

Analyse de contaminants organiques dans des matrices alimentaires complexes par la technologie QSight LC/MS/MS

Christophe CLARYSSE : SME Chromatography & Mass Spec Solutions EMEA

CECM Atelier du 26 Mars 2021



Agenda

- Pesticide Analysis
- Overview of pesticide workflow
 - Sample to result
- QSight flow-based mass spectrometry
- Selected applications
- Conclusions

Pesticides in foods and their regulation

- Regulation (EC) 396/2005
 - maximum residue limits (MRLs) are generally set at 0.01 mg/kg (with range from 0.001 – 100 mg/kg)

- SANTE 12682/2019
 - guidance document
 - performance requirements for analytical methods

Parameter	What/how	Criterion	Cross reference to AQC document
Sensitivity/linearity	Linearity check from five levels	Deviation of back-calculated concentration from true concentration $\leq \pm 20\%$	C14-C19
Matrix effect	Comparison of response from solvent standards and matrix-matched standards	*	C21-C29
LOQ	Lowest spike level meeting the identification and method performance criteria for recovery and precision	\leq MRL	G6 ⁸
Specificity	Response in reagent blank and blank control samples	$\leq 30\%$ of RL	C41
Recovery	Average recovery for each spike level tested	70-120 %	G3,G6
Precision (RSD _r)	Repeatability RSD _r for each spike level tested	$\leq 20\%$	G3, G6
Precision (RSD _{wr})	Within-laboratory reproducibility, derived from on-going method validation/verification	$\leq 20\%$	G3, G6
Robustness	Average recovery and RSD _{wr} , derived from on-going method validation/verification	See above	G6, C39-C44
Ion ratio	Check compliance with identification requirements for MS techniques	Table 3	Section D
Retention time		± 0.1 min.	D2

Challenges in multi-residue pesticide analysis in foods

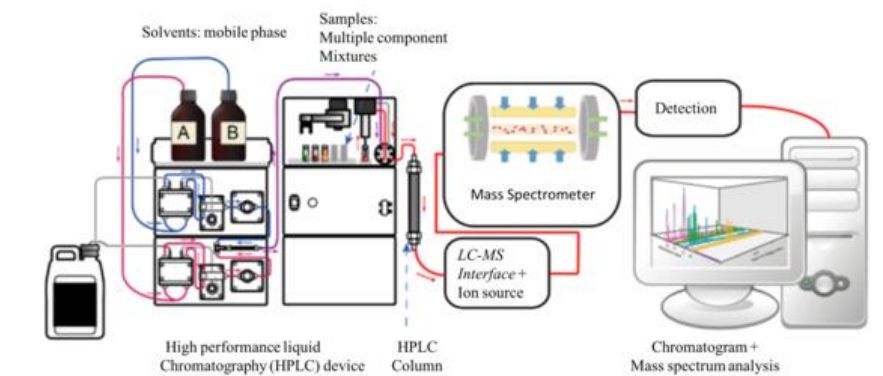


Changing legislation

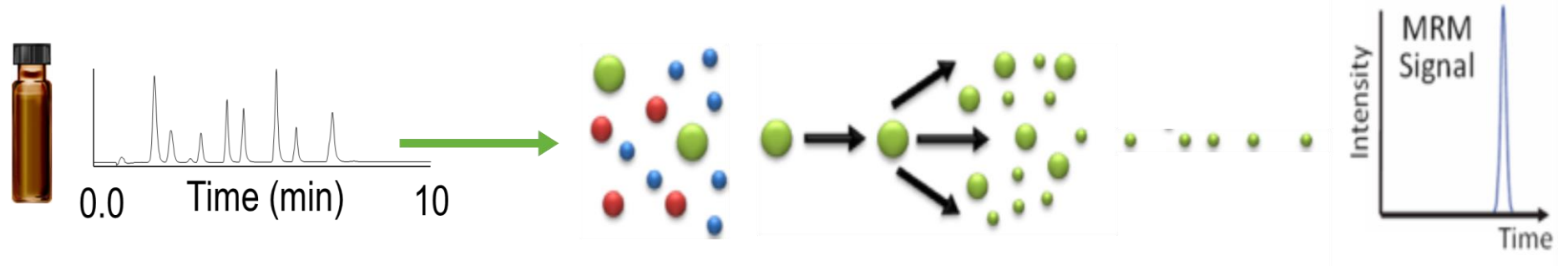
Variety of pesticides

Multitude of food matrixes

Instrument performance

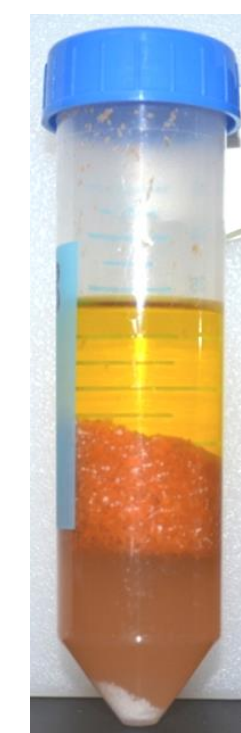
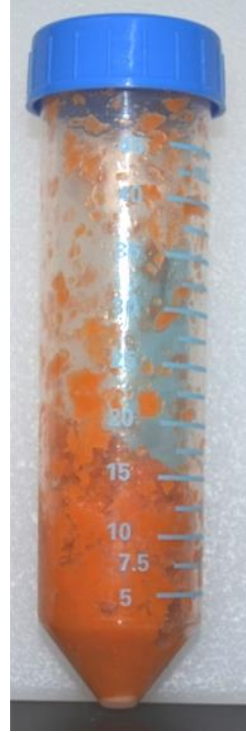


Analytical workflows by LC-MS/MS

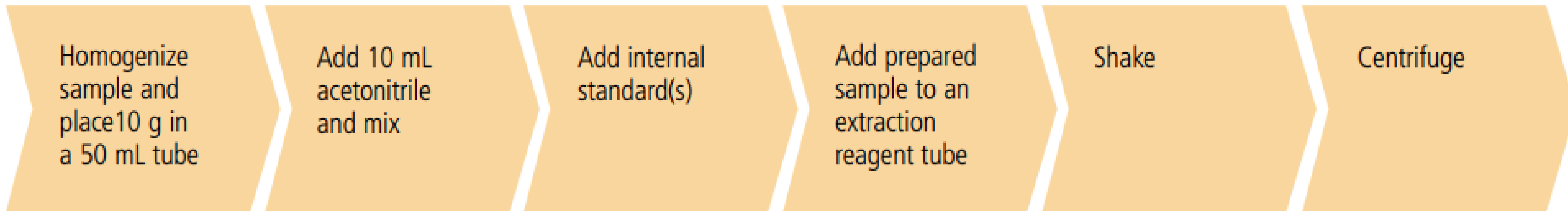


QuEChERS method is a simple two step procedure

...Quick Easy Cheap Effective Rugged Safe – Step 1: Extraction



Step 1: Extraction



Extraction Kits

Method	Vol.	Qty.	MgSO4	Na Acetate	Na Citrate	Na Citrate Sesquihydrate	NaCL	Part No.
AOAC 2007.01	50 mL	50	6 g	1.5 g				N9306900
EN 15662	50 mL	50	4 g		1 g	0.5 g	1 g	N9306901
Original	50 mL	50	4 g				1 g	N9306902



QuEChERS method is a simple two step procedure

...Quick Easy Cheap Effective Rugged Safe – Step 2: Clean-up



Step 2: Clean-Up

Transfer an aliquot of the supernatant to a clean-up tube



Shake

Centrifuge

Test supernatant directly by GC, GC/MS, LC, LC/MS



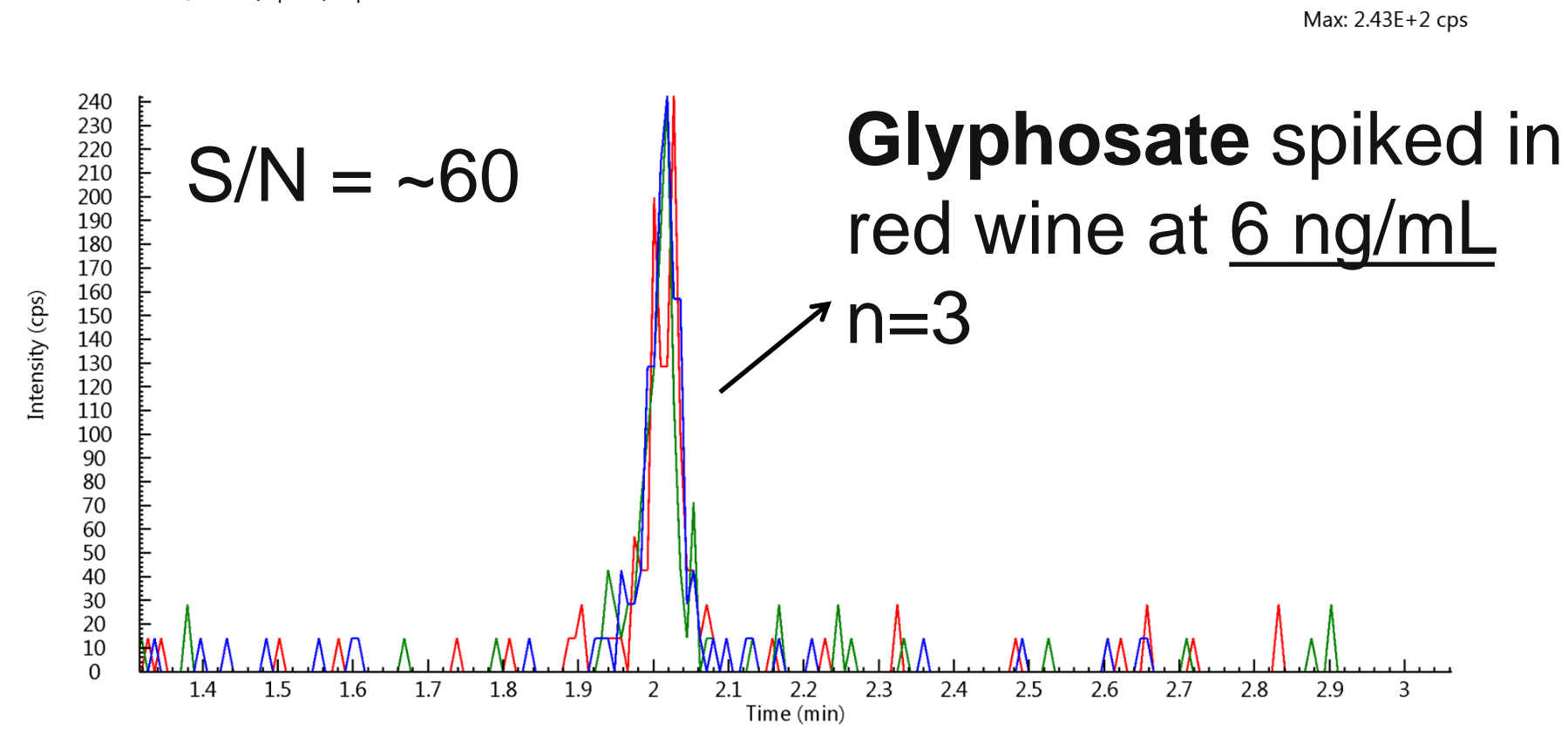
EN 15662 Clean-up Kits

Description	Vol.	Qty.	MgSO ₄ ¹	PSA ²	C18 ³	PGC ⁴	Part No.
Fruit & Vegetables 	2 mL	100	150 mg	25 mg			N9306920
Fruit & Vegetables	15 mL	50	900 mg	150 mg			N9306921
Fruit & Vegetables with Fats and Waxes 	2 mL	100	150 mg	25 mg	25 mg		N9306922
Waxed Fruit & Vegetables	15 mL	50	900 mg	150 mg	150 mg		N9306923
Pigmented Fruit & Vegetables 	15 mL	50	900 mg	150 mg		15 mg	N9306924
Pigmented Fruit & Vegetables	2 mL	100	150 mg	25 mg		2.5 mg	N9306925
High Pigmented Fruit & Vegetables 	2 mL	100	150 mg	25 mg		7.5 mg	N9306926
High Pigmented Fruit & Vegetables	15 mL	50	900 mg	150 mg		45 mg	N9306927

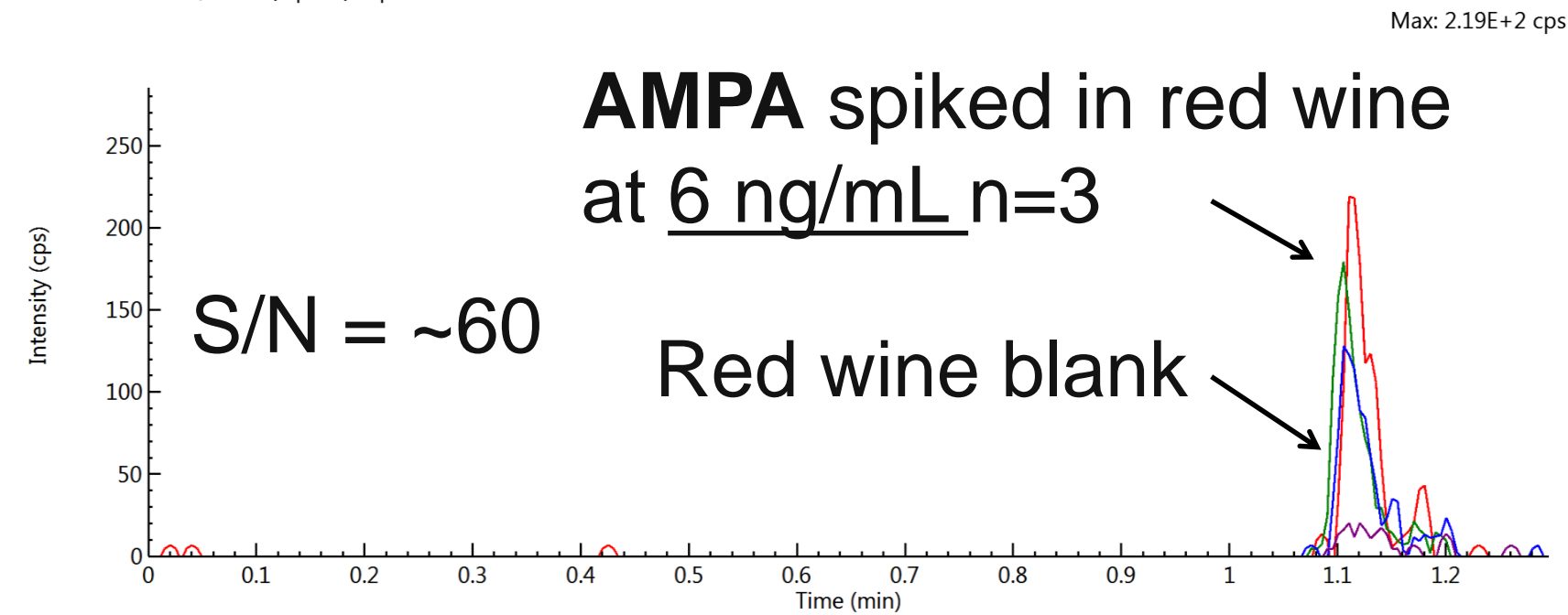


Special case: polar pesticides – Glyphosate, AMPA and others

11/1/2016: redwine-GB-6ppb0001_234156
EIC -MRM 167.50/63.00 (7 pairs) Exp 1



11/1/2016: redwine-GB-6ppb0001_234156
EIC -MRM 109.70/62.80 (4 pairs) Exp ""



No sample preparation



APPLICATION NOTE
Liquid Chromatography /
Mass Spectrometry

Authors:
Sharanya Reddy
Frank Kero
PerkinElmer, Inc.
Shelton, CT

Matteo Meglioli
Mosti Mondiale, Inc.
Quebec, Canada

Direct Analysis of Glyphosate in Wine with No Sample Preparation Using the QSight 220 LC-MS/MS System

Introduction

Glyphosate is an organophosphate herbicide that is used on crops to kill weeds and grasses. Its usage has multiplied with

the introduction of transgenic crops made resistant to glyphosate. Because of its rampant use, it is not surprising that glyphosate has been detected in variety of foods. Recently, the International Agency for Research on Cancer classified glyphosate as "probably carcinogenic in humans". In lieu of regulatory bodies setting limits on glyphosate in food, it has become imperative to develop robust and sensitive analytical methods for glyphosate detection. Since glyphosate is a very polar molecule, it does not retain well on a traditional reverse phase column making it very difficult to chromatographically separate from other components and detect. Methods involving derivatization with a hydrophobic moiety can help retain glyphosate on column, but, it also makes the process labor intensive and tedious. We present a study that involves direct analysis of glyphosate in wine on a mixed mode column with no sample dilution or extraction using a PerkinElmer QSight® 220 triple quadrupole mass spectrometer with a patented StayClean™ source, consisting of a hot surface induced desolvation (HSID)™ interface and a Laminar Flow Ion Guide™. Both the HSID and ion guide prevent any contaminants from entering the mass spectrometer, keeping it at its highest performance level and, thereby, maintenance free.



APPLICATION NOTE

Liquid Chromatography /
Mass Spectrometry

Authors:
Li-Zhong Yang, Zhuo Man, Xiangdong Zhou
PerkinElmer, Inc.
Shanghai, China

Feng Qin
PerkinElmer, Inc.
Bolton, Canada

Direct Analysis of Glyphosate and Similar Polar Pesticides in Oatmeal by UHPLC-MS/MS

Introduction

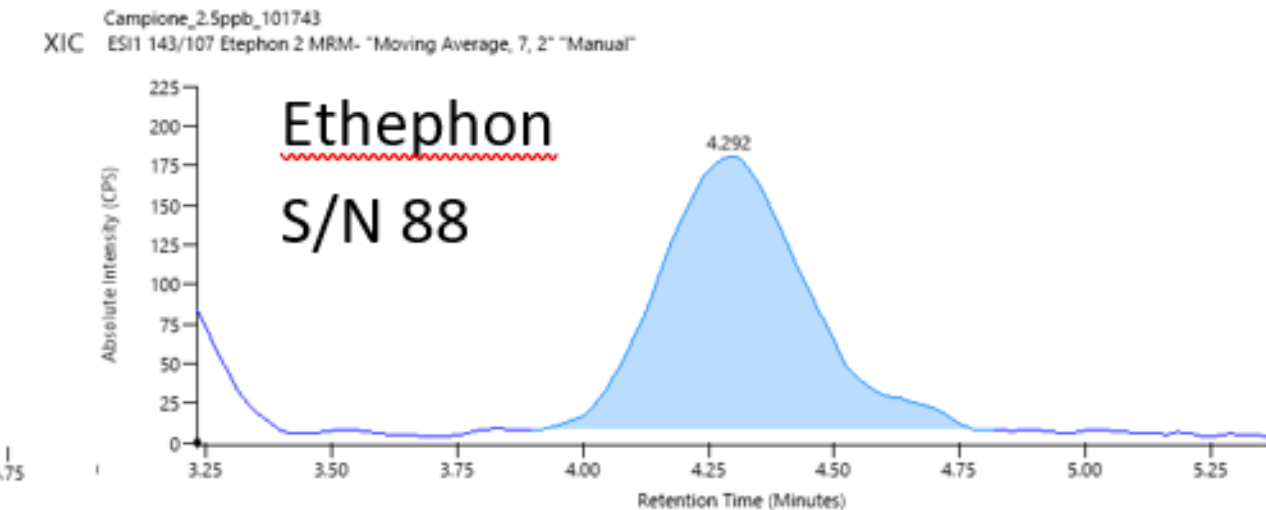
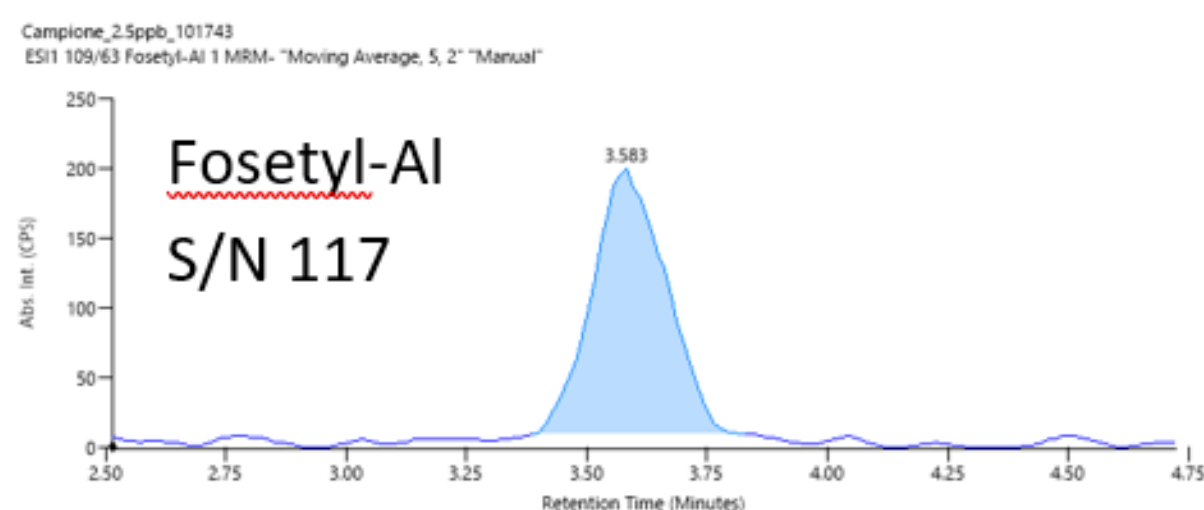
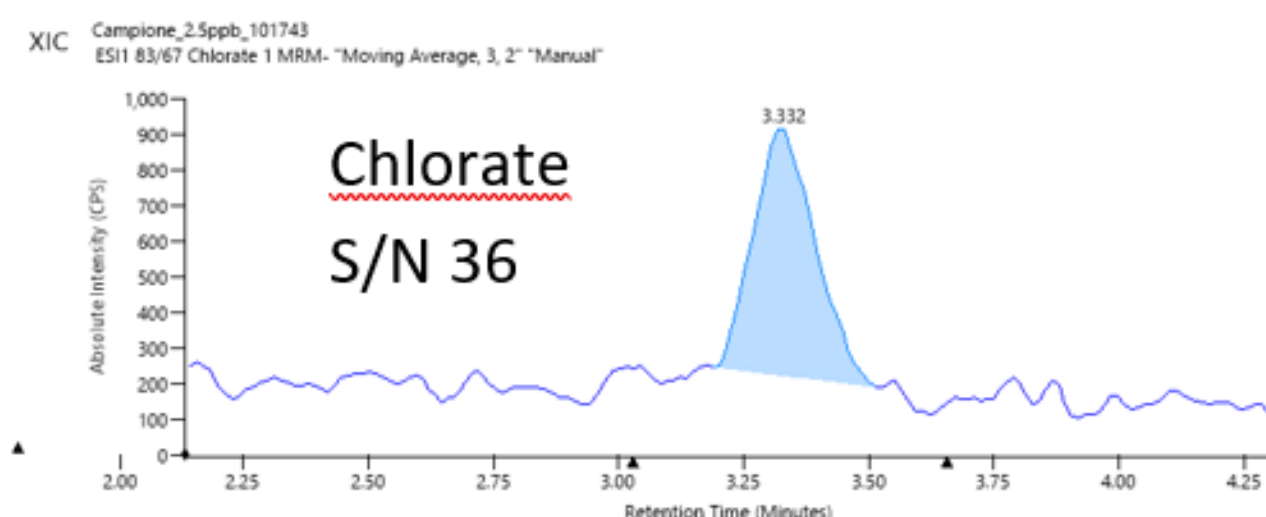
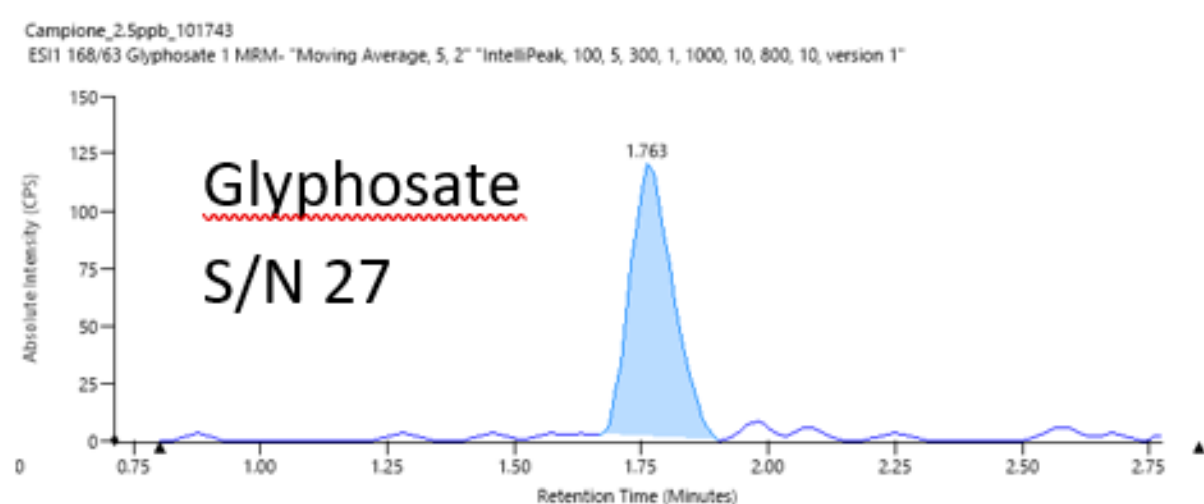
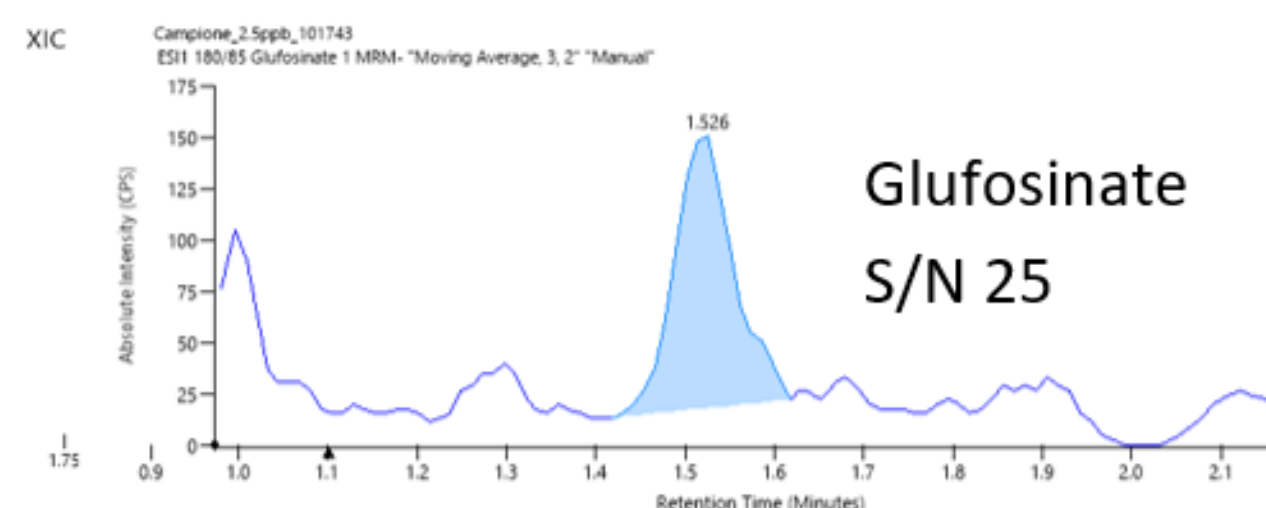
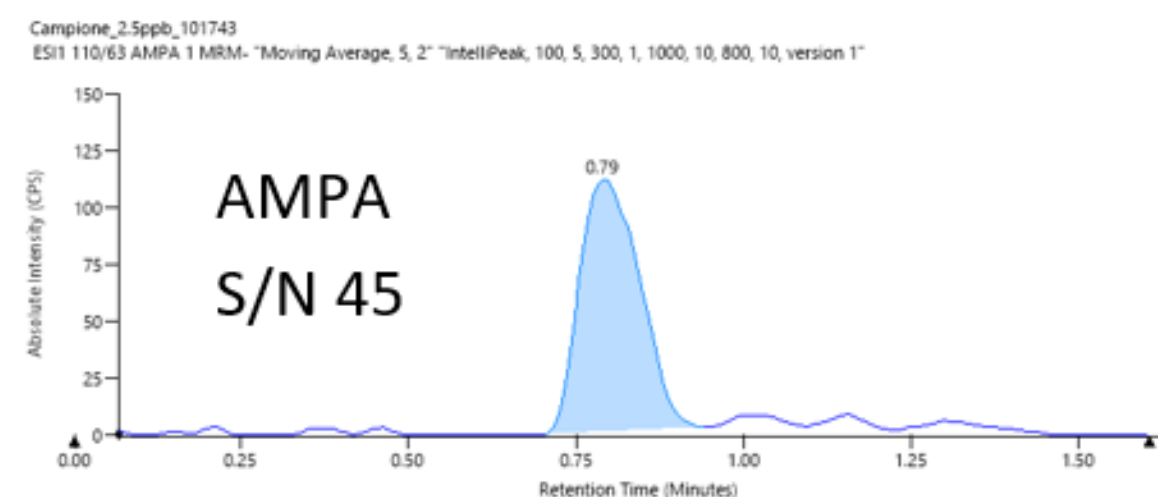
Glyphosate (N-(phosphonomethyl) glycine), an organophosphorus compound, is used to kill weeds (e.g. annual broadleaf weeds and

grasses) that compete with crops. Since its introduction to market approximately 40 years ago, glyphosate has become one of the world's most widely used herbicides due to its relatively low toxicity in comparison with other herbicides towards mammals. The adoption of glyphosate by farmers intensified after the introduction of genetically engineered "glyphosate tolerant" crops, such as corn and soybeans, that can withstand glyphosate treatment unlike the weeds the herbicide is meant to destroy. Like other pesticides, glyphosate is directly administered to food products and can come in contact with both food workers and the environment, resulting in the bio burden of exposure in uncontrolled regional populations. As a registered herbicide product under a number of regulatory organizations, glyphosate has been considered nontoxic with minimal risk to human health with persistent exposure at trace levels. However, recent toxicity evaluations by different organizations have put glyphosate at the center of a dispute. The World Health Organization's (WHO) International Agency for Research on Cancer classified it as "probably carcinogenic to humans" in March of 2015¹. However, in November of 2015, the European Food Safety Authority (EFSA) published a report claiming that there was no scientific evidence linking glyphosate to cancer².



Special case: polar pesticides – Glyphosate, AMPA and others

2.5 ppb in vegetable extract

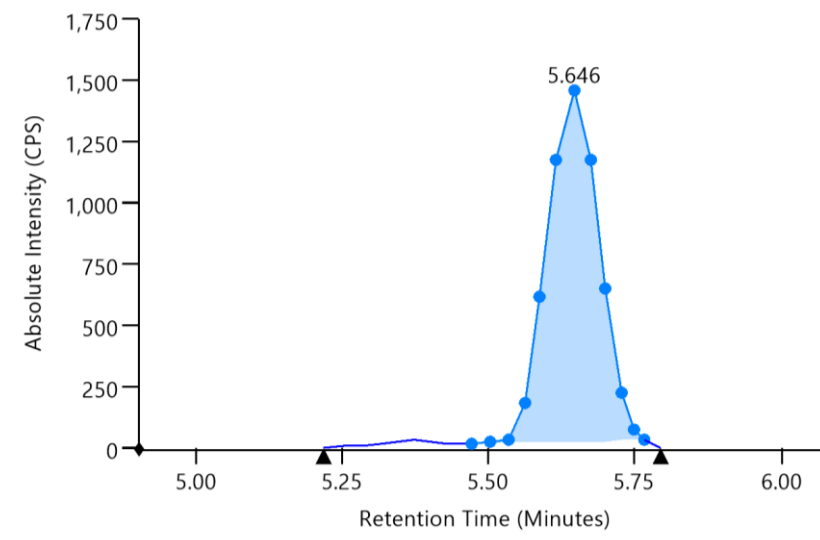


Method	Analyte	Commodity Group	Matrix	Spiking Level (mg/kg)	n	Mean Recov. %	RSD
M 1.3	AMPA	High water content + acidic	Grapes	0.02	12	110	9
	AMPA	Dry (cereals)	Barley	0.02	5	101	14
	AMPA	Dry (pulses)*	Lentil	0.1	10	95	17
	AMPA	Dry (cereals)	Wheat flour	0.1	5	119	6
	AMPA	High water content	Apple	0.02	17	100	12
	Cyanuric Acid	High water content	Cucumber	0.02	3	106	13
	Ethephon	Dry (cereals)	Barley	0.02	5	110	2
	Ethephon	Dry (cereals)	Wheat flour	0.1	5	85	6
	Ethephon	High water content	Apple	0.02	7	105	11
	Ethephon	High water content	Cucumber	0.02	3	101	11
	Ethephon	High water content + acidic	Grapes	0.01	5	104	4
	Fosetyl	High water content + acidic	Strawberry	0.1	6	94	4
	Fosetyl	Dry (cereals)	Barley	0.02	5	106	7
	Fosetyl	High water content	Apple	0.02	7	104	5
	Fosetyl	High water content	Cucumber	0.02	3	103	5
	Fosetyl	High water content + acidic	Grapes	0.01	5	105	2
	Glufosinate	High water content + acidic	Grapes	0.05	5	96	10
	Glufosinate	Dry (cereals)	Barley	0.02	5	101	13
	Glufosinate	Dry (cereals)	Wheat flour	0.1	5	85	5
	Glufosinate	High water content	Apple	0.02	7	106	8
	Glufosinate	High water content	Cucumber	0.02	3	115	4
	Glyphosate	High water content + acidic	Grapes	0.02	12	112	8
	Glyphosate	High water content + acidic	Grapes	0.02	5	102	6
	Glyphosate	Dry (cereals)	Barley	0.02	5	105	8
	Glyphosate	Dry (pulses)*	Lentil	0.1	11	107	18
	Glyphosate	High oil content, dry (oily seeds, nuts)*	Bean, Soya	0.1	10	95	10
	Glyphosate	High water content	Apple	0.02	16	93	12
	Glyphosate	High water content	Cucumber	0.02	3	94	3

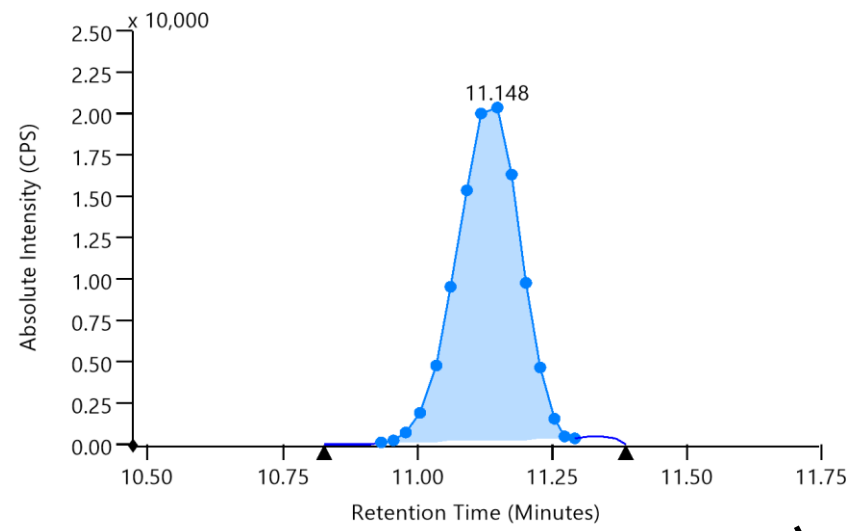
QuPpe Method 1.3: EURL
acidified methanol

Chromatographic separation

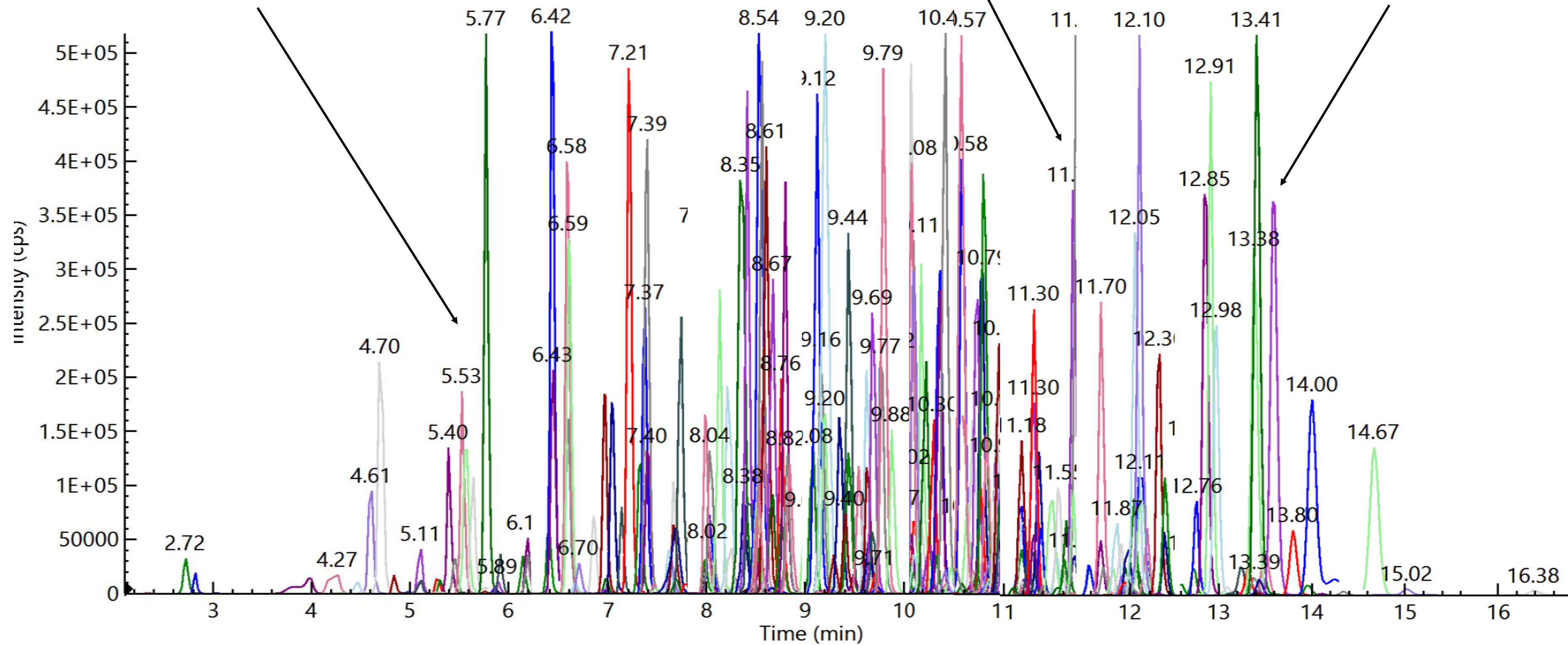
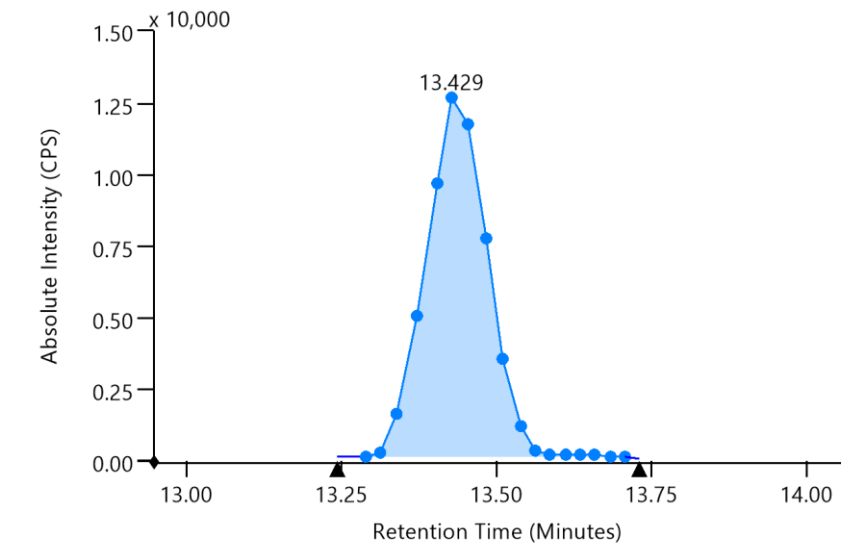
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ESI1 294/122 Amitraz 2 MRM+ "Moving Average, 3, 2" "Slope Max Area, 100, 5" XIC



std_10ppb_30122019_235430
ESI1 270/238 Alachlor 2 MRM+ "Moving Average, 3, 2" "Slope Max Area, 100, 5" XIC



std_10ppb_30122019_235430
ESI1 255/181 Fluroxypyr-1 MRM+ "Moving Average, 3, 2" "Slope Max Area, 100, 5" XIC



Time-managed multiple reaction monitoring (MRM) library, dwell time optimization and automated MS method creation

- 1: Experiment 1
- 2: Experiment 2
- 3: Experiment 3
- 4: Experiment 4
- 5: Experiment 5
- 6: Experiment 6
- 7: Experiment 7
- 8: Experiment 8
- 9: Experiment 9
- 10: Experiment 10
- 11: Experiment 11
- 12: Experiment 12
- 13: Experiment 13
- 14: Experiment 14
- 15: Experiment 15
- 16: Experiment 16
- 17: Experiment 17
- 18: Experiment 18
- 19: Experiment 19
- 20: Experiment 20
- 21: Experiment 21
- 22: Experiment 22
- 23: Experiment 23
- 24: Experiment 24
- 25: Experiment 25
- 26: Experiment 26
- 27: Experiment 27
- 28: Experiment 28
- 29: Experiment 29
- 30: Experiment 30
- 31: Experiment 31
- 32: Experiment 32
- 33: Experiment 33
- 34: Experiment 34
- 35: Experiment 35
- 36: Experiment 36
- 37: Experiment 37
- 38: Experiment 38
- 39: Experiment 39
- 40: Experiment 40
- 41: Experiment 41
- 42: Experiment 42
- 43: Experiment 43
- 44: Experiment 44
- 45: Experiment 45
- 46: Experiment 46
- 47: Experiment 47
- 48: Experiment 48
- 49: Experiment 49
- 50: Experiment 50
- 51: Experiment 51
- 52: Experiment 52
- 53: Experiment 53

Time Managed MRM

Current Catalog: System : Pesticides

New Load System : Pesticides Enter a compound catalog name Save As Delete

Selected	Name	Adduct	Q1 Mass	Polarity	RT	ΔRT	Fragment 1 Q2 Mass	CE1	EV1	CCL2-1	Fragment 2 Q2 Mass	CE2	EV2	CCL2-2	Fragment 3 Q2 Mass	CE3	EV3	CCL2-3	Fragment 4 Q2 Mass	CE4	EV4	
<input checked="" type="checkbox"/>	3-hydroxycarbofuran	+H	238.1	+	7.075	0.5	163	-23	25	-45	181	-19	25	-41	220	-13	25	-36				
<input checked="" type="checkbox"/>	acephate	+H	184.1	+	1.716	0.8	143	-12	25	-29	125	-25	25	-41	95	-30	25	-45				
<input checked="" type="checkbox"/>	acetamiprid	+H	223.2	+	8	0.5	126.1	-30	25	-49	99.1	-56	25	-73								
<input checked="" type="checkbox"/>	carbaryl (sevin)	+H	202.1	+	10.15	0.5	145	-38	25	-54	127	-42	25	-58	130	-55	25	-70				
<input checked="" type="checkbox"/>	carbofuran	+H	222.2	+	9.842	0.5	165.2	-16	25	-37	123.1	-28	25	-47								
<input checked="" type="checkbox"/>	chlorantraniliprole	+H	484	+	12.329	0.5	452.8	-20	25	-66	285.8	-18	25	-65								
<input checked="" type="checkbox"/>	clothianidin	+H	250	+	6.529	0.5	169.1	-16	25	-39	132	-26	25	-48								
<input checked="" type="checkbox"/>	cyromazine	+H	167.2	+	2.052	0.8	68	-30	25	-44	85	-30	25	-44	108	-29	25	-43				
<input checked="" type="checkbox"/>	diflubenzuron	+H	311	+	13.456	0.5	158.2	-20	25	-49	141.2	-48	25	-74	227	-8	25	-38				
<input checked="" type="checkbox"/>	dinotefuran	+H	203.1	+	3.033	0.7	114.1	-20	25	-38	129	-16	25	-35								
<input checked="" type="checkbox"/>	imazail	+H	297.1	+	14.087	0.7	159.2	-31	25	-58	201	-25	25	-52	255.1	-25	25	-52	161.2	-31	2	
<input checked="" type="checkbox"/>	imidacloprid	+H	256.2	+	7.411	0.5	209	-18	25	-42	175.2	-26	25	-49								
<input checked="" type="checkbox"/>	methamidophos	+H	142	+	1.331	0.5	94	-20	25	-32	112	-20	25	-32	124.9	-20	25	-32				

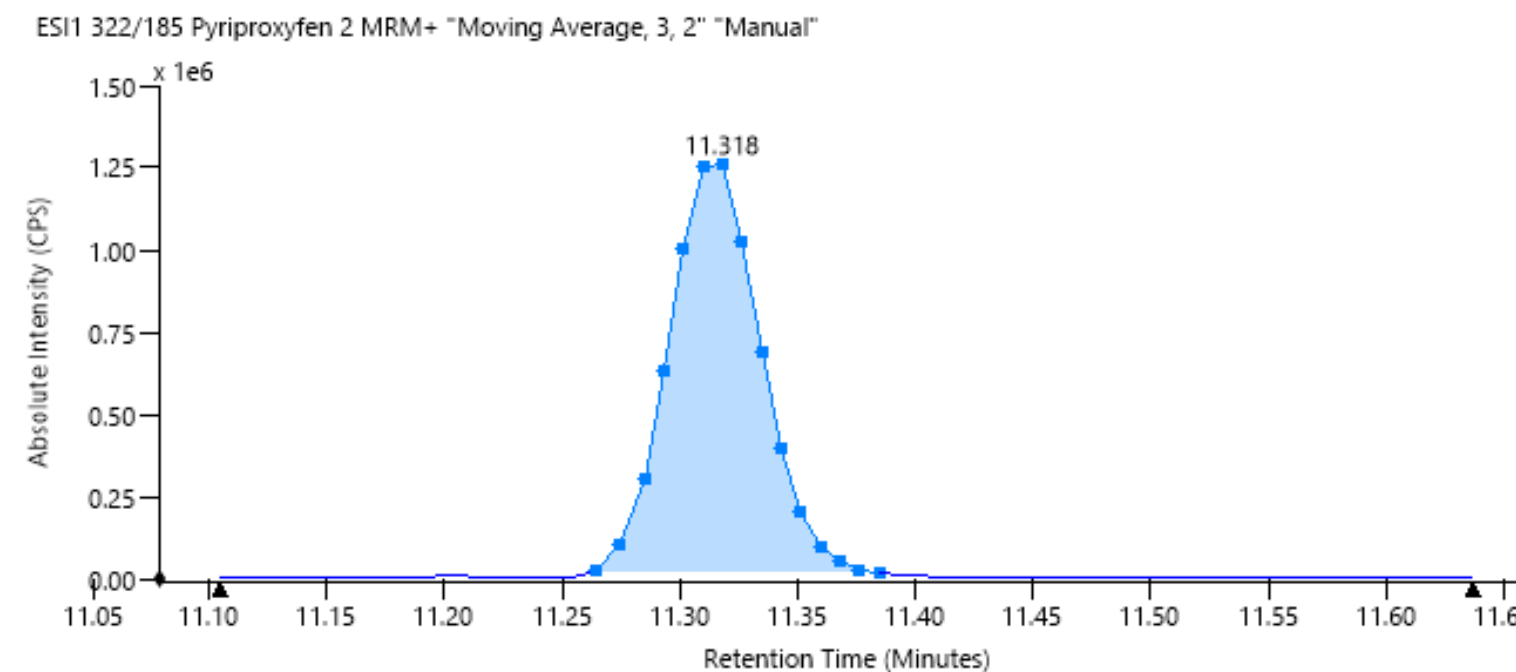
Synonyms

Transfer To Timed MRM

Anticipated Cycle Time (sec): 0.8

Polarity	Name	Q1 Mass	Q2 Mass	Expected RT (min)	Delta RT (min)
Positive	3-hydroxycarbofuran-1	238.1	163	7.075	0.5
Positive	3-hydroxycarbofuran-2	238.1	181	7.075	0.5
Positive	3-hydroxycarbofuran-3	238.1	220	7.075	0.5
Positive	acephate-1	184.1	143	1.716	0.8
Positive	acephate-2	184.1	125	1.716	0.8
Positive	acephate-3	184.1	95	1.716	0.8
Positive	acetamiprid-1	223.2	126.1	8	0.5
Positive	acetamiprid-2	223.2	99.1	8	0.5
Positive	carbaryl (sevin)-1	202.1	145	10.15	0.5
Positive	carbaryl (sevin)-2	202.1	127	10.15	0.5
Positive	carbaryl (sevin)-3	202.1	130	10.15	0.5
Positive	carbofuran-1	222.2	165.2	9.842	0.5
Positive	carbofuran-2	222.2	123.1	9.842	0.5
Positive	chlorantraniliprole-1	484	452.8	12.329	0.5
Positive	chlorantraniliprole-2	484	285.8	12.329	0.5
Positive	clothianidin-1	250	169.1	6.529	0.5
Positive	clothianidin-2	250	132	6.529	0.5
Positive	cyromazine-1	167.2	68	2.052	0.8

Apply Cancel



Dwell time optimization

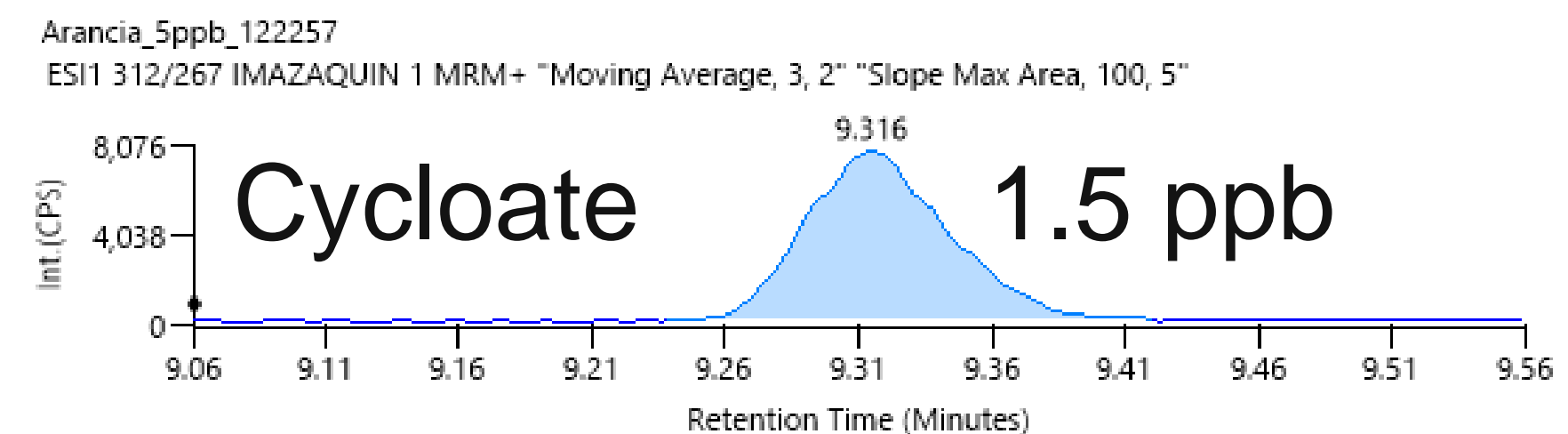
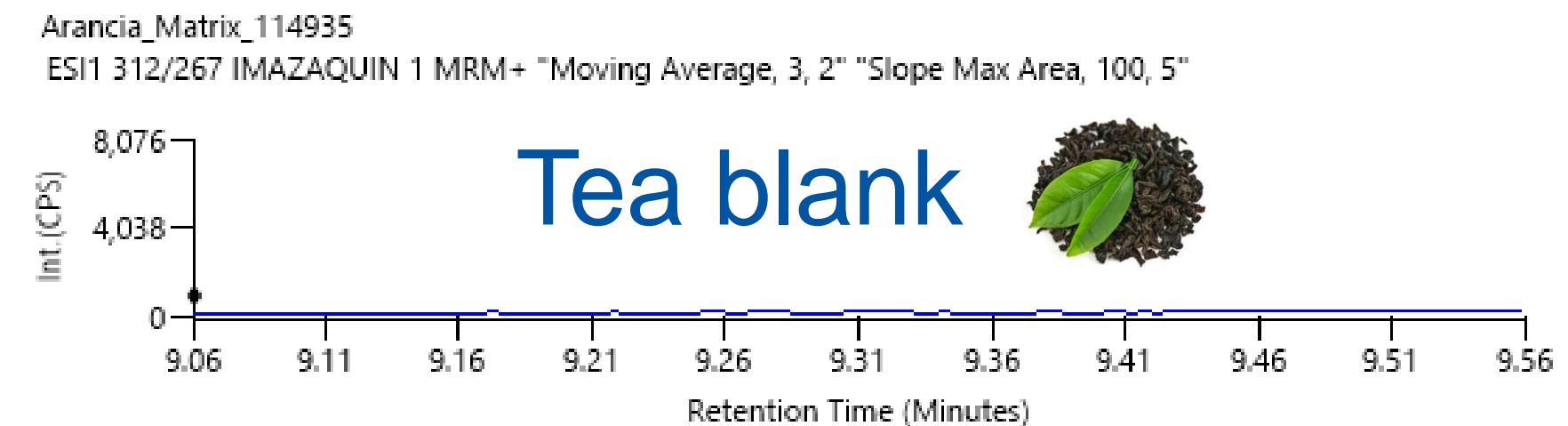
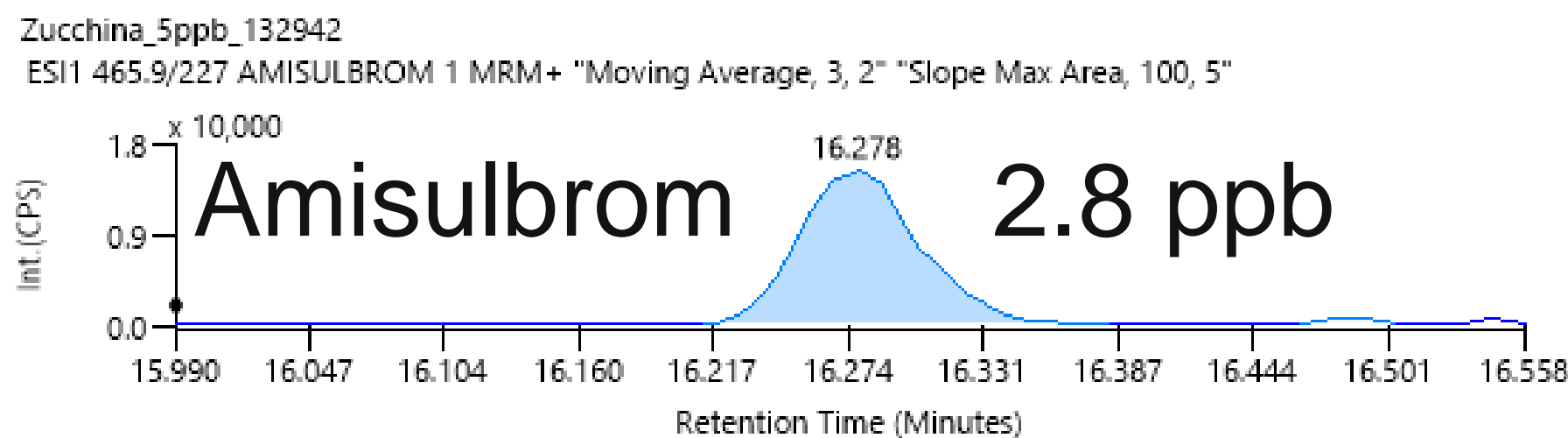
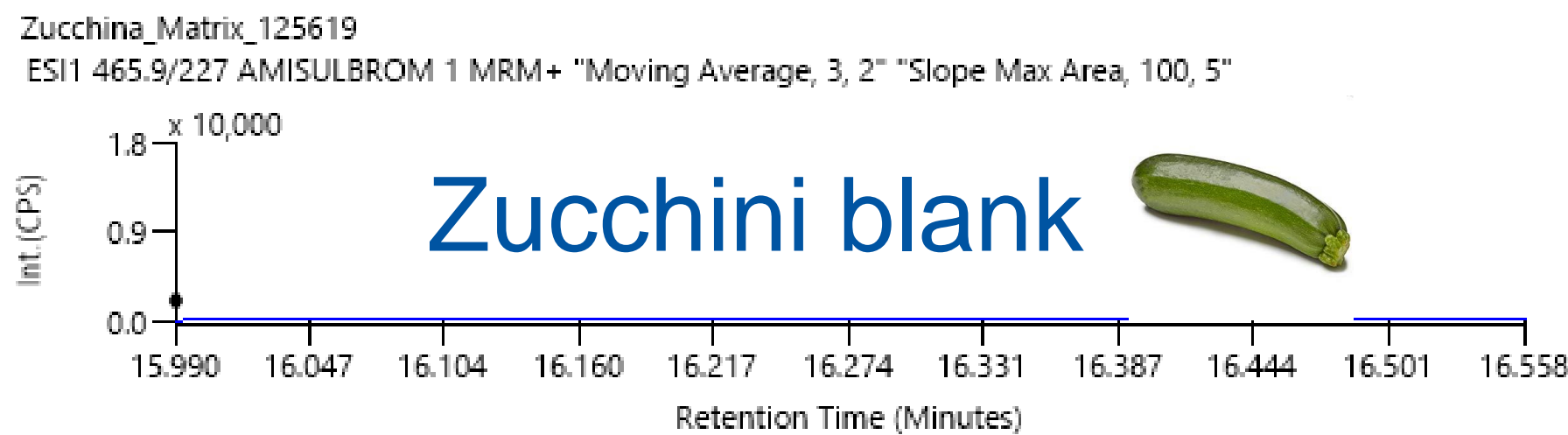
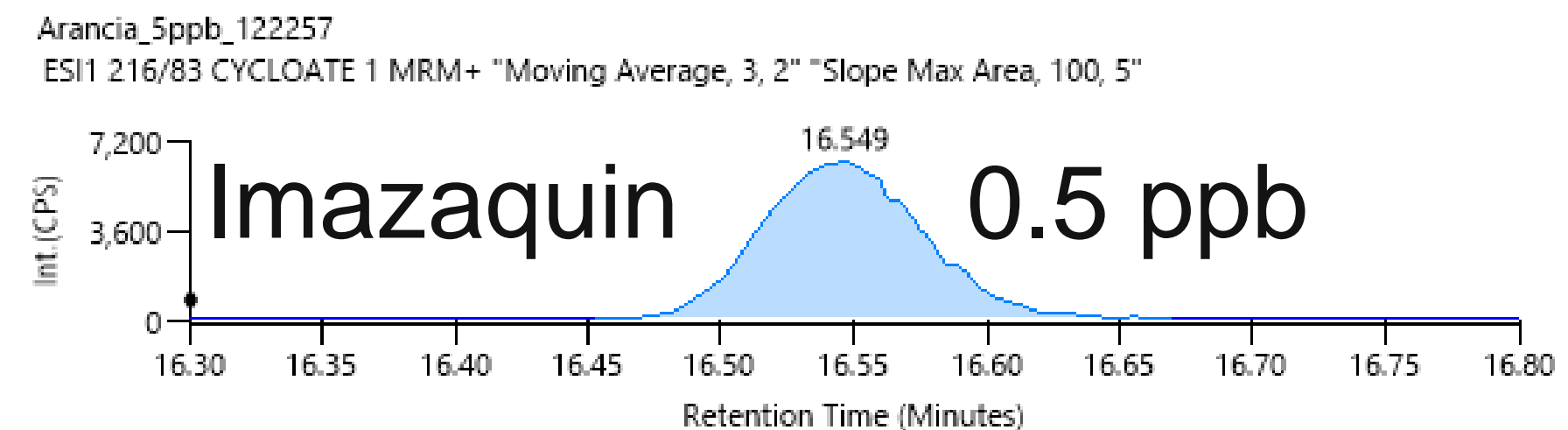
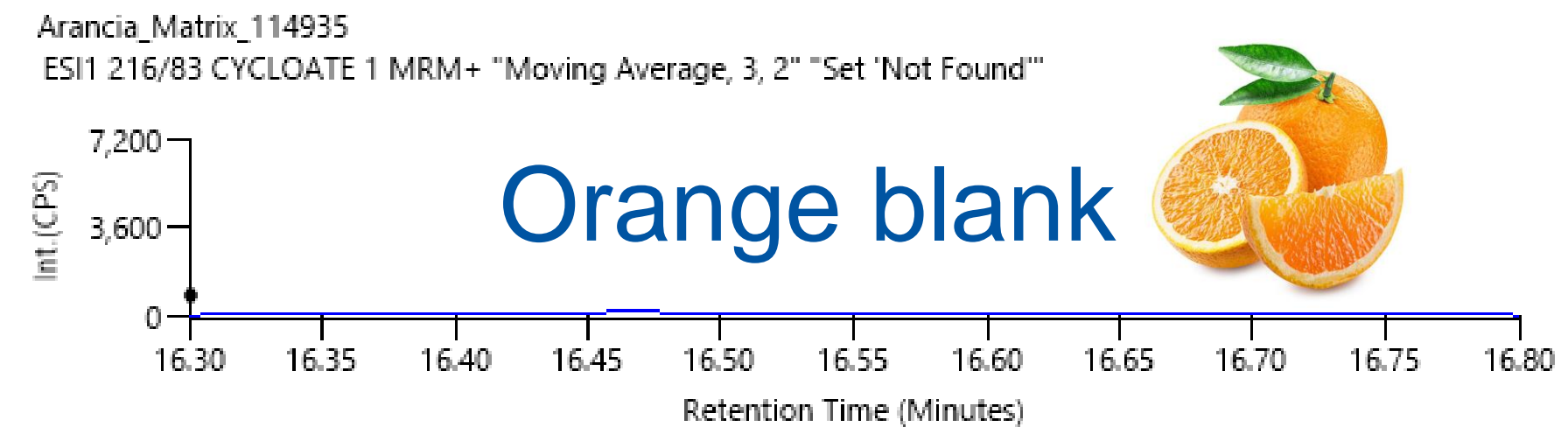
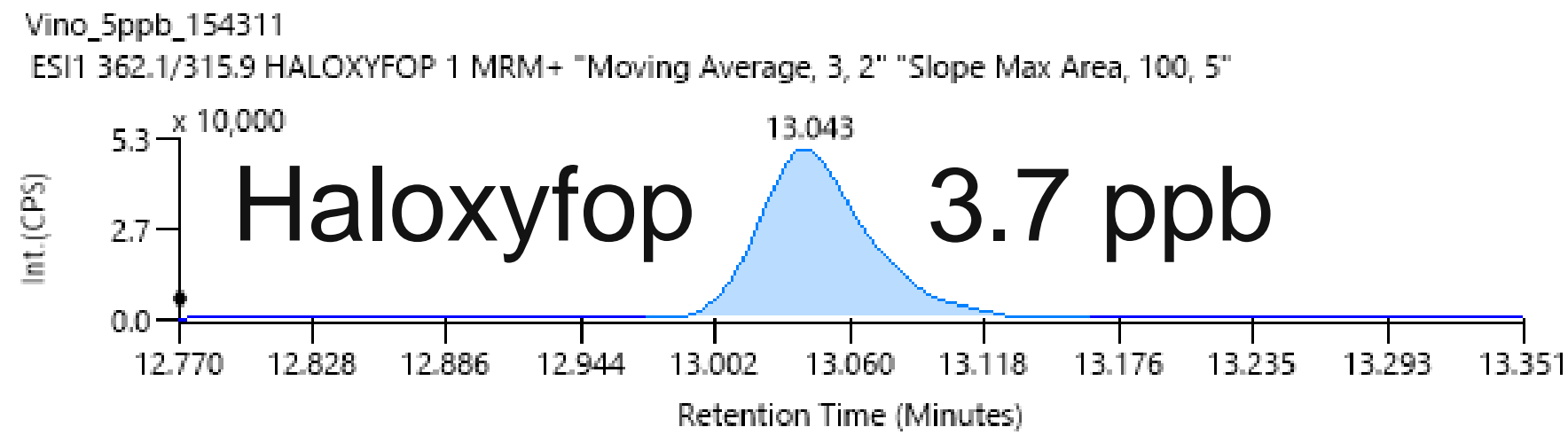
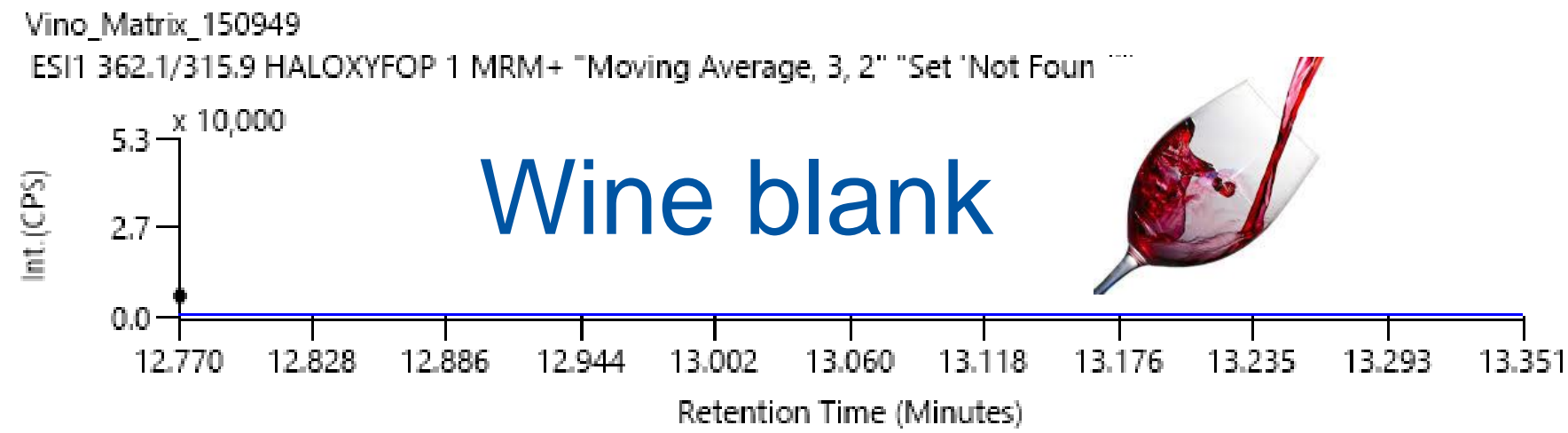
Peak width = 8 sec

Anticipated cycle time = 0.55 sec

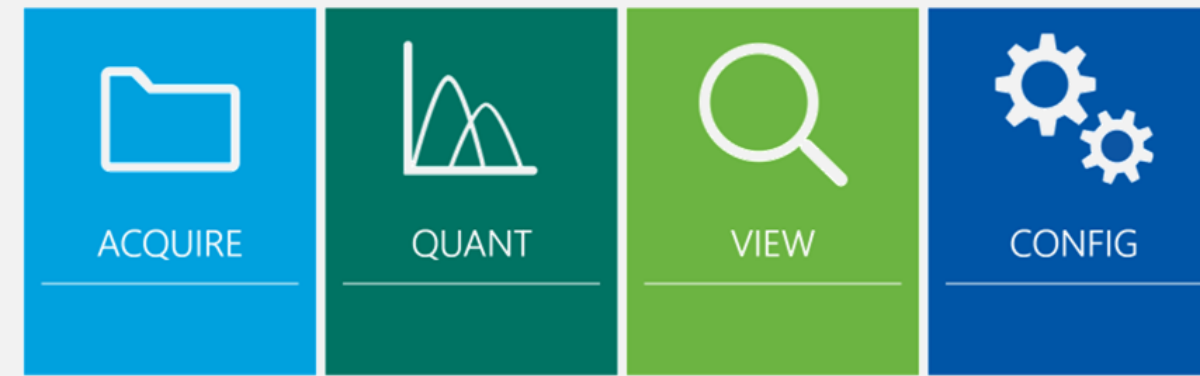
→ Data points across peak:

$8/0.55 = 15$ data points

Pesticides in foods – spiked blank samples



Simplicity™ Software



Home Configuration MS Method LC Method Batch List Creator Batch Acquisition Queue Quantitation Method Results Processor DataViewer

Project folder: C:\Users\Public\Instrument\Project_Pesticidi_Samer
 Quantitation Method: C:\Users\Public\Instrument\Project_Pesticidi_Samer\Quantitation Methods\Mastemizmqf
 Results by Analyte "Dimethomorph 1 (388.2/301.1)": each Ion Ratio column tooltip shows the average ratio

Group	Analyte Component	Component Type	Sample File Name	Include	Analyte Component	Mass Transitio	Known Concen	Dilution Factor	Concentration by A	Peak Area	Sample Accuracy %	Retention Time De	Concentration	CV% (Area)	Ion Ratio (165.1/30	Expected Ion Ratio	Inter	
81	360 Dichlorvos 1 (22...	Quantifier																
82	191 Dicrotophos 2 (...	Quantifier																
83	1530 Diethofencarb 1...	Quantifier																
84	2150 Difenoconazole...	Quantifier																
85	1000 Difloxuron 2 (...	Quantifier																
86	700 Diflubenzuron ...	Quantifier																
87	2510 Dimepiperate 2 ...	Quantifier																
88	841 Dimethenamid ...	Quantifier																
89	300 Dimethoate 1 (2...	Quantifier																
90	1320 Dimethomorph ...	Quantifier																
91	540 Dimethylphenyl...	Quantifier																
92	1211 Diniconazole 1 (...	Quantifier																
93	121 Diniconazole 2 (...	Quantifier																
94	2200 Dinitramine 1 (3...	Quantifier																
95	551 Dinoseb 1 (239...	Quantifier																

Standard Curve: "Concentration vs Area"
 Source "ESI1" Component "Dimethomorph 1 (388.2/301.1)"
 $y = 31,694x + 3,162.3$ $R^2 = 0.9986$ (ByArea, Linear, 1/X)

Sample: 1532_050818
 ESI1 388.2/301.1 Dimethomorph 1 MRM+ "Moving Average, 3, 2" "IntelliPeak, 100, 5, 1000, 1, 1000, 10, 1500, 10, version 1"

Dimethomorph
 39 ug/kg rucola sample

MS Pump AS Oven Q

Status Ready System is idle

Threshold Settings Dialog

Standard Curve Line Coloring
 R^2 0.980 0.950

Results Table Column Coloring
 Accuracy (%) 10.000 40.000
 RT Deviation (%) 10.000 20.000
 CV (%) 5.000 10.000

Peak Area
 Peak Height

Action Level Thresholds
 Calculated Concentration
 Apply thresholds to Concentration: by Area by Height

Expected Ion Ratio	Acceptable Difference (%)	EU values	US values
< 0.1	50	50 %	20 %
≥ 0.1 < 0.2	30	30 %	20 %
≥ 0.2 < 0.5	25	25 %	20 %
≥ 0.5	20	20 %	20 %

Results Table Concentration Column Coloring
 Extrapolation Cutoff (%) 20.000
 Within Standard Range
 0% < Extrapolation ≤ 20%
 Extrapolation > 20%

Apply to all analytes OK Cancel

Color coding for rapid identification of "positive" samples

<1 ug/kg <10 ug/kg >10 ug/kg (general MRL)

QSight Outside



LX50 UHPLC

Small Footprint, Vertical Design:
Compact 50 cm x 50 cm x 110 cm
no benchtop needed.



QSight Inside - Innovative and patented technologies – flow-based MS

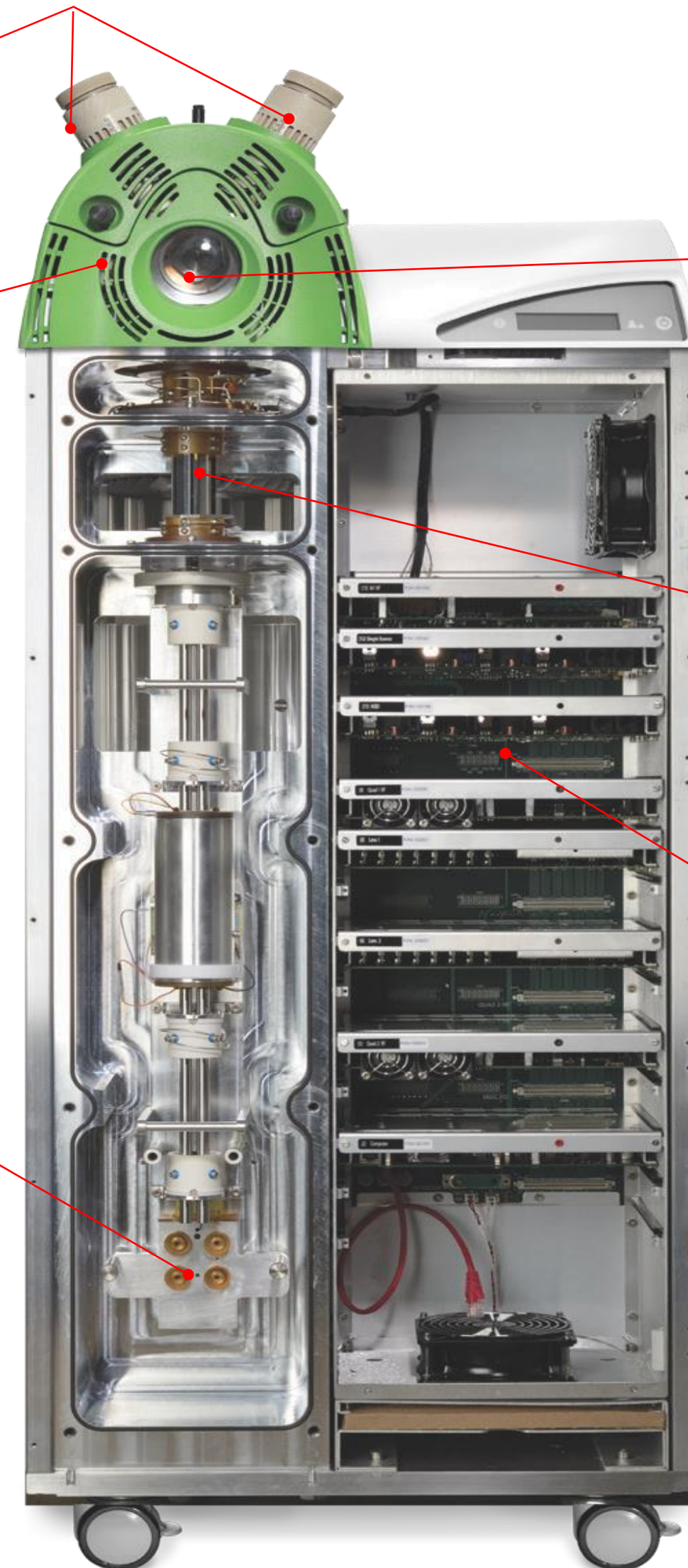


Dual Source: Two independent probes provide true multiplexing flexibility

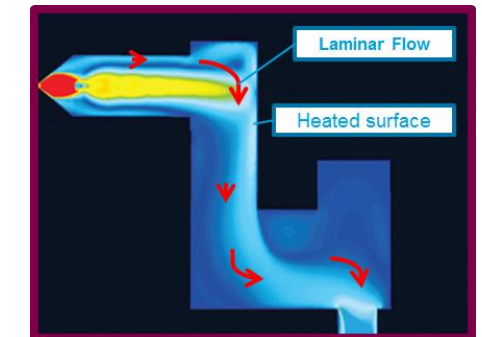
StayClean™ Source: Self cleaning design delivers maximum sensitivity and exceptional uptime



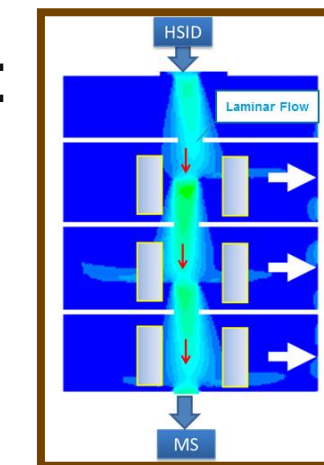
Unifield™ Detector: Patented technology counts positive and negative ions without high voltage switching



HSID™ Interface: Provides high S/N and reproducible results, with no optimization or regular maintenance



Laminar Flow Ion Guide™: Highly efficient field-free transmission

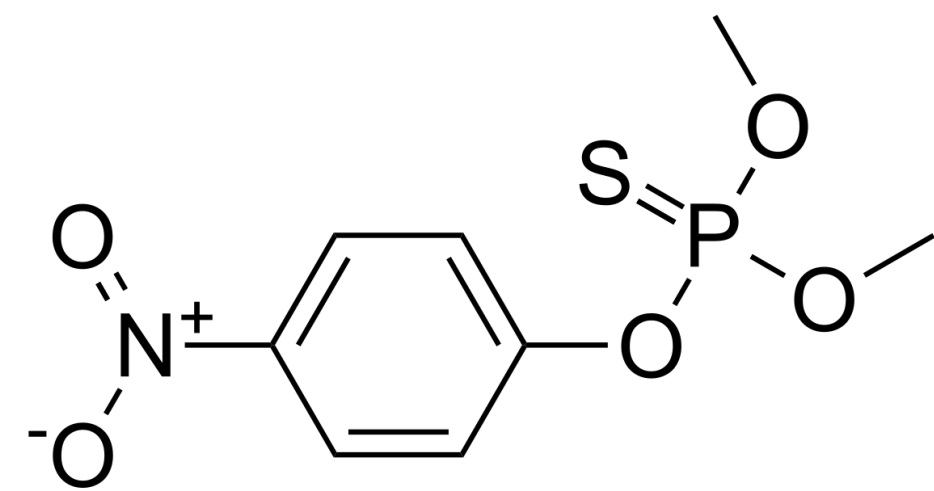


Modular: Plug-and-play design for ease of service

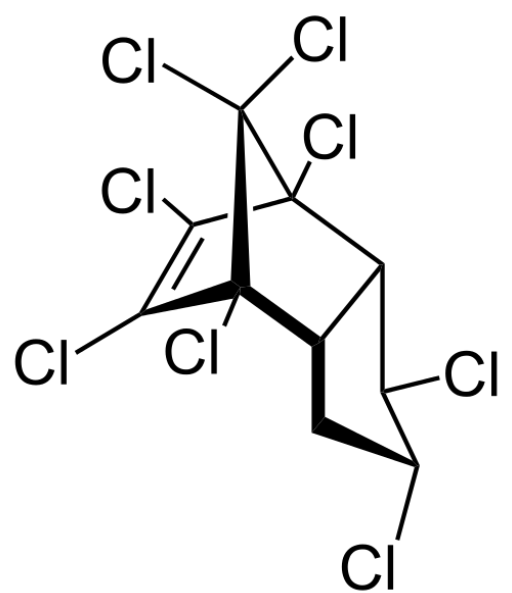
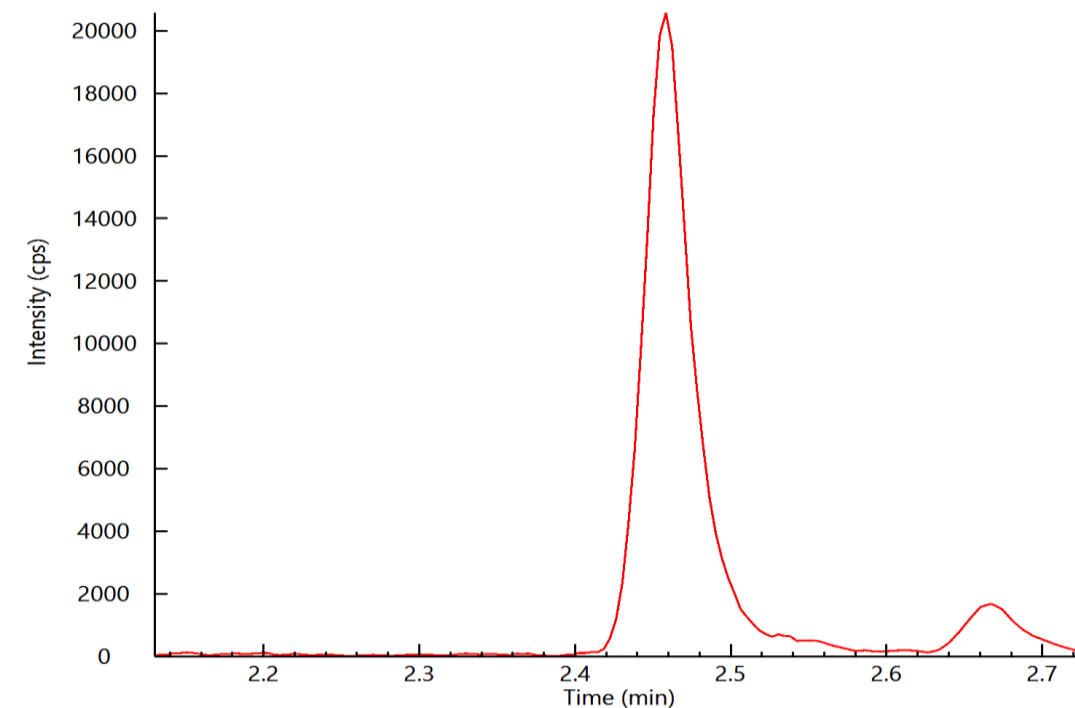


Selected Applications

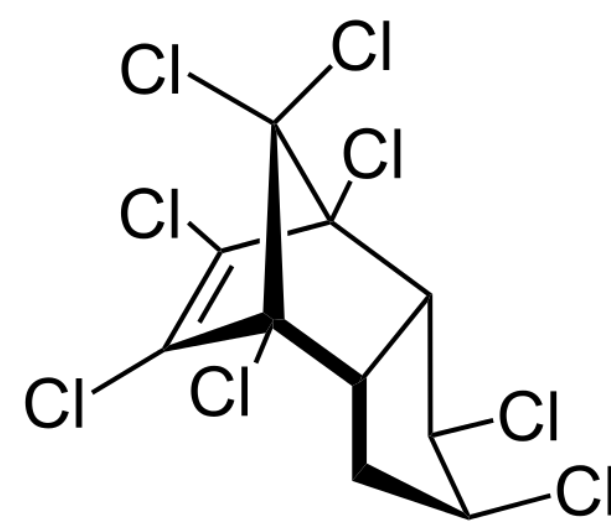
Dual Source: Use of APCI for GC amenable compounds



Methyl Parathion S/N = 200

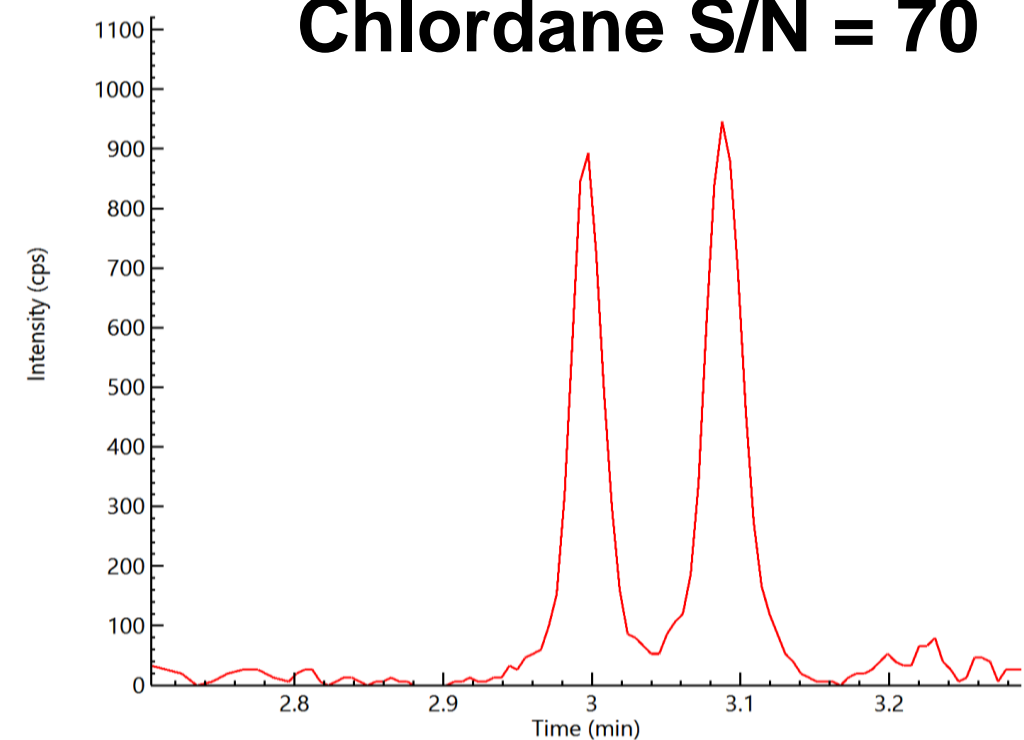


trans



cis

Chlordane S/N = 70



**Response for Four A
10x diluted Cannabis FI**



APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

Authors:

Avinash Dalmia, Saba Hariri, Jacob Jalali,
Erasmus Cudjoe, Toby Astill, Charlie Schmidt,
Feng Qin

PerkinElmer, Inc.
Shelton, CT
Toronto, ON

Charles Johnson
Joey Kingstad
Napro Research, Inc.
Sacramento, CA

Novel ESI and APCI LC/MS/MS Analytical Method for Testing Cannabis and Hemp Concentrate Sample Types

Introduction

As new adult-use and medicinal cannabis markets emerge in the US and Canada, the use of concentrate cannabis and CBD products (e.g. edibles, beverages, vape products, isolates, topicals,

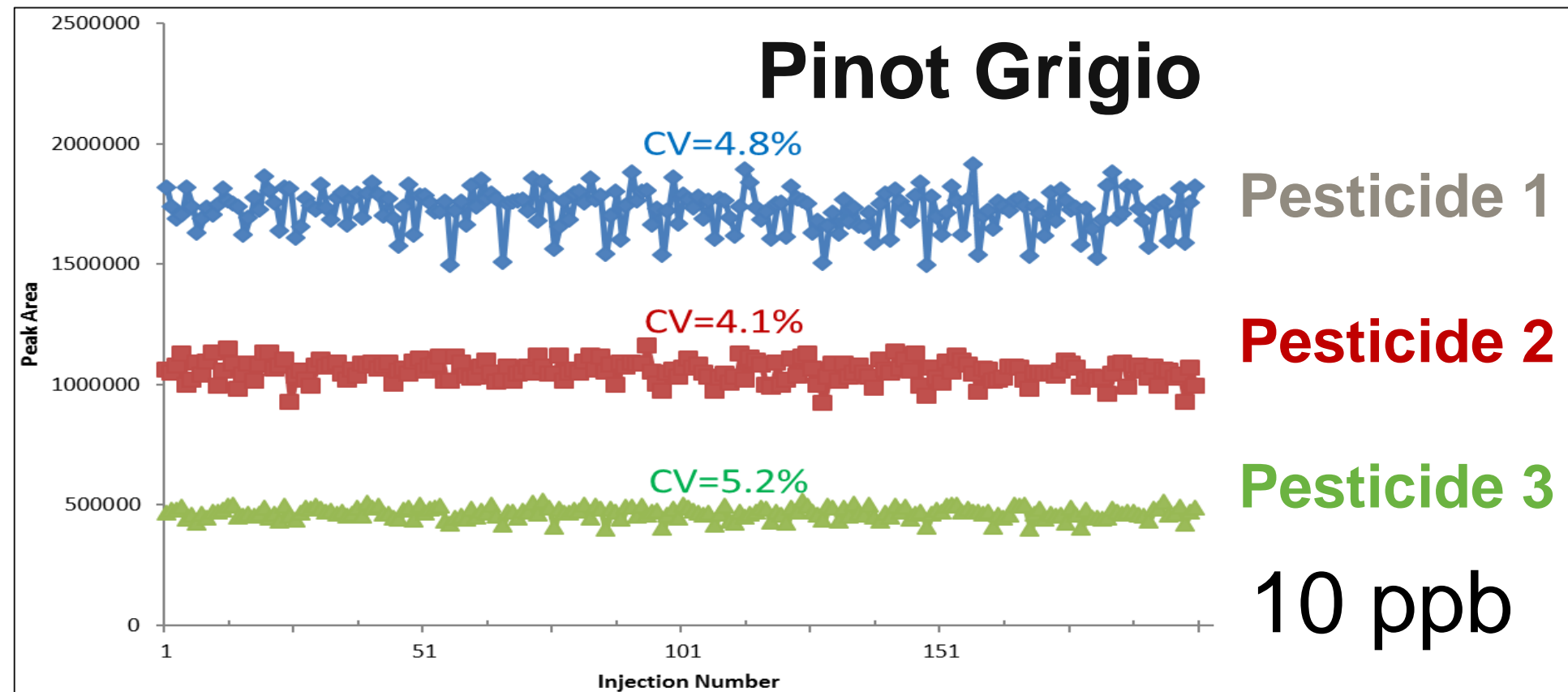
and waxes) continues to increase in popularity. According to market research, concentrates and their derivative products are expected to represent 50% of the consumer market by 2022.¹ This growth, and the diversity in sample type, presents an analytical challenge for testing laboratories. The concentrate matrix has a significant effect on the analytical method, owing to higher sample matrix effects caused by the increased concentration levels (up to 95% w/w) of cannabinoids in the sample. This effect influences the response of certain pesticide molecules, requiring laboratories to validate a pesticide method specific to the sample matrix type.

In this work, an LC/MS/MS method is presented for the analysis of 66 pesticides, including hydrophobic and chlorinated pesticides typically analyzed by GC/MS/MS, and five mycotoxins. Utilizing a cannabis concentrate matrix, the method features a simple solvent extraction, followed by analysis using an LC/MS/MS instrument with dual ESI and APCI sources. The analysis yielded excellent recoveries and detection limits, well below those specified by the State of California cannabis regulations, for all analytes.

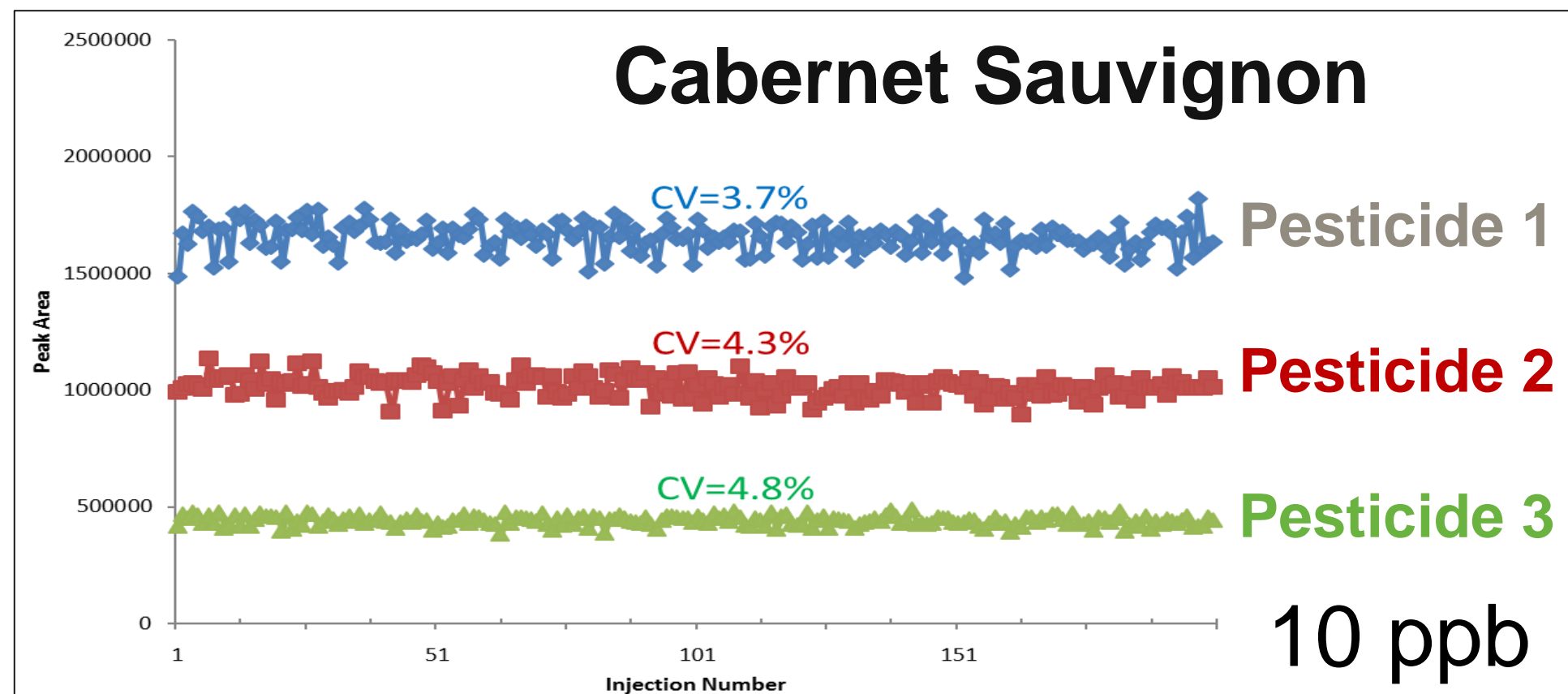
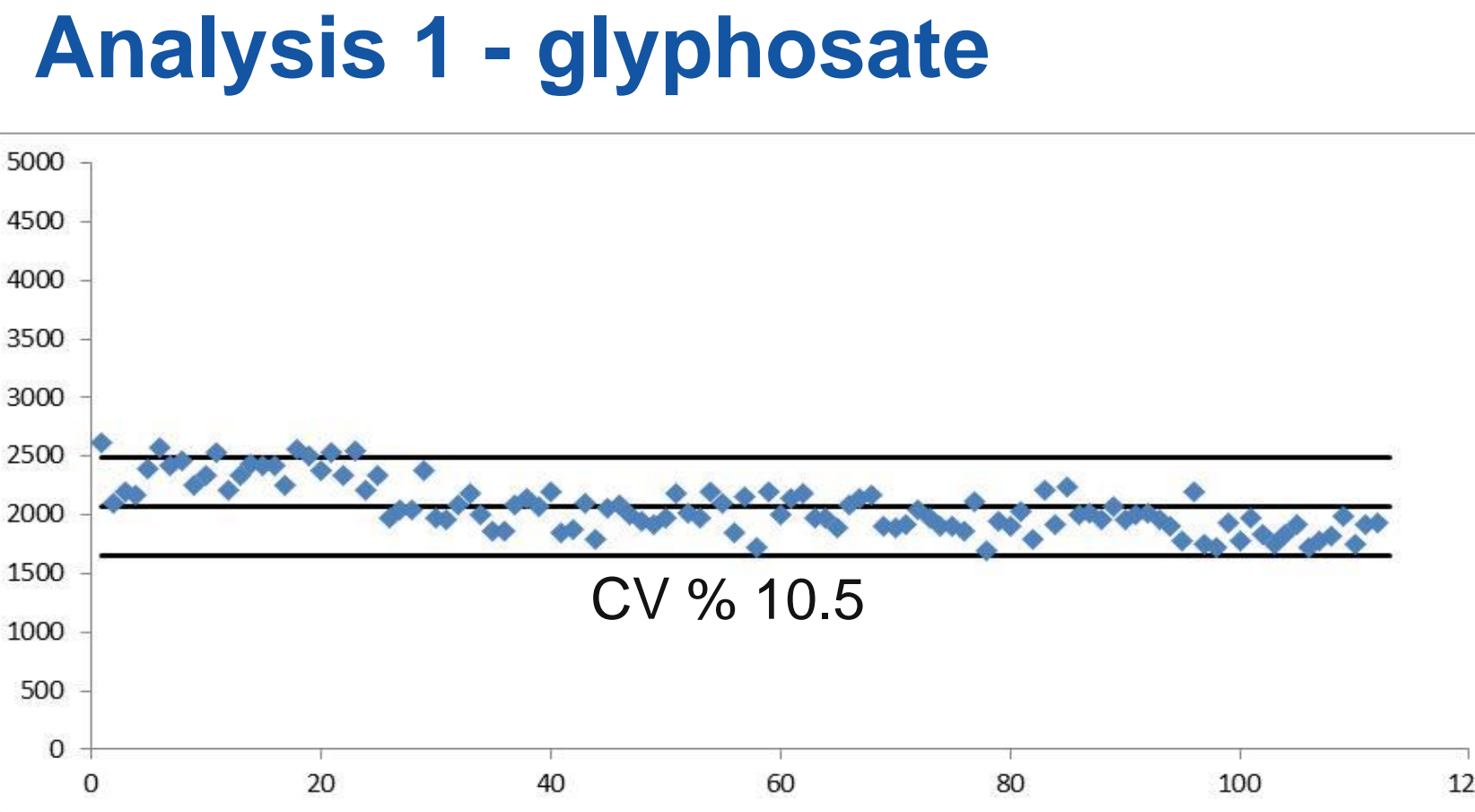
StayClean™/HSID™: “No Dilute” Just Shoot - pesticides in wine



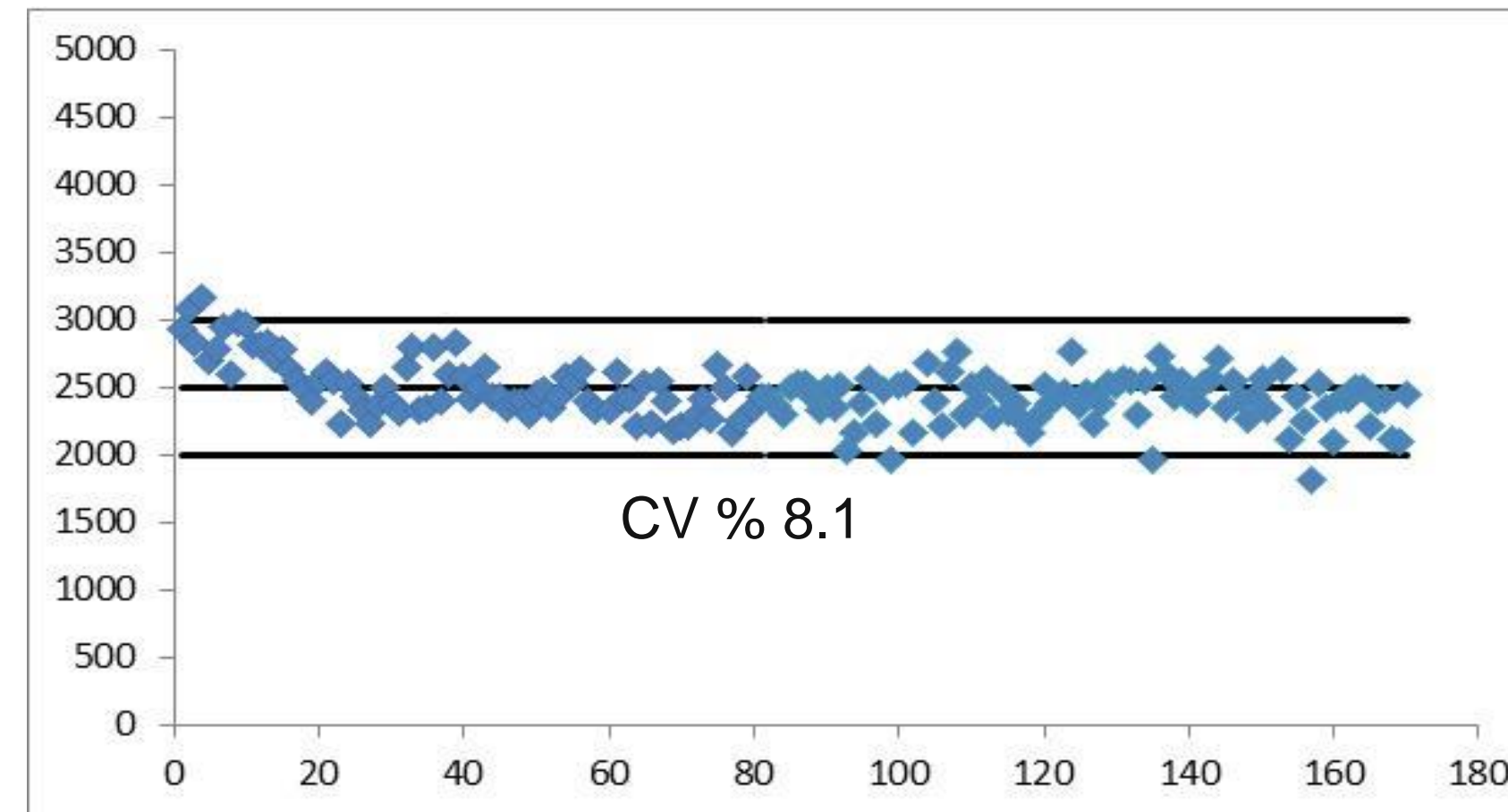
200 injections



270 injections

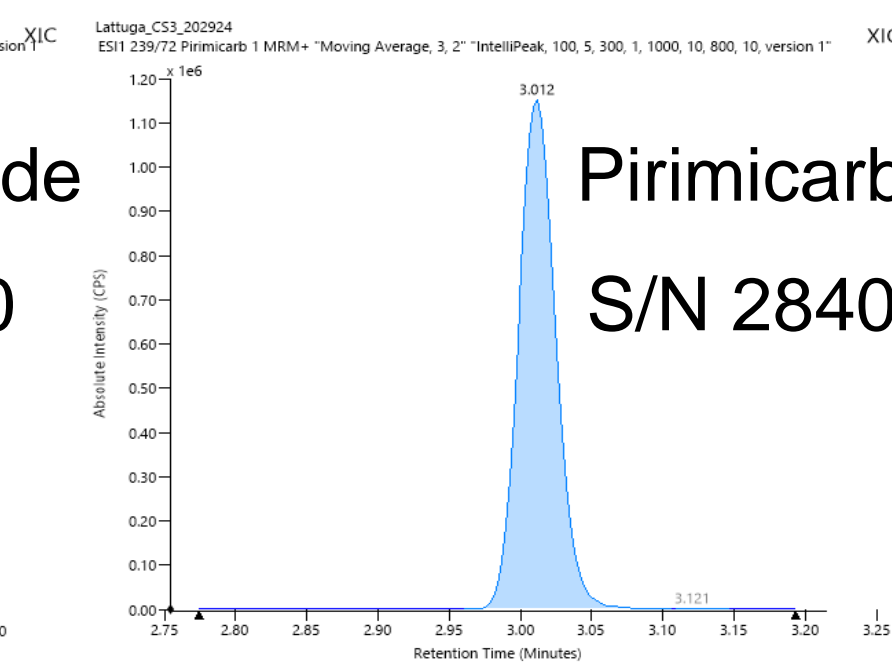
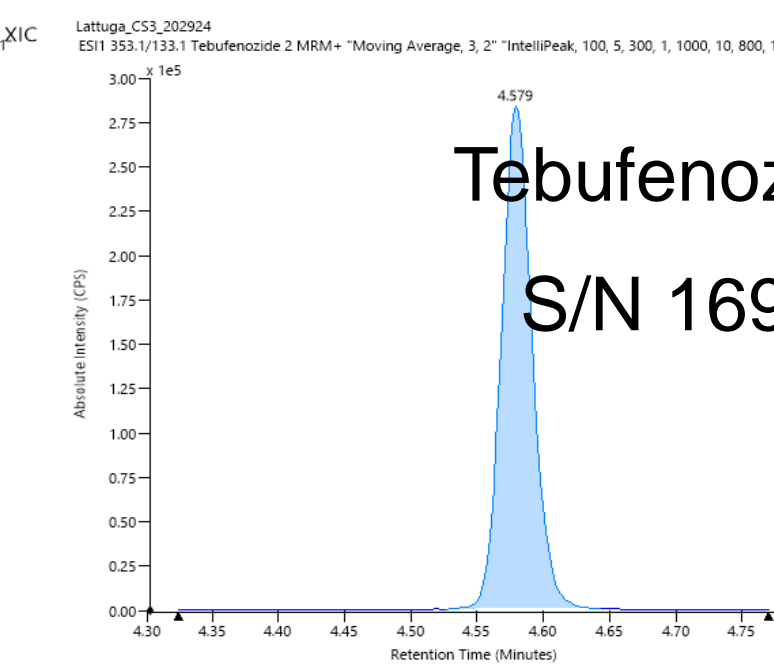
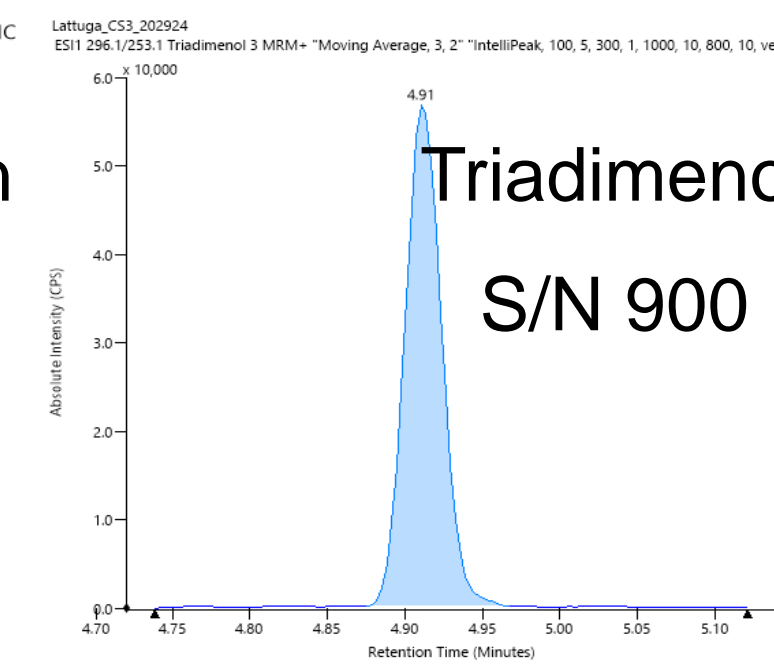
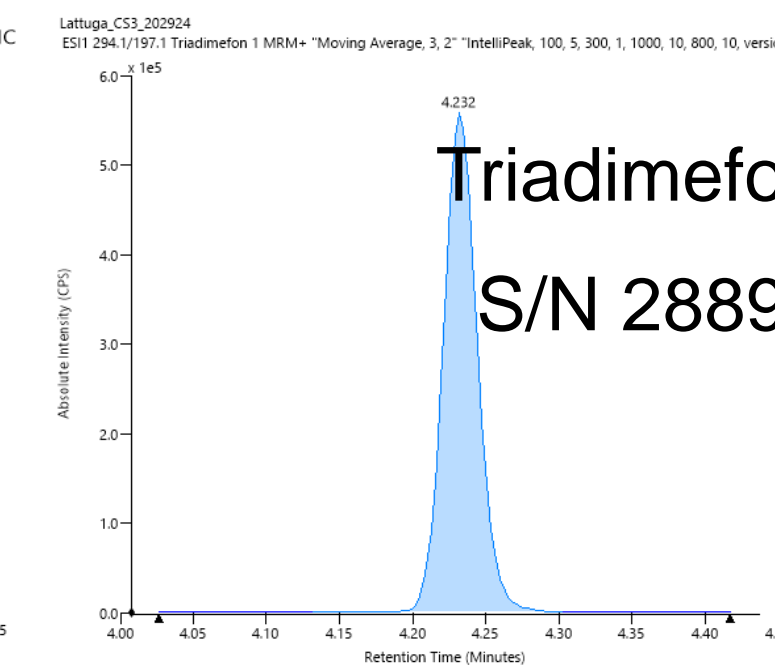
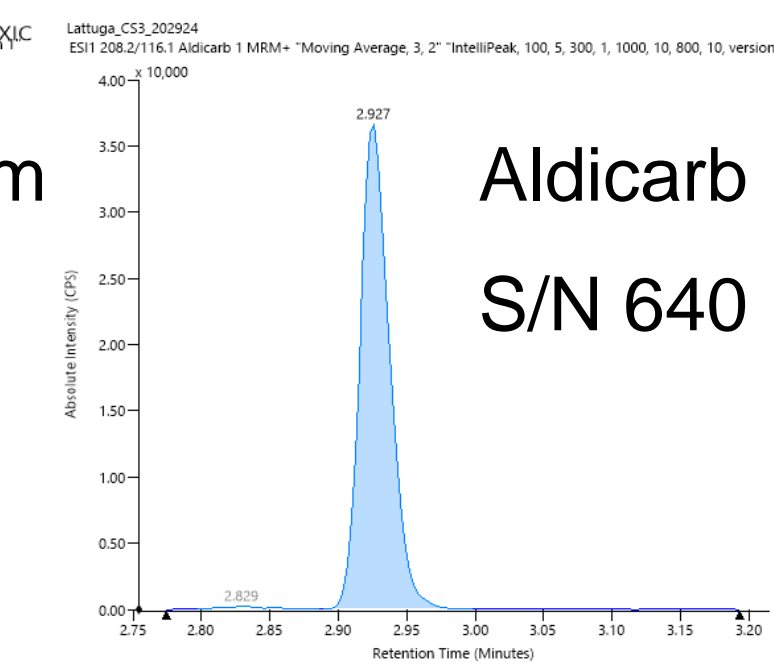
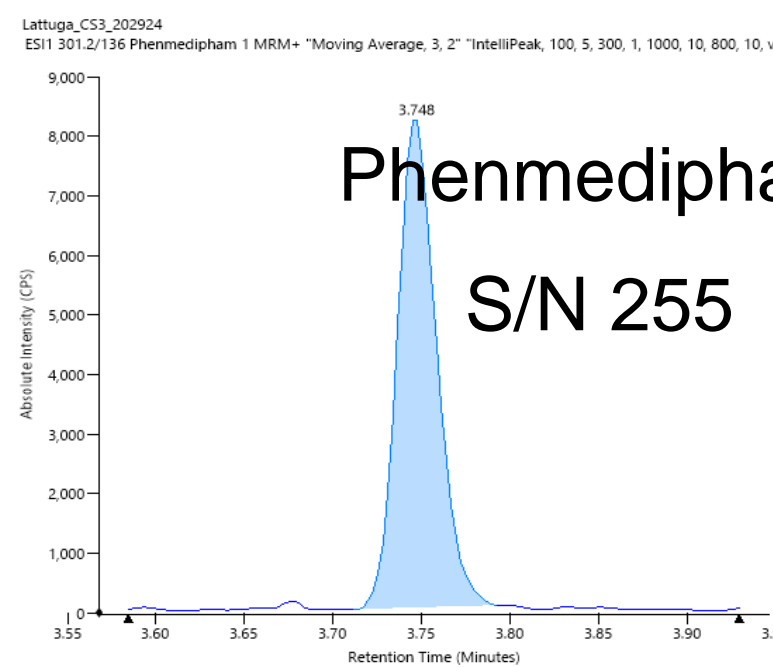
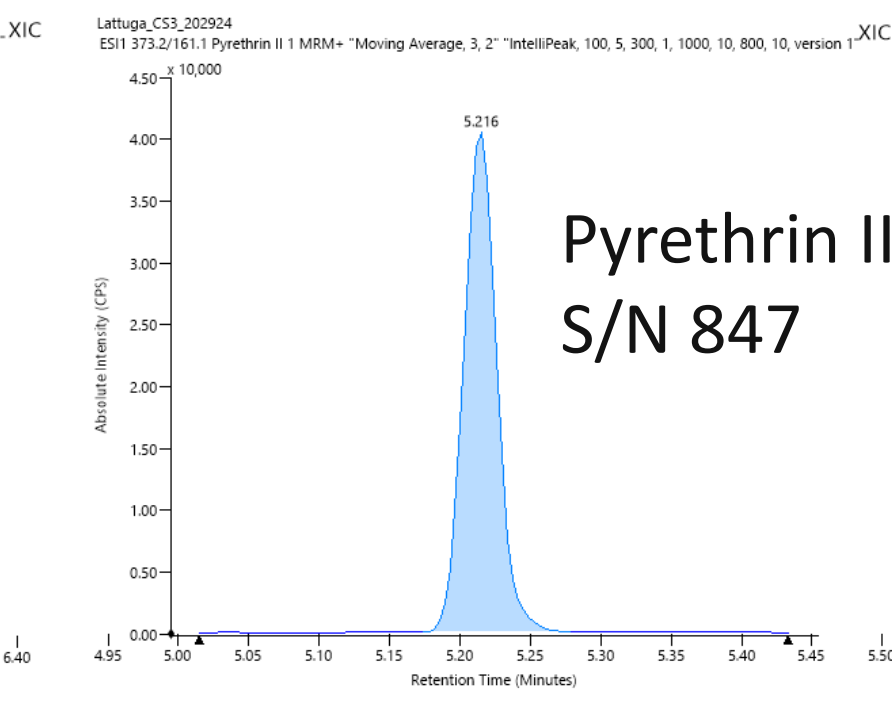
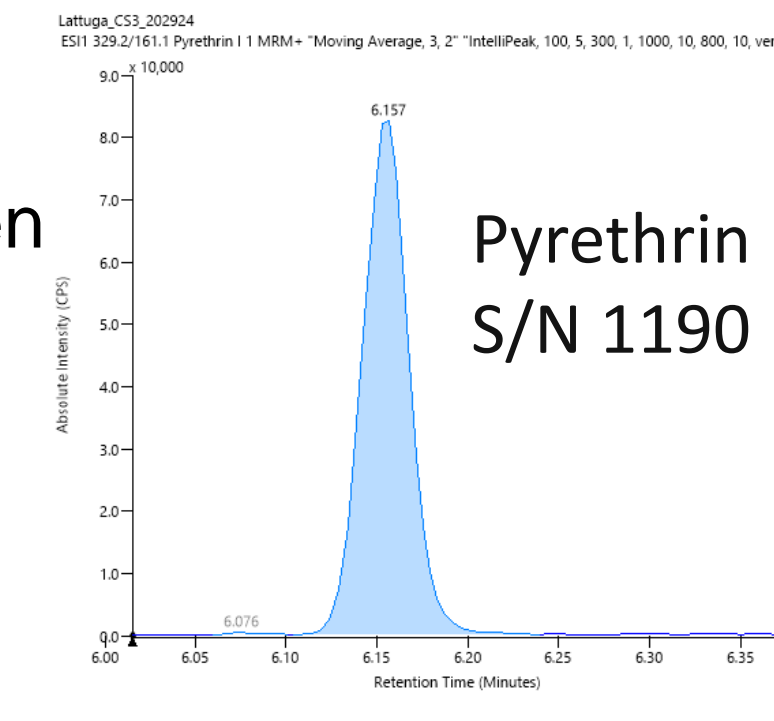
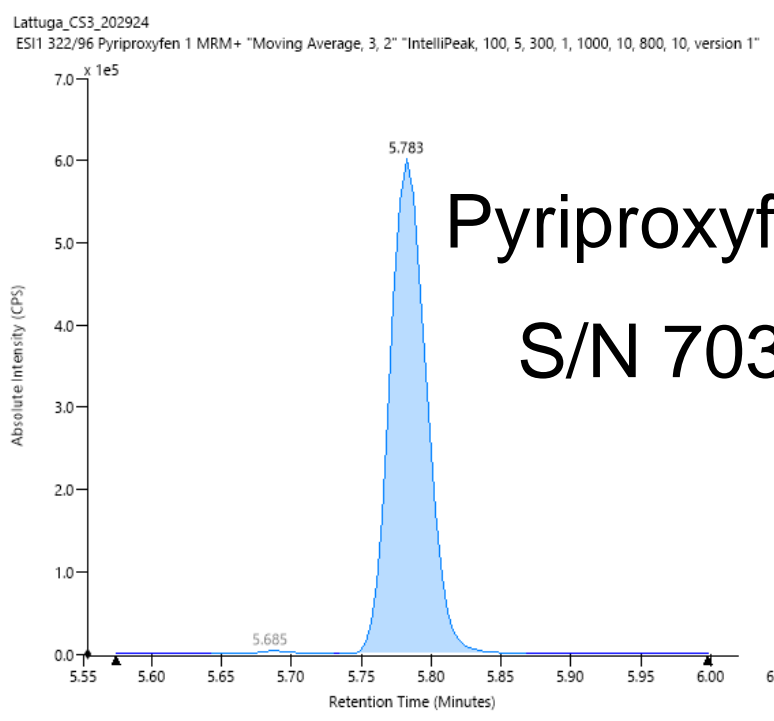
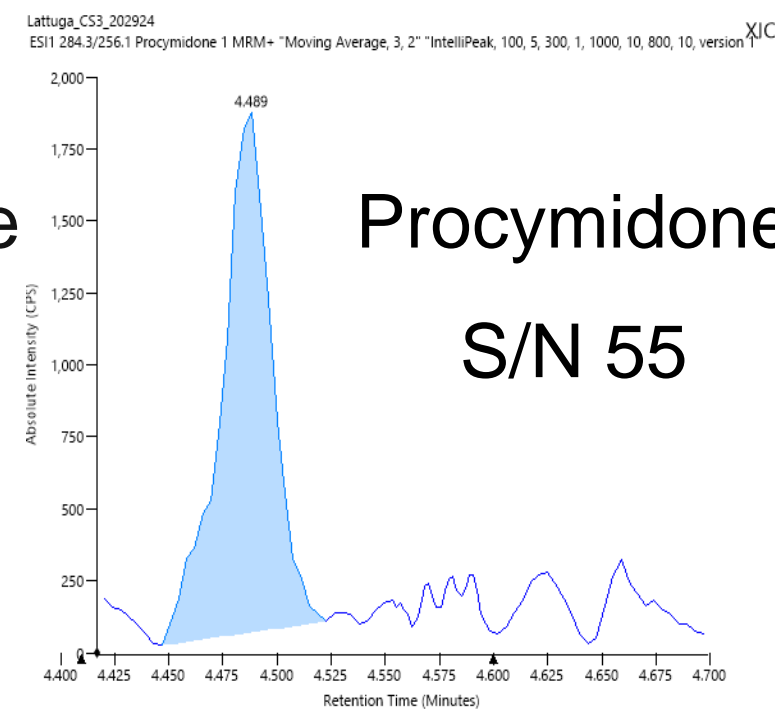
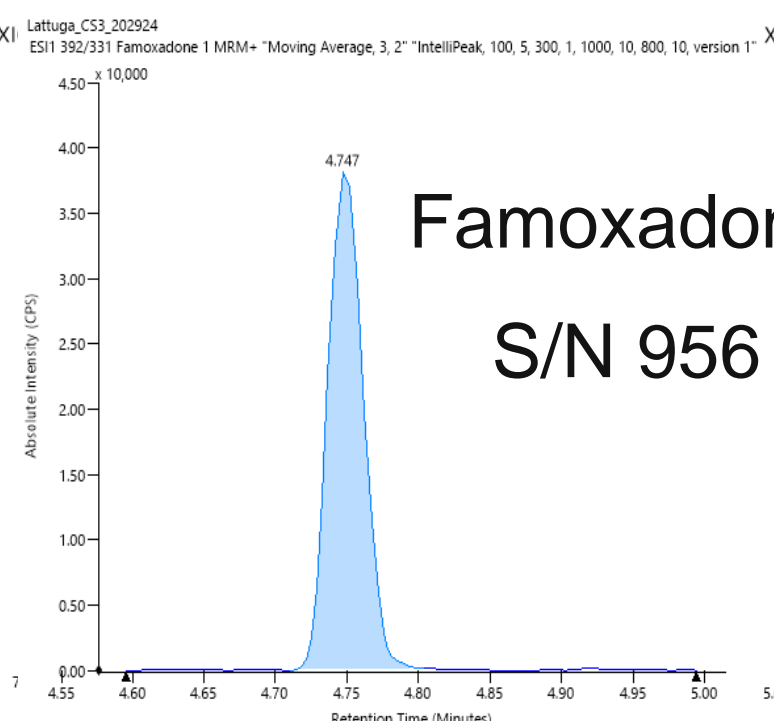
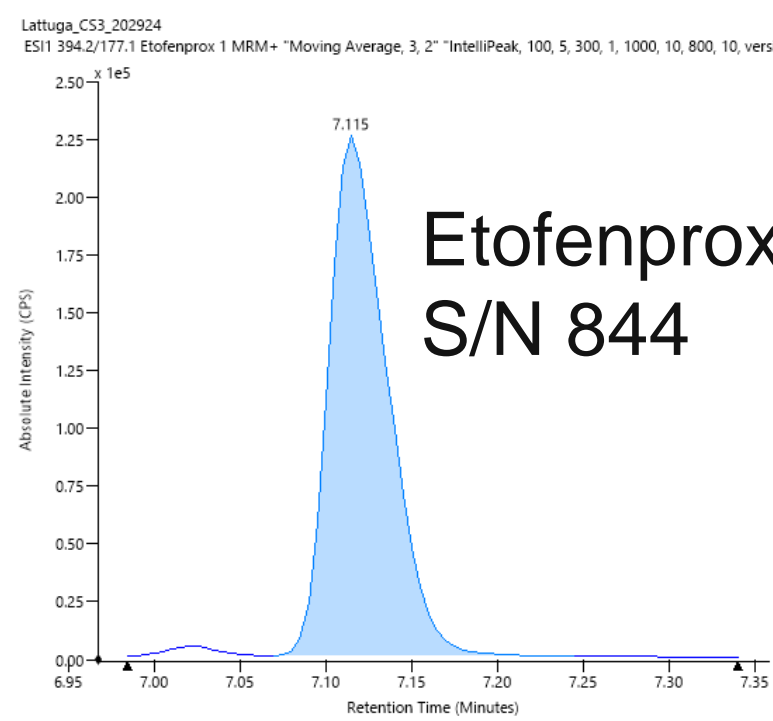
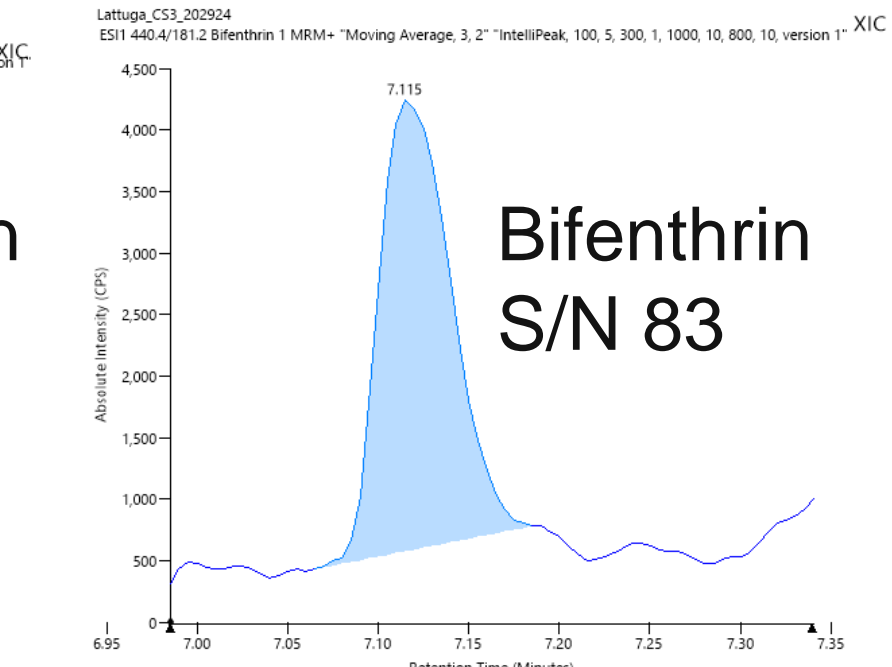
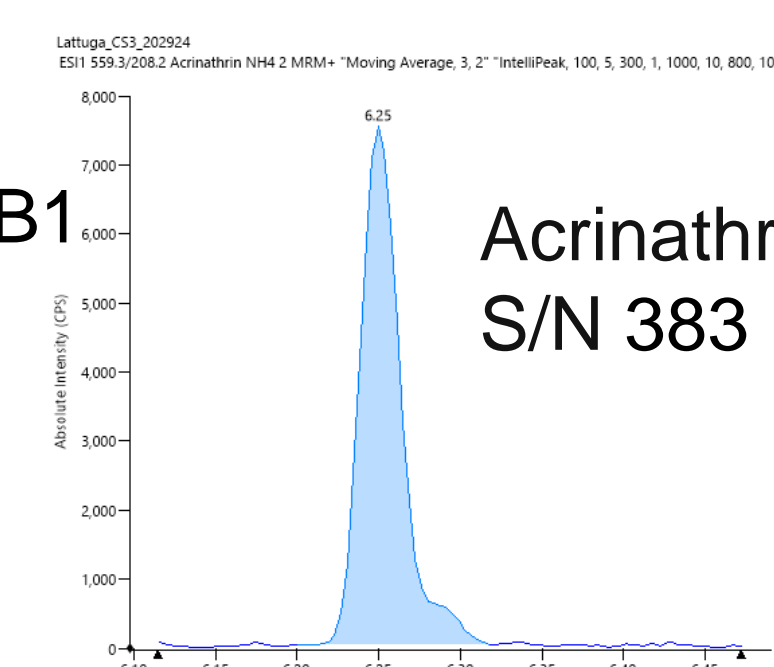
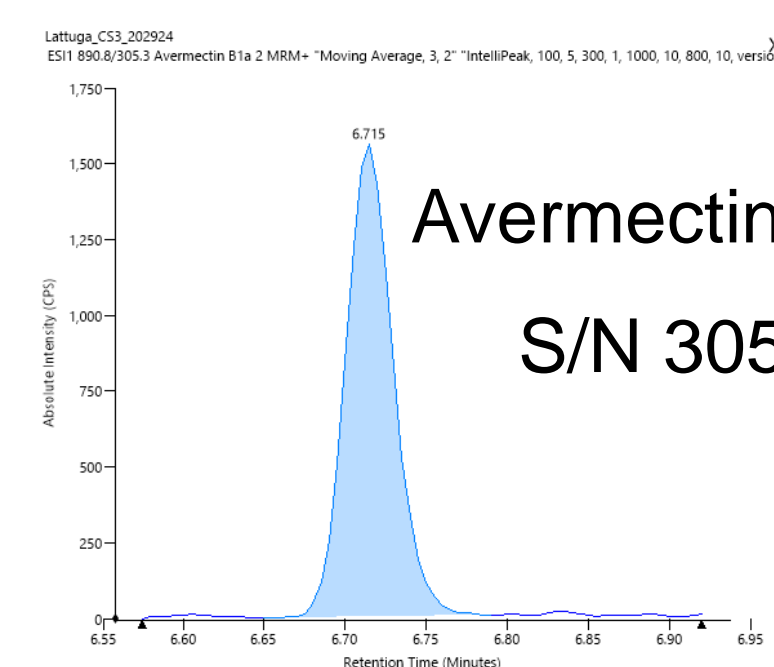
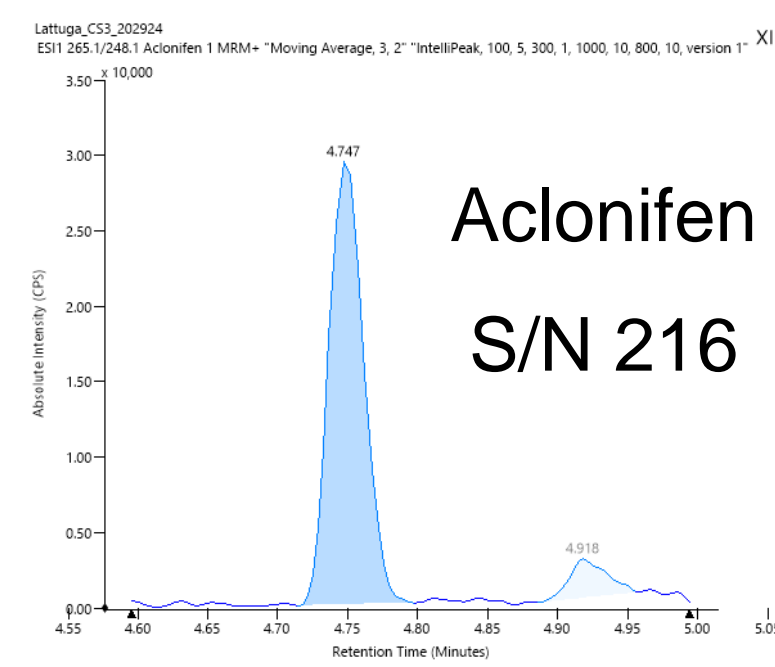
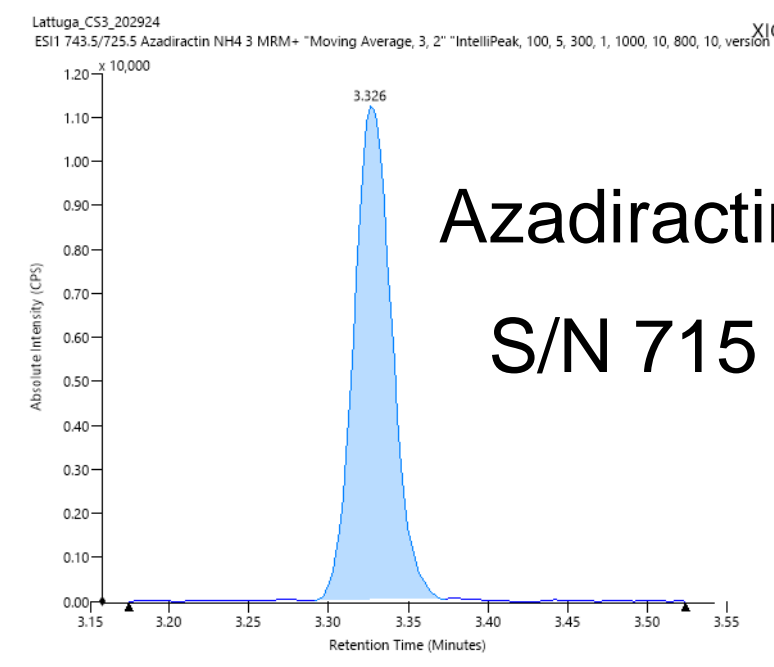
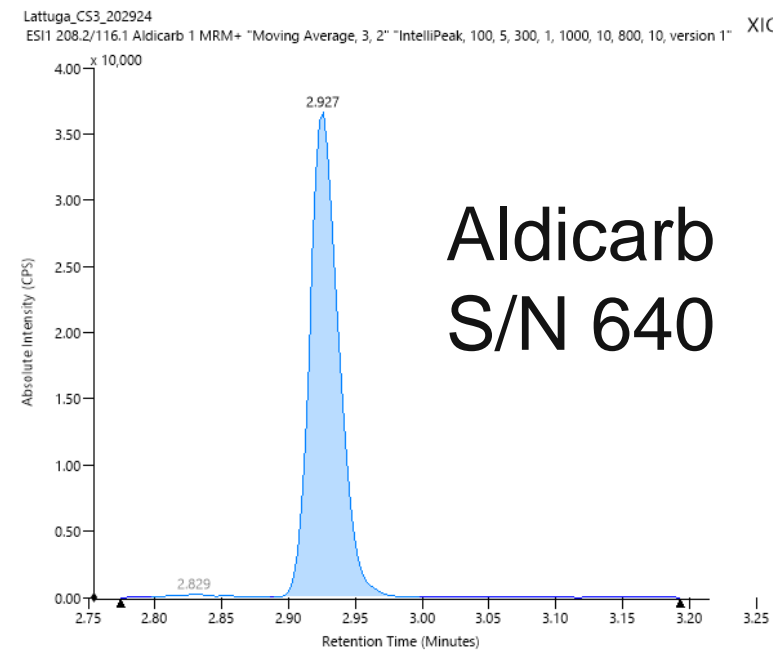


Analysis 2 - glyphosate 50 ppb



1. Dimethenamid, 2. Pyriproxyfen, 3. Benthiavalicarb-isopropyl

Laminar Flow Ion Guide™ - challenging pesticides – 10 ppb in lettuce



UniField Detector™: Multi-residue method with 500+ pesticides

Sample preparation:

- 10 g of vegetable/fruits (orange, apple, lettuce, endive, olives, black chickpeas)
- QuEChERS extraction and clean-up
- 45 mg of porous graphitic carbon (PGC) for highly pigmented fruit and vegetables
- IS addition

LC parameters:


- Flow Rate: 0.4 ml/min
- Column: C18, 2.7µm, 4.6x100mm
- Column Temperature: 40°C
- Sample Temperature: 10°C
- Injection volume: 10 µl
- Mobile Phase A: 9mM AcNH₄ in H₂O – ACN (90:10,v:v), 0.1 % FA
- Mobile Phase B: 1mM AcNH₄ in H₂O – ACN (10:90,v:v), 0.1 % FA

Step	Time (min)	%A	%B
1	0	100	0
2	10	0	100
3	15	0	100
4	16	100	0
5	20	100	0

MS parameters

- Instrument: QSight 220
- Source: Electrospray with polarity switching
- Spray Voltage: 5000V/-4800V
- Nebulizer Gas : 350
- Drying Gas : 120
- Source Temperature : 340°C
- HSID Temperature : 200°C
- Detection Mode: Time-managed MRM™





APPLICATION NOTE

Liquid Chromatography /
Mass Spectrometry

Authors:
Maria Laura Pati, Nicola Barbieri,
Alfredo Fantastico, Piero Pontrelli
S.A.Mer. Servizio Analisi Chimico-Merceologiche
Bari, Italy

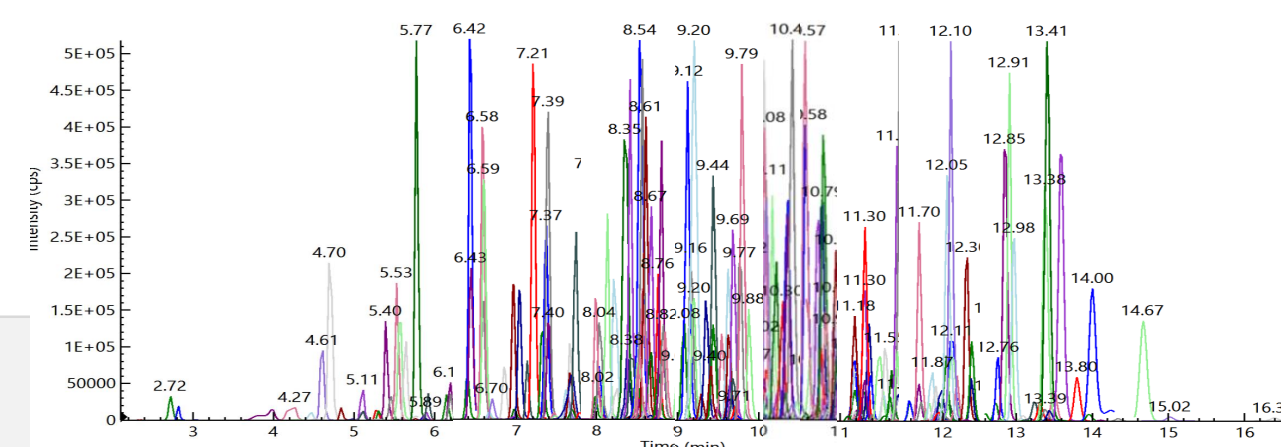
Aristide Ganci
Roberto Troiano
PerkinElmer, Inc.
Milan, Italy

Ignazio Garaguso
PerkinElmer, Inc.
Rodgau, Germany

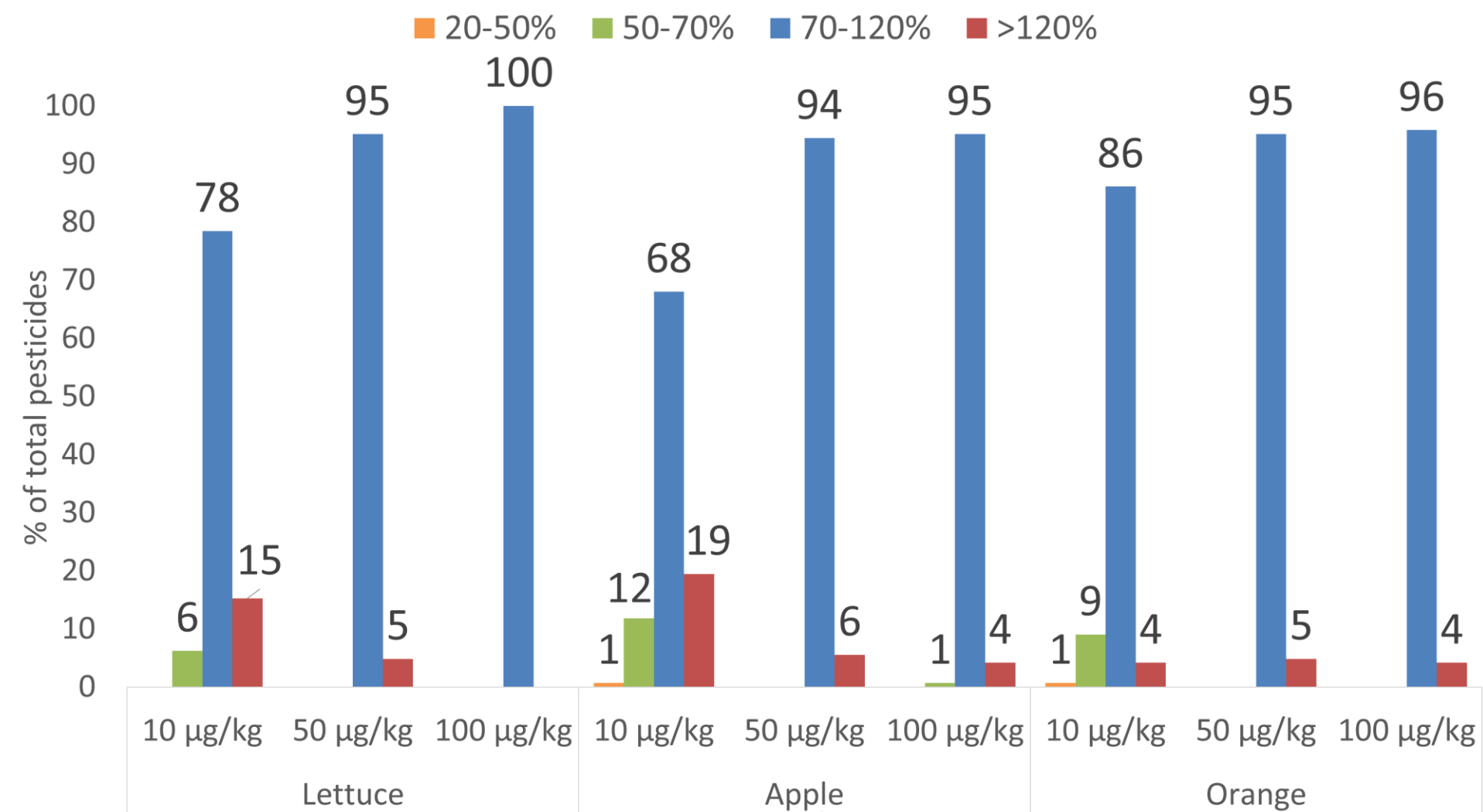
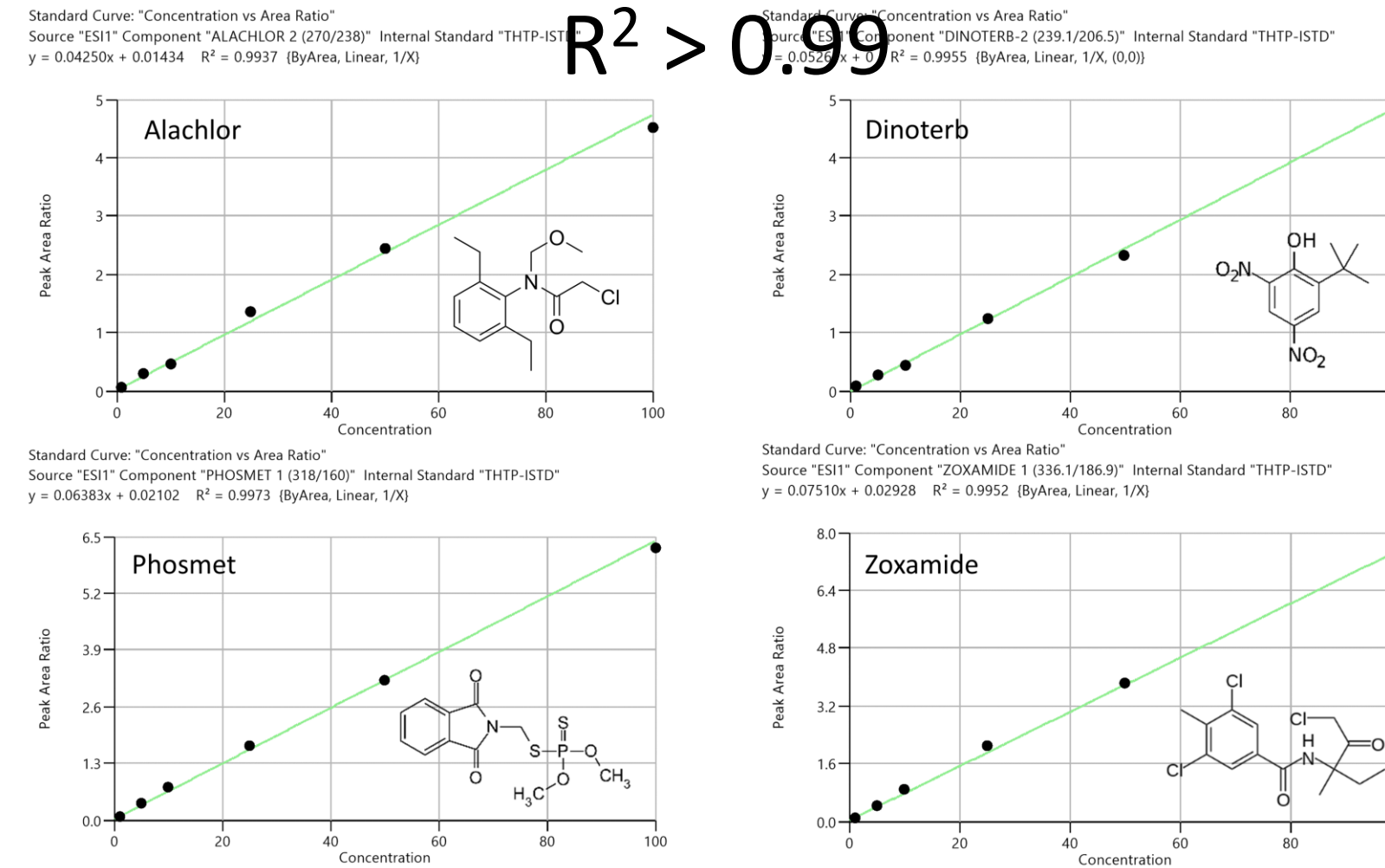
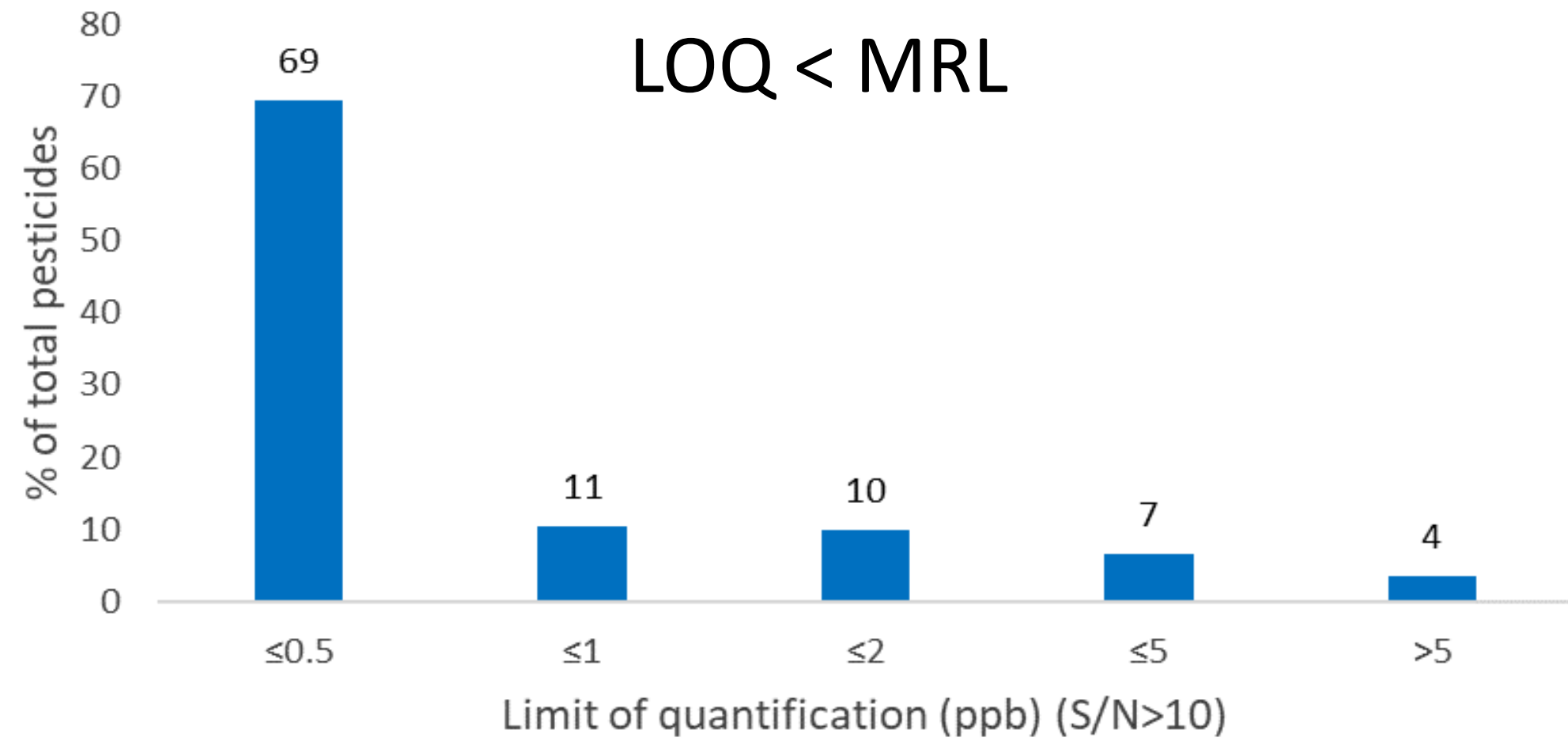
Multi-residue Analytical Method for the Confirmation and Quantification of 500+ Pesticides in Fruit and Vegetables

Introduction
Pesticides are a group of compounds containing hundreds of listed listed substances, most of which are regulated by governmental agencies. Their function is to prevent, destroy, or control harmful organisms or diseases, as well as protect plants or plant products during production, storage and transport. Pesticides are primarily utilized in the agricultural sector, and contain one or more active substances. From the point of application, pesticides can be transported through various media, and ultimately be deposited on plants and animals humans consume. While some of these compounds have not been found to be harmful, others may have toxic properties to humans and animals, as well as pose a danger to our environment and ecosystems.

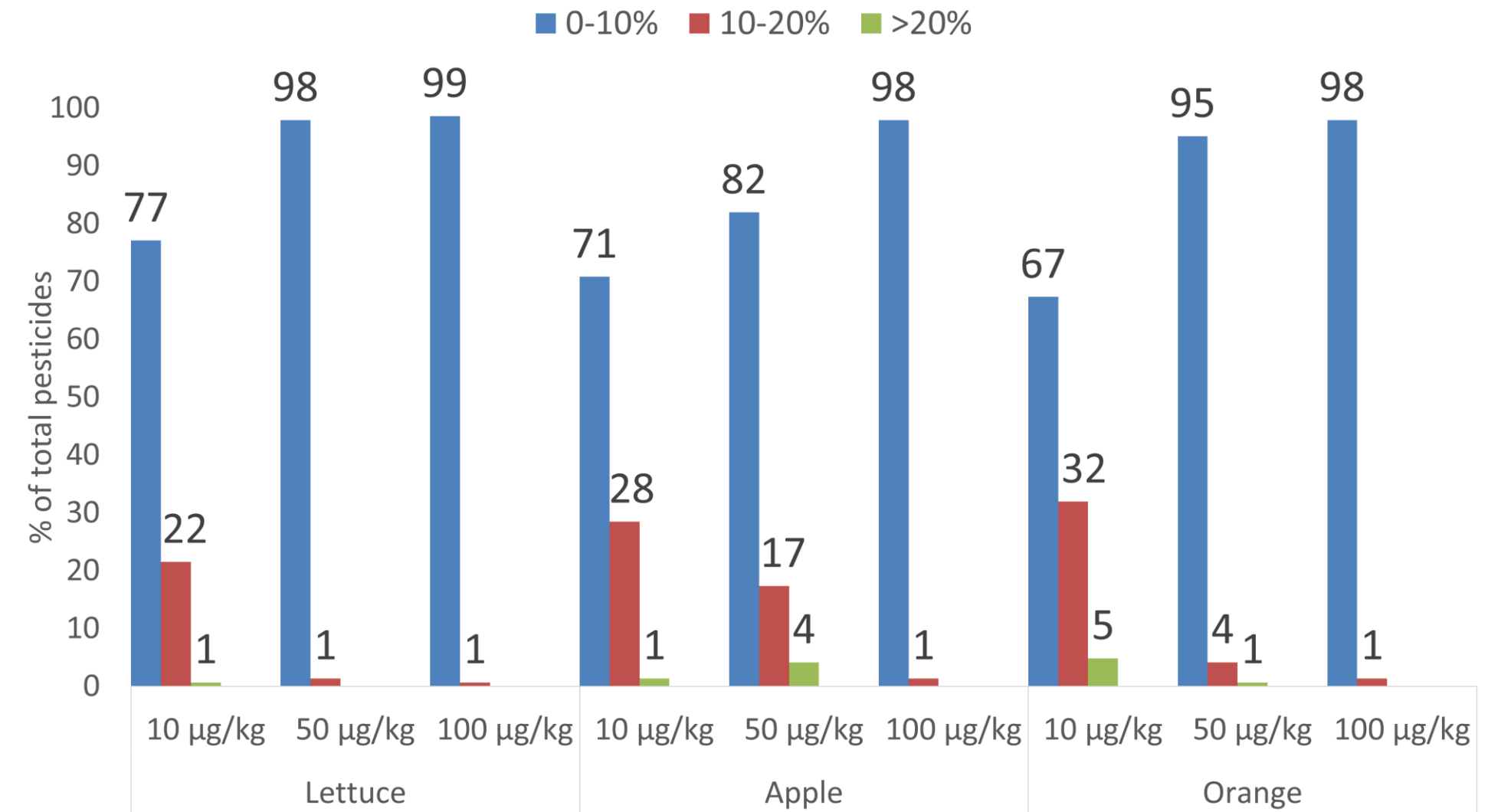
The European Commission (EC) has set maximum residue limits (MRLs) for pesticide residues in or on food and feed of plant and animal origin, as detailed in legislative framework Regulation (EC) 396/2005.¹ MRLs vary for given pesticides and food products, but generally, the MRLs are set at 0.01 mg/kg for many fruits and vegetables. For certain pesticides and matrices, different legally permitted concentrations have been set, mostly ranging from 0.001 – 100 mg/kg.² For pesticides not listed in the regulation, a default MRL of 0.01 mg/kg applies.¹



Method performance – LOQ, linearity, recovery and repeatability



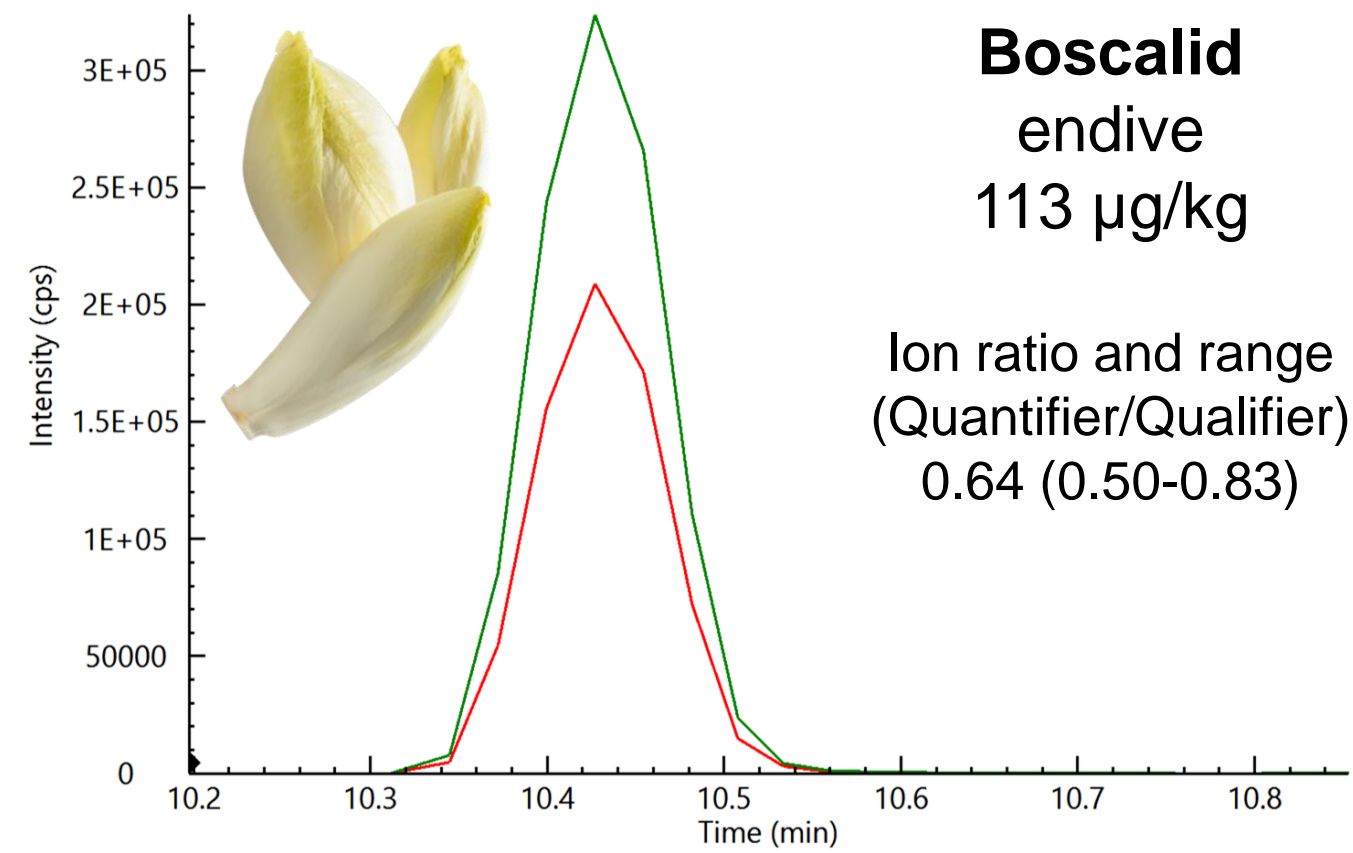
Recovery mostly 70-120%



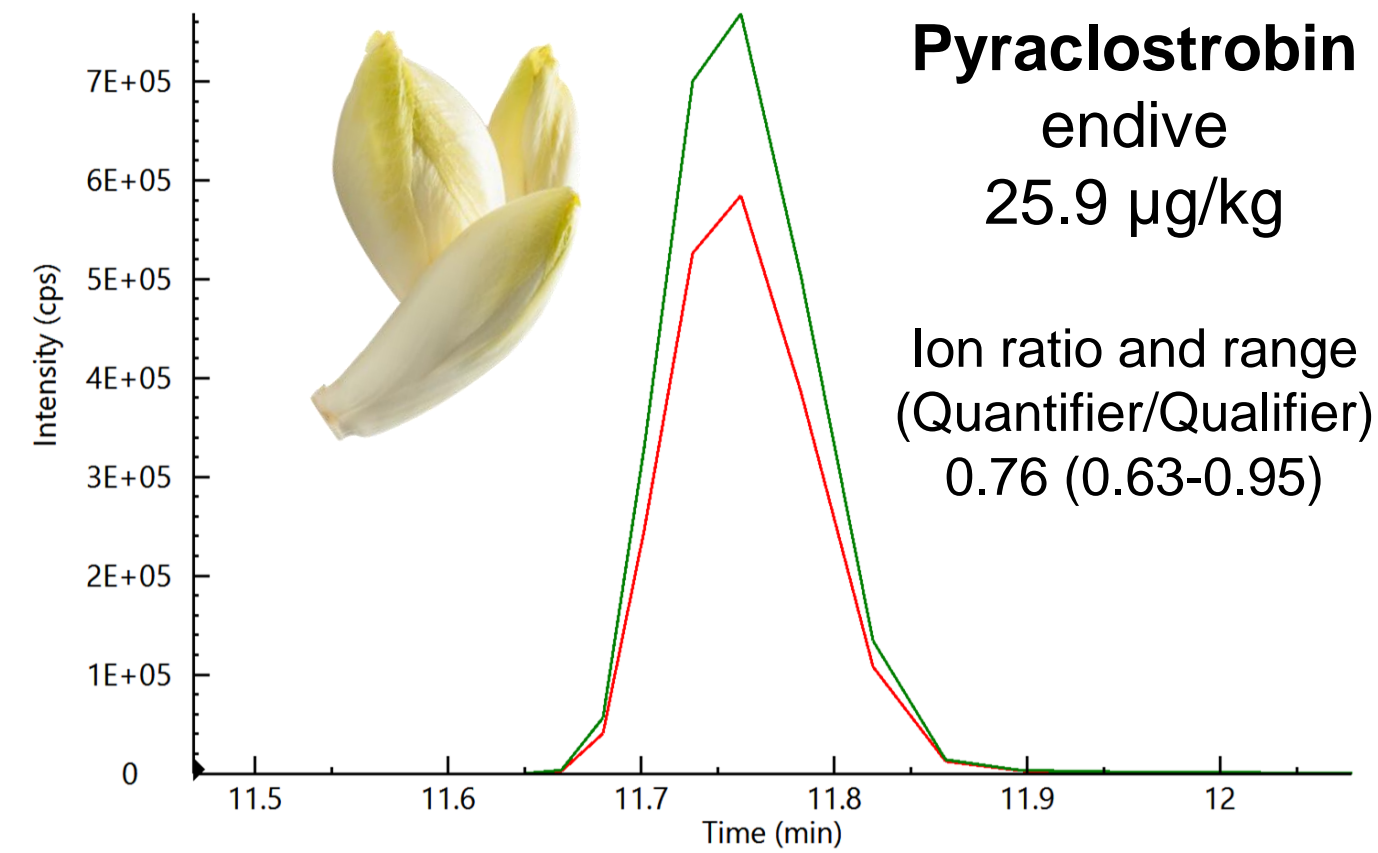
RSD < 20%

Examples of positively quantified pesticides at varying concentrations

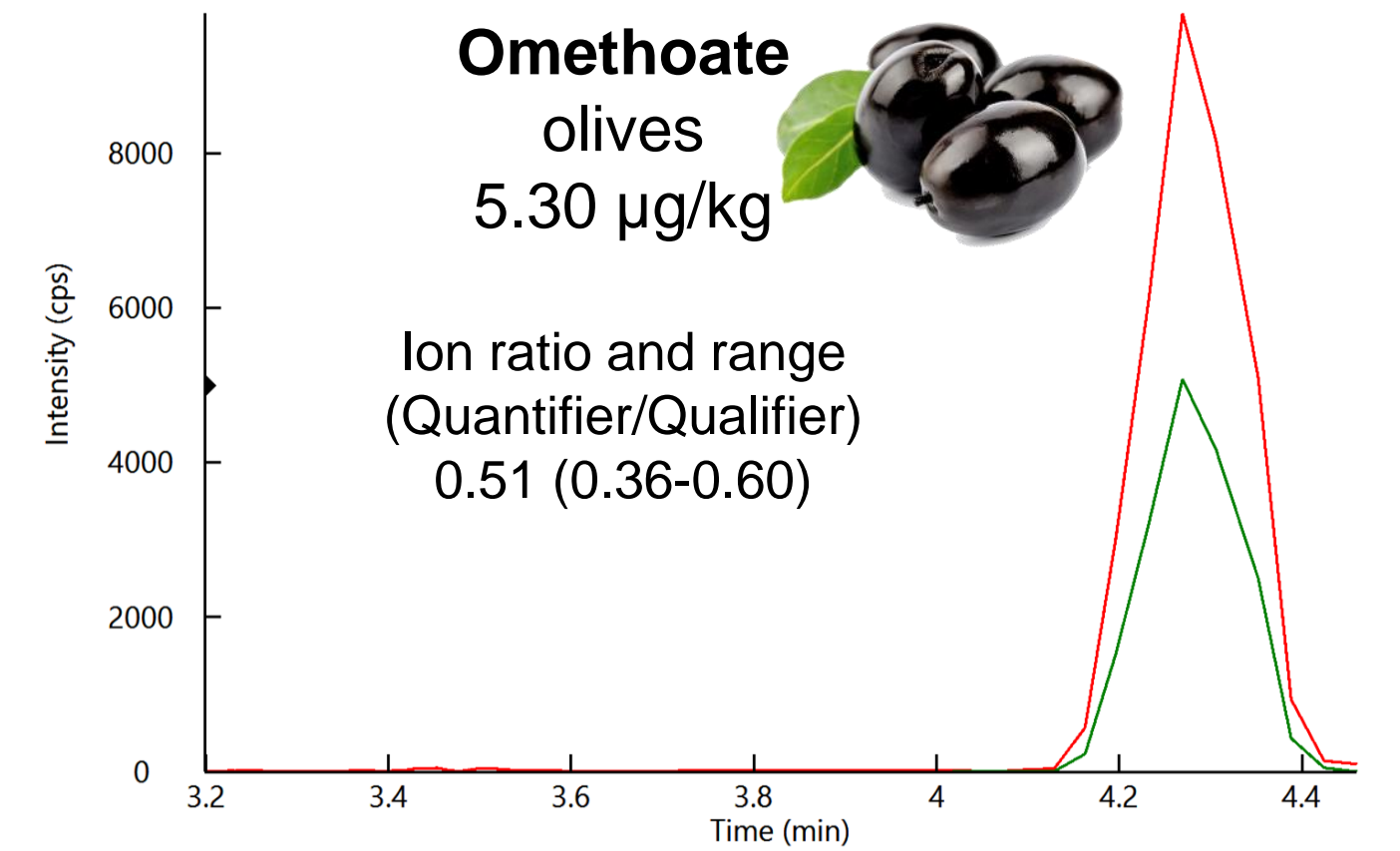
EIC +MRM 343.10/140.00 (34 pairs) EV: 25 V CC: -29 V Exp "Experiment 58" Boscalid 2
 Number of Scans: 24
 Smoothing Level: 1
 Max: 3.24E+5 cps



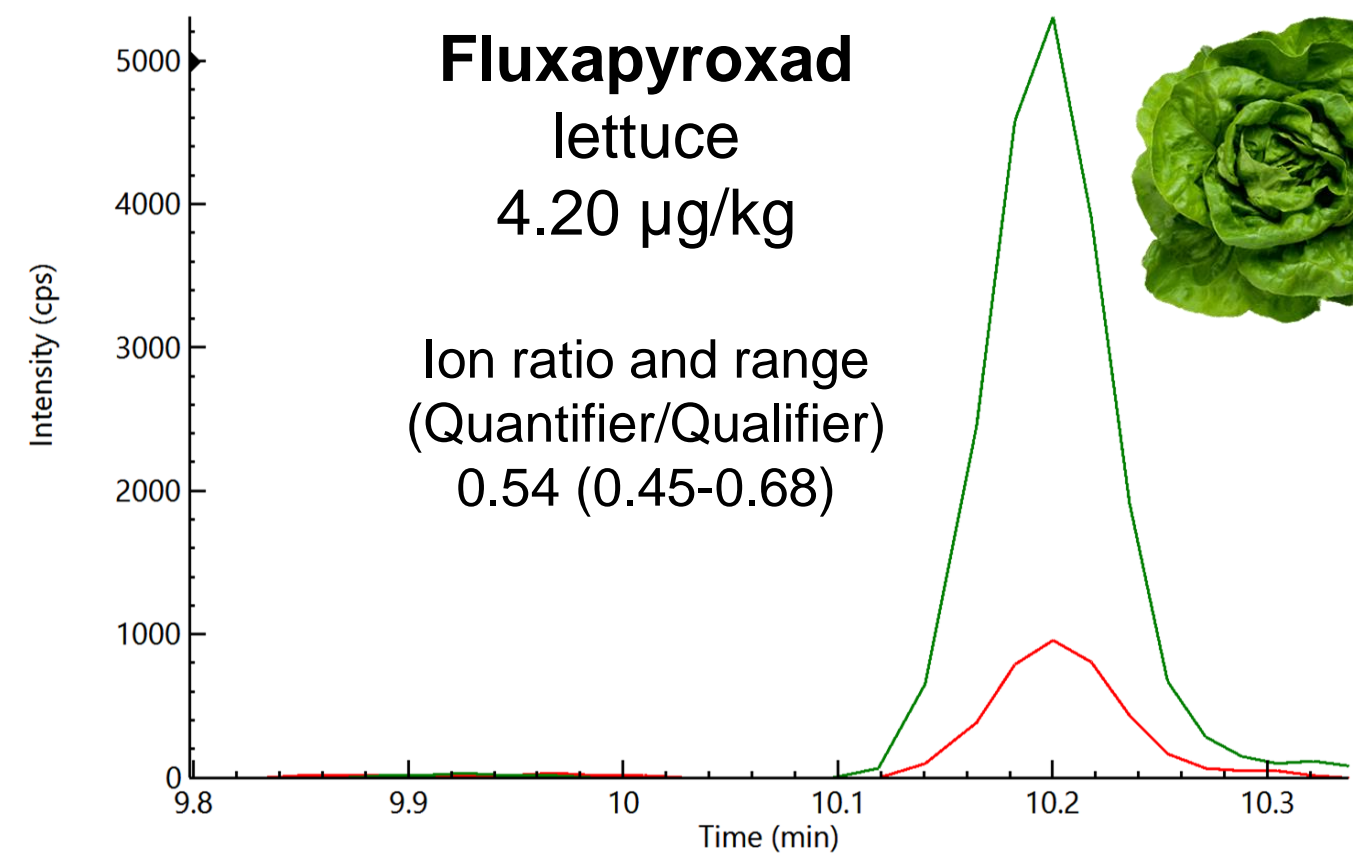
EIC +MRM 388.10/163.00 (20 pairs) EV: 25 V CC: -37 V Exp "Experiment 73" Pyraclostrobin 2
 Number of Scans: 22
 Smoothing Level: 1
 Max: 7.69E+5 cps



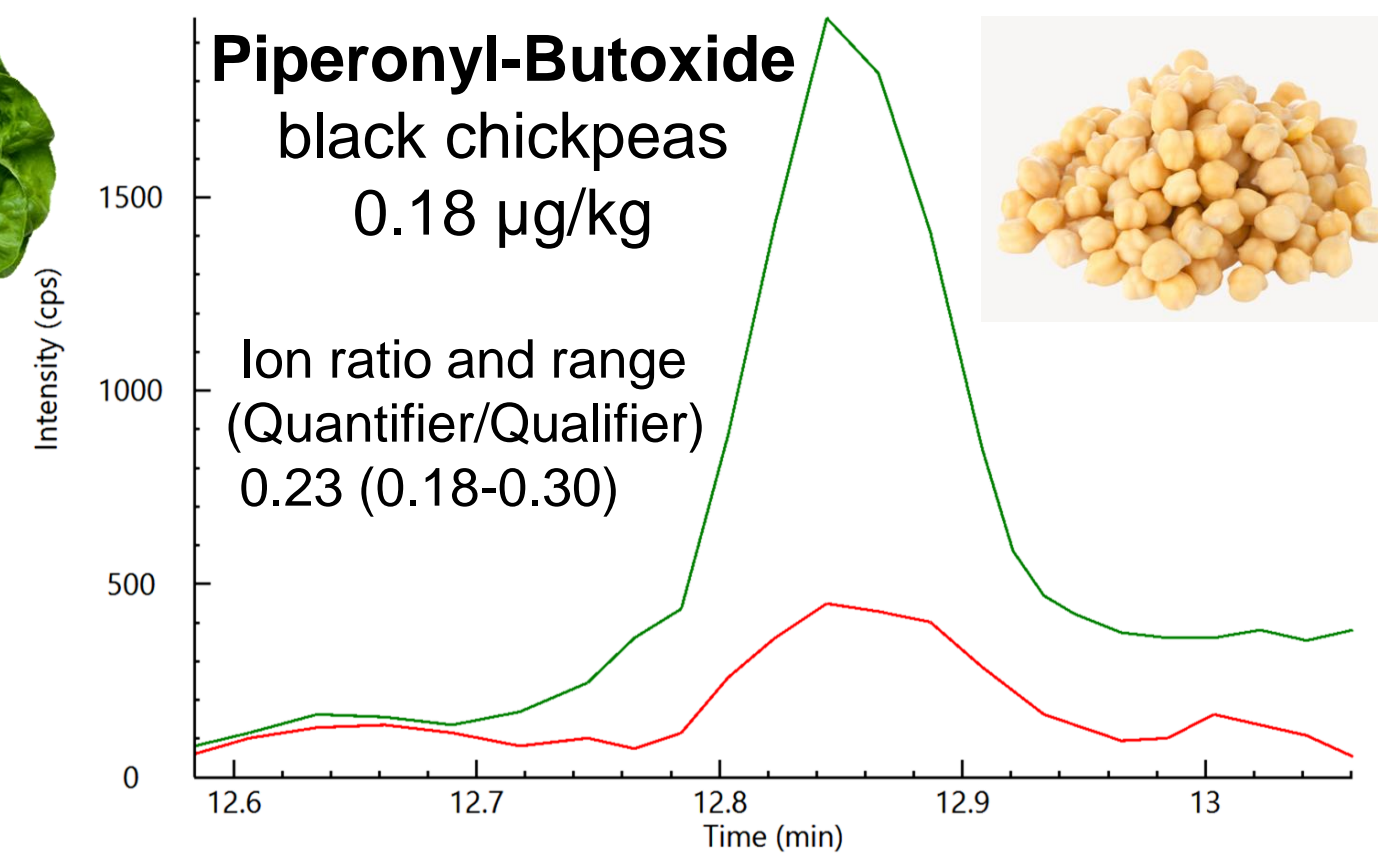
EIC +MRM 214.10/124.90 (6 pairs) EV: 10 V CC: -31 V Exp "Experiment 5" Omethoate 2
 Number of Scans: 68
 Smoothing Level: 1
 Max: 9.81E+3 cps



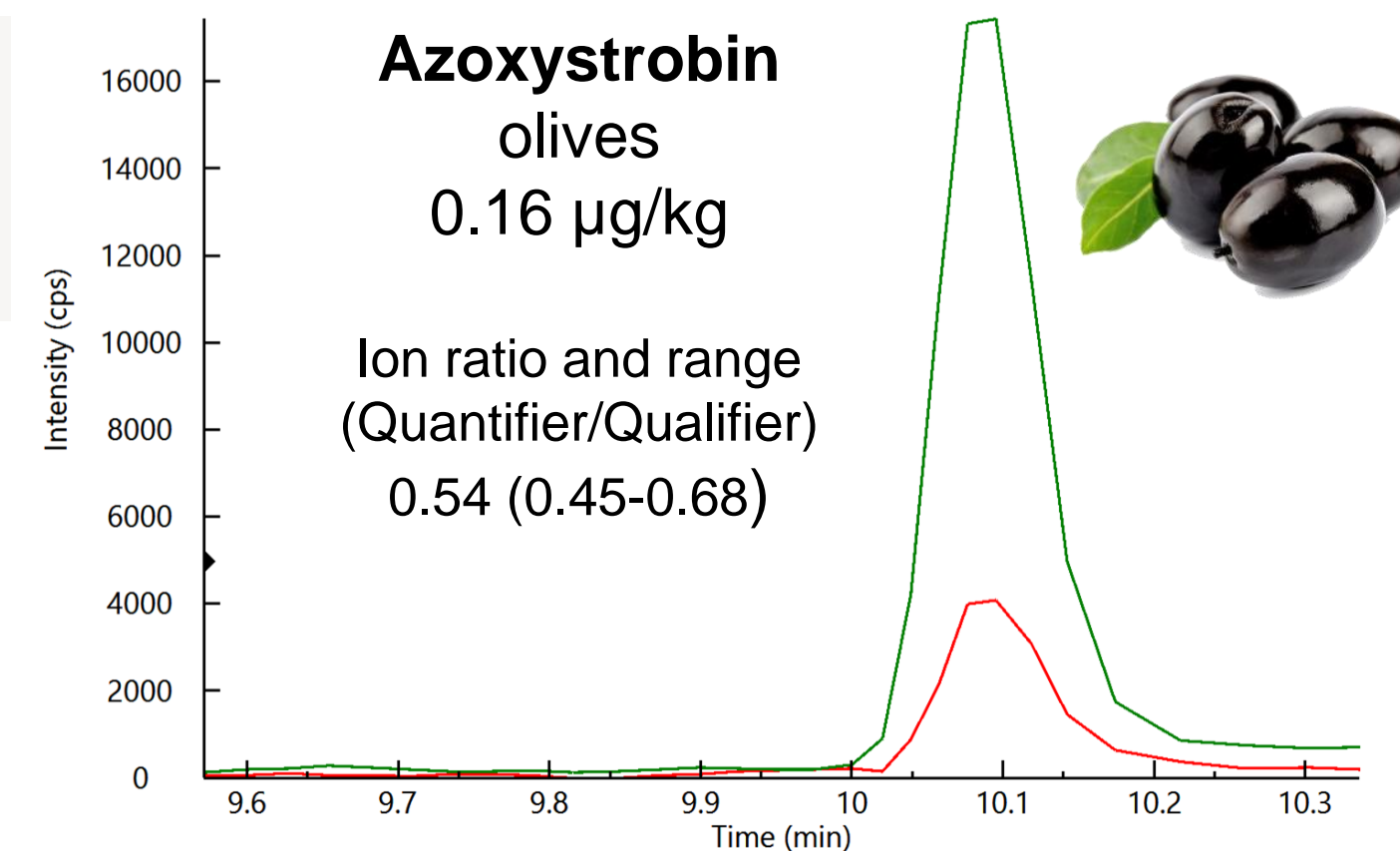
EIC -MRM 380.00/220.00 (2 pairs) EV: -1 V CC: 54 V Exp "Experiment 52" Fluxapyroxad 1
 Number of Scans: 29
 Smoothing Level: 1
 Max: 5.31E+3 cps



EIC +MRM 356.20/119.00 (6 pairs) EV: 12 V CC: -67 V Exp "Experiment 82" Piperonyl-Butoxide 2
 Number of Scans: 24
 Smoothing Level: 1
 Max: 5.32E+2 cps



EIC +MRM 404.10/344.10 (18 pairs) EV: 25 V CC: -35 V Exp "Experiment 53" Azoxystrobin 2
 Number of Scans: 29
 Smoothing Level: 1
 Max: 1.74E+4 cps



Overview of Application Notes for Pesticide Analysis in Foods



APPLICATION NOTE

QSight™ LC-MS/MS

Authors:
Narayan Kamble, Ph.D
Kailas Gavhane
Qin Feng
Dr. Umesh Talekar, Ph.D
PerkinElmer (India) Pvt. Ltd. Mumbai

Estimation of 136 pesticide residue in Black Pepper using QeChERS extraction technique and QSight™ LC-MS/MS.

Introduction
India is one of the largest producers of Spices in the world (40% of world production) with major exports being black pepper, cardamom, cumin, turmeric, etc. Kerala in the southern India is the major spice producers in the country and aptly known as 'The Land of Spices'. Amongst all spices, Black pepper is the most prominent one and is the third largest commodity with respect to production and export in the world. Due to its specific pungent aroma and flavor, Black pepper is used in various food preparations. Nowadays, black pepper cultivators are under threat due to the infestation of various diseases and pests.

Major diseases in Black pepper include foot rot, anthracnose, leaf rot, blight and basal wilt. *Lophobatis piperis*, *Diconocort heverii* and *Dactynotus piperis* are some of the major pests¹. Considering production of black pepper in India and import-export regulation of each country, existence of pesticide residue above maximum residue limit (MRL) will create major effect on export trade market. More than 2.5 million tons of pesticides are used worldwide per year, which includes insecticides, herbicides and fungicides for better production of black pepper². The uncontrolled use of pesticides has become a major concern for human health and environment. Therefore, accurate and sensitive analytical methods are needed.



APPLICATION NOTE


QSight™ LC-MS/MS

Authors:
Kailas Gavhane
Dr. Narayan Kamble
Qin Feng
Dr. Umesh Talekar
PerkinElmer (India) Pvt. Ltd. Mumbai

Identification and quantification of multiresidue pesticides in Tea Sample by QSight™ LC-MS/MS.

Introduction
"Camellia sinensis" botanical name of tea. India is one of largest producer and consumer of tea in the world. Tea is a popular beverage in India and throughout the world because of its pleasant aroma and flavor. In India the major tea-growing regions are in Northeast India – Assam, West Bengal, southern part of India Karnataka, Kerala and Tamilnadu¹. In recent years, to avoid the diseases and pest infestation like mites, black pepper cultivators are under threat due to the infestation of various diseases and pests.

caterpillar, leaf eaters² farmers widely employing different pesticides all over the agricultural sector including tea cultivation³. Worldwide increased level of pesticide use in agricultures becomes a major concern.
The use of pesticides benefits to increase crop yield, but simultaneously it increases the health risk of consumer⁴. International organizations like European Union (EU) proposed maximum residue limit (MRL) in the EU pesticide database⁵ which is based on the EC 396/2005⁶. EU regulation covers more than 450 MRLs for pesticides in tea. To prevent health risks, it is important to monitor the presence of pesticides and regulate their levels. To determine low levels of pesticides in tea, highly sensitive, selective and accurate analytical methods are needed.



APPLICATION NOTE

Liquid Chromatography / Mass Spectrometry

Authors:
Josh Ye, Feng Qin, Frank Kero, Craig Young, Jason Wisniewski, Jamie Foss
PerkinElmer, Inc.
Downers Grove, IL

"No Dilute" Just Shoot: Robustness of a QSight LC-ESI-MS/MS for Low Level Pesticide Residue Analysis in Wine

Introduction
Traditional analysis by chromatography and mass spectrometry often requires sample cleanup to minimize matrix effects and to avoid contamination of the ion source in the mass spectrometer. However, sample preparation is usually labor intensive and requires trained analysts with specialized skills. Strategies to redesign the front end of mass spectrometers to minimize source contamination and thereby avoid the need for extensive sample cleanup, led to the invention of a hot surface induced desolvation (HSD™) interface. The PerkinElmer QSight™ LC/MS/MS mass spectrometer contains the HSD interface coupled to a Laminar flow ion guide™, both of which prevent accumulation of contamination along the ion path making it a very sensitive and maintenance free instrument.



APPLICATION NOTE


Liquid Chromatography / Mass Spectrometry

Authors:
Arinash Dalmia, Sabu Hariri, Jacob Jalil, Erasmus Cudjoe, Toby Antill, Charles Schmidt, Feng Qin
PerkinElmer, Inc.
Shelton, CT
Toronto, ON
Charles Johnson
Joey Kingstad
Nipro Research, Inc.
Sacramento, CA

Novel ESI and APCI LC/MS/MS Analytical Method for Testing Cannabis and Hemp Concentrate Sample Types

Introduction
As new abuse and medicinal cannabis markets emerge in the US and Canada, the use of concentrate cannabis and CBD products (e.g. edibles, beverages, vape products, isolates, topicals, and waxes) continues to increase in popularity. According to market research, concentrates and their derivative products are expected to represent 50% of the consumer market by 2022. This growth, and the diversity in sample type, presents an analytical challenge for testing laboratories. The concentrate matrix has a significant effect on the analytical method, owing to higher sample matrix effects caused by the increased concentration levels (up to 55% w/w) of cannabinoids in the sample. This effect influences the response of certain pesticide molecules, requiring laboratories to validate a pesticide method specific to the sample matrix type.

In this work, an LC/MS/MS method is presented for the analysis of 66 pesticides, including



APPLICATION NOTE

Liquid Chromatography / Mass Spectrometry

Authors:
Josh Ye, Jingcun Wu, Feng Qin, Shixin Sun, Arinash Dalmia, Wilfried Reuter, Sergey Rakov, Jamie Foss and Frank Kero
PerkinElmer, Inc.
Waltham, MA

Analysis of 213 Pesticide Residues in Grapes by LC-MS/MS with Time-Managed MRM

Introduction
The grape crop is one of the most important fruit crops consumed

in the world. Grapes are consumed both as fresh and as processed products, such as wine, jam, juice, jelly, grape seed extract, raisins, vinegar and grape seed oil. A large variety of pesticides are used in grape production throughout its growing season to control pests and diseases in vineyards and to increase crop yields. Pesticide residue is a major concern for the stakeholders of the grape industry, due to more and more stringent regulations and safety standards in most countries. It is also a concern for the general consumers, due to increased demand for safer products. Therefore, to prevent health risks, it is important to monitor the presence of pesticides and regulate their levels in grapes.




APPLICATION NOTE

Liquid Chromatography / Mass Spectrometry

Authors:
Li Zhong Yang, Zhao Man, Xiangdong Zhao
PerkinElmer, Inc.
Shanghai, China
Feng Qin
PerkinElmer, Inc.
Shelton, Canada

Direct Analysis of Glyphosate and Similar Polar Pesticides in Oatmeal by UHPLC-MS/MS

Introduction
Glyphosate [N-(phosphonomethyl) glycine], an organophosphorus compound, is used to kill weeds (e.g. annual broadleaf weeds and grasses) that compete with crops. Since its introduction to market approximately 42 years ago, glyphosate has become one of the world's most widely used herbicides due to its relatively low toxicity in comparison with other herbicides towards mammals. The adoption of glyphosate by farmers intensified after the introduction of genetically engineered "glyphosate tolerant" crops, such as corn and soybeans, that can withstand glyphosate treatment unlike the weeds the herbicide is meant to destroy. Like other pesticides, glyphosate is directly administered to food products and can come in contact with both food workers and the environment, resulting in the bio burden of exposure in uncontrolled regional populations. As a registered herbicide product under a number of regulatory organizations, glyphosate has been considered nontoxic with minimal risk to human health with persistent exposure at trace levels. However, recent toxicity evaluations by different organizations have put glyphosate at the center of a dispute. The World Health Organization's (WHO) International Agency for Research on Cancer classified it as "probably carcinogenic to humans" in March of 2015¹. However, in November of 2015, the European Food Safety Authority (EFSA) published a report claiming that there was no scientific evidence linking glyphosate to cancer².



APPLICATION NOTE

Liquid Chromatography / Mass Spectrometry

Authors:
Josh Ye, PhD
Feng Qin, PhD
Shiranya Roddy, PhD
Frank Kero, PhD
PerkinElmer, Inc.
Waltham, MA

Analysis of Target Pesticide Residues in Berries with LC/MS/MS Coupled with a QeChERS Sample Preparation

Introduction
Pesticides are widely used in agriculture to protect plants from a variety of pests and to increase productivity. However,

the extensive use of pesticides can pose a health risk to humans and this has led to worldwide stringent regulations, for maximum allowable limits for these residues in foods. Among the routinely used testing methods, LC/MS/MS has become the method of choice, due to its high sensitivity, reliability and accuracy.
In the present study, a unique Laminar Flow UHPLC-ESI-MS/MS wide quad mass spectrometer was used to identify and quantitate 40 pesticides in four brands of non-organic berries. The QeChERS extraction method proved both rapid and reliable for extracting pesticide residues in the heavily pigmented berry samples.



APPLICATION NOTE

Liquid Chromatography / Mass Spectrometry

Authors:
Shiranya Roddy
Frank Kero
PerkinElmer, Inc.
Shelton, CT
Matteo Maghili
Motti Mondelli, Inc.
Quebec, Canada

Direct Analysis of Glyphosate in Wine with No Sample Preparation Using the QSight 220 LC-MS/MS System

Introduction
Glyphosate is an organophosphate herbicide that is used on crops to kill weeds and grasses. Its usage has multiplied with

the introduction of transgenic crops made resistant to glyphosate. Because of its rampant use, it is not surprising that glyphosate has been detected in variety of foods. Recently, the International Agency for Research on Cancer classified glyphosate as "probably carcinogenic in humans". In lieu of regulatory bodies setting limits on glyphosate in food, it has become imperative to develop robust and sensitive analytical methods for glyphosate detection. Since glyphosate is a very polar molecule, it does not retain well on a traditional reverse phase column, making it very difficult to chromatographically separate from other components and detect. Methods involving derivatization with a hydrophobic moiety can help retain glyphosate on column, but, it also makes the process labor intensive and tedious. We present a study that involves direct analysis of glyphosate in wine on a mixed mode column with no sample dilution or extraction using a PerkinElmer QeChERS 220 wide quad mass spectrometer with a patented StageClean™ source, consisting of a hot surface induced desolvation (HSD™) interface and a Laminar Flow Ion Guide™. Both the HSD and ion guide prevent any contaminants from entering the mass spectrometer, keeping it at its highest performance level and, thereby, maintenance free.



APPLICATION NOTE


Liquid Chromatography / Mass Spectrometry

Authors:
Jingcun Wu
Josh Ye
Erasmus Cudjoe
Feng Qin
Shixin Sun
PerkinElmer, Inc.
Woburn, Ontario, Canada

Analysis of Multi-Residue Pesticides in Rice by LC/MS/MS

Introduction
Rice is one of the most commonly consumed foods in the world. A

variety of pesticides have been used in rice production to control pests, weeds and diseases to increase crop yield. Pesticides applied in rice crops are often country/region specific due to the differences in legislation, weather and production system. Pesticide residue in rice not only affects the quality of the rice, but also threatens the health of general consumers. To prevent health risks, it is important to monitor the presence of pesticides and regulate their levels in rice. Several countries including the United States, China, Brazil, India, Japan and European Union (EU) have established maximum residue levels (MRLs) of pesticides for food and feed including rice.¹⁻⁴ The EU MRLs for pesticide residues in rice mostly range from 10 µg/kg to 8000 µg/kg depending on the pesticide.¹ To determine low levels of pesticides in rice, highly sensitive, selective and accurate analytical methods are needed. Due to the large number of pesticides potentially used in rice production, the use of multi-residue methods capable of determining many pesticides in one single run is the most efficient approach. Traditionally, pesticide residues were analyzed mainly by gas chromatography/mass spectrometry (GC/MS) methods,^{5,6} but GC is not a suitable technique for ionic and polar compounds, especially for compounds that are thermally labile in the GC injection port. Liquid chromatography tandem mass spectrometry (LC/MS/MS) has become the method of choice for pesticide analysis due to its high selectivity and sensitivity as well as its suitability for a wide range of compounds in various sample matrices.^{4,8}



APPLICATION NOTE

Liquid Chromatography / Mass Spectrometry

Authors:
Maria Laura Patti, Nicola Barbieri, Alfredo Fantastico, Piero Pontrelli
S.A.Mez. Servizio Analisi Chimico-Mercologiche
Fusi, Italy
Artidele Ganci
Roberto Troiano
PerkinElmer, Inc.
Milan, Italy
Ignazio Garagnon
PerkinElmer, Inc.
Rodgau, Germany

Multi-residue Analytical Method for the Confirmation and Quantification of 500+ Pesticides in Fruit and Vegetables

Introduction
Pesticides are a group of compounds containing hundreds of listed listed substances, most of which are regulated by governmental agencies. Their function is to prevent, destroy,

or control harmful organisms or diseases, as well as protect plants or plant products during production, storage and transport. Pesticides are primarily utilized in the agricultural sector, and contain one or more active substances. From the point of application, pesticides can be transported through various media, and ultimately be deposited on plants and animals humans consume. While some of these compounds have not been found to be harmful, others may have toxic properties to humans and animals, as well as pose a danger to our environment and ecosystems.
The European Commission (EC) has set maximum residue limits (MRLs) for pesticide residues in or on food and feed of plant and animal origin, as detailed in legislative framework Regulation (EC) 396/2005.¹ MRLs vary for green pesticides and food products, but generally, the MRLs are set at 0.01 mg/kg for many fruits and vegetables. For certain pesticides and matrices, different legally permitted concentrations have been set, mostly ranging from 0.001 – 100 mg/kg.² For pesticides not listed in the regulation, a default MRL of 0.01 mg/kg applies.¹

QSight 420 for Rapid, High Sensitivity Analysis of Marine Toxins Causing Diarrhetic Shellfish Poisoning in Mussels

Sheng-Suan (Victor) Cai, Senior Field Application Scientist

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HUMAN HEALTH • ENVIRONMENTAL HEALTH

Introduction/Background

- Why this method?
 - Method developed for Washington State Dept. of Health
 - Lock-out spec: LOQ: 50 ppt for DTX2, 200 ppt for OA and DTX1

- Marine toxins causing diarrhetic shellfish poisoning (DSP)

- Test Methods
 - Mouse Bioassay
 - HPLC-FLD, Derivatization
 - LC-MS/MS, High Sensitivity and Specificity

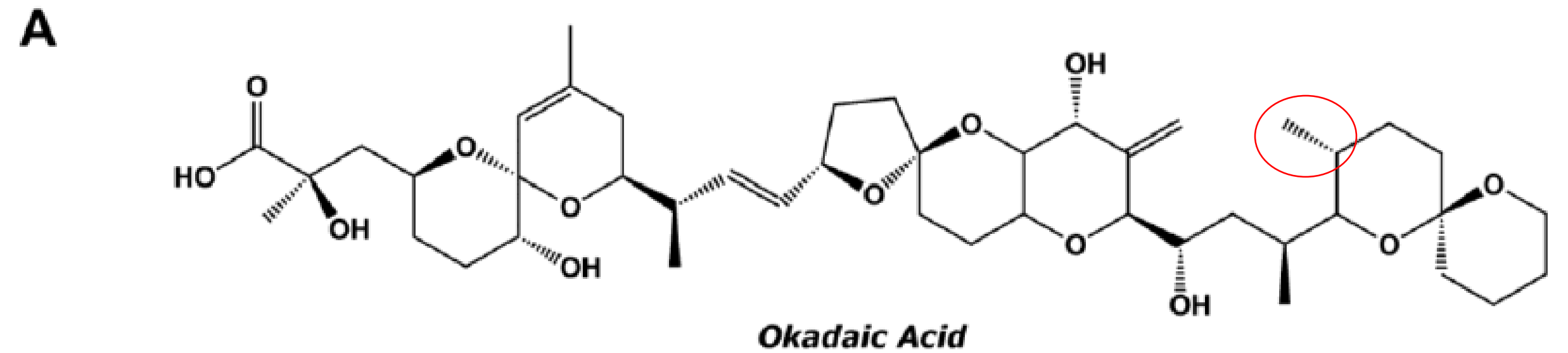
QSight 420 LX50 UHPLC-MS/MS System



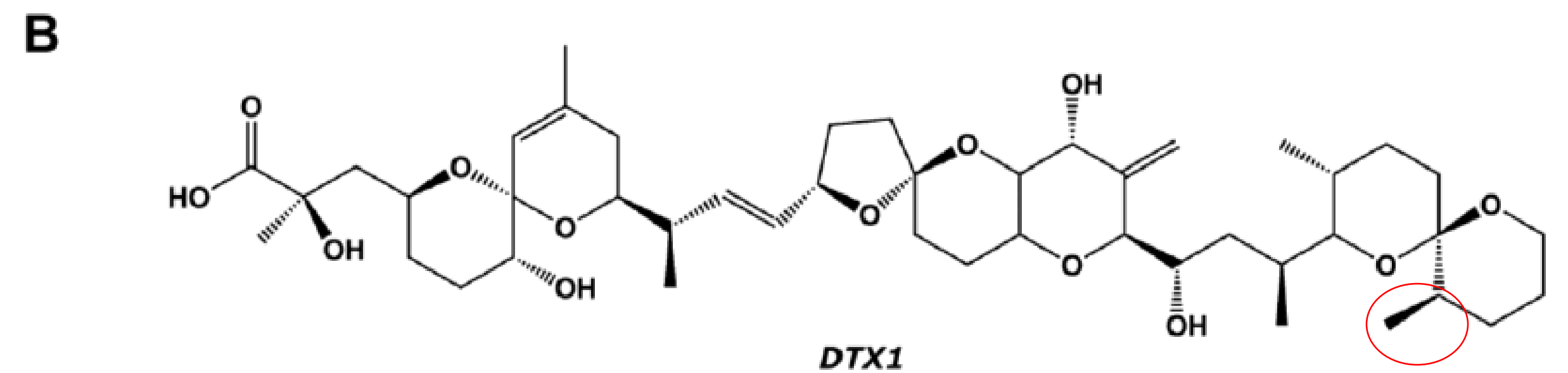
Triple Quad Mass Spec, Equipped with UHPLC and Dual ESI and APCI Ion Sources.

Target Analytes

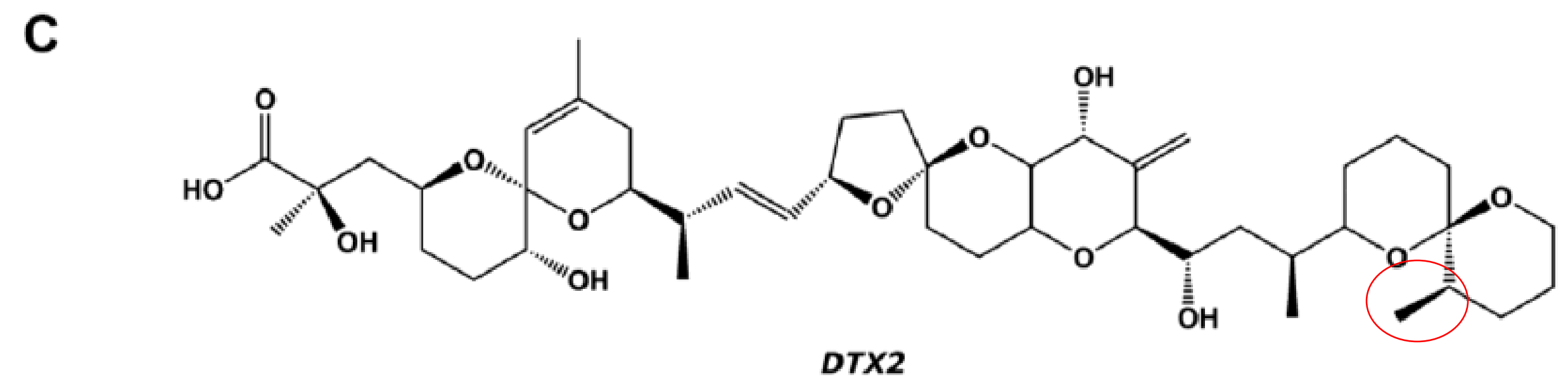
➤ A: Okadaic Acid (OA)



➤ B: Dinophysistoxin-1 (DTX1)



➤ C: Dinophysistoxin-2 (DTX2)



A and C are isomers, sharing exactly same MRM transitions.
B has an additional methyl group

MRM Transitions and Mass Dependent Parameters

Analyte	MRM Transition	Dwell Time (ms)	EV	CC L2	CC	Resolution (Q1:Q2)
OA	803.4 > 255.3	100	-136	352	54	Unit_Unit
OA	803.4 > 113.1	100	-136	268	76	Unit_Unit
DTX2	803.4 > 255.3	100	-110	328	54	Unit_Unit
DTX2	803.4 > 113.1	100	-131	244	73	Unit_Unit
DTX1	817.5 > 255.3	100	-118	290	54	Unit_Unit
DTX1	817.5 > 113.1	100	-124	260	81	Unit_Unit

- OA and DTX2 share same transitions, but fully separated by RT on column.
- Three mass dependent parameters optimized by AutoTune.
- EV = Entrance Voltage, CC L2 = Collision Cell Lense2, CC = Collision Energy.

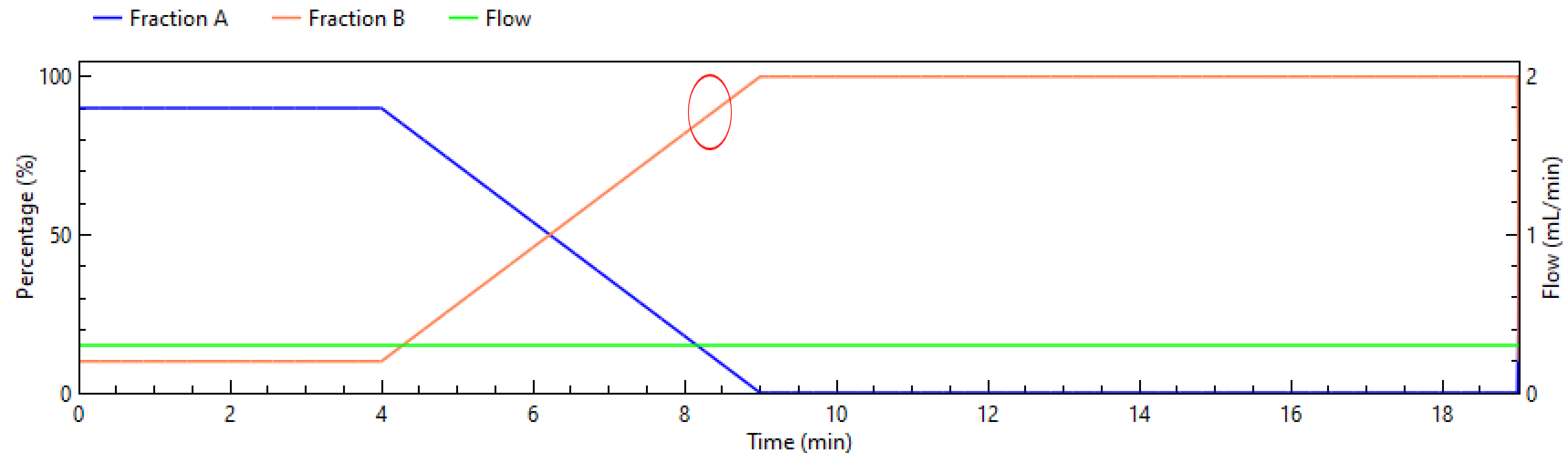
Rapid and Simple Sample Prep Procedure

- Weigh 1g of mussel homogenate in a 15-mL test tube.
- Add 1 mL methanol.
- Vortex for 2 min
- Centrifuge for 2 min at 3500 rpm.
- Put in a freezer at -10 °C for 30 min
- Centrifuge immediately for 2 more min at 3500 rpm.
- Transfer supernatant immediately to a 1.5-mL micro-centrifuge tube.
- Centrifuge at 14,000 rpm at 0 °C for 10 min.
- Transfer supernatant immediately to 0.22 µm Nylon filter and collect in a 2-mL HPLC injection vial for negative ESI UHPLC-MS/MS analysis.

- StayClean® ion source allows direct injection analysis of sample extracts with good data quality.

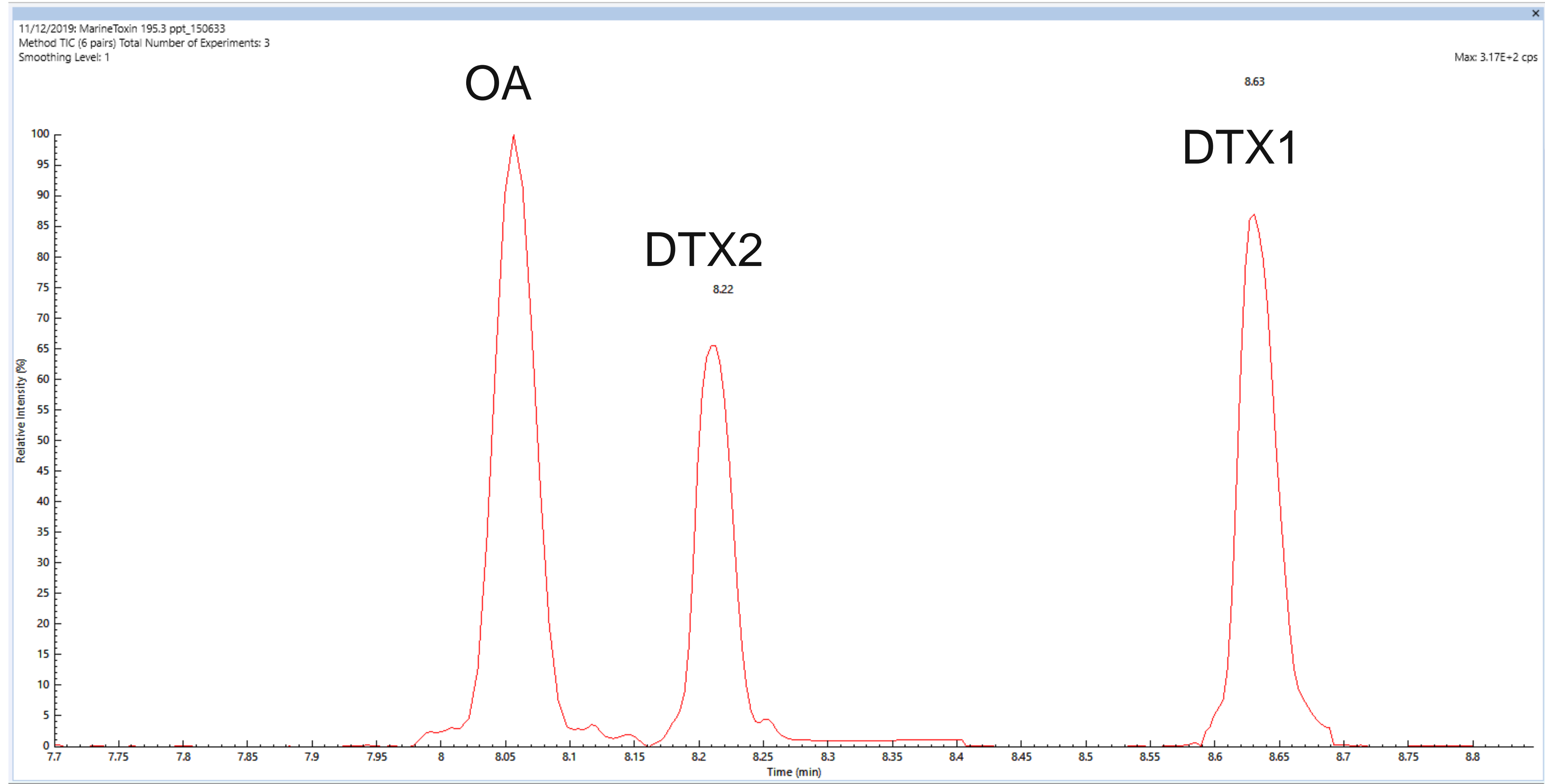
LC Conditions

- UHPLC column: Zorbax Eclipse Plus C₁₈ (2.1 x 50 mm, 1.8 μm).
- Mobile phase A: Water, B: Acetonitrile. Both contain 0.1% formic acid and 2 mM ammonium formate
- Wash solvent: 10% MeOH in Water, 250 μL
- Oven temp: 40°C, Flow rate: 0.3 mL/min
- Injection volume: 10 μL
- Gradient elution: Hold 10%B for 4 min. Linear gradient to 100%B in 5 min. Hold 100%B for 10 min.



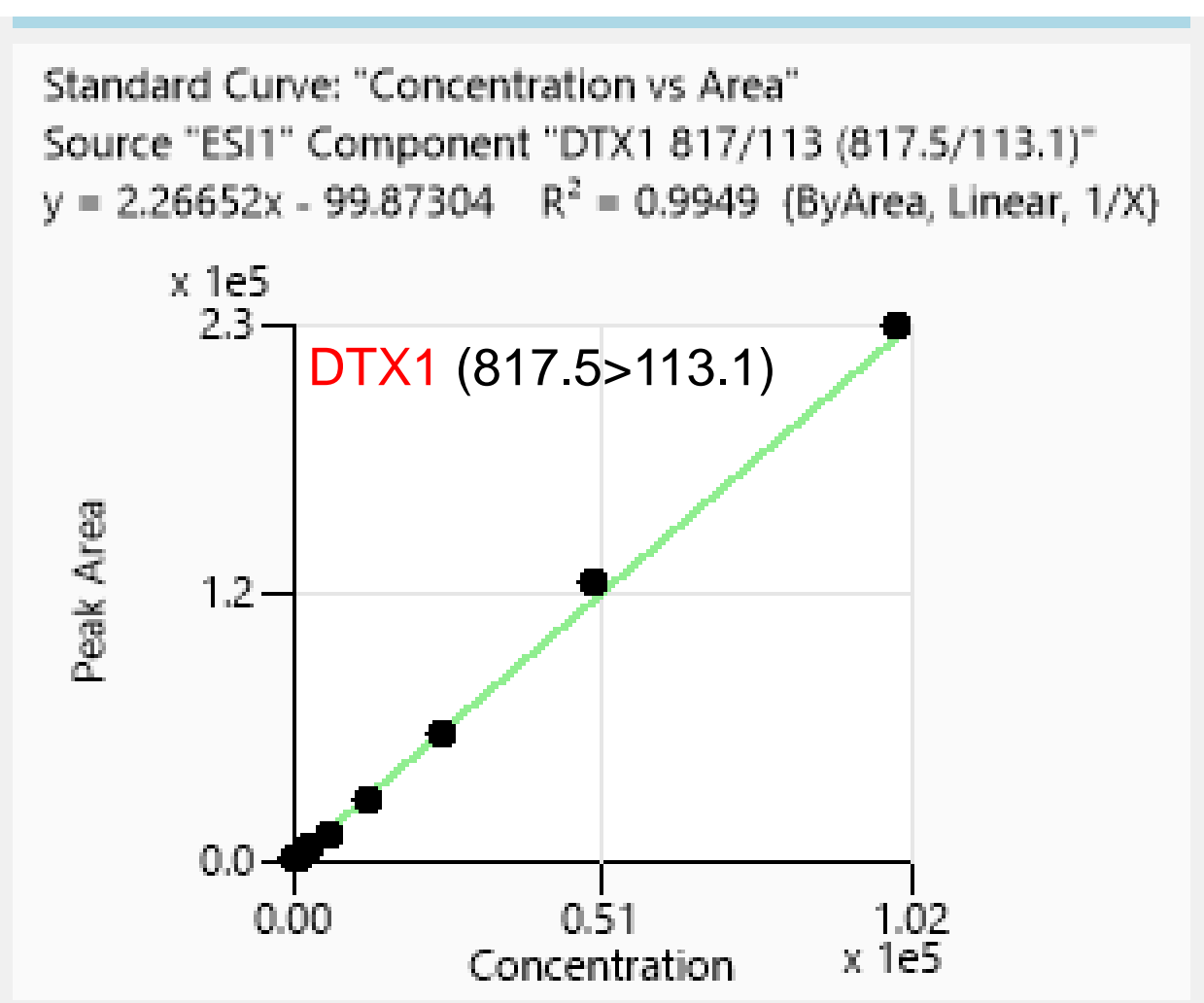
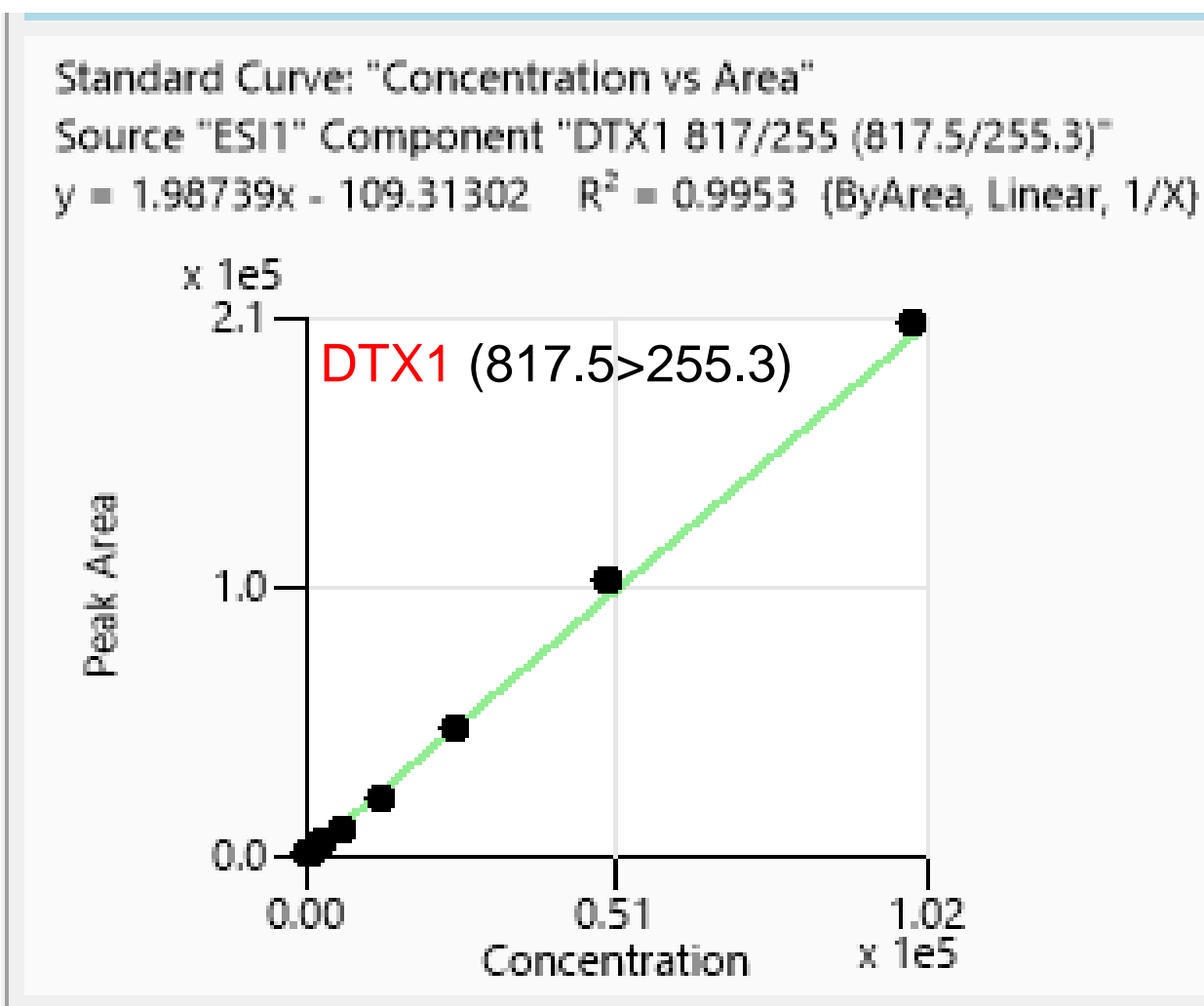
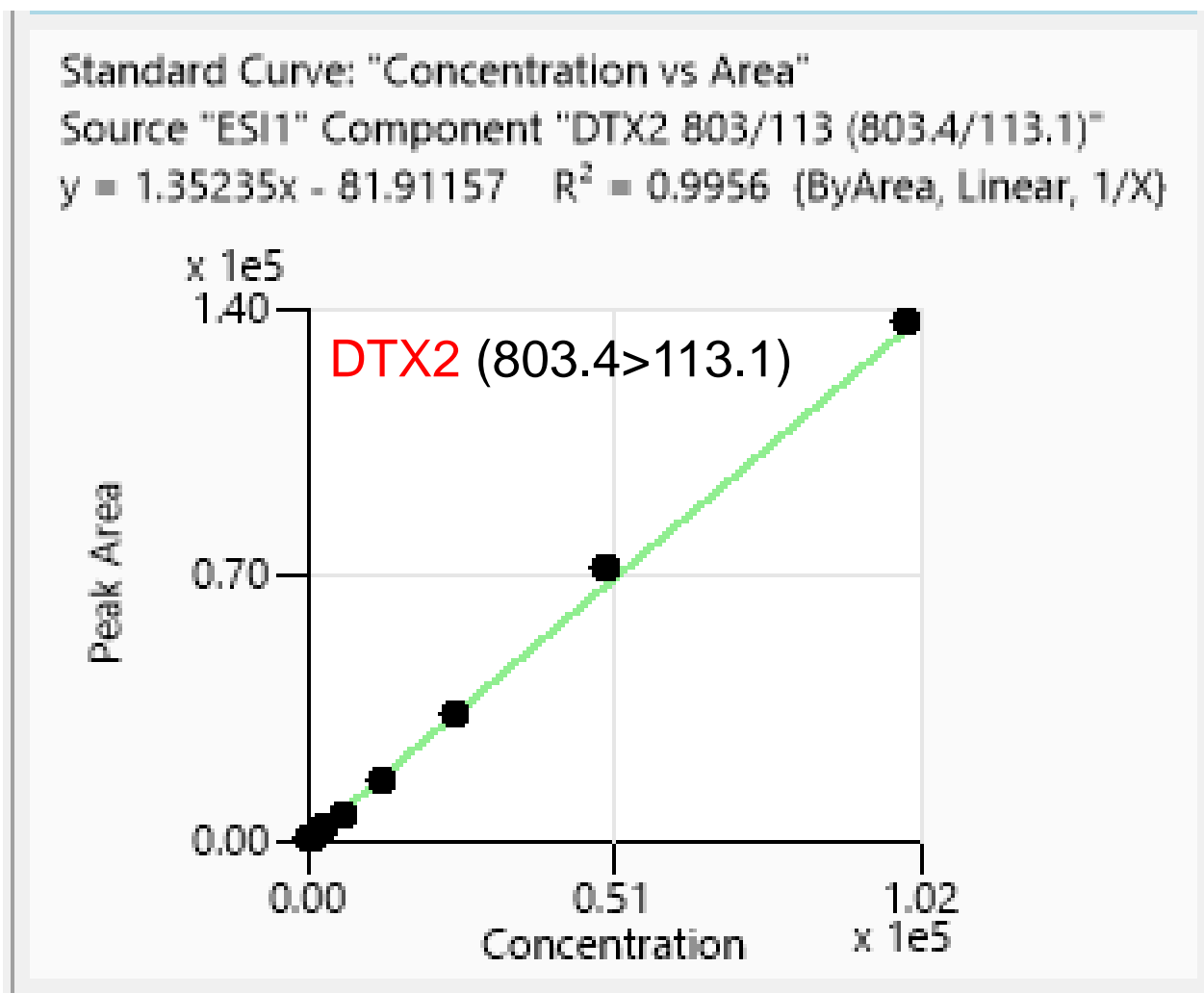
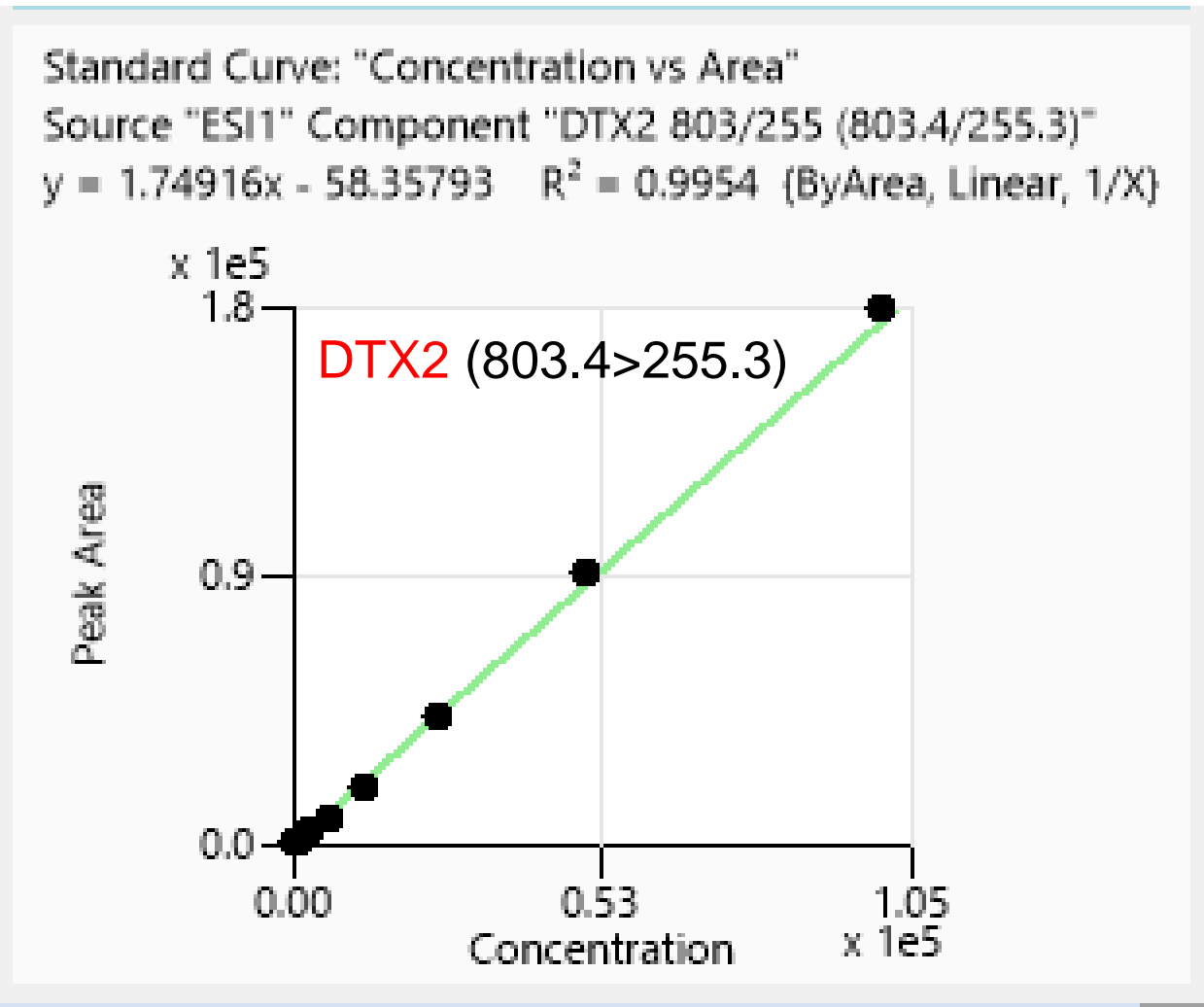
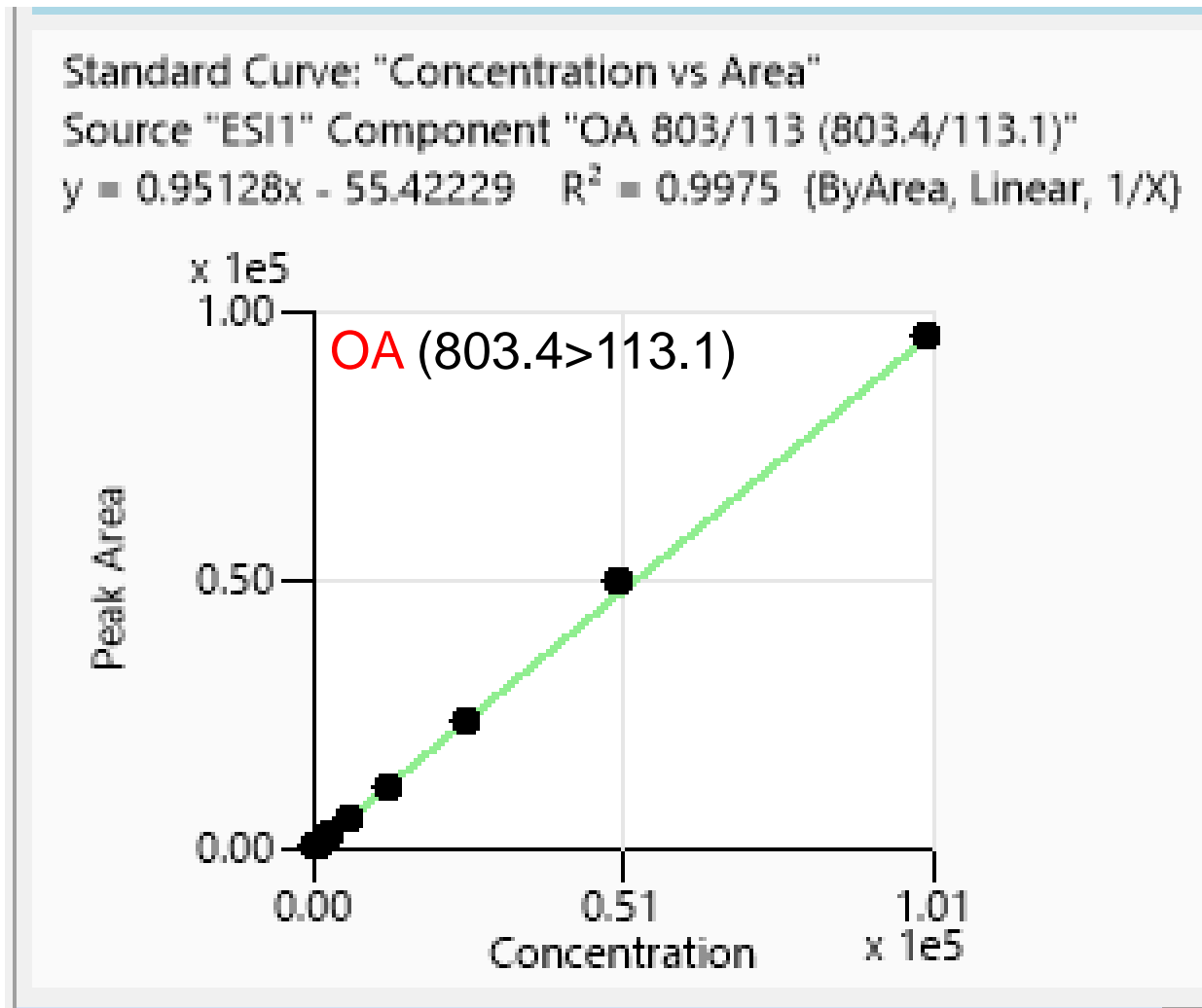
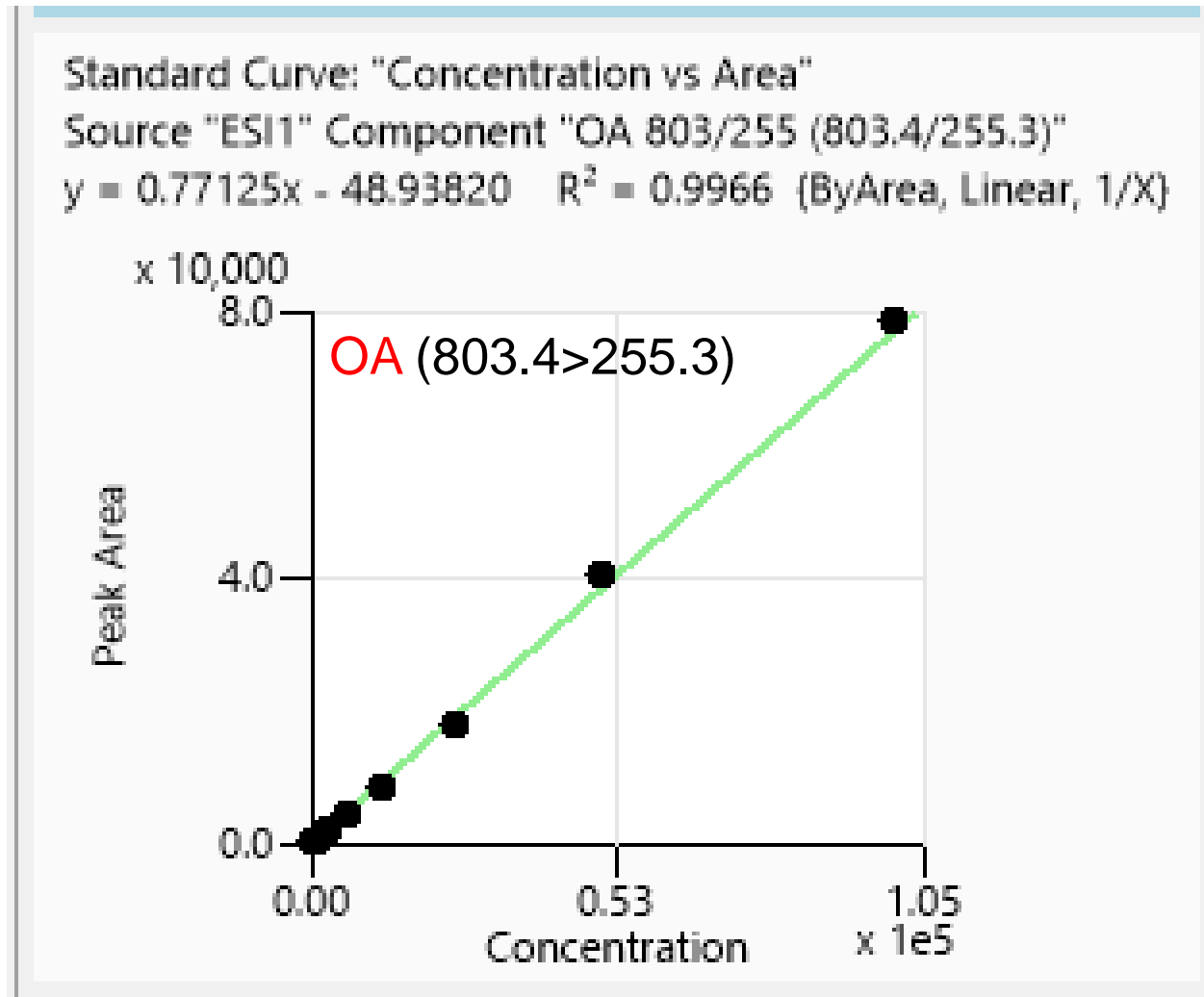
RT: OA = 8.05 min, DTX2 = 8.21 min, DTX1 = 8.63 min.

Column Separation, 195.3 ppt



RT: OA = 8.05 min, DTX2 = 8.22 min, DTX1 = 8.63 min. Peak Width = 6 sec. at base

Calibration Curves

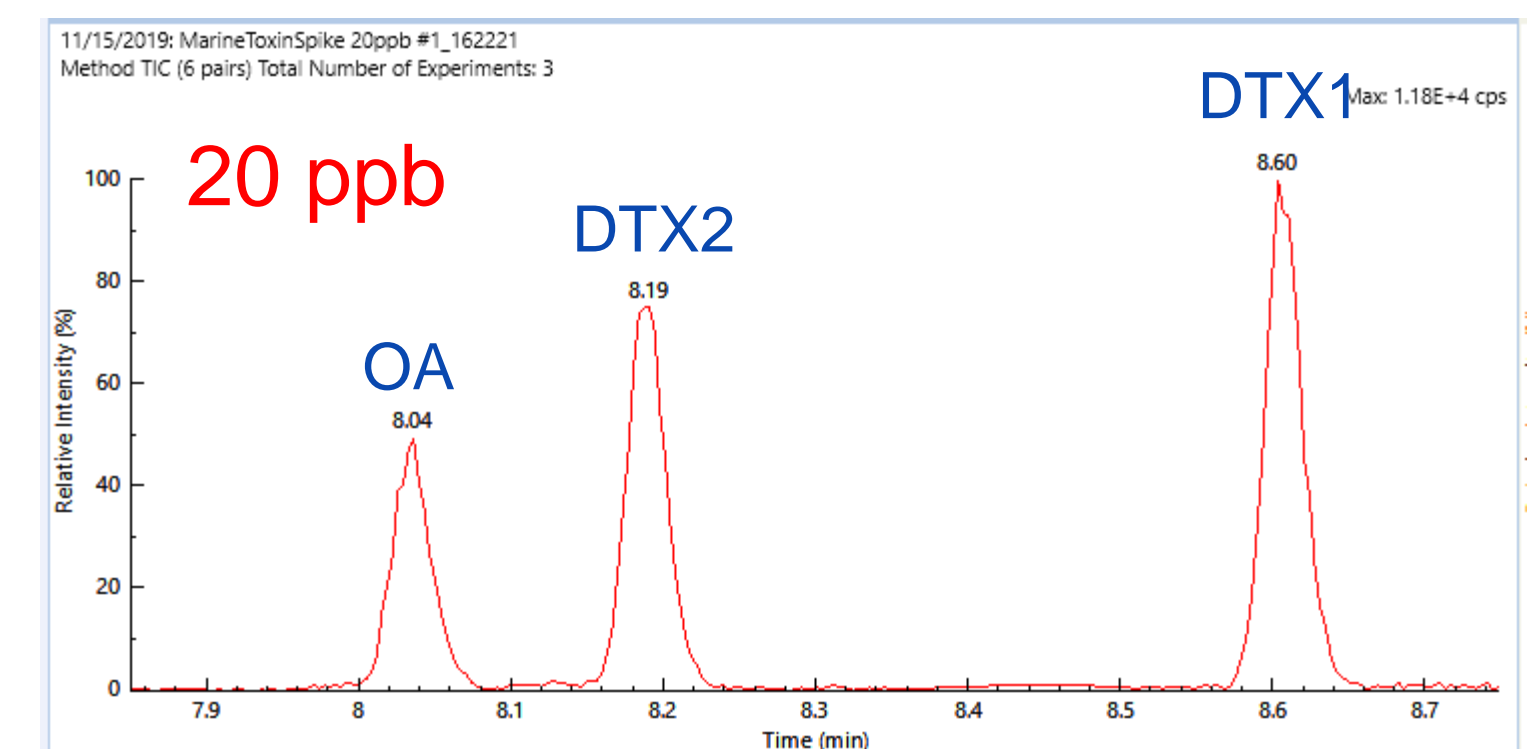
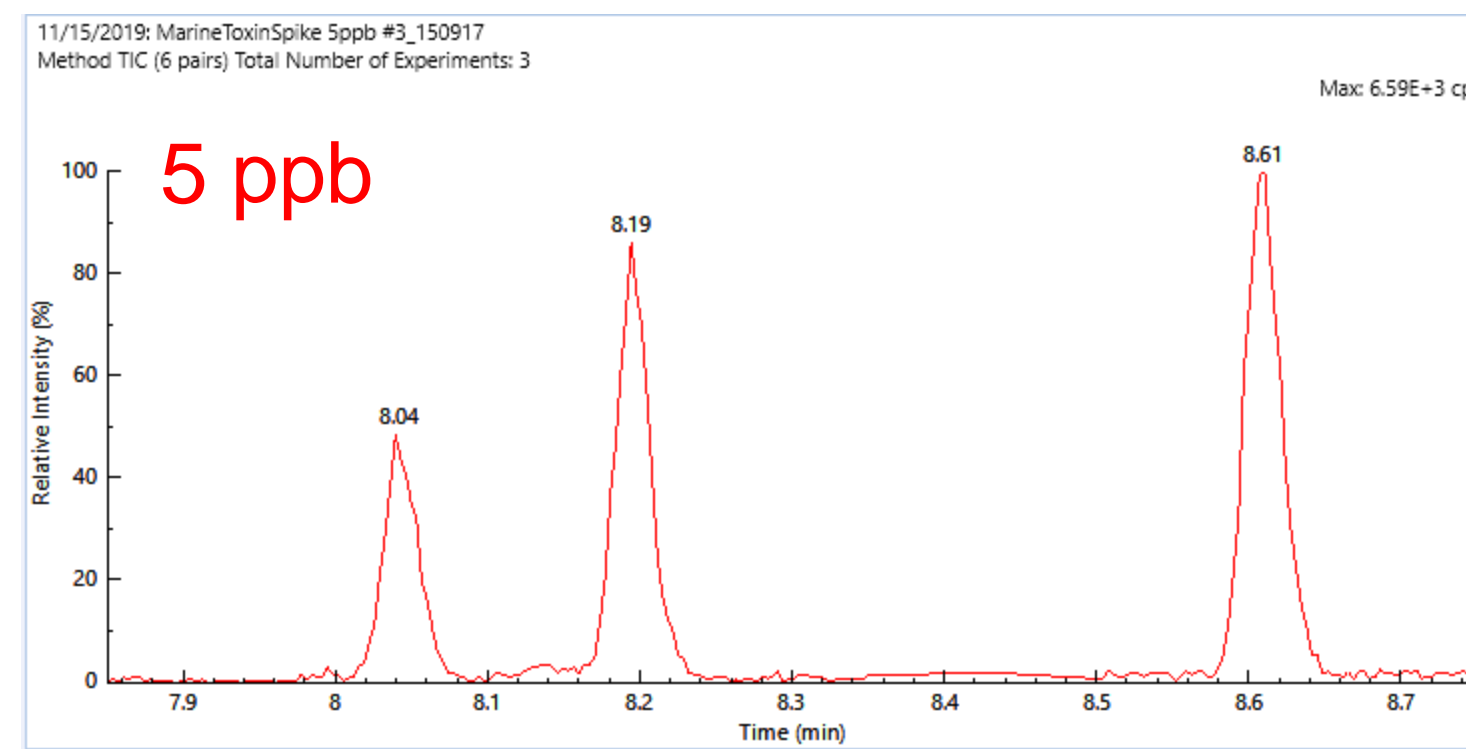
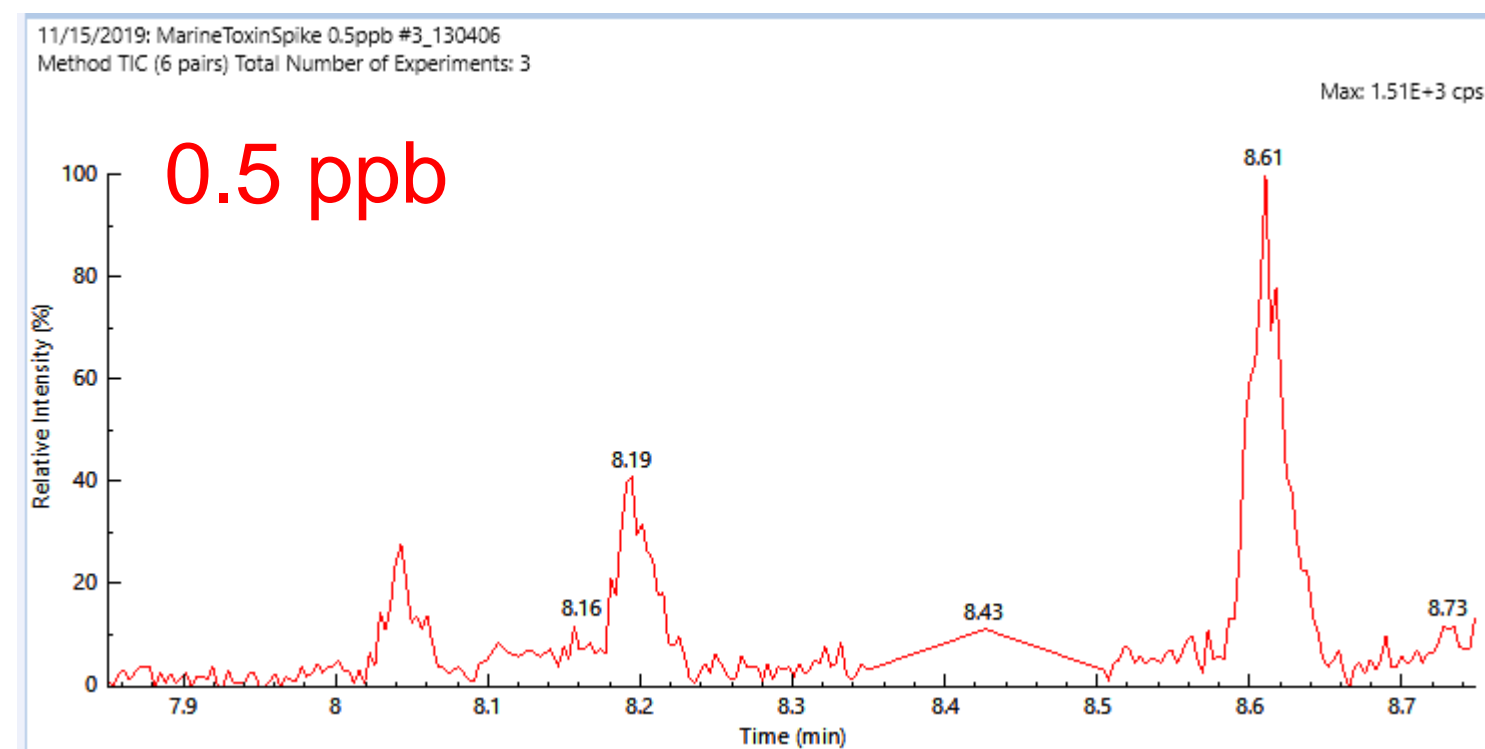
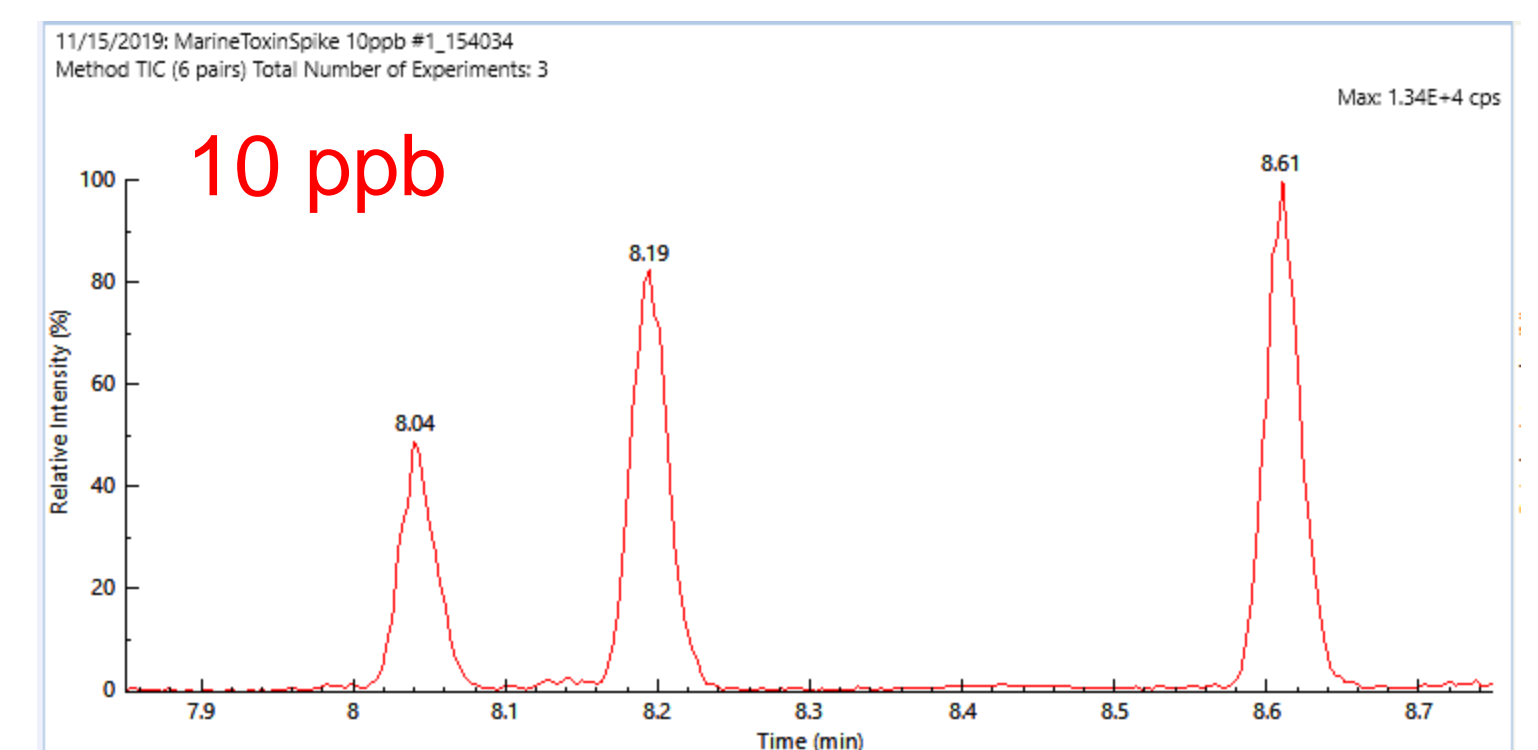
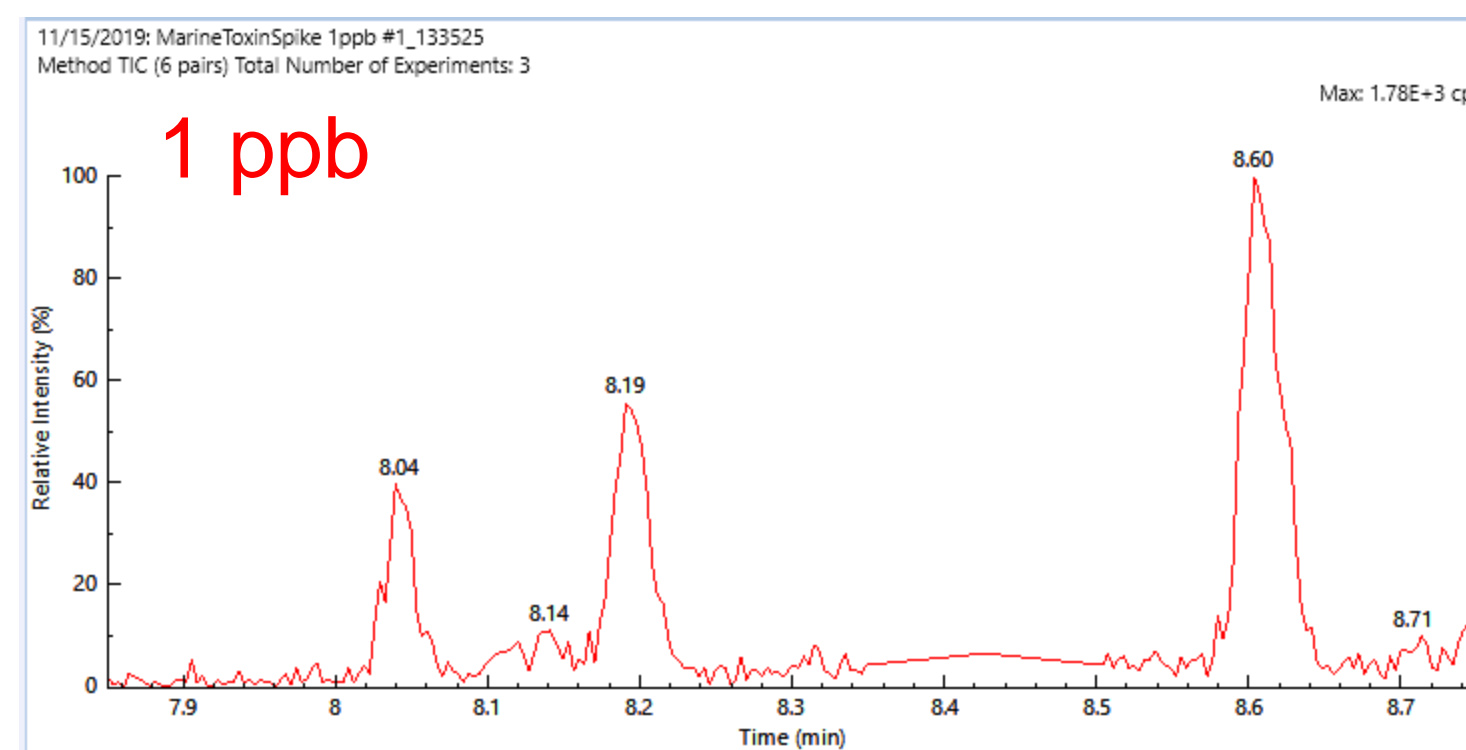
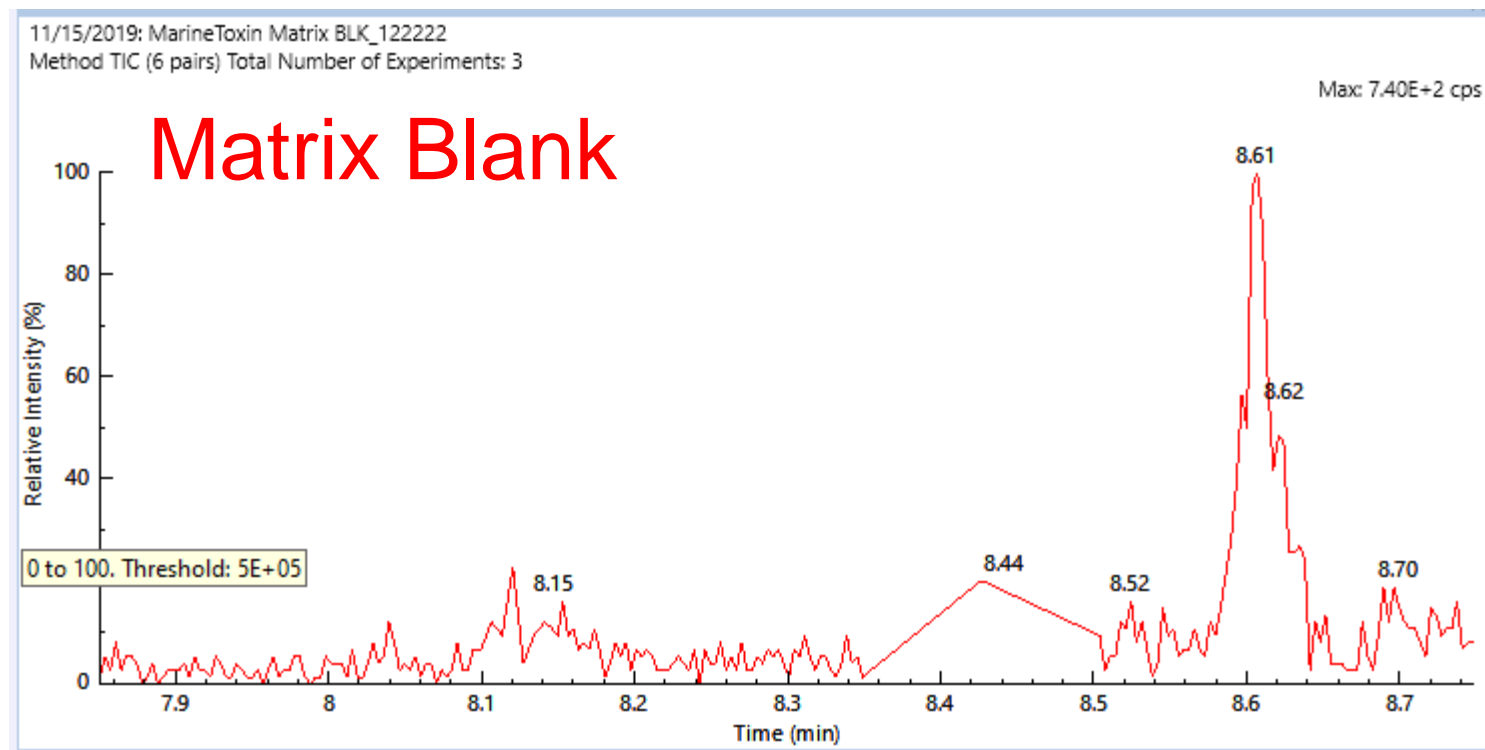


Linearity

Analyte	MRM Transition	Calibration Range (ppt)	Regression Equation	R ²
OA	803.4 > 255.3	97.7 - 100,000	$y = 0.77125x - 48.93820$	0.9966
OA	803.4 > 113.1	48.8 - 100,000	$y = 0.95128x - 55.42229$	0.9975
DTX2	803.4 > 255.3	24.4 - 100,000	$y = 1.74916x - 58.35793$	0.9954
DTX2	803.4 > 113.1	48.8 - 100,000	$y = 1.35235x - 81.91157$	0.9956
DTX1	817.5 > 255.3	48.8 - 100,000	$y = 1.98739x - 109.31302$	0.9953
DTX1	817.5 > 113.1	48.8 - 100,000	$y = 2.26652x - 99.87304$	0.9949

Calibration Range: Low ppt to 100 ppb. All R² ≥ 0.995

Matrix Blank vs. Matrix Spike (Without Smoothing)



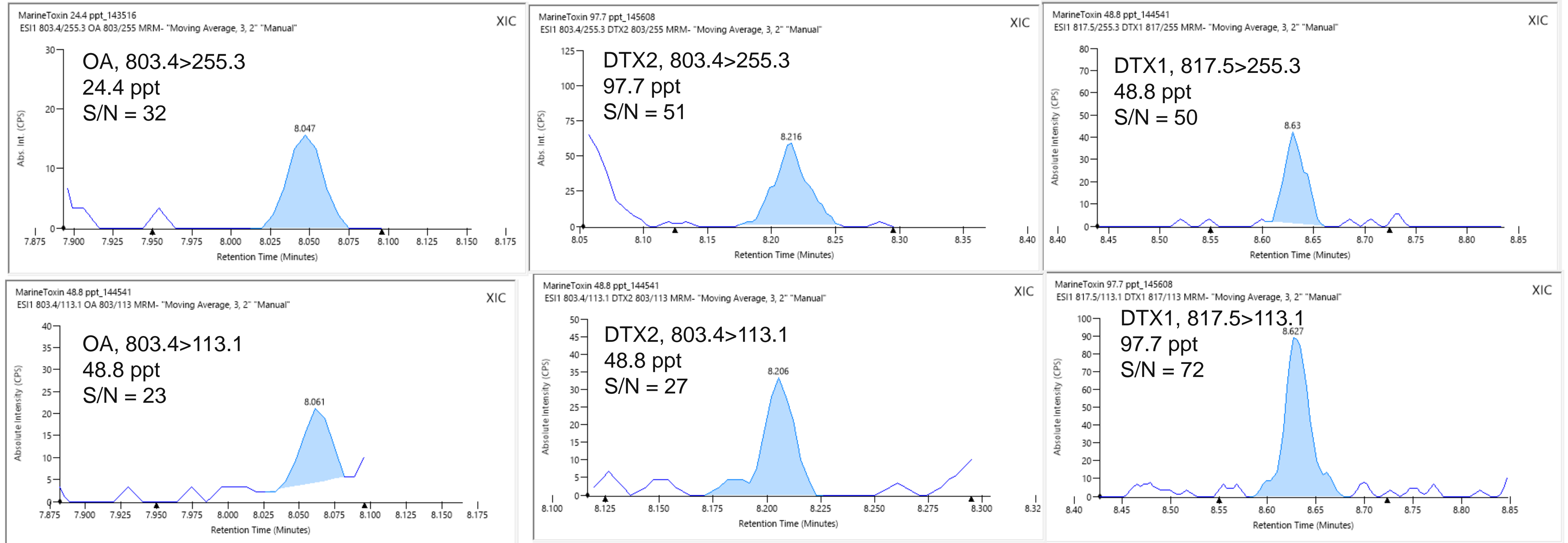
- Control samples contain low ppt level of analytes.
- OA = 91-107 ppt, DTX2 = 45-64 ppt, DTX1 = 277-299 ppt.
- **Combined action limits = 160 ppb (OA equivalent)**

Spiked Recovery

Analyte	MRM Transition	0.5 ppb		1 ppb		5 ppb		10 ppb		20 ppb	
		%Rec	StDev	%Rec	StDev	%Rec	StDev	%Rec	StDev	%Rec	StDev
OA	803.4 > 255.3	90.9	14.8	100.5	19.0	123.1	18.7	122.5	2.4	114.7	8.3
OA	803.4 > 113.1	88.6	20.1	105.1	10.8	121.0	19.7	116.9	6.3	112.3	9.2
DTX2	803.4 > 255.3	92.5	29.4	102.9	7.7	119.1	17.3	126.4	5.9	107.3	6.7
DTX2	803.4 > 113.1	75.6	15.7	84.2	3.0	112.3	15.4	109.3	10.8	102.8	8.6
DTX1	817.5 > 255.3	78.0	31.3	94.0	9.1	89.9	9.2	97.4	5.2	90.8	3.9
DTX1	817.5 > 113.1	77.6	36.6	92.8	5.7	94.3	12.0	97.1	4.4	91.3	6.5

- Spike level: 0.5, 1, 5, 10 and 20 ppb
- % Rec: Average of triplicate analyses
- StDev: Standard deviation of triplicate analyses.

Instrument Sensitivity (Low ppt level injections)



- Low ppt level injections of standard mix on column.
- Injection volume = 10 μ L
- SNR Method: Peak to Peak
- Smoothing Properties: Mean (3, 2). Noise Reduction

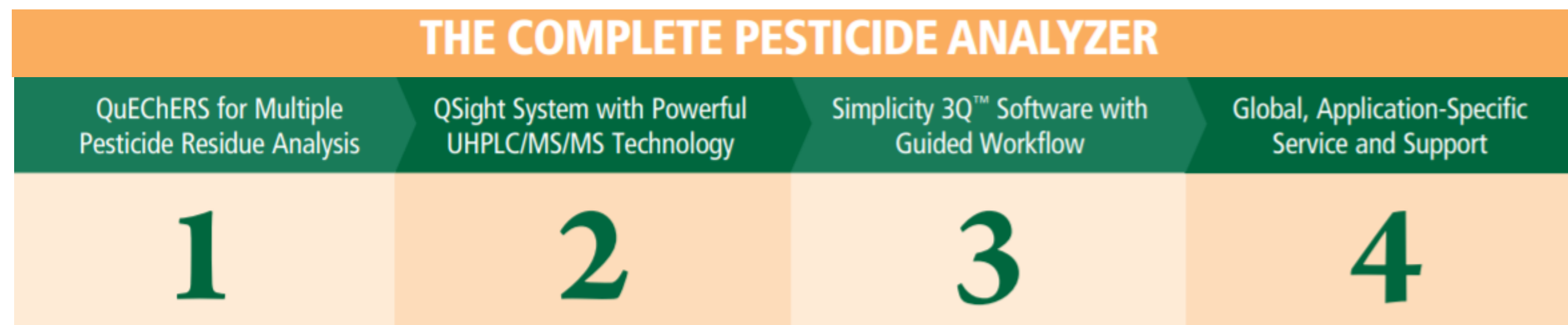
“Estimated” Limit of Quantification (LOQ, 10 x S/N)

Analyte	MRM Transition	RT (min)	LOQ (ppt)
OA	803.4 > 255.3	8.05	25
OA	803.4 > 113.1	8.05	26
DTX2	803.4 > 255.3	8.21	56
DTX2	803.4 > 113.1	8.21	20
DTX1	817.5 > 255.3	8.63	57
DTX1	817.5 > 113.1	8.63	26

- Control samples contain low ppt level of analytes.
- Low ppt level matrix spike tests difficult to perform.
- LOQ estimated from 0.5 ppb spike recovery tests. Background subtraction was performed for calculation of LOQ.

Conclusions

- Optimized workflow – from sample to result
- Sample preparation: easy/customized QuEChERS or... no sample preparation
- QSight flow-based mass spectrometry
 - Sensitive multi-residue method - EU MRL limits
 - Challenging contaminants in complex foods (ESI / APCI)
 - Instrument robustness and no frequent maintenance needed (StayClean™ / HSID™)





Merci pour votre attention

Christophe Clarysse - Christian Missitch