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Application Note

Evaluation of the Performance of a Method for the Determination of Highly Polar, Anionic Pesticides in Foodstuffs Using LC-MS/MS

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

Abstract

Waters previously developed a LC-MS/MS method for the determination of anionic polar pesticides in various food commodities. The method is based upon extraction with the established Quick Polar Pesticides method (QuPPe) from the EURL for Single Residue Methods (SRM) in Stuttgart and LC-MS/MS using the Anionic Polar Pesticide Column. This application brief shows the evaluation of the performance of this method in a selection of food commodities using an ACQUITY UPLC H-Class PLUS System and Xevo TQ-S micro Tandem Quadrupole Mass Spectrometer. Samples representing four different commodity groups (cucumber, rice, soyabean, and milk) were spiked with aminomethylphosphonic acid (AMPA), ethephon, 3-methylphosphinicopropionic acid (MPPA), N-acetylglufosinate (NAG), N-acetylglyphosate, fosetyl Al, glufosinate, glyphosate, and 2-hydroxyethanephosphonic acid (HEPA) at three concentrations. The trueness and repeatability (RSD_r) of the method, using internal standards, was determined to be within the range of 78 to 92% and 0.40–6.4%, respectively, across all four commodities. This demonstrated that the method could be suitable for checking compliance with MRLs and has the potential for screening at much lower concentrations, for example for food

business operators' due diligence testing.

Benefits

- The chromatographic method provides excellent peak shape integrity, maintains key analyte separations, and offers retention without resorting to derivatization or ion pair reagents
- The performance of the method, as demonstrated by this evaluation, provides users with evidence of its suitability for both official control and due diligence testing
- Implementation is supported across the globe utilizing our outcome-based support model to ensure customer success

Introduction

Quantitative multi-residue methods (***e.g.*** QuEChERS) can be used to determine hundreds of compounds in a variety of commodities at low levels. Some polar and ionic pesticides and their metabolites are not "amenable" to common multi-residue methods. These were typically treated as a series of selective single residue methods adding significant cost. The Quick Polar Pesticides (QuPPe) method allows the analysis of foodstuffs for highly polar pesticides not amenable to common multiresidue methods.[1] Waters has published results showing the performance of a method that successfully combined the use of the QuPPe with a chromatographic method using the Waters Anionic Polar Pesticide (APP) Column, which is made up of ethylene bridged hybrid (BEH) particles with tri-functionally bonded diethylamine (DEA) ligands. The combination of the hydrophilic surface and the anion-exchange properties of the ligand provides chromatographic characteristics well suited to the retention and separation of these highly polar anionic compounds.[2,3]

The EURL for Residues of Pesticides, Single Residue Methods, recently conducted an interlaboratory study to further validate the QuPPe approach for the determination of highly polar anionic pesticides in a selection of food commodities.[4] The scope of the analysis included the following analytes: AMPA, ethephon, MPPA, N-acetylglufosinate (NAG), N-acetylglyphosate, cyanuric acid, fosetyl Al, glufosinate, glyphosate, HEPA, and maleic hydrazide, for which stable isotope analogues were provided as internal standards. Waters took the opportunity to re-run the batches, prepared in representatives of four different commodity groups (cucumber, rice, soyabean, and milk), using a modification of our APP column Method B for all the analytes except cyanuric acid and maleic

hydrazide.[5] The analysis was carried out using an ACQUITY UPLC H-Class PLUS with FTN Sample Manager coupled to a Xevo TQ-S micro MS/MS System. The results of the evaluation of the performance of the method are reported here.

Results and Discussion

Chromatography

The method provides excellent chromatographic separation and retention of these highly polar compounds. Peak shapes and retention times for all the analytes were shown to be stable for all the commodities, analyzed across four days.

Sensitivity

Due to the relative response exhibited by each compound of interest, the standard solutions provided by the EURL, for both calibration and spiking, contained the analytes at different concentrations. As the weight of samples of cucumber and rice (10 g) was different to that of soyabean and milk (5 g), there was also a difference in the concentrations based upon commodity (Table 1). Figures 1 and 2 show typical chromatograms for all the highly polar, anionic pesticides and metabolites from the analysis of the lowest spikes, in rice and milk as examples, which shows that the method is typically capable of detection of these analytes in extracts at much lower concentrations or final extracts could be diluted further prior to LC-MS/MS. The exception was AMPA, the response for which was significantly suppressed in the presence of soyabean extract, so much so it could not be reliably determined in the spikes. This is not a significant failing as the EU MRL residue definition for glyphosate does not currently include the metabolite AMPA and even if it is updated in the future, the MRL for glyphosate in soyabean (20 mg/kg) is much greater than the concentrations evaluated here. One possible solution would be to dilute the extracts further prior to analysis.

	Concentration (mg/kg)							
	Cucumber and milk				Rice and soyabean			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Standard 1	0.025	0.014	0.010	0.005	0.050	0.028	0.020	0.010
Standard 2	0.049	0.030	0.020	0.010	0.098	0.060	0.040	0.020
Standard 3	0.098	0.060	0.040	0.020	0.196	0.120	0.080	0.040
Standard 4	0.123	0.074	0.050	0.025	0.246	0.148	0.100	0.050
Standard 5	0.246	0.148	0.100	0.050	0.492	0.296	0.200	0.100
Spike low	0.050	0.030	0.020	0.010	0.100	0.060	0.040	0.020
Spike med	0.100	0.060	0.040	0.020	0.200	0.120	0.080	0.040
Spike high	0.250	0.150	0.100	0.050	0.500	0.300	0.200	0.100

Group 1: Glyphosate, AMPA, n-Acetyl glyphosate
Group 2: Glufosinate
Group 3: MPPA, NAG, HEPA
Group 4: Ethephon, Fosetyl Al

Table 1. Concentrations of each analyte in the matrix-matched standards and spiked samples.

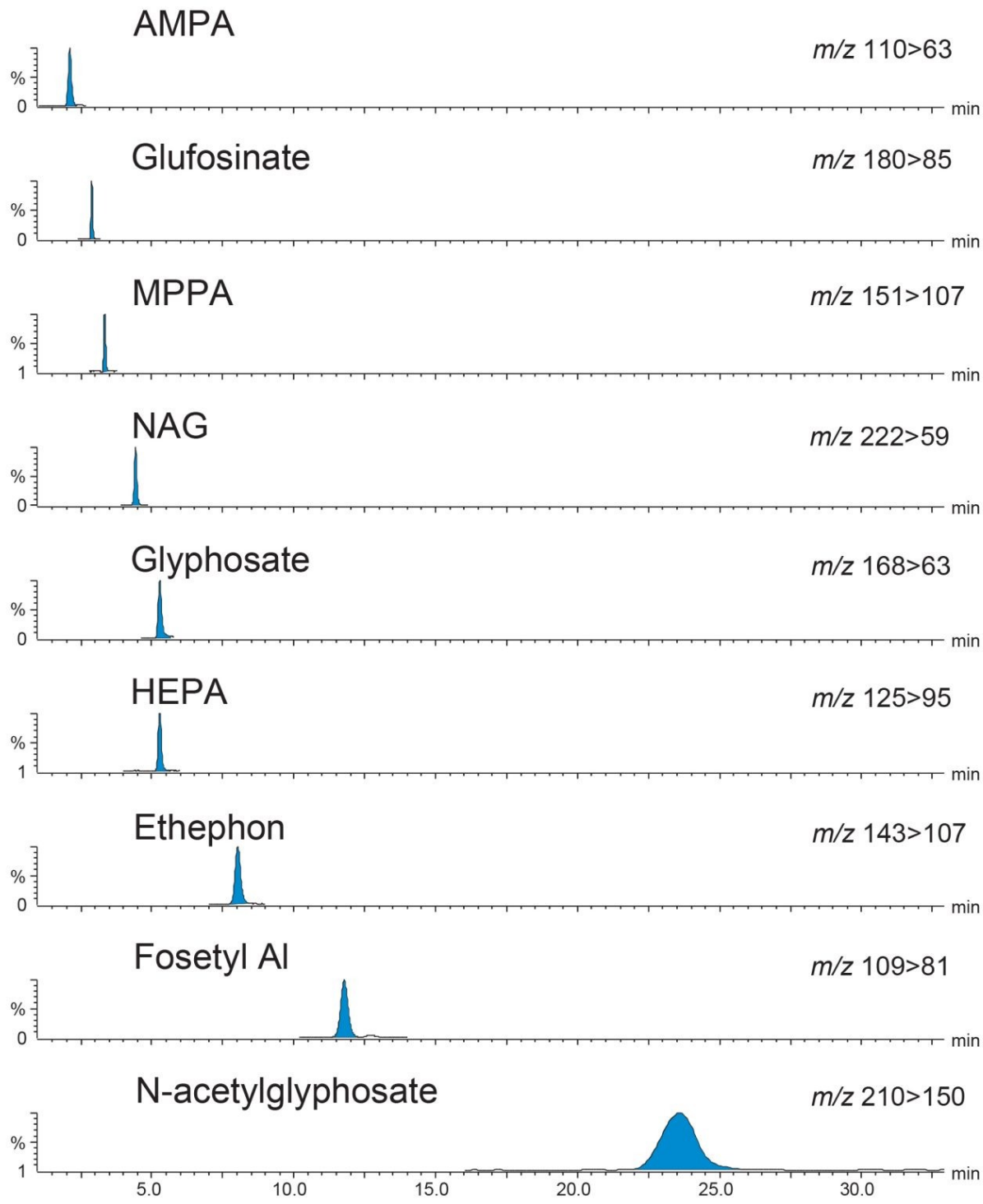

Figure 1. Chromatograms of all the highly polar, anionic pesticides, and metabolites from the analysis of the

lowest spike in rice (concentrations given in Table 1).

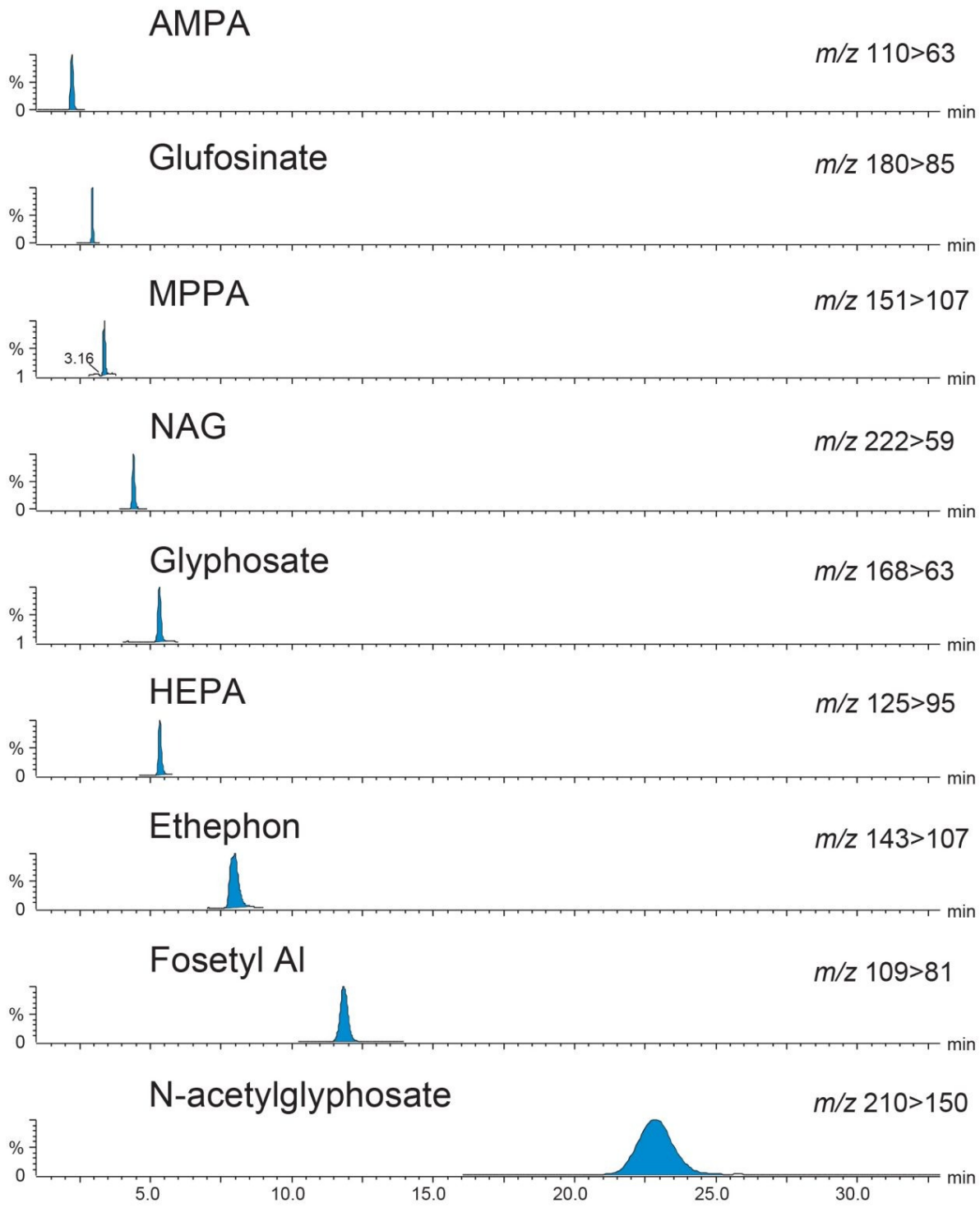

Figure 2. Chromatograms of all the highly polar, anionic pesticides, and metabolites from the analysis of the

lowest spike in milk (concentrations given in Table 1).

Selectivity, identification, and calibration criteria

Blank samples were prepared for each of the four commodity batches and analyzed. No signal was detected in the extracts that could lead to detection of false reporting of non-compliant samples. Some compounds were detected at trace levels but were estimated to be at concentrations much lower than the lowest standard. The two transitions for each analyte gave peaks with ion ratios and retention times within the recommended SANTE tolerances, when compared with the standards.[6] A 5-point calibration curve for each analyte, using the stable isotope analogue as an internal standard, was prepared in matrix extracts, and acquired on each day. Linear fit with 1/x weighing was applied and all correlation of determination (R^2) values from the calibration graphs were >0.99, with individual residuals all <20%, most much lower, demonstrating reliable quantification. Some examples of typical calibration curves in rice are given in Figure 3.

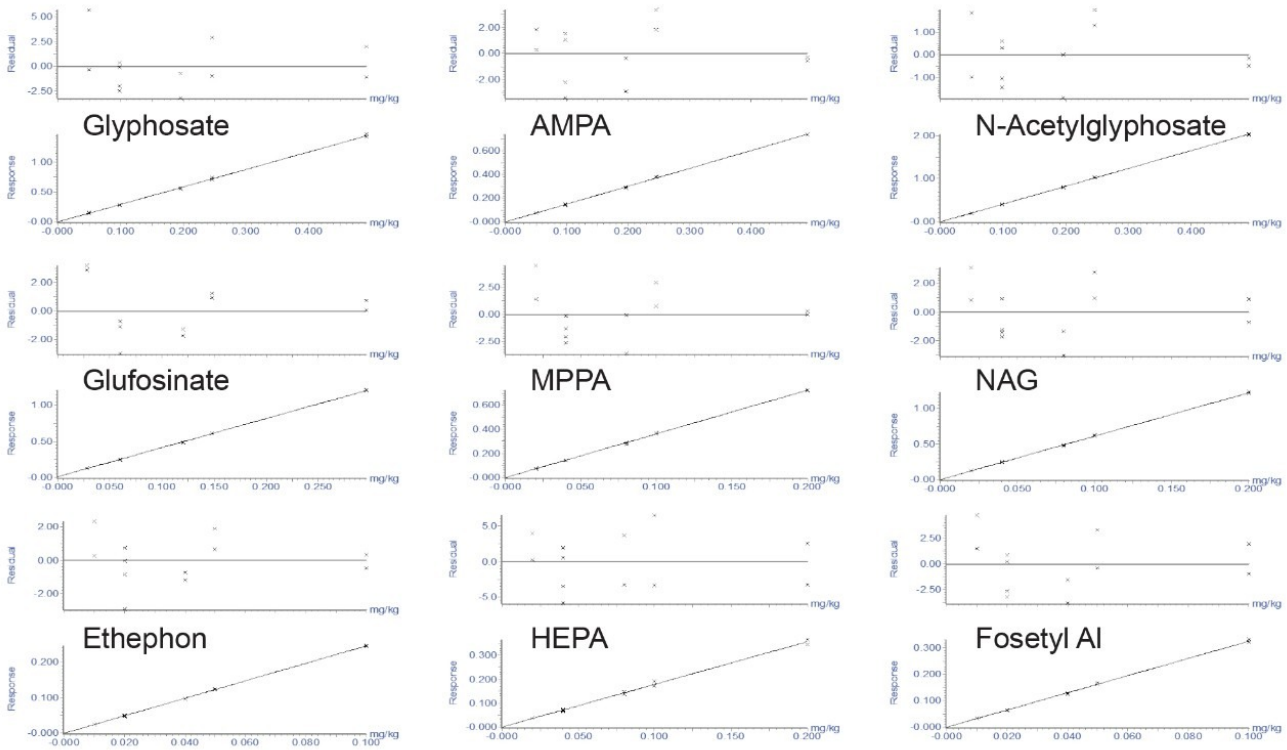

Figure 3. Calibration and residuals graphs for the analytes in rice.

Trueness and repeatability

The trueness, expressed by measured recovery after adjustment using the appropriate internal standard, was evaluated using the data from the analysis of the spiked samples from the four commodities. The mean measured recoveries for each set of five spikes, at the three concentrations, prepared and analyzed over four days, by three different analysts, were within the range 78 to 92% and hence were well within the criteria set out in the SANTE guidelines. The repeatability (RSD_r) of the method was also excellent for all analytes, at the three concentrations across all four commodities (0.40–6.4%). The trueness and repeatability data for each spiking level are shown in Tables 2 and 3 and Figure 4. Although the measured recoveries in the milk were consistently lower than the other commodities (mean 80%), and the repeatability values for some compounds in milk increased at the lowest spiking level, the values for these parameters remained acceptable and within the criteria set out in the SANTE guidelines. A summary of the other performance parameters evaluated is given in Table 4.

Compound		Cucumber		Milk	
	Conc. (mg/kg)	Trueness (%)	Repeatability (% RSD^r)	Trueness (%)	Repeatability (% RSD^r)
Glyphosate	0.05	88	1.6	80	4.4
	0.10	85	0.4	80	1.5
	0.25	89	0.6	79	1.7
AMPA	0.05	83	3.9	81	3.3
	0.10	87	4.5	78	1.3
	0.25	84	0.5	78	1.0
n-Acetyl glyphosate	0.05	88	0.6	82	4.4
	0.10	85	0.4	78	1.5
	0.25	89	0.4	78	0.9
Glufosinate	0.03	86	1.8	80	2.6
	0.06	85	1.7	81	2.1
	0.15	89	0.8	79	1.2
MPPA	0.02	90	1.8	83	3.7
	0.04	88	0.5	79	2.0
	0.10	90	0.8	80	1.2
NAG	0.02	89	0.7	84	3.4
	0.04	88	1.5	79	1.4
	0.10	91	1.0	79	1.4
Ethephon	0.01	92	1.7	84	6.4
	0.02	88	1.2	78	3.4
	0.05	92	1.6	81	1.4
HEPA	0.02	88	1.1	84	2.8
	0.04	87	1.1	83	2.2
	0.10	91	0.6	78	1.0
Fosetyl Al	0.01	89	0.5	81	3.4
	0.02	86	0.8	80	0.9
	0.05	91	0.6	79	0.5

Table 2. Summary of the measured recoveries (%) and repeatability (%RSD$_r$) from the analysis of spikes from cucumber and milk.

Compound		Cucumber		Milk	
	Conc. (mg/kg)	Trueness (%)	Repeatability (% RSDr)	Trueness (%)	Repeatability (% RSDr)
Glyphosate	0.10	87	1.1	86	0.5
	0.20	84	1.2	83	1.9
	0.50	87	0.4	85	1.3
AMPA	0.10	87	1.0		
	0.20	85	1.0		
	0.50	87	0.7		
n-Acetyl glyphosate	0.10	86	1.4	86	1.3
	0.20	85	1.4	83	1.2
	0.50	86	0.6	84	0.5
Glufosinate	0.06	86	2.2	83	5.0
	0.12	84	1.0	83	2.2
	0.30	86	0.8	85	2.3
MPPA	0.04	89	2.0	82	1.7
	0.08	86	2.2	80	1.9
	0.20	88	0.8	83	0.7
NAG	0.04	87	1.9	86	1.2
	0.08	85	1.1	85	1.1
	0.20	87	0.7	86	0.8
Ethephon	0.02	87	1.9	84	2.0
	0.04	84	1.0	84	2.0
	0.10	87	0.4	88	1.9
HEPA	0.04	86	1.3	86	1.3
	0.08	83	0.9	84	1.6
	0.20	87	1.3	86	0.9
Fosetyl Al	0.02	88	1.6	85	0.8
	0.04	85	1.3	82	1.1
	0.10	89	1.0	84	0.5

Table 3. Summary of the measured recoveries (%) and the repeatability (%RSD_r) from the analysis of spikes from rice and soyabean.

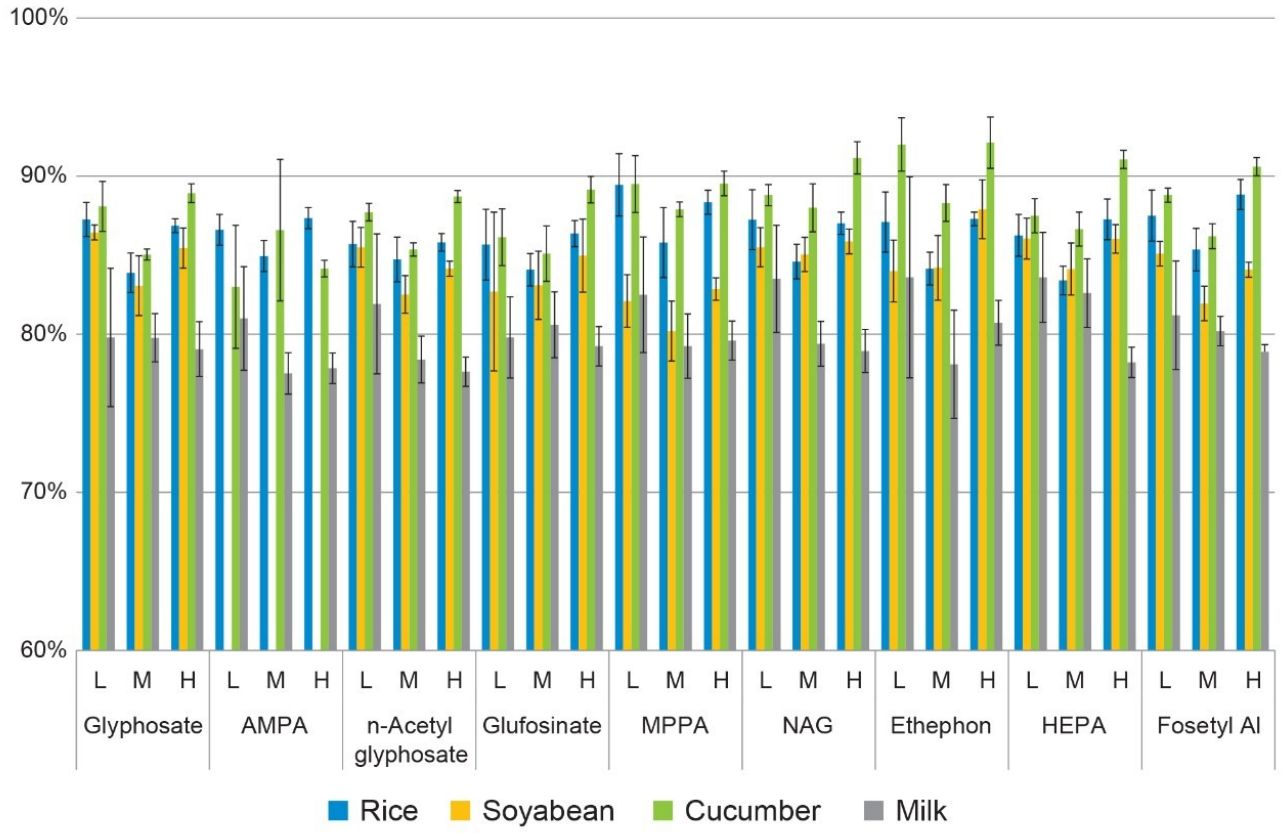

Figure 4. Summary of the measured recoveries (%) and repeatability (%RSD_r) from the analysis of spikes from cucumber, rice, soyabean, and milk.

Parameter	Correlation of determination (R^2)				Residuals (%)				Retention time across all batches	
	Cucumber	Milk	Soyabean	Rice	Cucumber	Milk	Soyabean	Rice	Mean RT (min)	RT RSD (%)
Glyphosate	0.9998	0.9986	0.9991	0.9990	<2.1	<5.0	<3.0	<5.7	5.28	1.2%
AMPA	0.9962	0.9986		0.9992	<8.0	<7.0		<3.4	2.14	2.7%
n-Acetyl glyphosate	0.9999	0.9987	0.9995	0.9997	<1.0	<5.8	<2.9	<2.0	23.3	1.6%
Glufosinate	0.9988	0.9993	0.9992	0.9996	<7.0	<4.2	<6.5	<3.2	2.89	1.4%
MPPA	0.9995	0.9980	0.9985	0.9993	<3.6	<7.5	<6.2	<4.5	3.34	1.1%
NAG	0.9995	0.9978	0.9996	0.9994	<2.9	<8.4	<4.6	<3.1	4.42	0.9%
Ethephon	0.9992	0.9961	0.9996	0.9997	<4.2	<10.1	<3.5	<2.9	8.01	0.9%
HEPA	0.9997	0.9989	0.9964	0.9967	<4.1	<5.0	<5.9	<6.5	5.28	1.0%
Fosetyl Al	0.9997	0.9953	0.9998	0.9980	<2.1	<3.9	<3.0	<4.7	11.77	1.3%

Table 4. Summary of the other performance parameters evaluated.

Conclusion

The results of the evaluation exercise have shown the method to be a sensitive and reliable means for the determination of a series of different highly polar, anionic pesticides, and metabolites, using a combination of the QuPPe method, chromatography from the Waters Anionic Polar Pesticide (APP) Column and determination on Xevo TQ-S micro. The method allows for a fast and reliable quantitation down to concentrations well below typical MRLs and was successfully assessed according to the SANTE guidelines, presenting satisfactory results in cucumber, rice, soyabean, and milk. The procedure can also be applied to other commodities after suitable validation. This cost-effective method can be easily implemented in routine testing laboratories, and this evaluation shows the method may be suitable for checking compliance with MRLs and has the potential for screening at much lower concentrations, for example for food business operators' due diligence testing.

Acknowledgements

Michelangelo Anastassiades and staff at the EURL for Pesticides using Single Residue Methods at Chemisches und Veterinäruntersuchungsamt (CVUA) Stuttgart.

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720007505, Jan 2022