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New Frontiers In Pesticide Residue Analysis

Michal Godula, Richard Fussell, and Inge de Dobbeleer
Thermo Fisher Scientific

Today's Topics

epa
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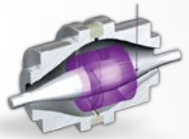
Screening and quantitation analysis by GC and LC Orbitrap



General Extraction Procedure

10 g of sample is weighed into Quechers extraction tube

+ 20 mL of water

+ 10 mL of ACN

shaking 10 min

Centrifugation 5 min @ 5000 rpm

Injection to GC-MS (after clean-up)
or LC-MS (clean-up optional)

Further details

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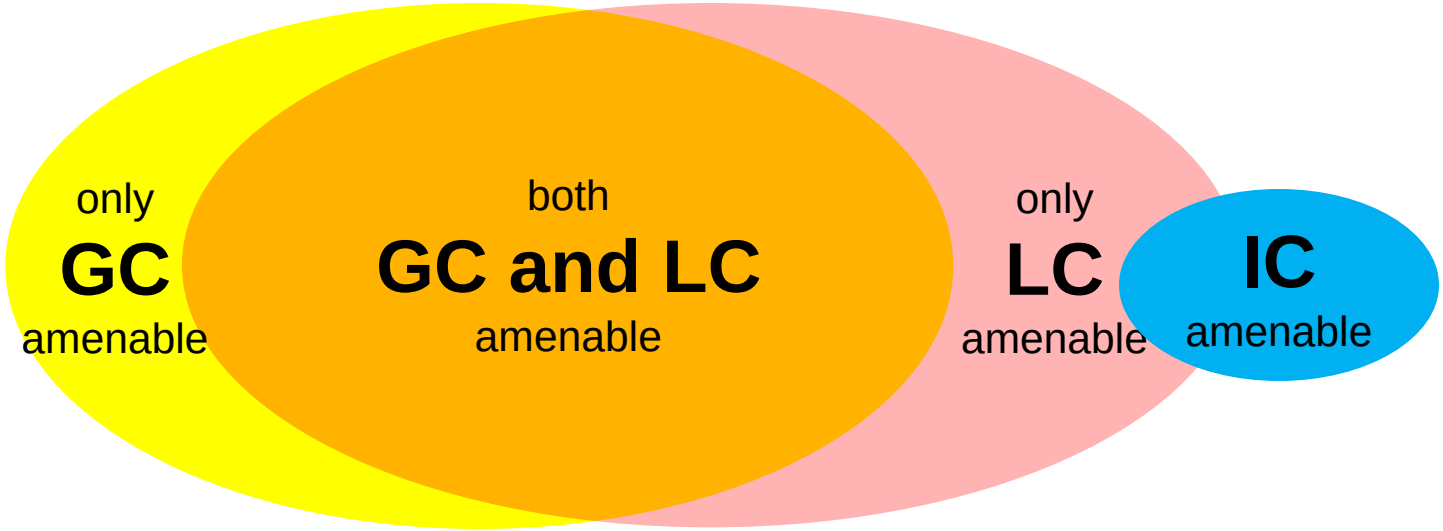
Current Routine Practice

- Chromatography with mass spectrometric detection



Current golden standard:
Targeted quantitative measurement by LC-MS/MS and GC-MS/MS
Multi-residue methods, ~150–250 analytes/method

Emerging:
Non-targeted measurement by LC and GC + full scan MS
for better coverage of pesticide scope and easier measurement

IC for easier separation of polar substances

MS Work Flow Options For The Analysis Of Pesticide Residues

QqQ targeted MS/MS analysis

HRAM non-targeted acquisition targeted +/or non-targeted analysis

- Quantification & identification
- Calibration and recovery spikes for all pesticides in the targeted acquisition method.
- **False negatives** do occur due to limited scope
- Occasionally **False positives** reported due to selectivity

Unknown analysis

- Peaks detected and chemical structure proposed on the basis of fragmentation data
- Synthesize pure compound and verify by further analysis

- Calibrate, identify and quantitate for expected residues (plus system check compounds)
- Increased 'scope' with less AQC
- Non target acquisition, targeted data processing against a user library or database with retention time (and/or against a database containing more compounds without retention time)
- Report identified residues only – require 2nd quantitative analysis
- Report residues screened, not found – must validate method first
- Detection of compounds without standards (e.g. transformation products)
- Retrospective analysis

High Resolution/Accurate (HRAM) Mass Mass Spectrometry

TSQ 8000 Evo GC-MS/MS Pesticide Analyzer

- Preconfigured performance leading Thermo Scientific™ TSQ™ 8000 Evo GC-MS/MS system featuring the award winning Thermo Scientific™ TRACE™ 1310 GC
- QuEChERS kit for sample preparation
- Pre-loaded acquisition methods
- 1000+ Pesticide compound database
- Thermo Scientific™ TraceGOLD GC column and consumable technology
- Thermo Scientific ™ TraceFinder™ EFS data processing Software
- AutoSRM & timed SRM (t-SRM)
- Pesticide Analyzer installation guide

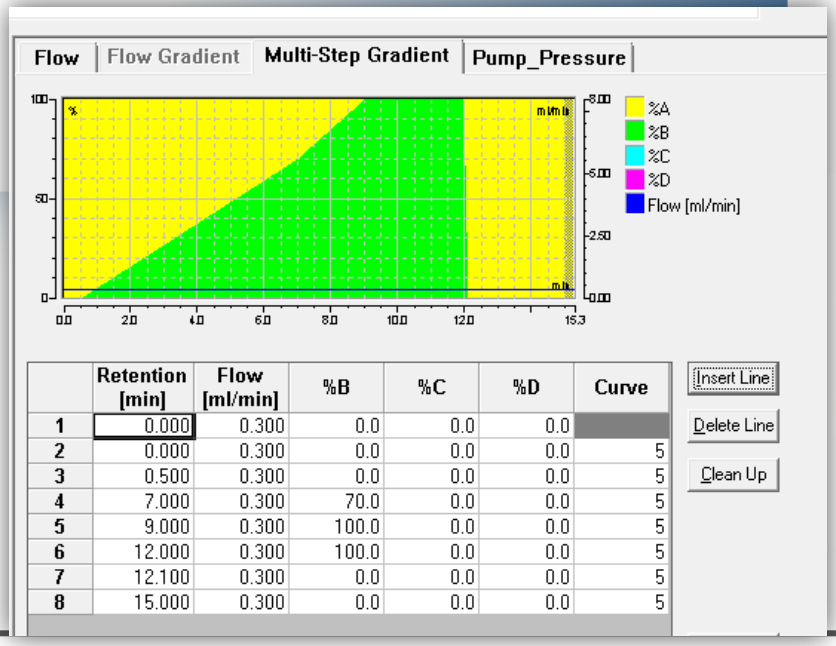
Thermo Scientific™ Endura™ LC-MS/MS Pesticide Explorer

- Thermo Scientific™ Pesticide Explorer™ package
- Package includes methods and SRM library
- 276 compounds completely validated, possible extension with SRMs available
- TraceFinder EFS data processing software
- Installation guide: get up and running fast
- Complete method support

Tested UHPLC Method

Thermo Scientific™ UltiMate™ 3000 RSLC system:

- Mobile phase:
  - A: Water:MeOH (98:2) + 5mM Ammonium formiate & 0.1% FA
  - B: MeOH:Water (98:2) + 5mM Ammonium formiate & 0.1% FA
- Injection volume: **1 µl**
- Column: **Thermo Scientifi™ Accucore™ aQ 100 mm x 2.1 mm x 2.6 µm**
- Column temperature: 25 °C
- Flow rate: 300 µl/min
- Run time: 15 min
- Gradient:



	Retention [min]	Flow [ml/min]	%B	%C	%D	Curve
1	0.000	0.300	0.0	0.0	0.0	
2	0.000	0.300	0.0	0.0	0.0	5
3	0.500	0.300	0.0	0.0	0.0	5
4	7.000	0.300	70.0	0.0	0.0	5
5	9.000	0.300	100.0	0.0	0.0	5
6	12.000	0.300	100.0	0.0	0.0	5
7	12.100	0.300	0.0	0.0	0.0	5
8	15.000	0.300	0.0	0.0	0.0	5

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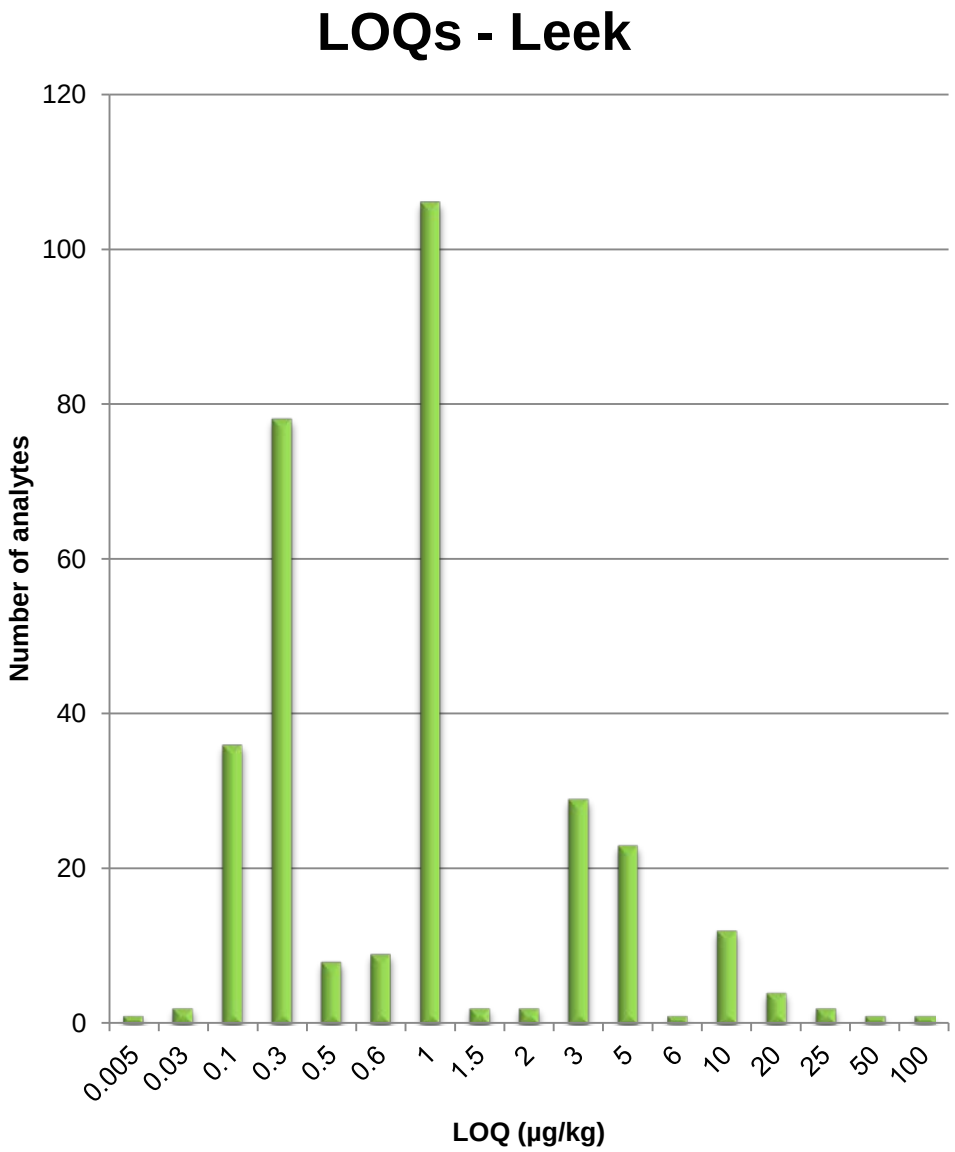
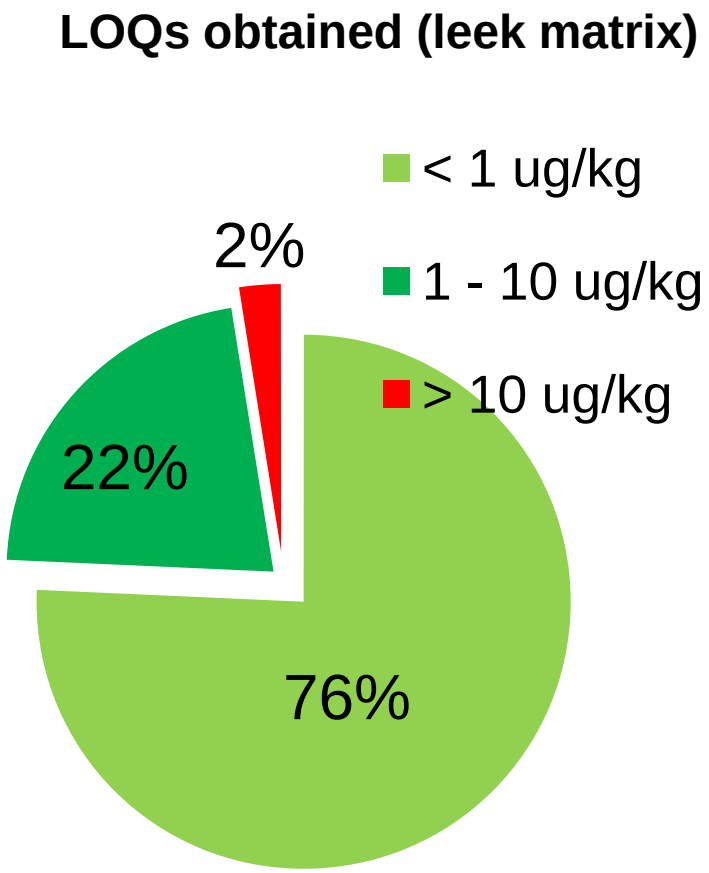
LC-MS/MS Method LOQs- Leek





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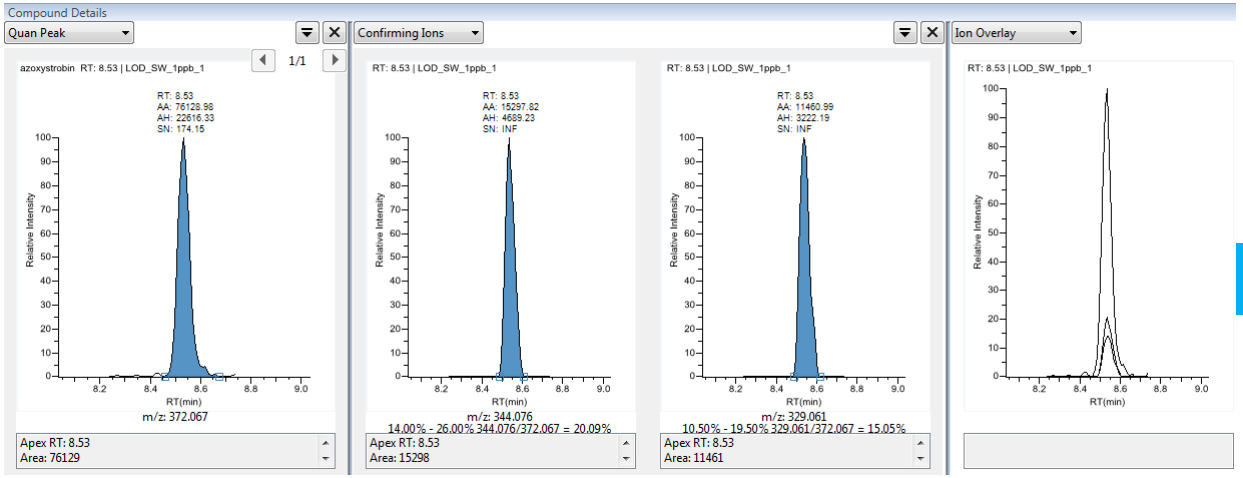
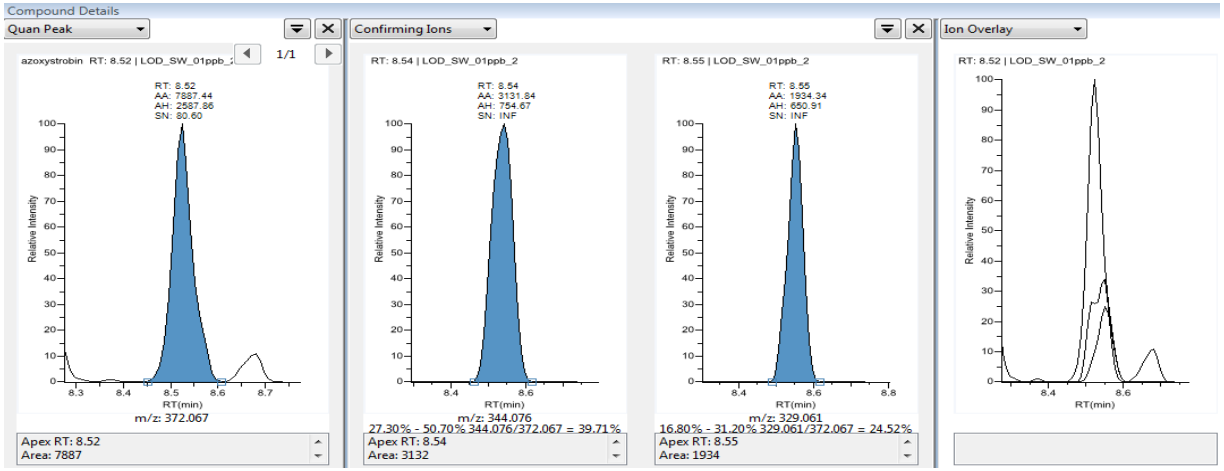
LC-MS/MS Method - Response For Azoxystrobin In Strawberry



1 µg/kg

LOD = 0.003 µg/kg
LOQ = 0.01 µg/kg



0.1 µg/kg

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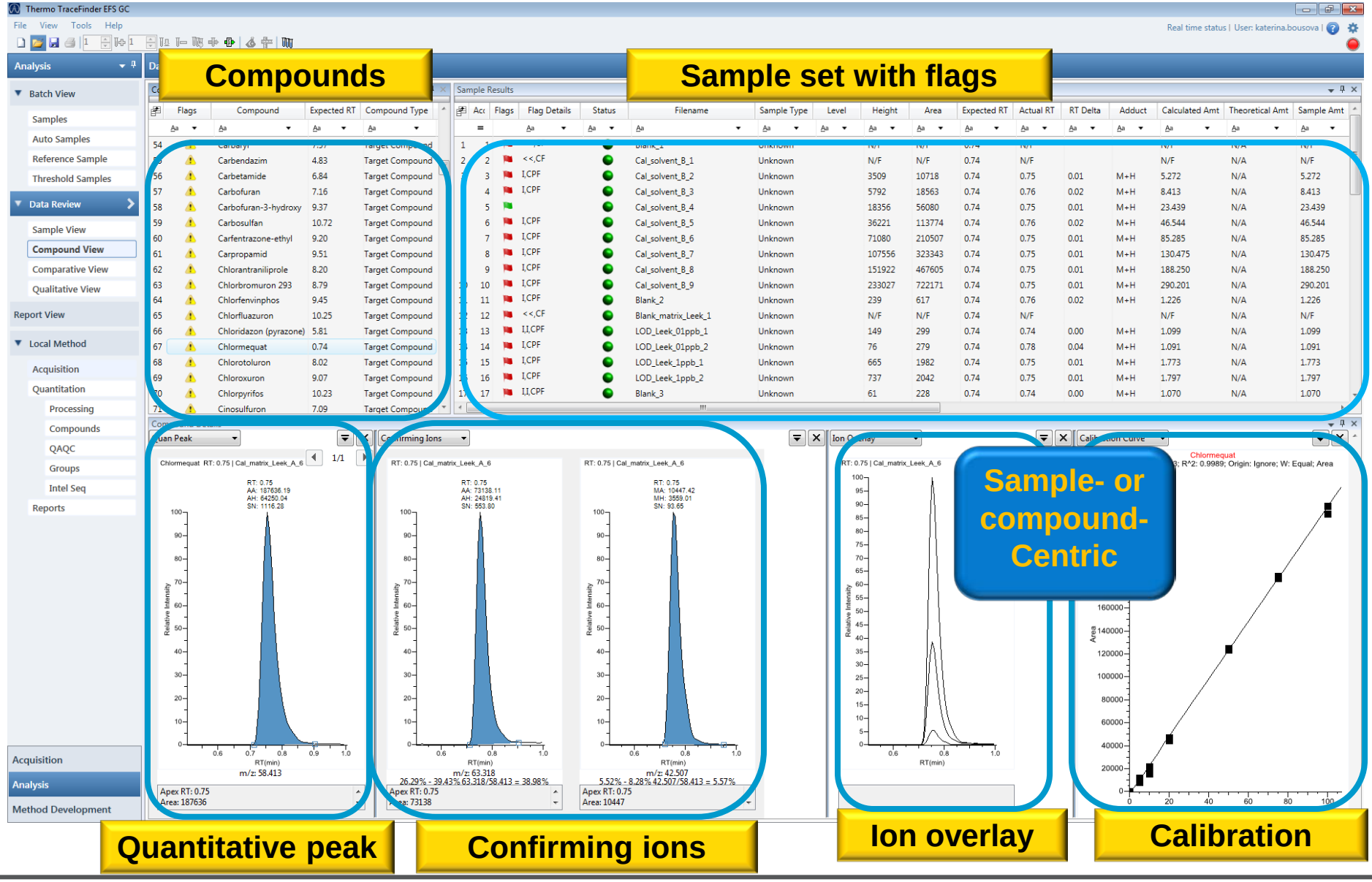
LC-MS/MS Method Accuracy

- Analysis of external certified reference material – FAPAS (3 different matrices)
- All results were in the acceptance range

Analyte	Fapas No.	Fapas matrix	Assigned value (µg/kg)	Acceptance range (µg/kg)	Measured value (µg/kg)	RSD (%)
Carbaryl	T19142	Melon puree	89.0	49.9-128.2	91.1	1.1
Diniconazol			52.3	29.3-75.3	59.7	9.0
Zoxamide			91.7	51.4-132.1	108.4	3.0
Pencycurone	T19140	Lettuce puree	73.2	41.0-105.4	45.9	6.0
Thiamethoxam			48.8	27.3-70.3	36.3	9.1
Azoxystrobin	19110	Lettuce puree	188.0	110-265	132.5	15.4
Dimetomorph (sum of isomers)			181.0	106-256	160.1	11.9
Propyzamide			197.0	116-277	195.1	16.5
Azoxystrobin	T0983	Wheat flour	383.0	241-524	361.2	1.7
Fenhexamid			110.0	61-158	125.4	10.4
Imazalil			161.0	93-229	157.2	8.2
Thiabendazole			88.0	49.3-126.7	67.6	7.3

Easy Data Review: TraceFinder



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Application Notes

Click here to download Method 63899

Method 63899

Validation of the Method for Determination of Pesticide Residues by Gas Chromatography – Triple-Stage Quadrupole Mass Spectrometry

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Thermo Fisher Scientific, Food Safety Response Center, Dreieich, Germany

Key Words
TraceFinder, TSQ, C
QuEChERS, Triple Q

1

Application Note 643

Fast Screening and Quantification of Pesticide Residues Using a Comprehensive LC-MS Solution: The Pesticide Explorer Collection – Standard Quantitation

Katerina Bousova[1], Ebru Sarikaya[1], Michal Godula[1], Claudia Martins[2], and Ed George[2]
[1]Thermo Fisher Scientific, Special Solution Center Europe, Dreieich, Germany
[2]Thermo Fisher Scientific, San Jose, California

Click here to download Application Note 643

Key Words
Pesticide Explorer Collection, European Regulation 396/2005, Commission Directive 2006/125/EC, European Commission 2002/657/EC, SANCO/12571/2013, European Commission 788/2012/EC, pesticide, food, QuEChERS, UltiMate 3000, TSQ Endura, TraceFinder

1

Experimental

Overview

The workflow overview from sample preparation through LC-MS/MS analysis is shown in Figure 1. Samples were homogenized and extracted according to the European EN 15662 QuEChERS protocol prior to injection into the LC-MS/MS system.[1,2] The ready-to-use QuEChERS

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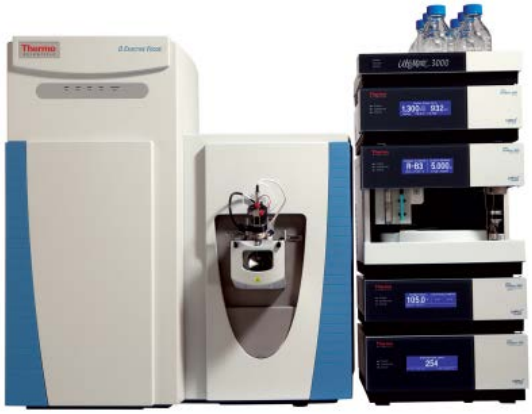
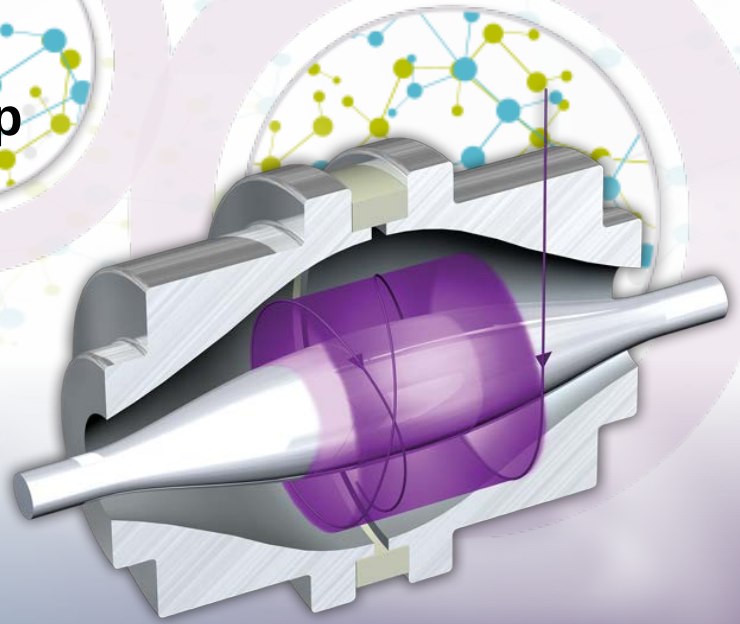
Complete Solution For Total Pesticide Analysis



Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap LC-MS/MS system

Q Exactive GC hybrid quadrupole-Orbitrap GC-MS/MS system



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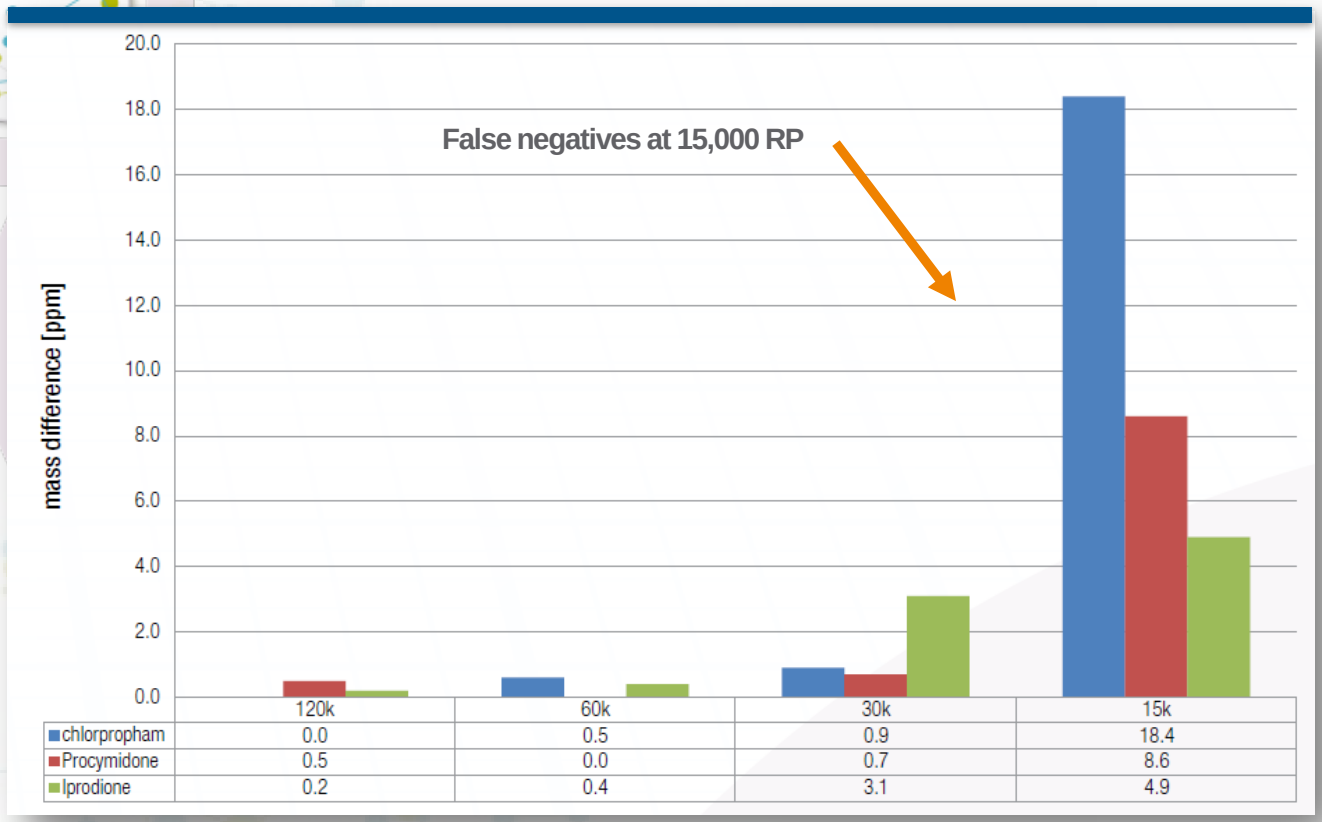
Q Exactive **GC System**

Source options:	EI and CI (NCI/PCI)
Scan range	*m/z* 30-3000
Resolving power:	4 settings, up to 120,000 FWHM @ *m/z* 200
Acquisition options:	*Non-target acquisition:* full scan
	Targeted acquisition: SIM, MS/MS, data-dependent MS/MS
	Combinations of the above...

Why Do We Need High Resolution In Pesticide Analysis?

High resolving power crucial to avoid false negatives



	120k	60k	30k	15k
chlorpropham	0.0	0.5	0.9	18.4
Procymidone	0.5	0.0	0.7	8.6
Iprodione	0.2	0.4	3.1	4.9

Secure screening

- 5 ppm identification threshold
- Higher resolving power allows confident detection and identification
- Mid- resolution of 15k causes false negatives
- Q Exactive GC routinely operates at 60k (FWHM @ m/z 200)

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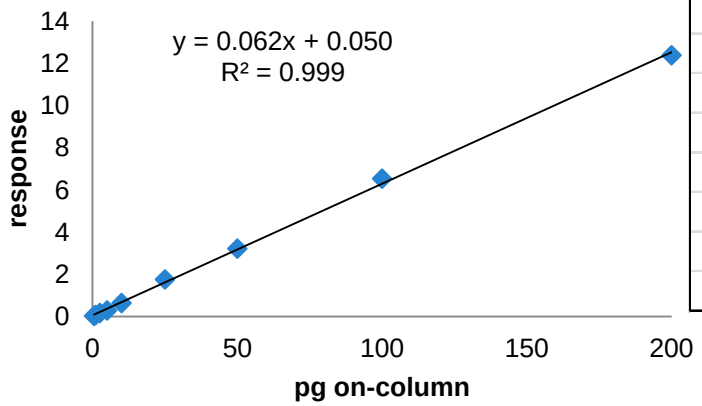
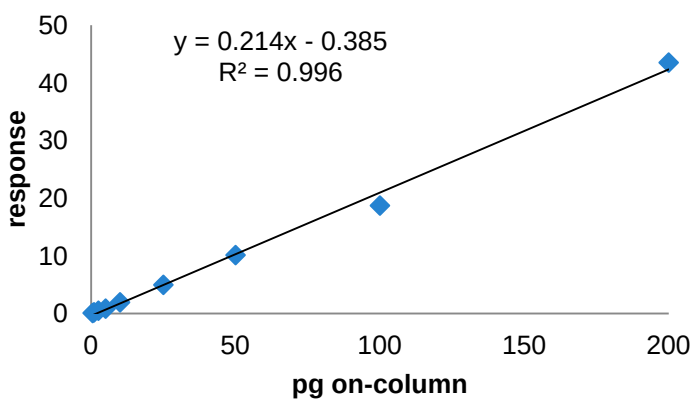
Linear Performance Of The Q Exactive GC

Bupirimate in tomato



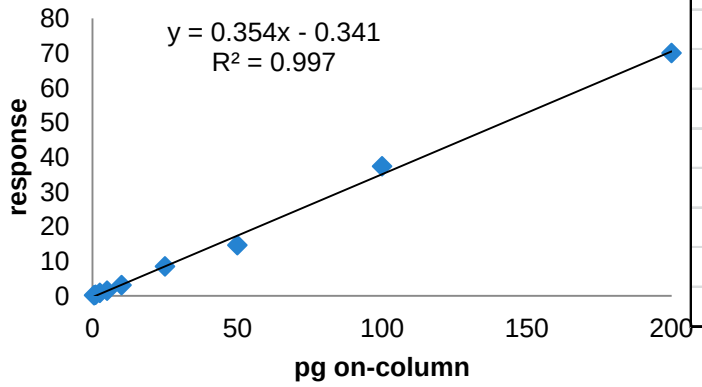
pg	deviation
0.5	-28%
1	0%
2.5	5%
5	-8%
10	3%
25	15%
50	5%
100	7%
200	1%

Diazinon in maize



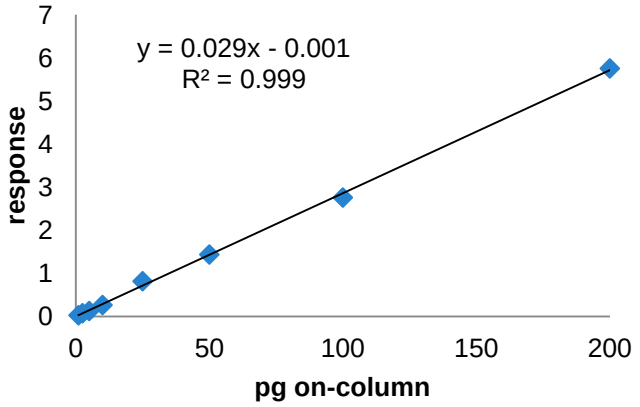
pg	deviation
1	-14%
2.5	4%
5	-11%
10	-5%
25	18%
50	4%
100	0%
200	4%

Tetramethrin in leek



pg	deviation
0.5	9%
1	-7%
2.5	5%
5	-10%
10	-6%
25	3%
50	-12%
100	13%
200	6%

α-endosulfan in tomato



pg	deviation
0.5	-34%
1	-4%
2.5	-1%
5	-12%
10	3%
25	6%
50	8%
100	0%
200	17%

Data courtesy of Dr Hans Mol, Rikilt Wageningen, UR

⇒ Adequate quantitative performance for majority of the pesticides tested

Why Electron Ionisation?

Most generic ionisation technique

Well established

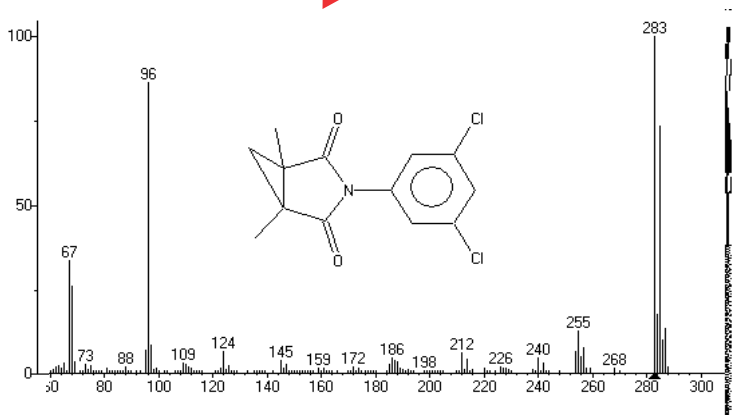
Hard ionisation => ionsation + fragmentation

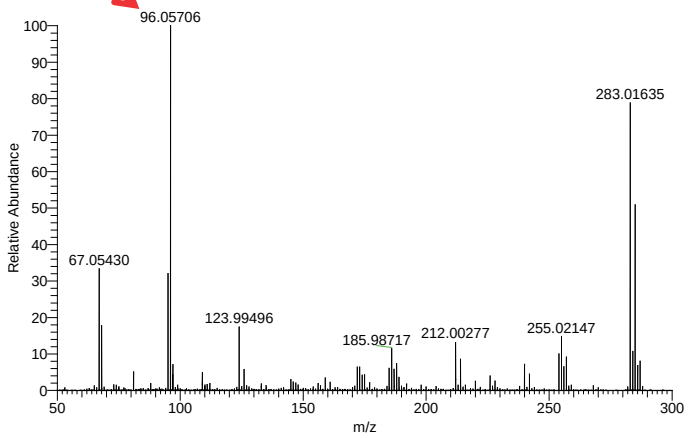
EI-MS spectra to a large extent instrument-independent

EI-MS libraries available (>242.000 spectra, mostly EI-quadrupole-MS)

Dedicated EI-MS libraries (pesticides, drugs..)

Existing libraries nominal m/z, development HR-libraries in progress





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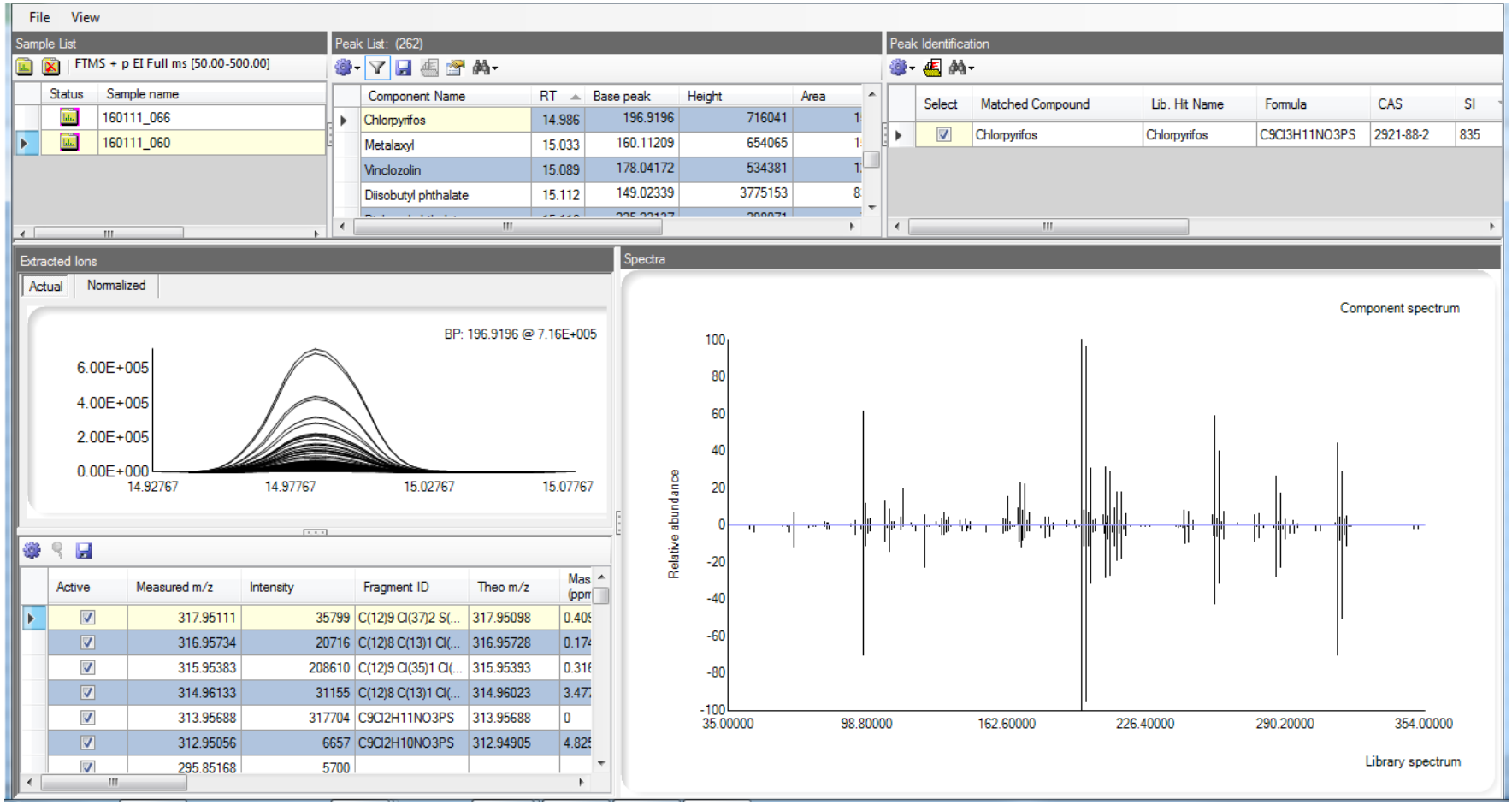
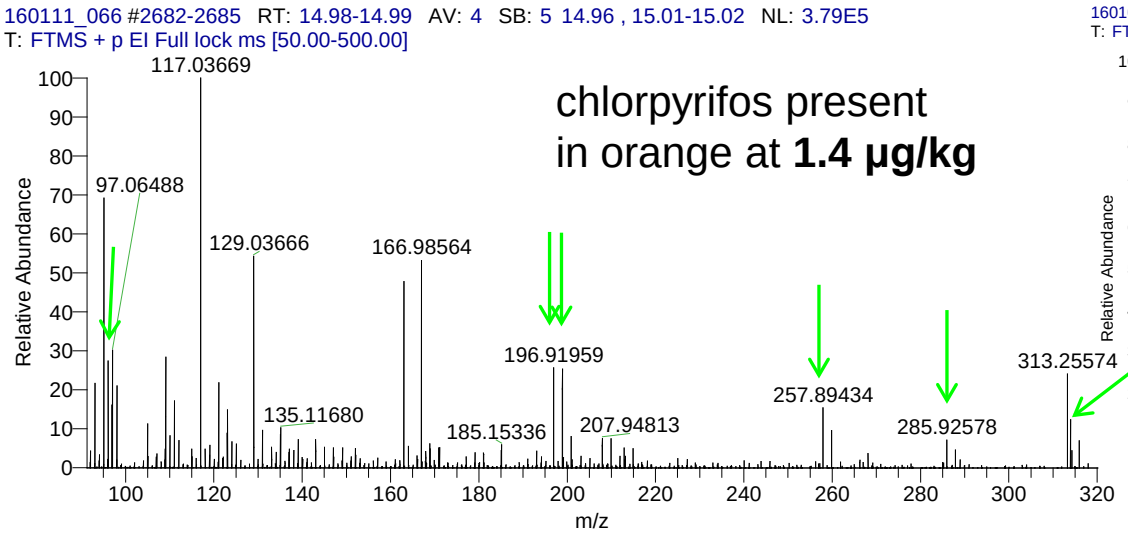
Qualitative Screening: Approach 1

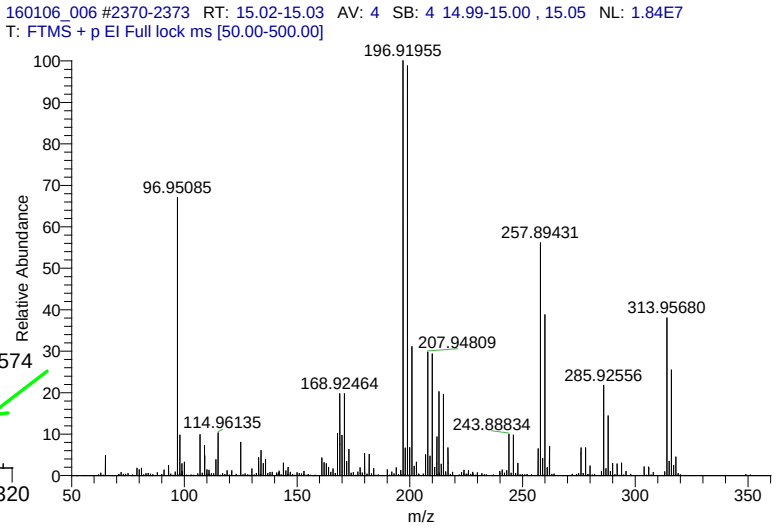
Orange spiked @ 10 µg/kg: **73% automatically found**

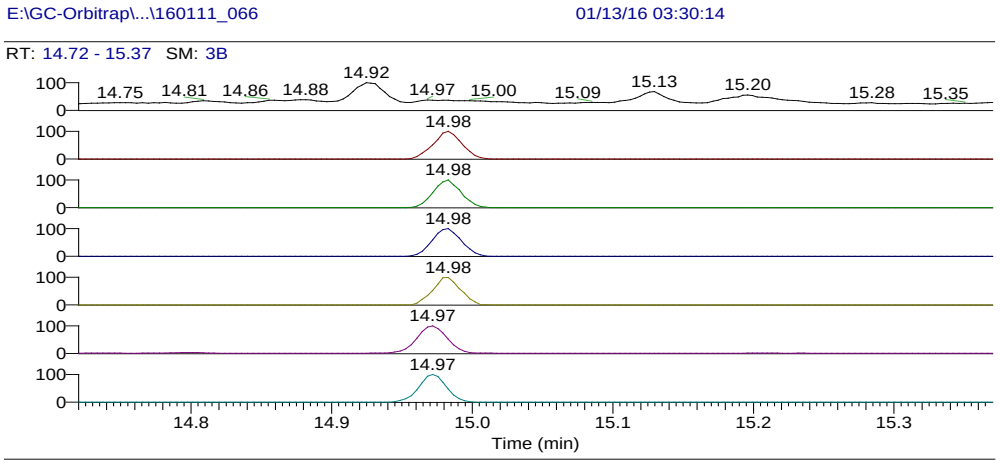


Data courtesy of Dr Hans Mol, Rikilt Wageningen, UR

Qualitative Screening: Approach 2







Data courtesy of Dr Hans Mol, Rikilt Wageningen, UR

Not found by library search but easily found using screening library in HR-AM

To the left

XIC at 5ppm of

- 196.91965
- 198.9167
- 257.89428
- 313.95688
- 117.03669
- 313.25574

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Qualitative Screening: First Conclusions

On the two 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

Analyst: manual review hits

+ allows screening for analytes not (yet) included in dedicated HR/AM database

- low µg/kg levels in complex matrices can be challenging

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

Analyst: manual review hits

+ better screening sensitivity

- needs database with t_r / exact masses

Data courtesy of Dr Hans Mol, Rikilt Wageningen, UR

Conclusions On GC Orbitrap Technology

- Scan speed: sufficiently fast for routine GC analysis
- Spectra: consistent over wide concentration range, NIST searchable
- Resolving power: 30/60K ensures reliable high mass accuracy at lower levels in simple/complex matrices, enables use of MEW of ±5 ppm => high selectivity
- System LOD in sub pg - low pg range
- Quantitative performance fit-for-purpose
- Identification: meets SANTE criteria at ≤10 µg/kg for the pesticides tested
- Scope / Sensitivity / Selectivity = + / + / +
- Screening based on 2-specific ions more sensitive than library matching
- Development databases / deconvolution software on-going

Further Details In…

Application Note 10448

High Efficiency, Broad Scope Screening of Pesticides Using Gas Chromatography High Resolution Orbitrap Mass Spectrometry

Dominic Roberts,1 Hans Mol,2 Marc Tienstra,2 Cristian Cojocariu,1 and Paul Silcock1
1Thermo Fisher Scientific, Runcorn, UK
2RIKILT – Wageningen UR, Wageningen, The Netherlands

Keywords
Accurate Mass, Complex Matrices, GC Orbitrap Mass Spectrometry, Pesticide Analysis, QuEChERS, Screening, TraceFinder Software

Introduction

Pesticides are used globally to improve the production and yields of agricultural crops and their use is essential to ensure a sufficient global food supply. However, this widespread use of pesticides and the potential for them to remain in the final product is of significant concern to consumers and to governments whose responsibility it is to ensure a safe food supply. Consequently, legislation exists to protect consumers from exposure to contaminated foods. This legislation requires that foods are monitored for both the type and quantity of the pesticide present, with each pesticide given a maximum residue limit (MRL) in a particular sample commodity. The list of compound and sample combinations is extensive, creating a challenge for accurate and reliable routine monitoring.

Laboratories are under ever-increasing pressure to screen samples for pesticides in a single analysis, with a fast turnaround time and at a competitive cost. Most existing laboratories rely on targeted analytical approaches using both gas chromatography and liquid chromatography coupled to mass spectrometry instrumentation. These techniques cover the wide range of chemical classes that need to be monitored and at the required levels of sensitivity and selectivity. However, they are limited to only those compounds in the target list, which are usually selected based on the residue definition and legislation requirements to demonstrate that the food is fit for consumption. These techniques require careful optimization of acquisition parameters for each compound and the monitoring of acquisition time windows to ensure detection of the analyte.

To increase the scope of the analysis, chemical screening methods using high-resolution, full-scan mass spectrometry have received significant attention in recent years. These methods use non-targeted acquisition, in which a generic full scan acquisition is run, followed by targeted data processing of a list of compounds within a database.

Although data interrogation is performed against a list of target compounds, retrospective data analysis is possible in order to identify new compounds that were not screened for at the time of acquisition. For this approach to be used in routine analysis, screening data processing software needs to be fast and accurate enough to detect residues at low concentrations with an acceptably low level of false negative results, as described in the European Union guidelines.[1] There is no recommendation for the number of false positives, but it is necessary for routine laboratories to keep this number as low as possible to minimize the time required for additional investigation. The majority of samples that pass through a laboratory are compliant with the legislation. Therefore, it is efficient to quickly screen compliant samples from those that are suspected to be contaminated. Following an initial screen, the suspect positive samples are reanalyzed using a second confirmatory method (e.g., GC-MS/MS) to confirm suspect positives and to accurately determine the concentration of the pesticide present. The confirmatory analysis contains a complete calibration series in an appropriate matrix that is not included in the screening analysis.

Always whats next.

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[Click here to download Application Note 10448]

Application Note 10449

Fast Screening, Identification, and Quantification of Pesticide Residues in Baby Food Using GC Orbitrap MS Technology

Cristian Cojocariu,[1] Dominic Roberts,[1] Michael T. Hetmanski,[2] Richard J. Fussell,[2] and Paul Silcock[1]
[1]Thermo Fisher Scientific, Runcorn, UK
[2]Food and Environment Research Agency (FERA), York, UK

Keywords
Baby Food, Exact Mass, Screening, Food Safety, GC Orbitrap, High Resolution GC-MS, Pesticide Analysis, Quantification, TraceFinder

Introduction

Pesticides are chemicals widely used to control a variety of pests, such as insects, plant pathogens, weeds, etc. The use of pesticides may result in residues in crops, therefore, strict regulations are in place to control the use of these chemicals and to ensure that concentrations do not exceed statutory maximum residue levels (MRLs).[1]

Pesticides are measured almost exclusively by liquid chromatography (LC) and gas chromatography (GC) analytical methodologies. GC coupled to a mass spectrometer (MS) as a detector is widely used in many pesticide residue laboratories, because many pesticides are not amenable to LC-MS or ionize poorly under soft ionization techniques. GC offers good separation efficiency and a choice of MS detectors, including single or triple quadrupoles. Quadrupole mass analyzers are selective, sensitive, and cost-effective instruments that operate at nominal mass resolution. When using quadrupole MS, the selectivity required to separate target pesticides from chemical background is achieved by by the use of either selected ion monitoring (SIM) or selected reaction monitoring (SRM). Both SIM and SRM are used in targeted experiments in which the mass spectrometer is pre-programmed using a list of preselected pesticides. However, targeting specific compounds during acquisition limits the scope of analysis and can result in false negative results (non-detection) for both unknown and untargeted compounds, which may be of concern with respect to food safety.

This limitation has led to increased interest in developing methods using MS analyzers that can operate in full scan with a higher mass resolving power than triple quadrupoles, but provide similar levels of selectivity and quantitative performance. Until now, high-resolution, accurate-mass GC-MS instruments have not gained wide acceptance due to their limited ability to provide full scan selectivity and quantitative performance comparable to triple quadrupole instruments operated in SRM.

In this work, we demonstrate the use of GC coupled to Orbitrap™ MS technology for fast, high throughput pesticide residues analysis in baby food samples, with an almost unlimited scope in the analysis through full scan acquisition. Quantitative performance comparable to triple quadrupoles and compliance with SANCO guidelines[2] will also be demonstrated.

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[Click here to download Application Note 10449]

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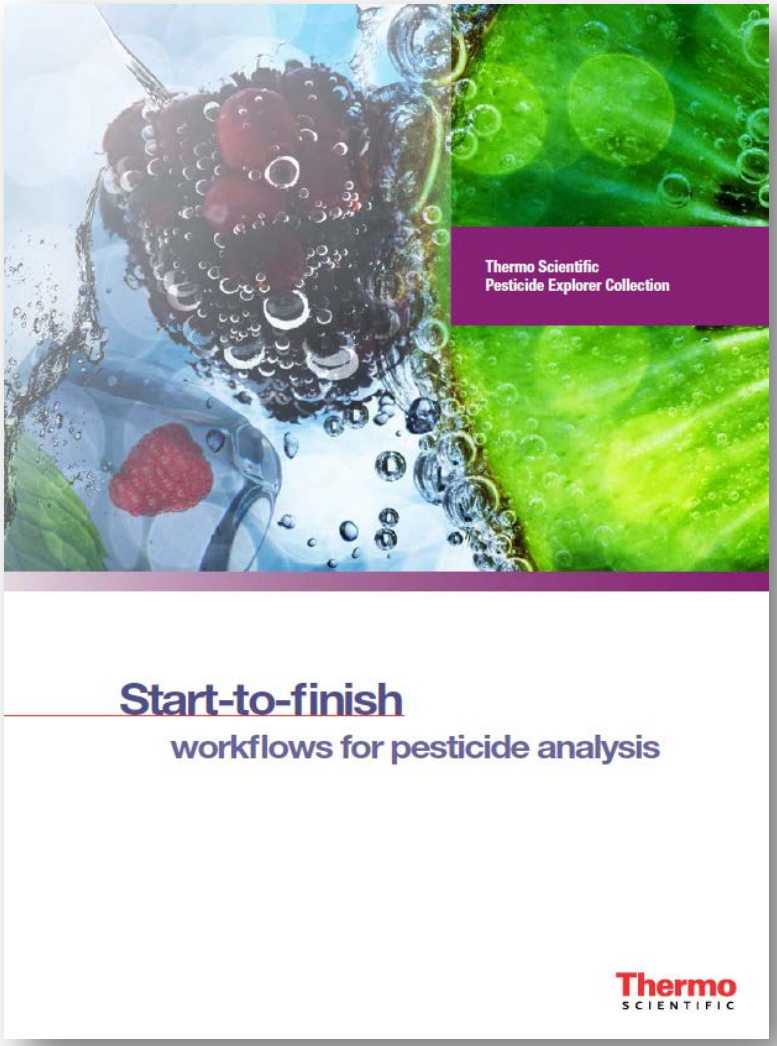
Pesticides: Non-target Analysis by UHPLC-HRAM-MS



Click here to download brochure

Configuration and complete start-up kit provides everything needed to perform robust, high resolution routine workflows for rapid screening and quantitation of pesticides, from the QuEChERS sample extraction kit to proven multi-class pesticide residue analysis methods.

Q Exactive Focus MS

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Q Exactive Focus Mass Spectrometer (MS)

Why use UHPLC-Q Exactive Focus

- ✓ High RP and stable accurate mass
- ✓Accurate mass assignment (MS and MS/MS of known and unknown analytes)
- ✓Analysis of both +ve & -ve compounds (polarity switching)
- ✓Quantification and identification of target compounds
- ✓Screening for `unexpected` components
- ✓Screening, Quantification and Identification in a single analysis?
- ✓Retrospective data analysis

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 in <1 sec (one full positive mode scan and one full negative mode scan at a resolution setting of 35,000)

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Various Acquisition Options

Non-target acquisition

without fragmentation (Full Scan)

with fragmentation in HCD cell

AIF = all-ion-fragmentation

vDIA = variable Data Independent Acquistion

Targeted acquisition

without fragmentation (SIM = Selected Ion Monitoring)

with fragmentation

ddMS/MS = data-dependent MS/MS with inclusion list

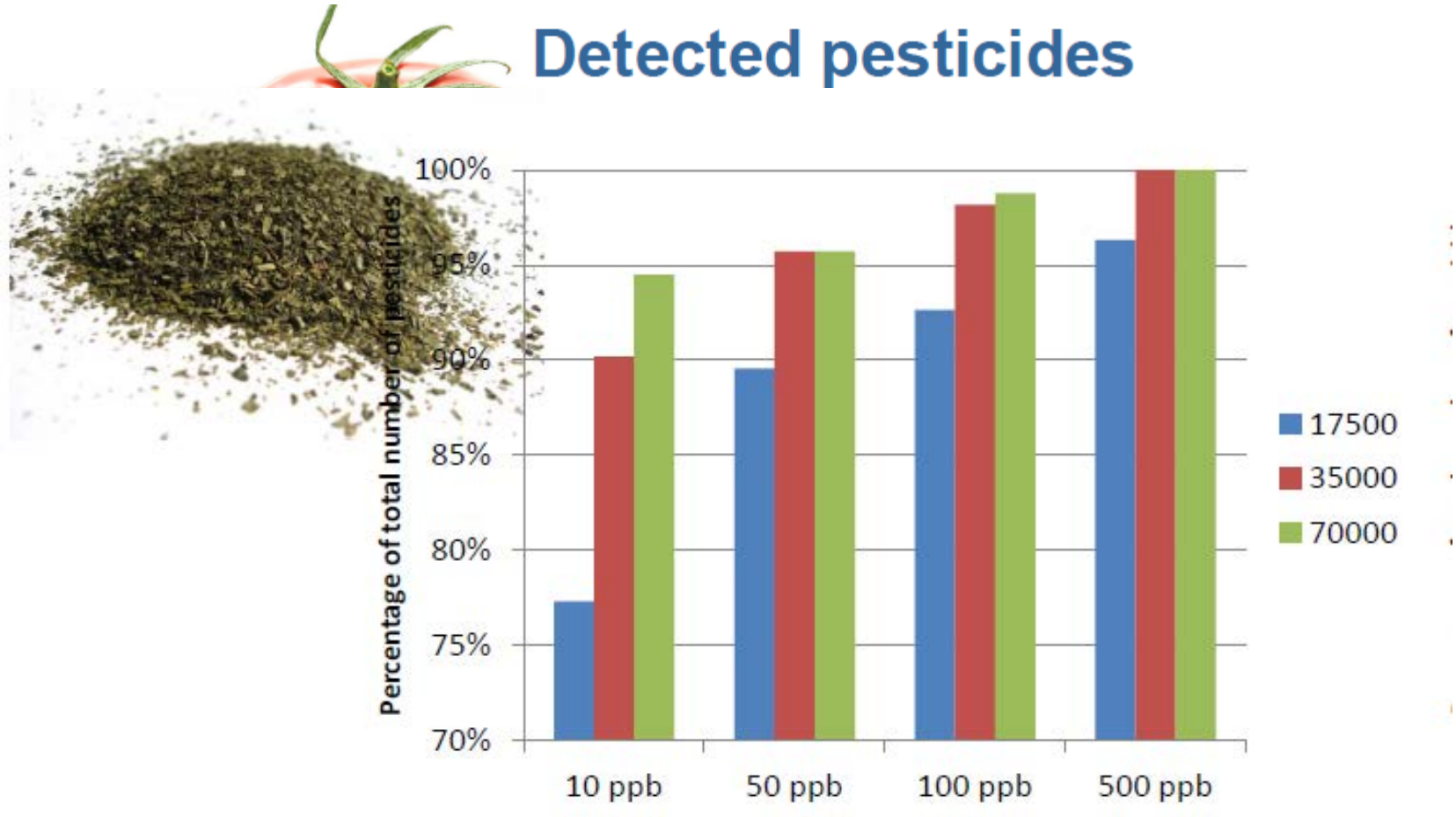
t-MS/MS = targeted MS/MS

PRM = Parallel Reaction Monitoring

Combinations of the above

vDIA is not available in the U.S.

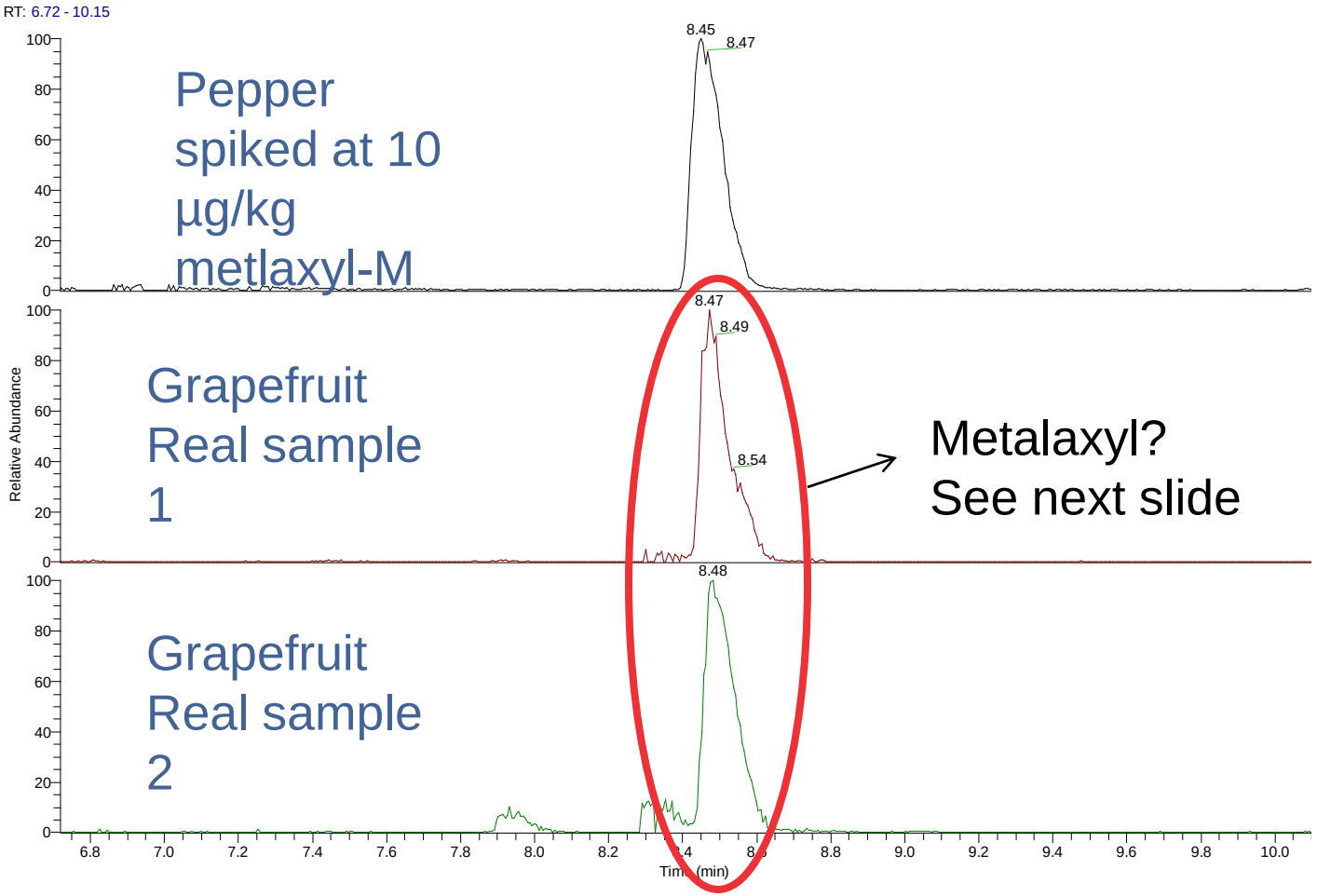
Does Resolution Matter?



RAFA 2013: Łukasz Rajski, María del Mar Gómez Ramos, Amadeo R. Fernández-Alba; EURL for Pesticide Residues in Fruits and Vegetables. Pesticide Residue Research Group. University of Almeria, Spain. e-mail: amadeo@ual.es

Analysis Of Fruit Samples By LC - Q Exactive MS/MS

Metalaxyl-M (XIC m/z 280.1543 ± 5 ppm). **Full scan MS**. Resolution 70000



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Analysis Of Fruit Samples By LC - Q Exactive MS/MS

Metalaxyl-M (XIC m/z 280.1543 ± 5 ppm).
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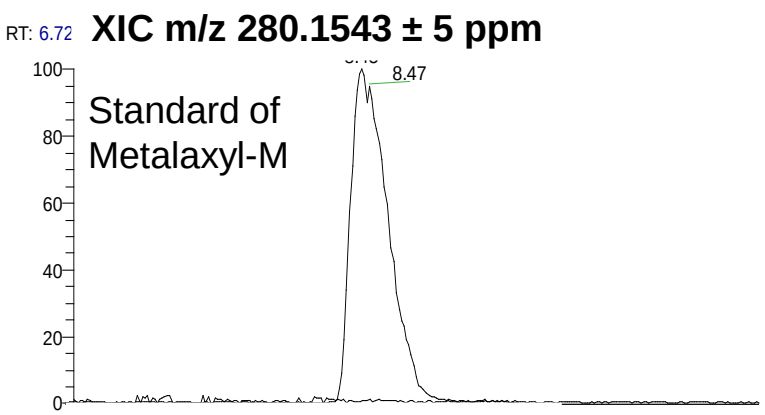

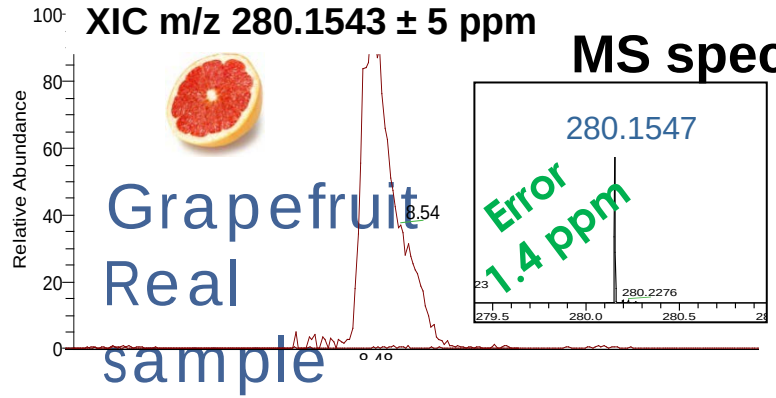

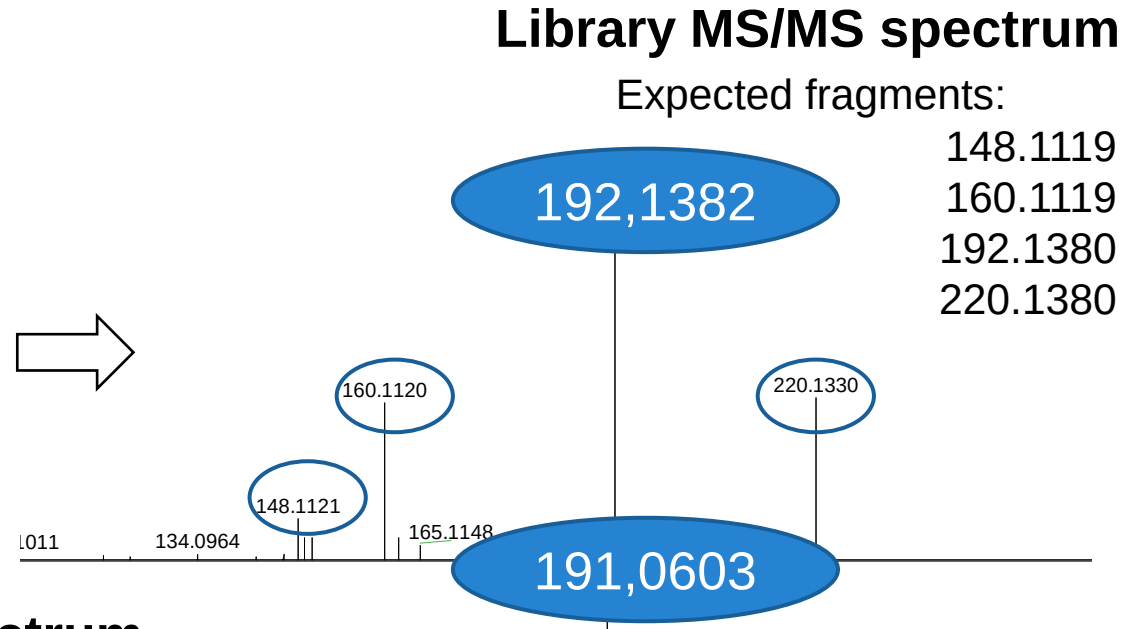

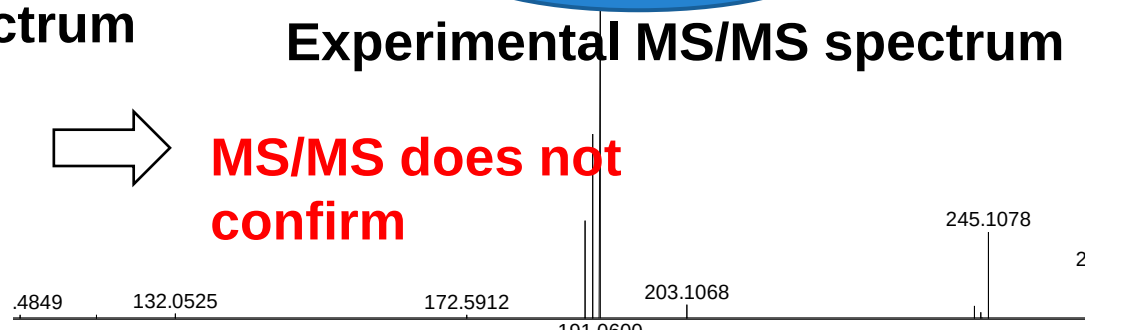

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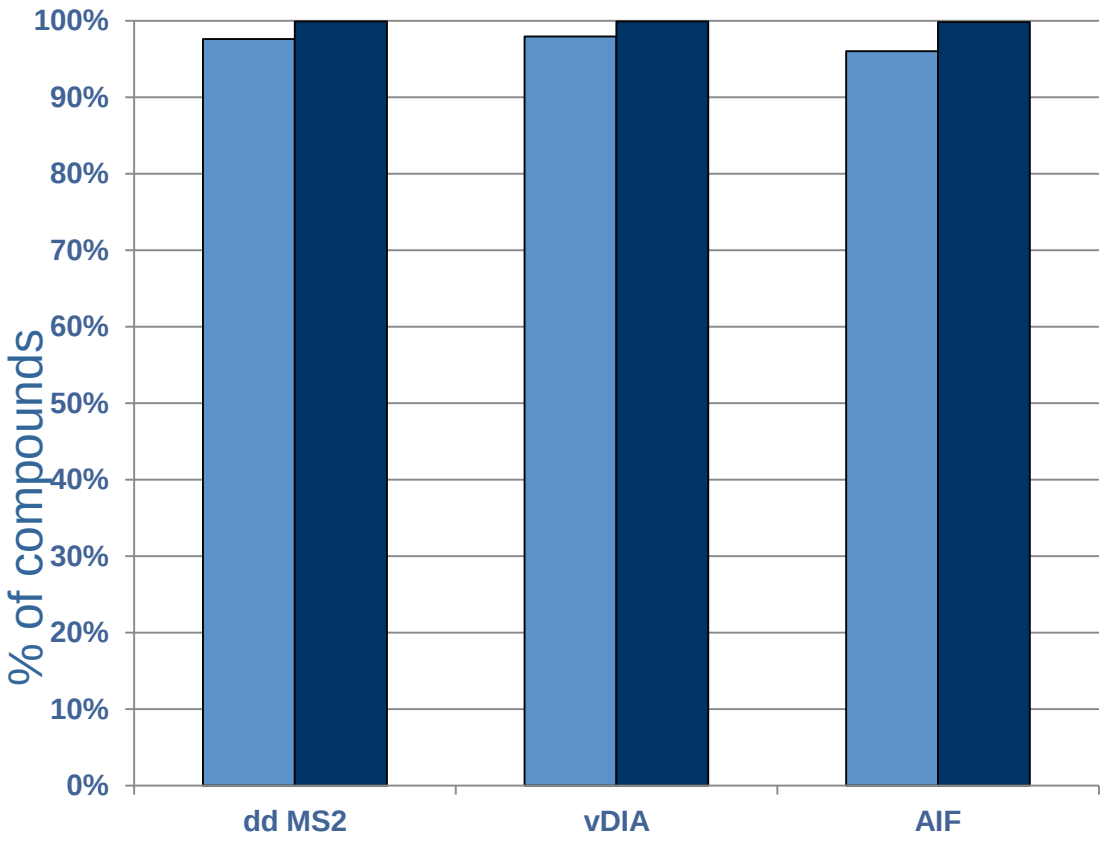
Identification Requires Fragments

166 pesticides x 11 matrices = 1826 results



0.01 mg/kg

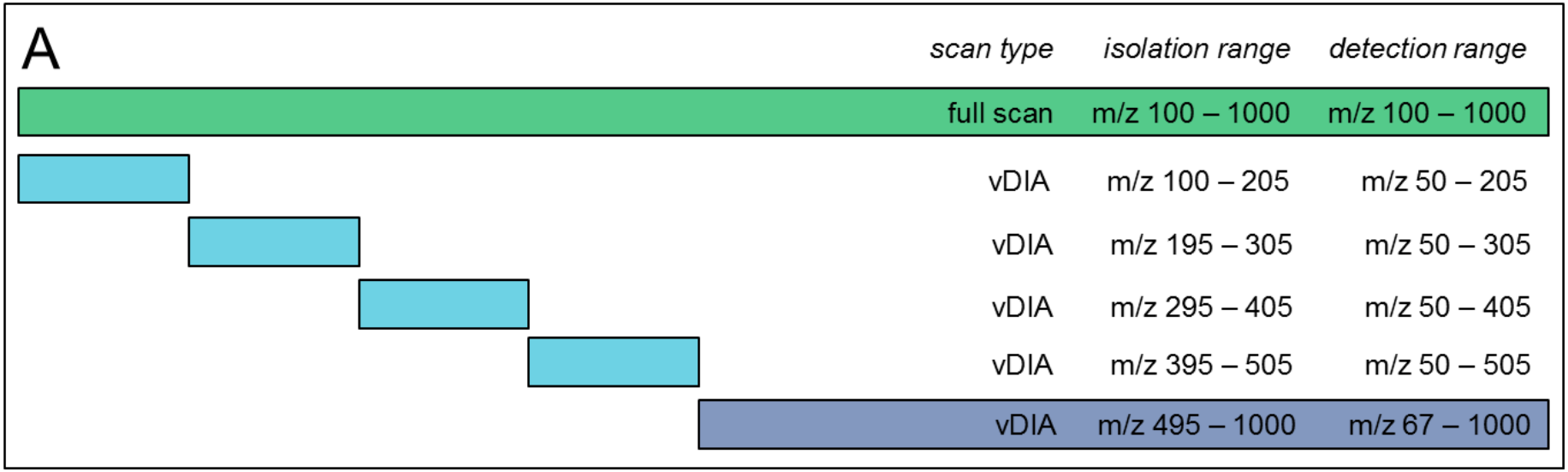
0.1 mg/kg

dd-MS2 typically based on 1 scan, vDIA and AIF on a number of scans

Slide courtesy of Amadeo Fernandez-Alba, University of Almeria, Spain

vDIA is not available in the U.S.

Alternative to All Ions Fragmentation (AIF)

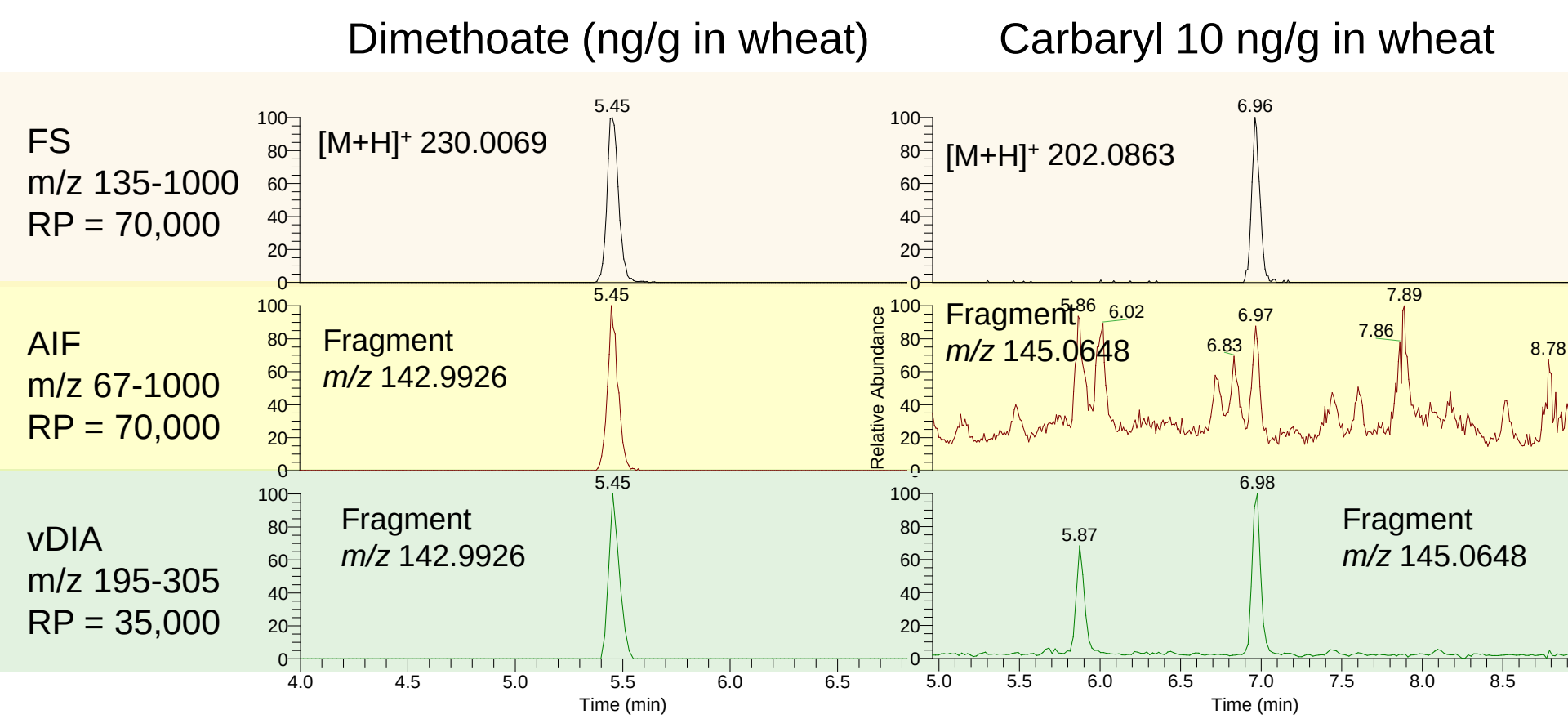


This method provides

- A complete record of data for mass range 100 to 1000 m/z in full scan as well as in MS/MS
- A cycle time of 720 ms or a scan speed of roughly 1.4 Hz on the full scan
- Excellent reproducibility
- Higher specificity and response for fragments compared to AIF

vDIA is not available in the U.S.

LC-Q Exactive Focus : All Ions Fragmenation (AIF) vs. vDIA

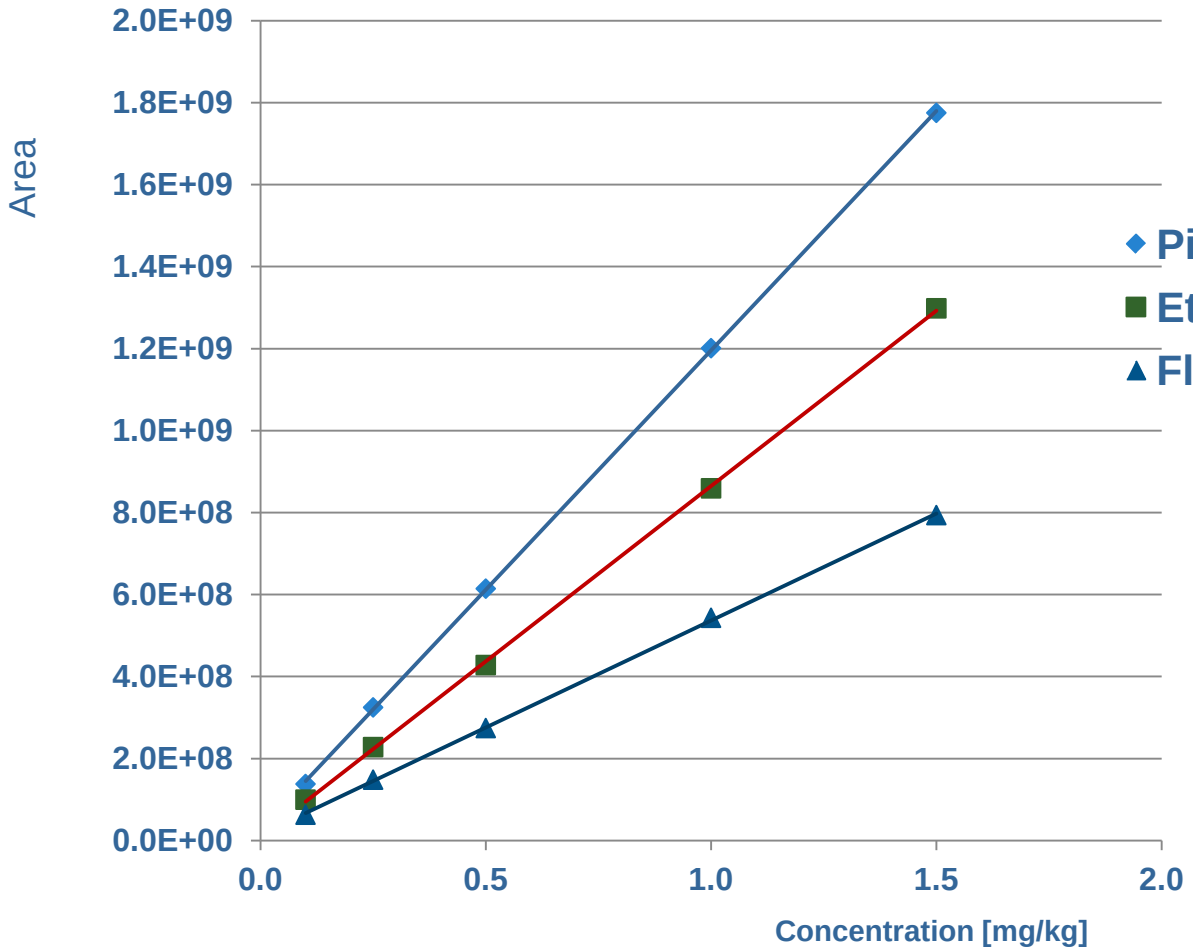


- vDIA provides improved selectivity & sensitivity for more reliable identification

Data courtesy of Dr Hans Mol, Rikilt Wageningen, UR

vDIA is not available in the U.S.

Linearity vDIA 5 Mass Segments



Spinach

Mass segments

120 - 205 m/z
195 – 305 m/z
295 – 405 m/z
395 – 505 m/z
495 – 1000 m/z

Slide courtesy of Amadeo Fernandez-Alba, University of Almeria, Spain

vDIA is not available in the U.S.

Independent Assessment Of Orbitrap Full Scan /vDIA Method

Food Additives & Contaminants: Part A, 2015
http://dx.doi.org/10.1080/19440049.2015.1085652

Taylor & Francis
Taylor & Francis Group

Simultaneous quantitative determination, identification and qualitative screening of pesticides in fruits and vegetables using LC-Q-Orbitrap™-MS

Paul Zomer* and Hans G.J. Mol

Natural Toxins & Pesticides, RIKILT Wageningen UR, Wageningen, the Netherlands

(*Received 18 May 2015; accepted 18 August 2015*)

- vDIA improves selectivity and response for fragment ions
- Quantitative validation at 10 & 50 ng/g in lettuce and orange
  (majority of 184 compounds met SANCO criteria for trueness & precision
- Qualitative validation for same 184 compounds)
  (SDL achieved for 134 compounds at 10 ng/g, & 39 at 50 ng/g
- vDIA fragment ratios compliant for 93% of 11,488 measurements
- Linearity fit-for purpose for 0-250 ng/ml- most deviation at high conc^{n}
- No false detects with automated data processing (minimal intervention)

"Based on these findings, the LC-full scan HRMS method can be considered as a fit-for-purpose alternative for methods based on LC-QqQ"

vDIA is not available in the U.S.

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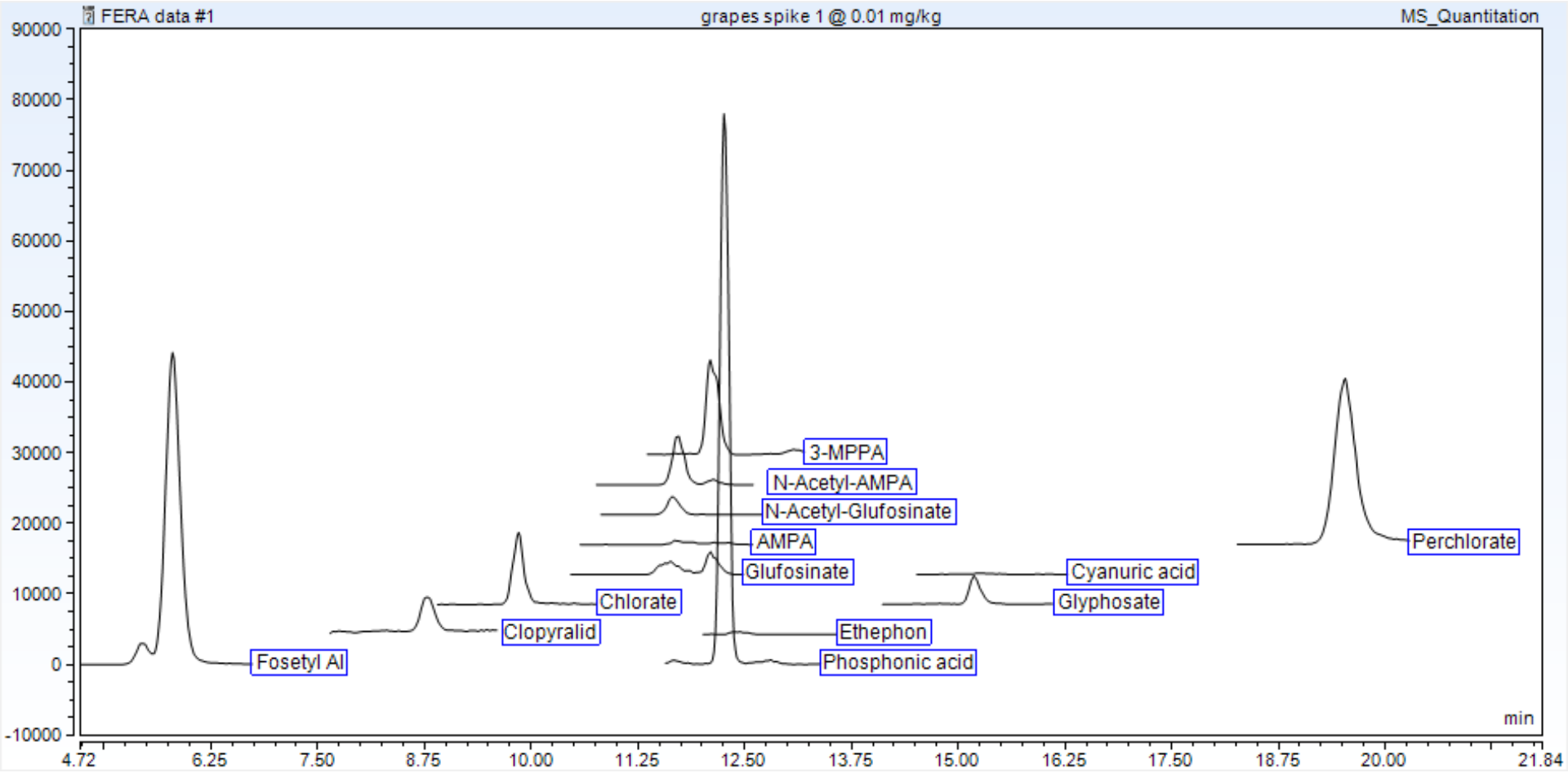
What Ever Your Challenge? We Have The Tools.....

- Instruments to future-proof your laboratory
- Instruments with high mass resolving power with excellent mass accuracy (selectivity)
- Instruments with highest level of sensitivity for target analysis
- Wide dynamic range (to be able to measure compounds at high and low concentrations
- Searchable databases and libraries (elemental composition, identity, etc.)
- Ready to go validated methods
- Centre of expertise for training

IC-MS/MS Pesticide Multi Residue Ion Chromatogram

- 10 µg/kg spike in grape (Fosetyl & Phosphonic acid @ 100 µg/kg)



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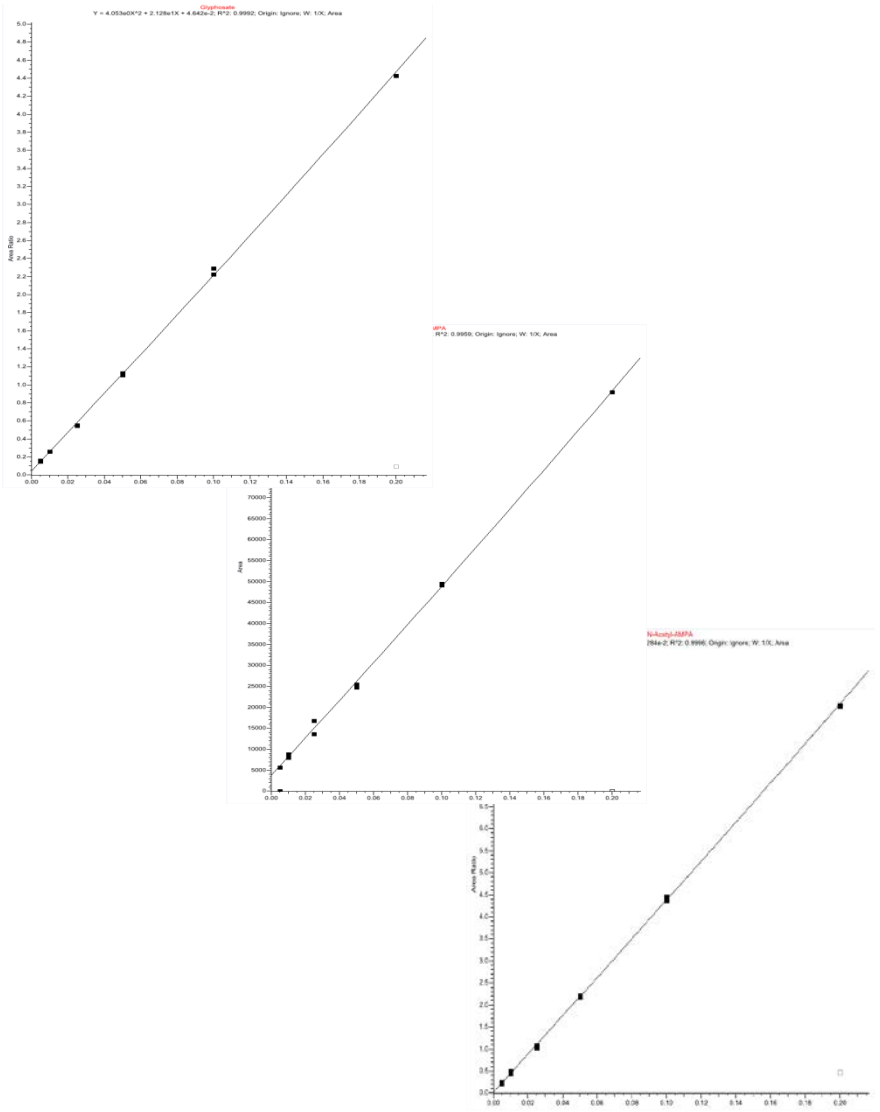
Glyphosate – Cereals (Flour)

Summary of validation results

Compound	Concn (µg/kg)	Mean % recovery (n=5)	Mean % RSD
Glyphosate (IS)	10	112	15
	50	108	12
	100	111	7
AMPA (no IS)	10	92	22
	50	98	13
	100	97	3
N-Acetyl-AMPA (no IS)	10	85	7
	50	82	10
	100	86	2



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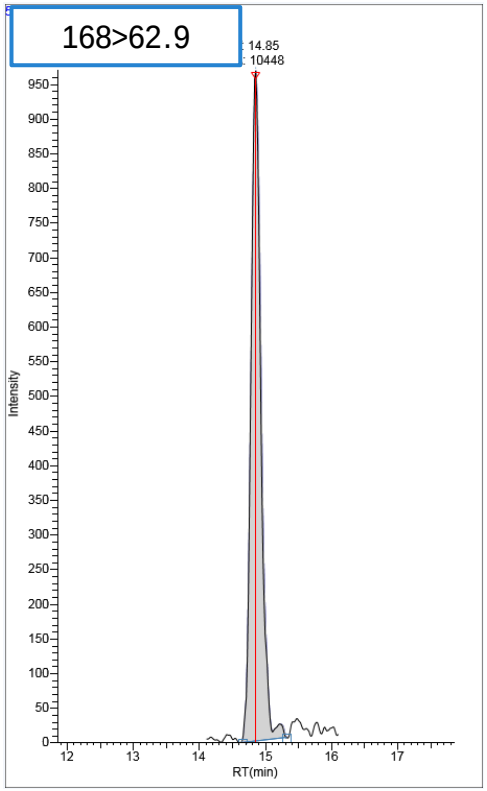
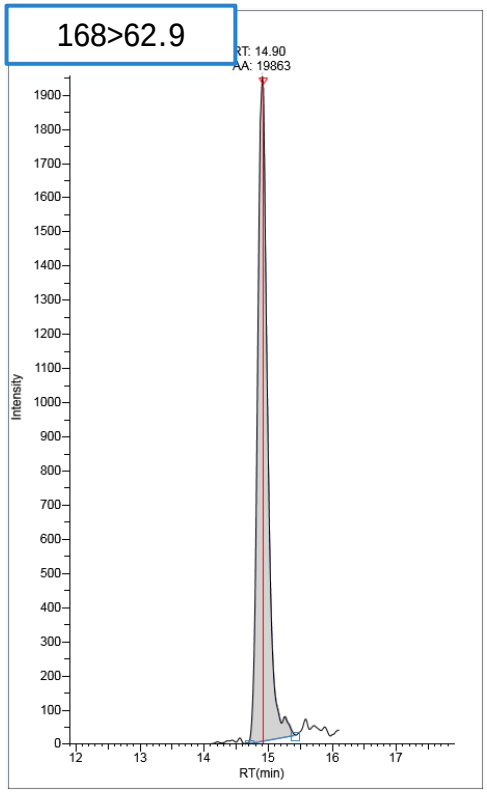
Glyphosate In Beer – No Extraction

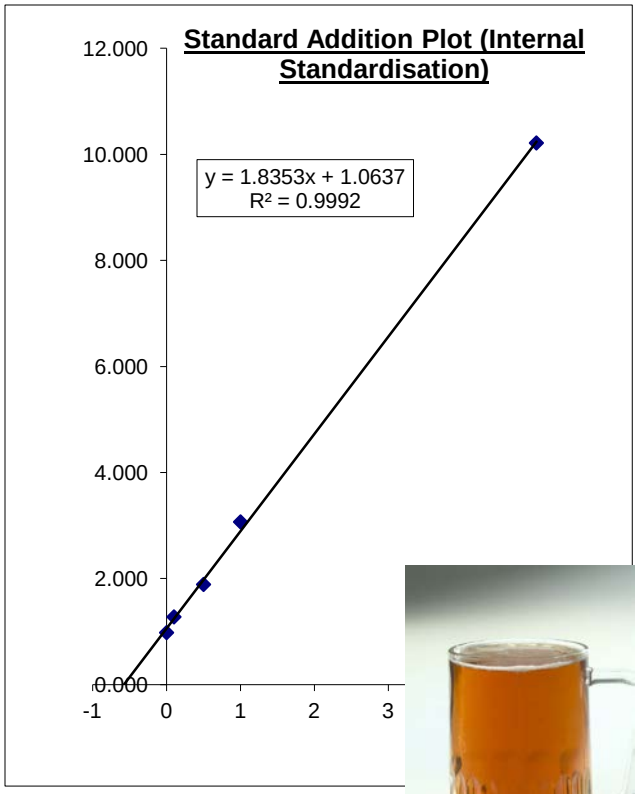
- Glyphosate incurred residue @ 0.58 µg/L

Glyphosate spike @ 0.5 µg/L

Calibration plot 0.1 – 5 µg/L spikes







- 1/10 dilution with water and internal standard added

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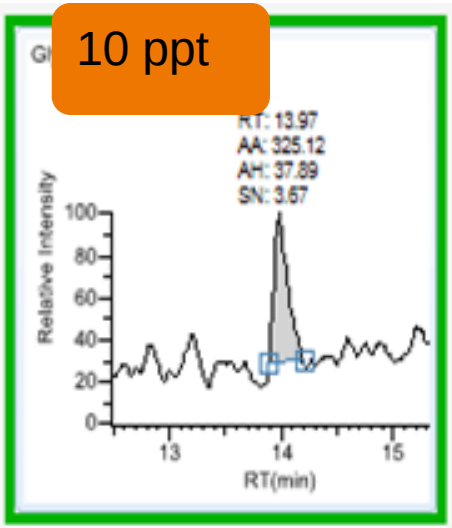
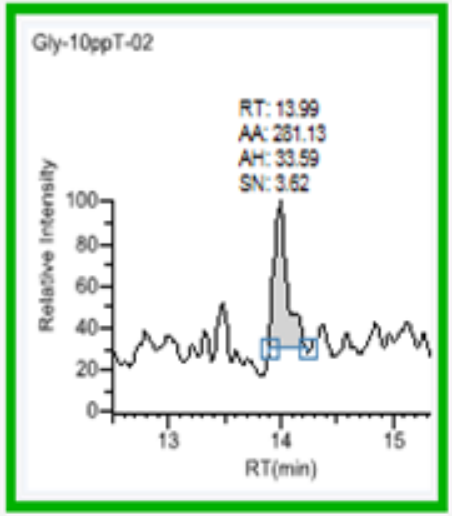
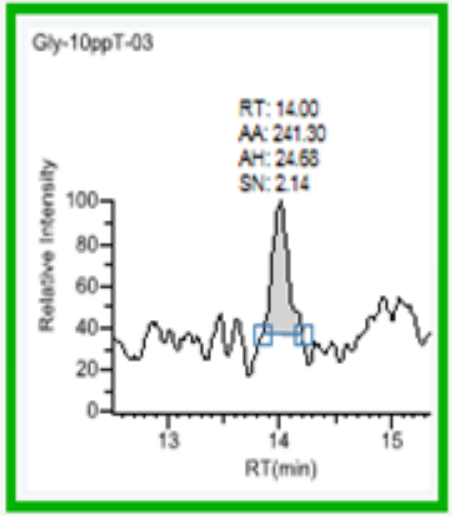
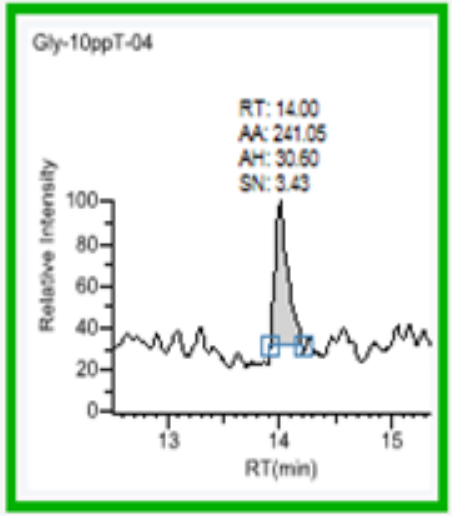
Glyphosate Detection Repeatability At LOQ 10 And 20 ppt

10 ppt

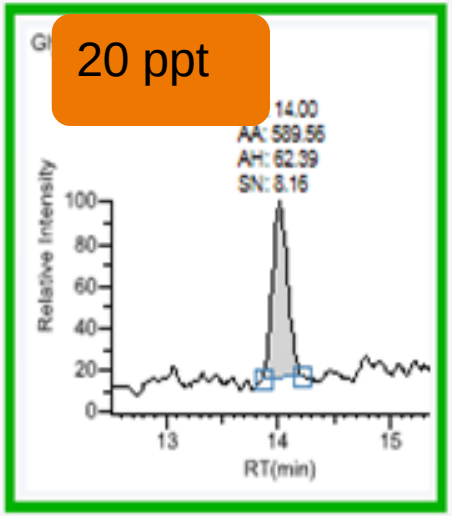


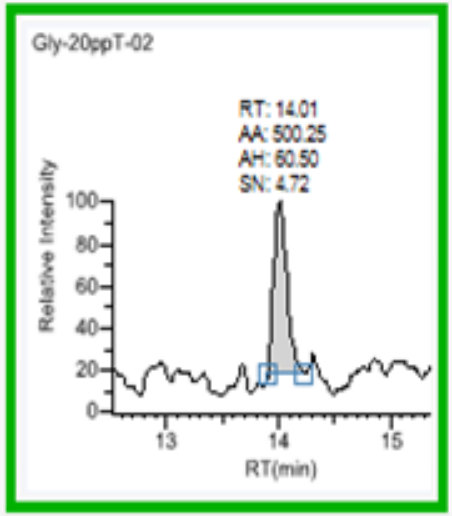


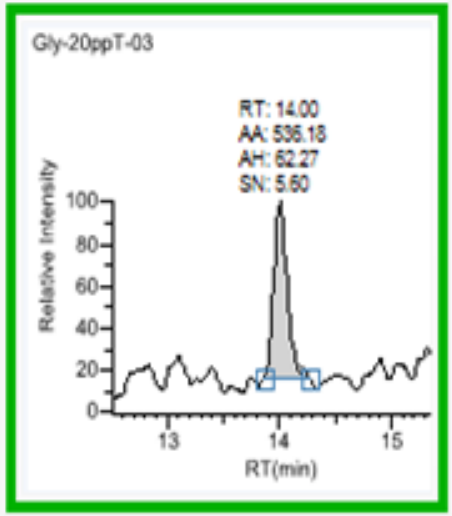


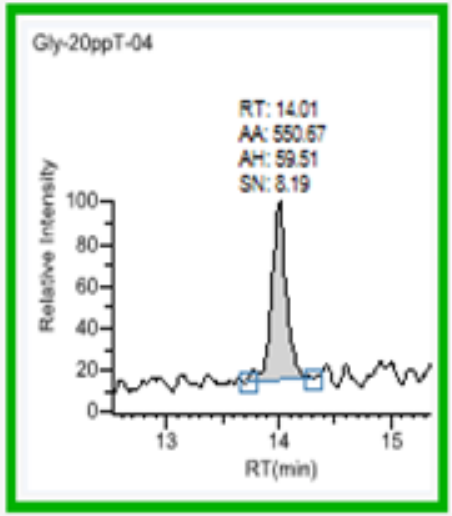


20 ppt



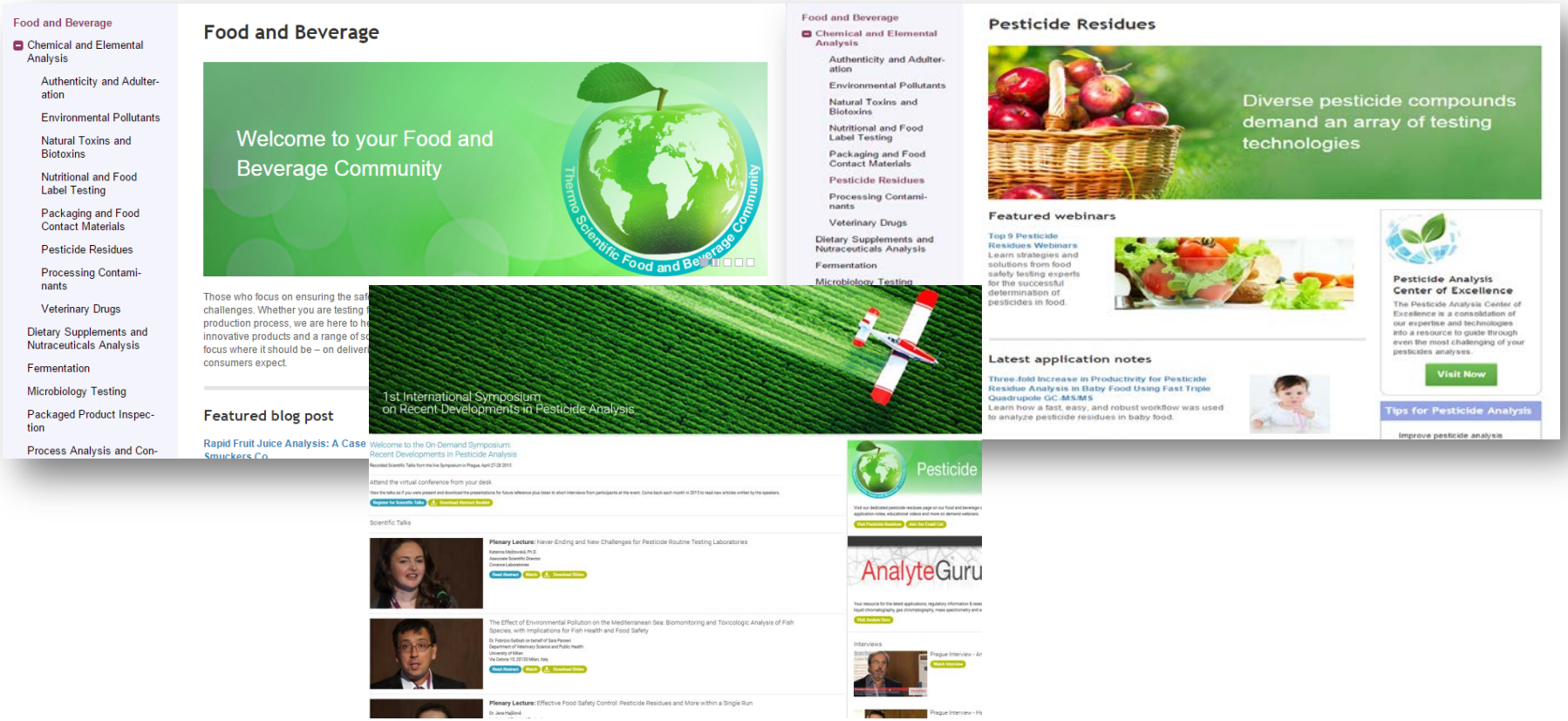






Thermo Scientific Food And Beverage Community

- View application notes, on-demand webinars, product information, and many more resources on our Pesticides and Food Communities pages
- www.thermofisher.com/food-beverage



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Method Validation (in-house)

EU Method performance criteria (SANTE /11945/2015)

http://ec.europa.eu/food/plant/docs/plant_pesticides_mrl_guidelines_wrkdoc_11945_en.pdf

Parameter	What/How	Criterion
Linearity	Calibration curve	Residuals < 20%
Matrix effect	Comparison response from solvent standards and matrix matched standards	-
LOQ	Lowest level for which trueness and precision criteria have been met	LOQ ≤ MRL
Specificity	Response in reagent blank and control samples	< 30% of LOQ
Precision (RSD_r)	Repeatability RSD	≤ 20%
Precision (RSD_{wR})	Within laboratory reproducibility	≤ 20%
Trueness (bias)	Average recovery for spike levels	70-120%

LC-MS/MS: Target Pesticides – Total **320 Compounds Tested**

2,4-D	Butafenacil	Desmetryn	Fenamiphos	Griseofulvin	Metconazole	Piperophos	Sulprofos
Abamectin	Butocarboxim	Dichlofenthion	Fenarimol	Halofenozide	Methabenzthiazuron	Pirimicarb	Tebuconazole
Acephate	Butocarboxim sulfoxide	Diclobutrazol	Fenazaquin	Halofenozide	Methamidophos	Pirimiphos-methyl	Tebufenozide
Acetamiprid	Butoxycarboxim	Dicrotophos	Fenbuconazole	Haloxyfop	Methiocarb	Pretilachlor	Tebufenpyrad
Acibenzolar-S-methyl	Carbaryl	Diethofencarb	Fenbutatin oxide	Haloxyfop-methyl	Methiocarb-sulfone	Primisulfuron-methyl	Tebuthiuron
Alachlor	Carbendazim	Difenacoum	Fenhexamid	Heptenophos	Methiocarb-sulfoxide	Prochloraz	Teflubenzuron
Alanycarb[1]	Carbetamide	Difenoconazole	Fenobucarb	Hexaconazole	Methomyl	Profenophos	Tepraloxydim
Aldicarb sulfone	Carbofuran	Diflubenzuron	Fenoxanil	Hexaflumuron	Methoprotryne	Promecarb	Terbacil[2,3]
Allethrin	Carbofuran-3-hydroxy[1]	Dimefuron	Fenoxycarb	Hexazinone	Methoxyfenozide	Prometon	Terbufos
Ametryn	Carbosulfan[1]	Dimethachlor	Fenpiclonil	Hexythiazox	Metobromuron	Prometryn	Terbumeton
Aminocarb	Carfentrazone-ethyl	Dimethametryn	Fenpyroximat	Imazalil	Metolachlor	Propamocarb	Terbuthylazine
Amitraz[1]	Carpropamid	Dimethenamid	Fensulfothion	Imazaquin	Metolcarb	Propanil	Terbutryn
Ancymidol	Chlorantraniliprole	Dimethoate	Fenthion	Imazethapyr	Metosulam	Propazine	Tetraconazole
Anilofos	Chlorbromuron	Dimethomorph	Fenthion-sulfoxide	Imibenconazole	Metoxuron	Propetamphos	Tetramethrin
Aramite	Chlorfenvinphos	Dimetilan	Fenuron	Imidacloprid	Metrafenone	Propiconazole	Thiabendazole
Atrazine	Chlorfluazuron	Dimoxystrobin	Flazasulfuron	Indanofan	Metsulfuron-methyl	Propoxur	Thiacloprid
Azaconazole	Chloridazon (pyrazone)	Diniconazole	Flonicamid	Indoxacarb	Mevinphos	Propyzamide	Thiamethoxam
Azamethiphos	Chlormequat	Dinotefuran	Florasulam	Ioxynil	Mexacarbate	Prosulfocarb	Thiazopyr
Azinphos-ethy	Chlorotoluron	Dioxacarb	Fluazifop	Iprovalicarb	Monocrotophos	Pymetrozine	Thidiazuron
Azinphos-methyl	Chloroxuron	Disulfoton	Fluazinam	Isocarbophos	Monolinuron	Pyraclostrobin	Thiobencarb
Azoxystrobin	Chlorpyrifos	Dithianon	Flubendiamide	Isoprocarb	Napropamide	Pyridalyl	Thiodicarb
Barban	Cinosulfuron	Dithiopyr	Flufenacet	Isoprothiolane	Naptalam	Pyridate	Thiofanox[2]
Bendiocarb	Clethodim	Diuron	Flufenoxuron	Isoproturon	Neburon	Pyrimethanil	Thiophanate-methyl
Benfuracarb[1]	Clofentezine	DNOC	Flufenoxuron	Isoxaben	Nicosulfuron	Pyroquilon	Tolfenpyrad
Benodanil	Clomazone	Dodemorph	Flumetsulam	Isoxadifen-ethyl	Nitenpyram	Pyroxsulam	Tralkoxydim
Benoxacor	Clopyralid	Epoxiconazole	Fluometuron	Isoxaflutole	Nuarimol	Quinoxyfen	Triadimefon
Bensulfuron methyl	Clothianidin	Esprocarb	Fluopicolide	Isoxathion	Ofurace	Quizalofop-ethyl	Triadimenol
Bentazon	Coumaphos	Etaconazol	Fluopyram	Kresoxim-methyl	Omethoate	Quizalofop-p	Triazophos
Benzoximate	Crotoxyphos	Ethiofencarb	Fluorochloridone	Lenacil	Oxadixyl	Resmethrin[2]	Trichlorfon
Benzoylprop-ethyl	Cumyluron	Ethiofencarb-sulfone	Fluoxastrobin	Malaoxon	Oxamyl	Rimsulfuron	Triclopyr
Bifenazate	Cyanazine	Ethiofencarb-sulfoxide	Fluquinconazole	Mandipropamid	Paclobutrazol	Rotenone	Tricyclazole
Bitertanol	Cyazofamid	Ethiprole	Fluroxypyr	MCPA	Parathion	Schradan	Tridemorph
Boscalid	Cycloate	Ethirimol	Flusilazole	Mefenacet	Penconazole	Sethoxydim	Trietazine
Brodifacoum	Cycluron	Ethofumesate	Flutriafol	Mepiquat chloride	Pencycuron	Simeconazole	Trifloxystrobin
Bromacil	Cyflufenamid	Ethoxyquin	Forchlorfenuron	Mepronil	Phenmedipham	Simetryn	Triflumizole
Bromoxynil	Cymoxanil	Etofenprox	Formetanate hydrochloride	Mesotrione	Phenthoate	Spinosad A	Triflumuron
Bromuconazole	Cyromazine	Etoxazole	Formothion	Metaflumizone	Phorate	Spiromesifen	Triforine
Bupirimate	Demeton-S-methyl sulfone	Etrimfos	Fosthiazate	Metalaxyl	Phoxim	Spirotetramat	Triticonazole
Buprofezin	Desmedipham	Famoxadone	Fuberidazole	Metamitron	Picoxystrobin	Spiroxamine	Vamidothion
Butachlor	Desmethyl-pirimicarb	Fenamidone	Furathiocarb	Metazachlor	Piperonyl butoxide	Sulfotep	Zoxamide

1 – Works only in solvent **2 – Doesn´t work in leek** **3 – Doesn´t work in flour**

MS/MS Instrumental Method

TRACE 1310 GC oven:

- **Carrier flow: 1.2 ml/min He**
- **Column: TG-5 SilMS 30m x 0.25 x 0.25mm**
- **PTV splitless** 1 µL

#	Rate [°C/min]	Temperature [°C]	Hold time [min]
Initial		40	1.5
1	25	90	1.5
2	25	180	0
3	5	280	0
4	10	300	5

TSQ 8000 Evo GC-GC/MS:

- Scan type: timed-SRM
- Ionisation: EI +
- MS transfer line temperature: 250 °C
- Ion source temperature: 300 °C
- Detector gain: 7
- Cycle time [s]: 0.3
- Minimum baseline peak width: 3 sec
- Desired scans per peak: 10
- Minimum dwell time: 0.001 sec
- Q1 resolution: normal

Method Validation: Linearity Assessment

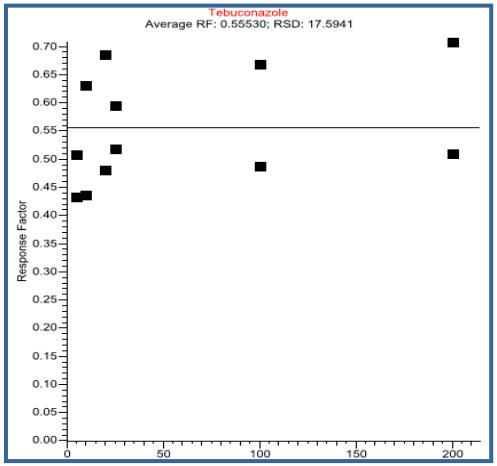
Evaluation: Mandel's linearity test, r^2>0.9850, residue plot

Acceptance criteria:

- residue plot RSD<20%

Residue plot RSD

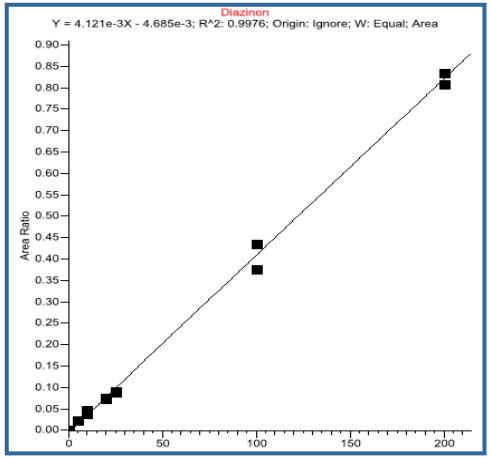
(in each matrices)



88% in acceptance range

Linear curve r^2 values

(for all matrices)



99% in acceptance range

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Method Recovery

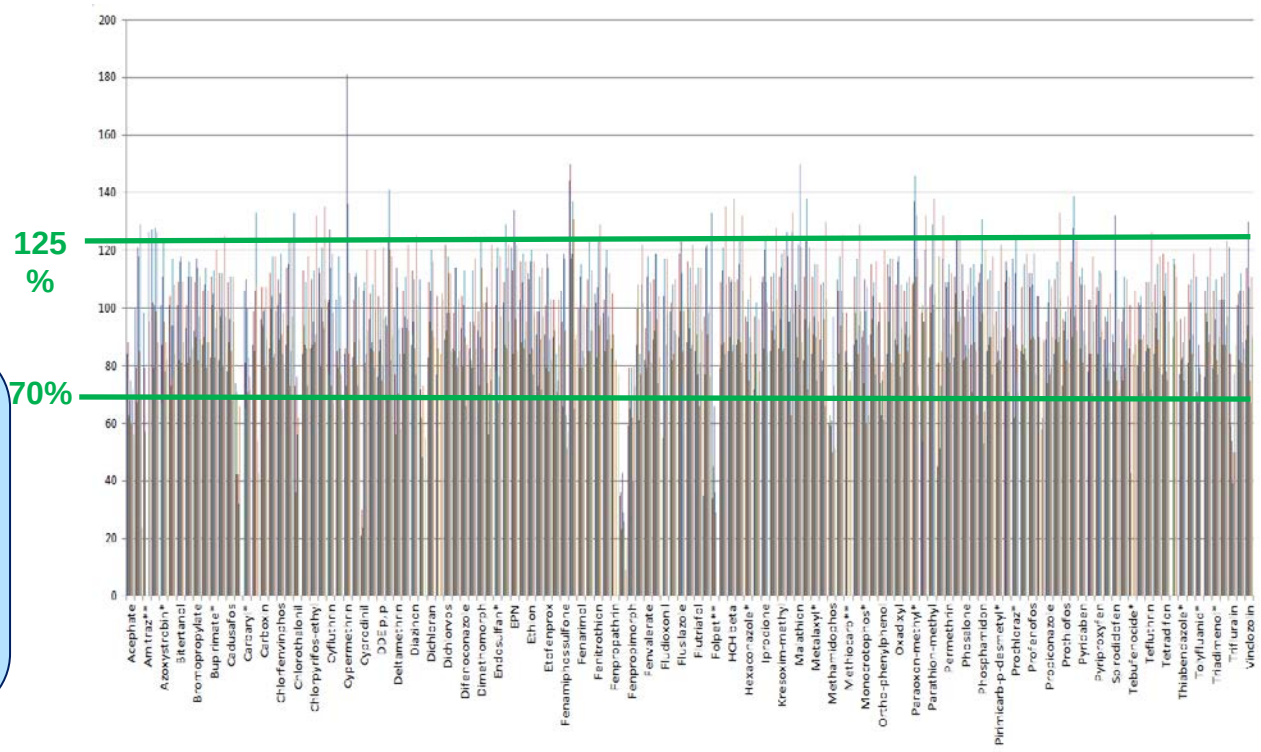
Evaluation: Method recovery at 3 levels (low, mid and high)

Acceptance criteria:

- Recovery 70-120%

Method recoveries at 3 levels (For all matrices)

92% acceptance



Out of range

folpet, fenpropidin, captan, carbofuran, methamidophos, trifluralin, cyprodinil, cypermetrhrin, fenamiphos-sulfoxide

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Method LOQ

Evaluation: IUPAC approach - signal to noise method

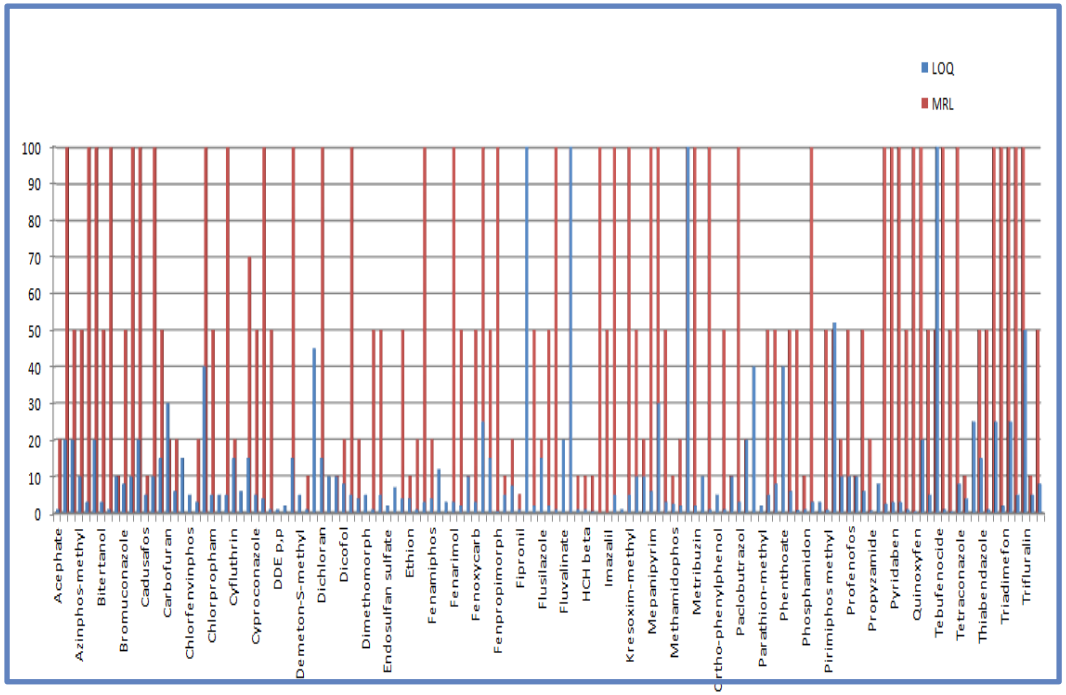
Acceptance criteria: LOQ ≤ MRL

97% in acceptance range

Out of target range:

Fenpropathrin (WF, LK), Tebufenocid (all),
Carbofuran* (SW), Methiocarb* (WF, LK), Oxadixil* (WF), Propargite* (WF, LK)

LOQ vs MRL in SB matrix



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