

Detection of Anionic Polar Pesticides in Food Samples Using the Xevo™ TQ Absolute With Sub $\mu\text{g}/\text{kg}$ Limits of Quantification

Stuart Adams, Gitte Barknowitz, Kari L. Organtini

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

The area of anionic polar pesticide analysis has been evolving over the past ten years where the adoption of generic extraction methods, such as the QuPPE method, have enabled laboratories to take a multi-residue approach for the analysis of these challenging analytes. With the enhanced negative ion sensitivity of the Xevo TQ Absolute Tandem Quadrupole Mass Spectrometer, limits of quantification of 0.5 $\mu\text{g}/\text{kg}$ in vegetable samples and 2 $\mu\text{g}/\text{kg}$ in cereal samples are achievable. Trueness was assessed over ten injections at 1 and 10 $\mu\text{g}/\text{kg}$ in cucumber matrix standards and at 10 and 50 $\mu\text{g}/\text{kg}$ in wheat flour matrix standards. Trueness in cucumber was between 91 to 117% with RSDs between 0.6 to 8.7% and between 96 to 104% in wheat flour with RSDs between 0.5 to 9.2%.

Benefits

- Performance of the Xevo TQ Absolute demonstrates enhanced sensitivity for the analysis of anionic polar pesticides achieving significantly lower method detection limits than previously demonstrated
-

- Reduced sample injection volume enables a reduction in sample matrix being introduced into the LC-MS/MS system
- Implementation is supported across the globe utilizing our outcome-based support model to ensure customer success

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 with 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. 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.

Experimental

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.

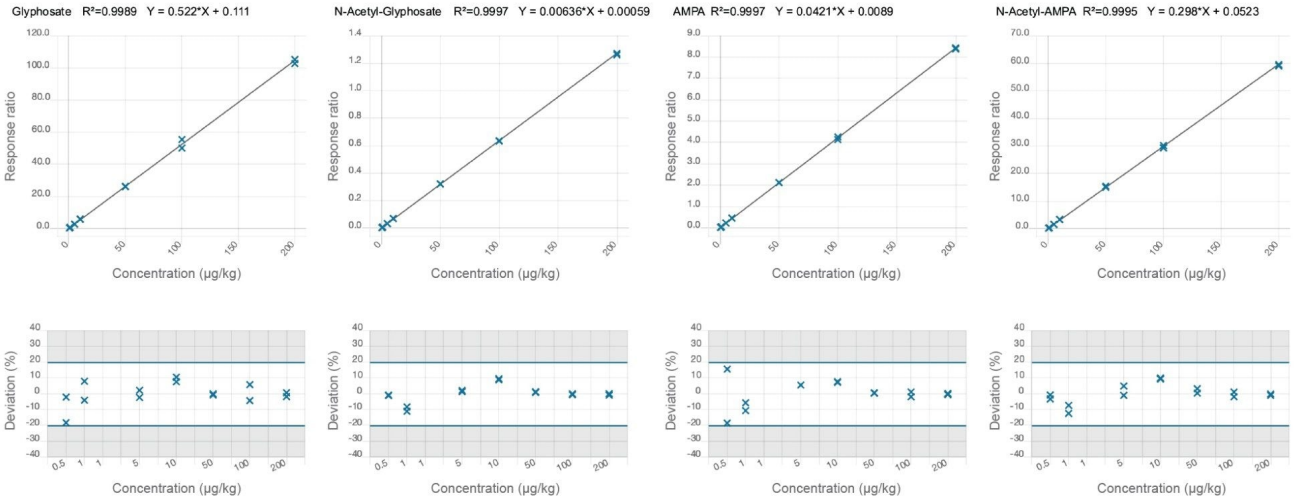
Chromatographic conditions used are listed in a previously released application note and utilize the Anionic Polar Pesticide Column.⁴

Results and 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}

Using an established Waters LC method, both linearity and limit of quantification were assessed on the Xevo TQ Absolute. 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.

1A.



1B.

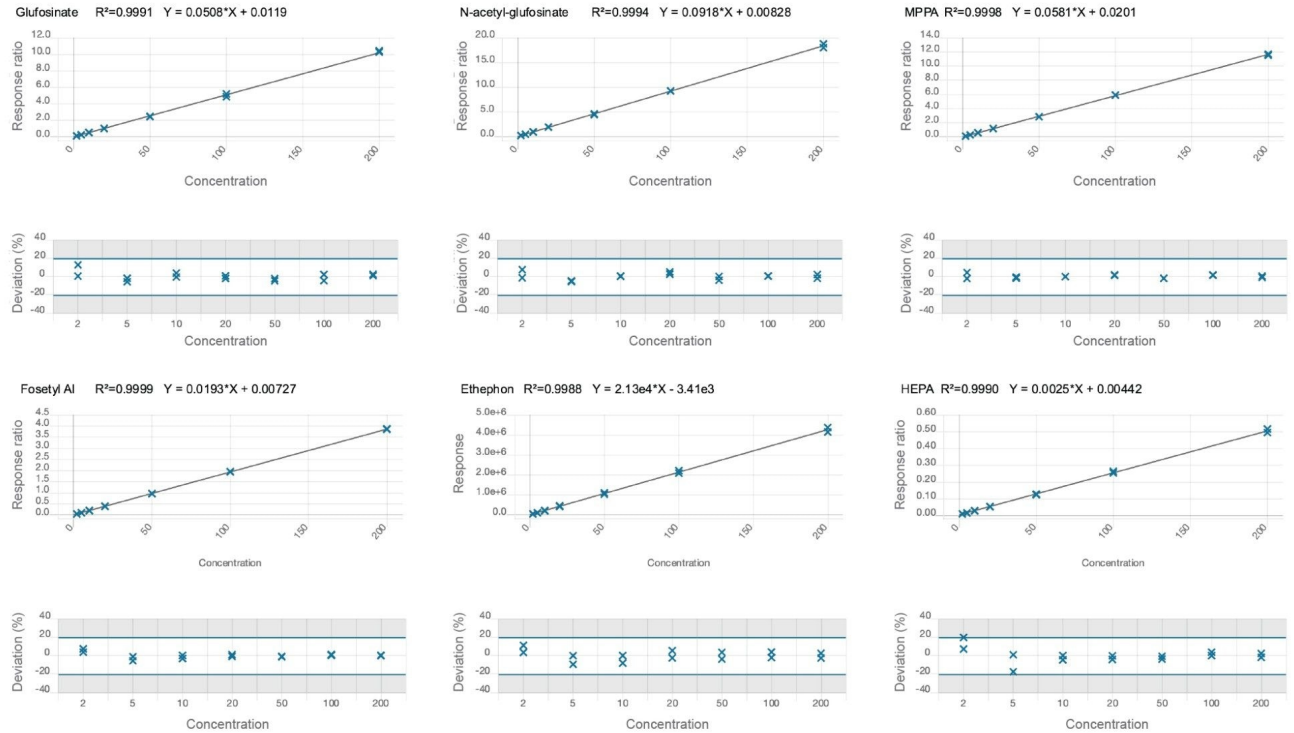


Figure 1A. Calibration and residual plots for anionic polar pesticides in cucumber 0.5–200 µg/kg (0.25 to 100 ng/mL in vial concentration) for Glyphosate, N-Acetyl-AMPA, AMPA, and N-Acetyl-AMPA. Figure 1B. Calibration and residual plots for anionic polar pesticides in wheat flour 2–200 µg/kg (0.25 to 25 ng/mL in vial concentration) for Glufosinate, N-Acetyl-Glufosinate, MPPA, Fosetyl AI, Ethephon, and HEPA.

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 1. The difference in sample limits of quantification observed between the 2 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		Wheat flour	
	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 1. 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 2 displays the results from these experiments which demonstrate that the Xevo TQ Absolute 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 2.

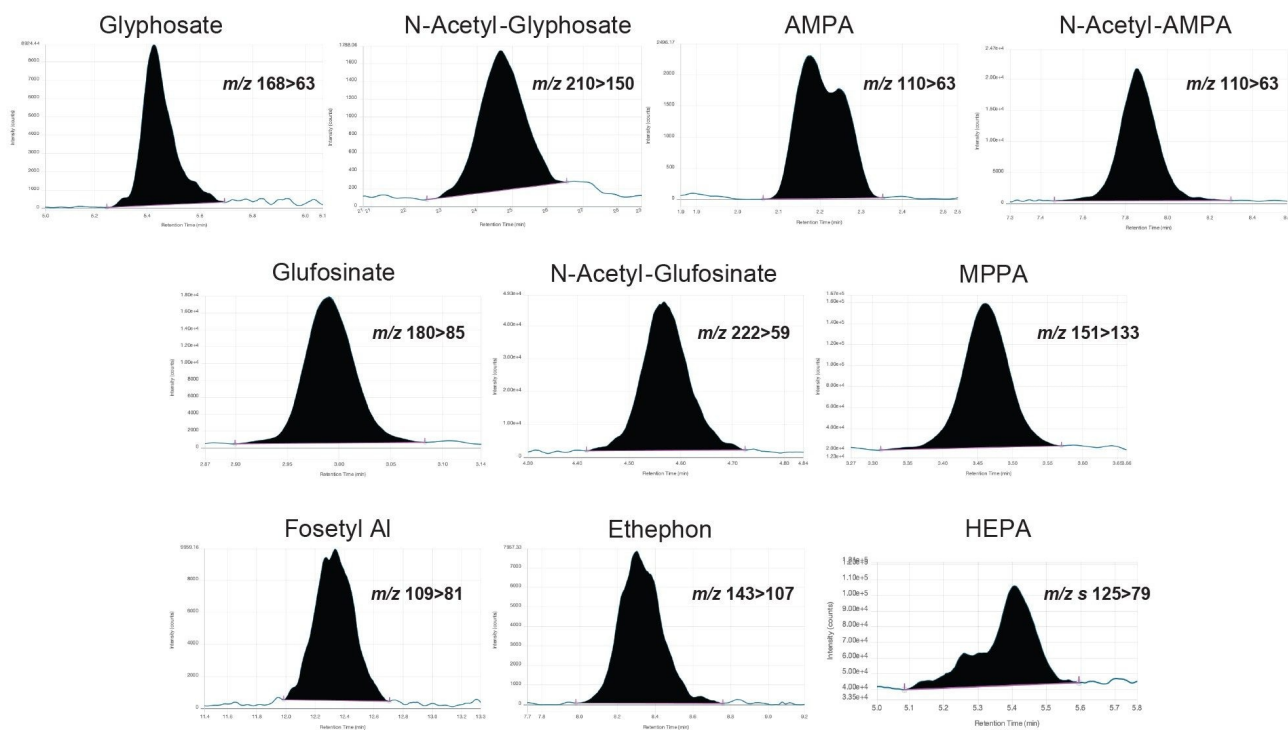


Figure 2. Chromatograms of the anionic polar pesticide and metabolites from the analysis of a cucumber matrix standard at 1 $\mu\text{g}/\text{kg}$ (in vial concentration 0.5 ng/mL).

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 AI	1	96	3.4	10	100	1.9
	10	105	1.1	50	96	1.0

Table 2. Summary of measured concentrations from a matrix standard and the repeatability of the measurement ($n=10$ at each concentration level).

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 AI which was 7%, as seen in Figure 3.

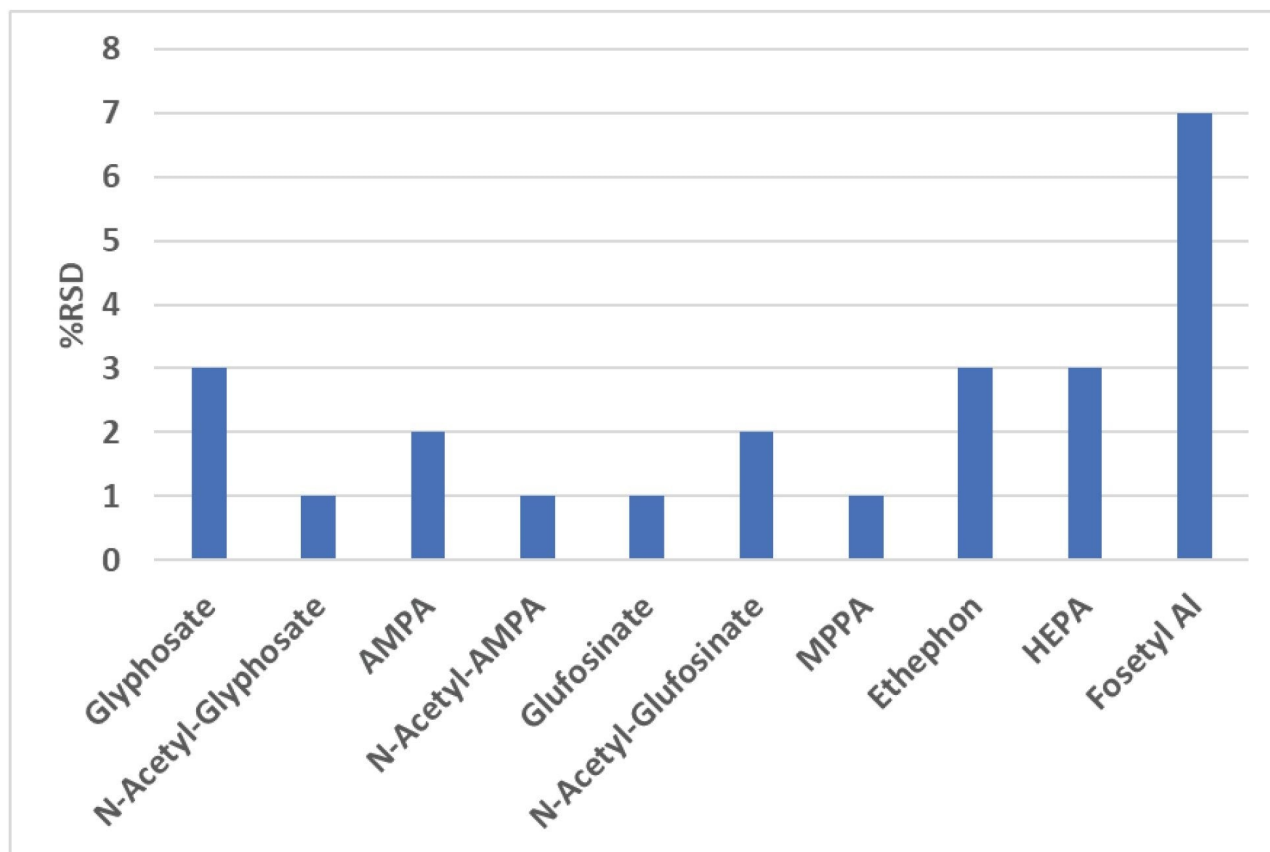


Figure 3. Peak area repeatability of the anionic polar pesticides on the Xevo TQ Absolute with a cucumber matrix standard ($n=30$) at $10 \mu\text{g}/\text{kg}$ ($5 \text{ ng}/\text{mL}$ in vial concentration).

Conclusion

There has been increasing demand to move to a multi-residue approach for the anionic polar pesticides which has been facilitated with the QuPPE generic extraction approach. With the move to a generic and simplified extraction technique, the demand for lower limits of quantification for these negative ionization analytes has been developed. The Xevo TQ Absolute with the enhanced sensitivity in negative ionization mode makes achieving significantly lower levels of quantification possible. This has resulted in achievable limits of quantification for the anionic polar pesticides in cucumber (representative vegetable samples) of $0.5 \mu\text{g}/\text{kg}$ and 2

µg/kg for all but AMPA, where 5 µg/kg was achieved, in the wheat flour (representative cereal sample).

References

1. M. Anastassiades, S. J. Lehotay, D. Stajnbaher, F. J. Schenck, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, *J. AOAC Int.*, 86 (2003) 412–431.
2. Dutch mini-Luke ("NL-") Extraction Method Followed by LC and GC-MS/MS for Multiresidue Analysis of Pesticides in Fruits and Vegetables, URL: <https://www.eurl-pesticides.eu/userfiles/file/NL-miniLuke-extraction-method.pdf> <<https://www.eurl-pesticides.eu/userfiles/file/NL-miniLuke-extraction-method.pdf>> .
3. M. Anastassiades; A.-K. Wachtler; D. I. Kolberg; E. Eichhorn; H. Marks; A. Benkenstein; S. Zechmann; D. Mack; C. Wi Idgrube; A. Barth; I. Sigalov; S.Görllich; D.Dörk and G. Cerchia. Quick Method for the Analysis of Highly Polar Pesticides in Food Involving Extraction with Acidified Methanol and LC - or IC MS/MS Measurement - I. *Food of Plant Origin* (QuPPE-PO-Method) –Version 12 (published on EURL-SRM website on July 23, 2021); URL: https://www.eurl-pesticides.eu/docs/public/tmpl_article.asp?CntID=887&LabID=200&Lang=EN <https://www.eurl-pesticides.eu/docs/public/tmpl_article.asp?CntID=887&LabID=200&Lang=EN> .
4. Hird S, Adams S, De-Alwis J. Evaluation of the Performance of a Method for the Determination of Highly Polar, Anionic Pesticides in Foodstuffs Using LC-MS/MS, Waters Application Note, [720007505](#), 2022.
5. De-Alwis J, Williams J, Hird S, Adams S. Evaluation of the Performance of an LC-MS/MS Method for the Determination of Anionic Polar Pesticides Residues in Crops and Foodstuffs Using an Interlaboratory Study, Waters Application Note, [720007154](#), 2021.
6. Kumar K P, Bhaska K, Gorella T, Wagh P. Determination of Anionic Polar Pesticides in Grapes using UPLC-MS with Anionic Polar Pesticide Column. Waters Application Note, [720006925](#), 2020.
7. Ross E, De-Alwis J, Adams S, Williams J, Shah D D. Determination of Anionic Polar Pesticides in High Water Foodstuffs. Waters Application Note, [720006645](#), 2019.

Featured Products

[Xevo TQ Absolute](#)

720007567, March 2022

© 2022 Waters Corporation. All Rights Reserved.

[Terms of Use](#)

[Privacy](#)

[Trademarks](#)

[Sitemap](#)

[Careers](#)

[Cookies](#)

[Cookie Preferences](#)