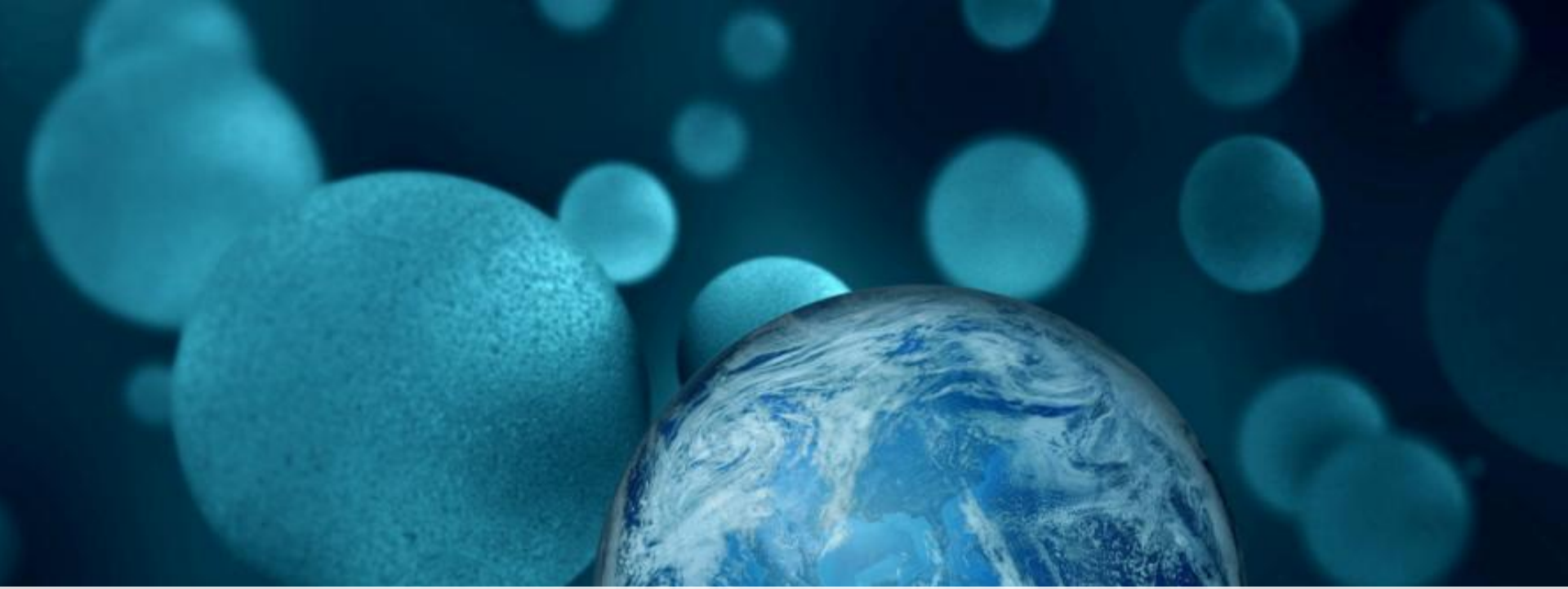


ThermoFisher SCIENTIFIC

Addressing Critical Analytical Challenges in Pesticide Residues Analysis with High Resolution Accurate Mass (HRAM) Mass Spectrometry

Richard J .Fussell

Outline

- Should laboratories consider migrating to full scan technologies such as Time-of Flight MS or Orbitrap MS technologies to provide a more comprehensive analysis of both the ‘expected’ and ‘unexpected’ residues?

- Features of Orbitrap MS technologies

- Can workflows based on non-targeted acquisition address the analytical challenges faced by pesticide residues laboratories?

Workflow- Analysis of Extracts

Thermo Scientific™ Endura™ Triple Quadrupole Mass Spectrometer

IC-(Dionex™ICS-5000⁺ Reagent-Free™ HPIC™ systems) MS/MS

QuEChERS
NL
SweEt

TSQ™ 8000 Evo Triple Quadrupole GC-MS/MS

Targeted analysis- triple quadrupole MS/MS

QuPPe
methanol/water
(polar pesticides)

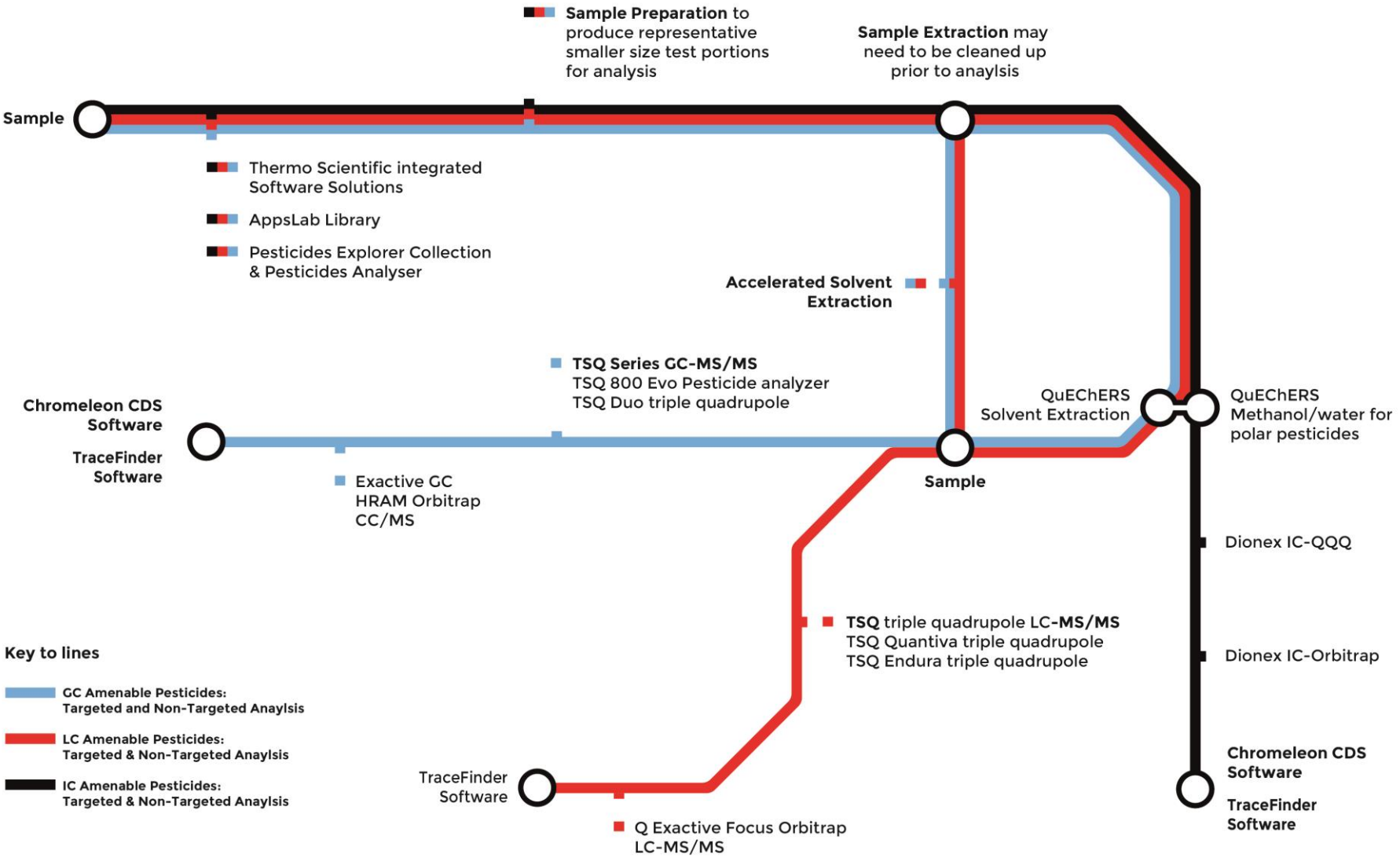
Simultaneous Targeted & Non-Targeted Analysis: Orbitrap-MS
(detection, quantification, identification, screening)

Exactive™ GC Orbitrap™ GC-MS

Q Exactive™ Focus hybrid quadrupole-Orbitrap MS

Dionex™ Integrion™ HPIC™ System- can be couped to coupled to Oribtrap MS

The Analysis Journey



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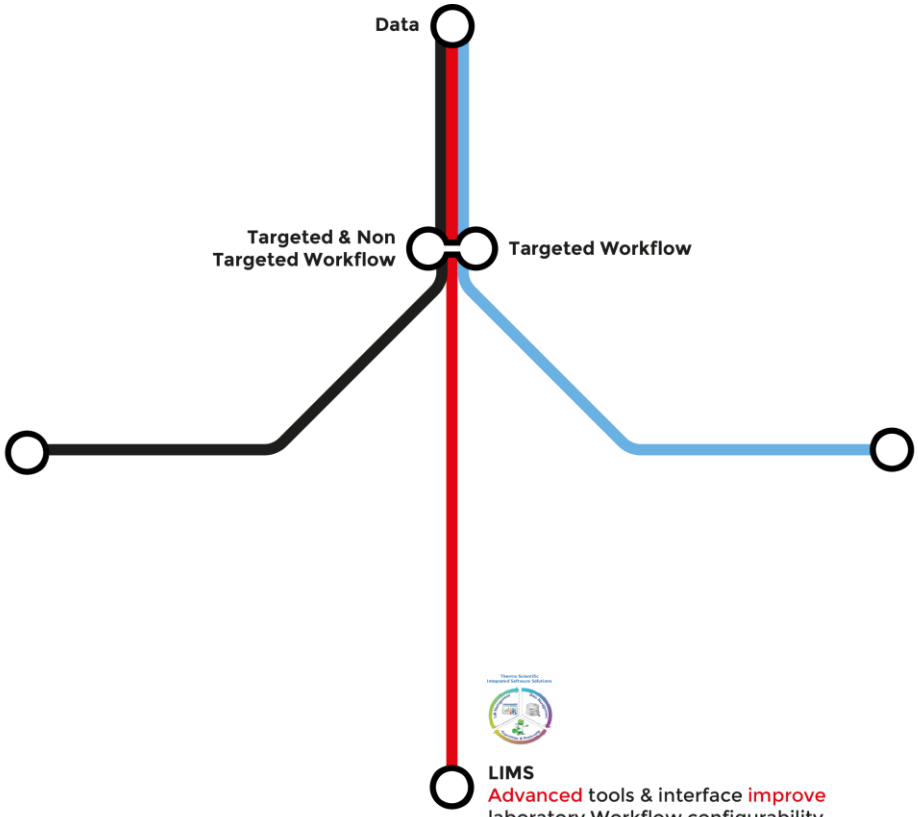
Data Processing, Storage and Management



TraceFinder
Perform targeted and untargeted screening, routine quantitation, and qualitative review of GC-MS, and LC-MS data. TraceFinder software is easy-to-use and compatible with GC-MS and LC-MS systems from Thermo Scientific. The software provides quantitative workflows and reports for food safety, applications.

Integrated with Thermo Fisher Scientific Tracefinder
mzCloud is a state of the art mass spectral database that assists analysts in identifying compounds for food safety applications. mzCloud™ features a freely searchable collection of high resolution/accurate mass spectra using a new third generation spectra correlation algorithm.

LIMS
Advanced tools & interface improve laboratory Workflow configurability. User-intuitive multiple sample login.

Chromeleon CDS software unifies workflows for chromatography and routine quantitative MS analysis, providing full integration of our gas chromatography (GC)-MS/MS and liquid chromatography (LC)-MS instruments. Quickly and easily process and report MS data in one application. Run your analyses in an enterprise environment, from method creation to quantitation & library-based compound identification.

Analytical Scientist LC-MS/MS Testimonial 2016

the Analytical Scientist

Exploring Pesticides Without Bugbears

Then and Now, with Mohamed Hamad, Director, Food Chemistry & Nutrition, Microbac Laboratories

"Made a decision to purchase the Thermo Scientific™ Pesticide Explorer Collection-standard method using the Thermo Scientific TSQ Endura™ Triple Quadrupole MS and the Thermo Scientific Vanquish™ UHPLC "

"First of all, you get a lot of value for money"

"You walk away with a complete workflow…. For quantification of nearly 300 pesticides"

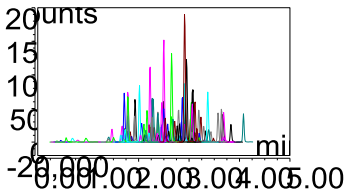
"We've been able to halve our turnaround time"

"Another key point is the absence of carry-over"

"We've experienced no problems operating 24/7"

Ongoing Developments in LC-QqQ MS

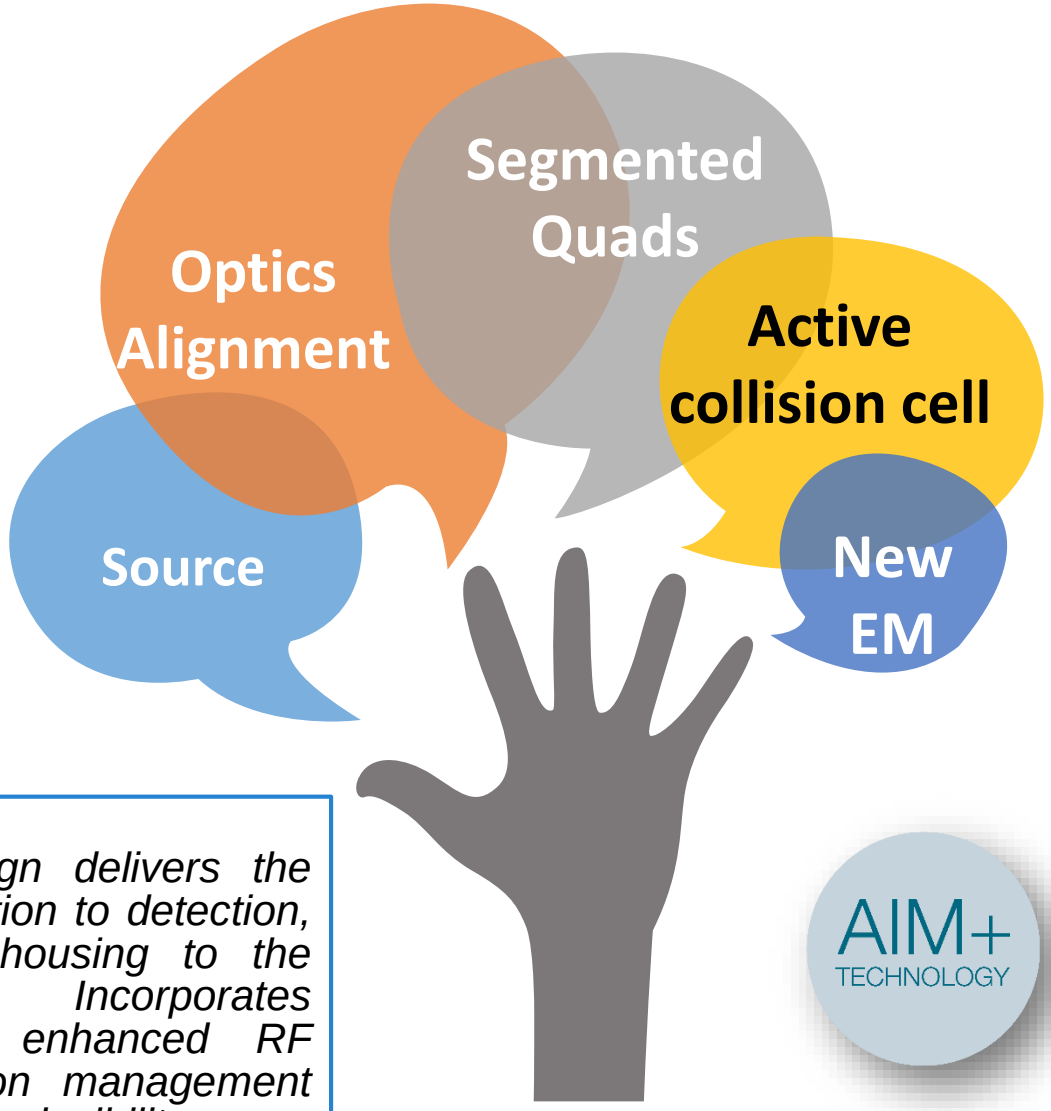
- Robust LC-MS Analysis of Pesticides with 1.0 mm ID column using the Vanquish Horizon UHPLC System



- 255 pesticides -Vanquish Horizon UHPLC system coupled to TSQ Endura™ Triple Quadrupole mass spectrometer
- The maximum system pressure was 1030 bar.
- Flow rate = 100µL/min

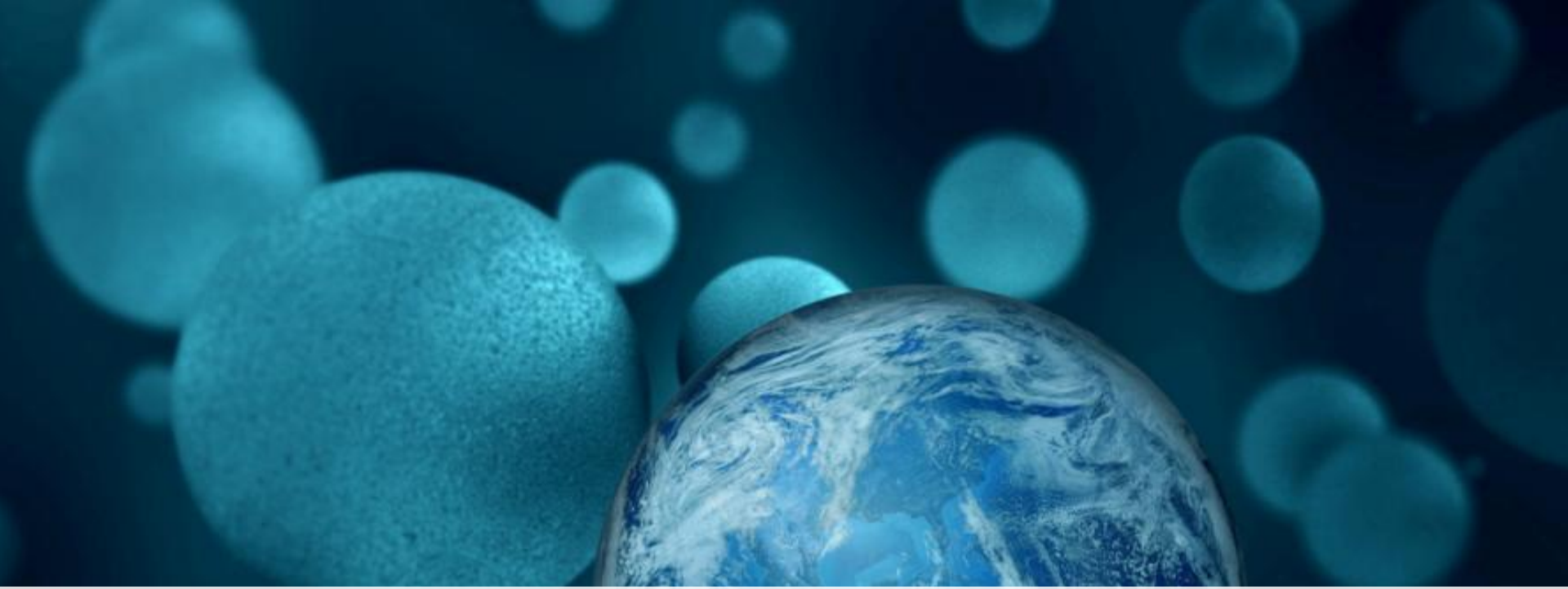
ASMS 2017: TSQ Quantis and Altis

TSQ Quantis



Active Ion Management Plus (*AIM+*)
The next step in precision design delivers the ultimate in ion management, inception to detection, from the OptaMax NG source housing to the enhanced electron multiplier. Incorporates segmented quadrupoles and enhanced RF Electronics to further optimize ion management precision, reliability, speed, and reproducibility.

ThermoFisher SCIENTIFIC



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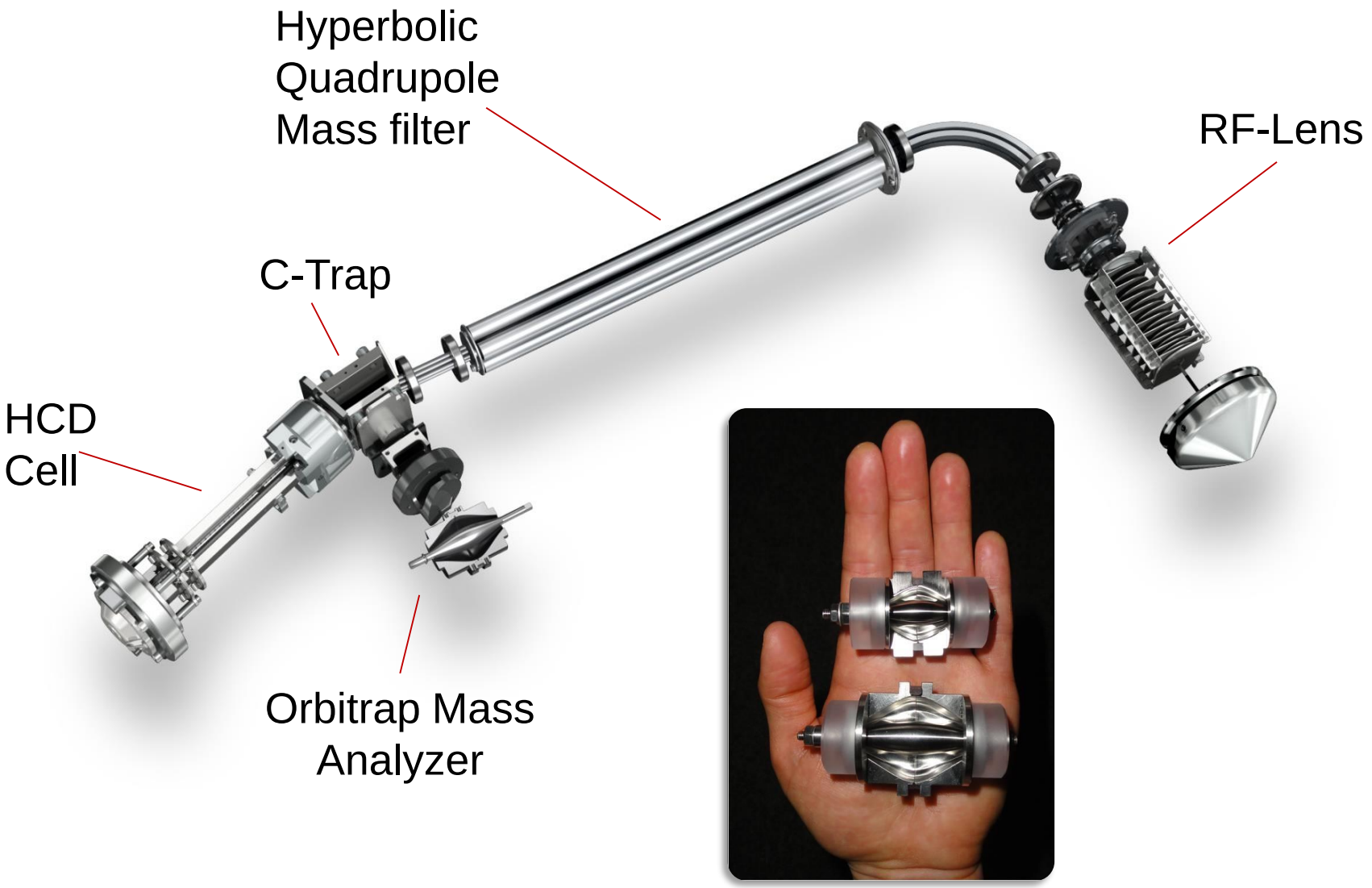
Enhanced Capabilities & Productivity with Orbitrap MS Technology

Orbitrap MS Geometry



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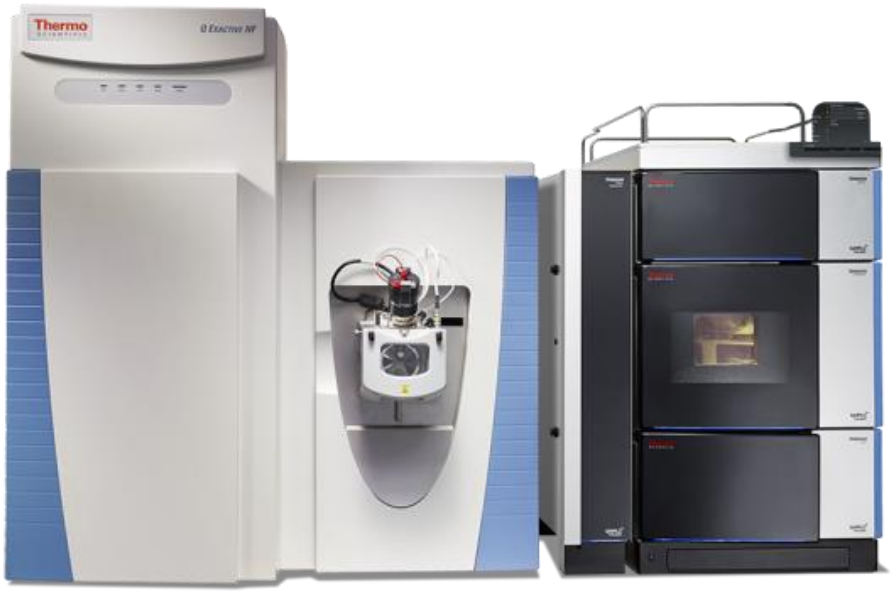
Redefining Routine LC-MS Analysis of Pesticides

Q Exactive Focus Hybrid Quadrupole-Orbitrap MS



vDIA is not available in the U.S.

Mass Range	50 < m/z < 2,000
Resolution @ m/z 200	17,500 at 12Hz 35,000 at 6 Hz 70,000 at 3 Hz
Top N	2
Mass Accuracy	< 1ppm RMS, Internal Calibration < 3ppm RMS, External Calibration
Polarity Switching	one full cycle –ve/+ve @ 35.000 RP in <1sec
Flexible Acquisition	Full scan dd-MS2 AIF vDIA PRM (SIM)

Mass Accuracy Does Matter!

Accurate mass improves the ability to confidently identify unknown compounds

ppm error = (mass error / MW) x 10^6

EXAMPLE:

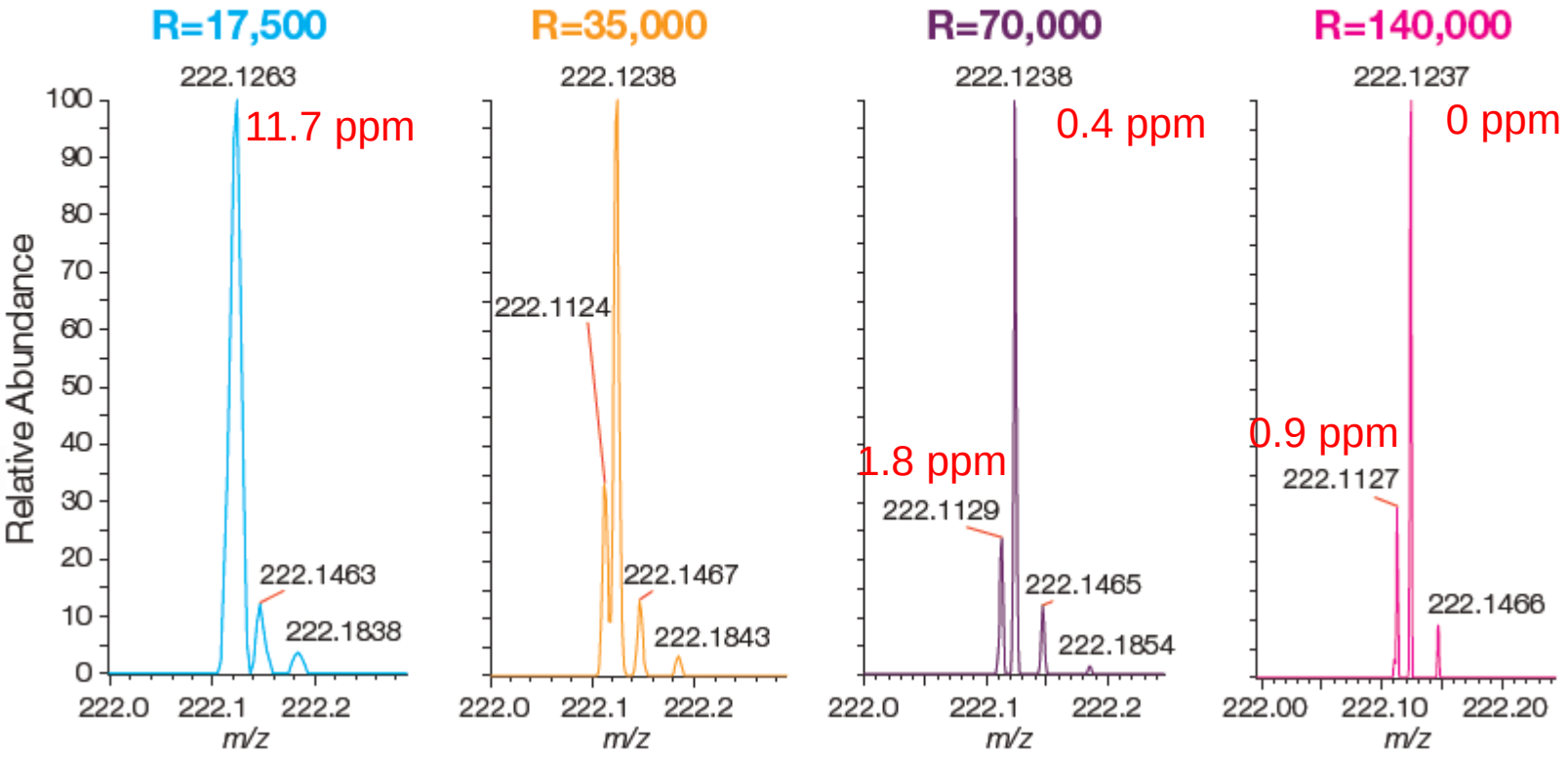
- Reserpine ($C_{33}H_{40}N_2O_9$) has a protonated ion at 609.28066

Mass Error (ppm)	# of Possible Chemical Formulae (using only C, H, N & O)
165 (0.1 daltons)	209
10	13
5	7
3	4
2	2
1	2
0.5 (0.0003 daltons)	1

HR/AM (High Resolution/Accurate Mass)

Mass accuracy correlated with Resolution

Carbofuran	$C_{12}H_{15}NO_3$	$M+H^+ = 222.1125$
Formetanate	$C_{11}H_{15}N_3O_2$	$M+H^+ = 222.1237$



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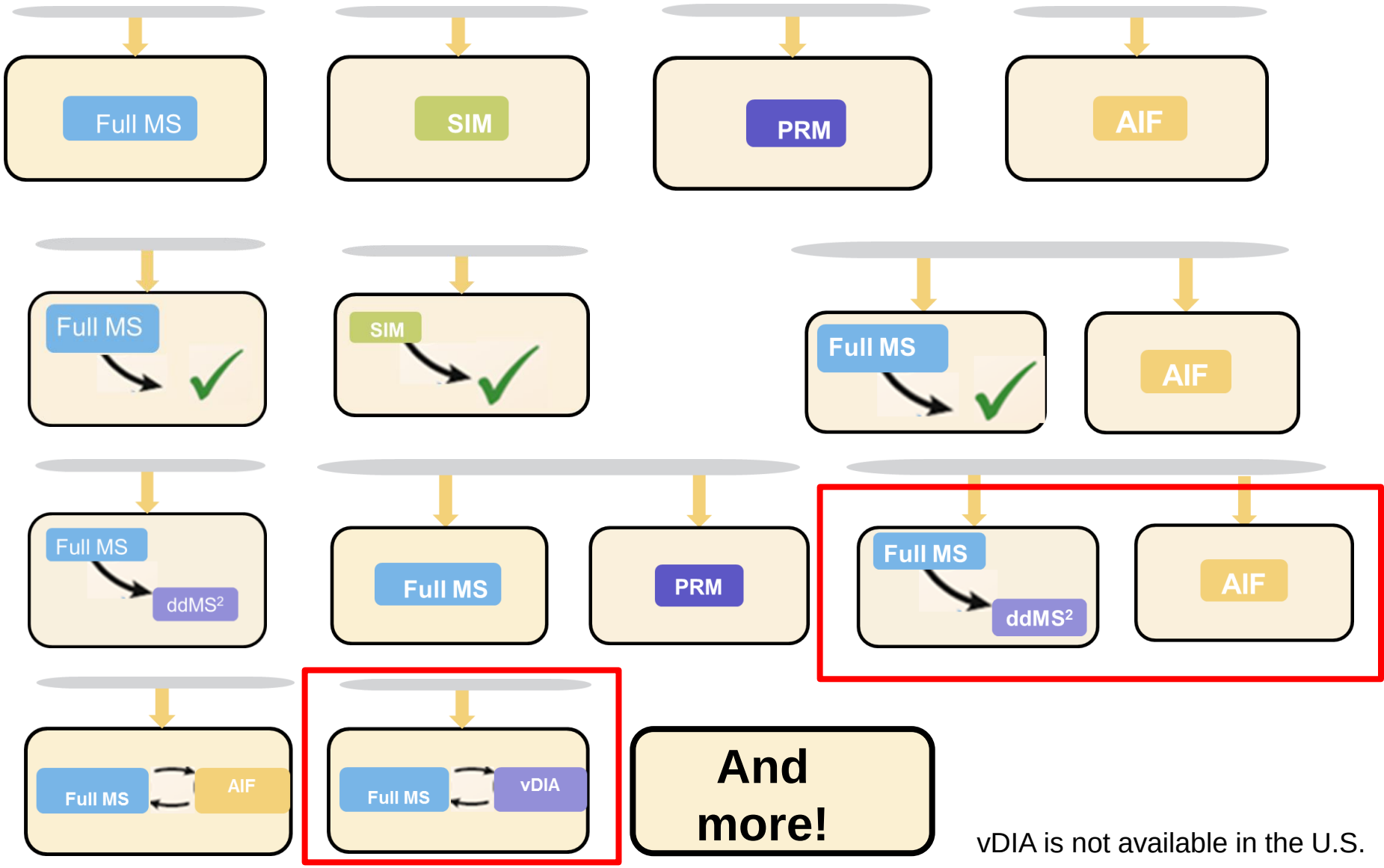
Acquisition: Q Exactive Focus MS System



vDIA is not available in the U.S.

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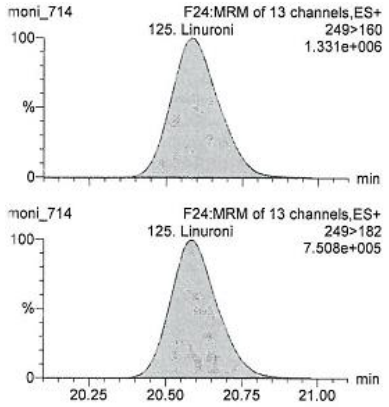
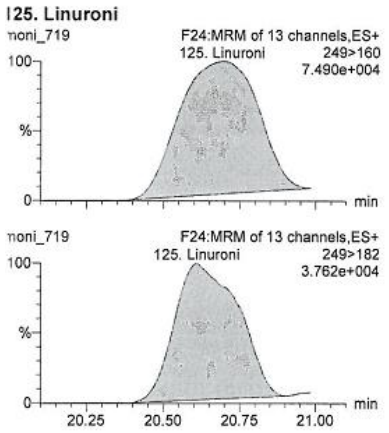
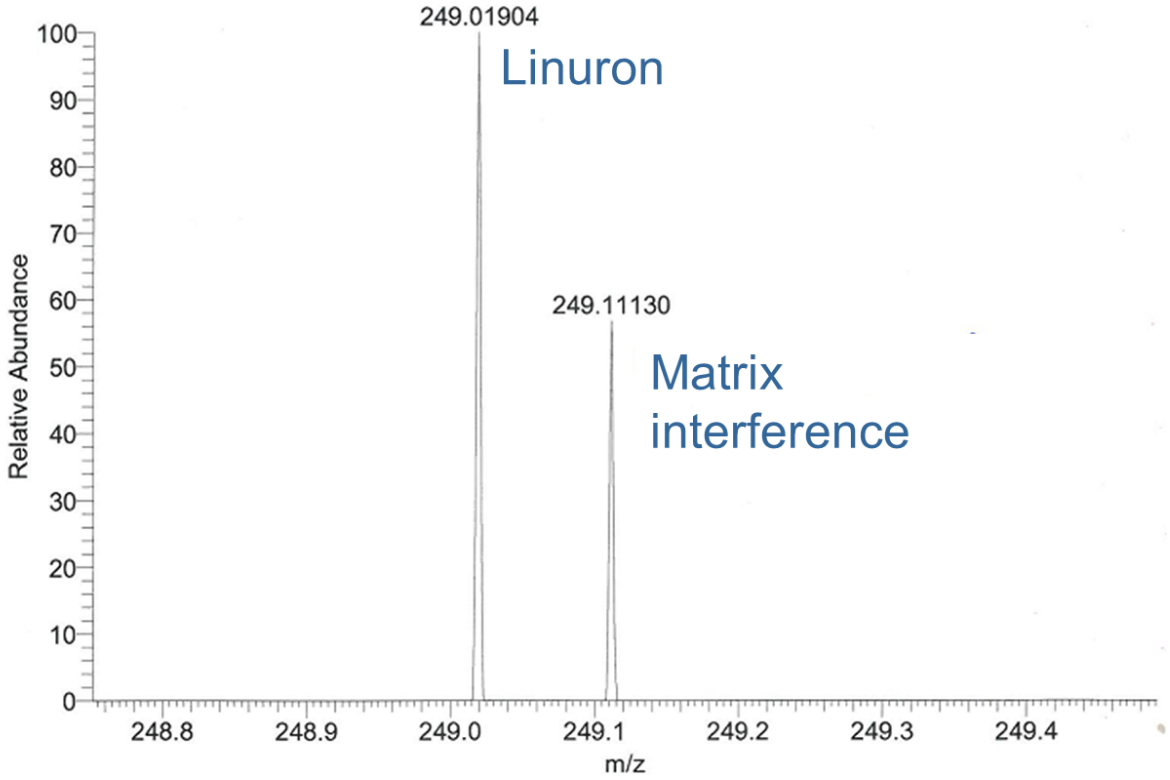
LTQ Orbitap MS System: Selectivity vs LC-QqQ

Standard in solvent (Ion ratio: 1.8)



Linuron (0.125 mg/kg in sample of coriander (Ion ratio: 2.4)

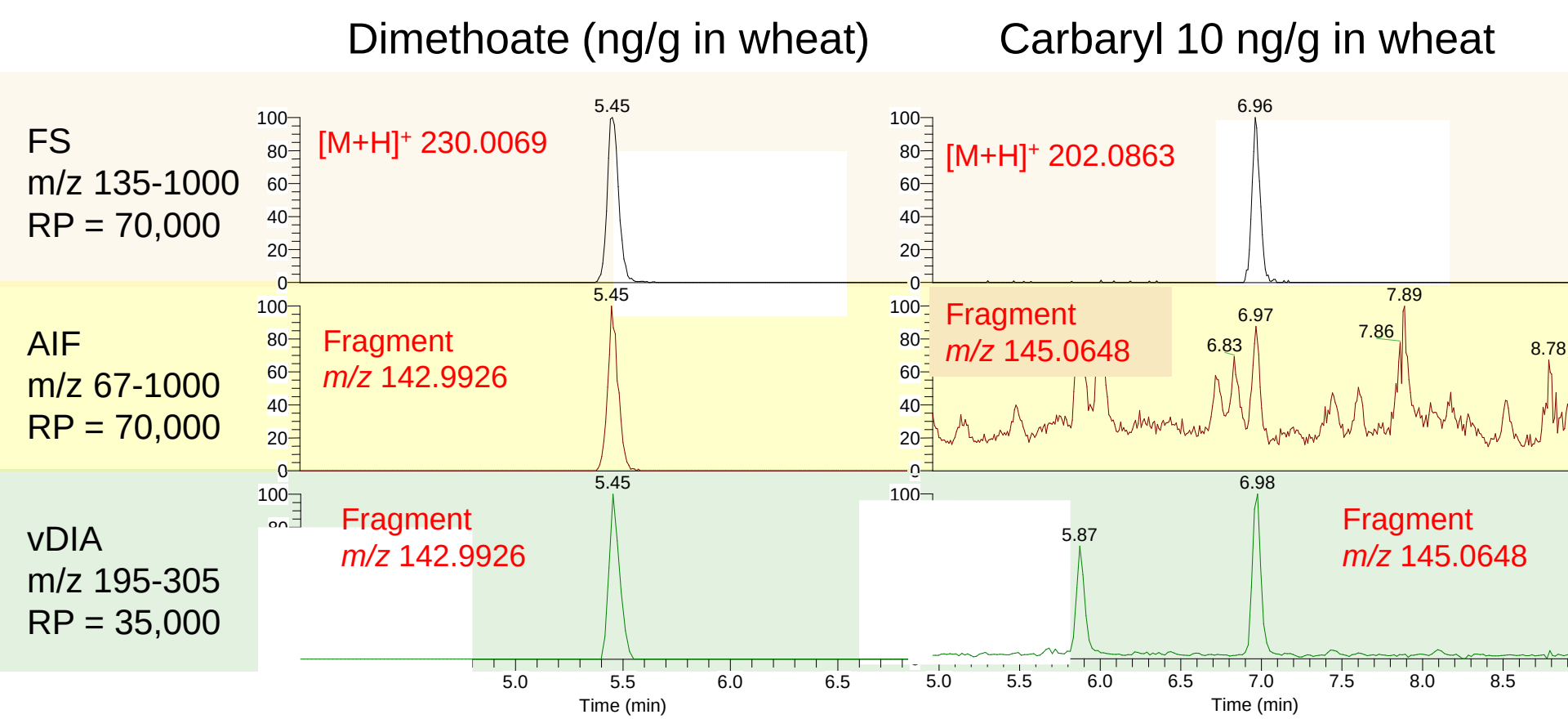




With permission of Prof. Amadeo Rodríguez Fernández-Alba, University of Almeria, Spain ,

ThermoFisher SCIENTIFIC

Q Exactive Focus MS System Selectivity: AIF vs. vDIA



- vDIA provides improved selectivity & sensitivity

vDIA is not available in the U.S.

Data from Hans Mol , Rikilt Wagenigen, UR

Redefining Routine Pesticides Analysis

Exactive GC system

Redefining Routine GC-MS

EI/CI; Full-scan; Timed-SIM

EI Spectra searchable against the NIST Library

Resolving Power

Up to 60,000 at *m/z* 200

Mass Accuracy

< 1ppm

Sensitivity

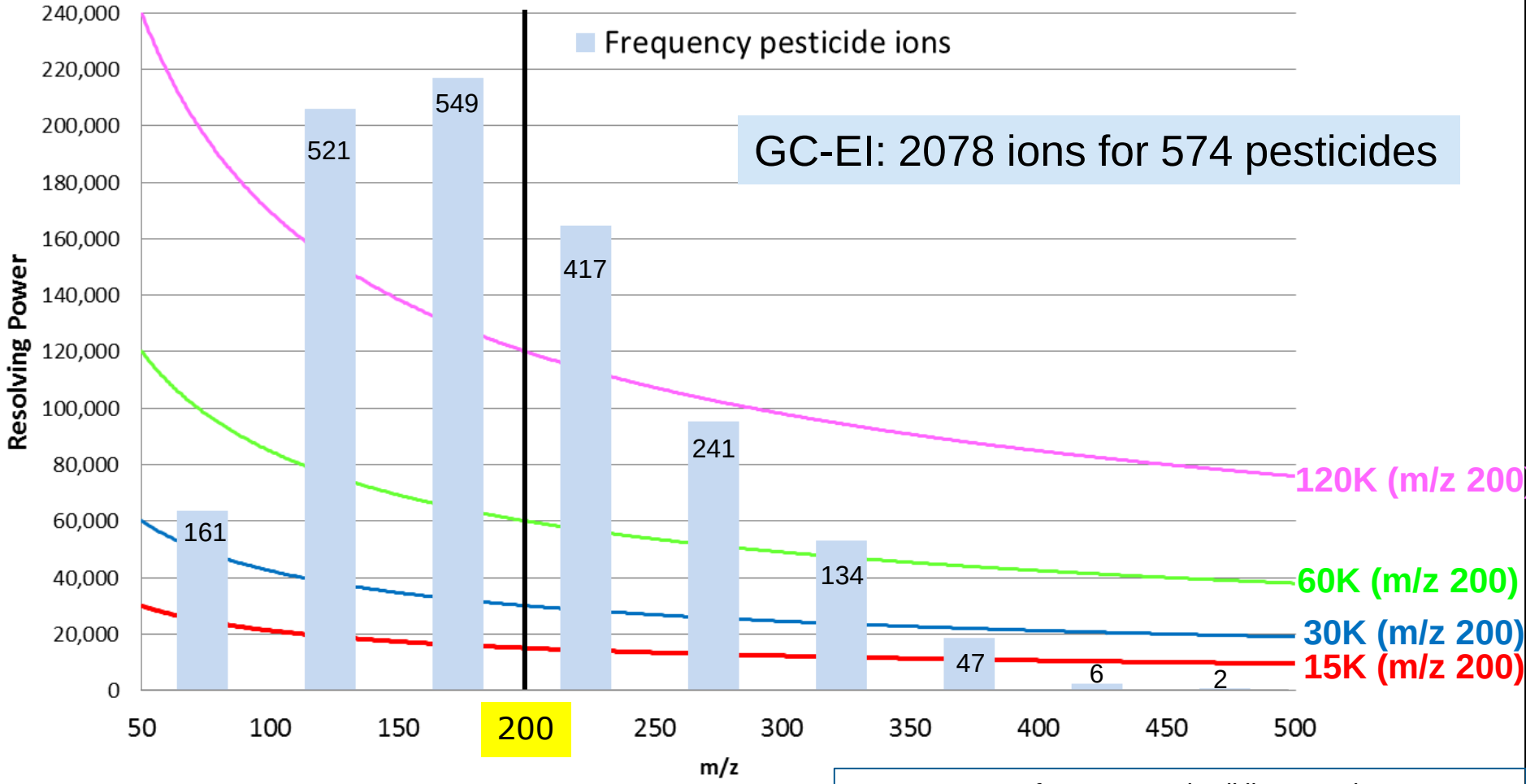
ppt

Dynamic Range

>6 orders

Orbitrap GC-MS Systems: Resolving Power

Resolving power ~1/√*m/z*



Data courtesy of Dr Hans Mol, Rikilt Wageningen, UR

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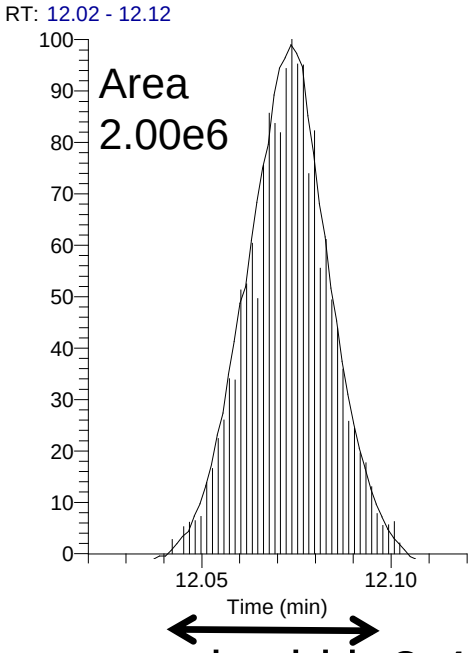
Challenges

- Selectivity (false detect and false negative rates)
- Sensitivity (dilution of matrix effects)
- Speed (acquisition, data processing or overall analysis)
- SANTE compliance
  - Linearity
  - Quantification
  - Recovery
  - Identification – retention time, ion ratios, mass accuracy
  - Compliance with MRLs
- Scope (the expected, suspected & unexpected = <false negatives)

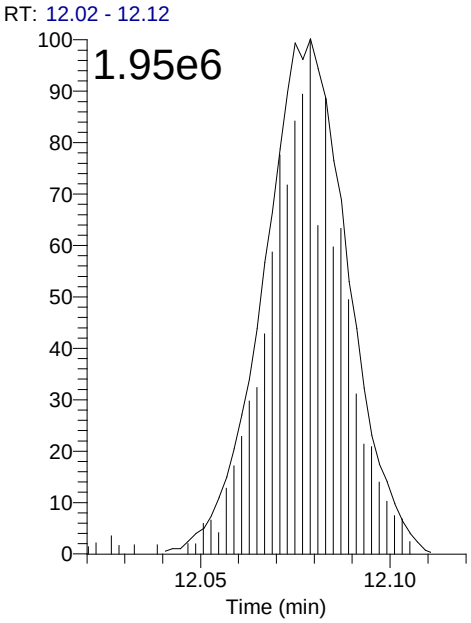
Q-Exactive GC-MS System- Speed of Acquisition

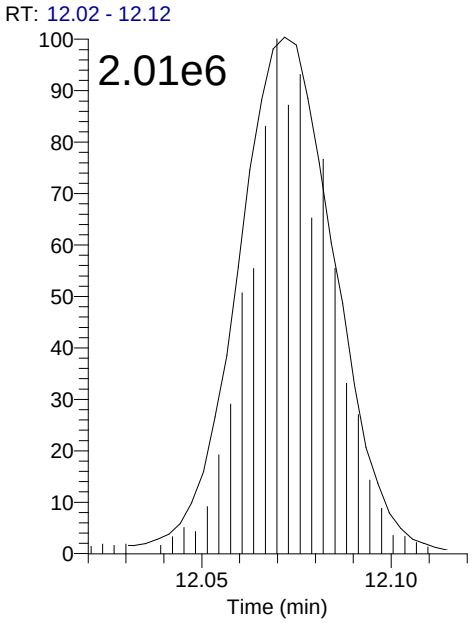
Scan speed ↔ Resolving power

- XIC *m/z* 180.93731; $C_6Cl_3H_4^+$
- Lindane in leek (10 µg/kg, 10 pg on-column)
- *m/z* 50-500; AGC 1E6



peak width 3-4 sec





15K ≈11-18 Hz

30K ≈8-14 Hz

60K ≈5-7 Hz

- Resolving power is trade-off with scan speed
- Scan speeds all compatible with GC as routinely applied in triple quad methods

Data courtesy of Dr Hans Mol, Rikilt Wageningen, UR

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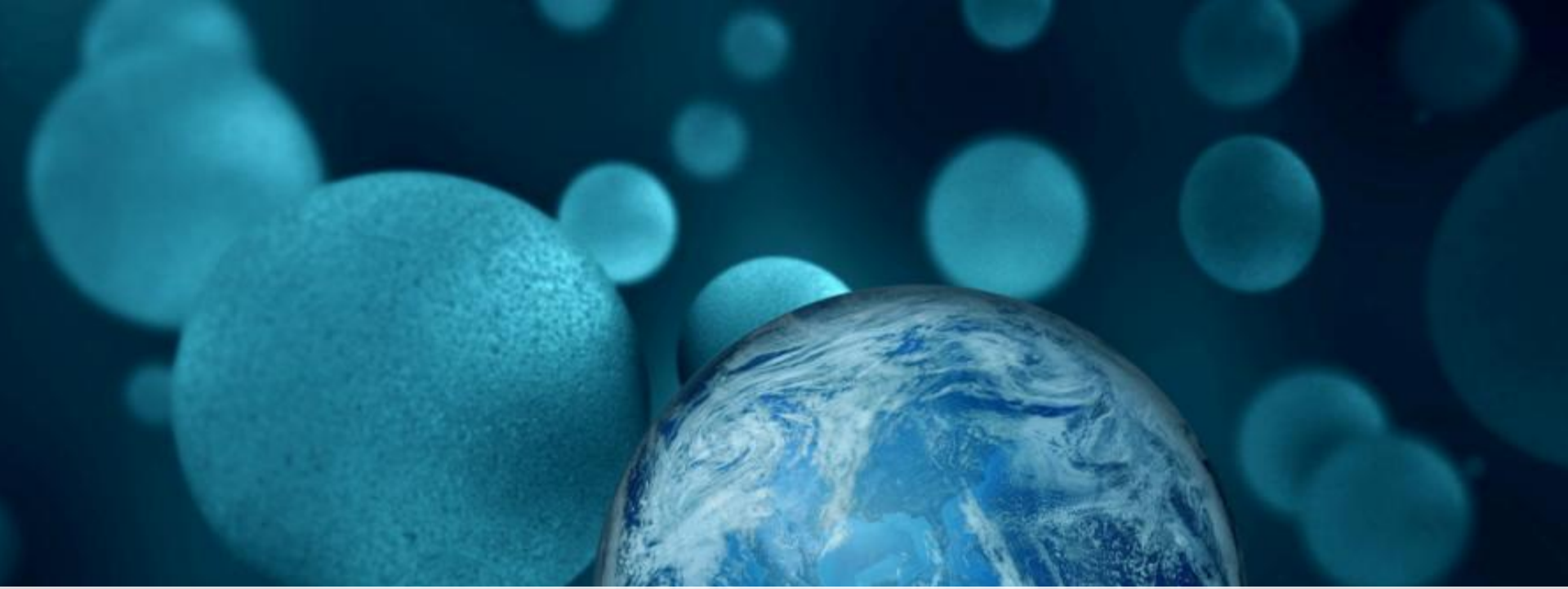

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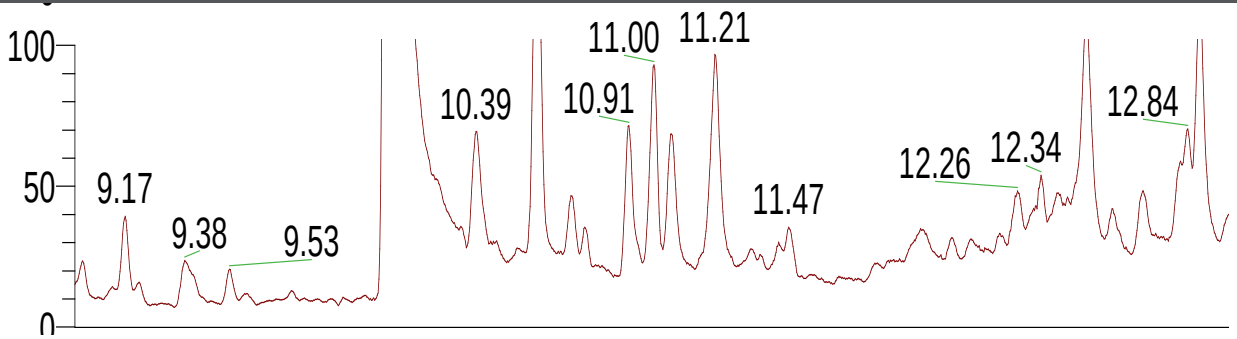
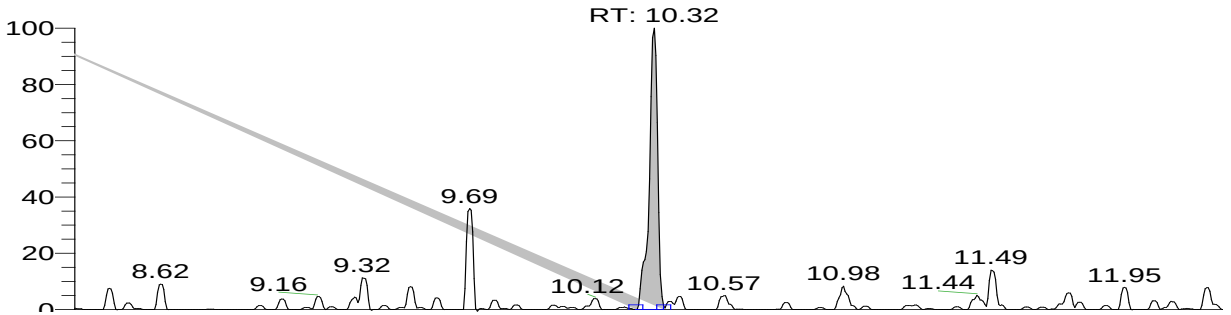
Thermo Scientific Exactive GC-MS System

Exactive GC System: Selectivity (chlorpropham in leek, 10 ppb)

Nominal mass
Full scan MS
XIC *m/z* ± 0.5 Da
127 ± 0.5 Da

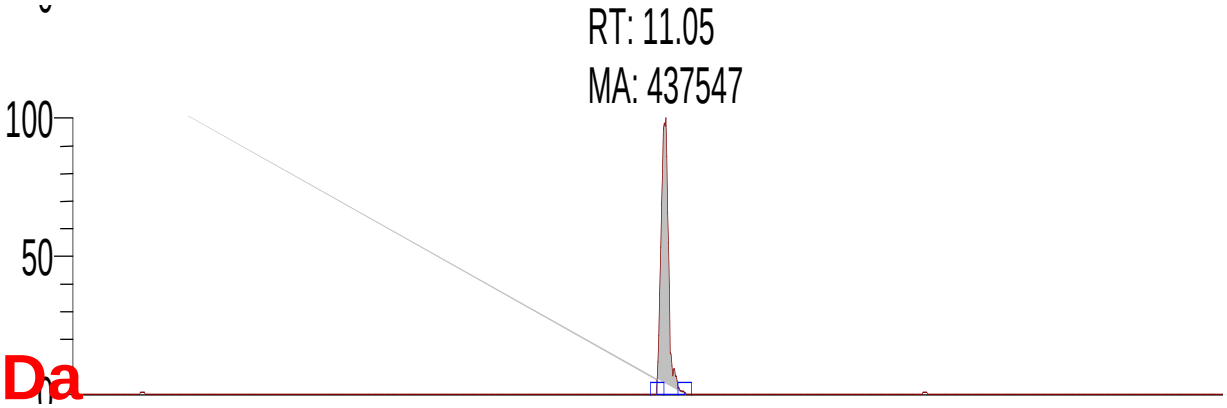


Nominal Mass
MS/MS
213 → 127



HRAM
Full scan MS
XIC *m/z* ± 5 ppm

127.01833 ± 0.0006 Da

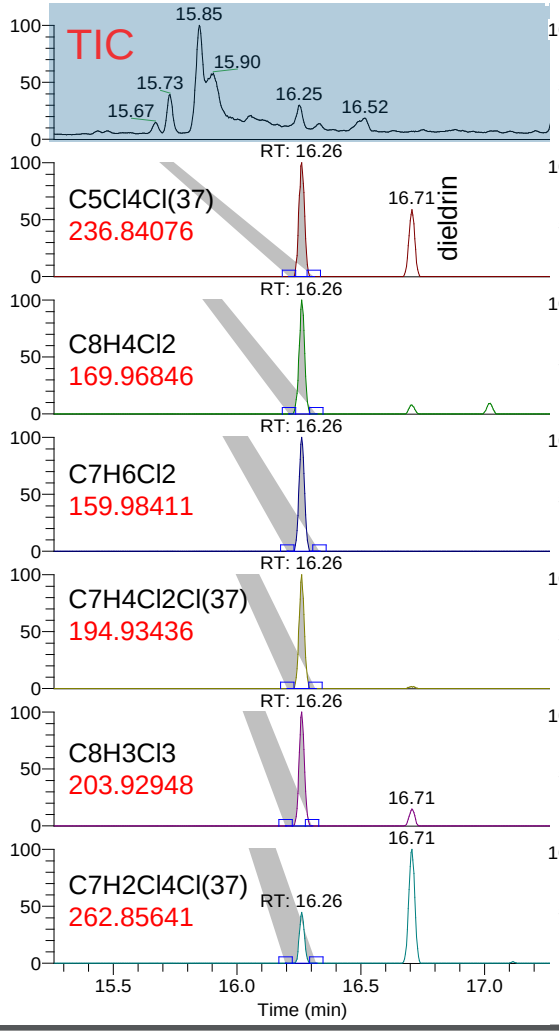


Data from Hans Mol , Rikilt Wagenigen, UR

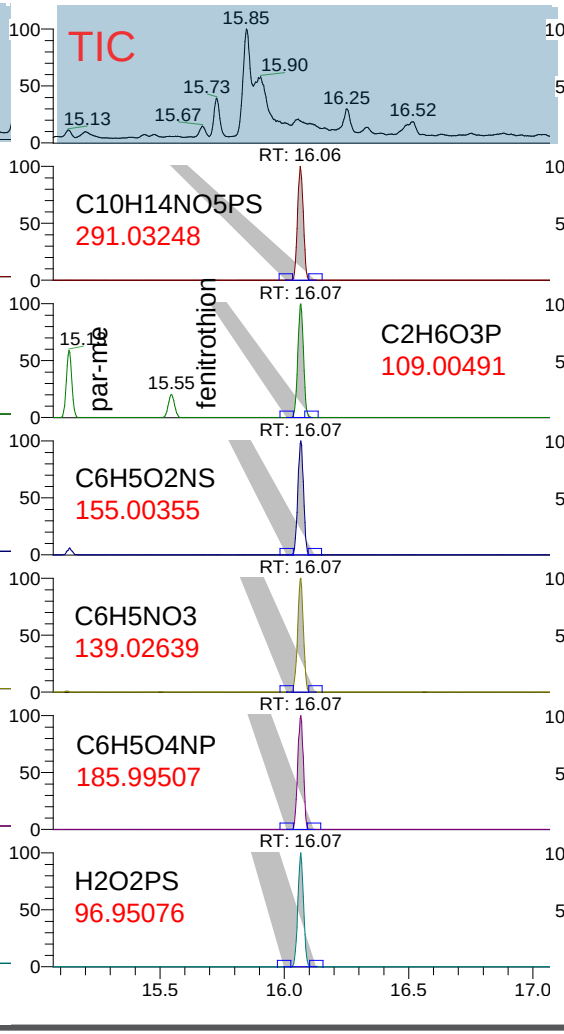
Exactive GC system: Selectivity

10 µg/kg in orange (10 pg on-column): 60.000 RP, MEW ±5 ppm

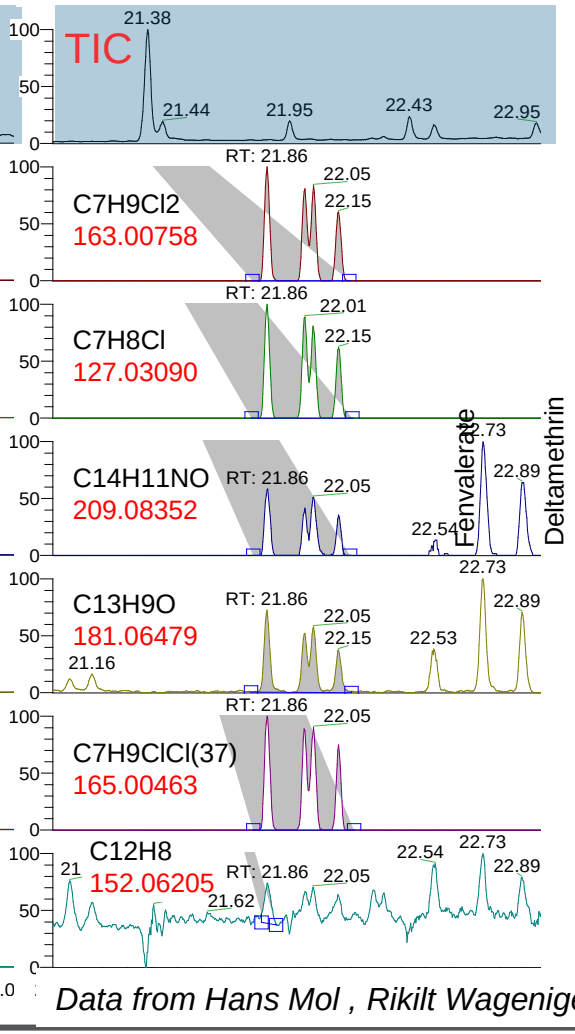
endosulfan-alpha



parathion



cypermethrin



Data from Hans Mol , Rikilt Wagenigen, UR

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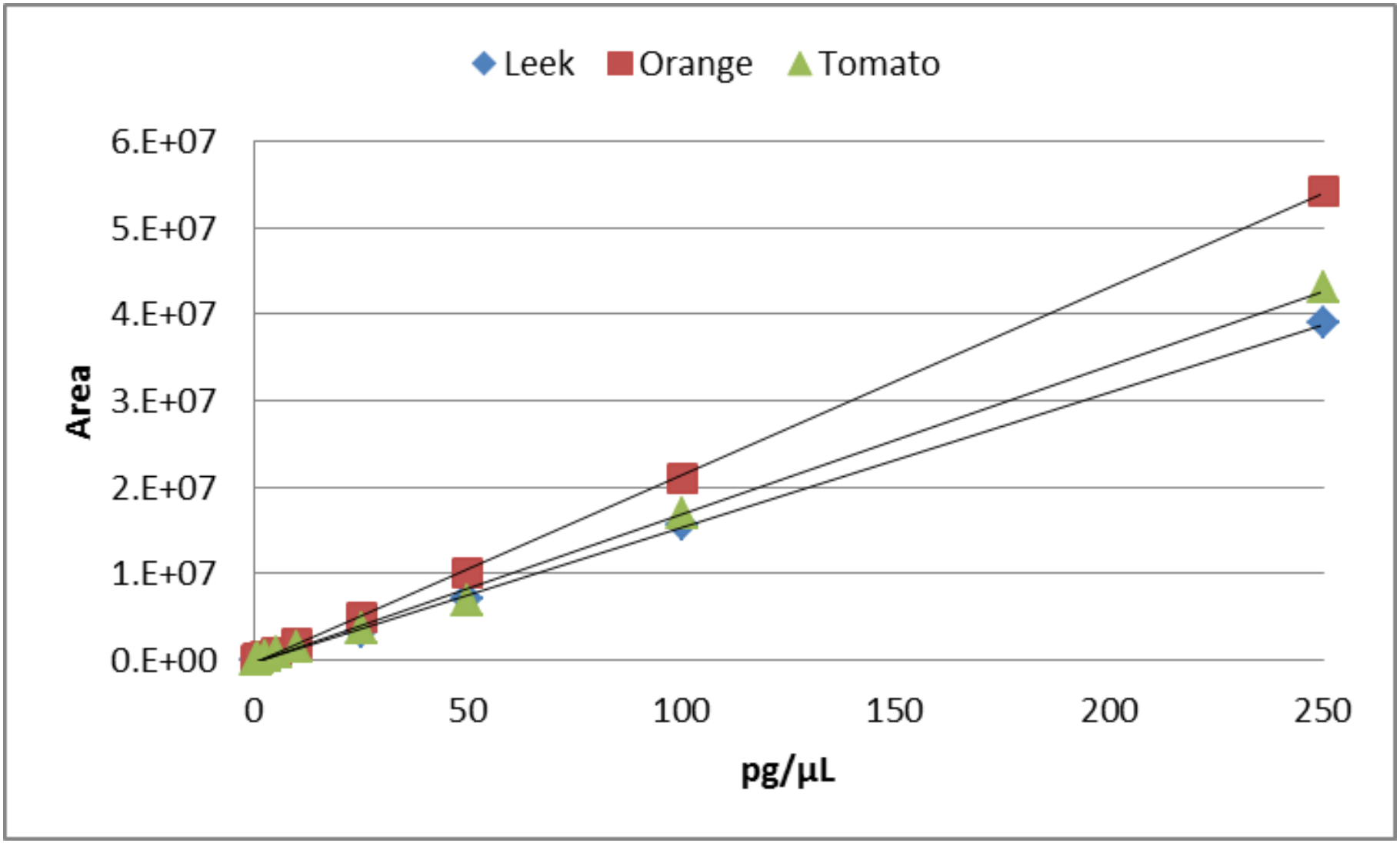
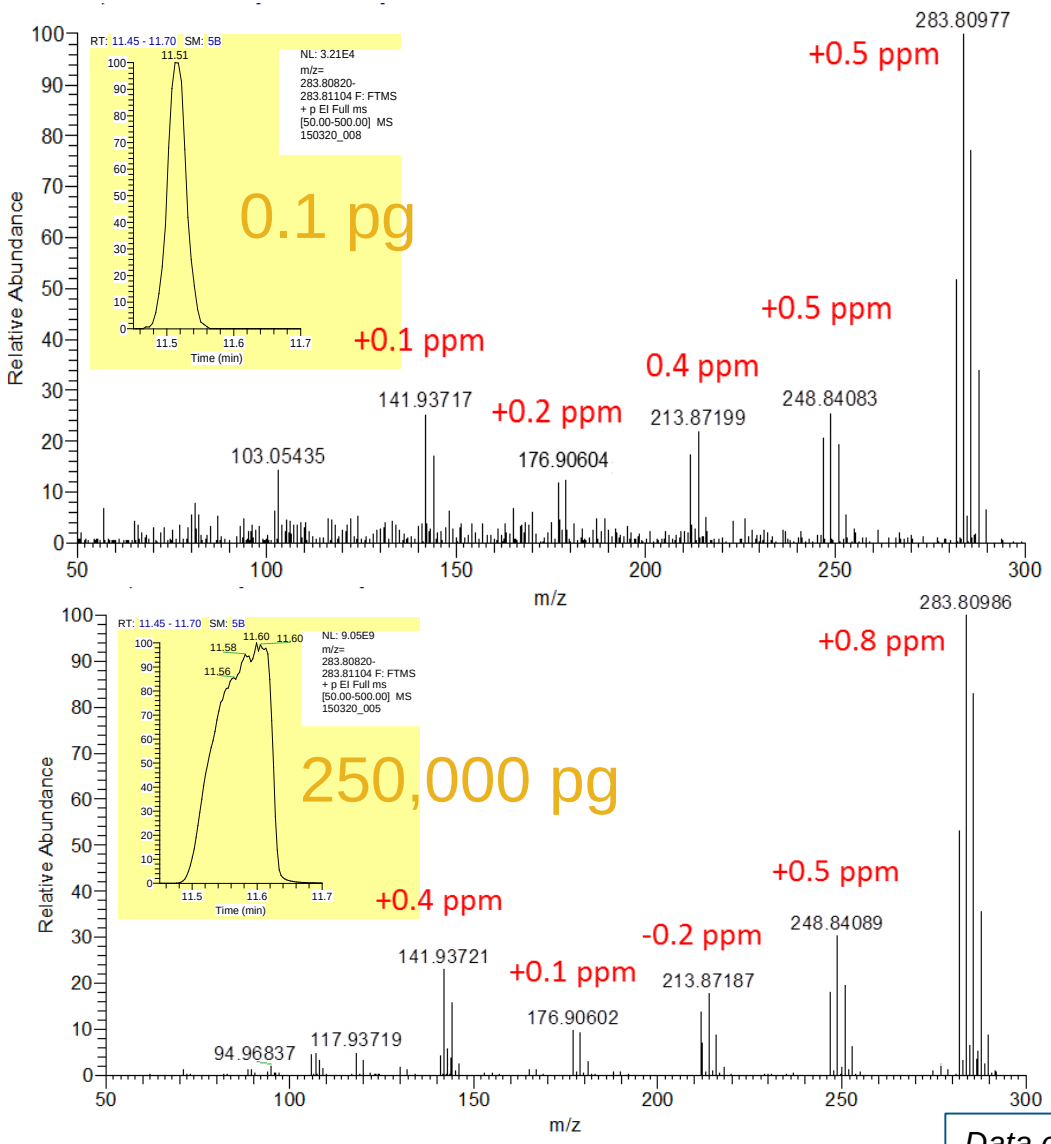
Q Exactive GC-MS: Linearity for Lindane



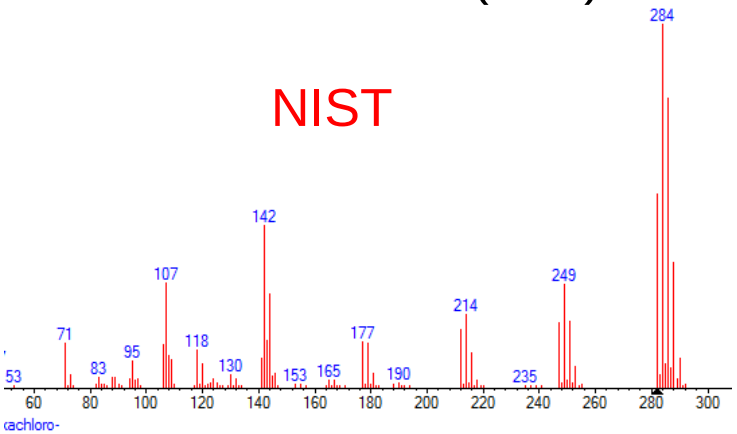
Data from Hans Mol , Rikilt Wagenigen, UR

Exactive GC system: Spectral Quality vs Amount Injected



Solvent standard
hexachlorobenzene (60K)

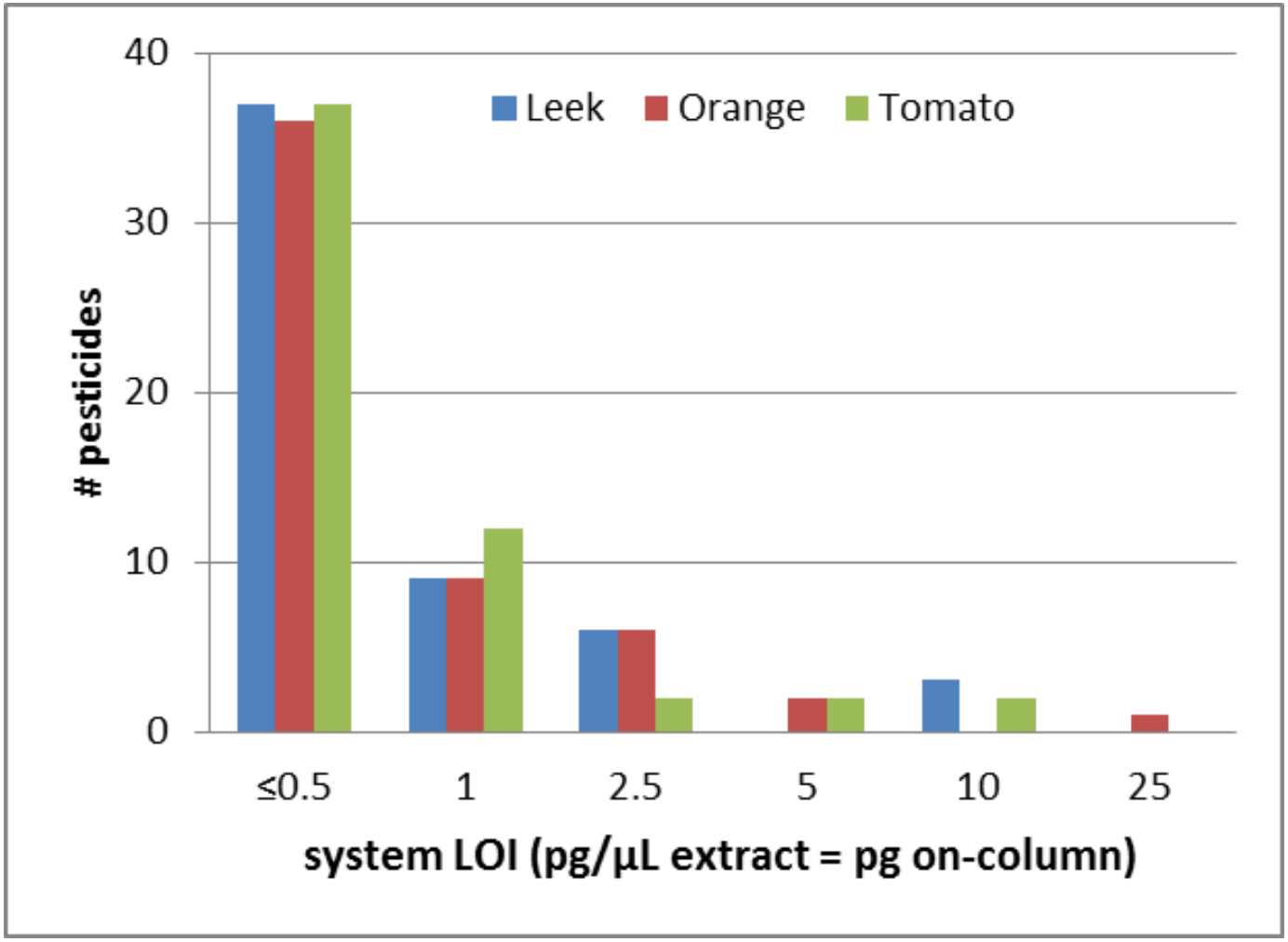
NIST



Data courtesy of Dr Hans Mol, Rikilt Wageningen, UR

ThermoFisher SCIENTIFIC

Exactive GC-MS System Sensitivity: Limit of Identification



QuEChERS - 1µL

Data courtesy of Dr Hans Mol, Rikilt Wageningen, UR

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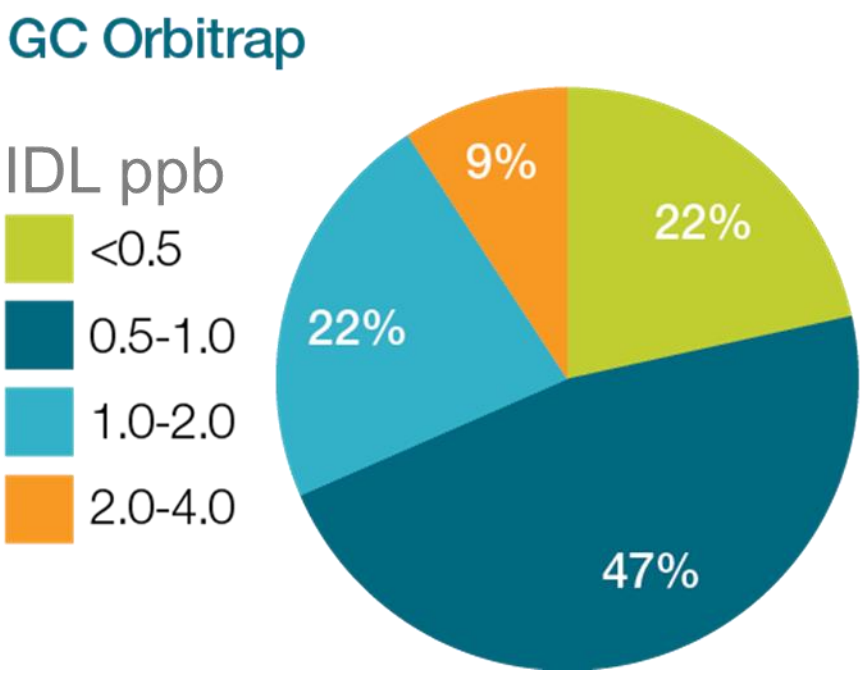
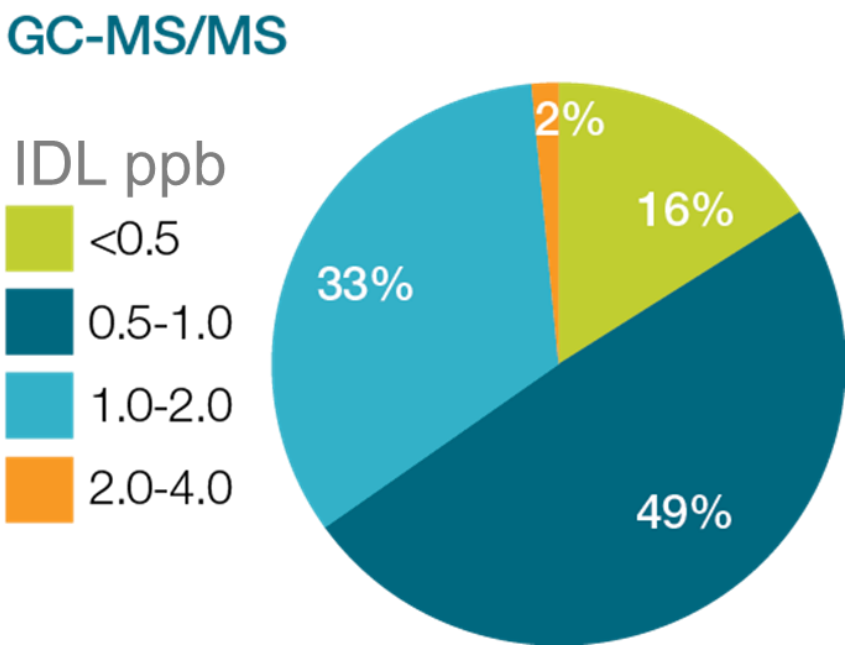
High Sensitivity Full-Scan

150 compounds in mixed vegetable matrix

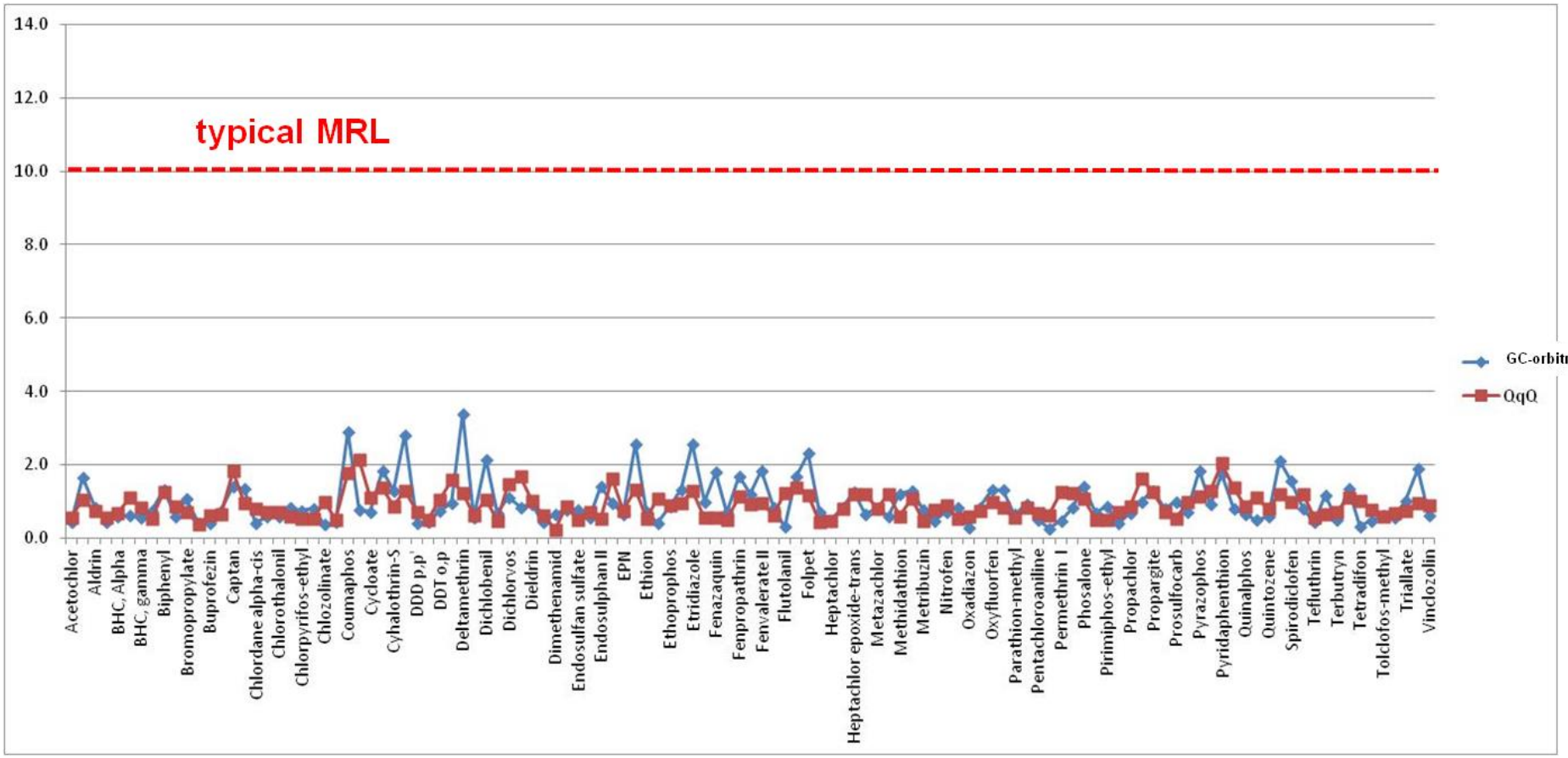




Triple-quadrupole-level sensitivity possible with a non-target acquisition

*Acquired on the Q Exactive GC system – the Exactive GC system provides equivalent performance.

Sufficient Sensitivity? Q Exactive GC-MS System vs GC- QqQ



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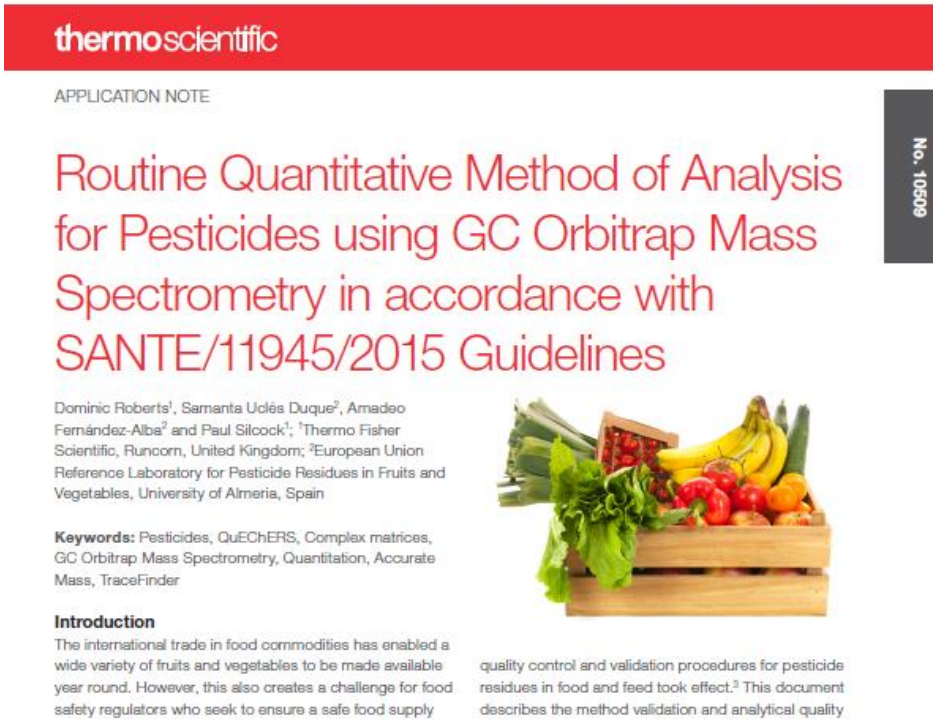
Thermo Scientific Application Note 10609



thermoscientific

APPLICATION NOTE

No. 10509

Routine Quantitative Method of Analysis for Pesticides using GC Orbitrap Mass Spectrometry in accordance with SANTE/11945/2015 Guidelines

Dominic Roberts[1], Samanta Uclés Duque[2], Amadeo Fernández-Alba[2] and Paul Silcock[1]; [1]Thermo Fisher Scientific, Runcorn, United Kingdom; [2]European Union Reference Laboratory for Pesticide Residues in Fruits and Vegetables, University of Almeria, Spain

Keywords: Pesticides, QuEChERS, Complex matrices, GC Orbitrap Mass Spectrometry, Quantitation, Accurate Mass, TraceFinder

Introduction

The international trade in food commodities has enabled a wide variety of fruits and vegetables to be made available year round. However, this also creates a challenge for food safety regulators who seek to ensure a safe food supply

quality control and validation procedures for pesticide residues in food and feed took effect.[3] This document describes the method validation and analytical quality

Identification Point	Tolerance	Primary ID	Optional additional ID
Retention time	0.1 minutes	✓	
Quant ion	5 ppm	✓	
Identification ion	5 ppm	✓	
Ion ratio	< 30%	✓	
Additional ions	5 ppm		✓
Isotopic pattern match	> 70%		✓
NIST spectral match	> 600		✓

51 pesticides were prepared in tomato, leek and orange at concentrations equivalent to 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 500 µg/Kg.

ThermoFisher SCIENTIFIC

Exactive GC-MS System: Automated Qualitative Screening Workflows

- Quantitative method with all associated AQC for usual suspects (~100)
- Automated qualitative screening: lower AQC burden for unexpected pesticides
- Same raw data files, but 2 different data processing approaches

1. Library: match of EI-spectra

deconvolution of HR spectra
⇒ cleaned spectrum
Library search (NIST, PEST library)

Software: Report if match (SI) > user threshold.
(The analysts manually review hits)

2. Database: RT + 2 exact masses

RT ± 0.5 min
XICs ± 5 ppm
2 ions (w/o ratio criterion)

Software:
Report if signal is found for both ions
(The analysts manually review hits)

10 µg/kg	found out of 50
Tomato	47
Orange	44
Leek	43

Data courtesy of Dr Hans Mol, Rikilt Wageningen, UR

Q Exactive GC-MS System: Non-targeted Data Processing

TRACE FINDER 4.0 SOFTWARE (Qual-Quant) PERFORMANCE

Stage	Time (aprox.)
Create batch	30 sec
Copy raw files	1 min 30 sec
Proccess files	2 min 30 sec
Manual revision	**60 min**
Data export	30 sec
Total time	**65 min**

- Time spent to evaluate:
  - 18 samples
  - 2 calibration curves by matrix matched standard (3 levels)
  - 2 recovery checks , 5 pure solvent

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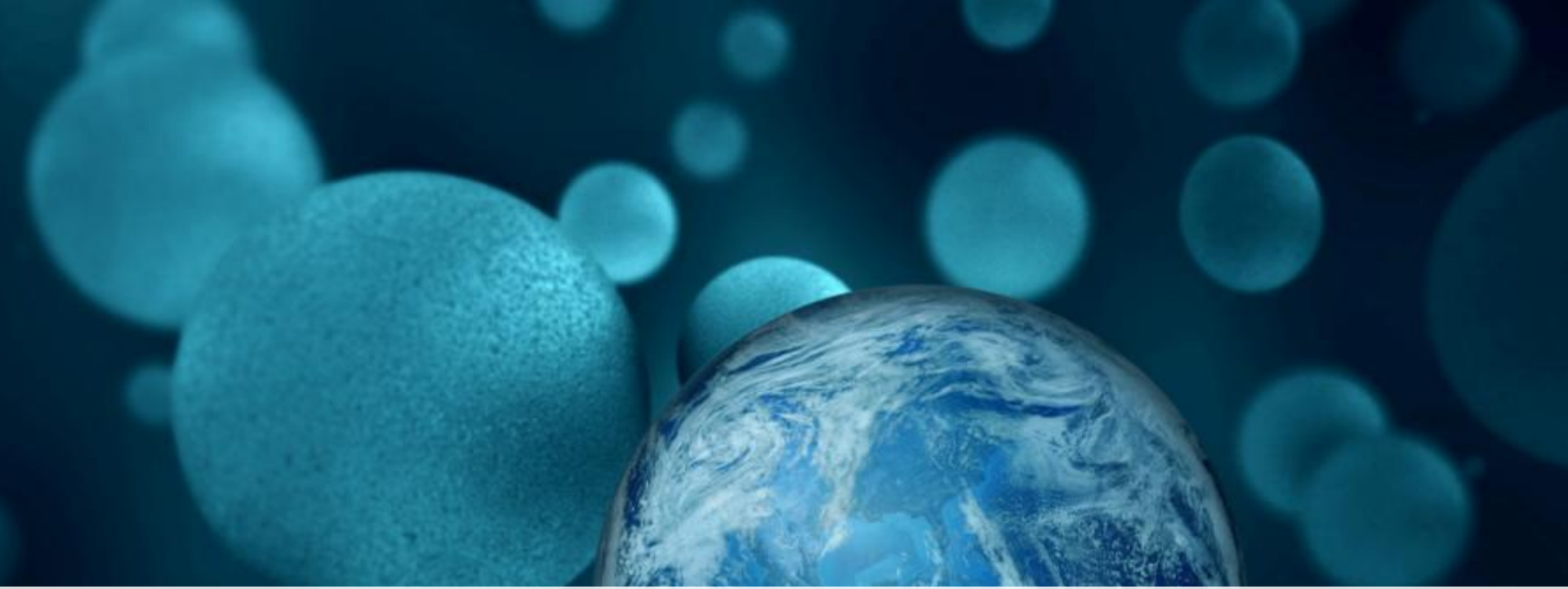

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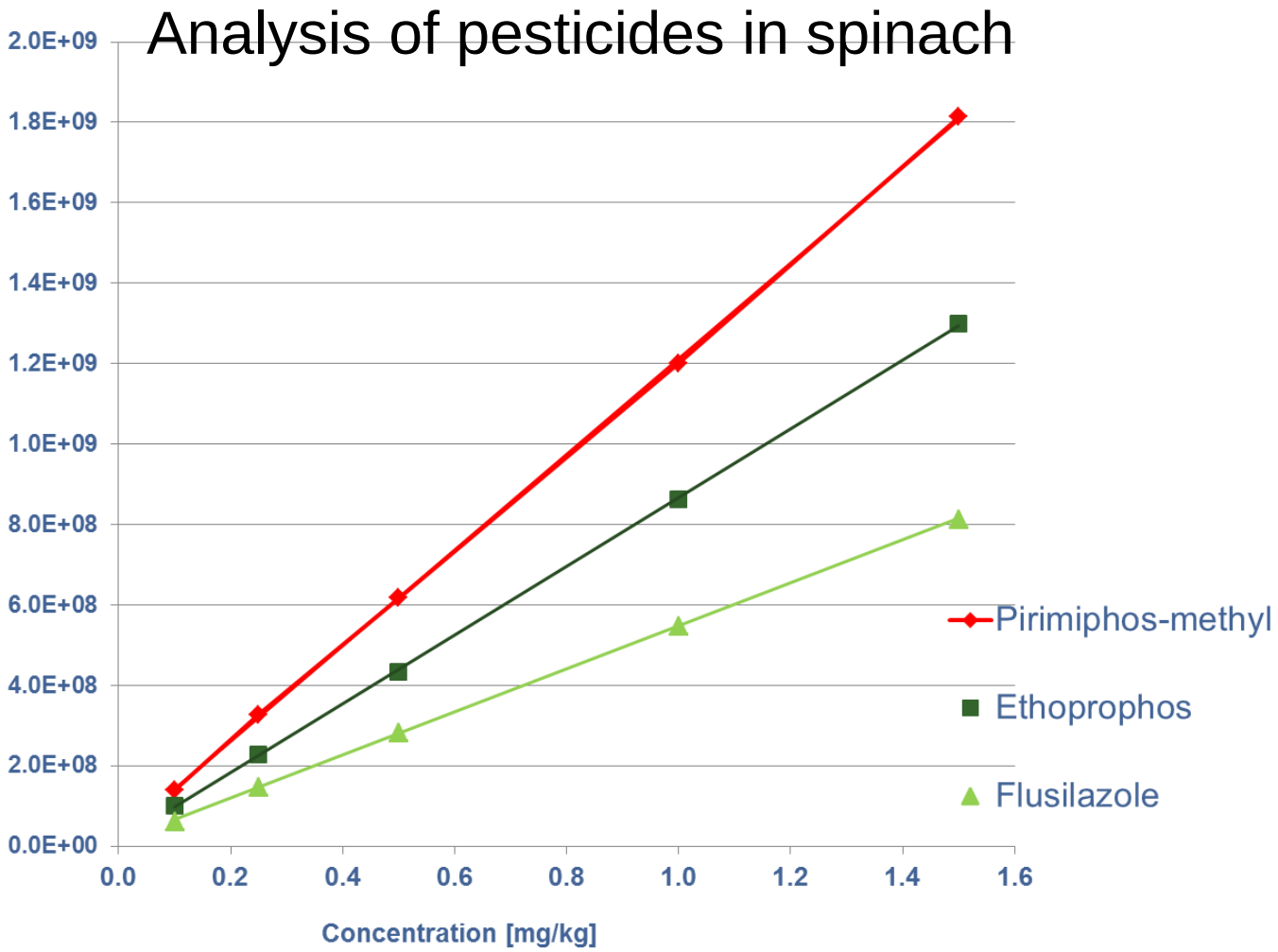
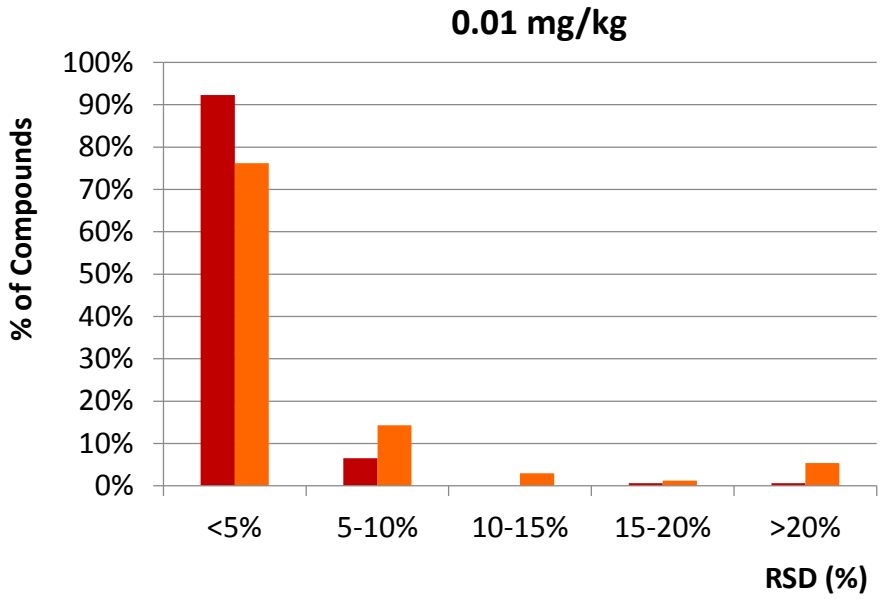
Thermo Scientific Q Exactive Focus MS System

Linearity: Q Exactive Focus MS System

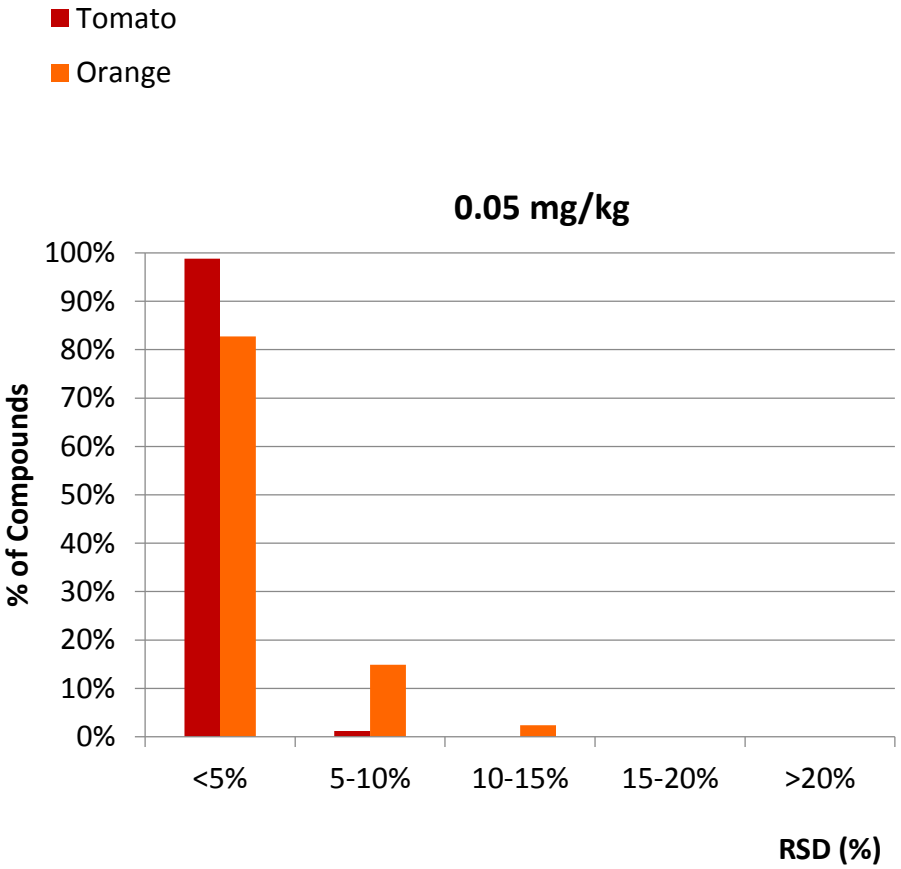


Data from Prof. Amadeo Rodríguez Fernández-Alba, University of Almeria, Spain ,

Validation: Q Exactive Focus MS System



Repeatability of Residue Concentrations



Courtesy Prof. Amadeo Rodríguez Fernández-Alba, University of Almeria, Spain

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ThermoFisher SCIENTIFIC

Quantification:LC - Q Exactive Focus MS vs LC - QqQ

		Concentration (mg/kg)		
Matrix	**Compound**	**LC-QqQ-MS/MS**	**LC-Q-Orbitrap-MS/MS**	**Diff (%)**
Conferencia Pear1	Boscalid	0.168	0.154	8
	Difenoconazole	0.027	0.029	-7
	Diflubenzuron	0.014	0.017	-21
	Imazalil	1.711	1.334	22
	Imidacloprid	0.047	0.052	-11
	Pyraclostrobin	0.114	0.096	16
	Tebuconazole	0.048	0.048	0
	Thiacloprid	0.037	0.036	3
	Trifloxystrobin	0.024	0.021	13
Conferencia Pear2	Boscalid	0.175	0.177	-1
	Difenoconazole	0.012	0.013	-8
	Fluopyram	0.027	0.026	4
	Imazalil	1.359	1.093	20
	Imidacloprid	0.053	0.061	-15
	Pyraclostrobin	0.067	0.063	6
	Tebuconazole	0.044	0.047	-7
Red Pepper	Flutriafol	0.019	0.013	32
Red Chili Pepper	Flutriafol	0.397	0.368	7
	Pirimicarb	0.034	0.034	0
Banana	Imazalil	0.915	0.788	14

Courtesy Prof. Amadeo Rodríguez Fernández-Alba, University of Almeria, Spain ,

The AQC Burden-LC-MS/MS

- 464 pesticides / 516 matrices /33,500 samples
- 239 pesticides in 0 samples
- 84 pesticides in < 10 samples
- Monitor 166 pesticides only with rigorous AQC

FS/dd-MS²/AIF-MS²

Q Exactive Focus MS System

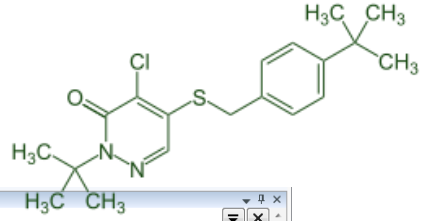
Courtesy Prof. Amadeo Rodríguez Fernández-Alba, University of Almeria, Spain ,

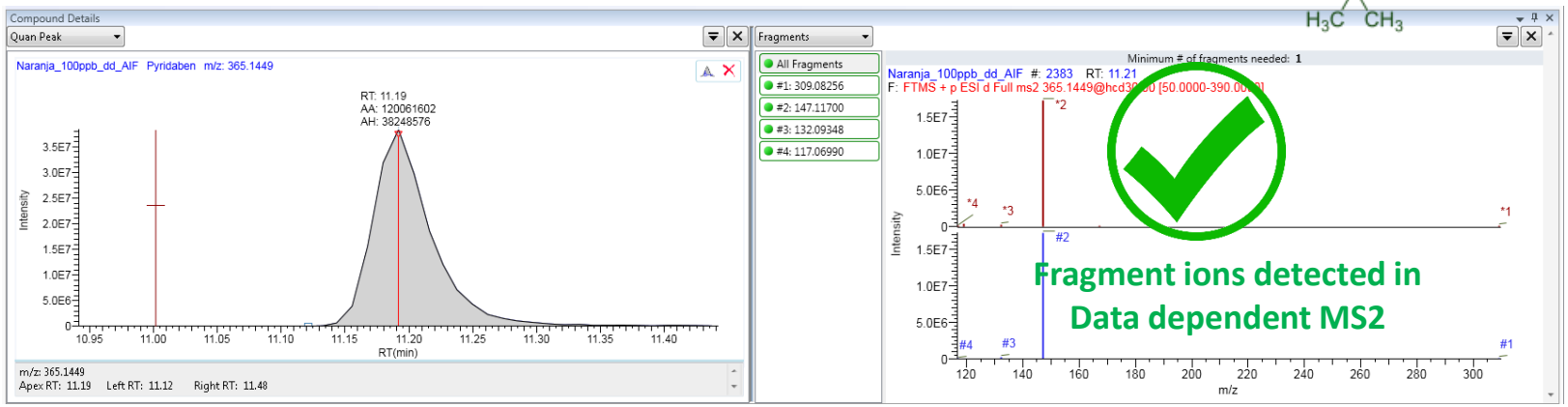
FS/dd-MS2/AIF-MS2

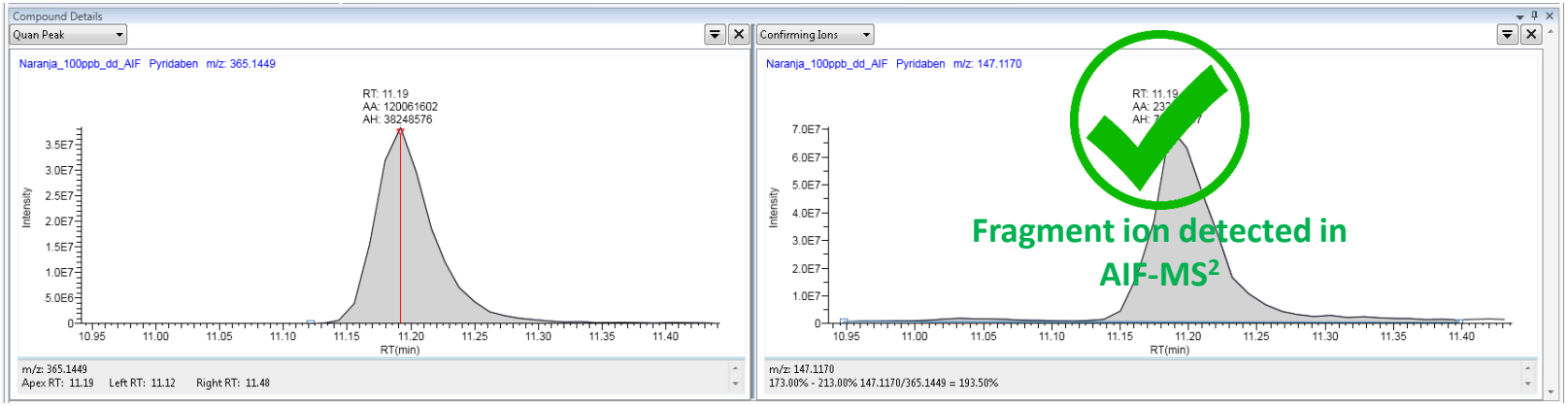
Pyridaben (target compound)
0.1 mg/kg in orange

MS

MS^2





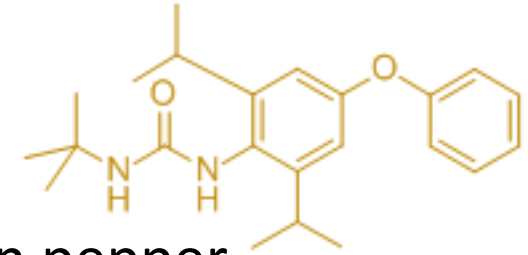


Courtesy Prof. Amadeo Rodríguez Fernández-Alba, University of Almeria, Spain ,

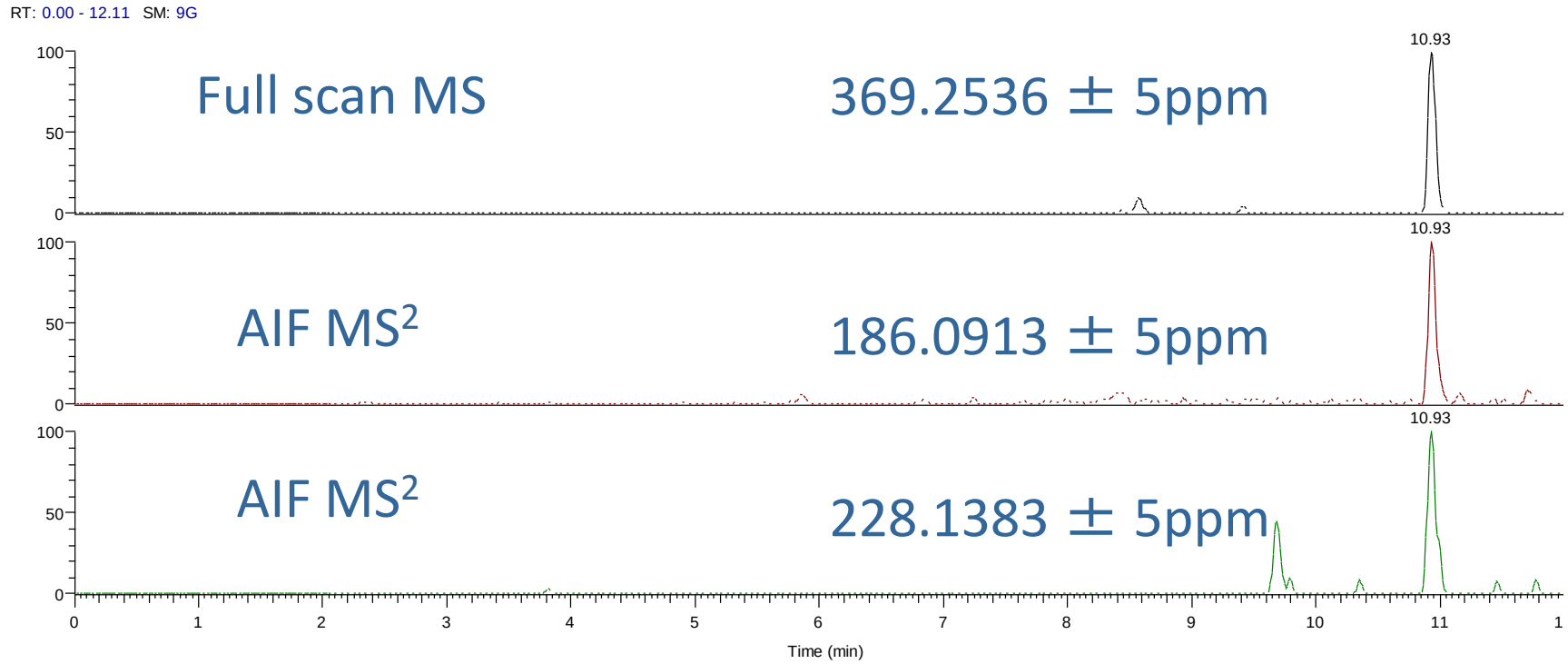
ThermoFisher SCIENTIFIC

Analysis of Real Samples: Diafenthiuron Metabolite in Pepper

DETECTION OF NON-TARGET PESTICIDES



Diafenthiuron urea (metabolite of diafenthiuron) in pepper
mass of protonated molecule = *m/z* 369.2536



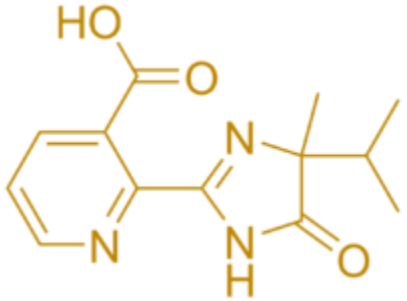
Courtesy Prof. Amadeo Rodríguez Fernández-Alba, University of Almeria, Spain ,

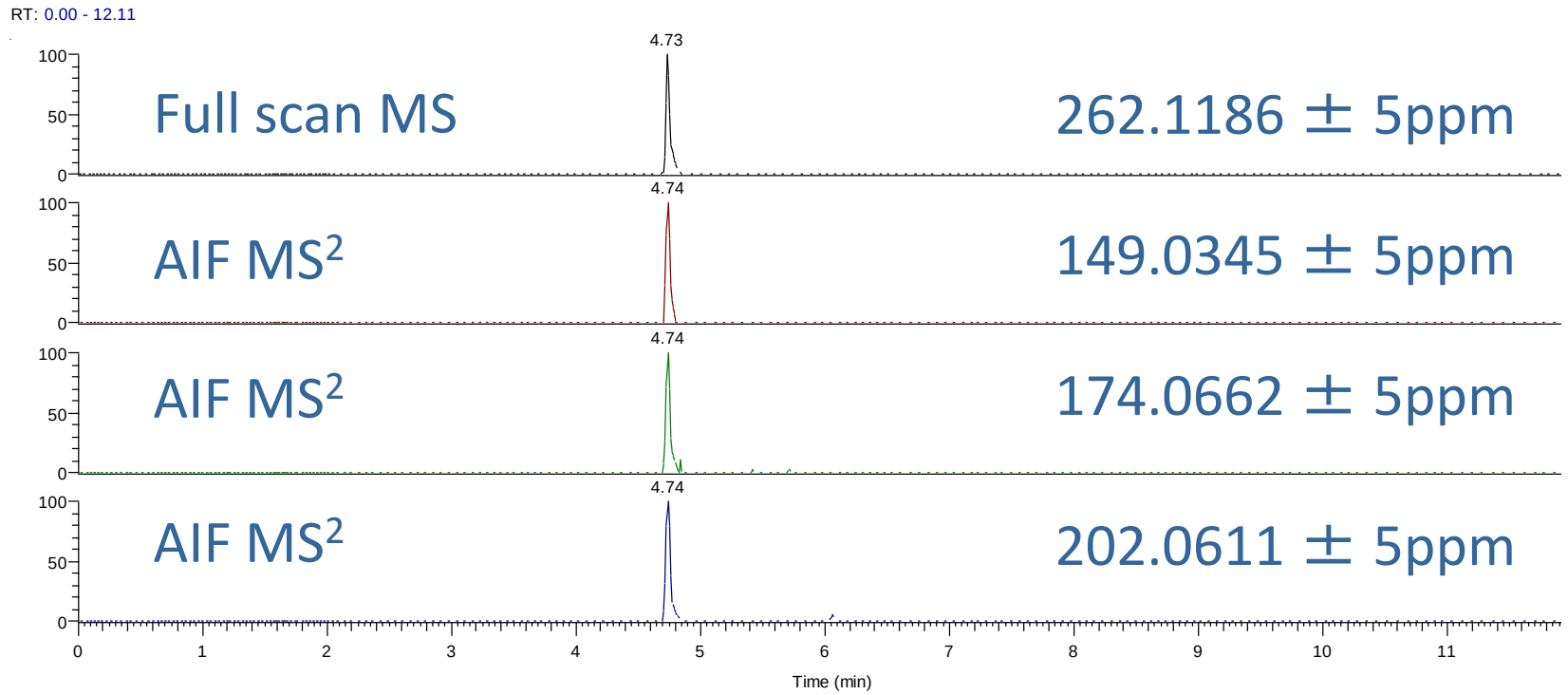
ThermoFisher SCIENTIFIC

Analysis of Real Samples: Imazapyr in Pears

DETECTION OF NON-TARGET PESTICIDES

- not included in EU Monitoring Program
- protonated molecule = *m/z* 262.1186





Courtesy Prof. Amadeo Rodríguez Fernández-Alba, University of Almeria, Spain ,

Summary (Orbitrap MS)

- 1. **Sensitivity / Selectivity** – ppt detection / > 60/70K Resolution
- 2. **Precision** and **Accuracy** (better than QQQ especially for difficult samples)
- 3. **Stability** – Mass accuracy for days without re-calibration
- 4. **>Scope** –targeted and non targeted workflows in one run
- 5. Analysis of **different classes of analytes-** subject to extraction
- 6. **User Friendly** – easier to learn than QqQ
- 7. **Results compliant with SANTE guidelines**
- 8. All of the above is assurance on **Productivity**

I hope that you are some of you are least better informed to be able to make the decision on which workflow is best for you

Thank You For Your Attention – Stay Connected with Us

Please come to our stand if you have further comments and questions.

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