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

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Agilent InfinityLab

Agilent

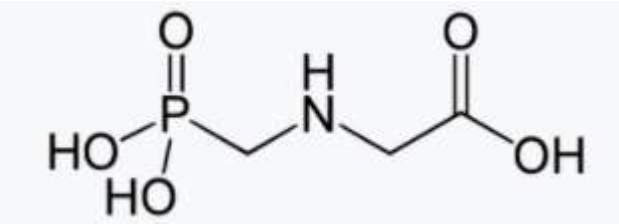
Glyphosate

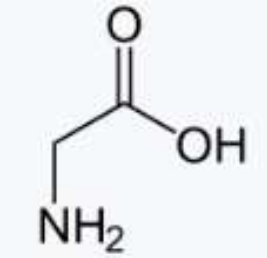
- Broad-spectrum herbicide first patented in the 1970s
- Roundup Ready™ crops introduced in the mid-1990s
- Widely used in fields and backyards

Pest Manag. Sci. 64:319–325 (2008)

The Challenging Analysis of Glyphosate

1. Highly Polar





- Synthetic amino acid
- Glycine analogue

Glyphosate **Glycine**

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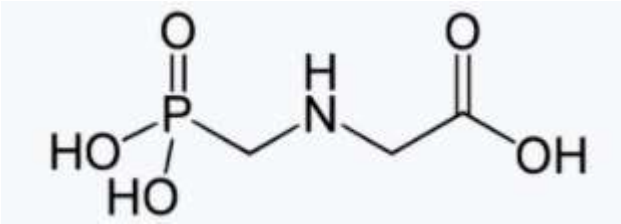

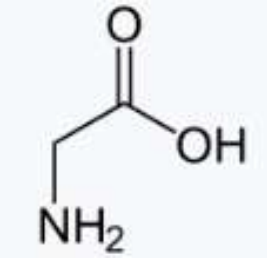

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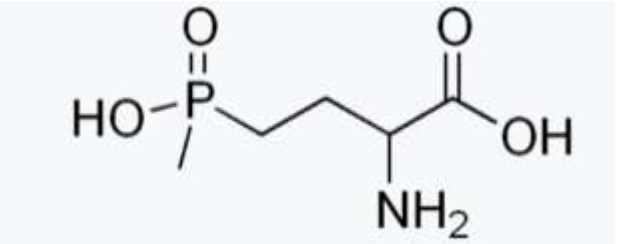

Glufosinate

The Challenging Analysis of Glyphosate

1. Highly Polar

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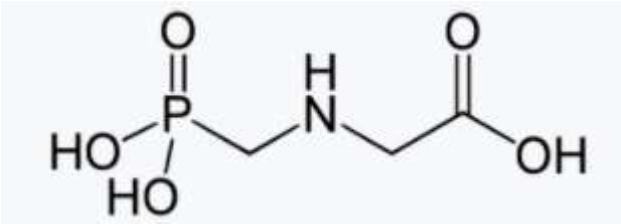
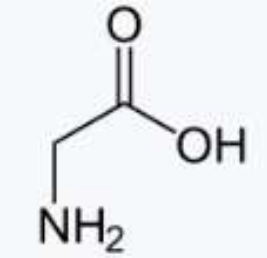
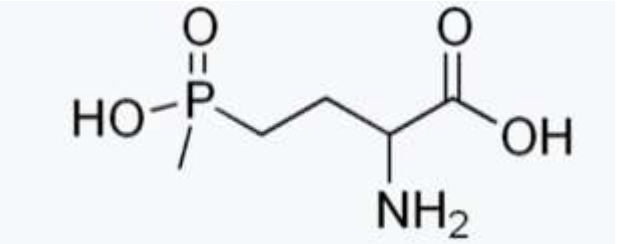
- Synthetic amino acid
- Glycine analogue
- Amino acid synthesis inhibitor



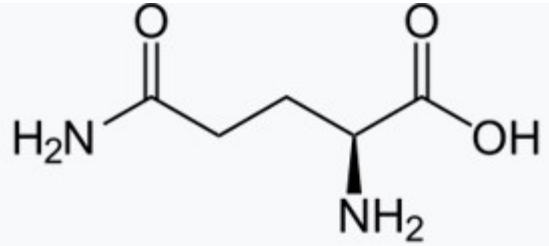
Glyphosate



Glycine



Glufosinate



Glutamine

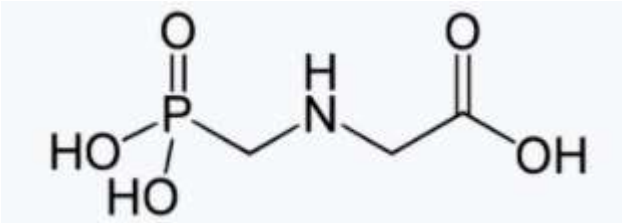
Agilent

The Challenging Analysis of Glyphosate

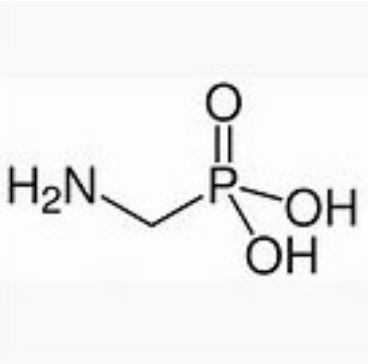
1. Highly Polar

Agilent InfinityLab

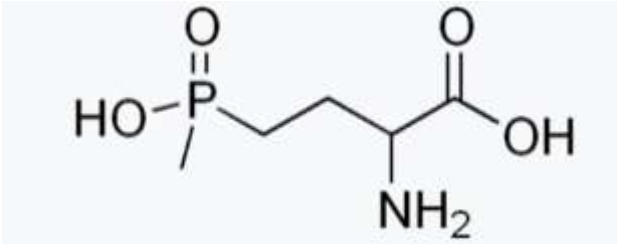
- Synthetic amino acid
- Glycine analogue
- Amino acid synthesis inhibitor
- Metabolized by bacteria in plants, soil and water



Glyphosate



AMPA
(Aminomethylphosphonic acid)



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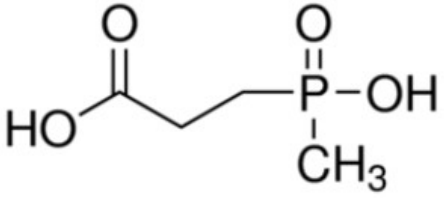

MPPA
(3-(methylphosphinico)propionic acid)

The Challenging Analysis of Glyphosate

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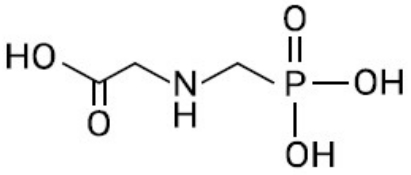
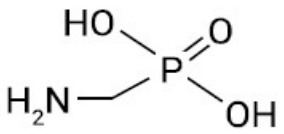
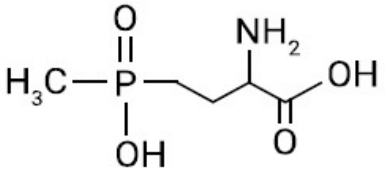


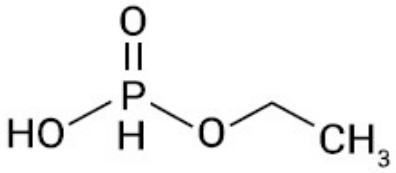
Glyphosate



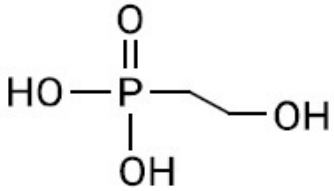
AMPA



Glufosinate

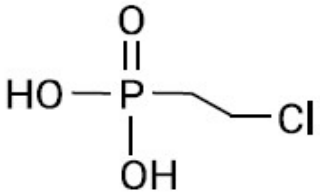


Fosetyl

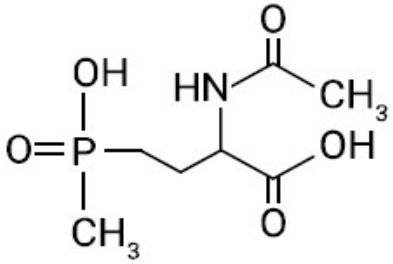


HEPA

2-hydroxyethyphosphonic acid

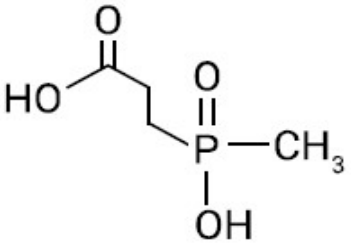


Ethephon



NAG

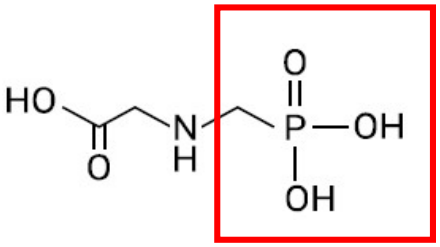
N-acetylglufosinate



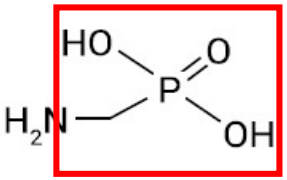
MPPA

The Challenging Analysis of Glyphosate

2. Chelating Agent



Glyphosate



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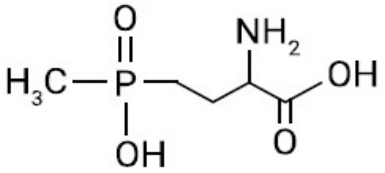
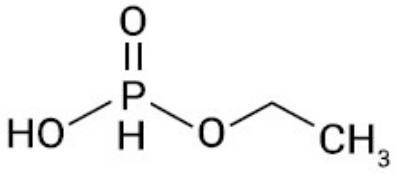
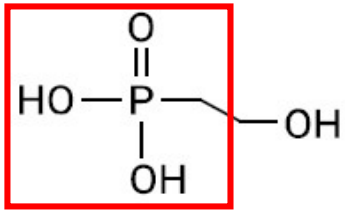


Glufosinate



Fosetyl



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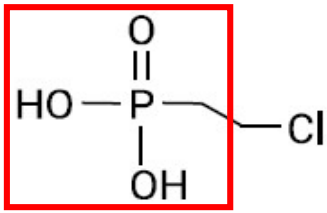

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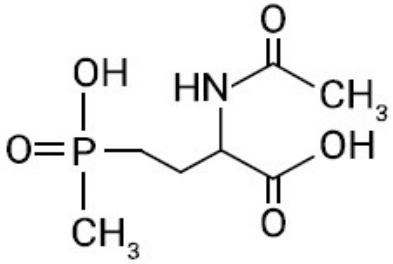

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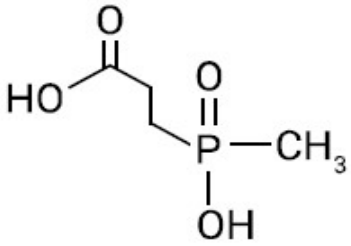

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The Challenging Analysis of Glyphosate

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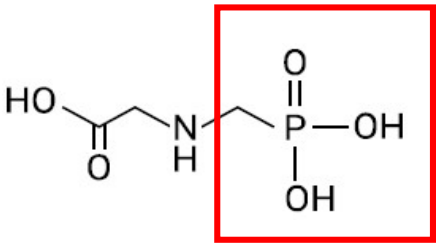
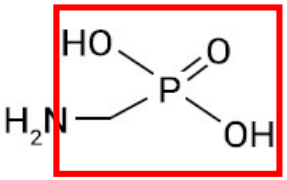
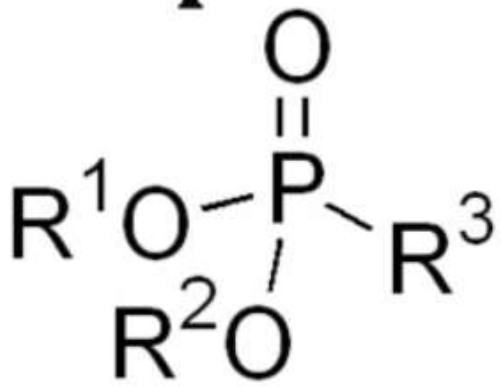


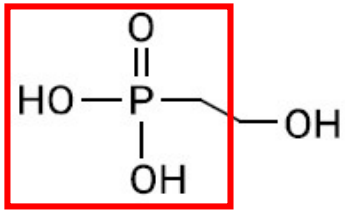
Glyphosate



AMPA

Phosphonate





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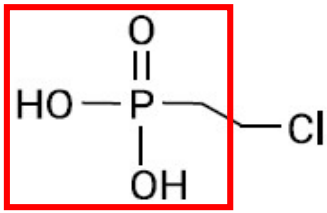


Ethephon

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

The Challenging Analysis of Glyphosate

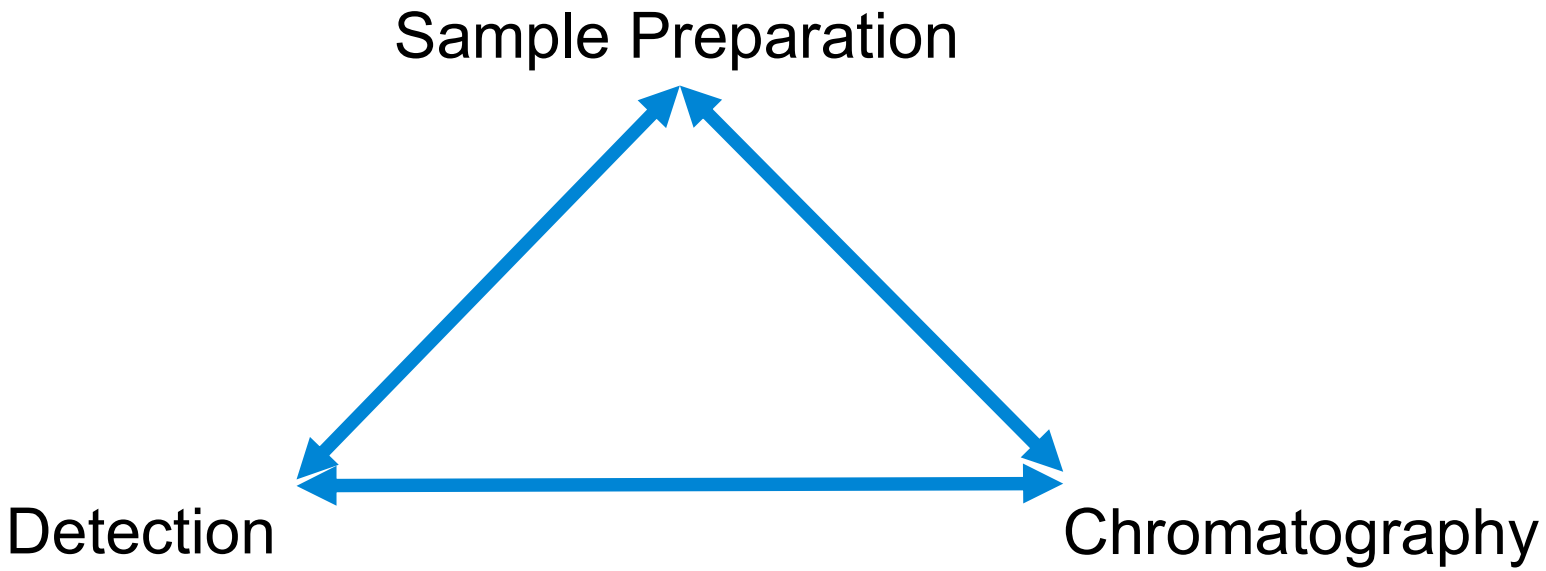
4. Separation Techniques in the Liquid Phase

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)

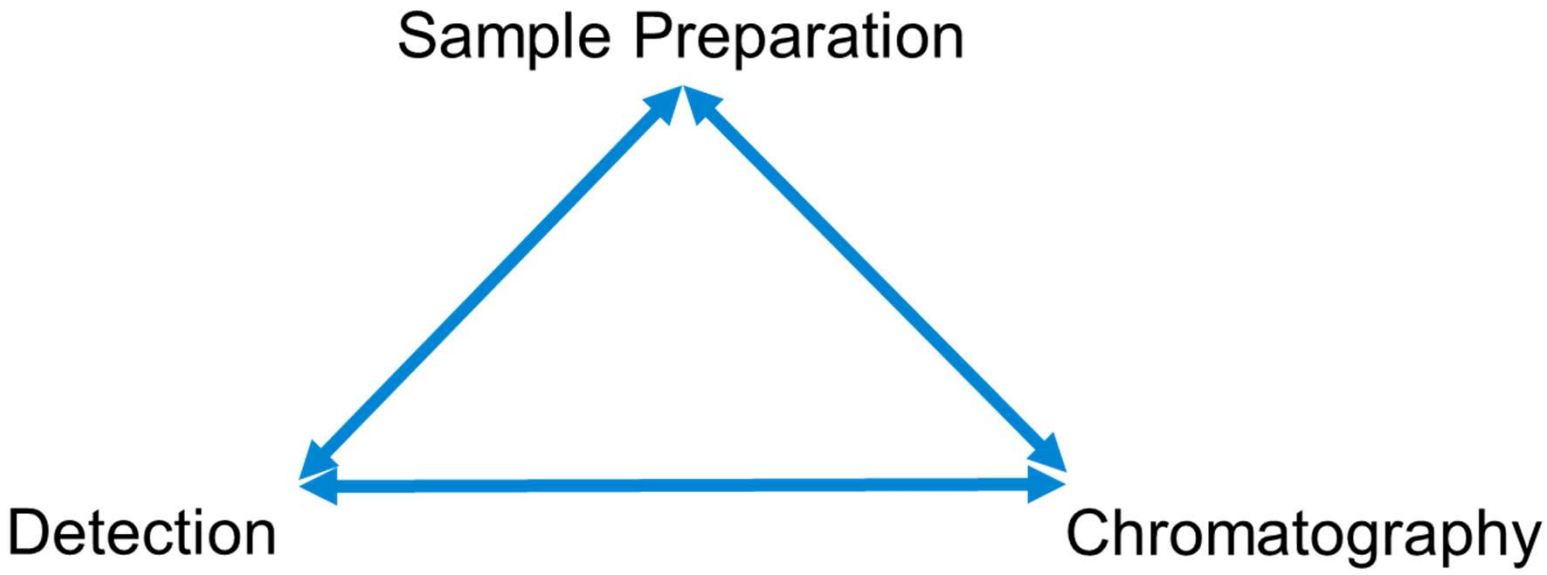
The Challenging Analysis of Glyphosate

The Three Interconnected Pillars

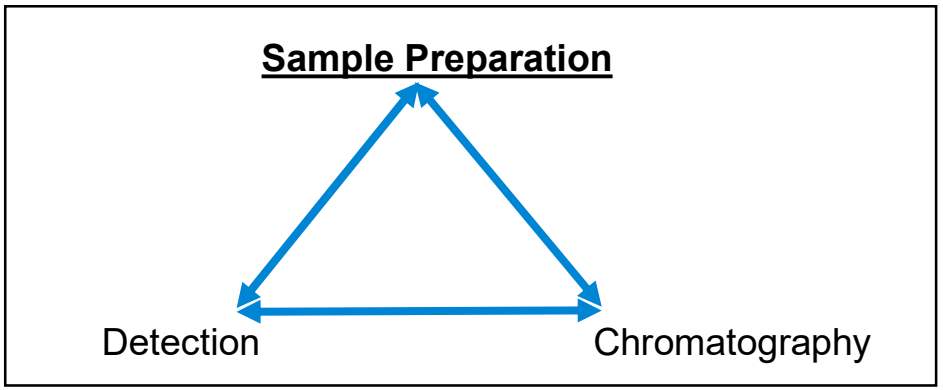


Objective

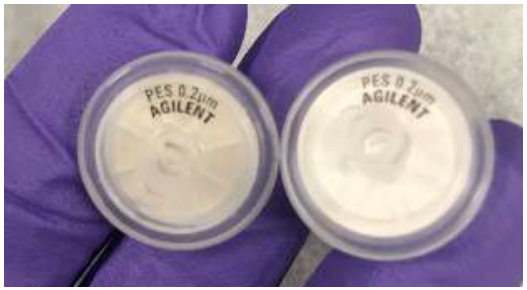
To develop a *sensitive* and *simple* methodology for the analysis of glyphosate and other polar pesticides, with perfectly aligned sample preparation, chromatography and mass spectrometry.



Sample Preparation – Surface Water

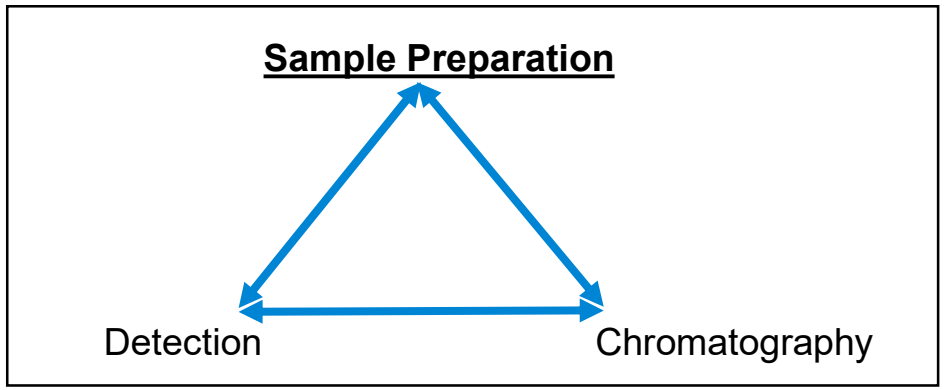


1. Collect in tube
2. Centrifuge at 5000 rpm for 5 min
3. Filter on 0.2 µm PES membrane



4. Acidify with concentrated formic acid (0.1 %)

Sample Preparation – Drinking Water

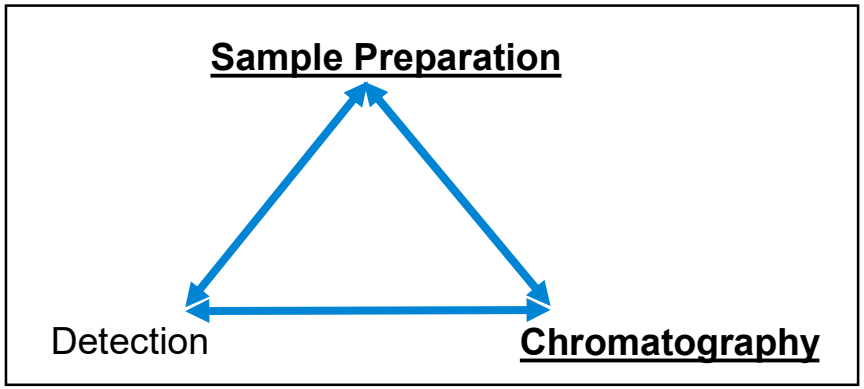


1. Filter on 0.2 µm PES membrane

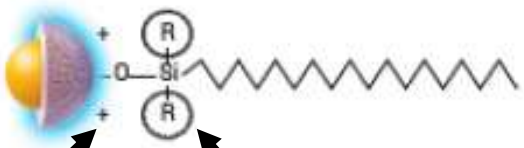
2. Acidify with concentrated formic acid (0.1%)

Chromatography

Introducing the InfinityLab Poroshell 120 CS-C18



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

Phosphonates are Metal Chelators

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

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analytical chemistry

Article

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

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Agilent Technologies, Santa Clara, California 95051, United States

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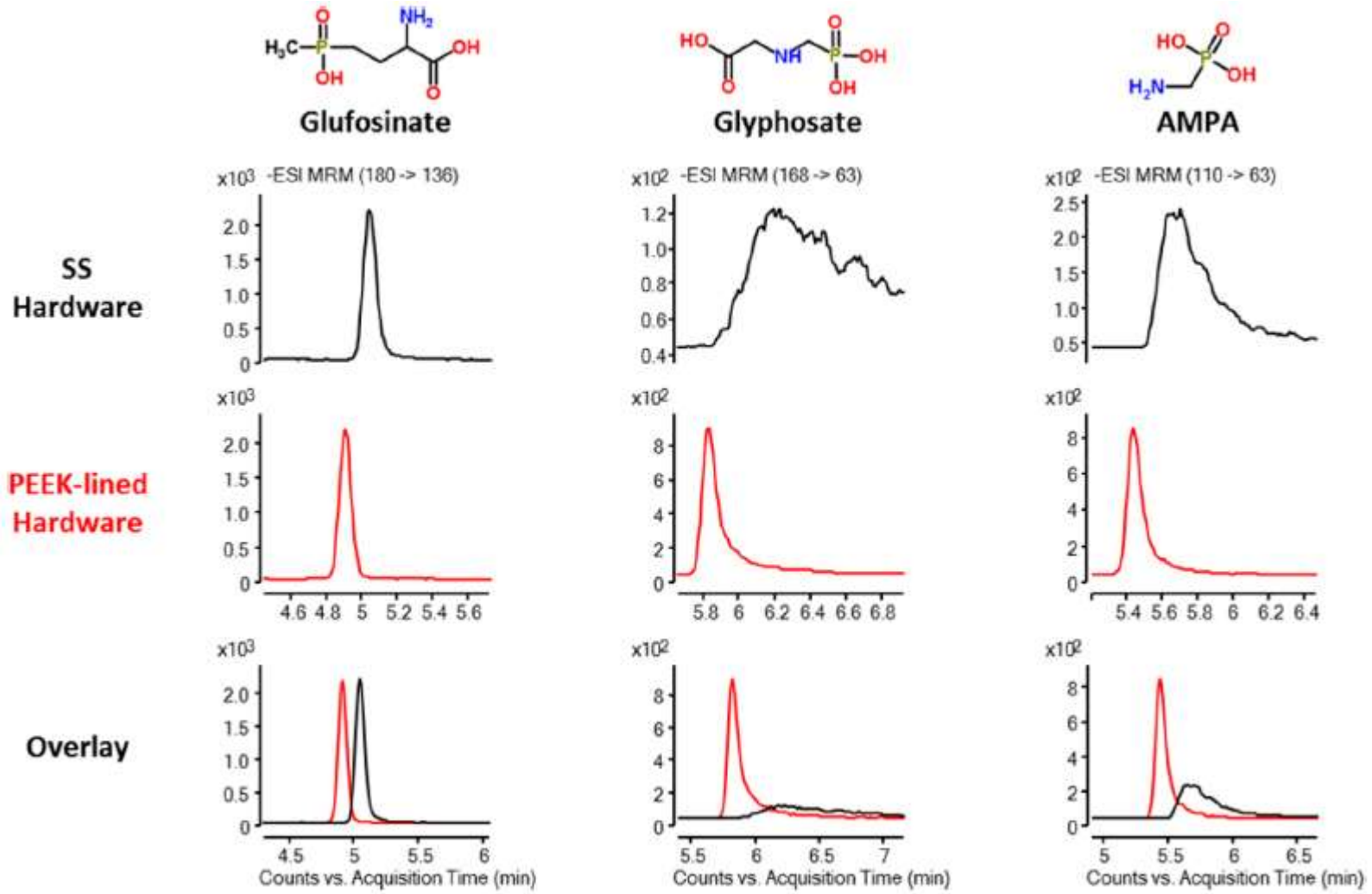
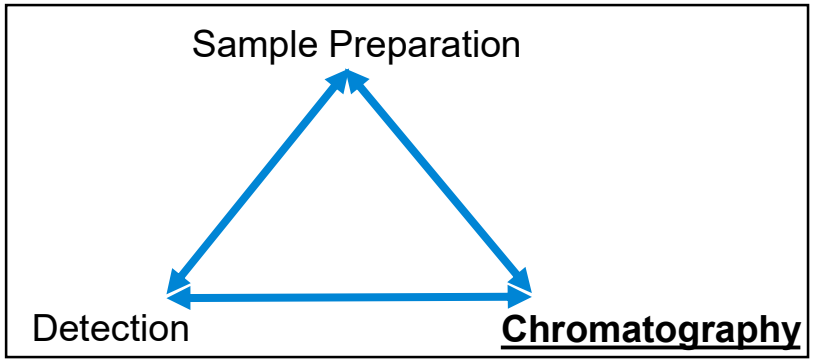
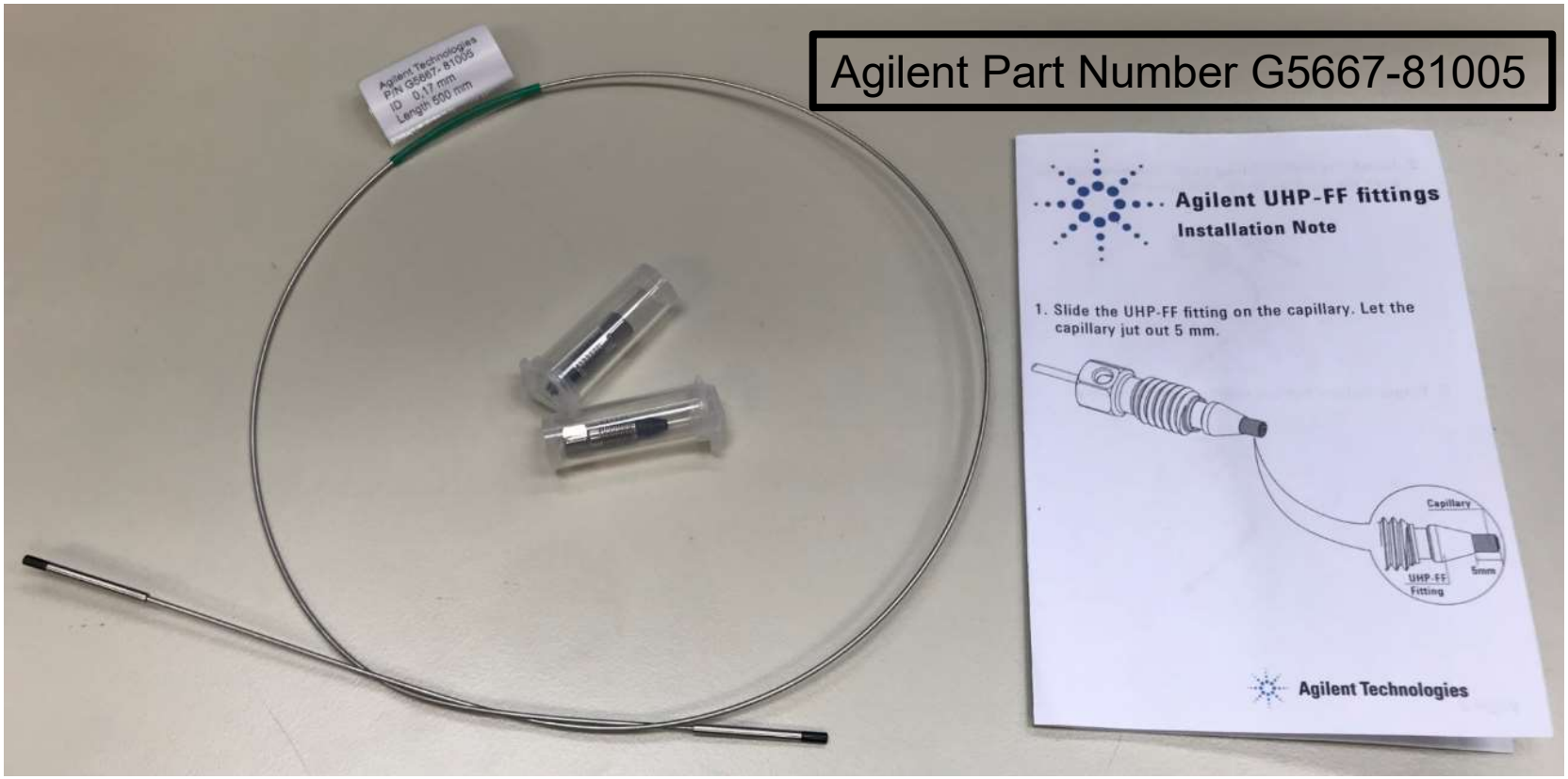


Anal. Chem. 2018, 90, 9457-9464

PEEK-lined Flow Path

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





PEEK-lined Flow Path

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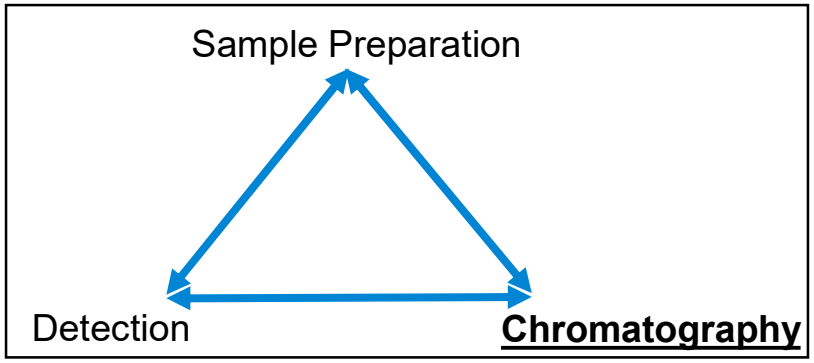
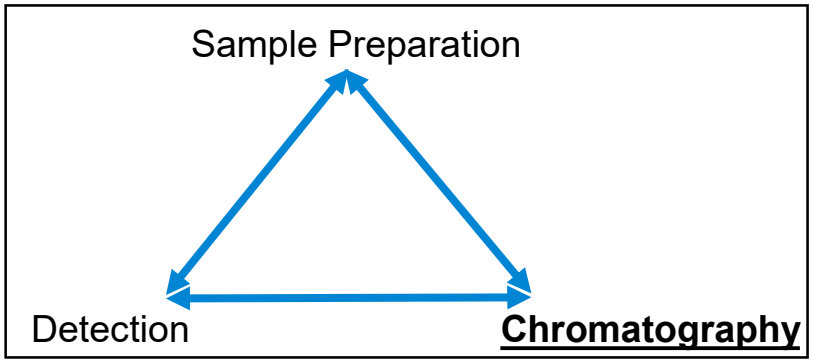
- InfinityLab Quick Turn Fitting with polymeric ferrule at Column Inlet



Agilent Part Number 5067-5966

PEEK-lined Flow Path

- PEEK needle seat and rotor seal
- PEEK-lined capillary from Multisampler to column inlet
- InfinityLab Quick Turn Fitting with polymeric ferrule at Column Inlet
- PEEK capillary from Column Outlet to MS



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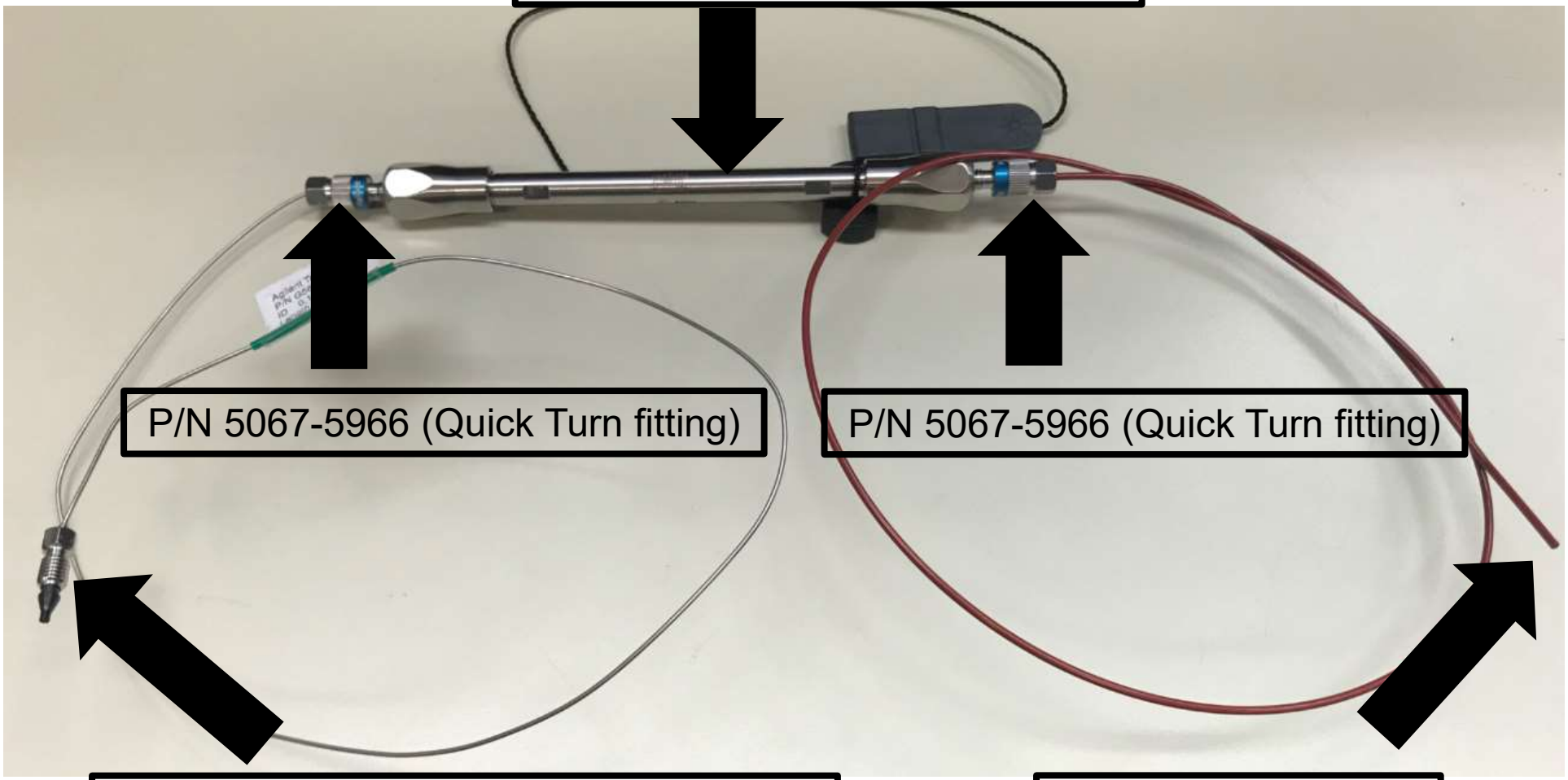
PEEK-lined Flow Path

Agilent InfinityLab



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Deactivator Additive in Mobile Phase

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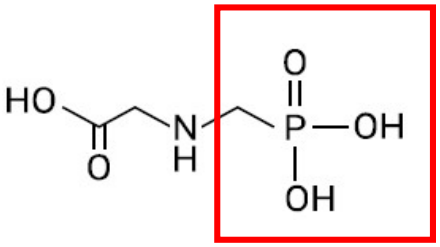
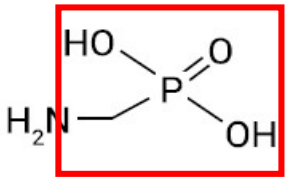
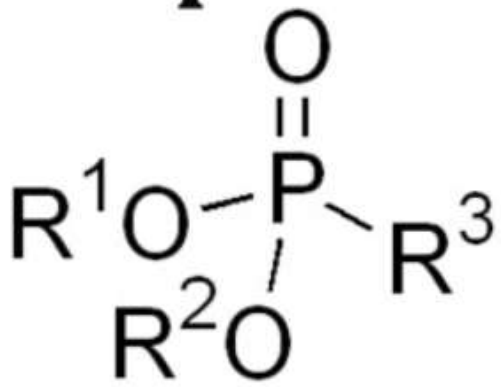


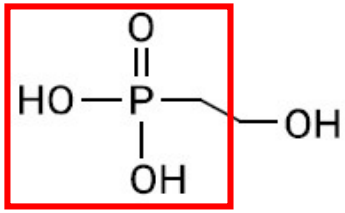
Glyphosate



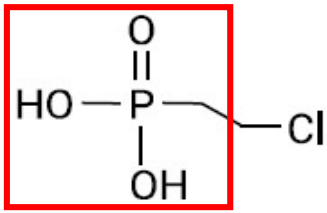
AMPA

Phosphonate





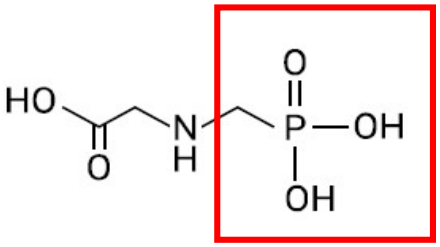
HEPA



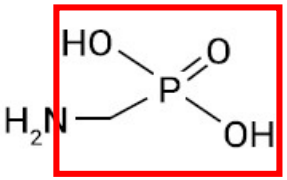
Ethephon

Phosphonates are Metal Chelators

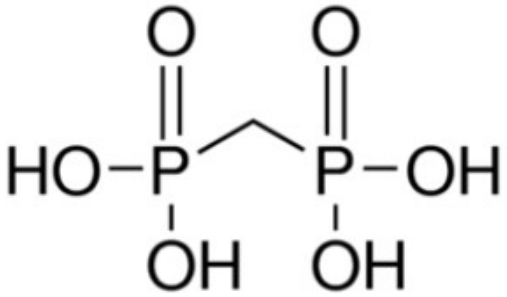
Deactivator Additive in Mobile Phase



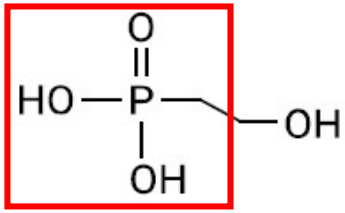
Glyphosate



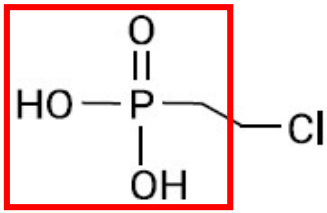
AMPA



Deactivator Additive
Agilent Part Number 5191-4506



HEPA



Ethephon

Hardware

1290 Infinity II Series UHPLC

- Multicolumn thermostat
- Multisampler with multiwash function
- Binary pump with built-in degasser

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

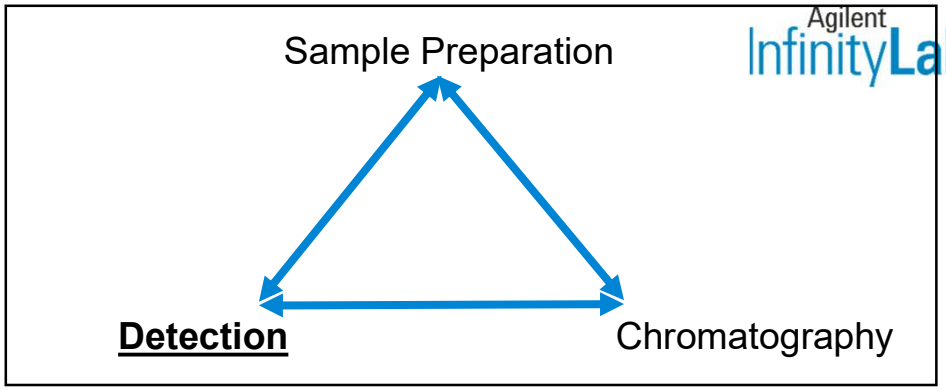
Mass Spec Settings

- Acquisition Mode : Dynamic MRM (dMRM)
- Source: Agilent JetStream ESI
- Polarity: Positive (+) or Negative (-)
- Capillary Voltage: 3000 V (+), 3500 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: 1500 V (+), 800 V(-)
- Q1 and Q2 Resolution: Unit (0.7 amu), optimized by Autotune
- Delta EMV: 0 V

Mass Spec Settings

Key point:

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



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

AMPA: 0.95 min

Glufosinate: 1.6 min

Glyphosate: 1.9 min

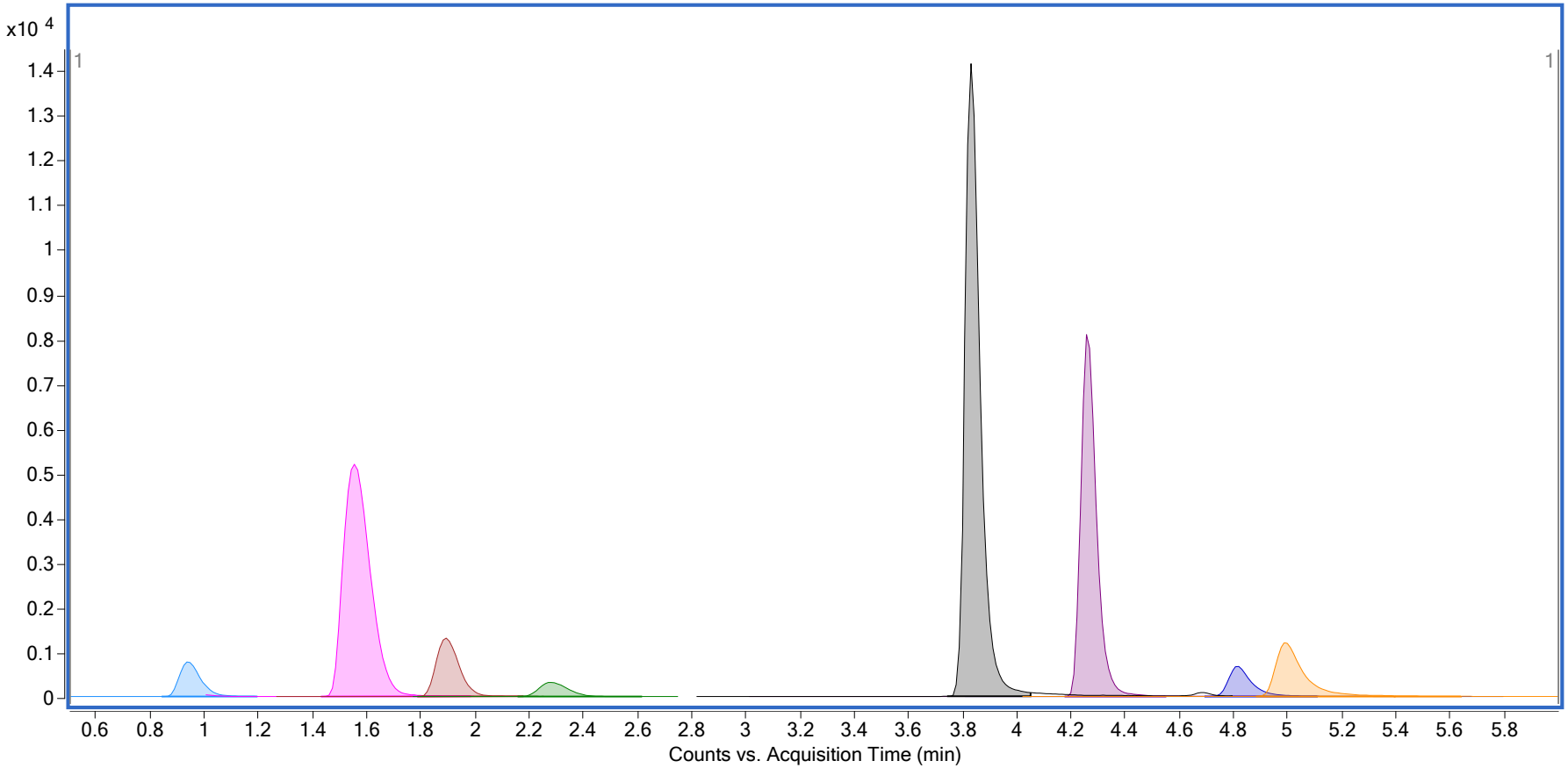
HEPA: 2.3 min

MPPA: 3.8 min

N-acetylglufosinate: 4.3 min

Ethephon: 4.8 min

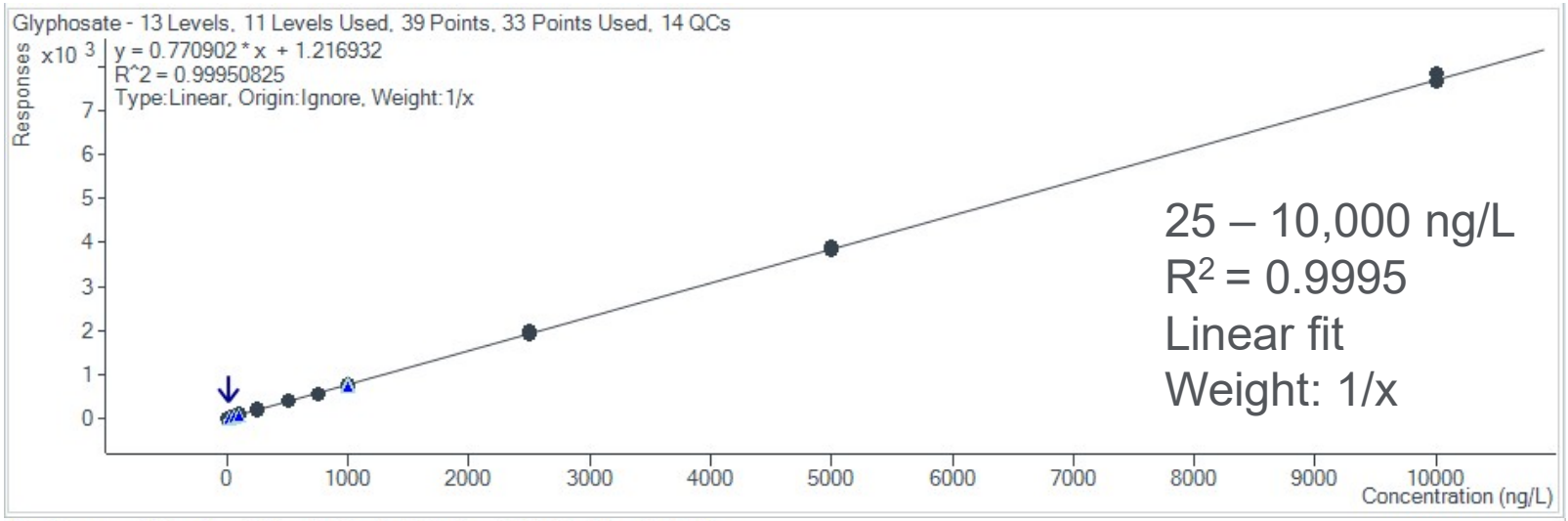
Fosetyl: 5.0 min

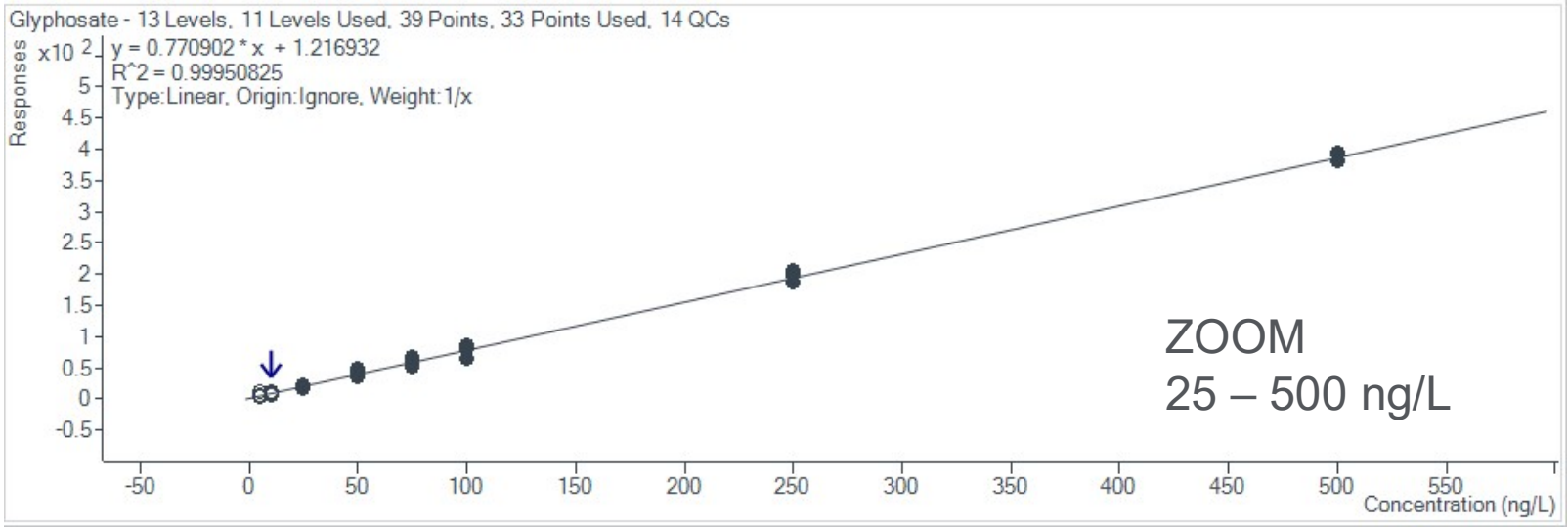


Results – Sensitivity and Linearity

		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

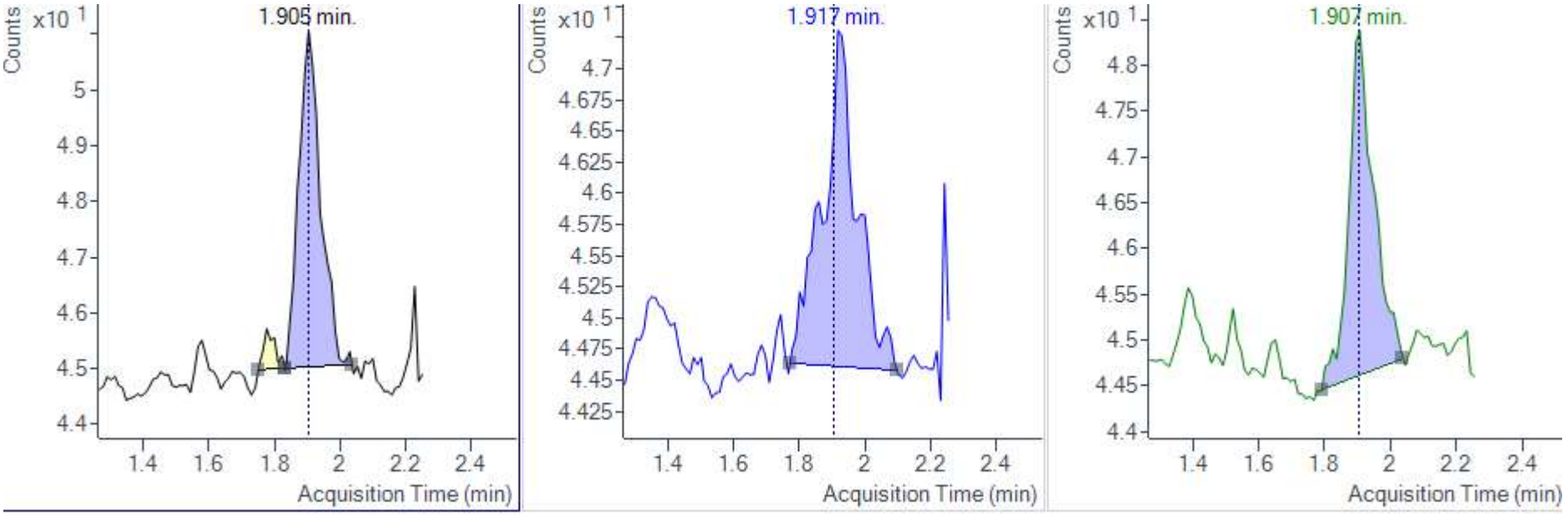




Results - Glyphosate

Agilent InfinityLab

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



Quantifier transition

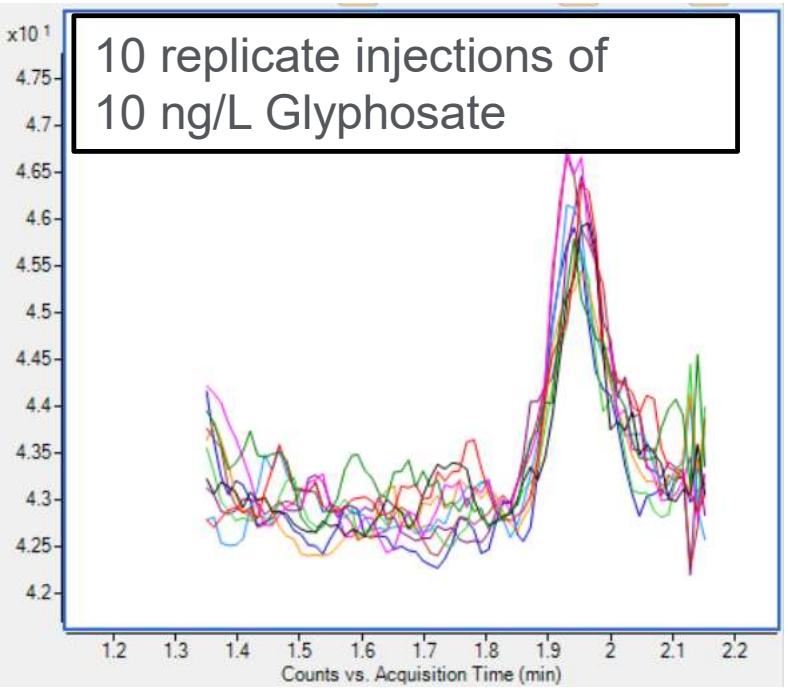
Qualifier transition

Qualifier transition

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

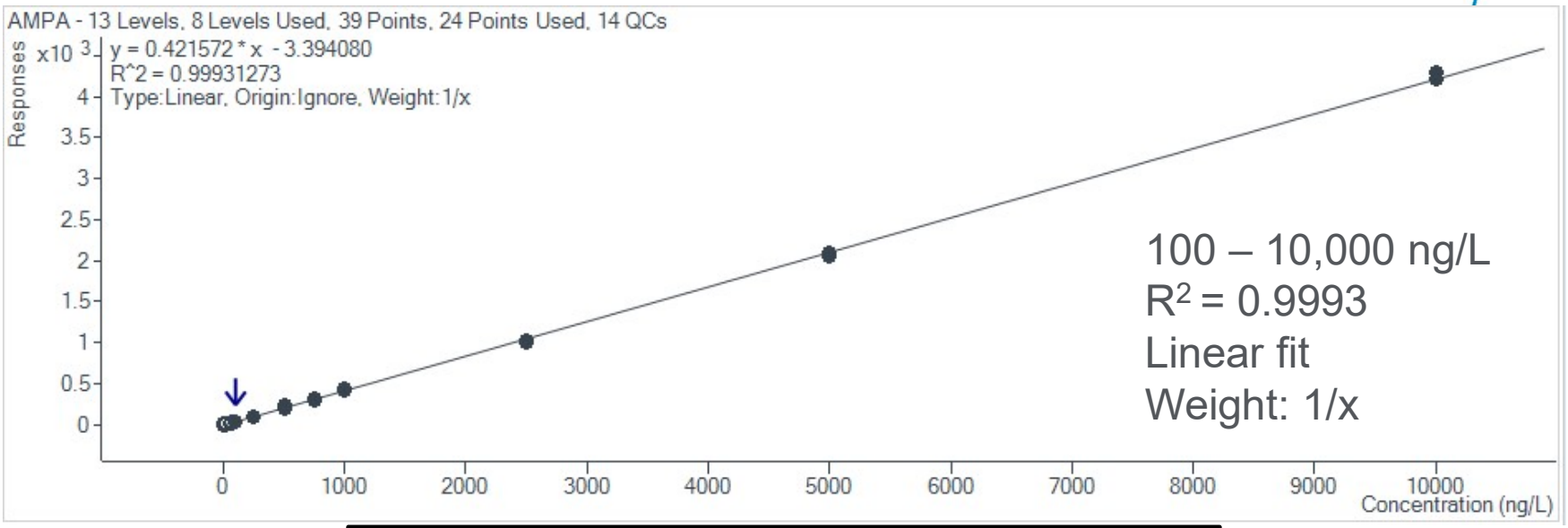


Results - Glyphosate

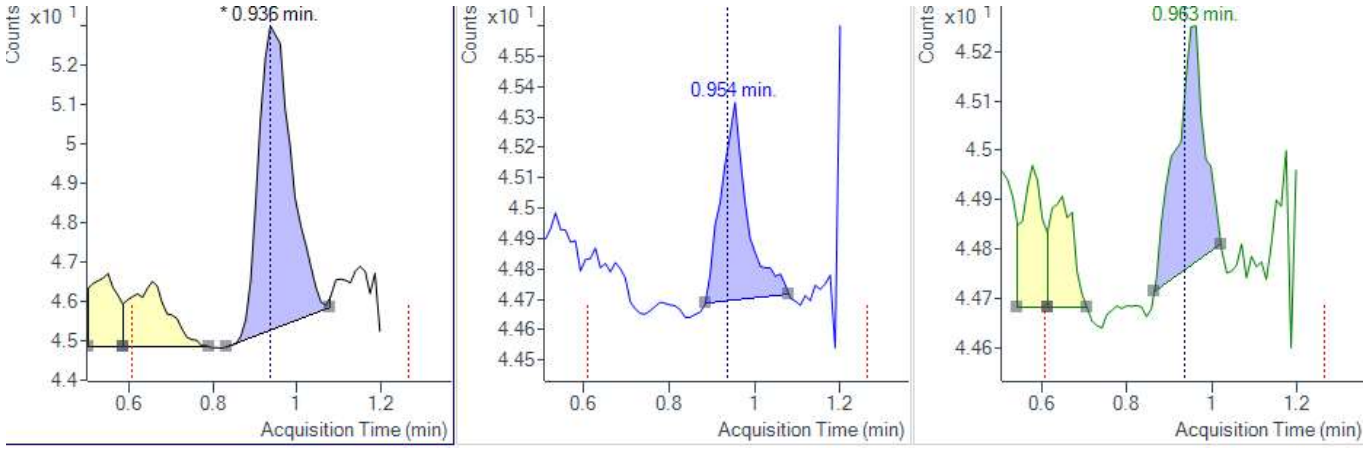
Agilent InfinityLab

			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

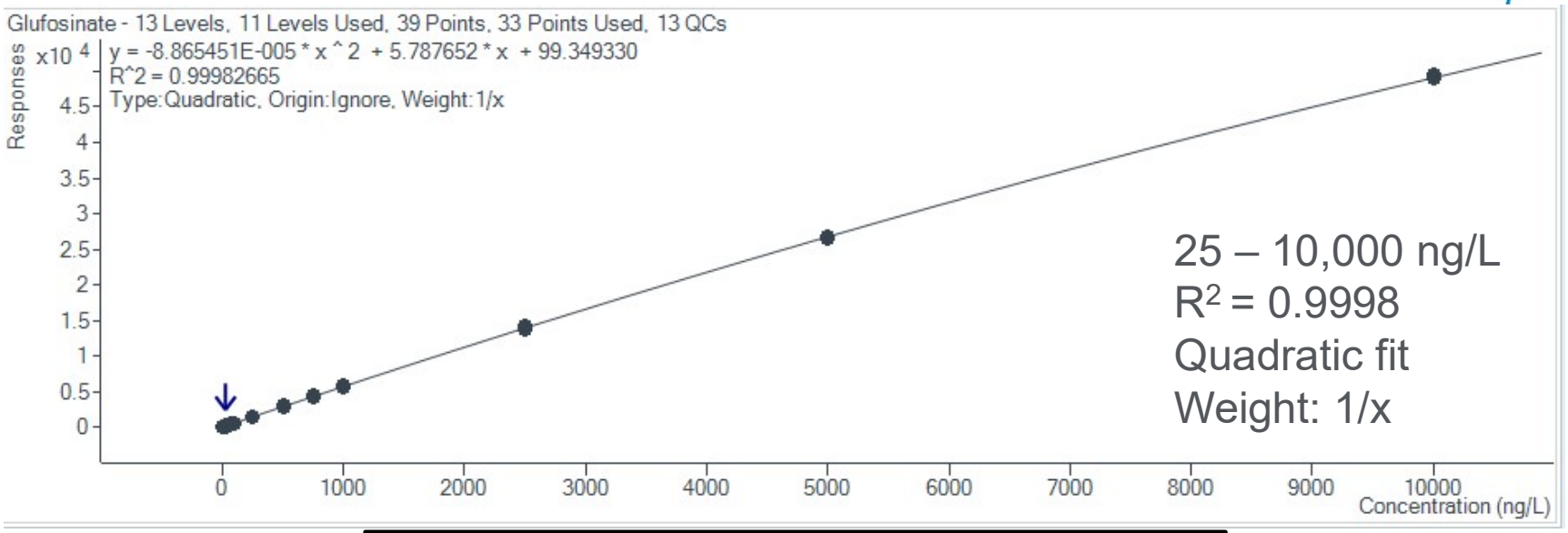


Limit of Quantitation (LOQ) = 100 ng/L (ppt)

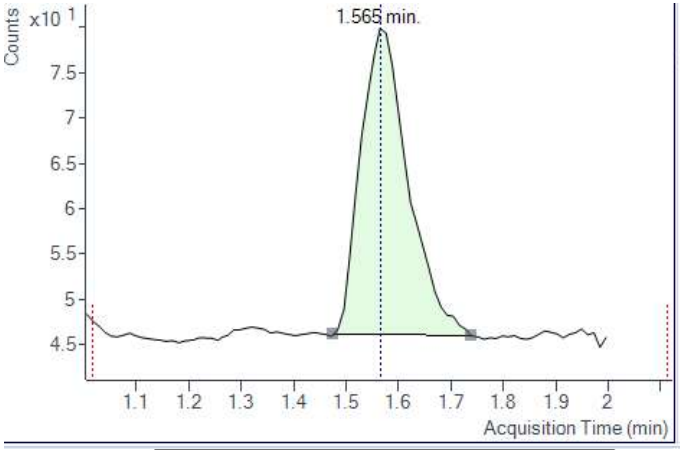


Quantifier transition | Qualifier transition | Qualifier transition

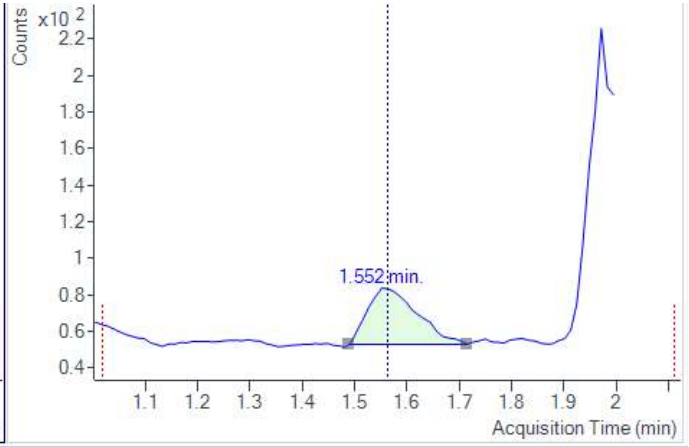
Results - Glufosinate



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

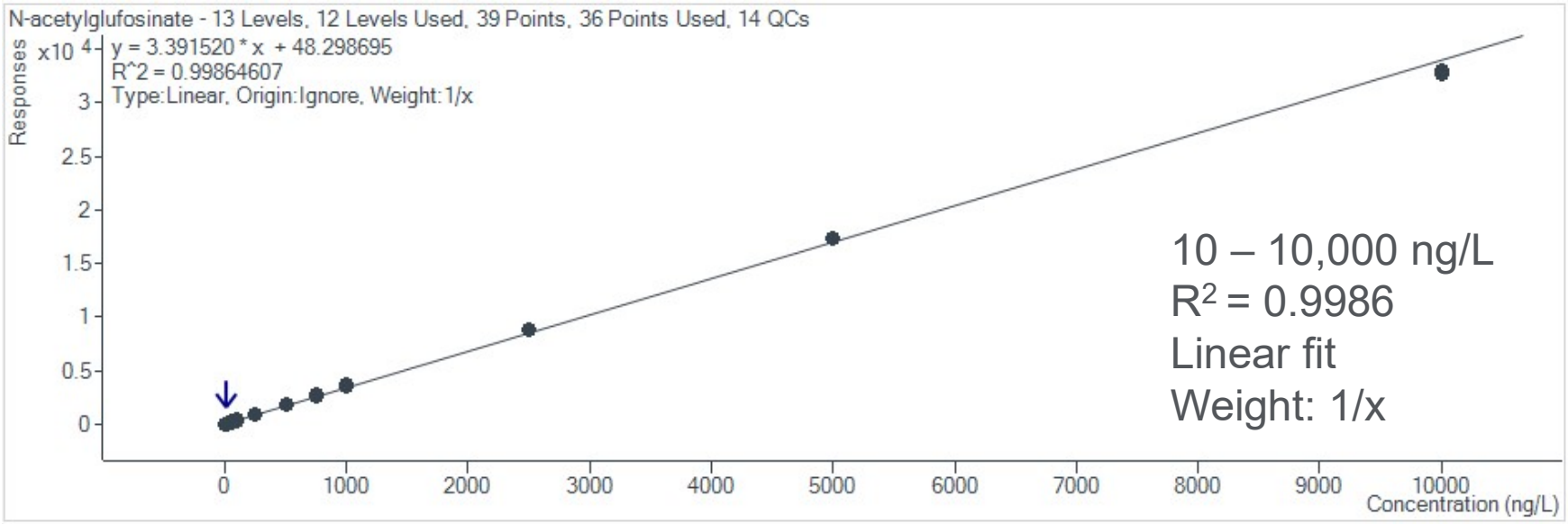


Quantifier transition

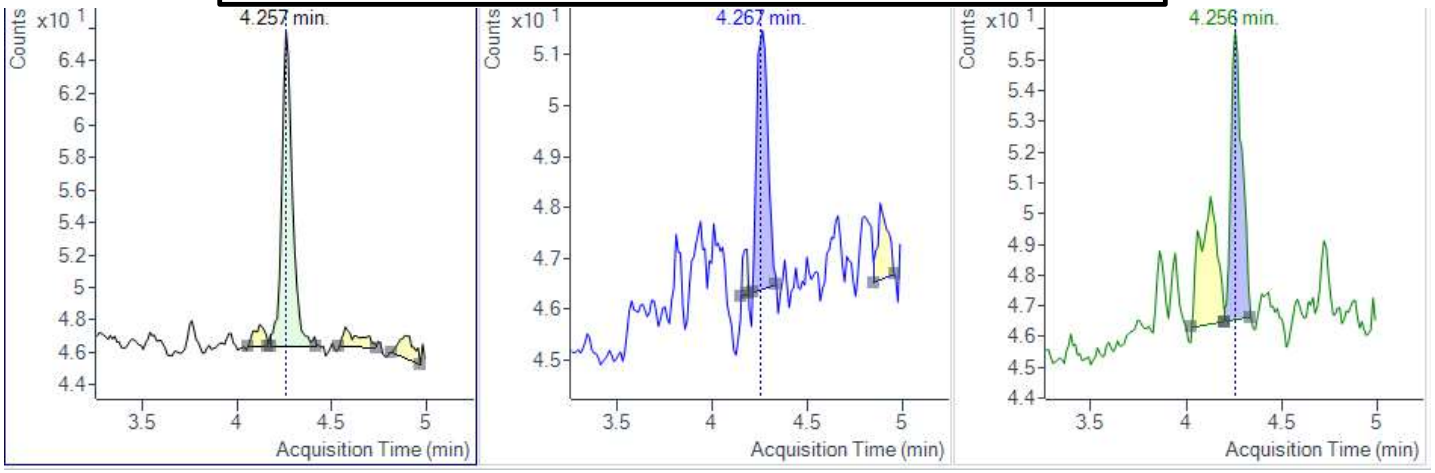


Qualifier transition

Results - NAG

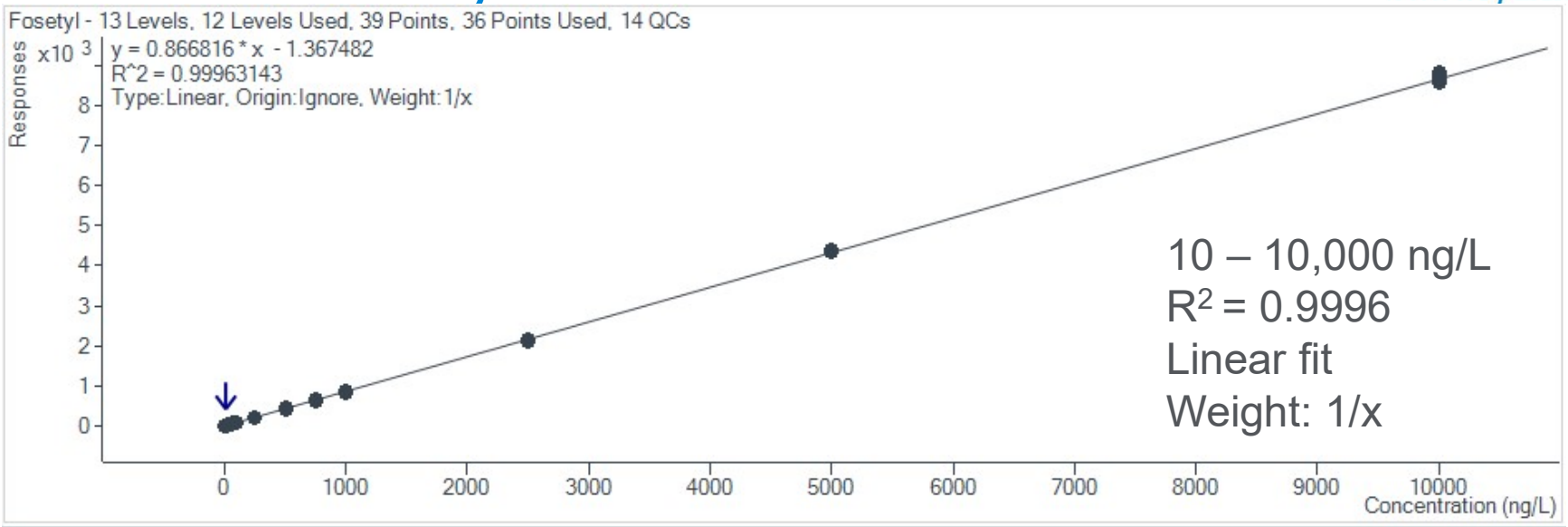


Limit of Quantitation (LOQ) = 10 ng/L (ppt)

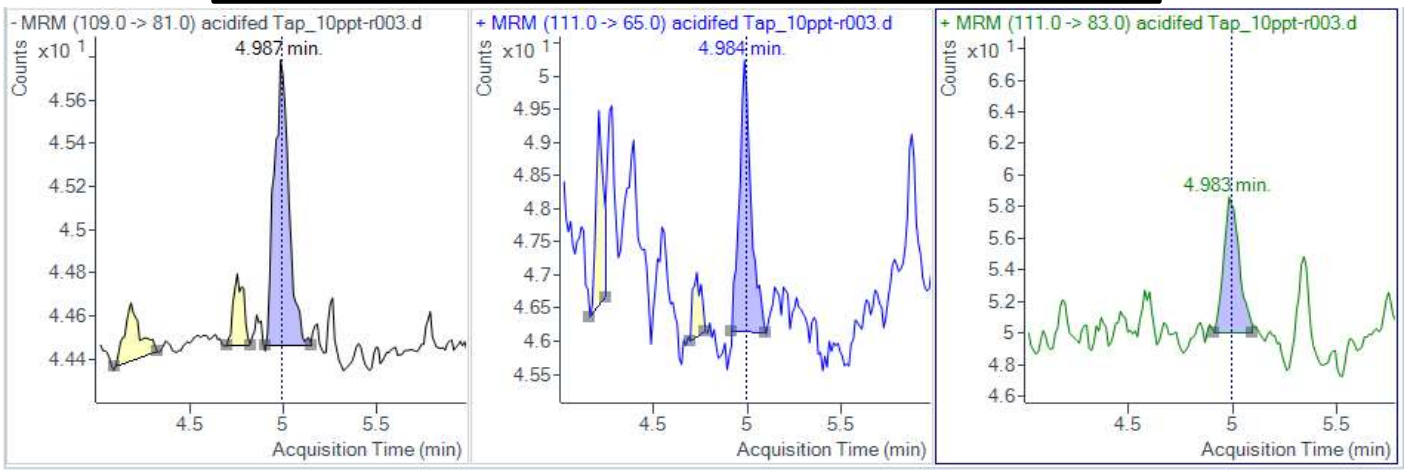


Quantifier transition | Qualifier transition | Qualifier transition

Results - Fosetyl

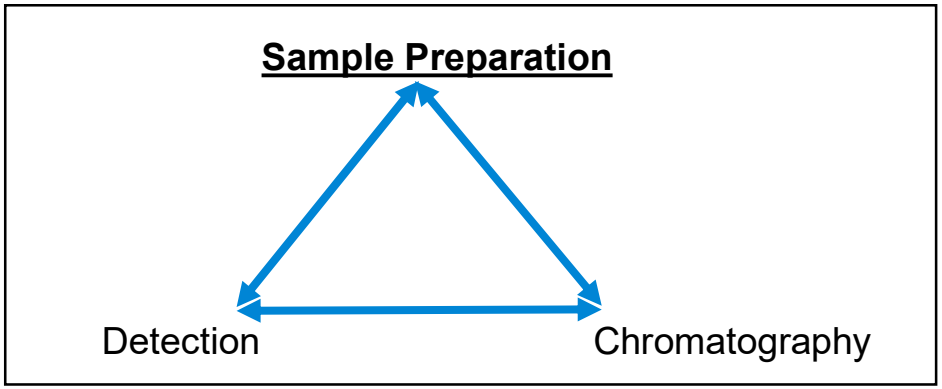


Limit of Quantitation (LOQ) = 10 ng/L (ppt)

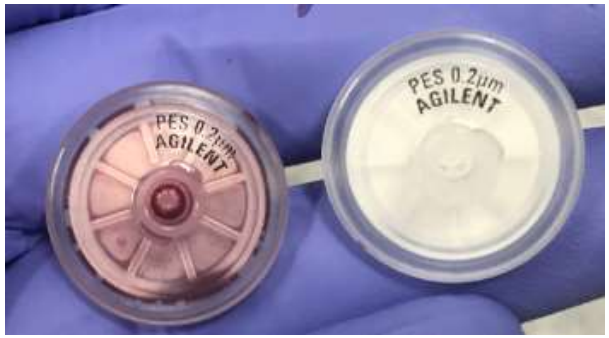


Quantifier transition | Qualifier transition | Qualifier transition

Sample Preparation – Wine



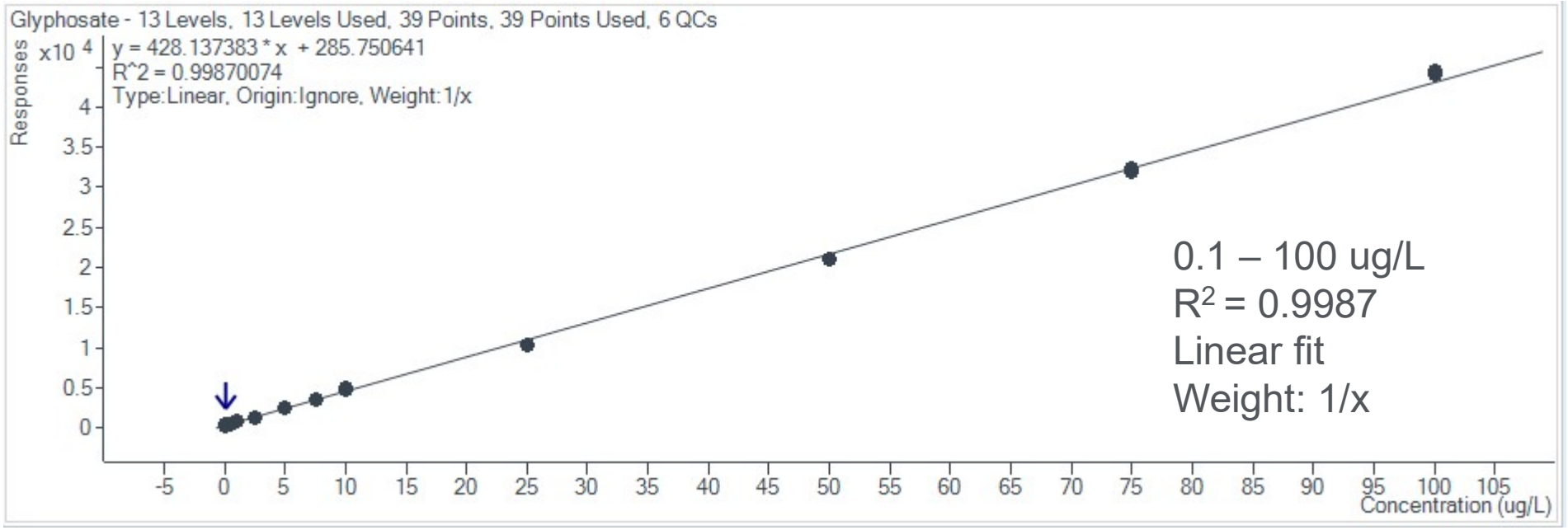
1. Filter on 0.2 µm PES membrane



2. Dilute 1 part of filtered wine with 9 parts of Type-1 water

3. Acidify with concentrated formic acid (0.1 %)

Results – Wine



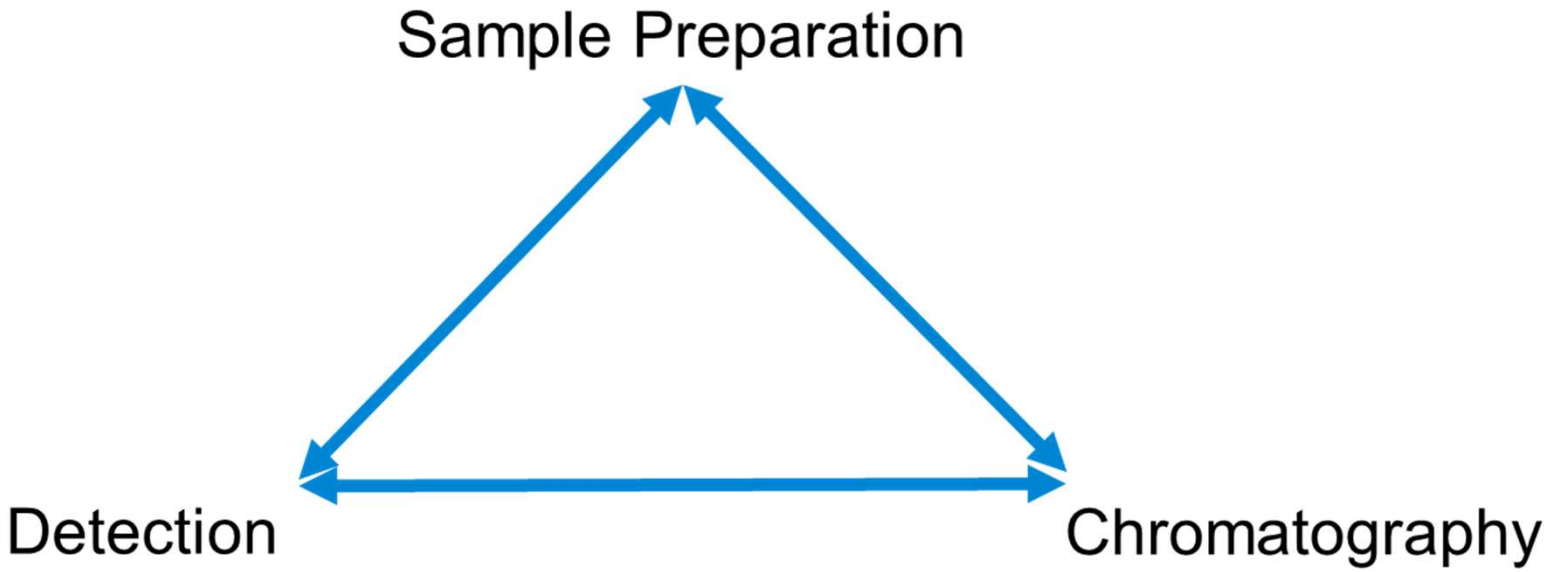
Compound	LOQ in vial (ug/L)	LOQ in wine (ug/L)
AMPA	1	10
Glufosinate	0.25	2.5
Glyphosate	0.1	1
HEPA	1	10
MPPA	0.25	2.5
NAG	0.1	1
Ethephon	0.25	2.5
Fosetyl	0.1	1

x10

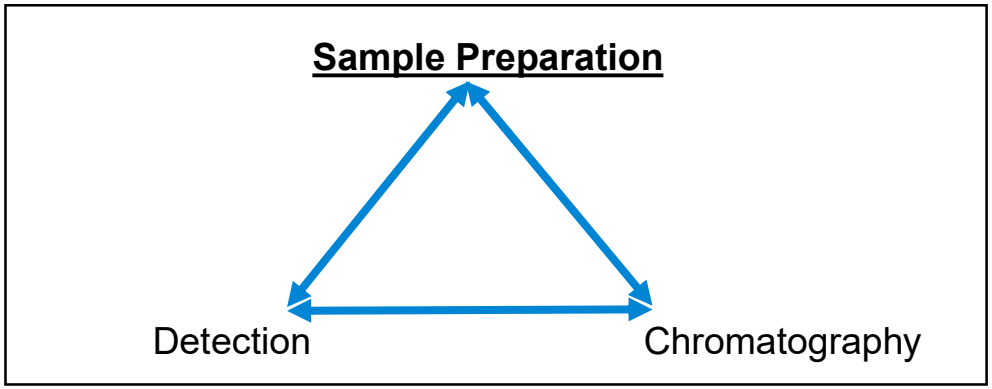
Need to account for dilution in LOQ calculation!

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:

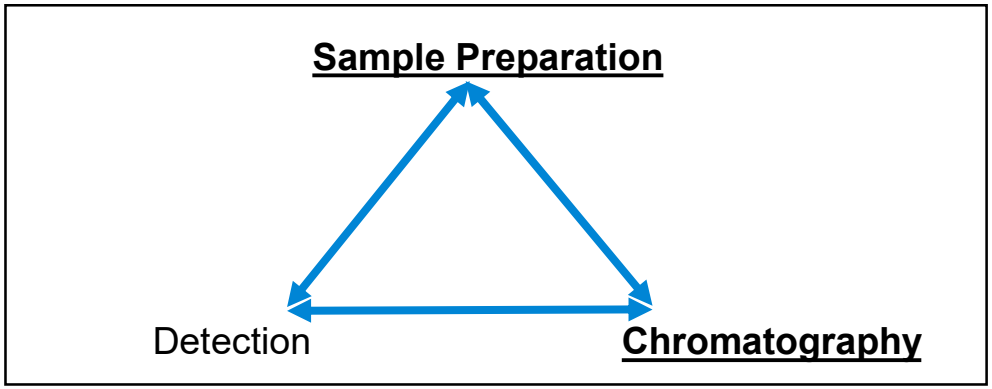


Summary



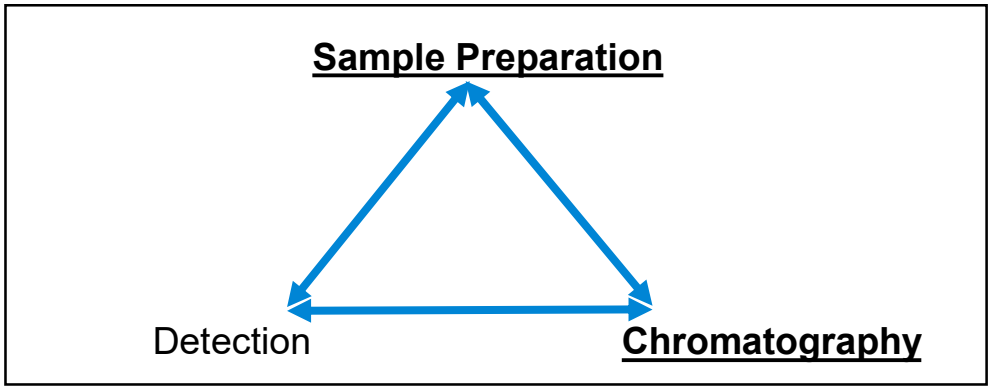
- Very quick and simple sample preparation, acidification identical to mobile phase system

Summary



- 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

Summary



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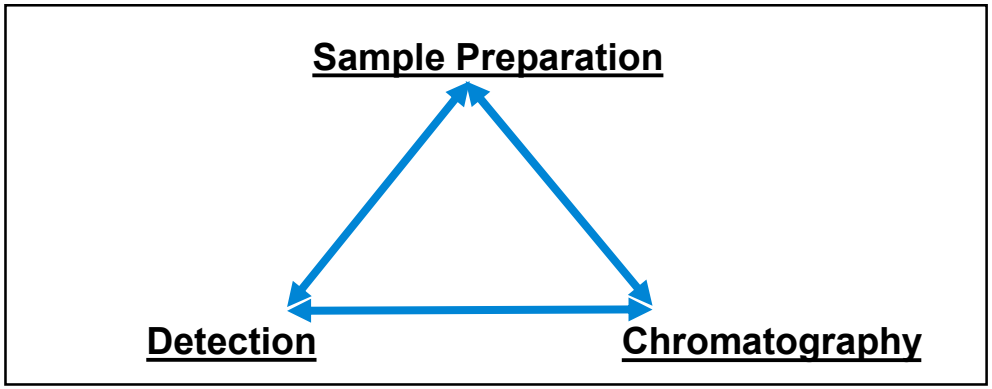
- Newly introduced InfinityLab Poroshell 120 CS-C18 column uses a novel reversed-phase packing; it is resistant to large injection volumes of aqueous extracts and offers good retention of these polar compounds in acidic conditions without sacrificing peak shape

Summary



- 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, 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

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Megan Marr
Sami Chanaa

Agilent

InfinityLab