

**EPA Method 538:
Determination of Selected Organic Contaminants
in Drinking Water
with Direct Aqueous Injection LC/MS/MS**

E. Michael Thurman and
Imma Ferrer
*Center for Environmental
Mass Spectrometry
University of Colorado
Boulder, CO, USA*

Abstract

EPA Method 538 is a new method from EPA for organophosphate pesticides in drinking water.

It uses direct aqueous injection; thus, no sample preparation is needed.

We use both UHPLC (Agilent 1290) and MS/MS (Agilent 6460) analysis for rapid analysis and sensitive detection with ng/L limits of detection.

A second MRM is added for more reliable identification.

Hypothesis

Direct injection of organophosphate pesticides (EPA Method 538) will work by UHPLC (Agilent Model 1290) and LC/MS/MS with Jetstream (Agilent Model 6460) with trace level detection at ng/L concentrations.

1. Introduction-Summary

1.1 EPA Method 538 (published in November 2009 by Shoemaker) deals with Organophosphate pesticides in drinking water (1) and one other contaminant, quinoline.

1.2 The method consists of 10 compounds: acephate, aldicarb, aldicarb sulfoxide, dicrotophos, diisopropylmethylphosphonate (DIMP), fenamiphos sulfone, fenamiphos sulfoxide, methamidophos, oxydemeton methyl, quinoline, and thiofanox with 5 labeled internal standards.

1.3 Direct aqueous injection is used with a large volume sample of 100 microliters; thus, no sample preparation is needed.

1.4 Because solid phase extraction (i.e. concentration of the sample is not carried out) suppression is minimized in the analysis.

1.5 Part-per-Trillion Detection Limits.

Introduction 1.1:

EPA Method 538: Determination of Selected Organic Contaminants in Drinking Water by Direct Aqueous Injection

by

Jody Shoemaker, EPA Cincinnati, OH

Shoemaker.jody@EPA.GOV

513-569-7298

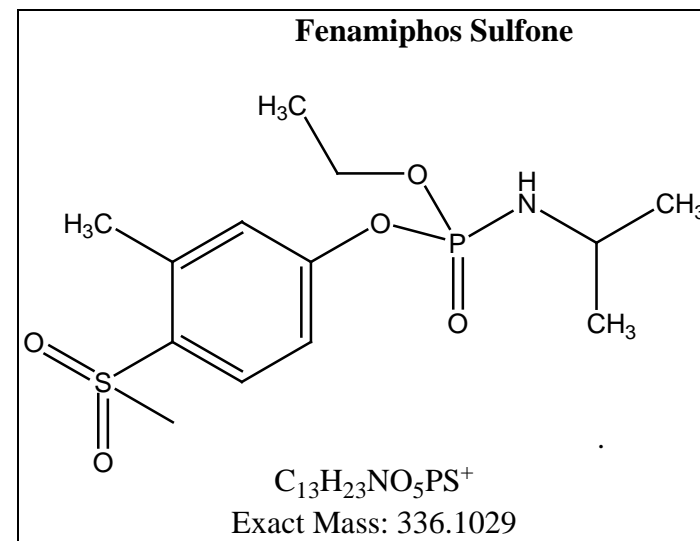
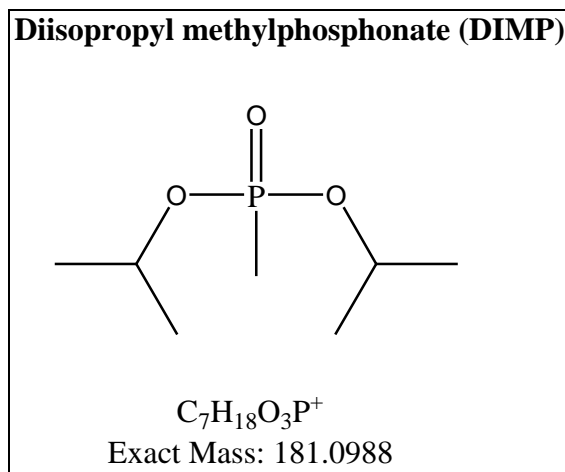
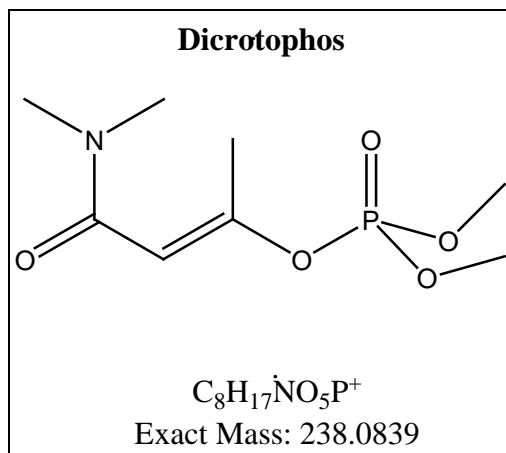
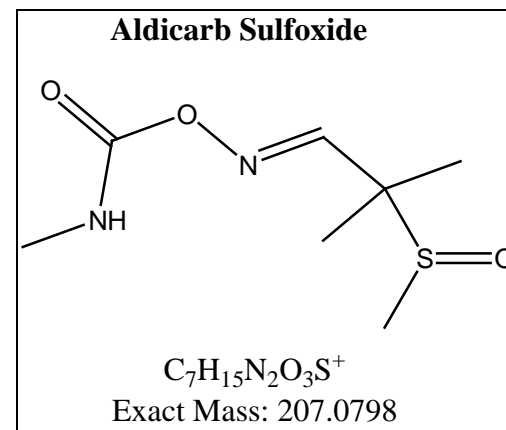
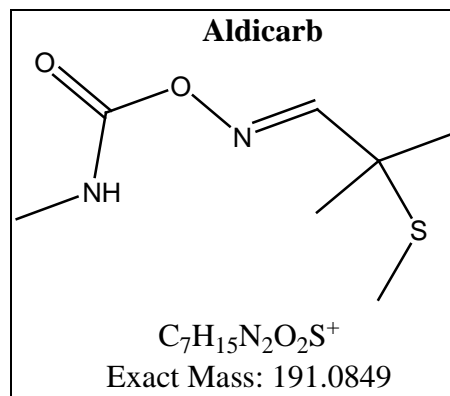
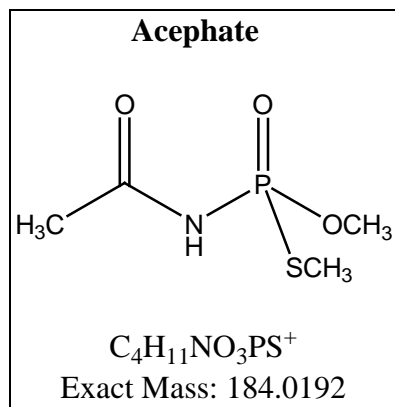
Introduction: 1.2.

Ten Organophosphates and Quinoline

<u>Analyte</u>	<u>Chemical Abstract Services Registry Number (CASRN)</u>
Acephate	30560-19-1
Aldicarb	116-06-3
Aldicarb sulfoxide	1646-87-3
Dicrotophos	141-66-2
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Fenamiphos sulfone	31972-44-8
Fenamiphos sulfoxide	31972-43-7
Methamidophos	10265-92-6
Oxydemeton-methyl	301-12-2
Quinoline	91-22-5
Thiofanox	39196-18-4

Introduction:1.3.

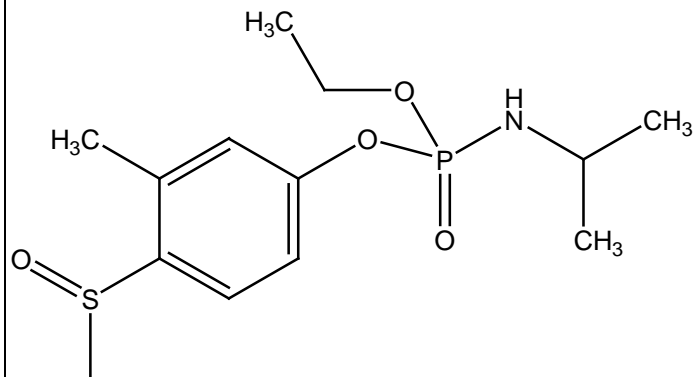
Organophosphate Structures



Introduction 1.3.

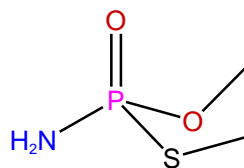
Organophosphate Structures

Fenamiphos Sulfoxide



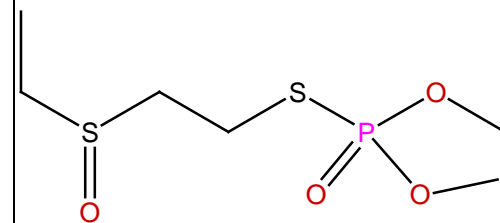
$C_{13}H_{23}NO_4PS^+$
Exact Mass: 320.108

Methamidophos



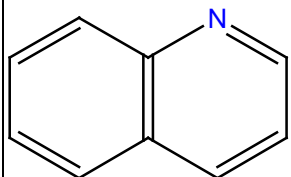
$C_2H_9NO_2PS^+$
Exact Mass: 142.0086

Oxydemeton-methyl



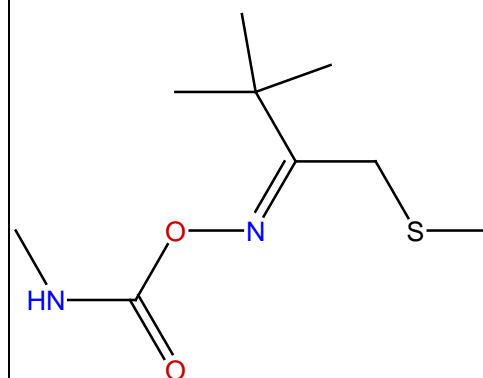
$C_6H_{16}O_4PS_2^+$
Exact Mass: 247.0222

Quinoline



$C_9H_8N^+$
Exact Mass: 130.0651

Thiofanox



$C_9H_{19}N_2O_2S^+$
Exact Mass: 219.1162

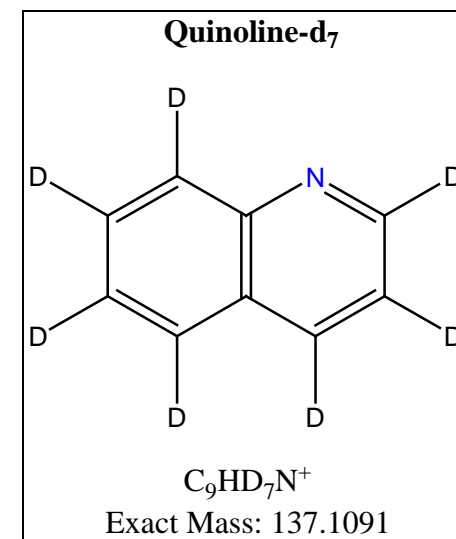
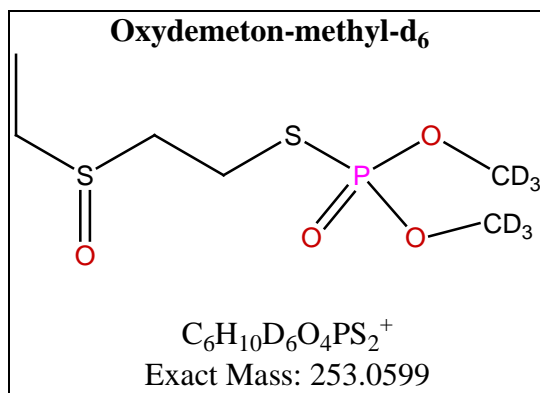
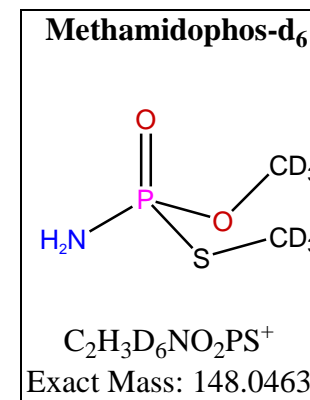
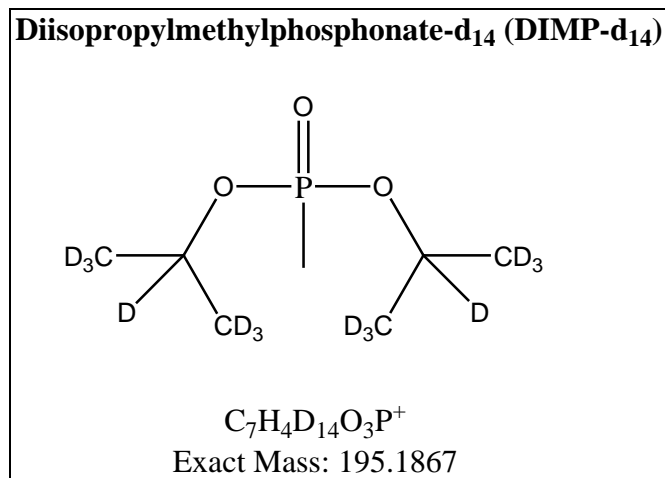
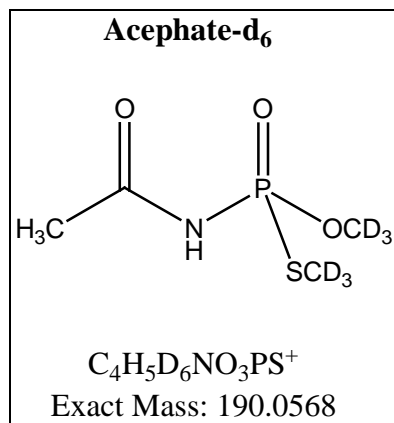
Introduction 1.4.

Five Deuterated Standards

Internal Standards
Methamidophos-d ₆
Acephate-d ₆
Oxydemeton-methyl-d ₆
Quinoline-d ₇
Diisopropyl methylphosphonate-d ₁₄ (DIMP-d ₁₄)

Introduction 1.4.

Deuterated Internal Standards



Introduction 1.5

No solid-phase extraction!

polymer.

2. Washing

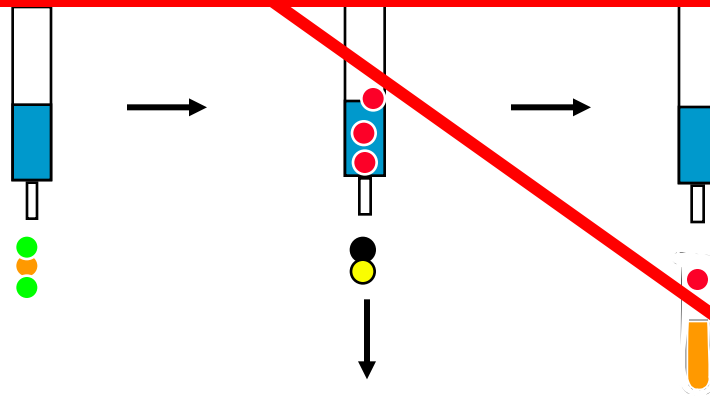
3. Elution

100-200 mL sample

ethanol
remove
S or
ticals

50% of the Time and Effort
Removed from Analysis

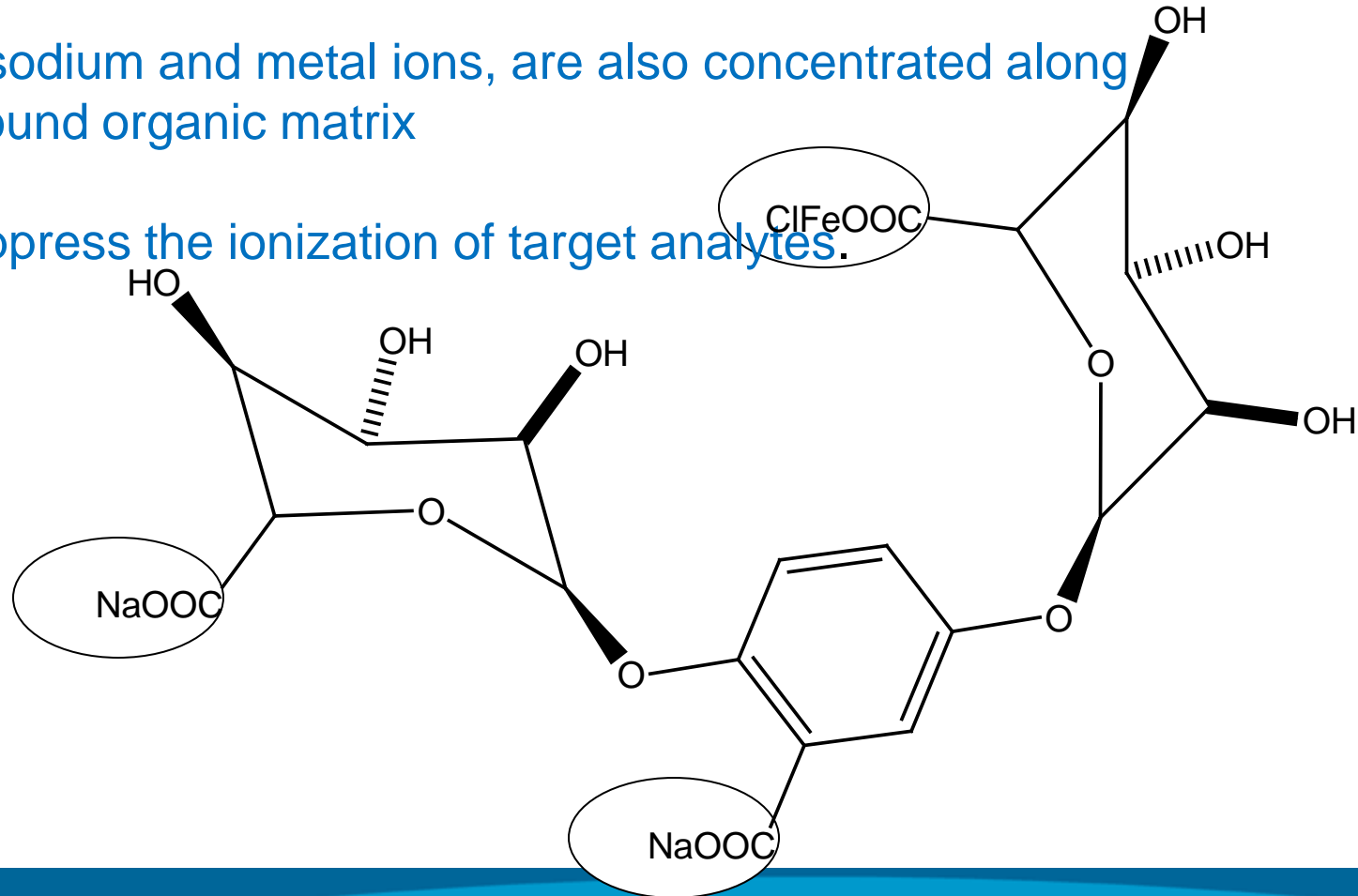
Cartridges:
- C18, polymeric
- Oasis HLB, OPT



Introduction 1.6

Minimizing Compound Suppression

- SPE increases background organic matrix by 200 to 500 fold
- Salts, such as sodium and metal ions, are also concentrated along with the background organic matrix
- These salts suppress the ionization of target analytes.



Introduction 1.6

Direct Aqueous Injection with the 1290 Infinity—UHPLC and 6460



2.0 Experimental Methods: Summary

1. Collect 40 mL sample and preserve with sodium omadine and ammonium acetate.
2. Aliquot 950 μL of sample and add 50 μL of internal standard.
3. Analyze by UHPLC/MS/MS with the Agilent 1290 LC and the Agilent Model 6460



Experimental Methods 2.1

MRM Transitions for QqQ Method

Compound Name	Precursor Ion	Product Ion	Dwell	Fragmentor	Collision Energy	Polarity
Acephate	206	165	10	90	5	Positive
Acephate	184	143	10	50	0	Positive
Aldicarb	213	116	10	90	5	Positive
Aldicarb	213	89	10	90	15	Positive
Aldicarb-Sulfoxide	229	166	10	70	5	Positive
Aldicarb-Sulfoxide	229	109	10	70	10	Positive
Dicrotophos	238	193	10	70	0	Positive
Dicrotophos	238	112	10	70	5	Positive
DIMP	181	139	10	70	0	Positive
DIMP	181	97	10	70	5	Positive
Fenamiphos-Sulfone	336	308	10	110	10	Positive
Fenamiphos-Sulfone	336	266	10	110	15	Positive
Fenamiphos-Sulfoxide	320	292	10	110	10	Positive
Fenamiphos-Sulfoxide	320	233	10	110	20	Positive
Methamidophos	142	125	10	70	10	Positive
Methamidophos	142	94	10	70	10	Positive
Oxydemeton-methyl	269	191	10	110	5	Positive
Oxydemeton-methyl	247	169	10	70	10	Positive
Quinoline	130	103	10	110	25	Positive
Quinoline	130	77	10	110	35	Positive
Thiofanox	241	184	10	90	5	Positive
Thiofanox	241	57	10	90	15	Positive

Experimental Methods 2.2

Transitions for Deuterated Standards

Compound	Transition	Frag.	CE
Acephate-d ₅	190→149	50	0
DIMP -d ₁₄	195→99	70	5
Methamidophos-d ₆	148→97	70	10
Oxydemeton-methyl-d ₆	253→175	70	10
Quinoline-d ₇	137→81	110	35



Experimental Methods 2.3

QqQ Experimental Source Parameters

Sample | Properties | Sampler | BinPump | TCC | **MS QQQ**

Tune file: atunes.TUNE.XML [Browse ...]

Stop time: No limit/As Pump 1 min

Ion source: ESI Agilent Jet Stream

Time filtering: Peak width 0.07 min

Time segments:

#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored
▶ 1	0	MRM	To MS	200	0	<input checked="" type="checkbox"/>

3.37 cycles/s 297.0 ms/cycle

Acquisition | **Source** | Chromatogram | Instrument | Diagnostics

Source parameters:

Gas Temp: 250 °C 250 °C

Gas Flow: 10 l/min 10.0 l/min

Nebulizer: 45 psi 45.0 psi

Sheath Gas Temp: 350 °C 350 °C

Sheath Gas Flow: 11 l/min 11.0 l/min

Capillary: Positive 4000 V Negative 3500 V 6453 nA

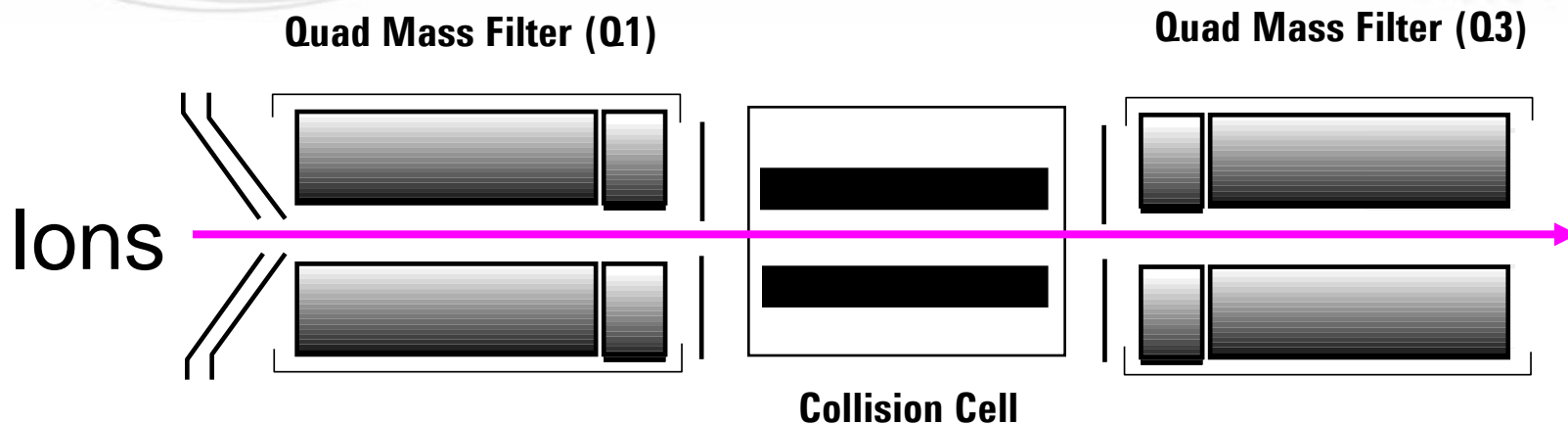
Nozzle Voltage: 0 V 1500 V

Chamber Current 0.16 µA

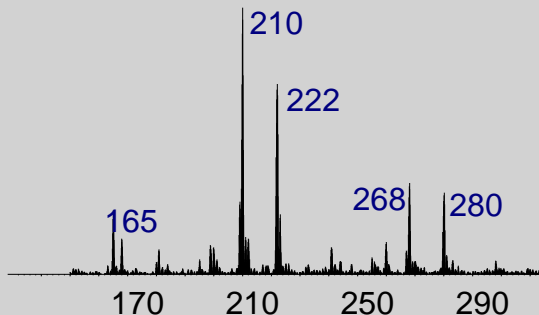
3.0 Results and Discussion

1. EPA Method 538: Organophosphate Pesticides in Drinking Water.
2. Direct Aqueous Injection; Thus, No Sample Preparation Needed.
3. UHPLC/MS/MS Analysis with Minimal Suppression.
4. Part-per-Trillion Detection Limits.

3.1 Multiple Reaction Monitoring



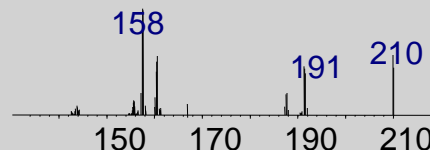
Spectrum with background ions (from ESI)



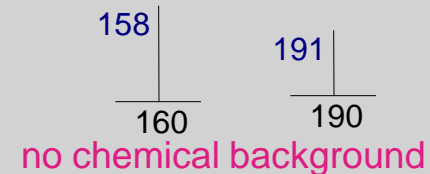
Q1 lets **only** target ion 210 pass through



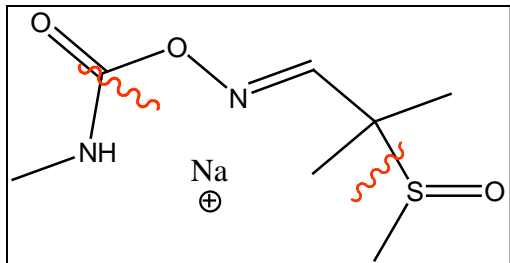
Collision cell breaks ion 210 apart



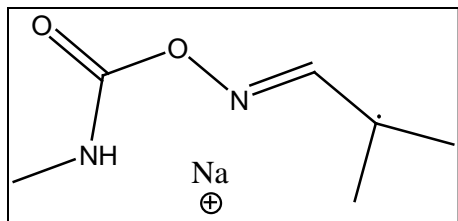
Q3 monitors **only** characteristic fragments 158 and 191 from ion 210 for quant and qual.



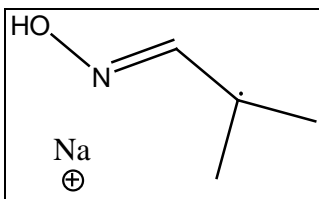
3.2 Example: Fragmentation Study of Aldicarb



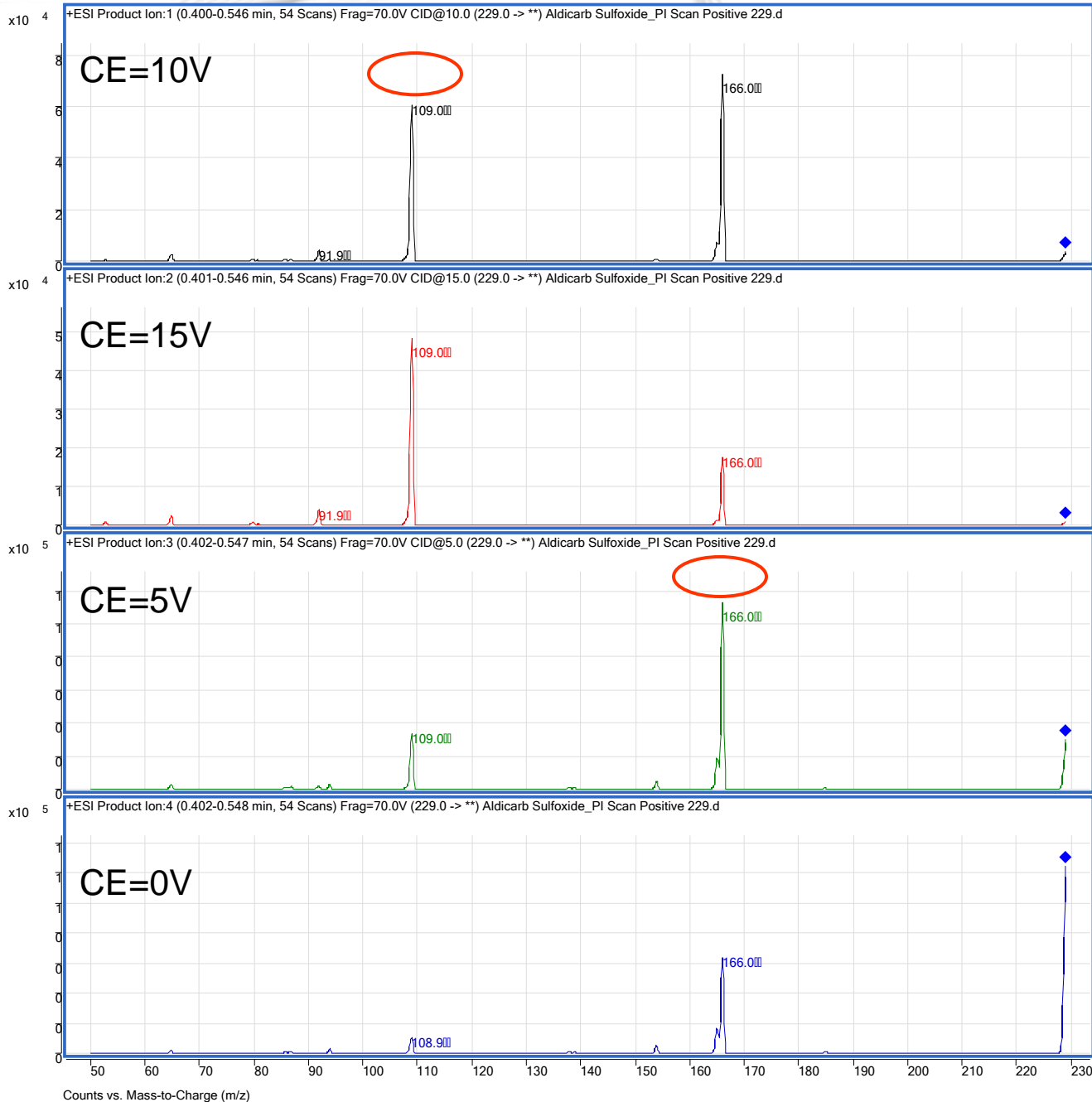
$[M+Na]^+ = 229$



$m/z = 166$

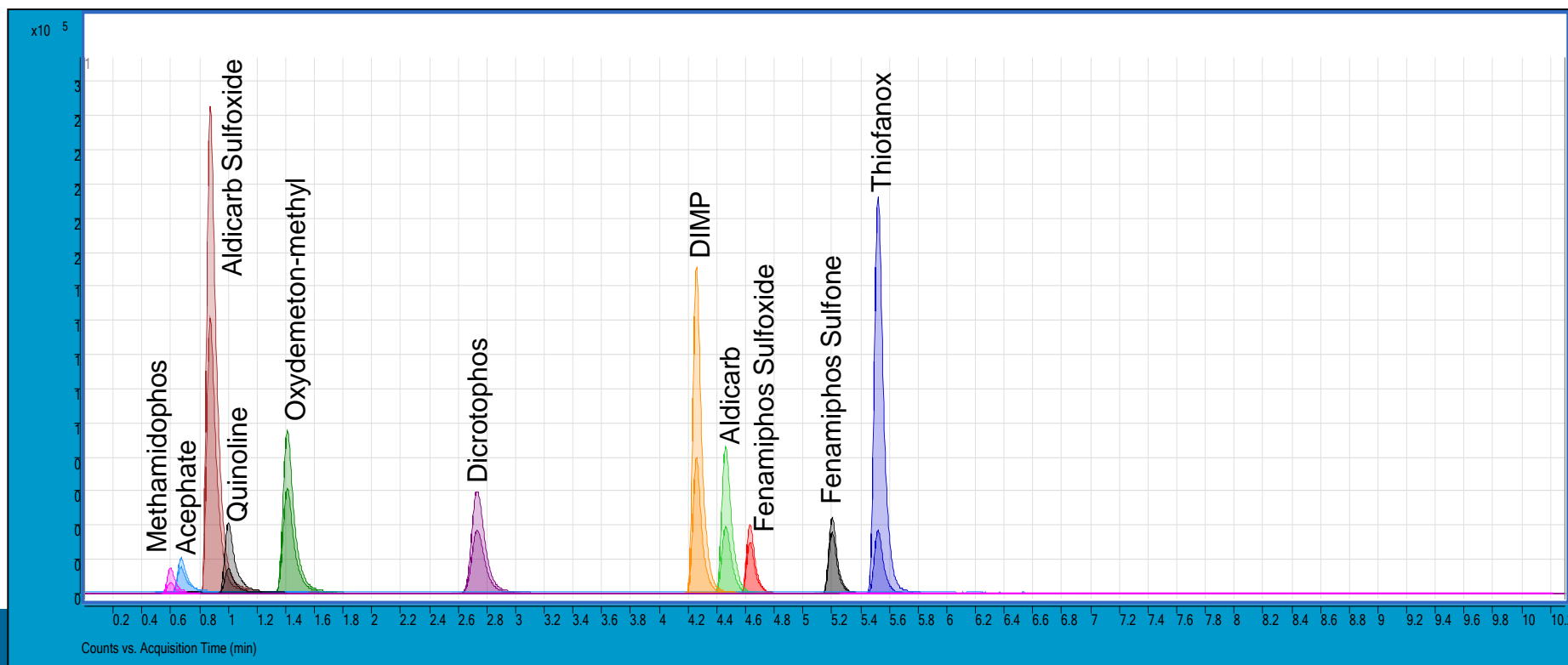


$m/z = 109$

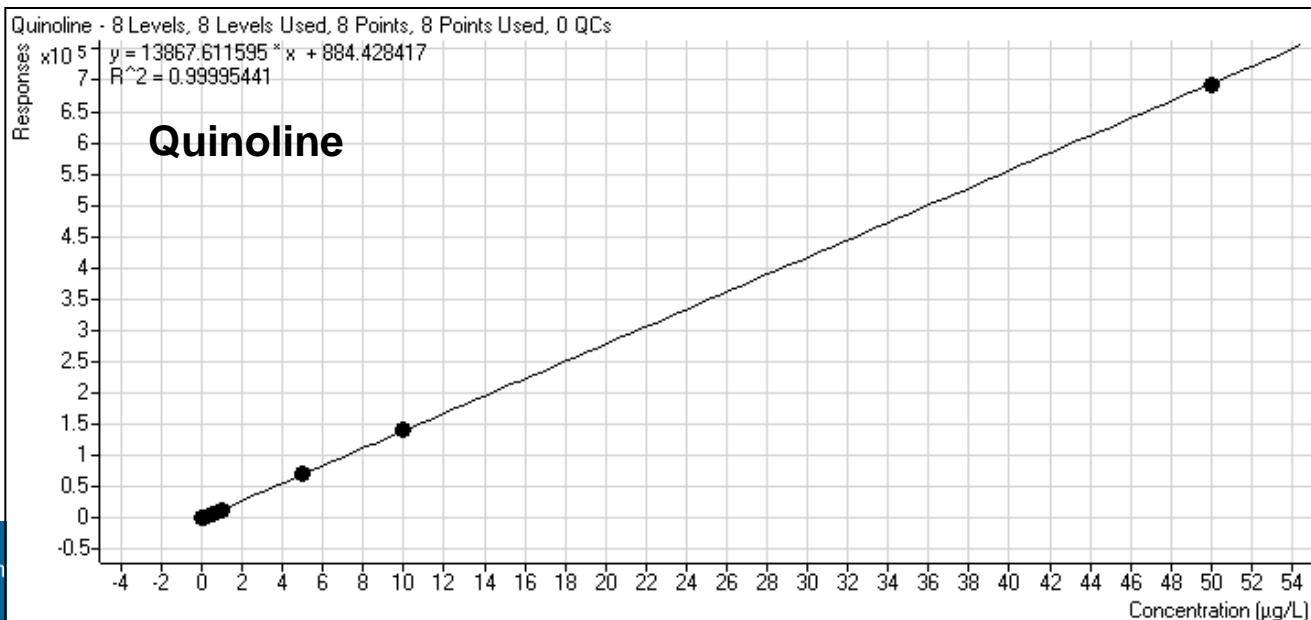
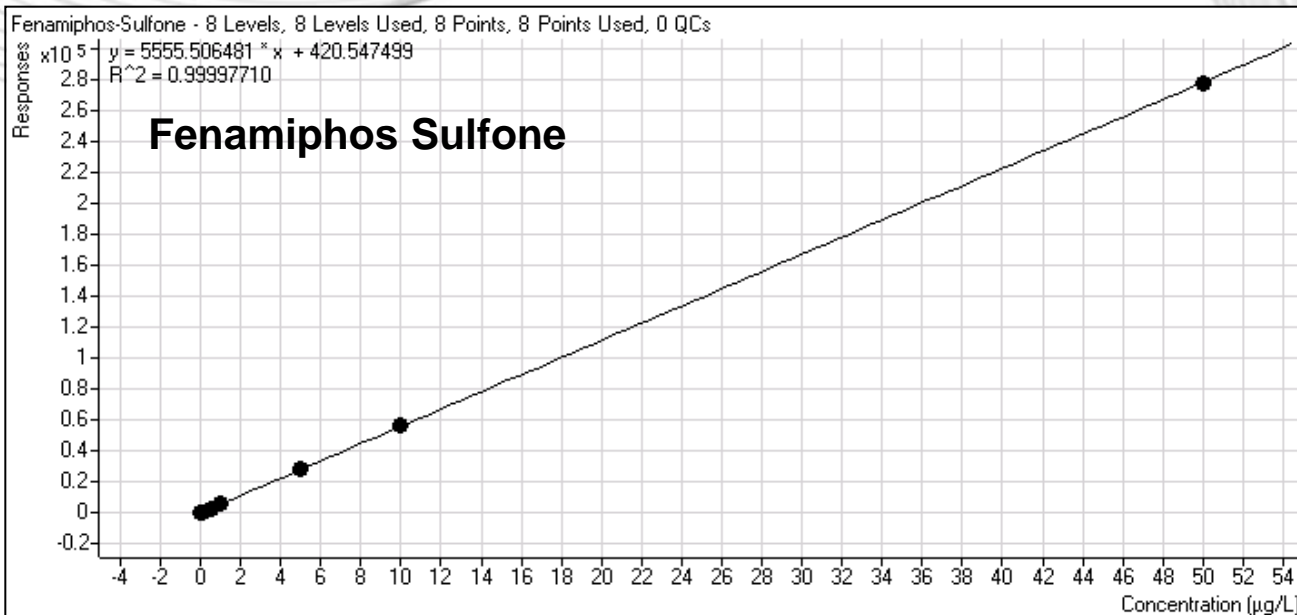


3.3 Extracted Ion Chromatograms of Compounds

Column: C₁₈ Eclipse Plus 2.1mm x 50mm, 1.8 um
Mobile phase: A=ACN, B=H₂O (0.1% Acetic Acid)
Flow-rate: 0.4 mL/min
Gradient: t=0min. 10% A/90% B
t=1.7min. 10% A/90% B
t=10 min. 100% B
Injection volume: 40uL



3.4 Examples of Standard Curves



3.5 EPA Method 538: Limits of Detection

Compound	LOD (ppt, ng/L)
Acephate	1000
Aldicarb	10
Aldicarb Sulfoxide	5
DIMP	50
Dicrotophos	50
Fenamiphos Sulfone	50
Fenamiphos Sulfoxide	50
Methamidophos	500
Oxydemeton-methyl	20
Quinoline	50
Thiofanox	10

4.0 Conclusions

1. EPA Method 538 works well on the Agilent Model 1290 UHPLC coupled to the Agilent Model 6460 LC/MS/MS.
2. 10 Organophosphates and quinoline are analyzed by direct aqueous injection and UHPLC/MS/MS.
3. A second MRM transition is added for QA/QC.
4. Detection Limits are in the ng/L range.
5. Method is robust and has low suppression.
6. Agilent has the competitive advantage in Direct Aqueous injection with UHPLC/MS/MS analysis.

References

1. Shoemaker, EPA Method 538: Determination of selected organic contaminants in drinking water by direct aqueous injection-liquid chromatography/Tandem Mass Spectrometry (DAI-LC/MS/MS): EPA/600/R-09/149.



References

2. EPA Method 538: Determination of selected Organic Contaminants in Drinking Water by Direct Aqueous Injection with the Agilent 6460 Triple Quadrupole LC/MS System. Michael Thurman and Imma Ferrer.

Agilent Application Note#5990-9670EN