Application Note

Water Analysis

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Measurement of Underivatized Glyphosate and Other Polar Pesticides in Surface and Drinking Water

Using reversed-phase chromatography and tandem mass spectrometry

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Abstract

The accurate quantitation of glyphosate and other highly polar pesticides at nanogram per liter (ng/L) levels in surface and drinking water has proven to be challenging, given the polar nature of these compounds. A simple yet effective methodology involving liquid chromatography coupled to tandem mass spectrometry (LC/TQ) is presented here. The method includes quick and effective sample preparation without derivatization, robust reversed-phase chromatography, and extremely sensitive mass spectrometry detection for routine analysis.

Key advantages

- Method detection limit (MDL) in drinking water: 10 ng/L (ppt) for glyphosate.
- No sample dilution; the method can handle large injection volumes of aqueous samples.
- Common acidic LC/MS mobile phases; no need for a dedicated LC/TQ system.

Introduction

Glyphosate is a synthetic, broad-spectrum herbicide widely used in both agricultural and residential sectors. Glufosinate is naturally produced by plants, but is also produced synthetically on an industrial scale. Both are degraded by bacteria in plants, soil, and water, to aminomethylphosphonic acid (AMPA) and 3-(methylphosphinico) propionic acid (MPPA), respectively. The accurate quantitation of these compounds and other polar pesticides (2-hydroxyethylphosphonic acid (HEPA), N-acetyl glufosinate (NAG), Ethephon, and Fosetyl) at sub-µg/L levels in surface and drinking water has proven difficult, with two main challenges to overcome.

The first challenge arises from the polar nature of these pesticides (Figure 1), which complicates sample preparation as well as their retention on a classical reversed-phase chromatographic column. Many approaches have been proposed and developed over the years, which align these two crucial steps for accurate quantitation–sample preparation and chromatography.[1] However, most existing methodologies are based on time-consuming, low-throughput approaches that require extensive sample preparation or expert-level analysts performing them to work in a routine environment.

Another challenge comes from the affinity of highly polar pesticides for trace metal in the HPLC flow path, which results in tailing peaks.[2] Trace metal can be found in multiple components within the flow path (mobile phases, glass containers, stainless steel (SST) tubing, etc.), and is difficult to remove completely and for good. Once again, multiple approaches have been used to contain trace metal in HPLC systems (passivation, chelation, the use of higher pH mobile phases, etc.), with different levels of success.

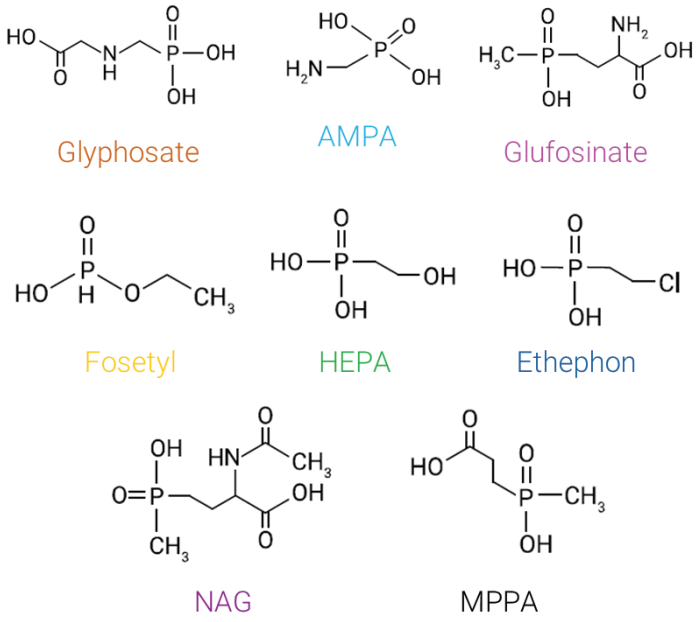

Figure 1. Analyte structures.

A simple, yet effective, methodology that overcomes these two challenges is presented in this work, encompassing quick sample preparation, robust reversed-phase chromatography, and sensitive mass spectrometry detection for routine analysis.

Experimental

Chemicals and reagents

- **Glyphosate:** Sigma-Aldrich (part number 45521-250MG)
- **AMPA:** Sigma-Aldrich (part number 324817-250MG)
- **Glufosinate-ammonium:** Sigma-Aldrich (part number 45520-100MG)
- **Fosetyl aluminum:** Toronto Research Chemicals Inc (part number F727450)
- **HEPA:** Toronto Research Chemicals Inc (part number H939650)
- **Ethephon:** Toronto Research Chemicals Inc (part number C366175)
- **NAG:** Toronto Research Chemicals Inc. (part number A178235)
- **MPPA:** Sigma-Aldrich (part number 31264-100MG)
- **Formic acid:** Agilent Technologies (part number G2453-85060)
- **Methanol:** Agilent Technologies (part number 5191-4497)
- **Agilent InfinityLab deactivator additive:** (Agilent Technologies part number 5191-4506)

Sample preparation

- Surface water samples were collected in 15 mL polypropylene (PP) tubes. The tubes were then centrifuged at 5,000 rpm for 5 minutes. Approximately 1.5 mL of each supernatant was then loaded into plastic syringes mounted with Agilent 0.2 µm polyethersulfone (PES) filters (part number 5190-5096) and filtered into a 1.5 mL PP tube.
- Drinking water samples were collected directly in plastic syringes mounted with Agilent 0.2 µm PES filters (part number 5190-5096) and filtered into a 1.5 mL PP tube.

Next, 999 µL of each filtrate was pipetted into a 2 mL HPLC vial and acidified with 1 µL of concentrated formic acid, for a final concentration of 0.1% formic acid. Each vial was then capped and briefly vortexed.

Standard preparation

Individual stock solutions of AMPA, Glyphosate, Glufosinate, MPPA, HEPA, NAG, Fosetyl, and Ethephon were prepared in 15 mL polypropylene tubes by diluting 10 mg of each standard in 10 mL of ultrapure (Type 1) water.

Table 1. Preparation of matrix matched calibrators following a serial dilution approach.

Calibrant Level	Pesticide Concentration (µg/L)
13	10
12	5
11	2.5
10	1
9	0.75
8	0.5
7	0.25
6	0.1
5	0.075
4	0.05
3	0.025
2	0.01
1	0.005

Calibration curves were matrix-matched with acidified (0.1% formic acid) surface or acidified drinking water. Blank matrix was prepared in sufficient volumes as described in the sample preparation section. This blank matrix was then also used to prepare a common pesticide stock (1,000 µg/L) in a 1.5 mL PP tube by mixing 1 µL of each of the eight individual pesticide stocks with 992 µL blank matrix. An extra common stock of 100 µg/L was later prepared in the same blank matrix, then 13 calibrants ranging from 0.005 to 10 µg/L were prepared following a serial dilution approach. Labs can reduce calibration points based on their individual QA/QC requirements.

Instrument conditions

- Agilent 1290 Infinity II high speed pump G7120A
- Agilent 1290 Infinity II multisampler G7167B with tray cooling option
- Agilent 1290 Infinity II integrated column compartment G7116B

Table 2. UHPLC conditions.

UHPLC Method Parameters			
Run Time	8 min		
Re-equilibration Time	12 min		
Column	Agilent InfinityLab Poroshell 120 CS-C18, 2.1 × 150 mm, 2.7 µm (p/n 693775-942)		
Mobile Phase A	0.1% Formic acid + 5 µM Agilent InfinityLab deactivator additive in ultrapure water		
Mobile Phase B	0.1% Formic acid in methanol		
Injection Volume	25 µL		
Needle Wash	0.1% Formic acid in methanol in flush port for 3 seconds		
Multisampler Temperature	4 °C		
Column Temperature	40 °C		
Flow Rate	0.35 mL/min		
Gradient	Time (min) 0 1.5 2 4 4.1 8	% Mobile Phase A 99.9 99.9 80 60 0 0	% Mobile Phase B 0.1 0.1 20 40 100 100

Table 3. Mass spectrometry parameters.

Agilent 6470A Triple Quadrupole LC/MS				
Acquisition Mode	Dynamic MRM (dMRM)			
Ion Source	Agilent Jet Stream ESI			
Polarity	Positive (+) or Negative (–)			
Capillary Voltage	3,000 V (+), 3,500 V (–)			
Drying Gas Flow	11 L/min			
Drying Gas Temperature	220 °C			
Nebulizer Pressure	30 psi			
Sheath Gas Temperature	300 °C			
Sheath Gas Flow	11 L/min			
Nozzle Voltage	1,500 V (+), 800 (-)			
Q1 and Q2 Resolution	Unit (0.7 amu), optimized by Autotune			
Delta EMV	0 V			
MRM Transitions	**Compound**	**Quantifier**	**Qualifier 1**	**Qualifier 2**
	AMPA	112 → 30 (+)	110 → 79 (–)	110 → 63 (–)
	Glufosinate	182 → 56 (+)	182 → 136 (+)	
	Glyphosate	170 → 88 (+)	170 → 60 (+)	170 → 42 (+)
	HEPA	125 → 79 (–)	127 → 81 (+)	127 → 109 (+)
	MPPA	153 → 79 (+)	153 → 135 (+)	
	NAG	224→ 56 (+)	224→ 164 (+)	224 → 136 (+)
	Ethephon	145 → 63 (+)	145 → 91 (+)	143 → 107 (–)
	Fosetyl	109 → 81 (–)	111 → 83 (+)	111 → 65 (+)

Results and discussion

Analytical chemists need the three pillars of sample preparation, chromatography, and mass spectrometry to be optimally aligned to achieve very low detection levels. This alignment has always been a challenge for polar pesticides like glyphosate. Numerous approaches have been proposed over the years, but the methodology presented in this application note presents a favorable alignment between the three pillars.

First, using this method, starting materials of aqueous nature are either not or minimally diluted, thereby enabling lower detection limits. Avoiding lengthy treatments of large volumes of starting materials helps to reduce labor and consumable costs while boosting throughput capacity. Derivatization is also not required, which reduces the use of environmentally hazardous solvents, provides time savings, and increases ease of implementation.

Second, the aqueous nature of the matrices is a perfect fit for the Agilent InfinityLab Poroshell 120 CS-C18 column, which was developed using a bonded phase chemistry. This phase was created by applying a positive charge to the silica surface and then functionalizing the particle with a C18 bonded phase, thereby offering retention capacity for highly polar, ionizable molecules. The result is a column that is totally compatible with aqueous mobile phase conditions and samples. Moreover, this compatibility enables the injection of large volumes of aqueous samples without sacrificing peak shape. The use of one of the most common LC/MS conditions (0.1% formic acid) additionally makes for an easy transition from one application to another on the same LC/MS system without the need for a dedicated system or lengthy rinsing and conditioning. Importantly these conditions also allow the use of positive mode transitions for most pesticides, thereby increasing sensitivity.

Third, the potential presence of trace metal in the UHPLC flow path, detrimental to the peak shape of phosphonate-containing compounds, is addressed in two ways:

– The incorporation of 5 µM InfinityLab deactivator additive (Figure 3) in the mobile phase system provides constant trace metal-chelating that is not detrimental to positive- or negative-mode ionization and does not accumulate in the mass spectrometer ion path.

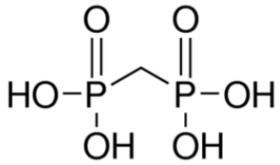

Figure 3. Structure of the main ingredient in the Agilent InfinityLab deactivator additive.

– The removal of most SST in the flow path from the sample's point of entry to the mass spectrometer. PEEK-lined tubing (part number G5667-81005) was used to replace the standard SST tubing between the injection valve of the multisampler and the column inlet. This tubing was connected to both the injection valve and the column with Agilent Quick Turn fittings (part number 5067-5966), which use polymeric ferrules.

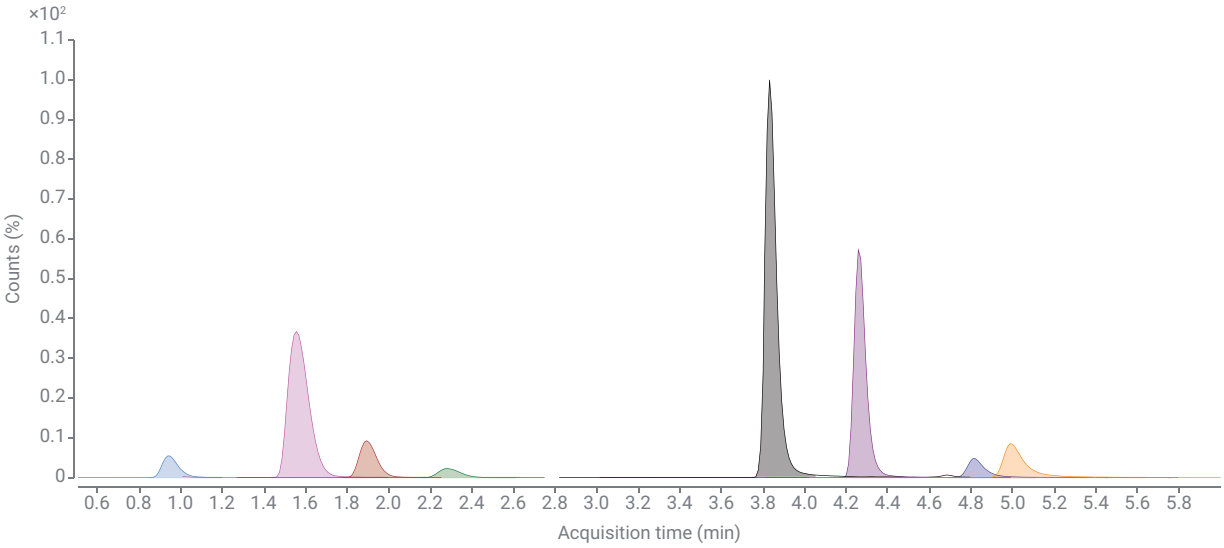

Figure 2. Typical chromatogram for the eight pesticides at 10 µg/L. Retention times are as follows: AMPA = 0.95 minutes, Glufosinate = 1.6 minutes, Glyphosate = 1.9 minutes, HEPA = 2.3 minutes, MPPA = 3.8 minutes, NAG = 4.3 minutes, Ethephon = 4.8 minutes, Fosetyl = 5.0 minutes.

Lastly, the 6470 triple quadrupole LC/MS enables the acquisition of positive and negative polarity signals for the same compound, in the same dynamic MRM window. Depending on the matrix, a given compound may show better sensitivity (higher signal or lower noise) either in positive or negative polarity, thereby making this a versatile approach for quantitation. The highly sensitive 6470 also provides highly reproducible peak areas, even in the low ng/L (ppt) range, as can be seen in the Method Detection Limit (MDL) calculations for glyphosate.

The highlights of the results obtained using drinking water from the Montreal, Canada city network and surface water collected from the St.Lawrence river (Montreal, Canada), are shown in Table 4.

Table 4. Calibration results (each calibrator was injected in triplicate).

Compound	Curve Type	Drinking Water			River Water		
		Linearity Range (ng/L)	Number of Calibrator Levels	R^2	Linearity Range (ng/L)	Number of Calibrator Levels	R^2
AMPA	Linear	100 to 10,000	8	0.9993	100 to 10,000	8	0.9993
Glufosinate	Quadratic	25 to 10,000	11	0.9998	25 to 10,000	11	0.9998
Glyphosate	Linear	25 to 10,000	11	0.9995	25 to 10,000	11	0.9997
HEPA	Linear	50 to 10,000	10	0.9995	50 to 10,000	10	0.9994
MPPA	Linear	50 to 10,000	10	0.9991	50 to 10,000	10	0.9986
NAG	Linear	10 to 10,000	12	0.9986	10 to 10,000	12	0.9984
Ethephon	Linear	25 to 10,000	11	0.9990	25 to 10,000	10	0.9989
Fosetyl	Linear	10 to 10,000	12	0.9996	10 to 10,000	11	0.9996

Calibration curves and linearity range

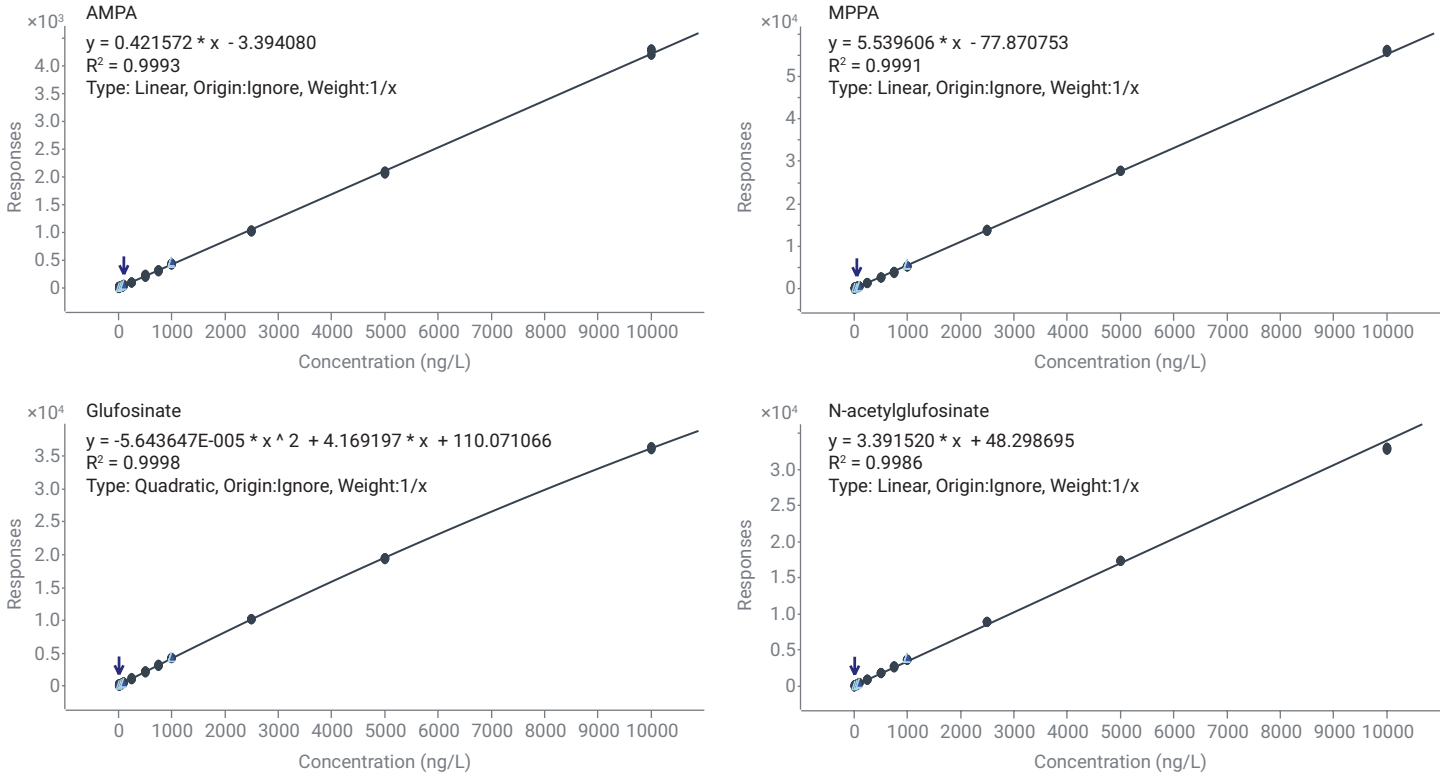

Figure 4. Typical calibration curves for each pesticide (continued on next page).

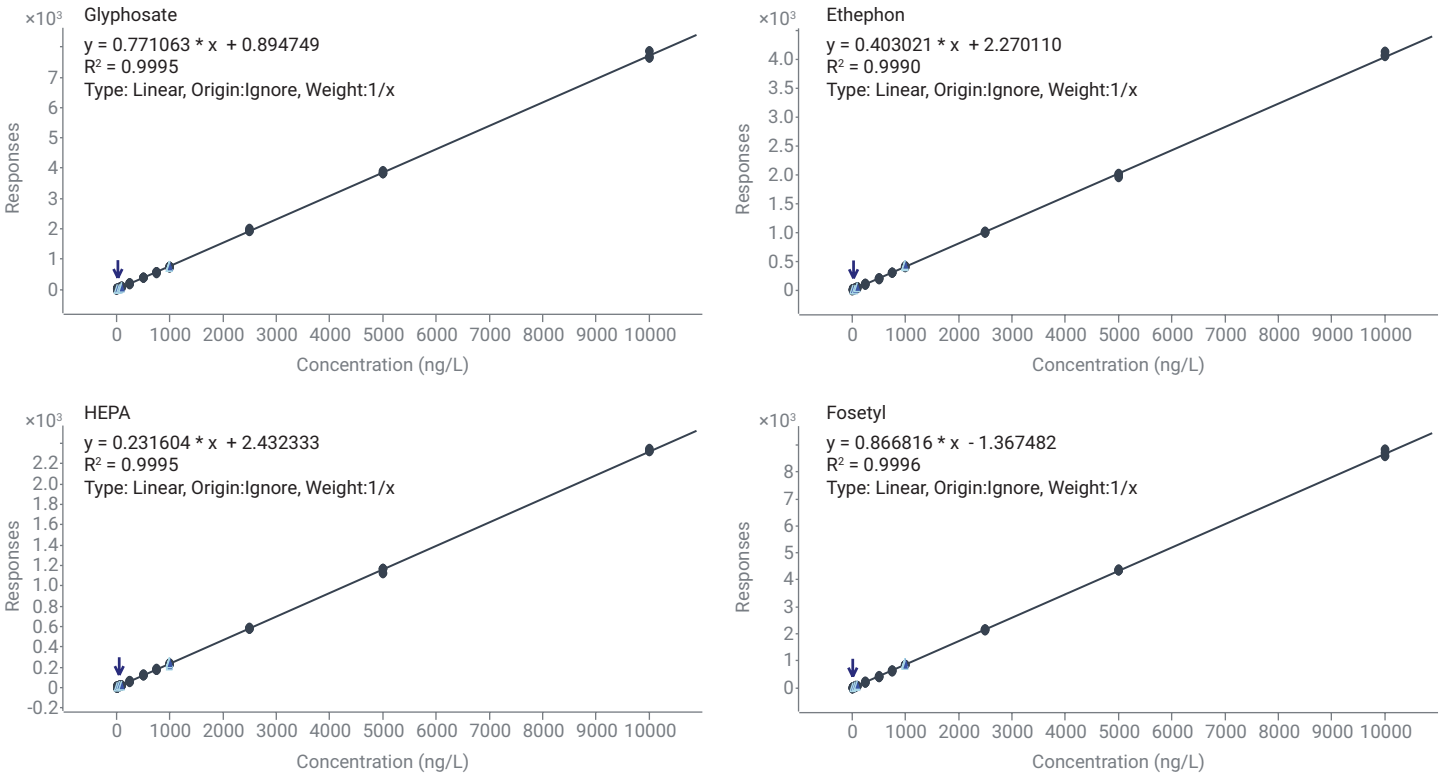

Figure 4. Typical calibration curves for each pesticide (continued from previous page).

Accuracy and chromatograms at the limit of quantitation

Table 5. Calibration results (each calibrator was injected in triplicate).

	Drinking Water		River Water	
Compound	LOQ (ng/L)	Accuracy at LOQ (Averaged from Three Replicates)	LOQ (ng/L)	Accuracy at LOQ (Averaged from Three Replicates)
AMPA	100	102.9%	100	87.6%
Glufosinate	25	107.2%	25	85.6%
Glyphosate	25	96.8%	25	108.6%
HEPA	50	110.9%	50	105.8%
MPPA	50	120.3%	50	118.9%
NAG	10	82.6%	10	93.4%
Ethephon	25	92.5%	50	121.7%
Fosetyl	10	108.9%	25	96.6%

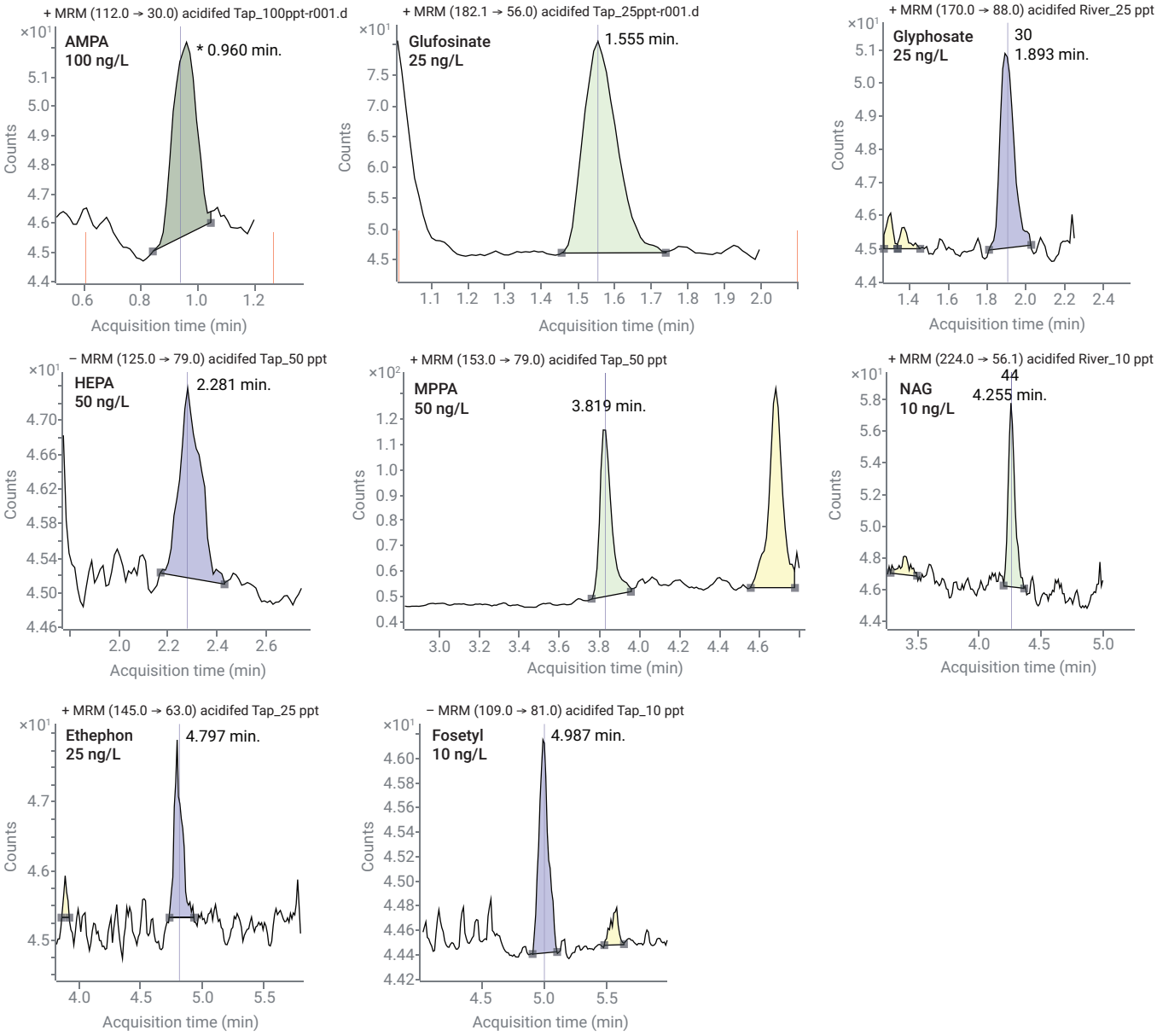

Figure 5. Typical chromatograms for each pesticide at its respective LOQ.

Glyphosate MDL

The calculation of an MDL is based on the reproducibility statistics for a series of replicate injections, determining the on-column concentration where one is 99% confident a sample is unambiguously and reproducibly distinguished from baseline noise following EPA guidelines.[3] The glyphosate MDL was calculated with the following formula, using four distinct 10 ng/L calibrants on four separate days and four different columns from four different lots:

MDL = (Concentration on column) × (%RSD/100) × (t-statistic)

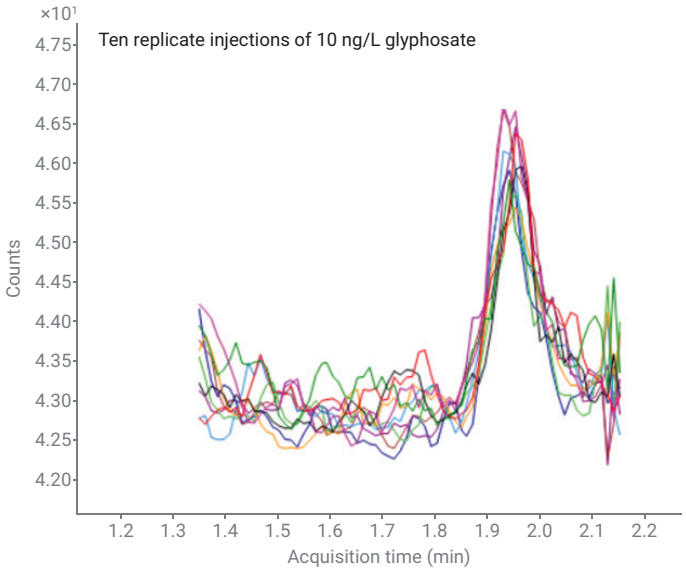

Figure 6. Ten overlaid replicate injections of a glyphosate calibrant at 10 ng/L.

Table 6. MDL calculations for glyphosate.

			Day1	Day2	Day3	Day4
		User Input Injection Units	**User Input Sample Amount (Concentration or Amount On-Column)**			
		ng/L (on-column)	10	10	10	10
		Replicate number	**User input response (no manual integration)**			
Minimum	Recommended	Replicate1	13	15	20	11
		Replicate2	19	30	15	12
		Replicate3	15	26	25	10
		Replicate4	17	14	16	15
		Replicate5	16	20	18	11
		Replicate6	16	13	11	15
		Replicate7	29	25	10	12
		Replicate8	23	15	10	11
		Replicate9	14	22	14	7
		Replicate10	21	17	16	9
		Calculated parameters				
		%RSD (CV)	26.7%	29.7%	30.5%	21.7%
		Critical t-value (t)	2.821	2.821	2.821	2.821
		MDL (ng/L)	7.5	8.4	8.6	6.1

Conclusion

The workflow presented in this application note offers an Agilent solution for the analysis of underivatized glyphosate and seven other polar pesticides in surface and drinking water, with well-aligned sample preparation, chromatography, and mass spectrometry.

The potential presence of trace metal within the flow path, responsible for peak tailing of polar compounds, was minimized with the addition of the Agilent InfinityLab deactivator additive in the mobile phase and the use of PEEK-lined tubing between the multisampler and the column.

The Agilent InfinityLab Poroshell 120 CS-C18 column uses a novel reversed-phase packing; it is resistant to large injection volumes of aqueous extracts and offers good retention of these polar compounds in acidic conditions without sacrificing peak shape.

The Agilent 6470 triple quadrupole LC/MS offers great sensitivity, reproducibility, and linearity, all suitable for the quantitative analysis of these challenging analytes in surface and drinking water.

Acknowledgments

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References

1. Zweigenbaum, J.; Lucas, D.; Cali, A. Direct Analysis of Glyphosate, AMPA, and Other Polar Pesticides in Food. *Agilent Technologies application note*, publication number 5994-0796EN, **2019**.
2. Hsiao, J. J. *et al.* Improved LC/MS Methods for the Analysis of Metal-Sensitive Analytes Using Medronic Acid as a Mobile Phase Additive. *Analytical Chemistry* **2018**, *90(15)*, 9457–9464.
3. US EPA, Procedures for Detection and Quantitation – Documents. https://www.epa.gov/cwa-methods/procedures-detection-and-quantitation-documents

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