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Measurement of Underivatized Glyphosate and Other Polar Pesticides in Multiple Matrices Using Reversed-Phase Liquid Chromatography and Tandem Mass Spectrometry

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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-acetylglufosinate (NAG), Ethephon, Fosetyl) at sub-µg/L levels in surface water, and low-µg/L levels in other matrices, has proven difficult.

The first challenge associated with the aforementioned compounds arises from their very polar nature (Figure 1), which renders them incompatible with classical reversed-phase chromatography. The second challenge comes from their affinity for trace metal in the HPLC flow path, which results in tailing peaks[1].

A simple, yet effective, methodology overcoming those two challenges will be presented encompassing quick sample preparation, very robust reversed-phase chromatography and sensitive mass spectrometry detection for routine analysis.

Experimental

The need for very low detection levels calls for optimized alignment between sample preparation, chromatography, and mass spectrometry.

Sample Preparation

Water samples

Samples were simply centrifuged, then filtered on a 0.2µm polyethersulfone (PES) membrane, and finally acidified with concentrated formic acid to 0.1%.

Wine samples

Samples were simply diluted 10-fold with 0.1% formic acid in nanopure water.

Honey samples

0.5g of honey was weighed in a 15mL tube, then 5mL of 0.5% formic acid in nanopure water was added, and the tube was vortexed for 30 min. A portion of the solution was then filtered on a 0.2µm polyethersulfone (PES) membrane, and 100µL of the filtrate was diluted with 900µL of 0.1% formic acid in nanopure water.

Experimental

Chromatography

Standard Infinity II 1290 LC modules were employed (high-speed binary pump, multisampler, column thermostat).To minimize peak tailing, acidic mobile phase conditions were used along with the InfinityLab Deactivator Additive (p/n 5191-4506) and a PEEK-lined stainless steel capillary (p/n G5667-81005) between the multisampler and the column.

A PEEK-lined prototype column built with a new superficially porous reversed phase packing (maximum pressure: 600 bar) was used under the following conditions:

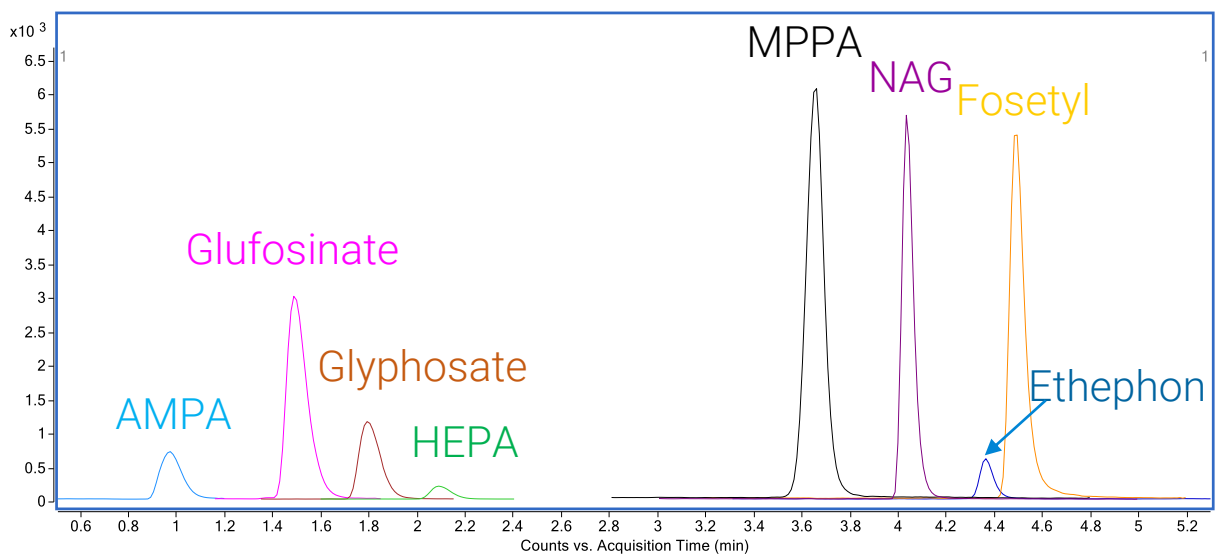
LC Conditions		
Column Temp	40°C	
Injection Vol	25µL	
Flow Rate	0.35mL/min	
Run Time	8min	
Mobile Phase A	0.1% formic acid and 5µM deactivator additive in water	
Mobile Phase B	0.1% formic acid in methanol	
Gradient	Time (min) 0 1.5 2 4 4.1 8	% B 0.1 0.1 20 40 100 100

Mass Spectrometry

An Agilent 6470A Triple Quadrupole LC/MS System was used with dual polarity detection using the following conditions:

MS and Source Conditions	
Acquisition Mode	Dynamic MRM
Ion Source	Agilent Jet Stream ESI
Drying Gas Temp	220°C
Drying Gas Flow	11L/min
Nebulizer Pressure	30psi
Capillary Voltage	3000V (+), 3500V (-)
Sheath Gas Temp	300°C
Sheath Gas Flow	11L/min
Nozzle Voltage	1500V (+), 800V (-)

Results and Discussion



Compound	Retention Time	Polarity of MRM Transitions
AMPA	0.97 min	(+) and (-)
Glufosinate	1.49 min	(+)
Glyphosate	1.79 min	(+)
HEPA	2.09 min	(+) and (-)
MPPA	3.66 min	(+)
NAG	4.03 min	(+) and (-)
Ethephon	4.36 min	(+) and (-)
Fosetyl	4.49 min	(+) and (-)

Figure 1: Typical chromatogram observed after spiking each of the 8 analytes at 5µg/L in water, with tabulated compound information.

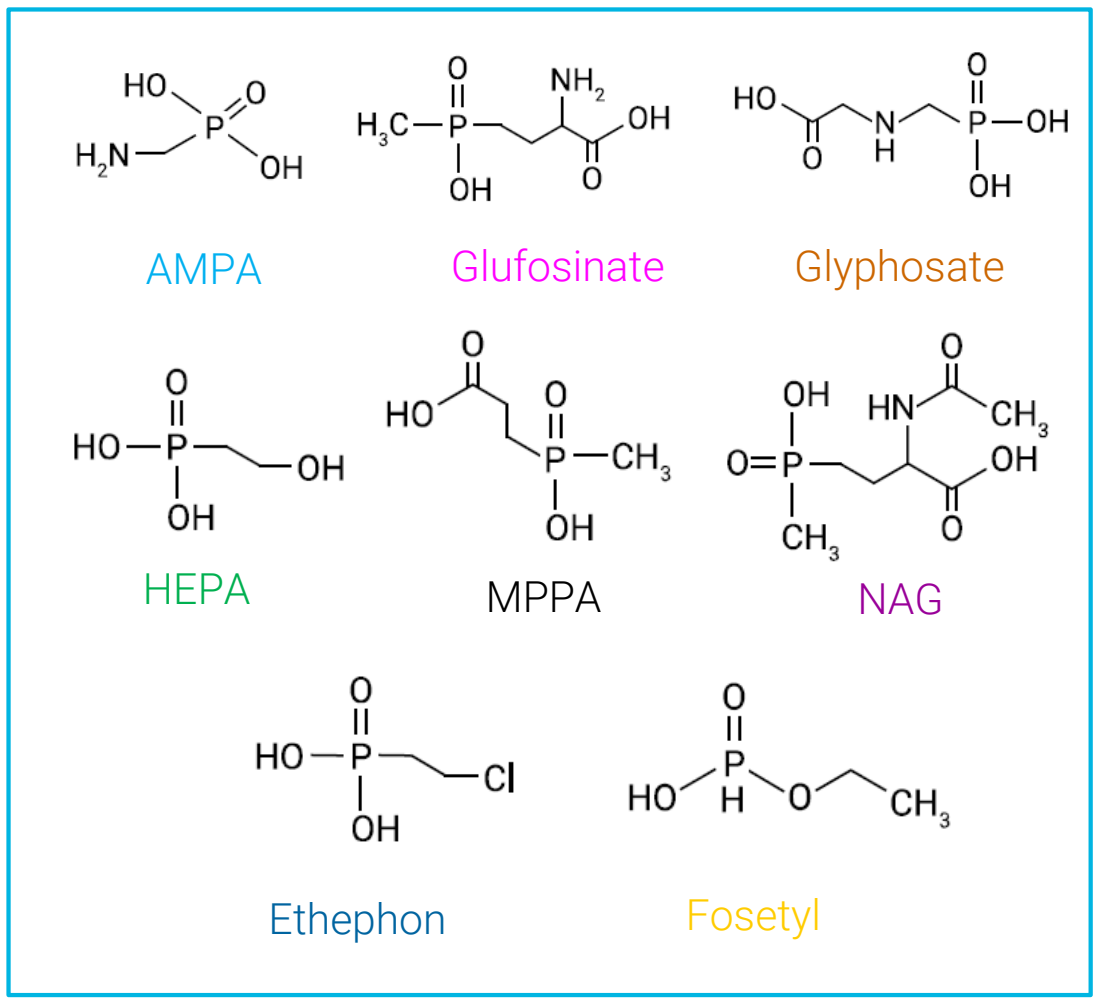


Figure 2: Analyte structures

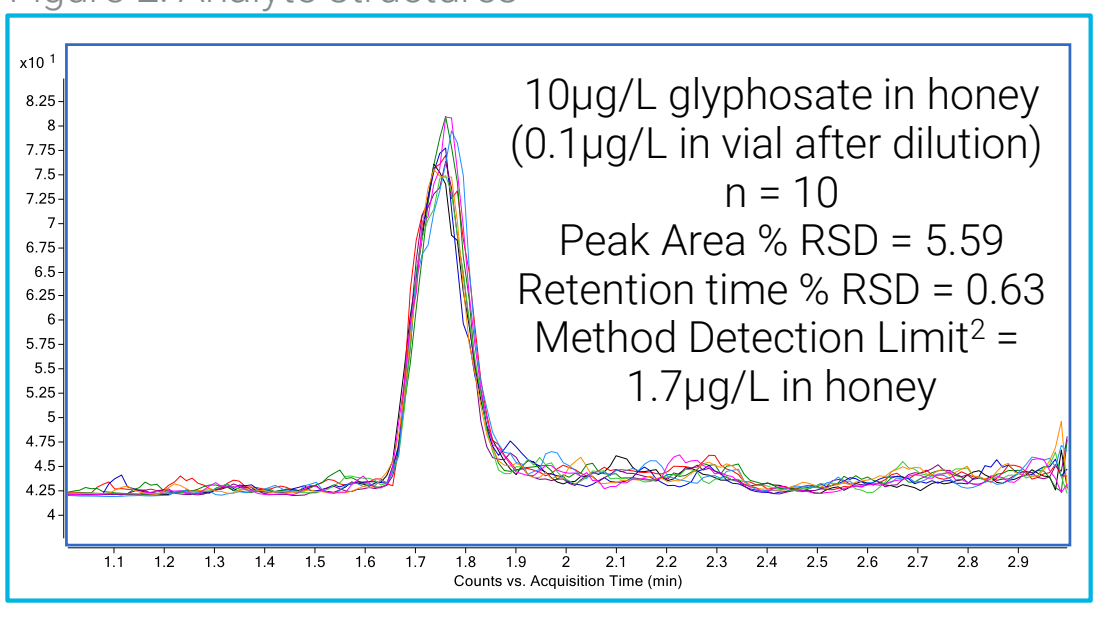


Figure 3: 10 overlaid replicate injections of glyphosate spiked at 10µg/L in honey (0.1µg/L in vial)

Sample Preparation Highlights

- Sample preparation is minimal, very fast and fully aligned with chromatographic and mass spectrometry conditions.
- Starting materials are either not or minimally diluted, which enables better sensitivity.

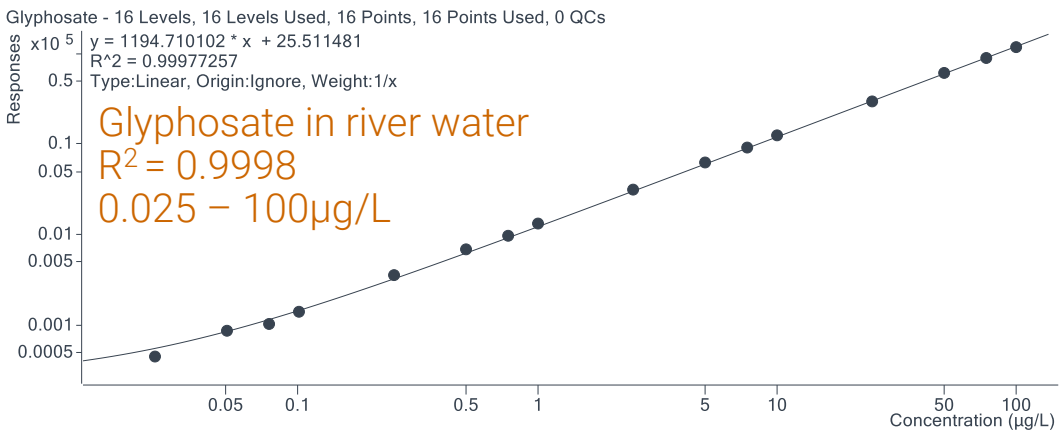
Chromatographic Highlights

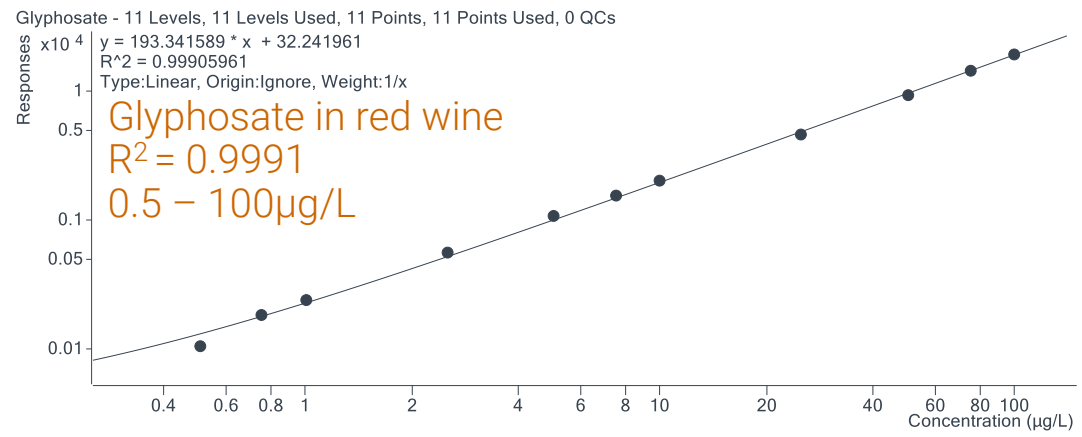
- The column is compatible with near 100%-aqueous conditions, which is ideal for the separation of highly polar molecules with strong solubility in water.
- The gradient was optimized to balance retention, separation, and capacity for high throughput.
- A large injection volume (25µL) is possible due to the aqueous extracts that are fully compatible with the mobile phase system at injection time.
- Peak shape for all compounds is maintained through the concentration range.
- No peak tailing is observed, helped by the PEEK flow path and the Deactivator additive.
- The large injection volume enables lower limits of quantitation.

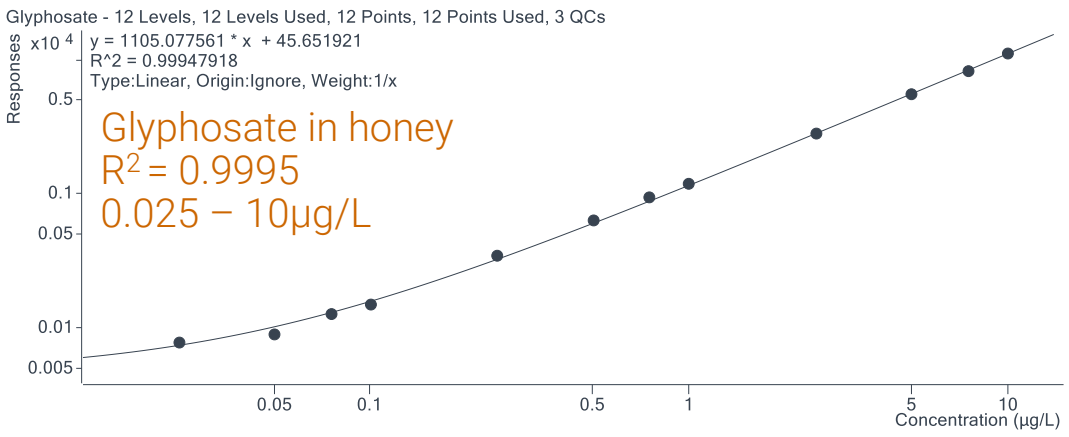
Mass Spectrometry Highlights

- The 6470 LC/TQ enables the acquisition of positive and negative polarity signals for the same compound, in the same Dynamic MRM windows.
- 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 peak areas are highly reproducible even in the ppt range.

Results and Discussion







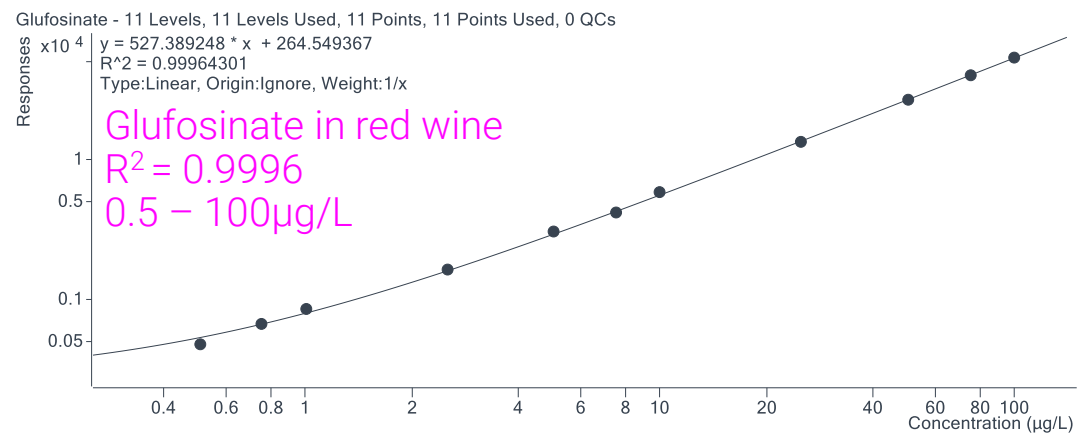


Figure 4: Matrix-matched calibration curves for glyphosate in river water, honey and red wine, and for glufosinate in red wine. All axes are in logarithmic scale for display purposes. Regressions are calculated for a linear fit with a 1/x weighing. Concentration ranges are in-vial; equivalent to starting material concentration of 0.025 – 100µg/L for river water, 2.5 – 1000µg/L for honey, and 5 – 1000µg/L for red wine.

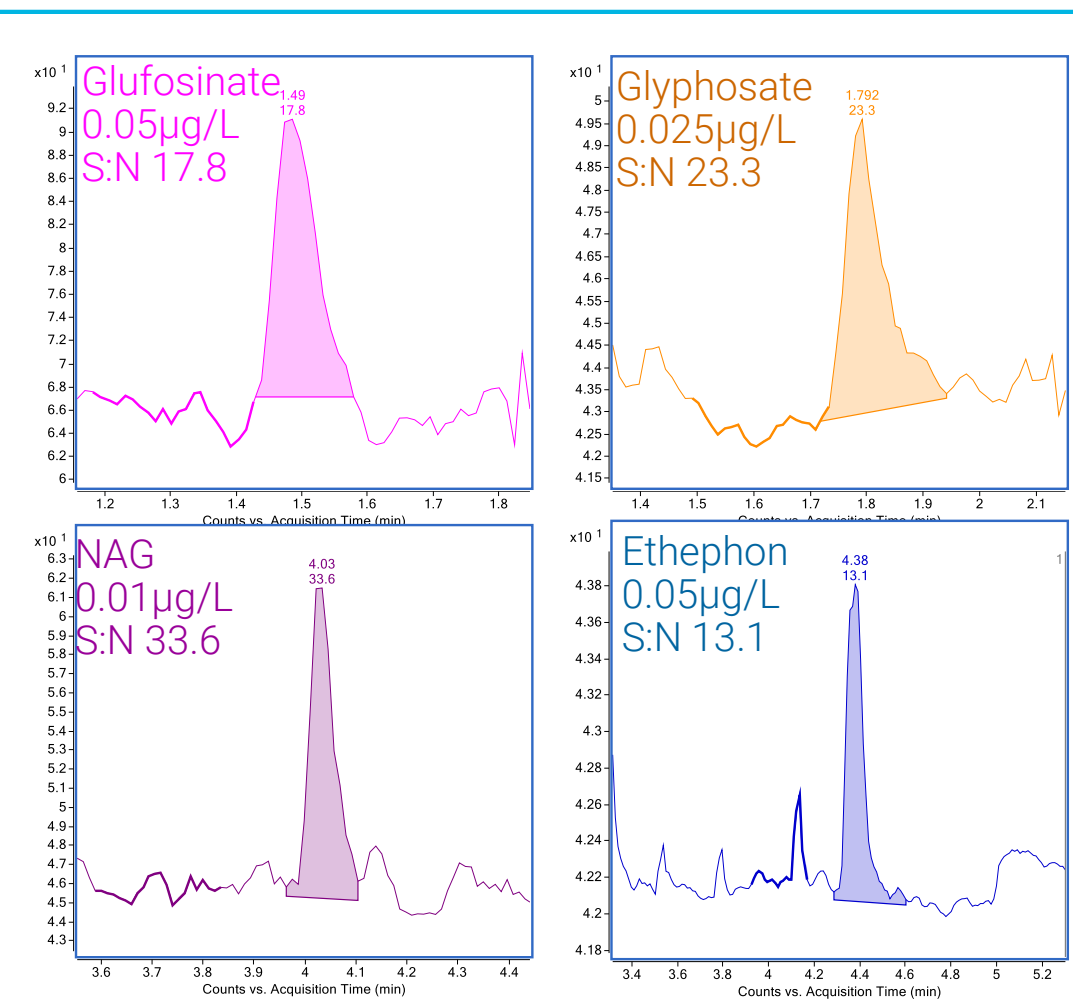


Figure 5: Examples of sub-µg/L chromatograms for 4 analytes in water. Signal-to-noise (S:N) ratios were calculated with Auto-RMS noise definition, with (bold) noise region of 0.25 min.

Conclusions

- This methodology offers a solution for the analysis of underivatized glyphosate and seven other polar pesticides with well-aligned sample preparation, chromatography and mass spectrometry.
- The usage of a PEEK-lined flow path along with the Deactivator Additive results in non-tailing peaks.
- The 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 without sacrificing peak shape nor retention time stability.
- The Agilent 6470A Triple Quadrupole LC/MS System demonstrated great sensitivity, reproducibility and linearity, all suitable for the quantitative analysis of the analytes in water, wine, and honey matrices.

Reference

[1]Jordy J. Hsiao et al., *Anal. Chem.* 2018, 90, 9457−9464

[2]https://www.epa.gov/sites/production/files/2016-12/documents/mdl-procedure_rev2_12-13-2016.pdf

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