Application Note

Environmental

A Direct Aqueous Injection Method for Polar Pesticides in Drinking and Nonpotable Water

A fast, simple method using the Agilent 6495D LC/TQ

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Abstract

A comprehensive liquid chromatography/triple quadrupole mass spectrometry (LC/TQ) method was developed for the quantitation of three polar pesticides: glyphosate, aminomethylphosphonic acid (AMPA), and glufosinate. The method also included various metabolites with the intention to accelerate and simplify routine laboratory water testing. Compound transitions and optimized parameters were applied to the analytical method. Method suitability was demonstrated using an Agilent 1290 Infinity II LC system coupled to an Agilent 6495D LC/TQ on drinking water, surface water, and ground water, using direct aqueous injection.

Method performance was evaluated based on instrument limit of detection (LOD), limit of quantification (LOQ), calibration curve linearity, recovery, and precision using calibration standards up to 0.5 µg/L. All the analytes demonstrated linearity with $R^2 \geq 0.995$. The method precision was assessed using relative standard deviation (RSD). The RSD at 0.030 and 0.100 µg/L for all compounds were within the limit of 12.5%. The mean recoveries for all the target analytes were within the limits of 75 to 125%.

Introduction

Glyphosate is a broad-spectrum systemic herbicide and, by volume, is one of the most widely used herbicides throughout the world. It is an organophosphorus compound, specifically a phosphonate. There is a great demand for a sensitive method at the low ppb level for food, and even lower levels for environmental water analysis. Reliable sample preparation and analysis are needed to routinely analyze glyphosate and its major metabolite, AMPA. However, glyphosate and its metabolites have high polarity and chelating properties, so they can be challenging to extract from food or water and for analysis. Of concern is the affinity of these compounds to stainless steel and other surfaces, making system-to-system reproducibility difficult. This application note shows the analysis of glyphosate, plus eight other metabolites and polar pesticides. All the pesticides are phosphonates. Fosetyl aluminum, a postharvest fungicide, is also important to include, as it can be mistaken for AMPA. The analysis uses a 1290 Infinity II LC coupled to a 6495D LC/TQ for the analysis of water samples to 10 ppt.

Experimental

Chemicals and reagents

LC/MS-grade solvents and analytical reagents were used for this study.

Standards and solutions

Ready-to-use, custom premixed, and individual standards were acquired where available; neat compounds were sourced where custom mixes were not available.

Two intermediate standard mixes were prepared from stock standards and used for the rest of the experiments. Working standards diluted from mixes 1 and 2 were used for the preparation of prespiked calibration and quality control (QC) samples.

A separate mixture of an internal standard (IS) containing stable isotope-labeled compounds was prepared from a custom mix.

Calibration standards were prepared in matrix water and ultrapure water. Serial dilutions were performed to prepare six calibration concentration levels. Calibration standards were prepared fresh and stored in a refrigerator at 3 °C if not used immediately.

Sample preparation

Samples were collected from three sources: river water, bore-hole water, and drinking water.

Samples were prepared by spiking with the relevant level of additive and transferred as 1 mL to an LC/MS amber vial. The only pretreatment was the addition of sodium thiosulphate to remove residual chlorine from drinking water samples.

Instrumentation

Chromatographic separation was performed using a Metrohm A Supp 5 column (part number 6.1006.520) installed on an Agilent 1290 Infinity II LC system.

The individual modules of the 1290 Infinity II LC system included:

- Agilent 1290 Infinity II high-speed pump (G7120A)
- Agilent 1290 Infinity II autosampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)

A 6495 LC/TQ with an Agilent Jet Stream (AJS) electrospray ion source was operated in dynamic multiple reaction monitoring (dMRM) mode. This mode allows additional MRM transitions if future development is required for additional compounds. The LC/TQ autotune was performed in both unit and wide modes. All data acquisition and processing were performed using Agilent MassHunter software (version 12).

Results and discussion

For each compound, MRM transitions, as well as collision energies and ionization polarity, were optimized using Agilent MassHunter Optimizer software. The two or three most abundant product ions per compound were selected automatically, where available. Depending on the fragmentation behavior of the individual compound, two or three MRM transitions were selected per compound. This selection was done for confident identification and confirmation by the LC/TQ. The most abundant fragments were defined as primary transitions.

The chromatographic method was optimized using the Metrohm A Supp 5 column, which resulted in good separation and distribution of polar pesticides analyzed within an 18.2-minute HPLC gradient. The flow rate offered effective desolvation of target ions using the AJS ion source.

The selected cycle time ensured that sufficient data points were collected across the chromatographic peaks for reproducible quantitation and confirmation of results.

Verification of method performance and validation

The method performance criteria were assessed and completed a validation based on linearity, method sensitivity, recovery, and precision. The three real matrix samples were spiked at a blank, low, and high levels. The water matrices used included drinking water, surface water, and ground water. All compounds met the requirements including precision (< 12.5%), bias (< 25%), uncertainty of measurement (UoM) (< 60%), LOD (< 0.01 µg/L), and LOQ (< 0.03 µg/L).

Chromatography for the three main polar pesticides showed excellent peak shape, retention, and sensitivity at 0.01 and 0.03 µg/L in ultrapure water. In real water samples, there was some suppression of the peaks due to interferences from the matrix. Because of this suppression, a real matrix or a synthetic water can be used to make matrix-matched calibration standards to account for the suppression. Also, the use of internal standards can allow for correction in the final concentrations.

Table 1. Basic LC method setup.

Parameter	Value
Column	Metrohm A Supp 5, 4 × 150 mm
Flow Rate	0.7 mL/min
Column Oven Temperature	40 °C
Injection Volume	100 µL
Stop Time	18.2 min

Table 2. Compound transitions of each compound with MS parameters.

Compound Name	Precursor (*m/z*)	MS1 Resolution	Product (*m/z*)	MS2 Recolution	CE (V)	iFunnel Mode	Polarity
AMPA	110	Unit	79	Wide	33	Fragile	Negative
AMPA	110	Unit	63	Wide	22	Fragile	Negative
AMPA ^{13}C ^{15}N	112	Unit	63	Wide	22	Fragile	Negative
Ethephon	143	Unit	107.1	Wide	5	Fragile	Negative
Ethephon	107	Unit	79	Wide	15	Fragile	Negative
Ethephon D_4	111	Unit	79	Wide	15	Fragile	Negative
Fosetyl Al	109	Unit	81	Wide	12	Fragile	Negative
Fosetyl Al	109	Unit	63	Wide	40	Fragile	Negative
Fosetyl Al D_5	114	Unit	82	Wide	12	Fragile	Negative
Glufosinate	182	Unit	136	Wide	10	Fragile	Positive
Glufosinate	182	Unit	56	Wide	29	Fragile	Positive
Glufosinate	180	Unit	63.1	Wide	53	Fragile	Negative
Glufosinate D_3	185	Unit	56	Wide	29	Fragile	Positive
Glyphosate	170	Unit	88	Wide	5	Fragile	Positive
Glyphosate	168	Unit	63	Wide	40	Fragile	Negative
Glyphosate $^{13}C_2$ ^{15}N	173	Unit	91	Wide	5	Fragile	Positive
N-Acetyl AMPA	152	Unit	110	Wide	30	Fragile	Negative
N-Acetyl AMPA	152	Unit	63	Wide	40	Fragile	Negative
N-acetyl Glyphosate	210	Unit	149.9	Wide	12	Fragile	Negative
N-acetyl Glyphosate	210	Unit	63	Wide	37	Fragile	Negative
NAG	222	Unit	180	Wide	16	Fragile	Negative
NAG	222	Unit	63	Wide	68	Fragile	Negative
NAG D_3	225	Unit	63	Wide	68	Fragile	Negative

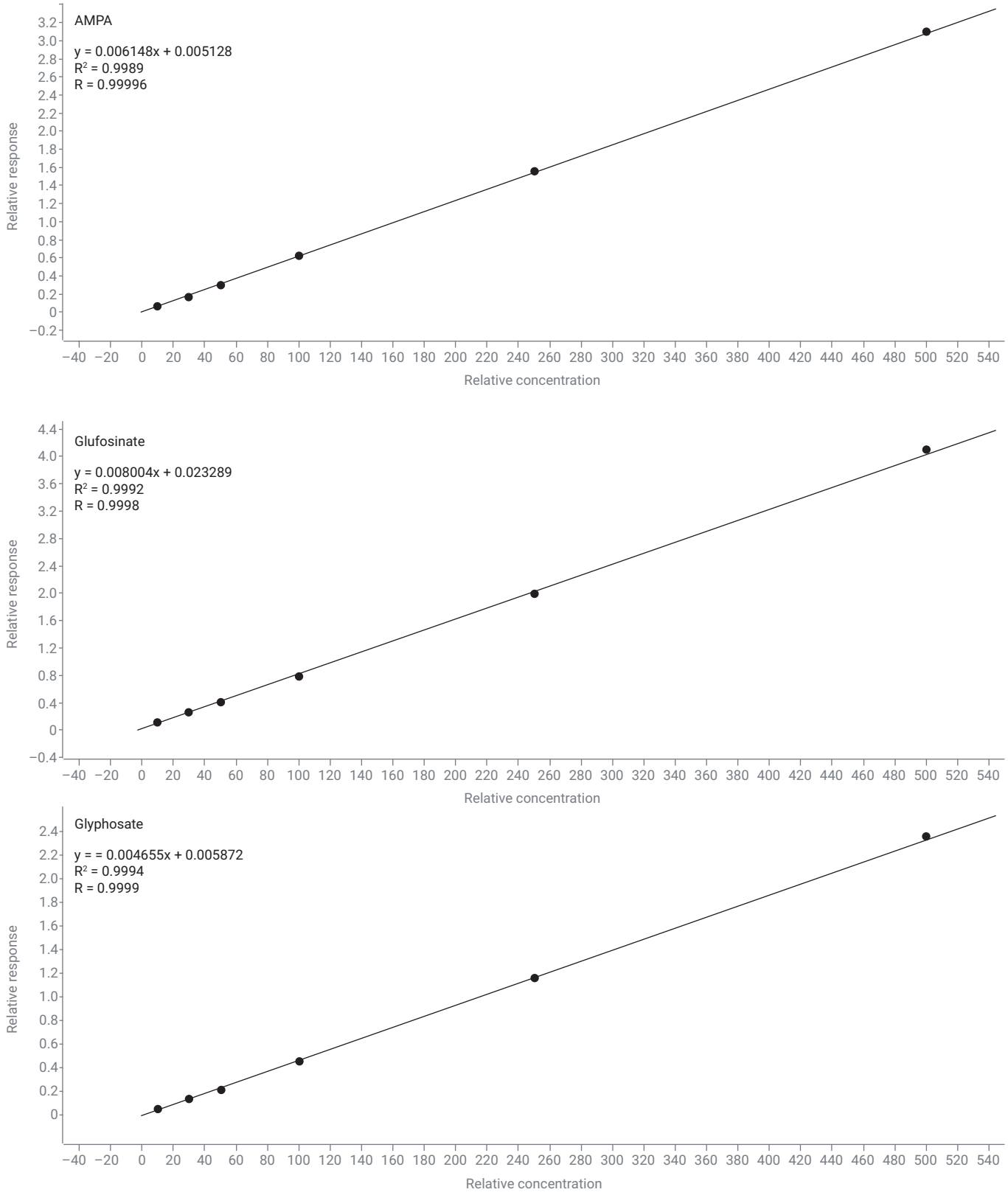

Figure 1. Calibrations for glyphosate, AMPA, and glufosinate, from 0.01 to 0.5 µg/L.

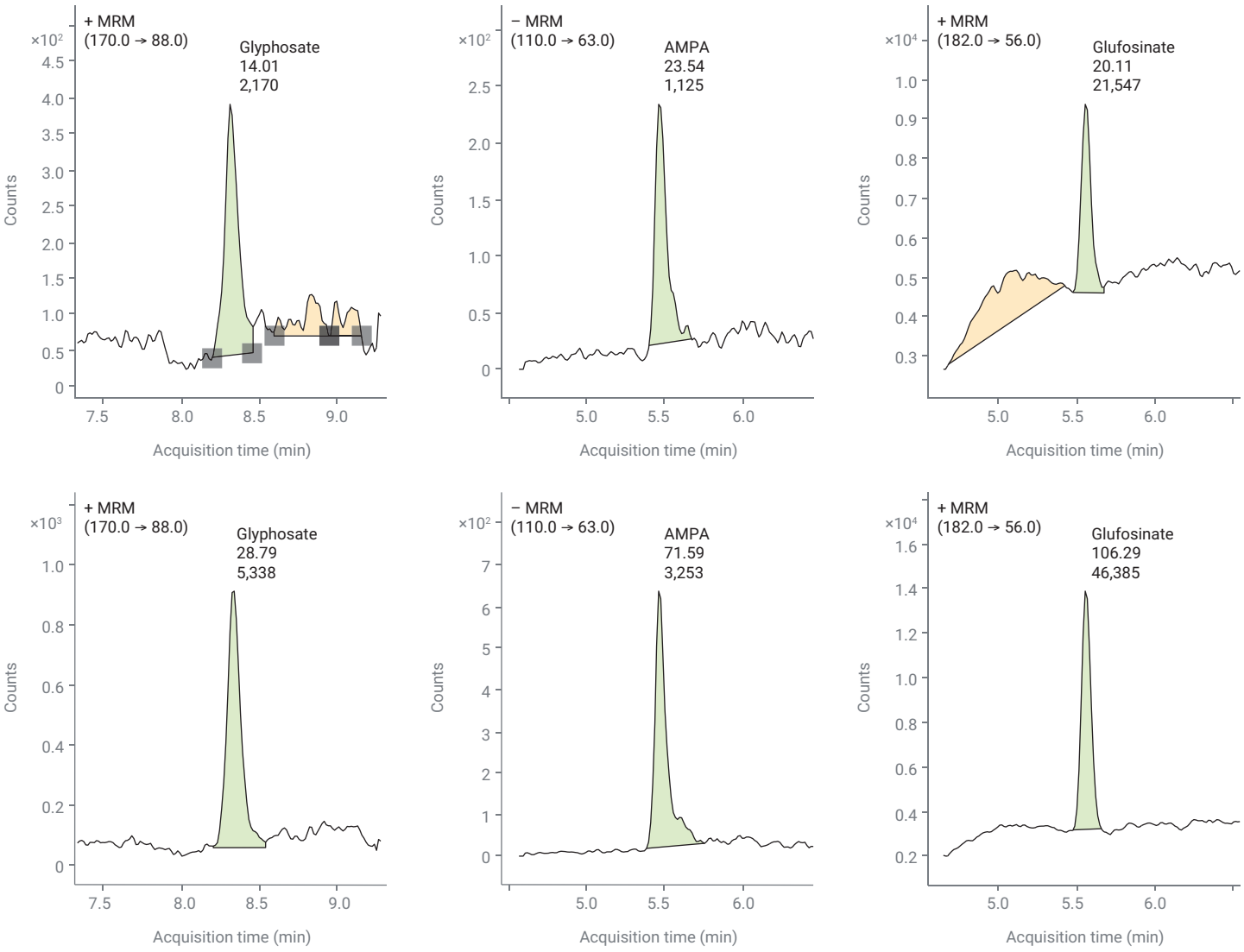

Figure 2. Chromatography at 0.01 and 0.03 µg/L for glyphosate, AMPA, and glufosinate in ultrapure water. Signal-to-noise ratio and area are listed.

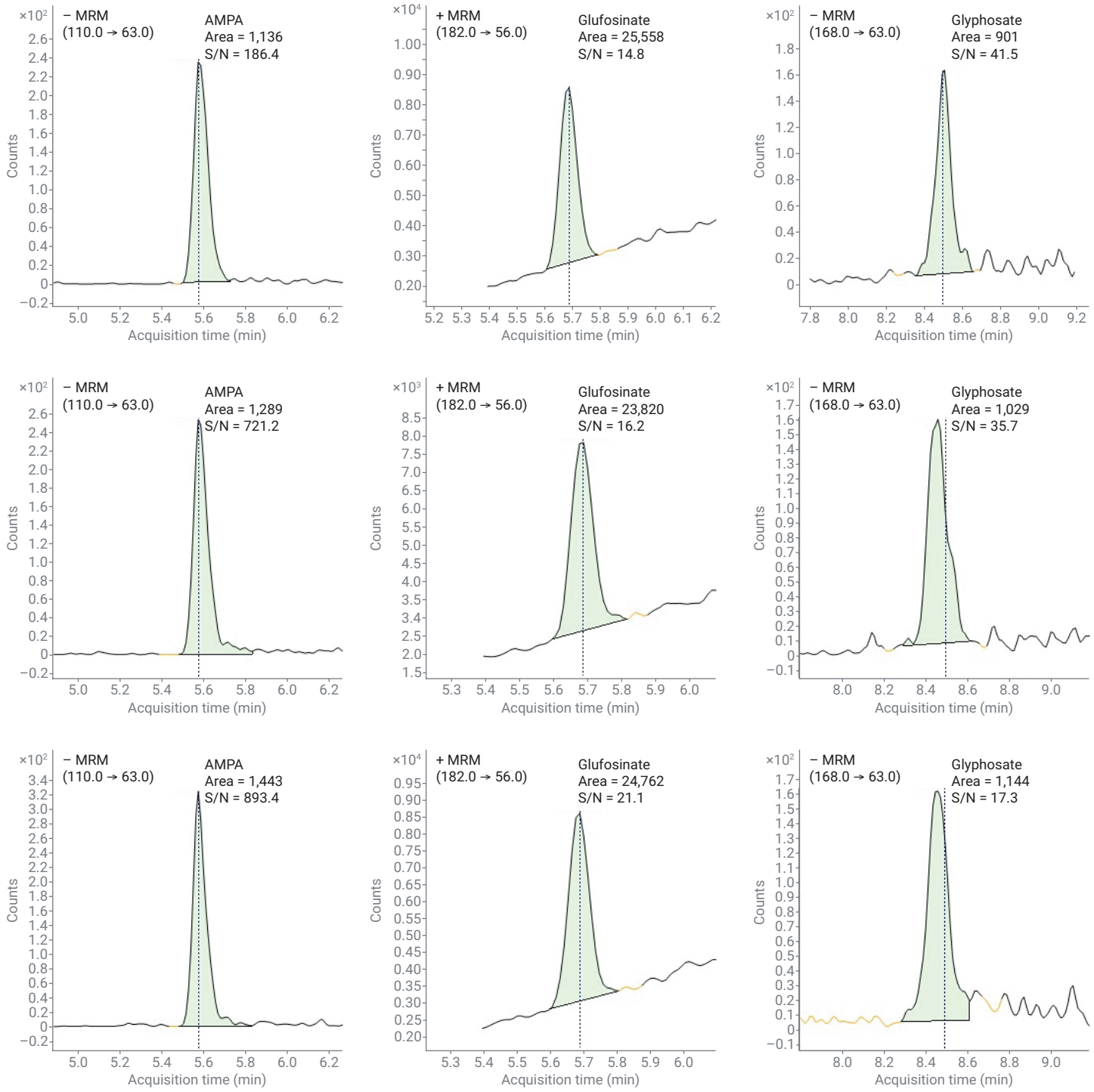

Figure 3. Table 5. Chromatography at 0.03 µg/L for glyphosate, AMPA, and glufosinate in bore hole water, river water, and drinking water. Signal-to-noise ratio and area are listed.

Instrument reproducibility and stability

A group of 200 injections of real samples were run overnight to test the long-term stability of the method. The method showed no change in sensitivity throughout the analysis and the retention times were very stable.

Due to the buffers used as the mobile phase, the spray shield was also monitored for buildup of any residue. Since the method diverts to waste when the compounds of interest are not being detected, the build up of residue on the spray shield and vacuum shield over the 200 injections, which was ~60 hours run time, was minimal.

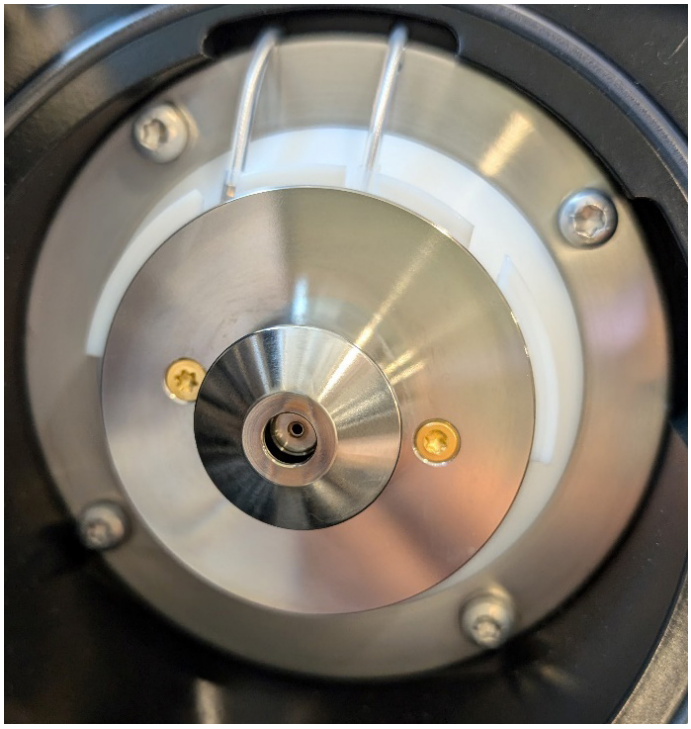

Figure 5. Spray shield after 200 injections.

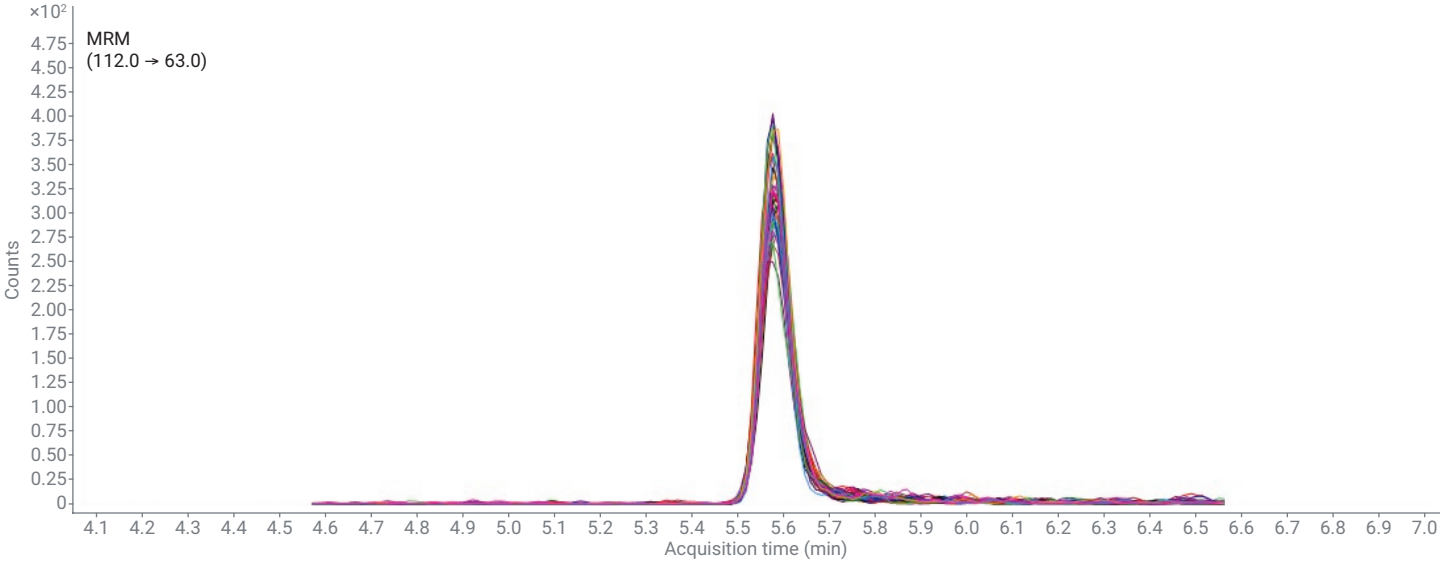

Figure 4. AMPA internal standard overlaid from 200 injections of real matrix samples.

Table 3. Data for %RSD and recoveries.

		AMPA Results		Glufosinate Results		Glyphosate Results	
		Area	Final Concentration ng/L	Area	Final Concentration ng/L	Area	Final Concentration ng/L
Standard in Milli-Q	Sample Number	12	12	12	12	12	12
	Mean	2,422.3	29.7	47,280.4	30.2	1,036.0	30.5
	SD	135.2	0.4	2,078.4	1.0	101.2	0.7
	30 ng %RSD	5.6	1.2	4.4	3.4	9.8	2.4
	30 ng % Recovery	NA	98.9	NA	100.8	NA	101.6
Ground Water	Sample Number	12	12	12	12	12	12
	Mean	1,138.3	28.2	25,544.8	29.8	1,024.3	28.5
	SD	51.9	0.4	904.1	0.6	90.0	1.5
	30 ng %RSD	4.6	1.4	3.5	1.9	8.8	5.3
	30 ng % Recovery	NA	93.9	NA	99.2	NA	95.1
Surface Water	Sample Number	12	12	12	12	12	12
	Mean	1,124.8	28.8	23,938.8	28.8	1,055.3	29.3
	SD	70.2	1.5	491.8	0.7	60.7	0.7
	30 ng %RSD	6.2	5.1	2.1	2.3	5.7	2.5
	30 ng % Recovery	NA	96.1	NA	96.1	NA	97.8
Drinking Water	Sample Number	12	12	12	12	12	12
	Mean	1,314.6	28.6	25,347.8	29.5	1,156.3	29.2
	SD	74.5	1.0	926.1	1.0	40.1	0.5
	30 ng %RSD	5.7	3.5	3.7	3.3	3.5	1.7
	30 ng % Recovery	NA	95.4	NA	98.3	NA	97.4

Conclusion

This application describes a highly sensitive and reproducible method for the fast and reliable quantitation of polar pesticides in water by direct injection. The dMRM method was created and optimized using Agilent MassHunter software and allows the addition of more MRM transitions if future development is required for additional compounds.

An Agilent 1290 Infinity II LC coupled to an Agilent 6495D LC/TQ was used for the analysis. The 18.2-minute LC gradient method using a Metrohm A Supp 5 column offered good chromatographic separation and retention time distribution of all targets. The LC/TQ data acquisition was in dMRM mode with fast polarity switching for the most efficient use of instrument cycle time. The method performance was verified based on requirements for calibration curve linearity, instrument LOD, recovery, and precision. The results demonstrate the capability of the quantitative analytical method for polar pesticides in water by direct injection. The pesticide metabolite data are listed in the Appendix.

Appendix

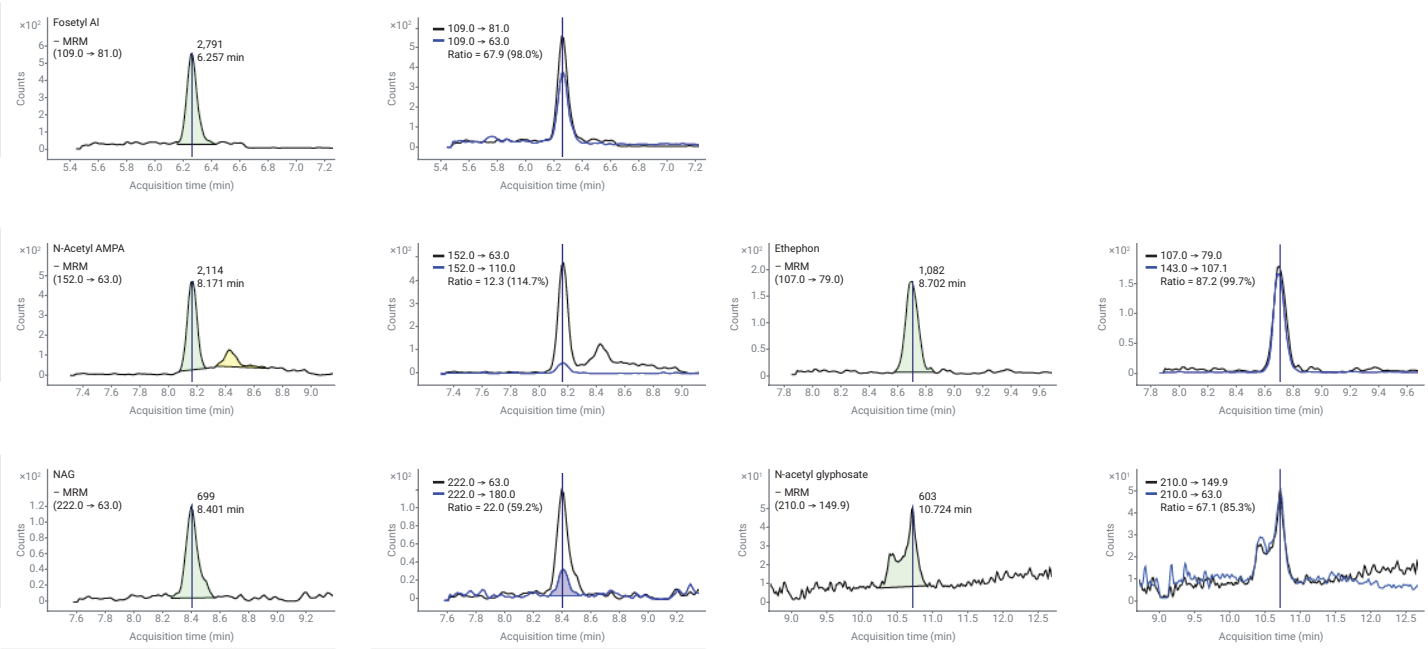

Figure A1. Chromatography at 0.03 µg/L in real matrix water (ground water).

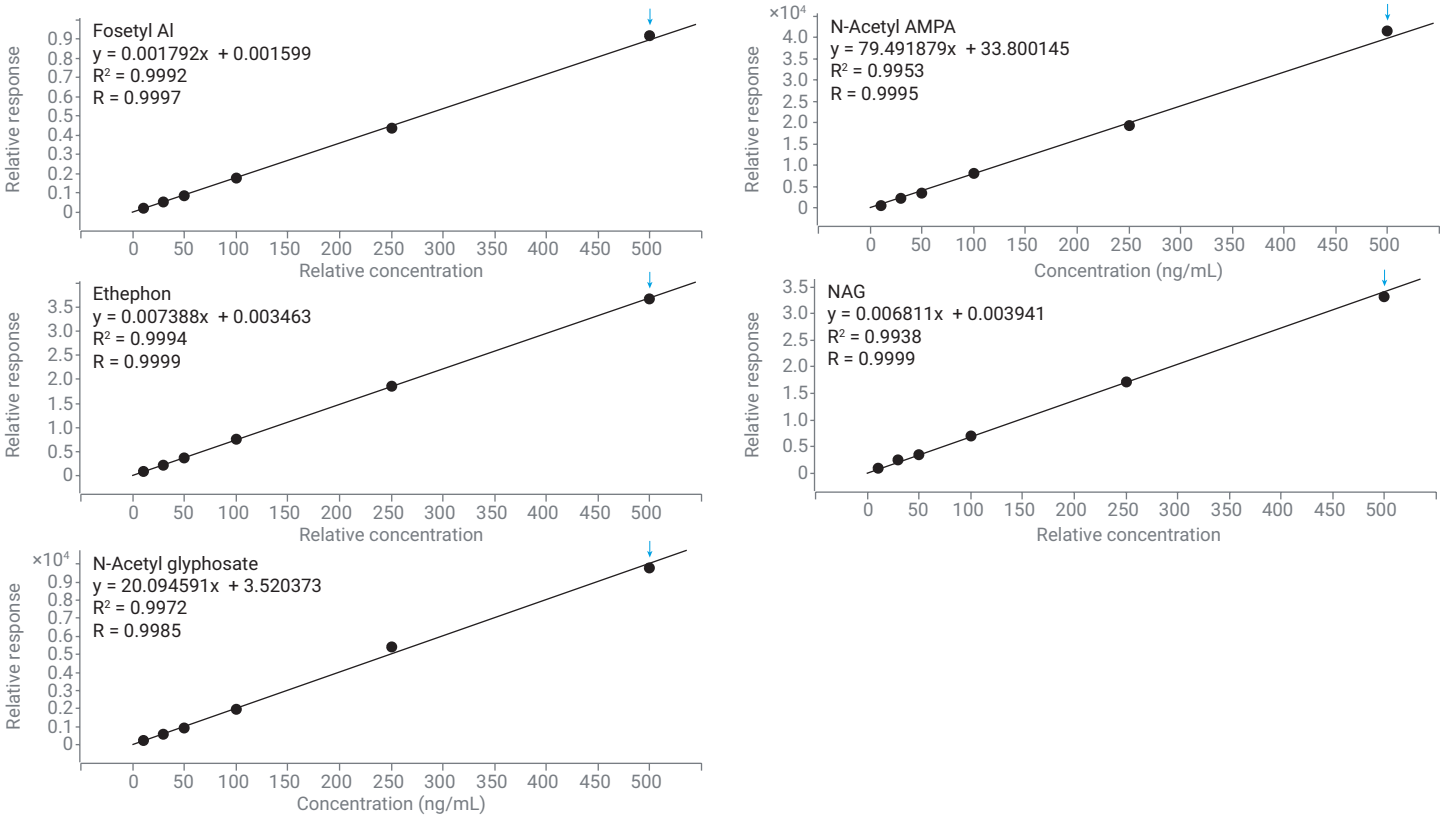

Figure A2. Calibration curves.

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