

Measurement of Underivatized Glyphosate and Other Polar Pesticides in Aqueous Matrices Using LC-MS/MS

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Agilent Technologies

Glyphosate

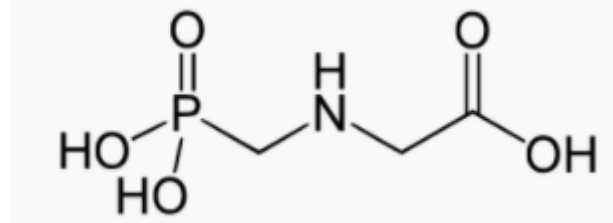
- Broad-spectrum herbicide first patented in the 1970s
- Roundup Ready™ crops introduced in the mid-1990s
- Widely used in fields and backyards



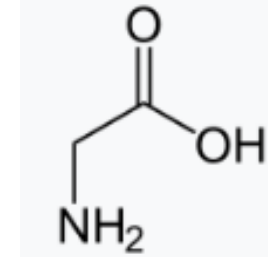
The Challenging Analysis of Glyphosate

1. Highly Polar

- Synthetic amino acid
- Glycine analogue



Glyphosate

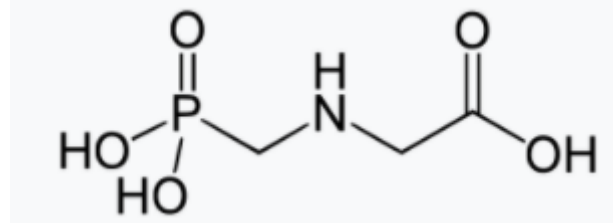


Glycine

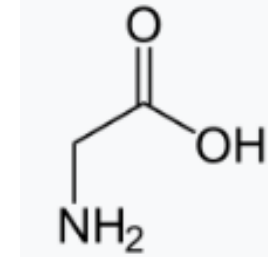
The Challenging Analysis of Glyphosate

1. Highly Polar

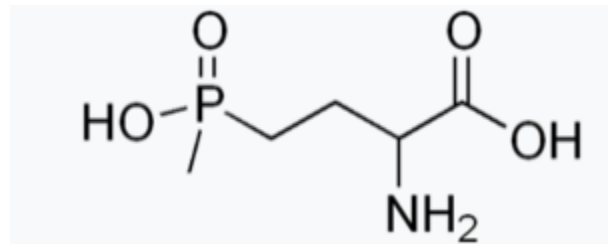
- Synthetic amino acid
- Glycine analogue
- Amino acid synthesis inhibitors



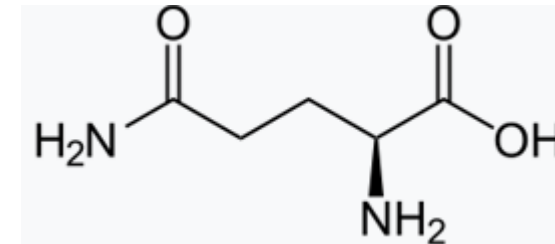
Glyphosate



Glycine



Glufosinate

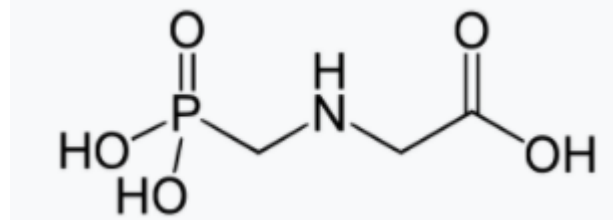


Glutamine

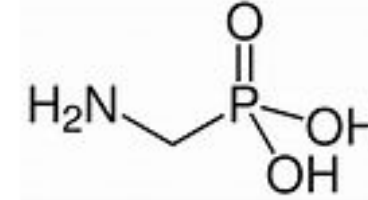
The Challenging Analysis of Glyphosate

1. Highly Polar

- Synthetic amino acid
- Glycine analogue
- Amino acid synthesis inhibitors
- Metabolized by bacteria in plants, soil and water

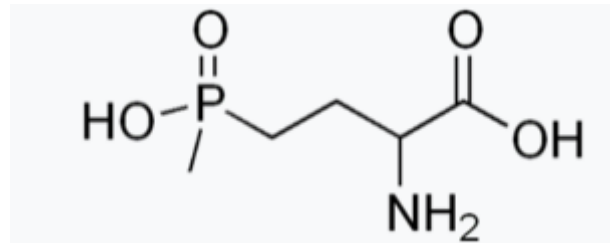


Glyphosate

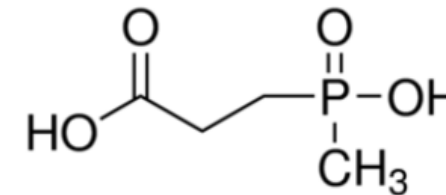


AMPA

(Aminomethylphosphonic acid)



Glufosinate

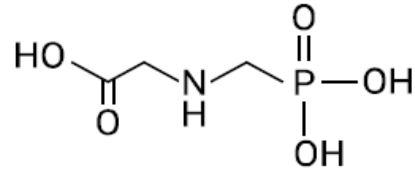


MPPA

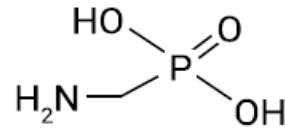
(3-(methylphosphinico)propionic acid)

The Challenging Analysis of Glyphosate

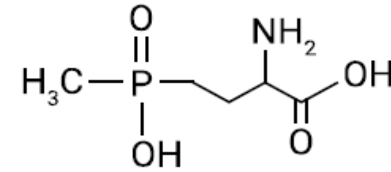
1. Highly Polar



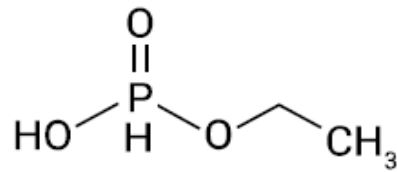
Glyphosate



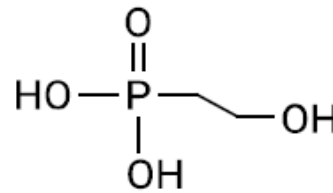
AMPA



Glufosinate

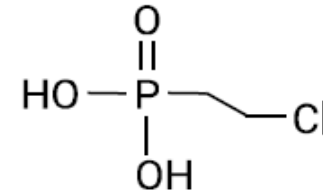


Fosetyl

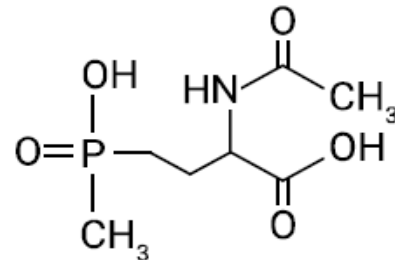


HEPA

2-hydroxyethylphosphonic acid

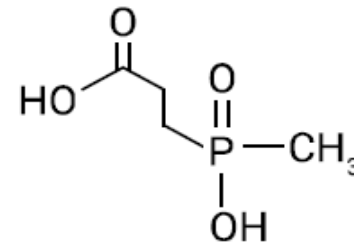


Ethephon



NAG

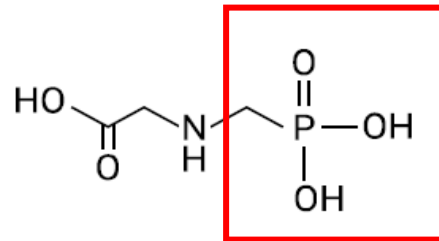
N-acetylglufosinate



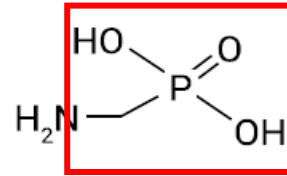
MPPA

The Challenging Analysis of Glyphosate

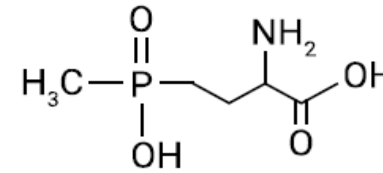
2. Chelating Agent



Glyphosate

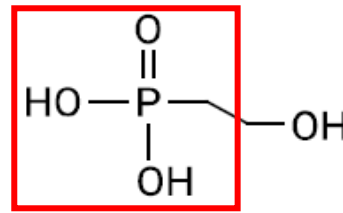


AMPA

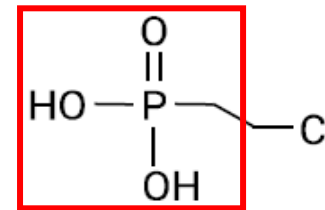


Glufosinate

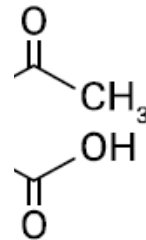
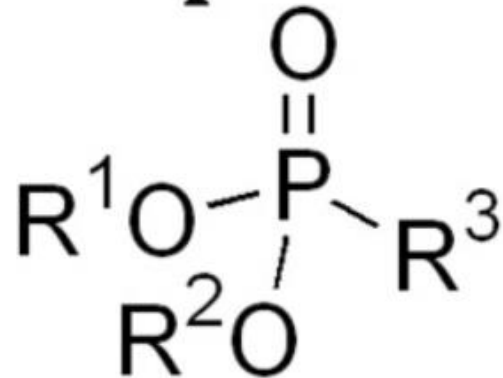
Phosphonate



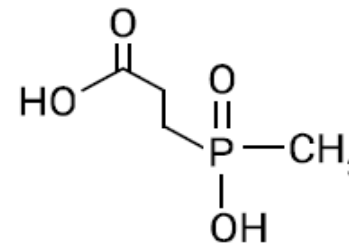
HEPA



Ethephon



NAG



MPPA

The Challenging Analysis of Glyphosate

3. Various Matrices and Regulatory Limits, Multiple Extraction Techniques

1. Liquid-liquid extraction MeCl:water, derivatization with FMOC-Cl
2. Extract with water, cleanup on SAX, elute with 1 N HCl, rotovap to dryness, derivatize with TMOA in glacial acetic acid, dried again and taken up in 9:1 water:methanol
3. Extract with water, pass through Plexa SPE and inject
4. QuPPE
5. Buffered extraction with PAX, elution with 1% formic acid
6. Extract with 50mM acetic acid and 10 mM EDTA, pass through Oasis HLB, inject
7. 50 mM acetic acid 10 mM EDTA, pass through an SEC cartridge

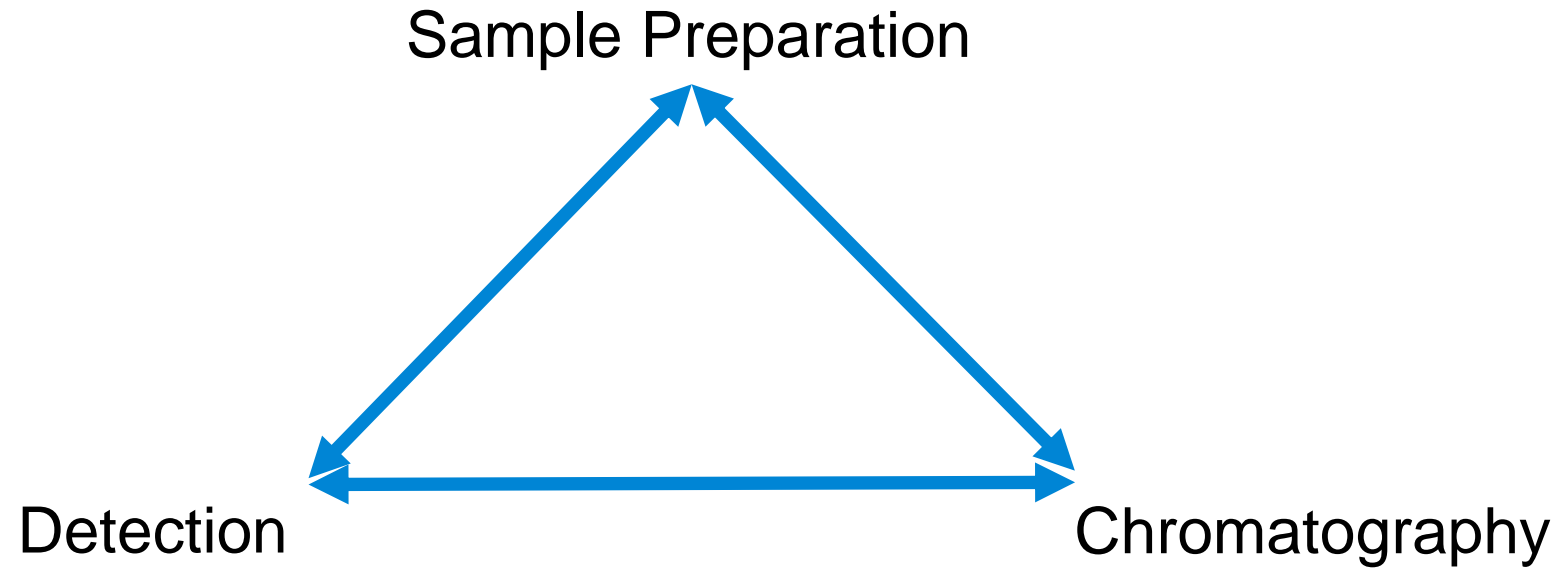
The Challenging Analysis of Glyphosate

4. Separation Techniques in the Liquid Phase

1. HILIC silica based
2. iHILIC polymer based
3. Reversed-phase chromatography
4. Anion exchange chromatography with suppresser column
5. Anion exchange without suppressor column
6. Cation exchange chromatography
7. Mixed mode chromatography
8. Ion pair chromatography (with reversed-phase column)

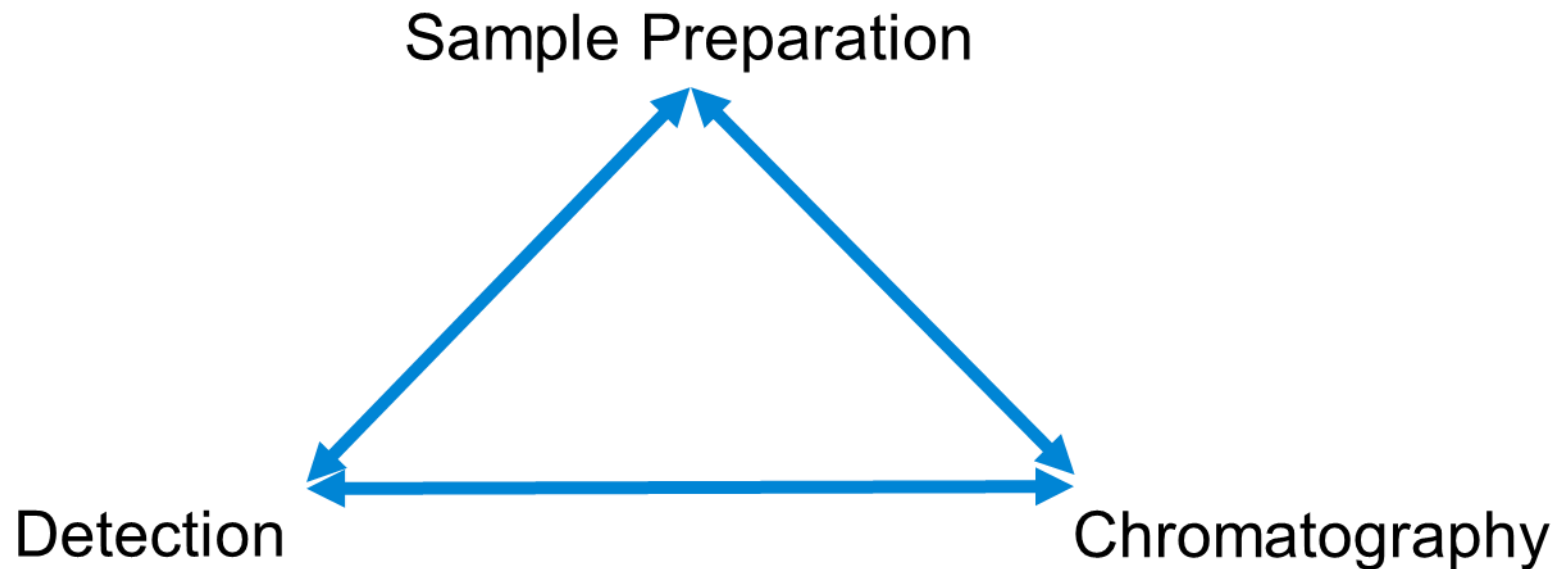
The Challenging Analysis of Glyphosate

The Three Interconnected Pillars

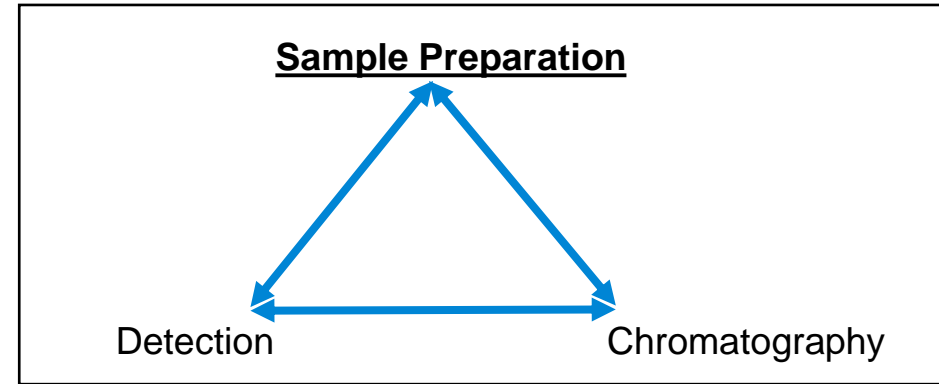


Objective

To develop a sensitive and simple methodology for the analysis of glyphosate and other polar pesticides, with perfectly aligned sample preparation, chromatography and mass spectrometry.



Sample Preparation – Drinking Water

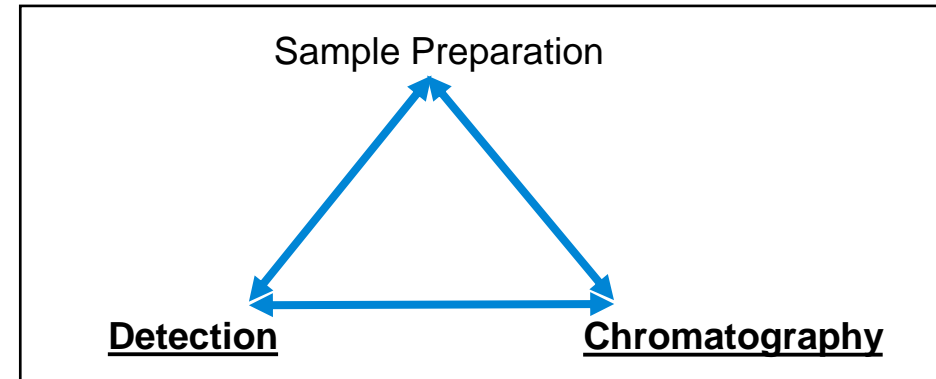


1. Filter on 0.2 μm PES
2. Acidify with concentrated formic acid (0.1%)

UHPLC Conditions

- Run Time: 8 min
- Column: Agilent InfinityLab Poroshell 120 CS-C18, 2.1 × 150 mm, 2.7 μm
- Mobile Phase A: 0.1 % formic acid + 5uM Infinity Lab Deactivator Additive in water
- Mobile Phase B: 0.1 % formic acid in methanol
- Injection Volume: 25 uL
- Multisampler Temperature: 4°C
- Column Temperature: 40°C
- Flow: 0.350 mL/min
- Gradient:

Time	Mobile Phase A	Mobile Phase B
0.00 min	99.9	0.1
1.50 min	99.9	0.1
2.00 min	80	20
4.00 min	60	40
4.10 min	0	100
8.00 min	0	100



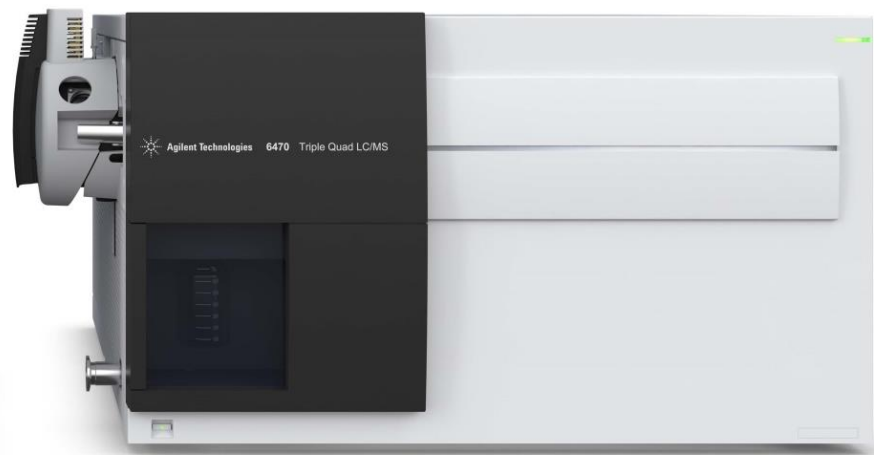
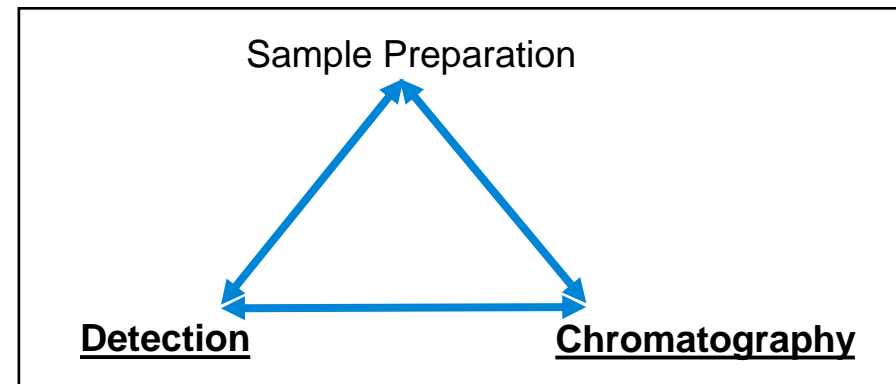
Key points:

- **Typical LCMS mobile phases**
- **Aqueous mobile phase allows large injection of aqueous samples**

- Needle wash: 0.1% formic acid in methanol

Hardware

1290 Infinity II Series UHPLC Coupled to 6470 TQ



Results – Typical Chromatography

AMPA: 0.95 min

Glufosinate: 1.6 min

Glyphosate: 1.9 min

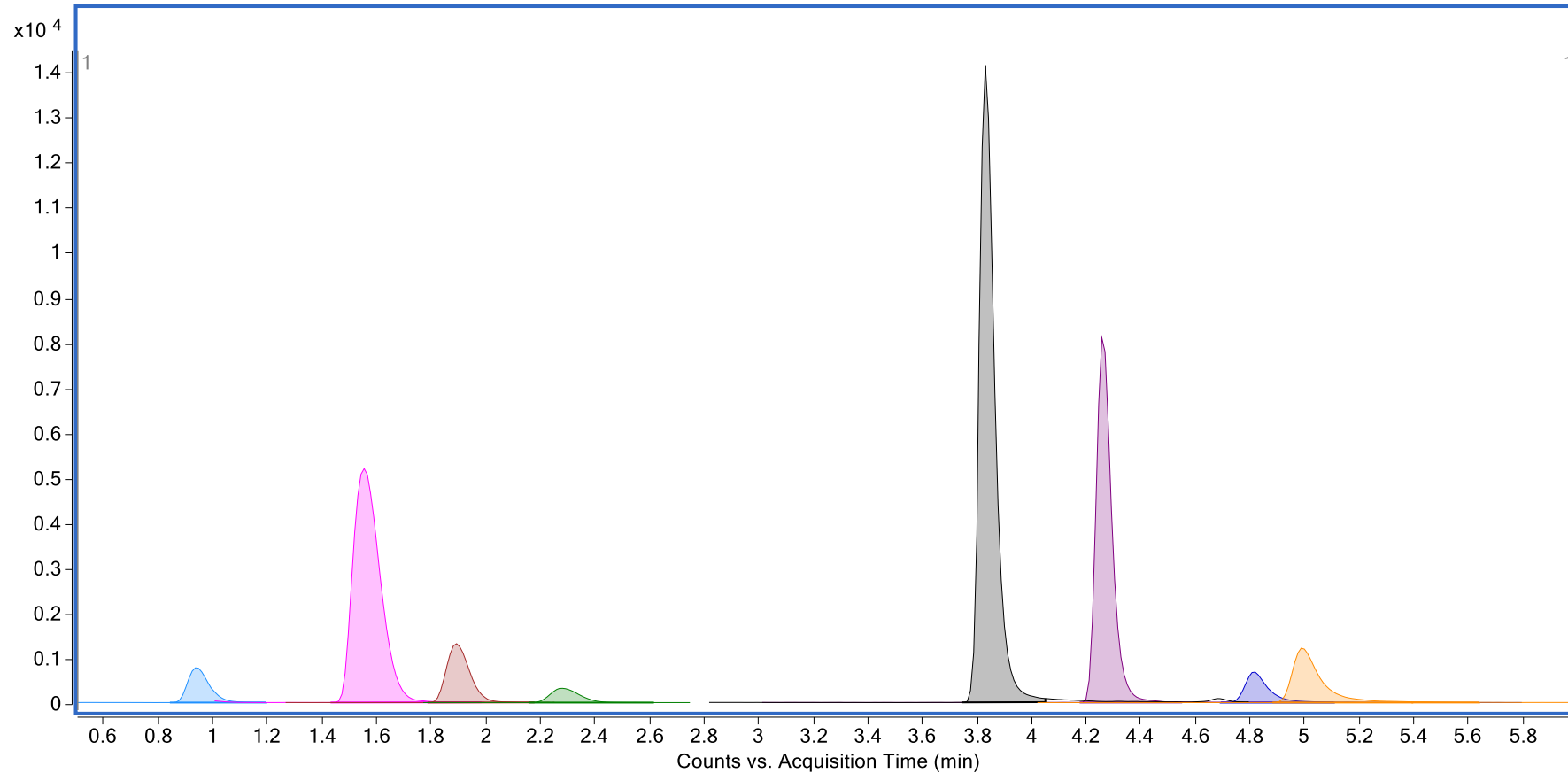
HEPA: 2.3 min

MPPA: 3.8 min

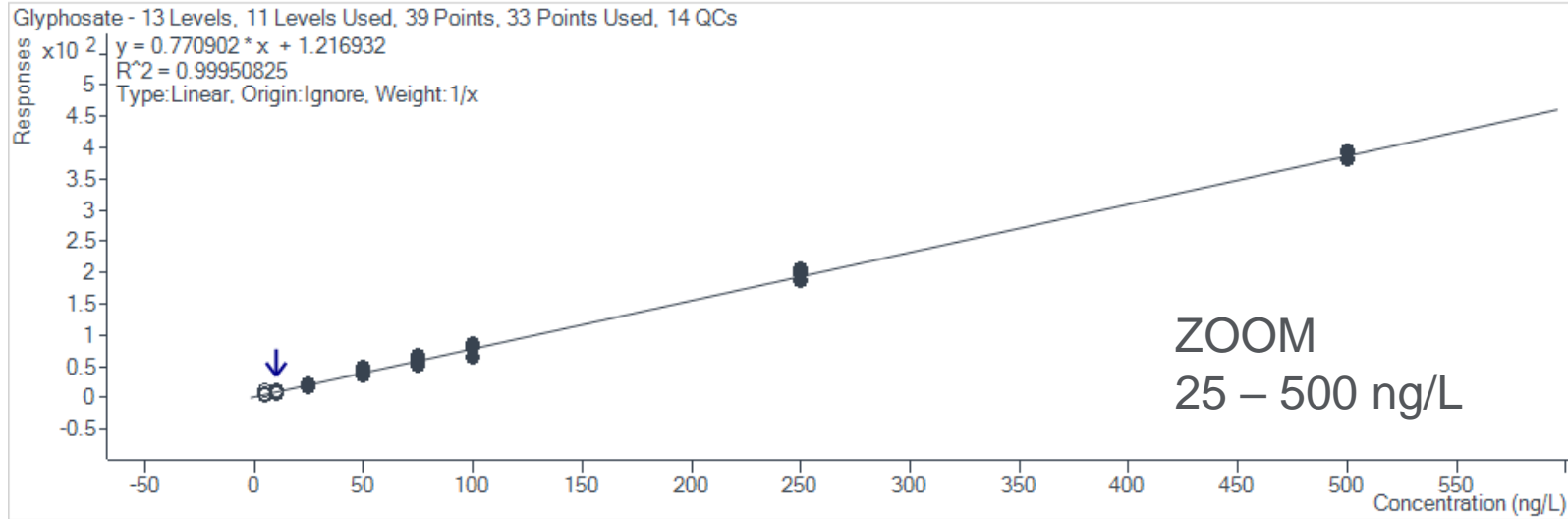
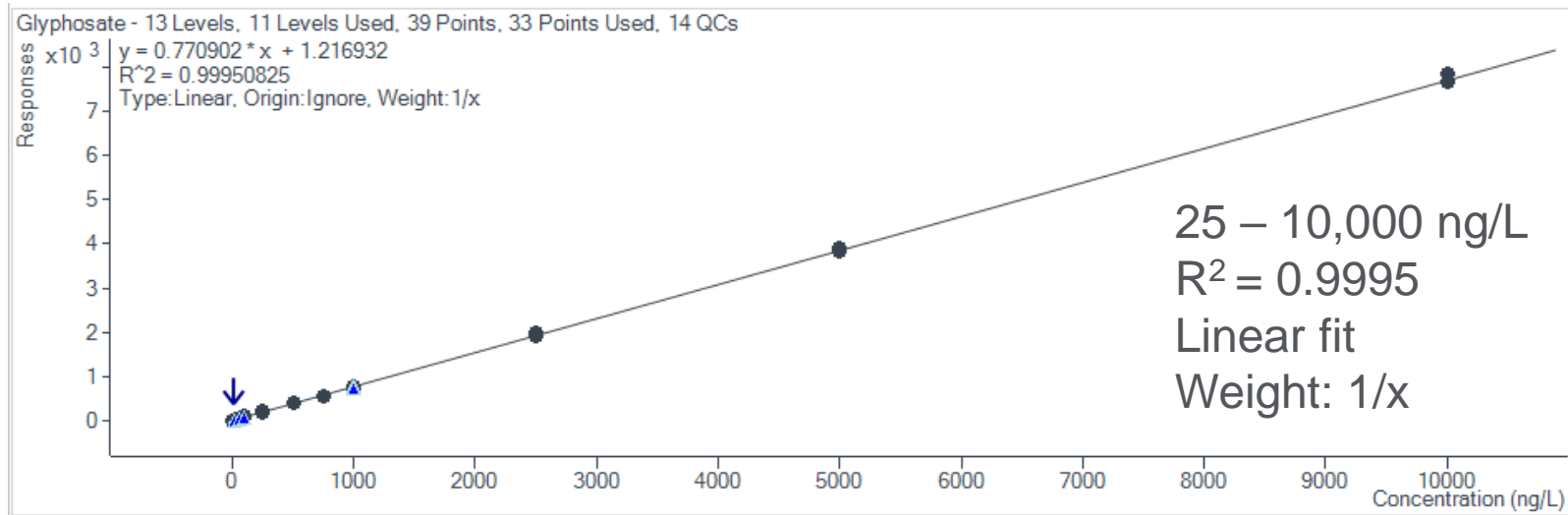
N-acetylglufosinate: 4.3 min

Ethephon: 4.8 min

Fosetyl: 5.0 min



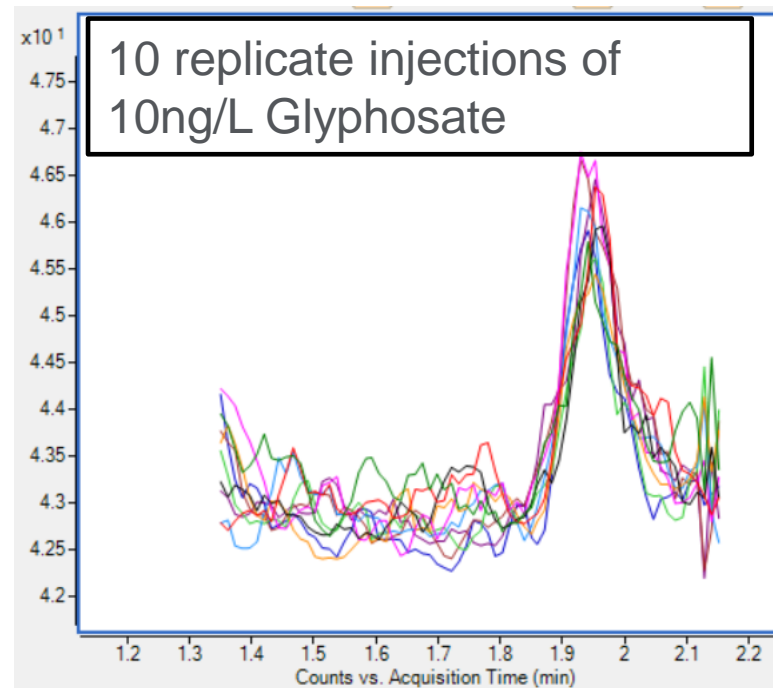
Results - Glyphosate



Results - Glyphosate

- The calculation of a Method Detection Limit (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*.

- **US EPA, Clean Water Act Analytical Methods, Procedures for Detection and Quantitation**

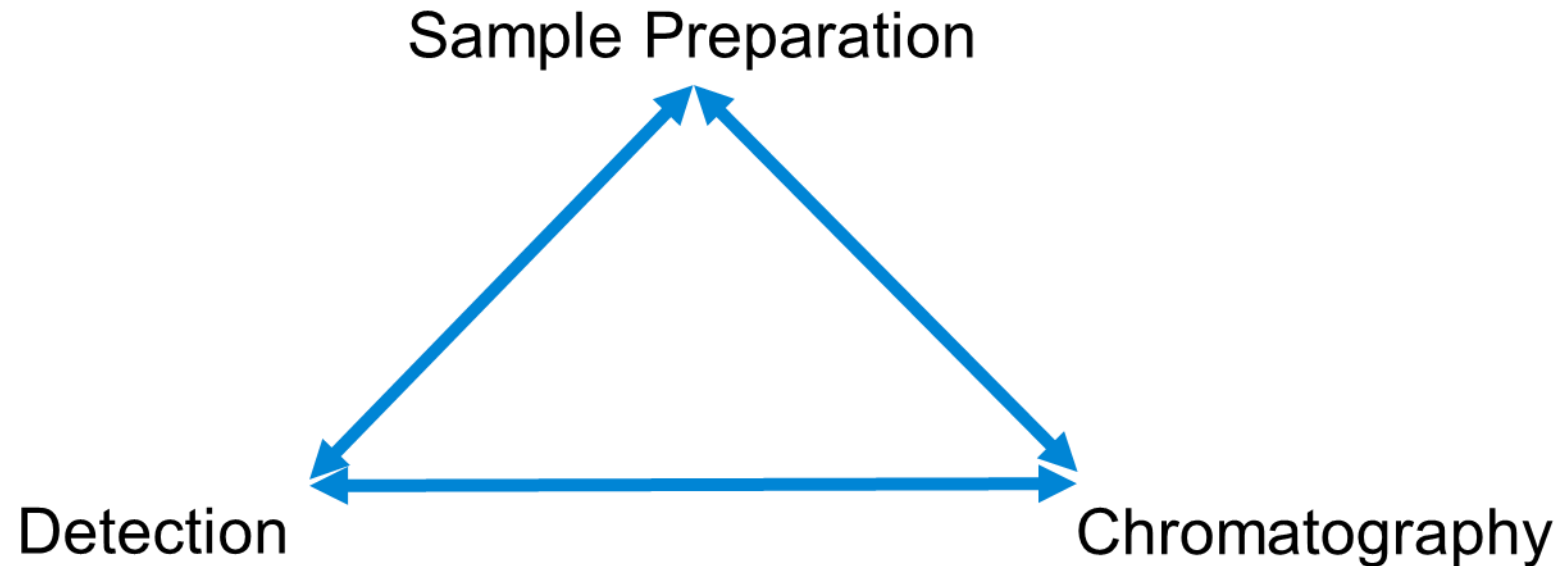


Results - Glyphosate

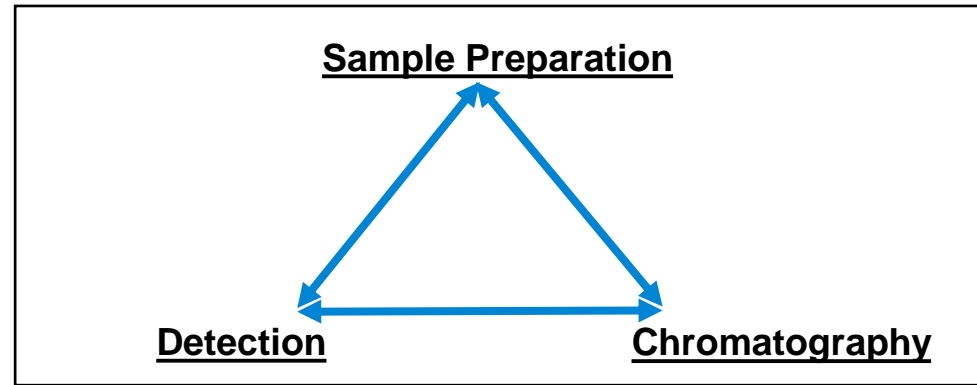
			Column 1	Column 2	Column 3	Column 4
		ng/L (on-column)	10	10	10	10
		Replicate #	User Input Response (no manual integration)			
<i>Minimum</i>	<i>Recommended</i>	Replicate1	13	15	20	11
		Replicate2	19	30	15	12
		Replicate3	15	26	25	10
		Replicate4	17	14	16	15
		Replicate5	16	20	18	11
		Replicate6	16	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				
		Mean (\bar{x})	18.3	19.7	15.5	11.3
		Standard Deviation (s)	4.877	5.851	4.720	2.452
		%RSD (CV)	26.7%	29.7%	30.5%	21.7%
		# Replicates (n)	10	10	10	10
		Degrees of Freedom (df)	9	9	9	9
		Critical t-value (t)	2.821	2.821	2.821	2.821
		MDL (ng/L)	7.5	8.4	8.6	6.1

Summary

- An all-Agilent solution for the analysis of underivatized glyphosate and seven (7) other polar pesticides in aqueous matrices, with perfectly-aligned sample preparation, chromatography and mass spectrometry:



Summary



- Very quick and simple sample preparation, acidification identical to mobile phase system
- Newly introduced 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 System offers great sensitivity, reproducibility and linearity, and along with MassHunter software, is compatible with dual polarity transitions for a given analyte

Additional Resources

Columns and Supplies Shopping Cart for Polar Pesticide Application:

- [View here.](#)

InfinityLab Poroshell 120 Product Page:

- <https://www.agilent.com/en/product/small-molecule-columns/reversed-phase-hplc-columns/infinitylab-poroshell-120>

InfinityLab Poroshell 120 Ordering Guide:

- Publication number [5991-9123EN](#)

InfinityLab Poroshell 120 CS-C18 Flyer:

- Publication number [5994-2720EN](#)

Agilent Environmental Solutions:

- <https://www.agilent.com/en/solutions/environmental>

Acknowledgements

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Any Questions?