25	2
N-Acetyl-Glyphosate	0.25	0.5	0.25	2
AMPA	0.25	0.5	0.63	5
N-Acetyl-AMPA	0.25	0.5	0.25	2
Glufosinate	0.25	0.5	0.25	2
N-Acetyl-Glufosinate	0.25	0.5	0.25	2
MPPA	0.25	0.5	0.25	2
Ethephon	0.25	0.5	0.25	2
HEPA	0.25	0.5	0.25	2
Fosetyl Al	0.25	0.5	0.25	2

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

Trueness and repeatability for the analysis of the polar pesticides was assessed for both cucumber and wheat flour matrices by repeatedly injecting a matrix standard and quantifying the response against a calibration graph generated from bracketed calibration standards. Table 3 displays the results from these experiments which demonstrate that

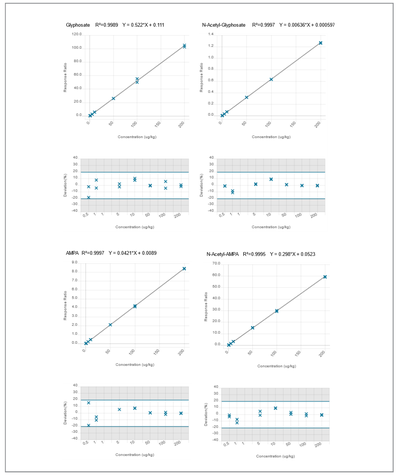

Figure 1. Calibration and residual plots for anionic polar pesticides in cucumber 0.5–200 µg/kg (0.25 to 100 ng/mL in vial concentration) for Glyphosate, N-Acetyl-AMPA, AMPA, and N-Acetyl-AMPA.

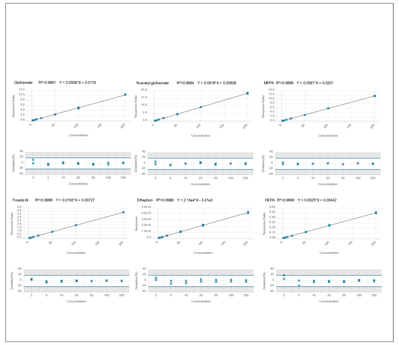

Figure 2. Calibration and residual plots for anionic polar pesticides in wheat flour 2–200 µg/kg (0.25 to 25 ng/mL in vial concentration) for Glufosinate, N-Acetyl-Glufosinate, MPPA, Fosetyl Al, Ethephon, and HEPA.

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).

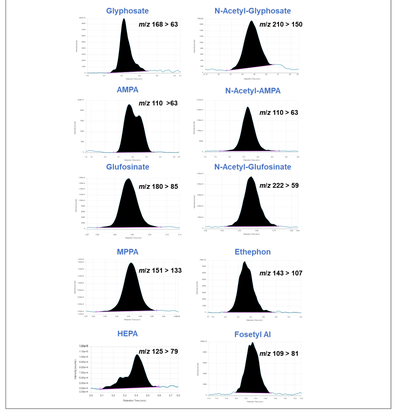

Figure 3. Chromatograms of the anionic polar pesticide and metabolites from the analysis of a cucumber matrix standard at 1 µg/kg (in vial concentration 0.5 ng/mL).

the Xevo TQ Absolute system is capable of accurately quantifying residues of anionic polar pesticides at concentrations of 1 µg/kg in cucumber (a representative vegetable matrix) and at 2 µg/kg in wheat flour (a representative cereal matrix) with AMPA slightly higher in wheat flour at 5 µg/kg. Example chromatograms for the anionic polar pesticides in cucumber matrix at 1 µg/kg are displayed in Figure 3.

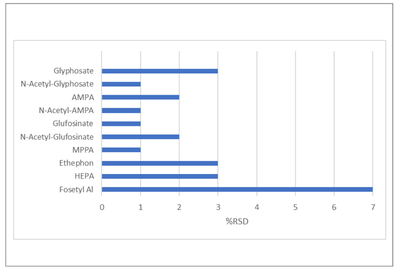

Figure 4. Peak area repeatability of the anionic polar pesticides on the Xevo TQ Absolute system with a cucumber matrix standard (n=30) at 10 µg/kg (5 ng/mL in vial concentration).

An additional experiment was carried out to investigate response repeatability of the analytes by a series of injections of a single cucumber matrix standard at 10 µg/kg (5 ng/mL in vial concentration). The peak areas were plotted to ensure that a stable response was achieved across a typical analytical batch of thirty injections. The response was not adjusted by internal standard response and peak area response from the native analyte was used. The RSDs for the peak areas over the series of thirty injections was generally 3% or lower except for Fosetyl-Al which was 7%, as seen in Figure 4.

DISCUSSION

Extraction method performance for the QuPPe extraction is well documented and demonstrates that this extraction process is suitable for quantification work in the analysis of polar pesticides.3,4,5,6 Chromatographic method performance has been established and documented using the Anionic Polar Pesticide Column.4,5,6 By using a high sensitivity mass spectrometer such as the Xevo TQ Absolute system lower limits can be achieved for this challenging analysis as demonstrated by the results presented in Table 2. Across a "normal" calibration range of 0.5–200 µg/kg this method approach gives linear calibrations for all the anionic polar pesticides studied and is generally regarded as the preferred calibration approach for pesticide residue analysis.

The results demonstrate how a lower limit of quantification can be achieved but the extra sensitivity of the method can be used to lower the method injection volume, whilst maintaining current method performance limits. With this approach there would be an expected increase in method and system robustness as with the reduced injection volume, less matrix would be 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.**
- **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.**

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