Evolution of Glyphosate & Polar Pesticide Analysis in Aqueous Matrices Using LC-TQ

Tarun Anumol, Ph.D.
Director, Global Environment & Food Markets

Jean-Francois Roy & Jarod Grossman
LC/MS Application Scientists

Agilent Technologies Inc.

Glyphosate

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

Pest Manag. Sci. 64:319–325 (2008)

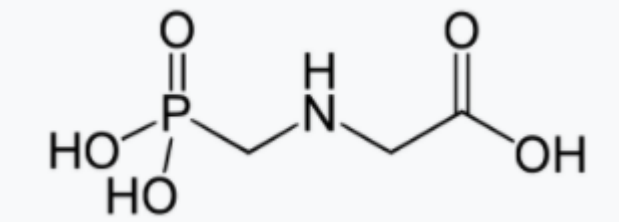
Glyphosate & AMPA Presence in water

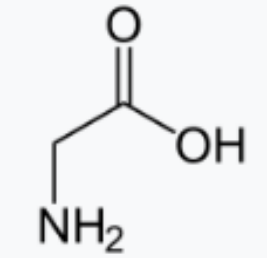
- Presence detected in several US streams and rivers[1]
- Detected in European groundwater sources[2]
- Maximum allowable concentration in drinking water set by the European Community for several polar pesticides of 0.1 µg/L

[1] Battaglin et al., Glyphosate, Other Herbicides, and Transformation Products in Midwestern Streams. *JAWRA Journal of the American Water Resources Association* **2005,** *41*, (2), 323-332.
[2] B. Schmidt, B. Siegemund, H. Ehses, E. Zietz, Proceedings of XI Symp. Pesticide Chem., Sept. 13–15, **1999**, p. 591.

The Challenging Analysis of Glyphosate

1. Highly Polar





- Synthetic amino acid
- Glycine analogue

Glyphosate **Glycine**

The Challenging Analysis of Glyphosate

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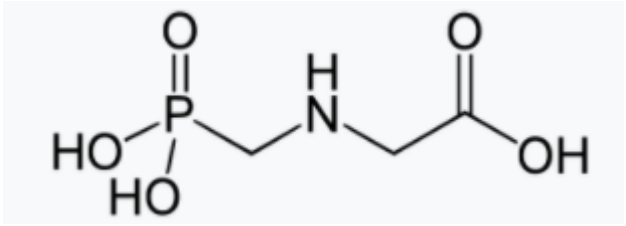

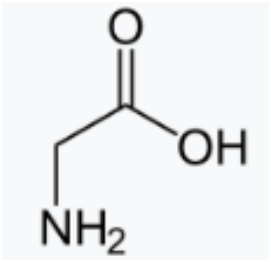

- Synthetic amino acid
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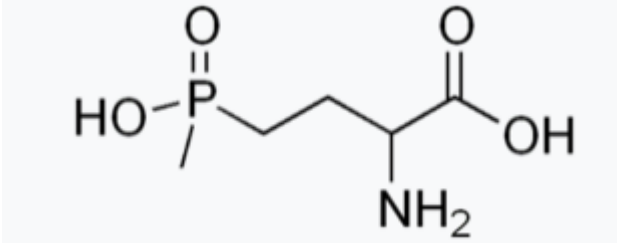
Glyphosate **Glycine**

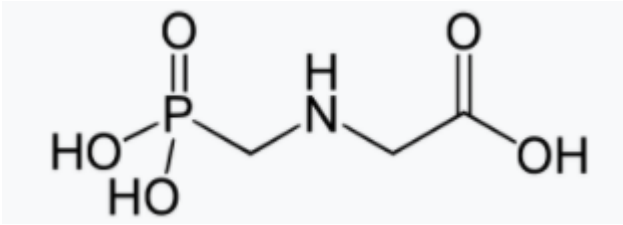


Glufosinate

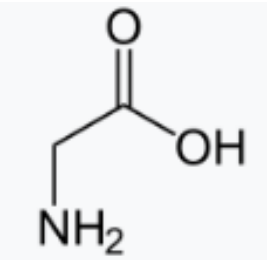
The Challenging Analysis of Glyphosate

1. Highly Polar

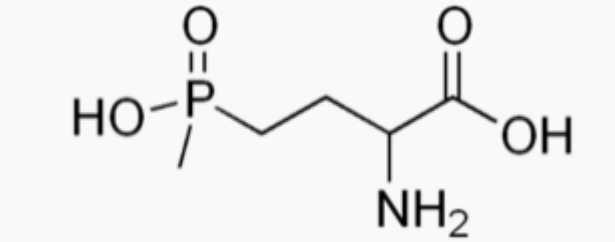
- Synthetic amino acid
- Glycine analogue
- Amino acid synthesis inhibitor



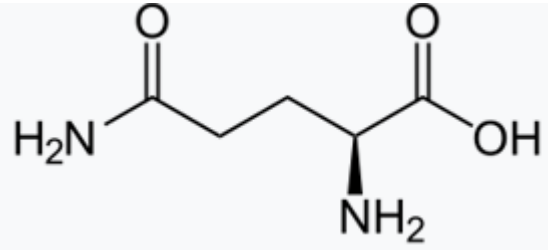
Glyphosate



Glycine



Glufosinate

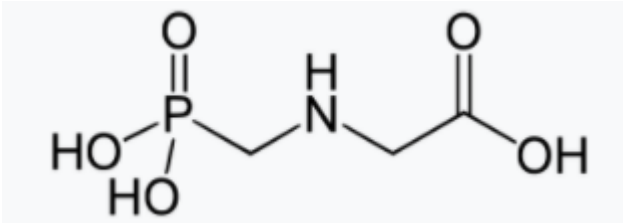


Glutamine

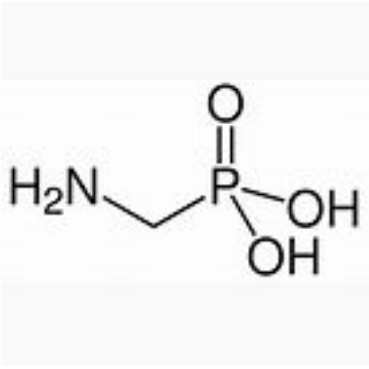
The Challenging Analysis of Glyphosate

1. Highly Polar

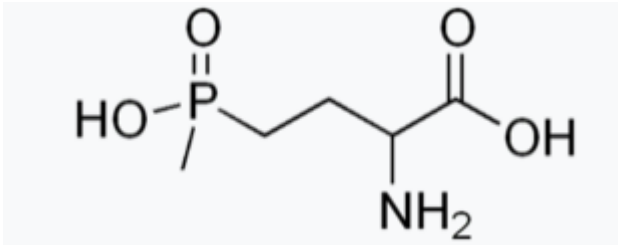
- Synthetic amino acid
- Glycine analogue
- Amino acid synthesis inhibitor
- Metabolized by bacteria in plants, soil and water



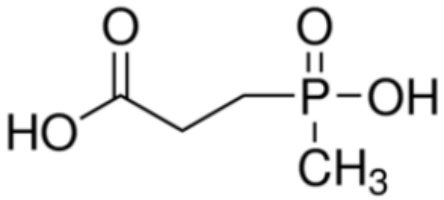
Glyphosate



AMPA
(Aminomethylphosphonic acid)



Glufosinate

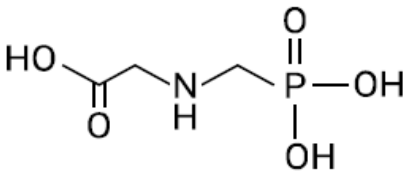


MPPA
(3-(methylphosphinico)propionic acid)

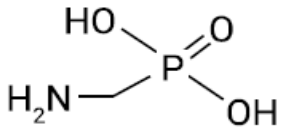
The Challenging Analysis of Glyphosate

1. Highly Polar

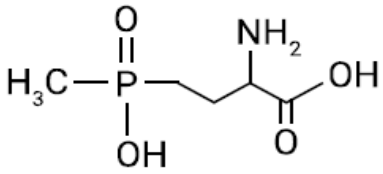
Agilent InfinityLab



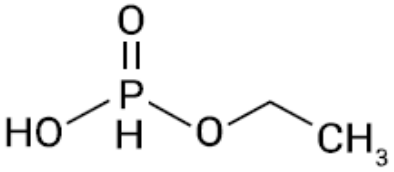
Glyphosate



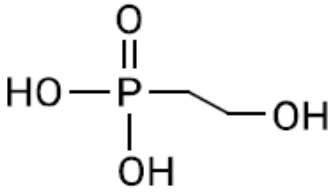
AMPA



Glufosinate

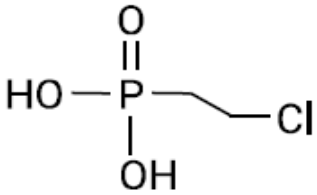


Fosetyl

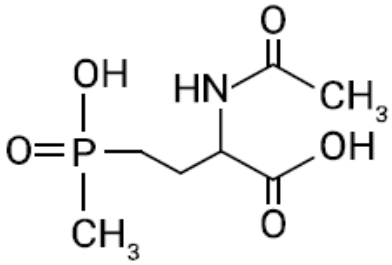


HEPA

2-hydroxyethyphosphonic acid

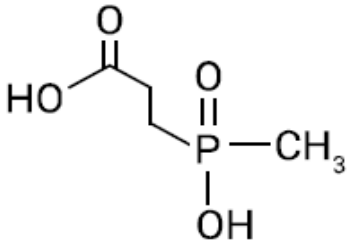


Ethephon



NAG

N-acetylglufosinate



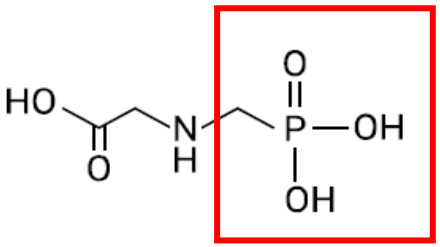
MPPA

Agilent

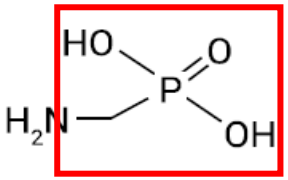
The Challenging Analysis of Glyphosate

2. Chelating Agent

Agilent InfinityLab



Glyphosate



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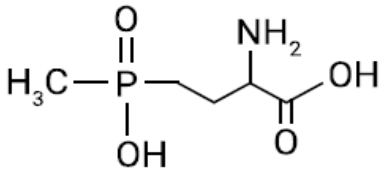
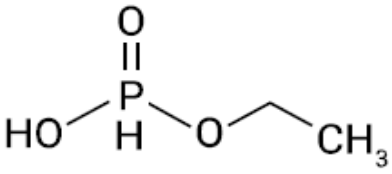
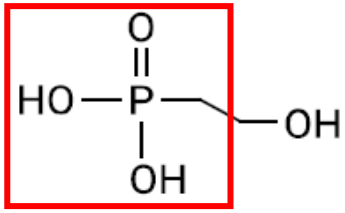


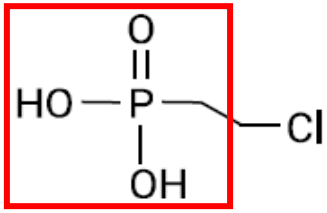
Glufosinate



Fosetyl



HEPA



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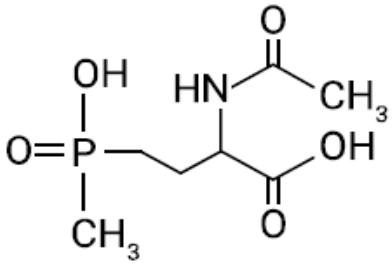

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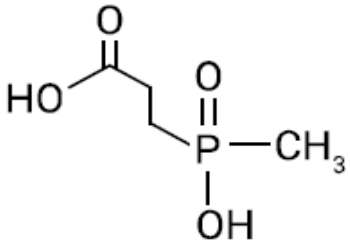

MPPA

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The Challenging Analysis of Glyphosate

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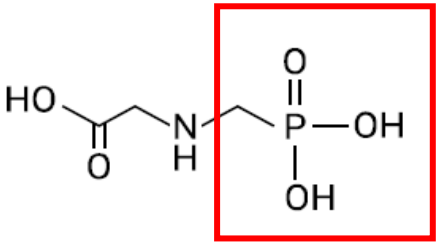
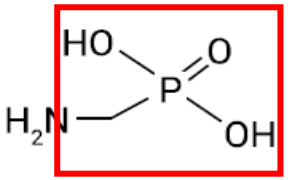
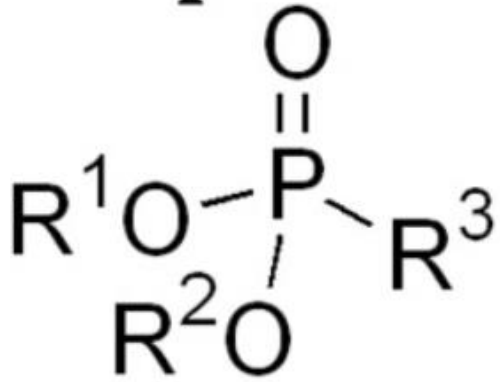


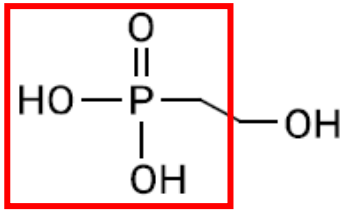
Glyphosate



AMPA

Phosphonate





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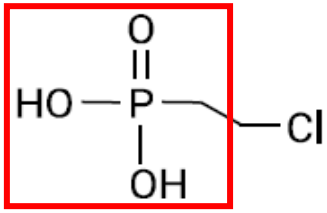


Ethephon

The Challenging Analysis of Glyphosate

3. Various Matrices and Regulatory Limits, Multiple Extraction Techniques

Agilent InfinityLab

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

Derivatization **SPE** **QuPPe** **Direct Inject**

The Challenging Analysis of Glyphosate

4. Separation Techniques in the Liquid Phase

Agilent InfinityLab

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)

HILIC **Reverse Phase** **Ion/Ionic**

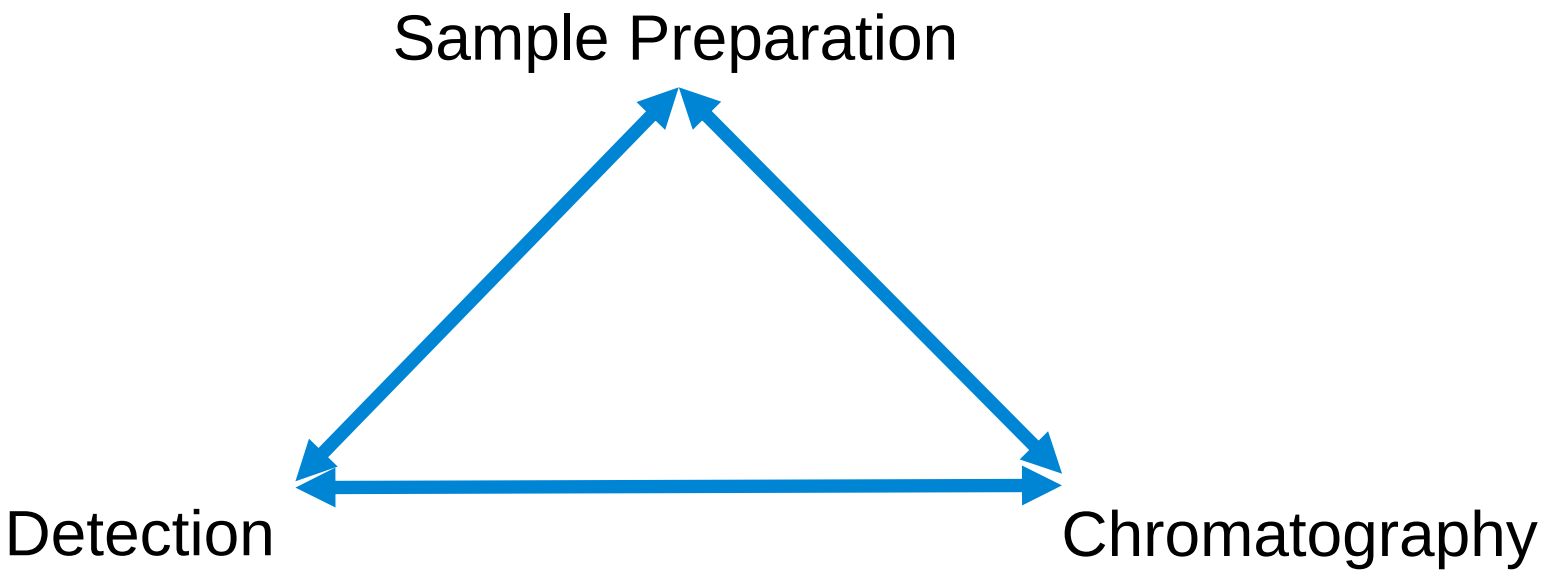
Chromatography Choice

Agilent InfinityLab

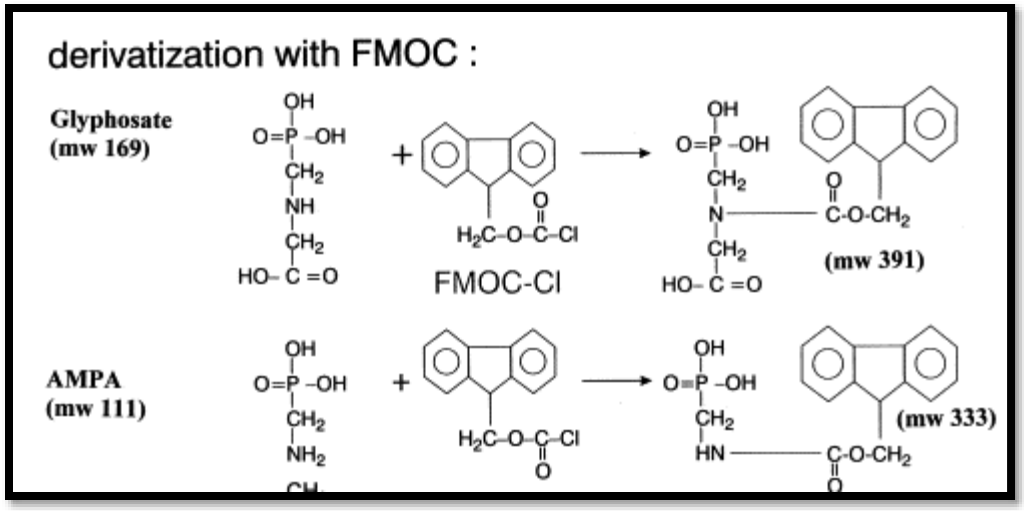
HILIC	Ion/Ionic
Reverse-Reverse Phase	Good Separation
Sufficient Resolution	Separation of Anions
Low injection volume	New/Special Technique in lab
Sensitivity	Separation of Anions
Long-term reproducibility	Chromatographic Resolution
	‘Salting’ in the MS

The Challenging Analysis of Glyphosate

The Three Interconnected Pillars



Derivatization reaction with FMOC
20+ years of use

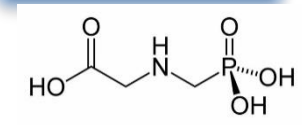


*Adapted from: Vreeken, R.J., et al., Journal of Chromatography A, 1998. **794**(1–2): p. 187-199.*

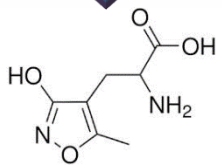
Derivatization Workflow Automation with Online SPE

Agilent InfinityLab

Collect 25 mL sample → Derivatization → Flexcube 900 uL → 6460 LC/MS/MS



Glyphosate

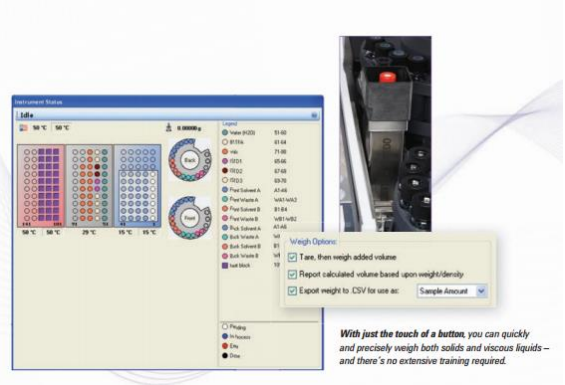


AMPA

- 25 ml sample
- 250 uL of 10 ug/L surrogate
- 1 ml EDTA
  1 ml Borate buffer
- 0.5 ml of 20 mM FMOC
- Heat at 100 C for 30 min
- 50 uL Phosphoric Acid

Automated Sample Prep (Derivatization) 7696 Workbench

Agilent InfinityLab



Agilent

Separation Parameters
Agilent 1290 LC & Flexcube

LC Column: Poroshell EC 120 C-18, 3x50 mm, 2.7 µm
Flowrate: 0.35 mL/min
Injection Volume: 900 µL
Column Compartment Temperature: 30ºC

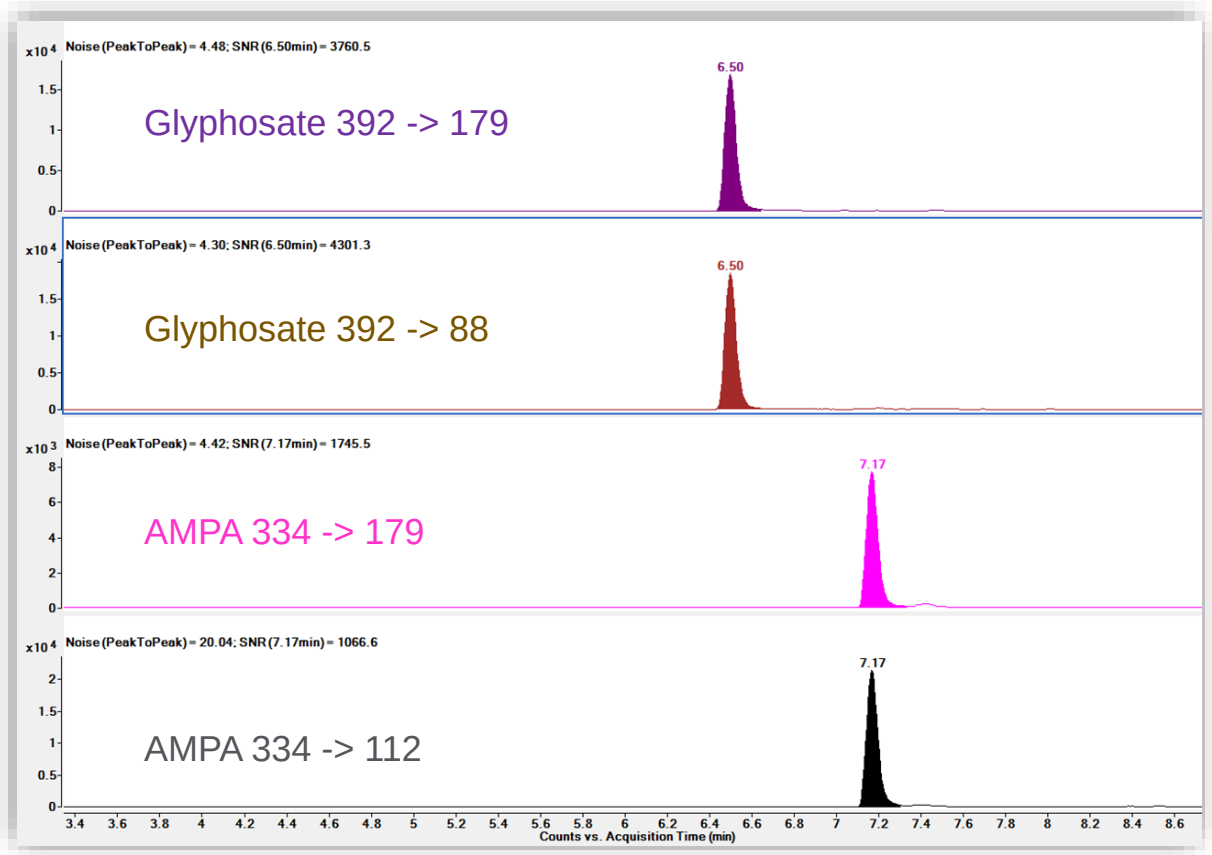
LC Mobile Phase:

A – Water + 5mM Ammonium Acetate
B – Acetonitrile

Flexcube Solvents:

A - [95/5(v/v): HPLC Water/Acetonitrile] + 0.1% Acetic Acid
B - 1/1/1 (v/v/v): Acetonitrile/Methanol/Isopropanol

Sample Chromatogram



10 ng/L in Mili-Q water

Method Performance

Analyte	Ground Water			
	20 ng/L spike (n=5)		100 ng/L spike (n=5)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Gylphosate	88.6	2.7	93.4	2.4
AMPA	98.9	7.7	94.1	4.5

Analyte	Surface Water			
	20 ng/L spike (n=5)		100 ng/L spike (n=5)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Gylphosate	95.2	1.1	98.7	1.6
AMPA	78.4	2.4	84.5	2.5

- Recoveries for AMPA and glyphosate in both waters was 75-100%
- RSD (%) was <10% in all spikes

Limit of detection and quantification (LOD/LOQ)

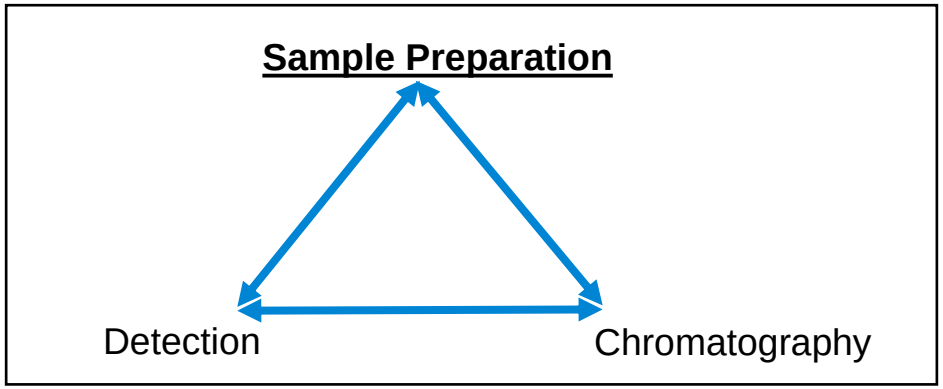
Agilent InfinityLab

Limit of Detection (LOD): S/N>3 for most abundant transition
Limit of Quantification (LOQ): S/N>9 for both transitions

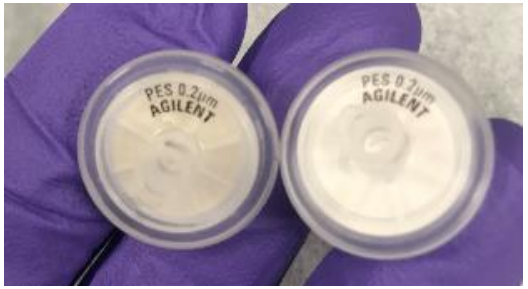
Analyte	S/N for 0.2 ng/L standard	Expected LOQ (ng/L)	Expected LOD (ng/L)
Glyphosate (Q1)	25.8	0.1	0.025
Glyphosate (Q2)	18.1		
AMPA (Q1)	13.1	0.2	0.045
AMPA (Q2)	10.6		

‘Messy’ method – derivatization is not environmentally friendly
24-hour process from start to finish
Not suitable for wider range of polar pesticides

Sample Preparation – Surface Water

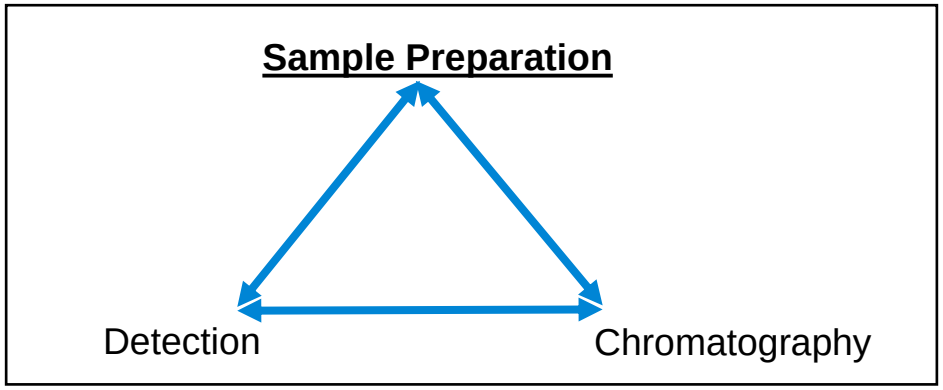


1. Collect in tube
2. Centrifuge at 5000 rpm for 5 min
3. Filter on 0.2 µm PES membrane



4. Acidify with concentrated formic acid (0.1 %)

Sample Preparation – Drinking Water



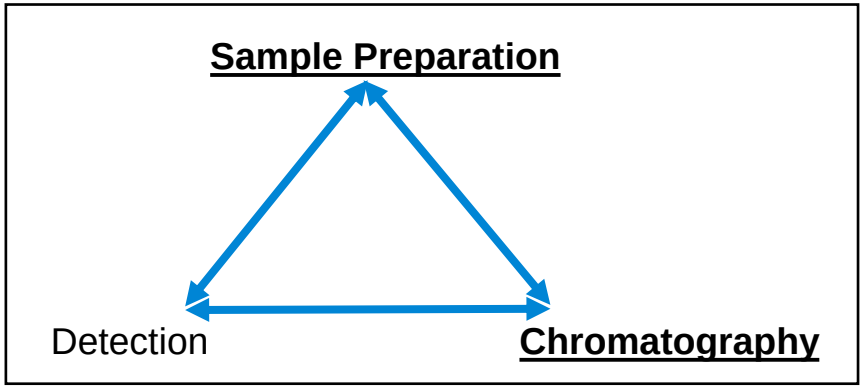
1. Filter on 0.2 µm PES membrane

2. Acidify with concentrated formic acid (0.1%)

Chromatography

Introducing the InfinityLab Poroshell 120 CS-C18

Agilent InfinityLab



2.7 µm Poroshell superficially porous particles for UHPLC performance at lower pressure

Unique chemistry for pH stability from 1-11

Charged surface C18 for good peak shape with formic acid and unique selectivity

Agilent

Phosphonates are Metal Chelators

Presence of Trace Metal Will Lead to Tailing, Poor Sensitivity and Variability

Agilent InfinityLab

analytical chemistry

Article

Cite This: *Anal. Chem.* 2018, 90, 9457−9464

pubs.acs.org/ac

Improved LC/MS Methods for the Analysis of Metal-Sensitive Analytes Using Medronic Acid as a Mobile Phase Additive

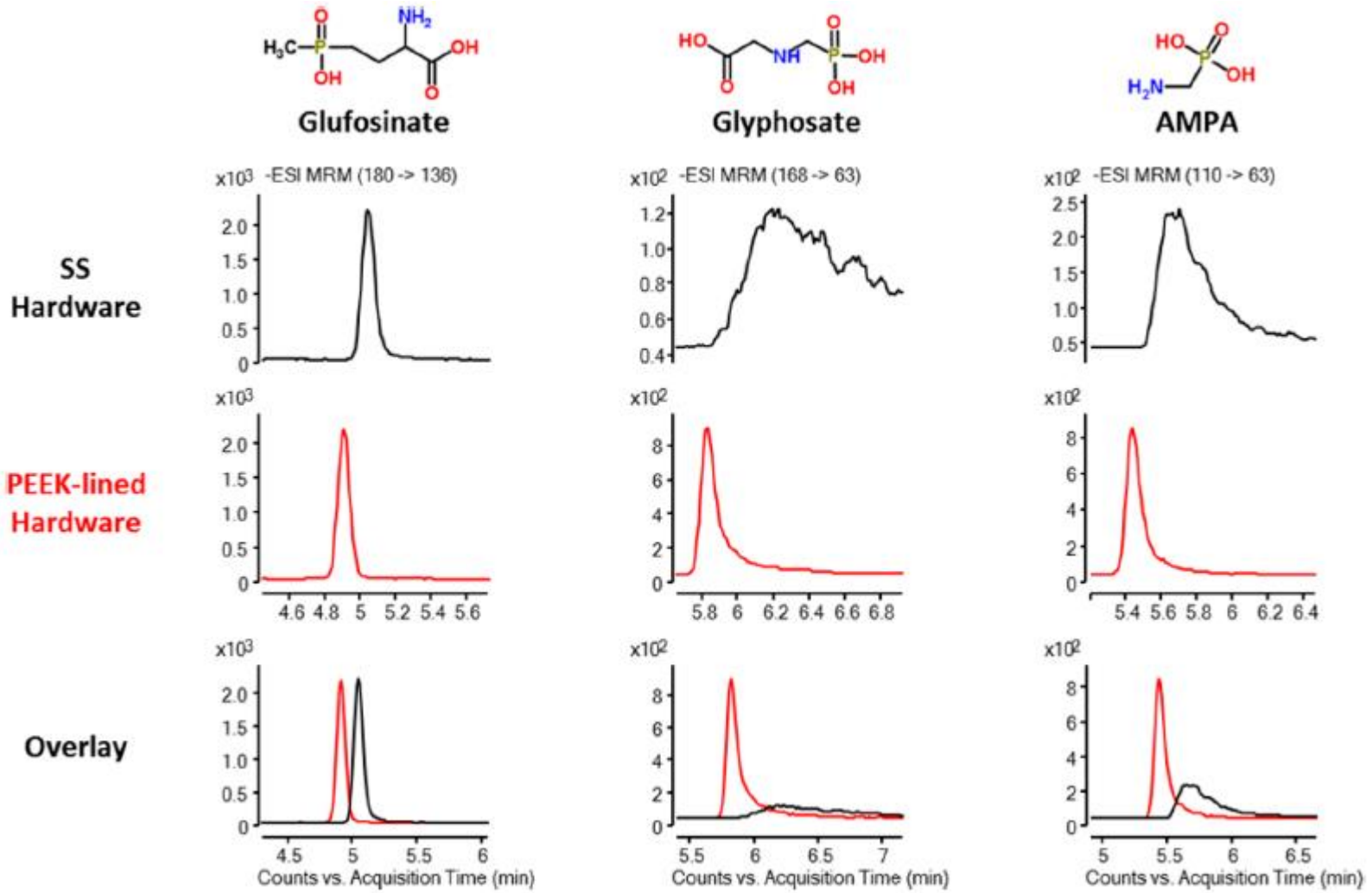
Jordy J. Hsiao,* Oscar G. Potter, Te-Wei Chu, and Hongfeng Yin

Agilent Technologies, Santa Clara, California 95051, United States

Phosphonates are Metal Chelators

Presence of Trace Metal Will Lead to Tailing, Poor Sensitivity and Variability

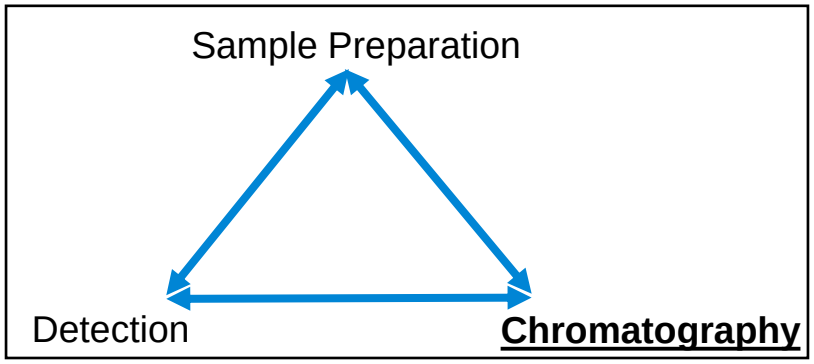
Agilent InfinityLab

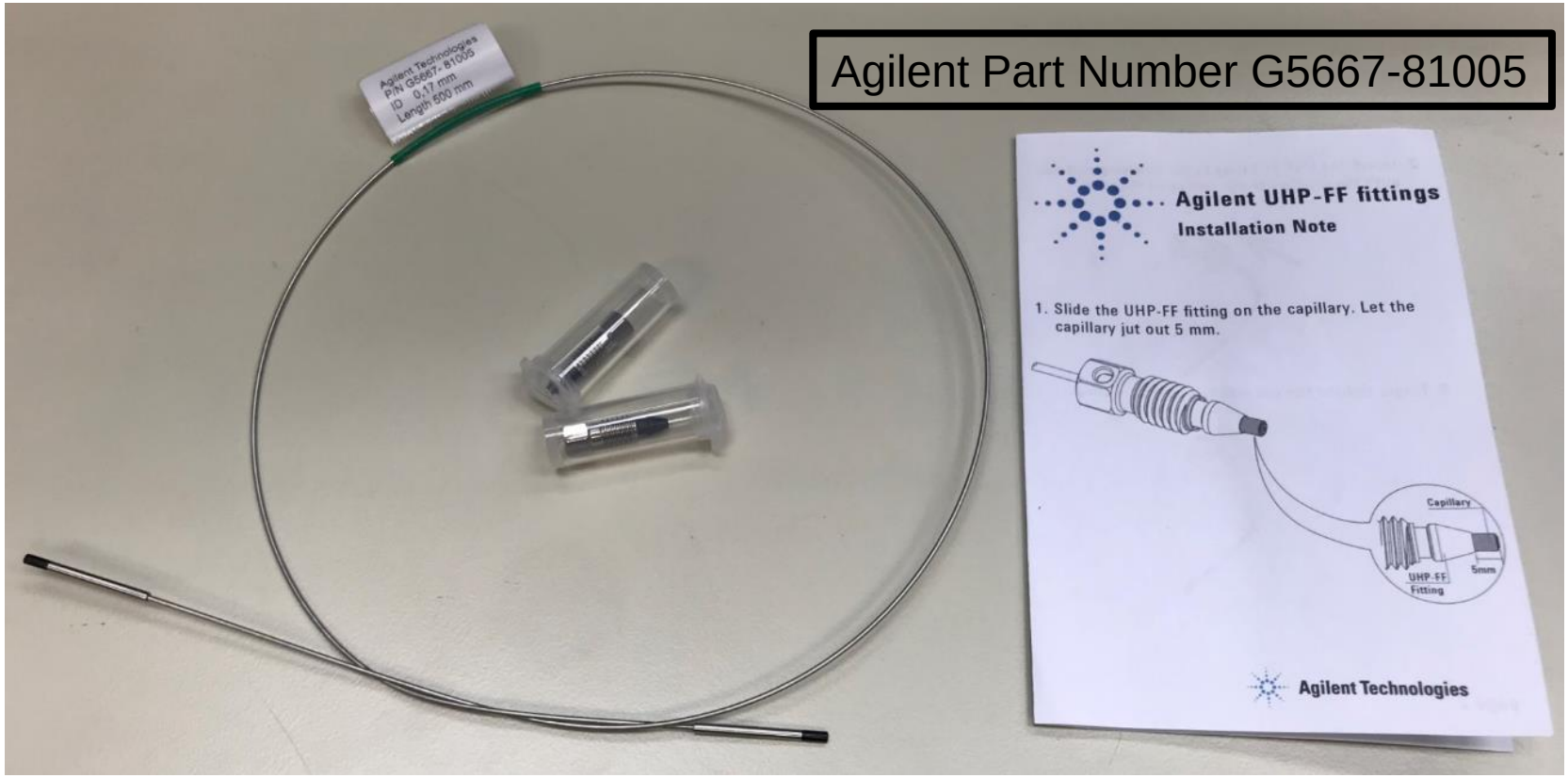


Anal. Chem. 2018, 90, 9457-9464

PEEK-lined Flow Path

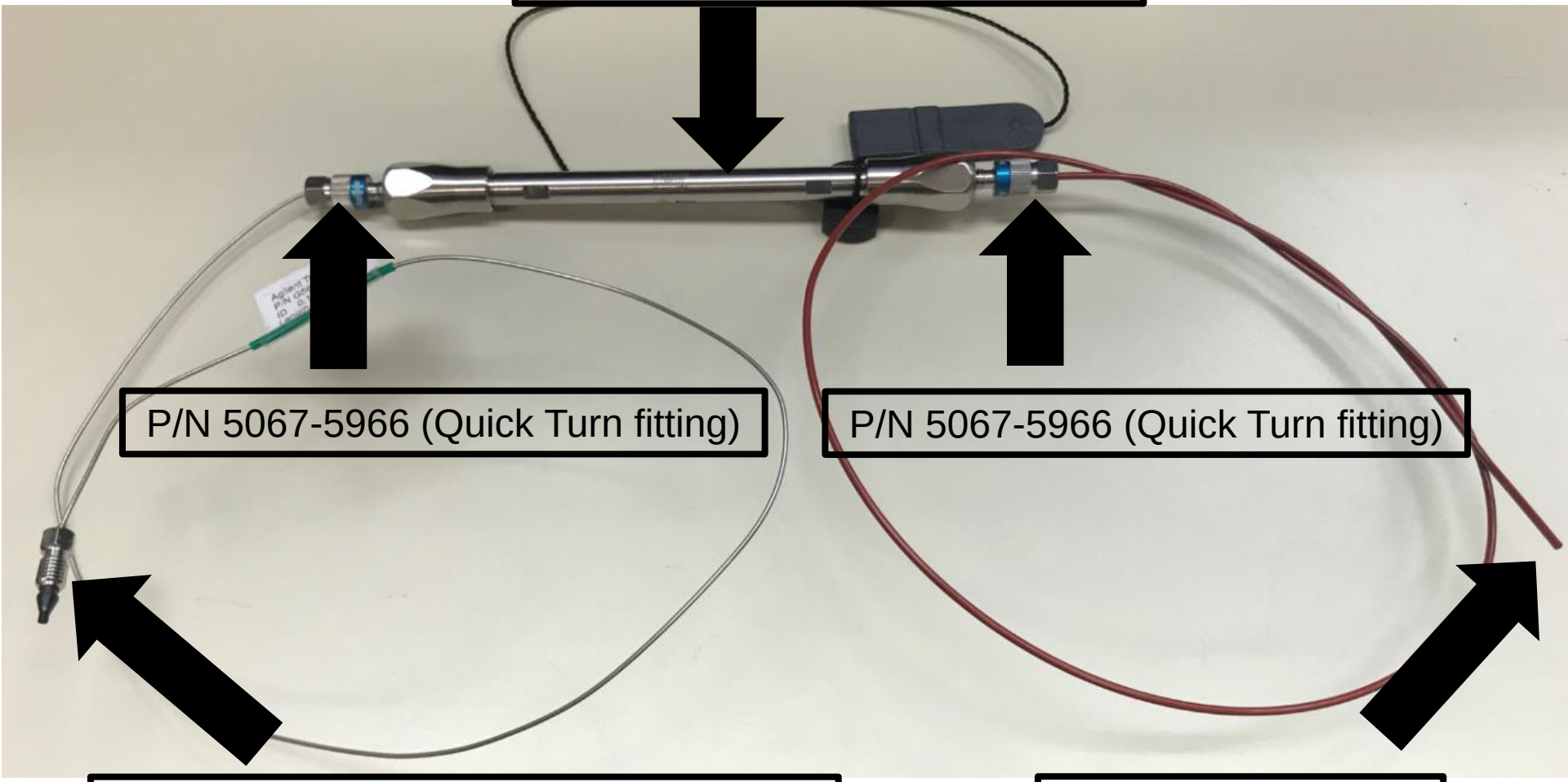
- PEEK needle seat and rotor seal
- PEEK-lined capillary from Multisampler to column inlet





PEEK-lined Flow Path

Agilent InfinityLab



Phosphonates are Metal Chelators

Deactivator Additive in Mobile Phase

Agilent InfinityLab

analytical chemistry

Article

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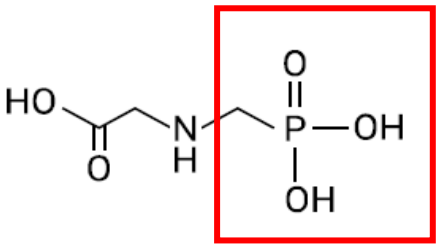
Improved LC/MS Methods for the Analysis of Metal-Sensitive Analytes Using Medronic Acid as a Mobile Phase Additive

Jordy J. Hsiao,* Oscar G. Potter, Te-Wei Chu, and Hongfeng Yin

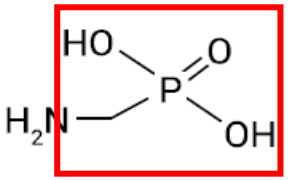
Agilent Technologies, Santa Clara, California 95051, United States

Phosphonates are Metal Chelators

Deactivator Additive in Mobile Phase

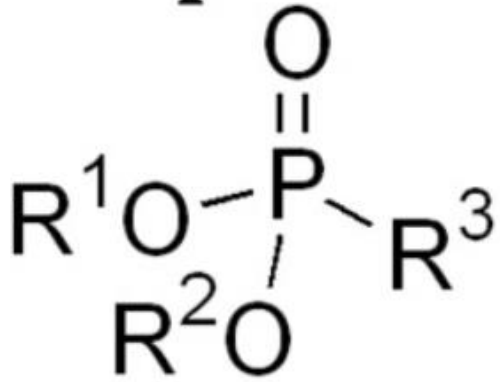


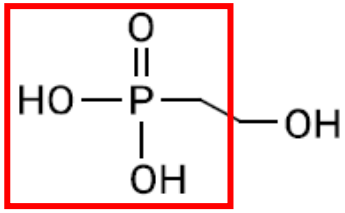
Glyphosate



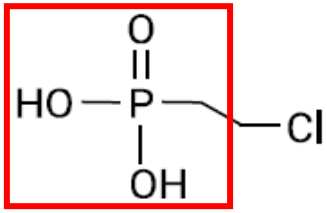
AMPA

Phosphonate





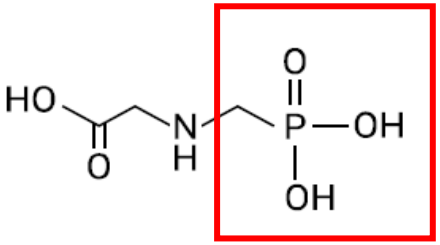
HEPA



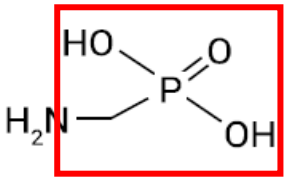
Ethephon

Phosphonates are Metal Chelators

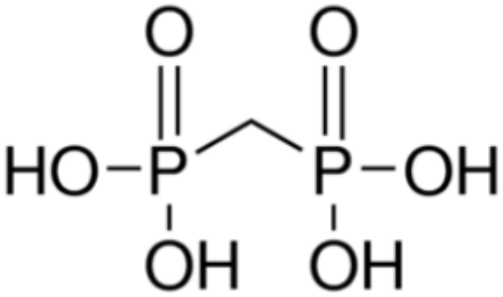
Deactivator Additive in Mobile Phase



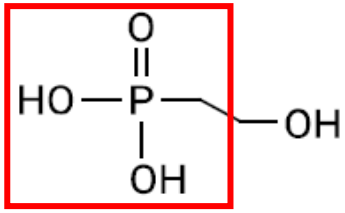
Glyphosate



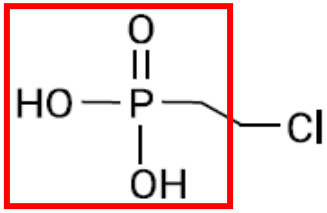
AMPA



Deactivator Additive
Agilent Part Number 5191-4506



HEPA



Ethephon

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 + 5 uM 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

- Needle wash: 0.1 % formic acid in methanol

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

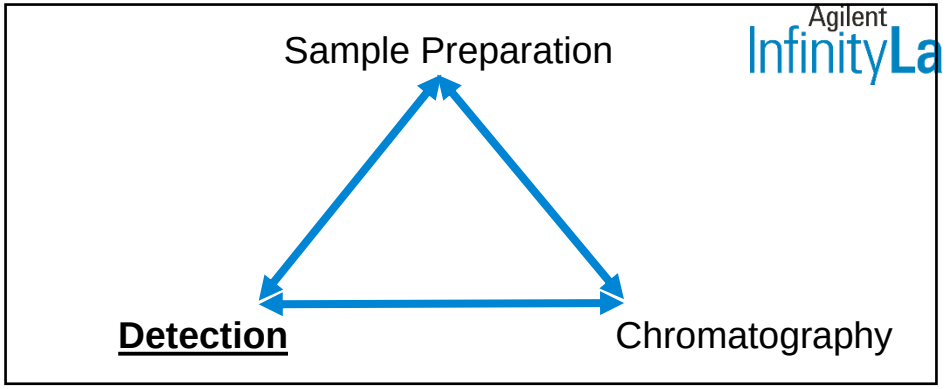
Hardware

1290 Infinity II Series UHPLC Coupled to 6470 TQ

Mass Spec Settings

Key point:

- **Positive and negative polarity transitions are no problem for instrument and software!**



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Compound	Quantifier Transition	Qualifier Transition 1	Qualifier Transition 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 – Typical Chromatography

Agilent InfinityLab

AMPA: 0.95 min

Glufosinate: 1.6 min

Glyphosate: 1.9 min

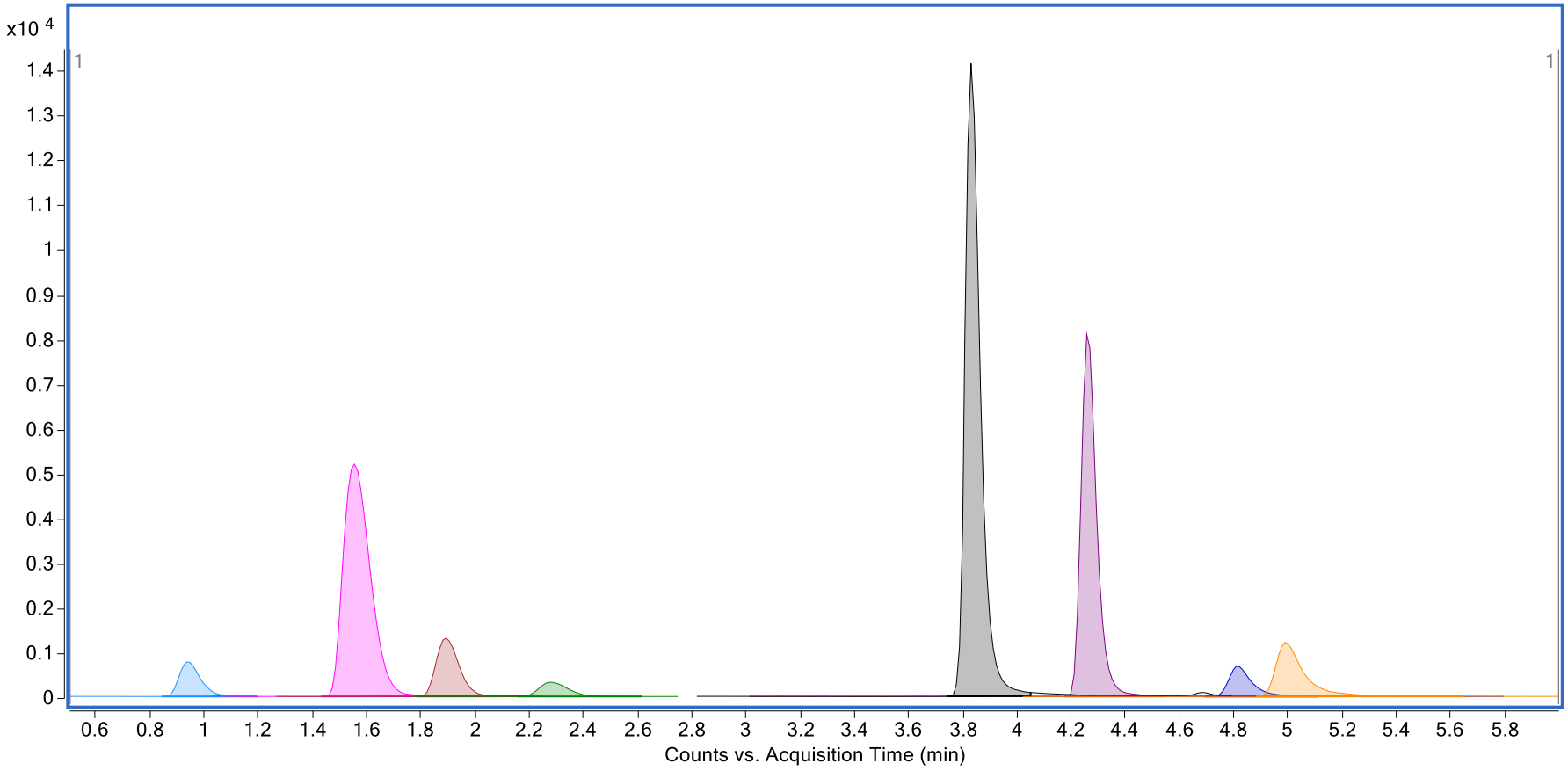
HEPA: 2.3 min

MPPA: 3.8 min

N-acetylglufosinate: 4.3 min

Ethephon: 4.8 min

Fosetyl: 5.0 min



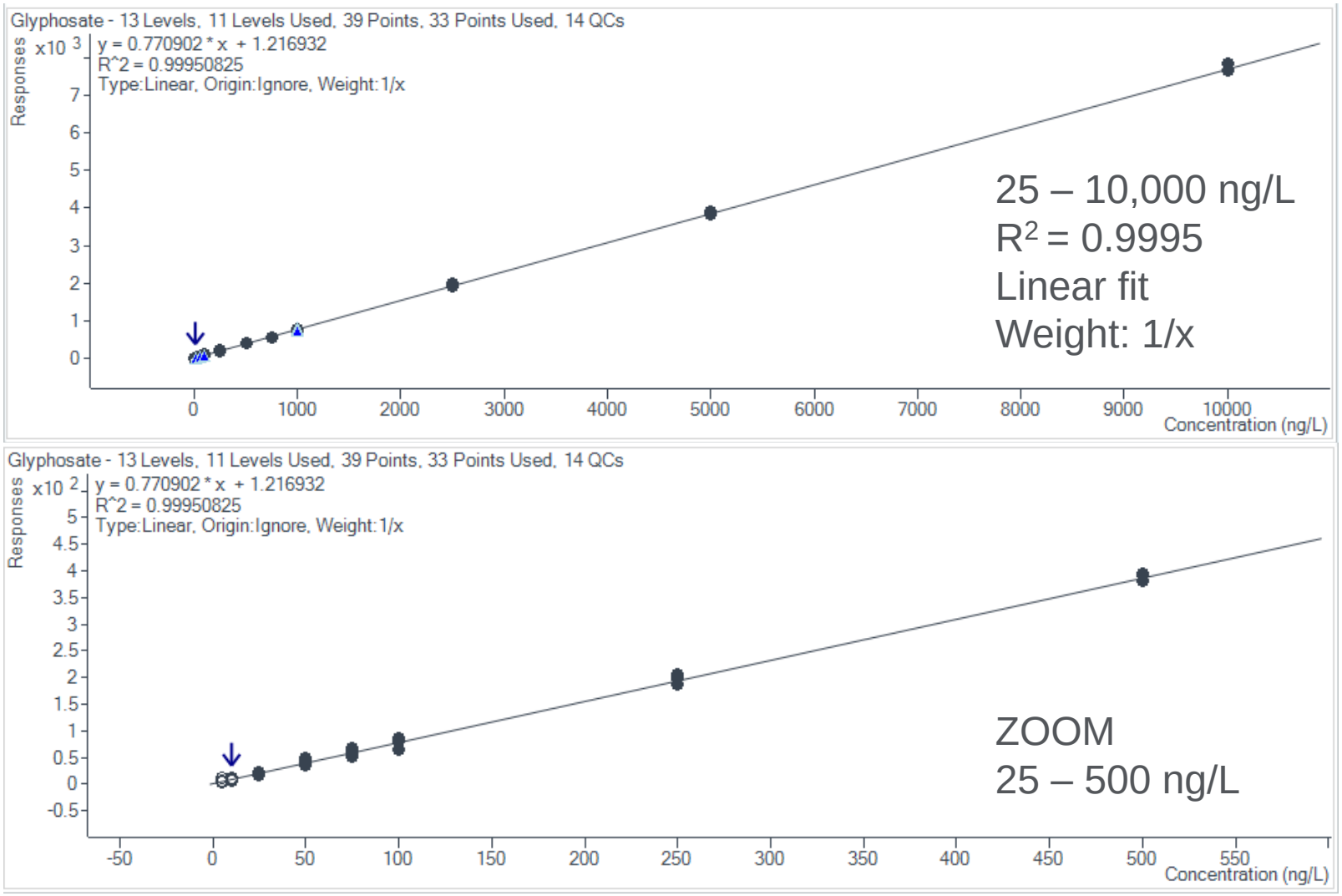
Agilent

Results – Sensitivity and Linearity

Agilent InfinityLab

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

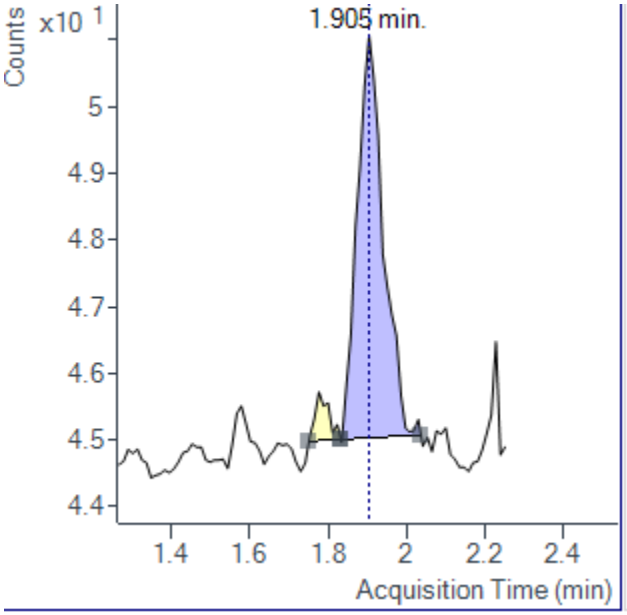
Results - Glyphosate

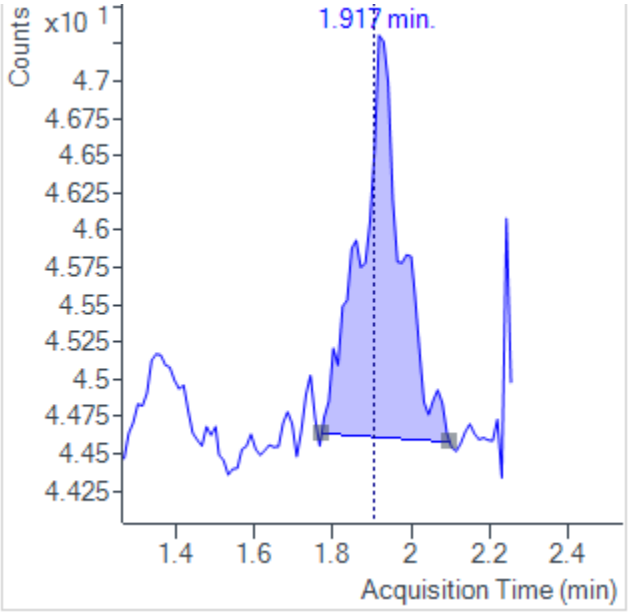


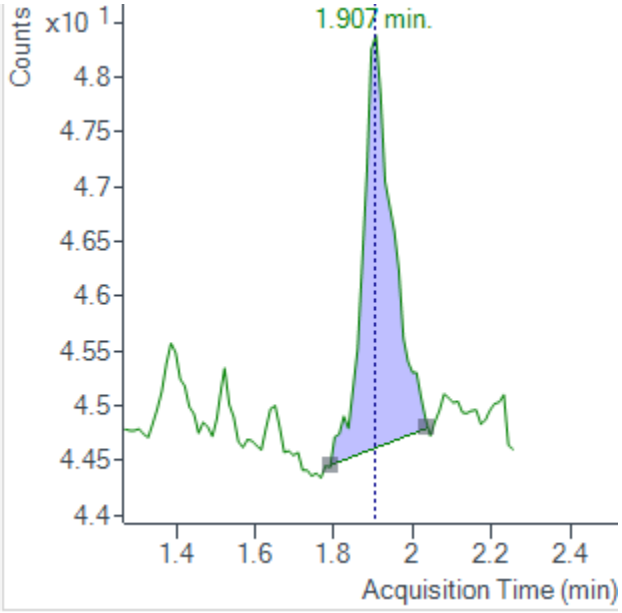
Results - Glyphosate

Agilent InfinityLab

Limit of Quantitation (LOQ) = 25 ng/L (ppt)







Quantifier transition

Qualifier transition

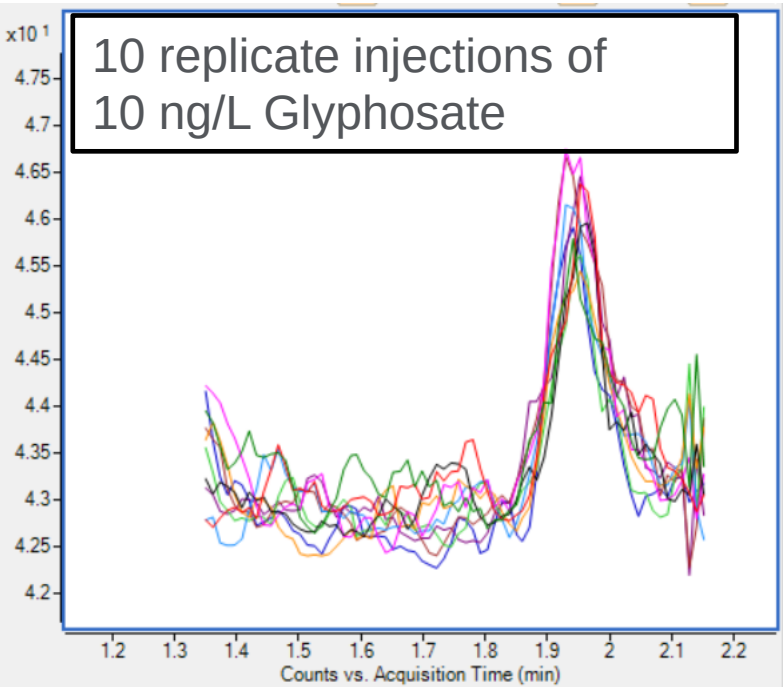
Qualifier transition

Agilent

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



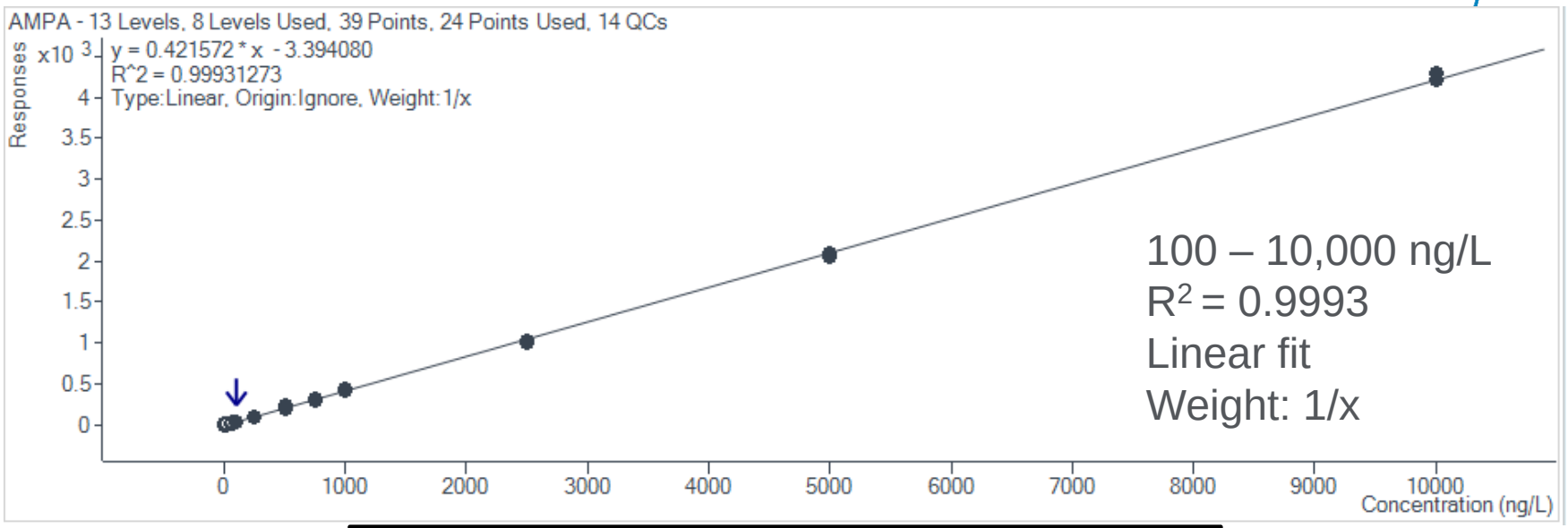
Results - Glyphosate

Agilent InfinityLab

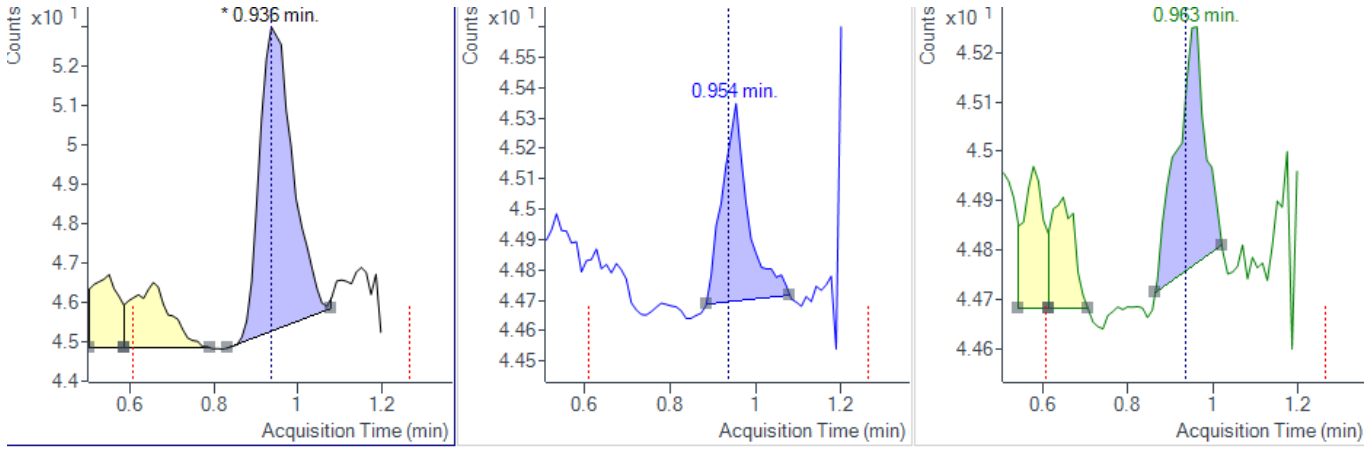
			Column 1	Column 2	Column 3	Column 4
		ng/L (on-column)	10	10	10	10
		Replicate #	**User Input Response (no manual integration)**			
Minimum	*Recommended*	Replicate1	13	15	20	11
		Replicate2	19	30	15	12
		Replicate3	15	26	25	10
		Replicate4	17	14	16	15
		Replicate5	16	20	18	11
		Replicate6	16	13	11	15
		Replicate7	29	25	10	12
		Replicate8	23	15	10	11
		Replicate9	14	22	14	7
		Replicate10	21	17	16	9
		Calculated Parameters				
		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

Results - AMPA

Agilent InfinityLab



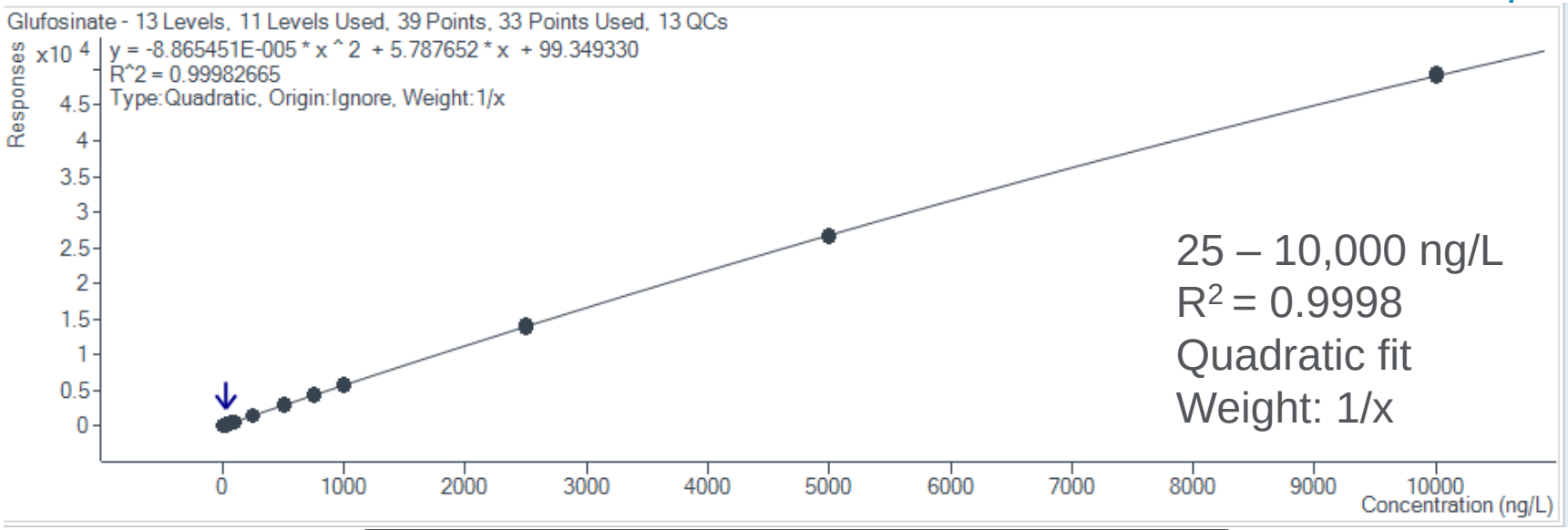
Limit of Quantitation (LOQ) = 100 ng/L (ppt)



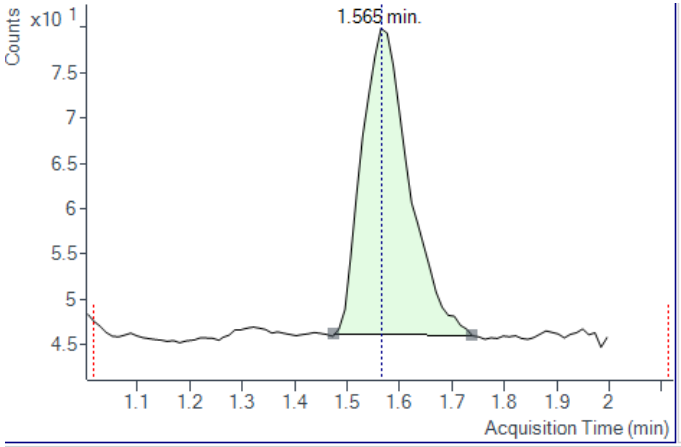
Quantifier transition | Qualifier transition | Qualifier transition

Results - Glufosinate

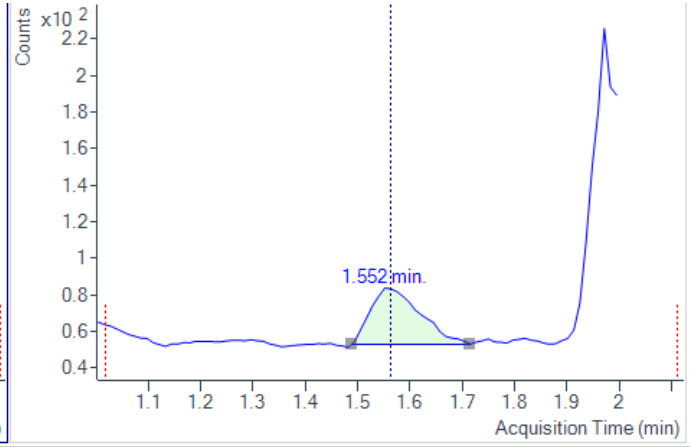
Agilent InfinityLab



Limit of Quantitation (LOQ) = 25 ng/L (ppt)



Quantifier transition

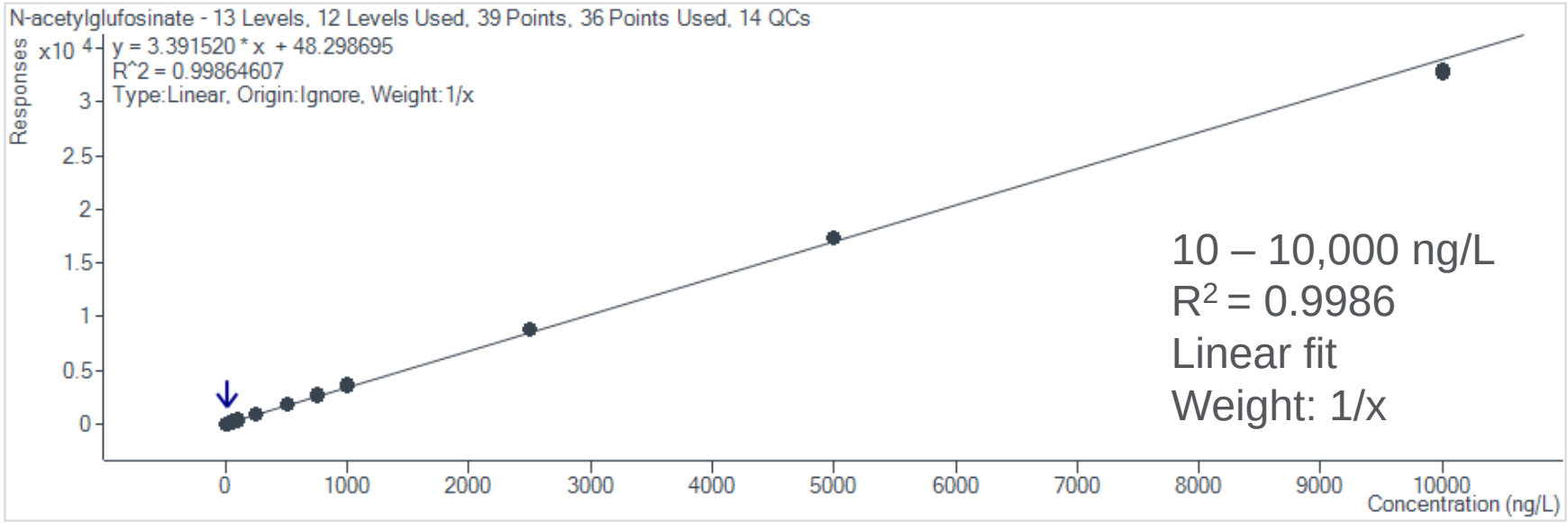


Qualifier transition

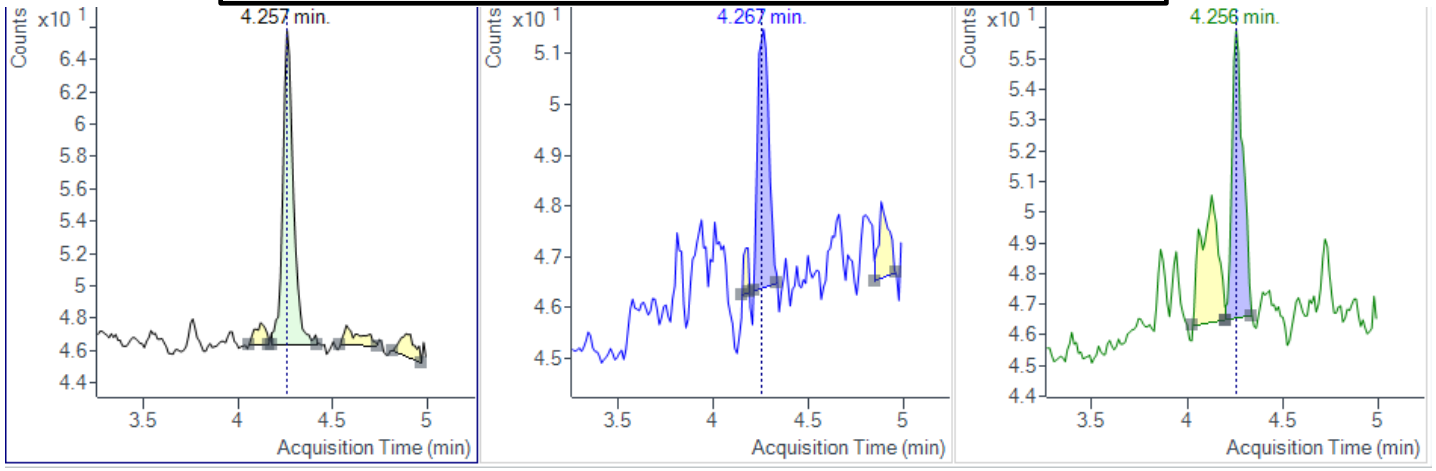
Agilent

Results - NAG

Agilent InfinityLab



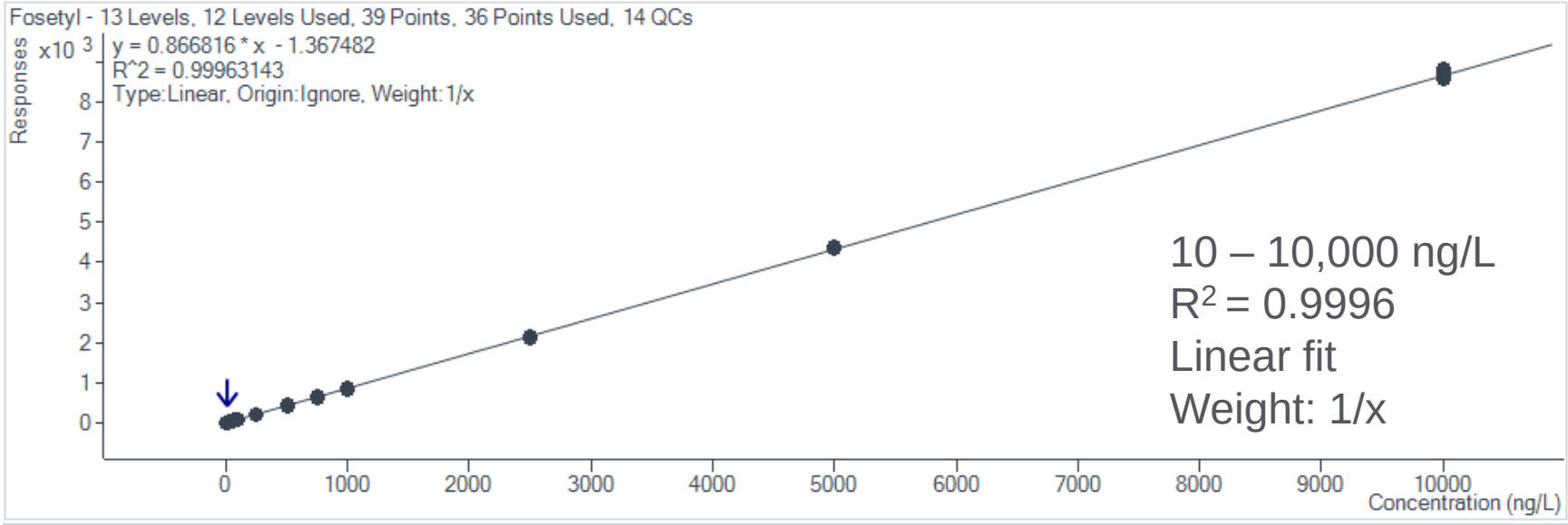
Limit of Quantitation (LOQ) = 10 ng/L (ppt)



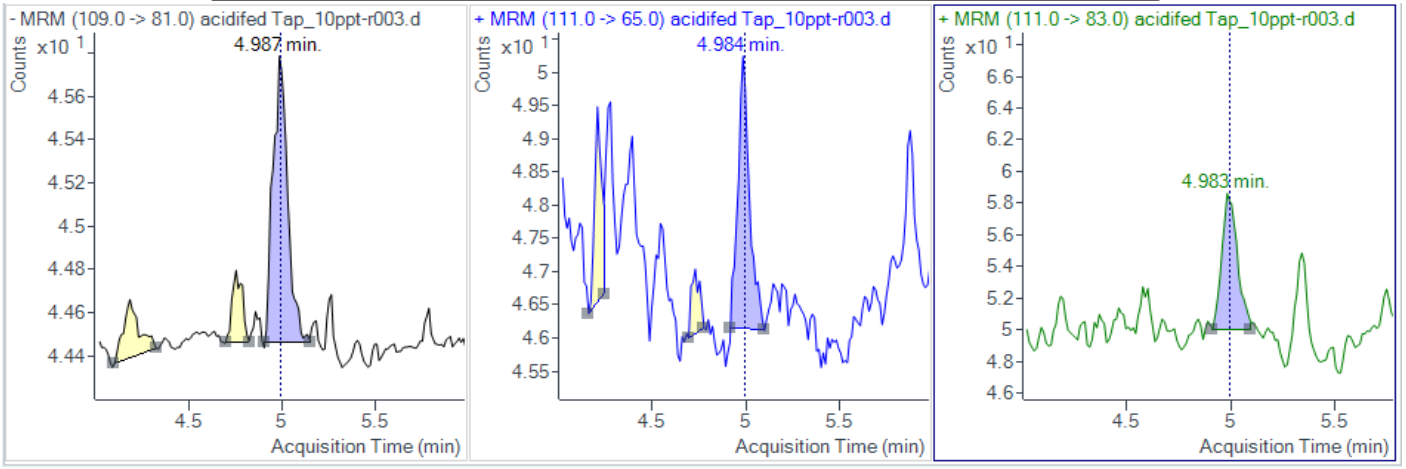
Quantifier transition | Qualifier transition | Qualifier transition

Results - Fosetyl

Agilent InfinityLab

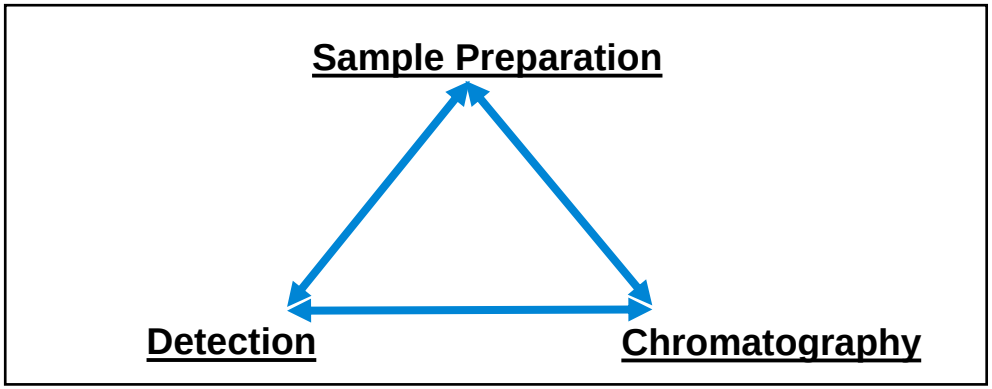


Limit of Quantitation (LOQ) = 10 ng/L (ppt)



Quantifier transition | Qualifier transition | Qualifier transition

Summary



- Polar pesticide analysis has come a long way with advancements in sample prep., & analysis
- Very quick and simple sample preparation, acidification identical to mobile phase system
- Sources of potentially problematic trace metal are removed from flow path by using PEEK components; any remaining trace is chelated with Deactivator Additive, which does not accumulate in system and is not detrimental to positive or negative ionization
- 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, additional sensitivity can be achieved with a 6495

Additional Resources

Agilent InfinityLab

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