



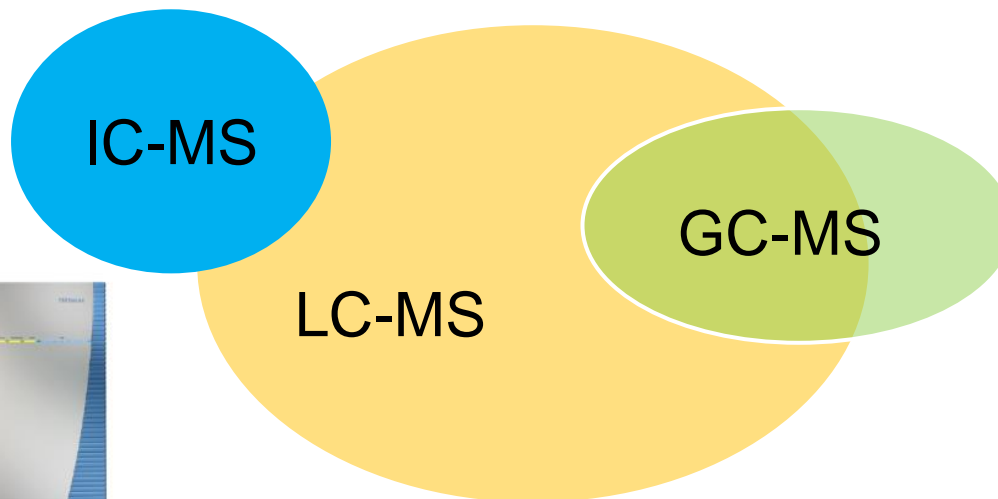
**ThermoFisher**  
S C I E N T I F I C

## Jak na problémy v reziduální analýze pomocí moderní hmotnostní spektrometrie?

*Michal Godula, Ph.D.*  
*Special Solutions Center*  
*Thermo Fisher Scientific*

The world leader in serving science

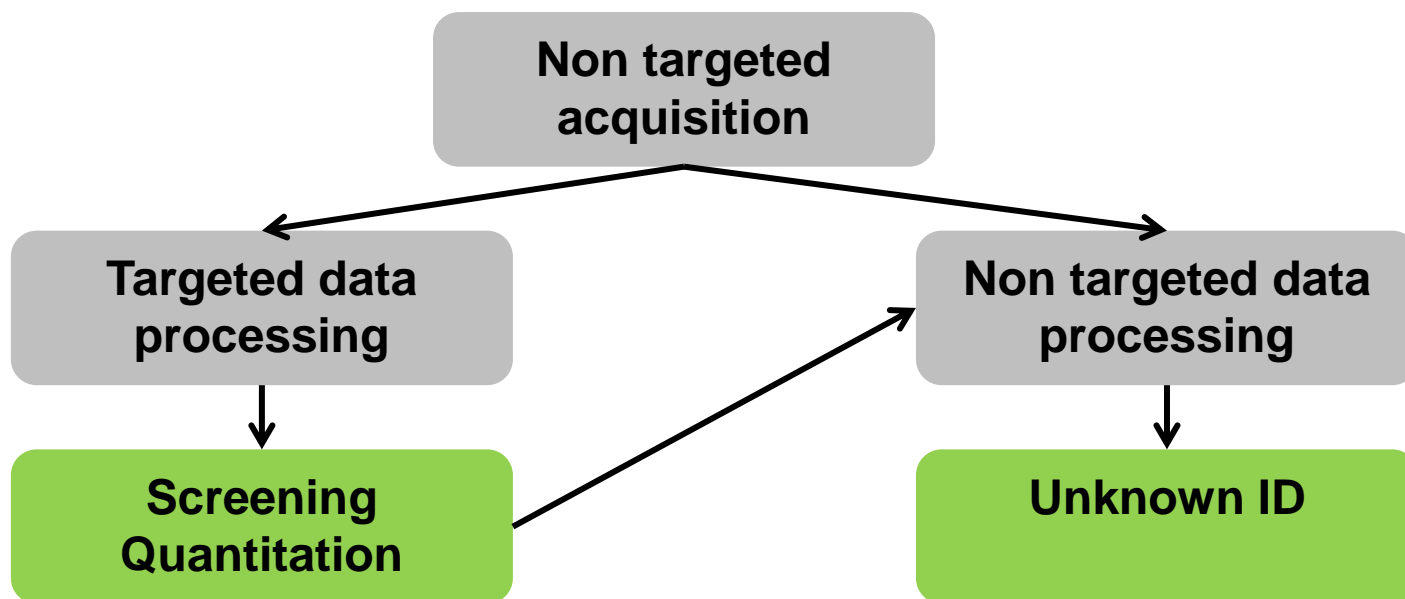
# Where are we?



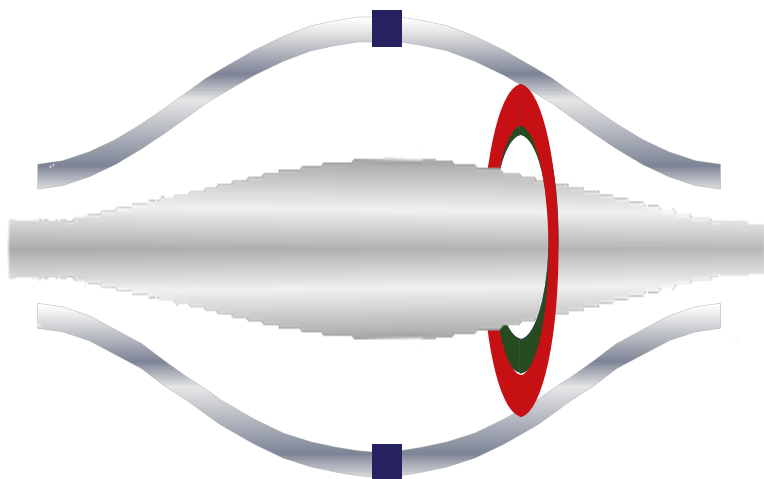
- **Targeted acquisition with Triple Quadrupole MS/MS:**
  - Needed selectivity and sensitivity
  - Robustness
  - Targeted approach only
  - False positives
  - Limited scope
  - Unknowns?

# Why to vote for high resolution MS?

- **Non-targeted acquisition:**
  - Similar sensitivity as triple quadrupole MS/MS
  - Increased scope of the analysis through full-scan
  - Simple setup of full-scan acquisition – no tuning
  - Flexibility – screen and confirmation on one platform
  - Perform targeted live or retrospective data processing



# Orbitrap: The Performance Leader since 2006



## ● What Orbitrap provides?

- Fundamental difference to other HRAM instruments
- Parameter measured is **frequency**, not time/voltage/etc
- Resolution up to 1M\* for more accurate  $m/z$
- Less prone to ambient conditions changes
- Stability within <1-2 ppm during several days
- No need for lock mass in “routine work”
- Small footprint
- Easy setup

## ● Which applications?

- accurate identification, structural analysis, and quantification of organic molecules, lipids, carbohydrates, peptides & proteins in complex mixtures

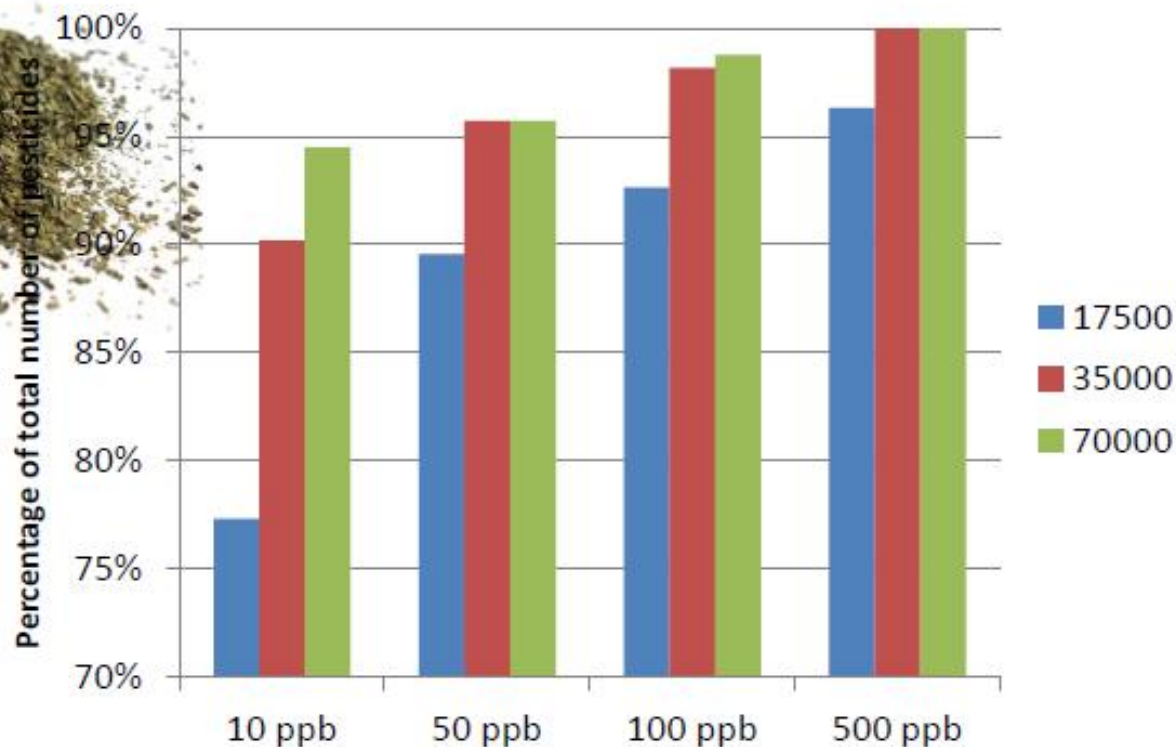
Unmatched ultrahigh resolution,  
accurate mass performance

*\*ASMS 2017 : Performance metrics and example applications: Orbitrap mass spectrometry at resolving power 1M*

# Does resolution matter?

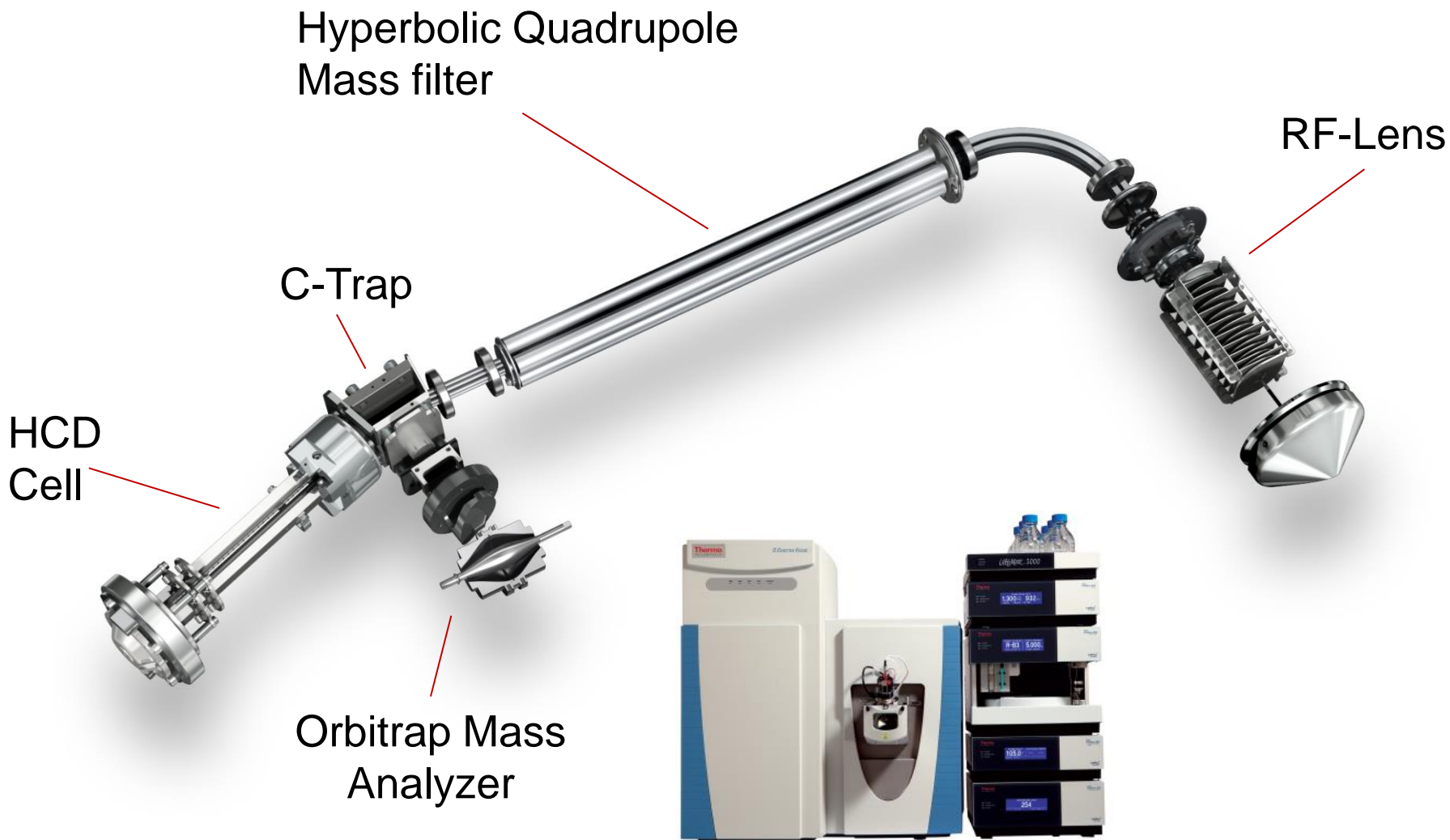


## Detected pesticides



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 Almería, Spain. e-mail: amadeo@ual.es

# Q Exactive MS Family



# Flexibility for Routine/Research Work

## Full MS(SIM)/data dependent MS/MS

- Post-acquisition extracted ion chromatograms of parent ions
- Relies on high resolution (selectivity) and MS/MS (confirmation)
- Useful for targeted screening and quantitative methods (QQQ like)

Targeted Workflow

## Full MS/ All Ion Fragmentation – vDIA\*

- Minimum method development
- Great for screening purposes, but quantitation, too
- Fully retrospective!

Non targeted workflow!

## PRM (Parallel Reaction Monitoring)

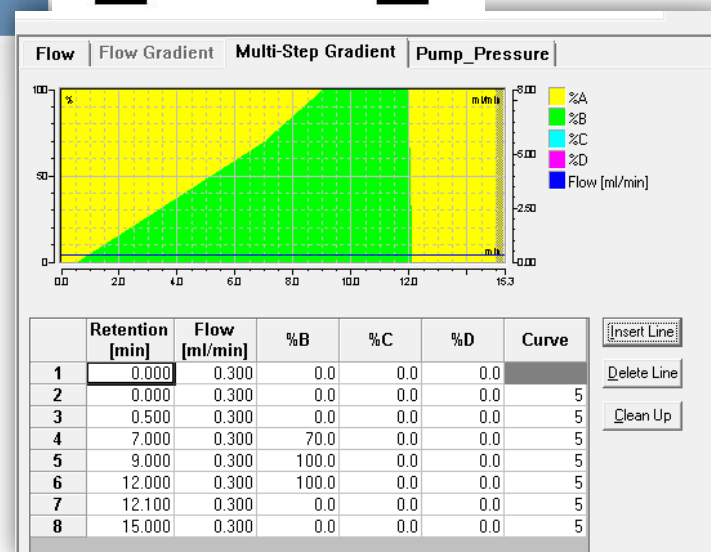
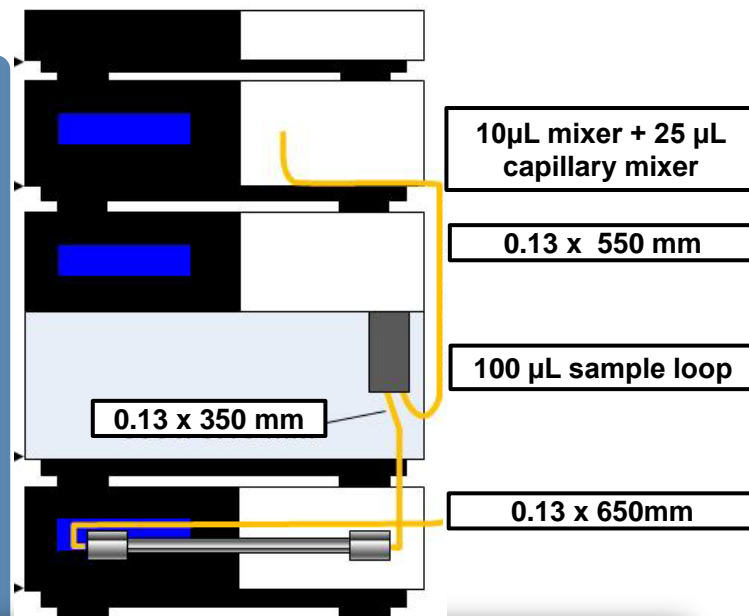
- SRM like -> fragment transitions acquired
- Scheduled target list (Rt,  $m/z$ , collision energy)
- Most sensitive and selective even in highly complex matrices

Maximum sensitivity!

# UHPLC Conditions


## Thermo Scientific™ UltiMate® 3000 RSLC :

- Column: **Accucore aQ 100 mm x 2.1 mm x 2.6 μm**
- Column oven T: 25 °C
- Injection Volume: **1 μL**
- Mobile phase A:  
Water 5mM NH<sub>4</sub>COOH, 0.1% HCOOH
- Mobile phase B:  
MeOH 5mM NH<sub>4</sub>COOH, 0.1% HCOOH
- Flow rate: 300 μL/min





# Q Exactive Conditions – ddMS<sup>2</sup> experiment

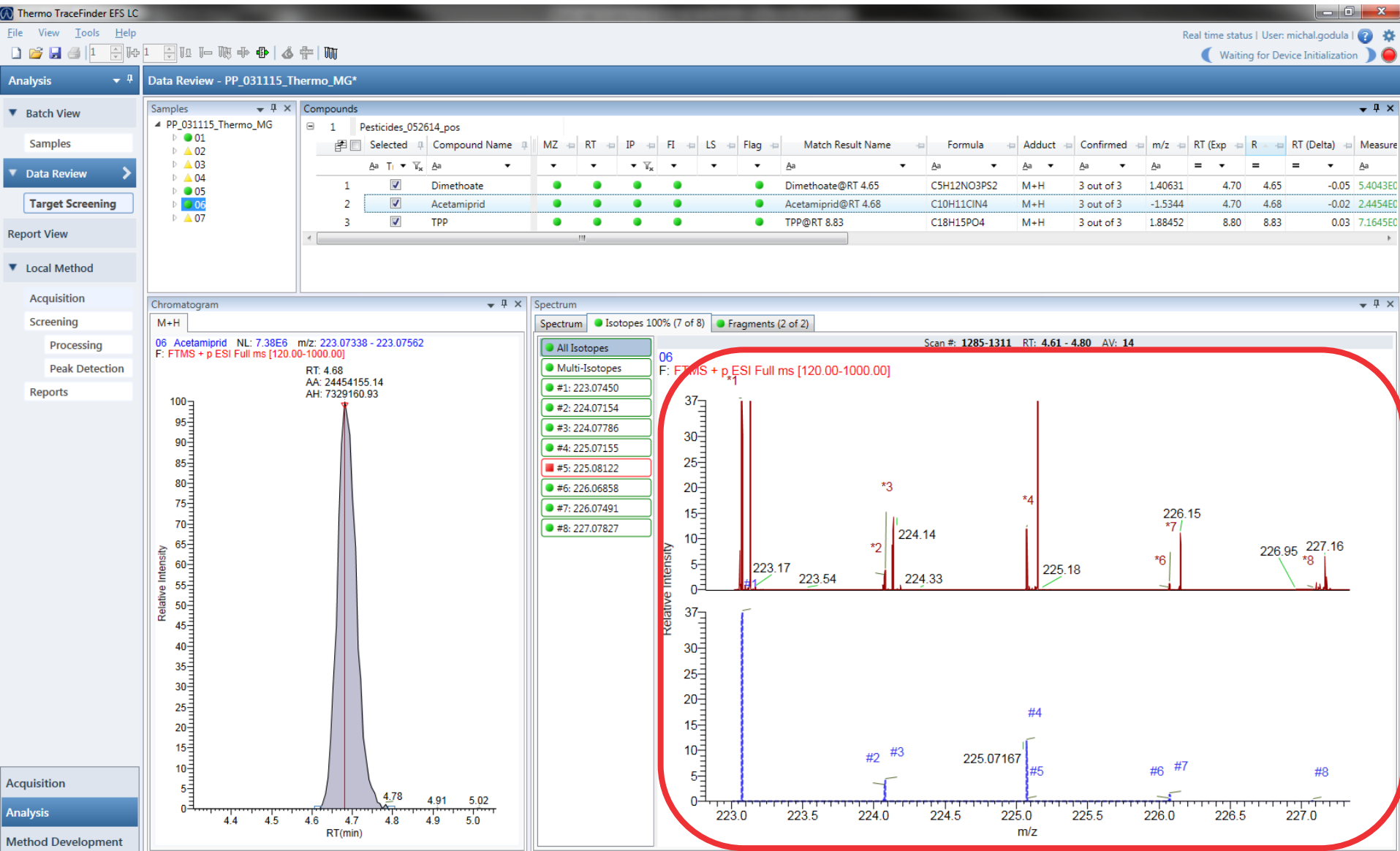
- full MS:resolution **70,000 FWHM**
- scan range **120-1200 m/z**
- ddMS<sup>2</sup>:resolution **17,500 FWHM**
- Inclusion list **on** 
- isolation **1 amu**
- exclusion **5 s**

## List of target analytes

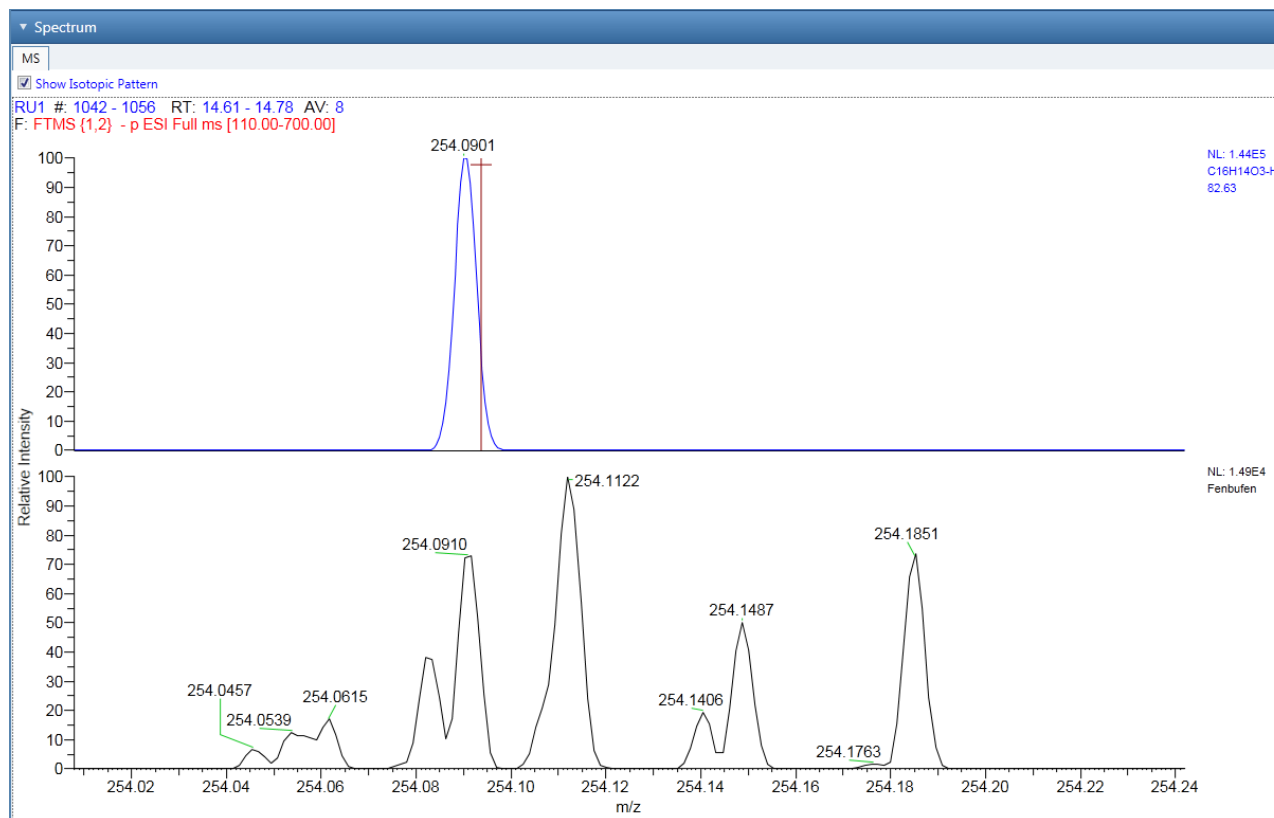
	Mass [m/z]	Polarity	Start [min]	End [min]	NCE	CS [z]	Comment
1	184.01860	Positive	2.23	3.23	35 %		Acephate
2	223.07400	Positive	6.15	7.15	35 %		Acetamidiprid
3	270.12500	Positive	8.47	9.47	35 %		Acetochlor
4	564.12110	Positive	10.11	11.11	35 %		Acrinathrin
5	270.12500	Positive	8.47	9.47	35 %		Alachlor
6	208.11090	Positive	6.49	7.49	35 %		Aldicarb
7	240.10070	Positive	3.99	4.99	35 %		Aldicarb sulfone
8	207.07920	Positive	3.65	4.65	35 %		Aldicarb sulfoxide
9	228.12720	Positive	7.50	8.50	35 %		Ametyrn
10	216.10050	Positive	7.42	8.42	35 %		Atrazine
11	890.52550	Positive	10.21	11.21	35 %		Avermectin-b 1a
12	743.25160	Positive	7.18	8.18	35 %		Azadirachtin
13	346.04380	Positive	8.41	9.41	35 %		Azinphos-ethyl
14	318.01250	Positive	7.81	8.81	35 %		Azinphos-methyl
15	404.12350	Positive	7.80	8.80	35 %		Azoxystrobin
16	326.17450	Positive	8.78	9.78	35 %		Benalaxyl
17	224.09120	Positive	6.92	7.92	35 %		Bendiocarb
18	451.09810	Positive	9.95	10.95	35 %		Beta-cyfluthrin
19	440.15930	Positive	10.65	11.65	35 %		Bifenthrin
20	338.18580	Positive	8.94	9.94	35 %		Bitertanol
21	343.03940	Positive	8.01	9.01	35 %		Boscalid
22	261.02280	Positive	6.89	7.89	35 %		Bromacil
23	375.96080	Positive	8.28	9.28	35 %		Bromuconazole
24	317.16360	Positive	8.29	9.29	35 %		Bupirimate
25	306.16290	Positive	9.43	10.43	35 %		Buprofezin
26	271.09440	Positive	9.11	10.11	35 %		Cadusafos
...							
...							
...							
556	336.03194	Positive	8.84	9.84	35 %		Zoxamide



# Screening Example – Acetamidrid in honey sample



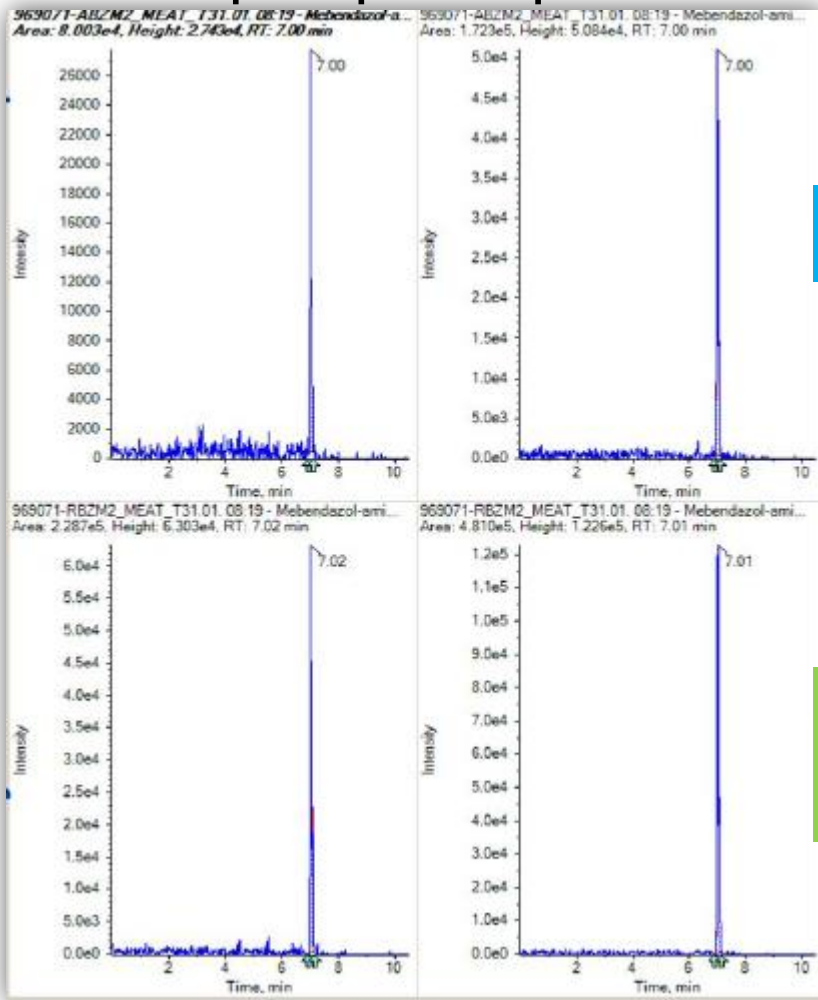
# Isotopic pattern fitting is influenced by resolution



**Mismatched isotopic pattern will lead to false negative!**

# Preventing false positives with HRAM acquisition

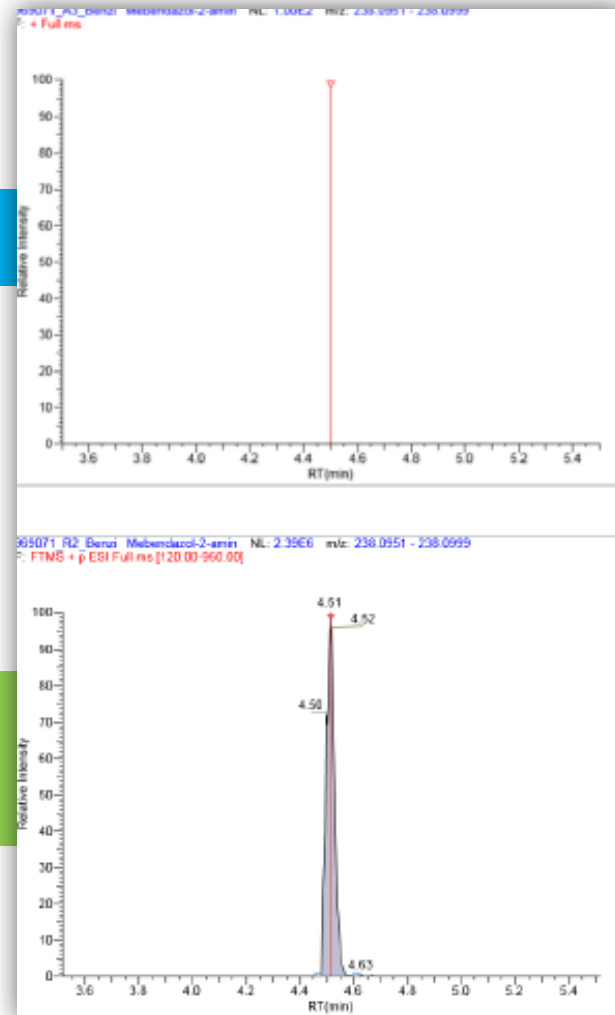
## Triple quadrupole



Sample

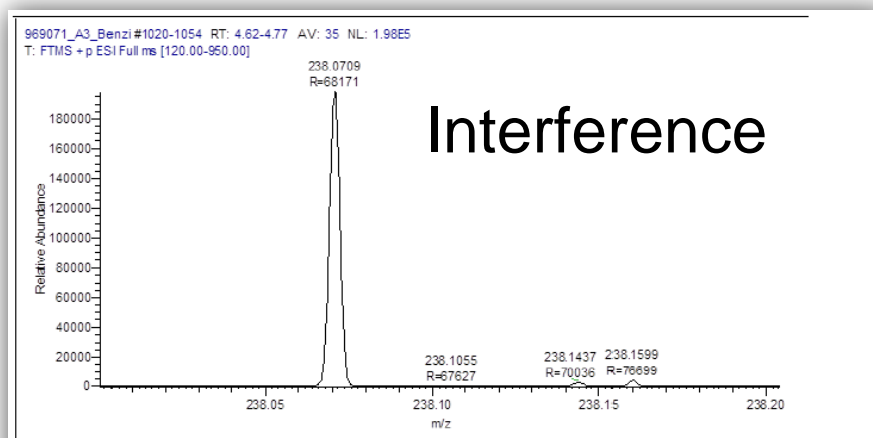
QC sample

## HRAM

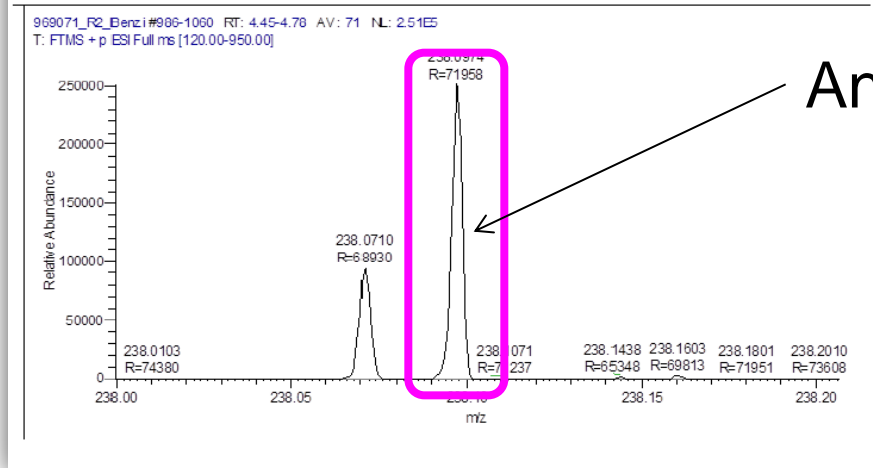


# Preventing false positives with HR acquisition

Sample



QC



# 3 ways of Quantitation/Screening for Routine Work

## Full MS(SIM)/data dependent MS/MS

- Post-acquisition extracted ion chromatograms of parent ions
- Relies on high resolution (selectivity) and MS/MS (confirmation)
- Useful for targeted screening and quantitative methods (QQQ like)

Targeted Workflow!

## Full MS/ All Ion Fragmentation – vDIA\*

- Minimum method development
- Great for screening purposes, but quantitation, too
- No false negatives
- Fully retrospective

Non targeted workflow!

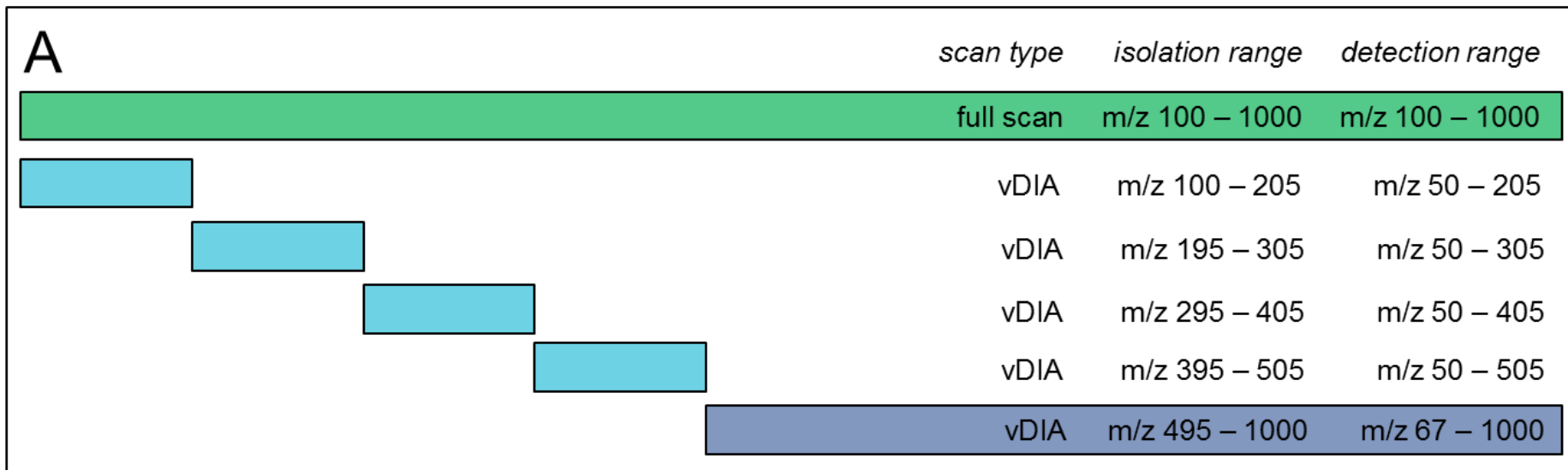
## PRM (Parallel Reaction Monitoring)

- SRM like -> fragment transitions acquired
- Scheduled target list (Rt,  $m/z$ , collision energy)
- Most sensitive and selective even in highly complex matrices

Maximum sensitivity!

# Q Exactive Focus: Variable Data Independent Analysis (vDIA)

## Alternative to All Ions Fragmentation (AIF)



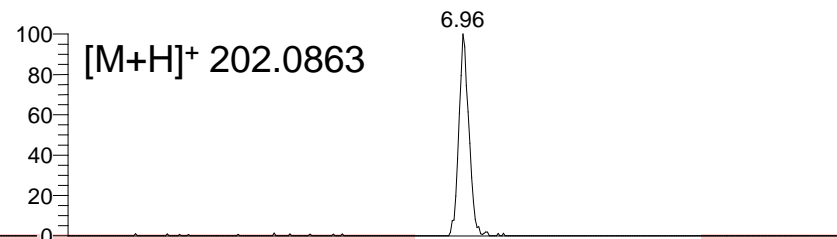
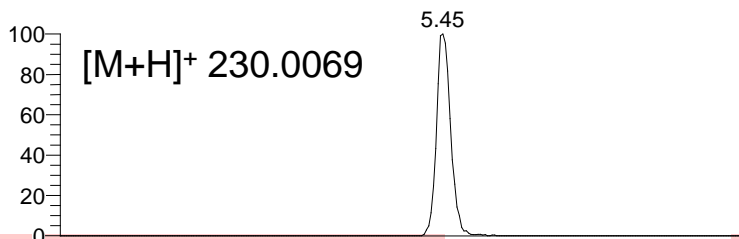
- A complete record of data in full scan as well as in MS/MS
- A cycle time of 1.4 Hz
- Excellent reproducibility
- Higher specificity and response for fragments compared to AIF

# Q Exactive Focus : All Ions Fragmentation (AIF) vs. vDIA

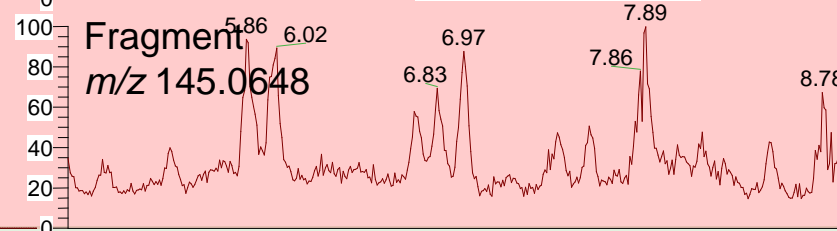
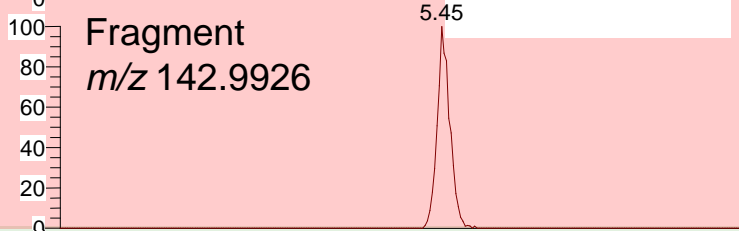
Dimethoate 10 ng/g in wheat

Carbaryl 10 ng/g in wheat

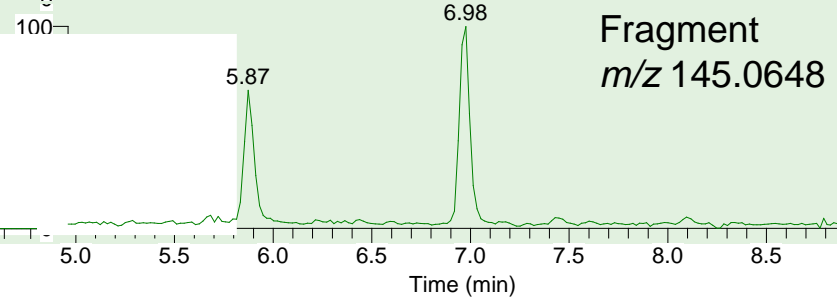
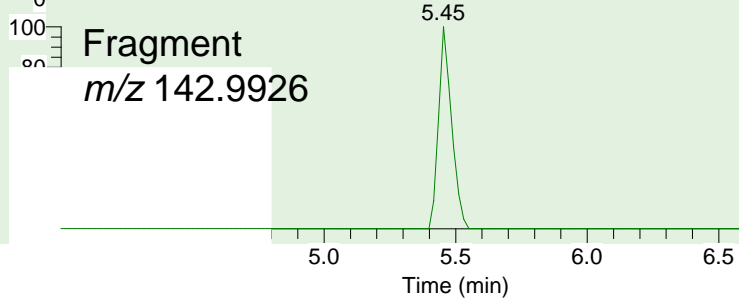
Full Scan  
m/z 135-1000  
RP = 70,000



AIF  
m/z 67-1000  
RP = 70,000



vDIA  
m/z 195-305  
RP = 35,000



Data courtesy of Dr Hans Mol, Rikilt Wageningen, UR



# Results of vDIA – Sensitivity in Tea for 330 compounds

thermo scientific

APPLICATION NOTE 665

Pesticide residues screening analysis in tea and honey using a Q Exactive Focus High-Resolution Mass Spectrometer

# 3 ways of Quantitation/Screening for Routine Work

## Full MS(SIM)/data dependent MS/MS

- Post-acquisition extracted ion chromatograms of parent ions
- Relies on high resolution (selectivity) and MS/MS (confirmation)
- Useful for targeted screening and quantitative methods (QQQ like)

Targeted Workflow!

## Full MS/ All Ion Fragmentation – vDIA\*

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Non targeted workflow

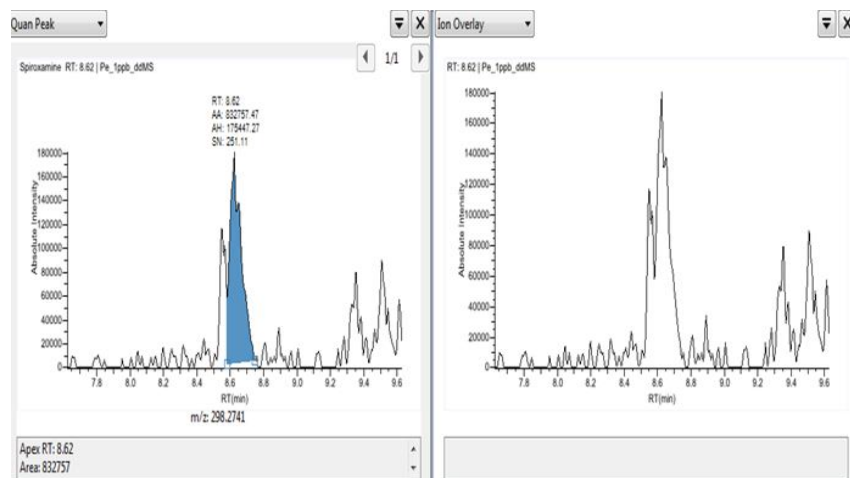
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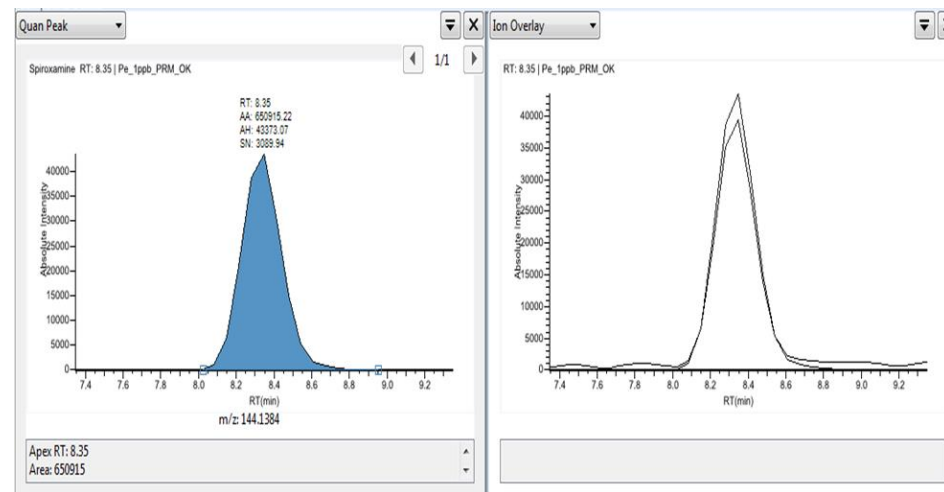
Maximum Sensitivity!

# PRM Provides Maximum Sensitivity – Spiroxamine 1 ppb

## Full Scan-ddMS2



## PRM



# Q Exactive GC



Source options:

Scan range

Resolving power:

Acquisition options:

**EI and CI (NCI/PCI)**

***m/z* 30-3000**

4 settings, up to **120,000 FWHM @ *m/z* 200**

***Non-target acquisition:* Full Scan**

*Targeted acquisition:* SIM, MS/MS, data-dependent MS/MS

*Combinations of the above...*

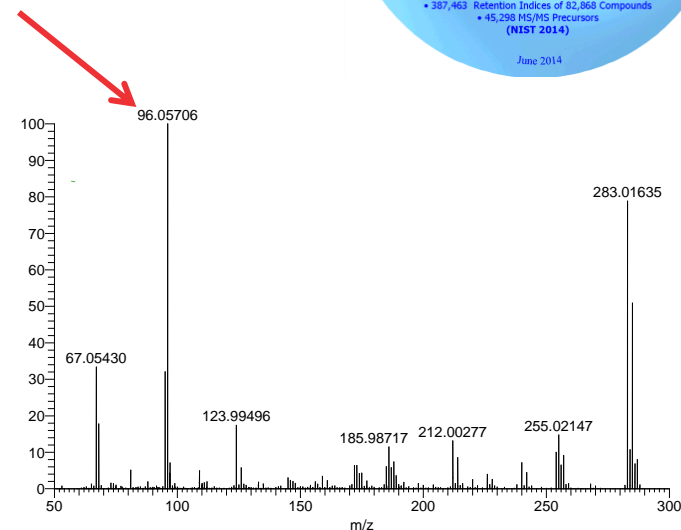
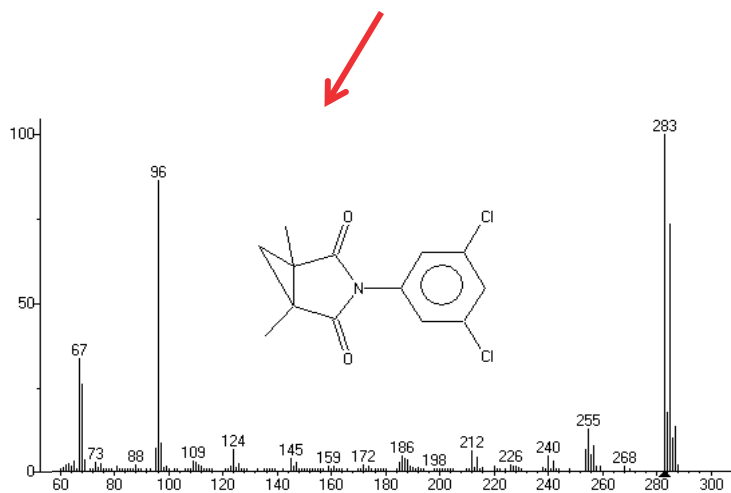
# Why Electron Ionisation?

EI-MS spectra to a large extent instrument-independent

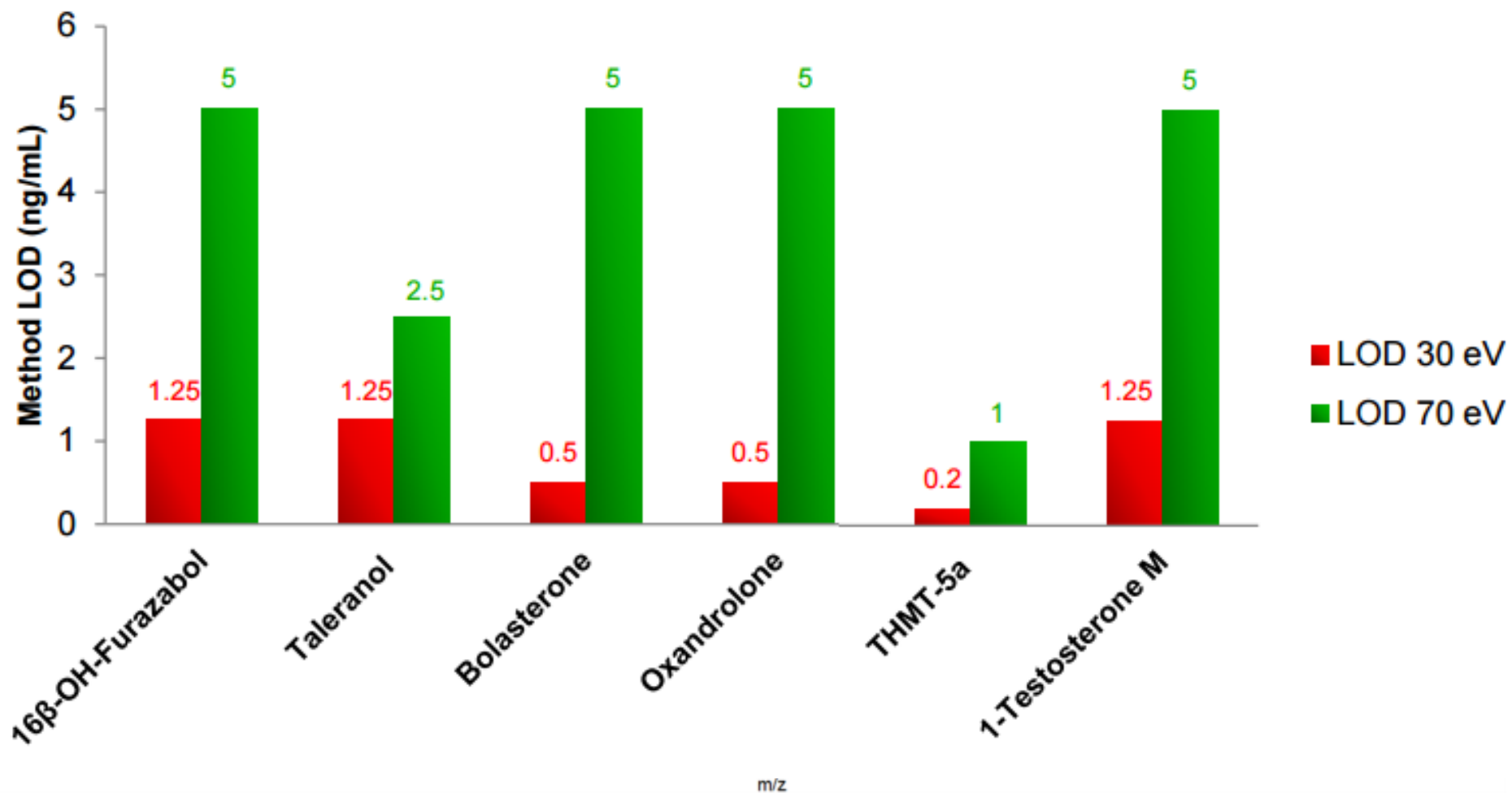
EI-MS libraries available (>242.000 spectra, mostly EI-quadrupole-MS)

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

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



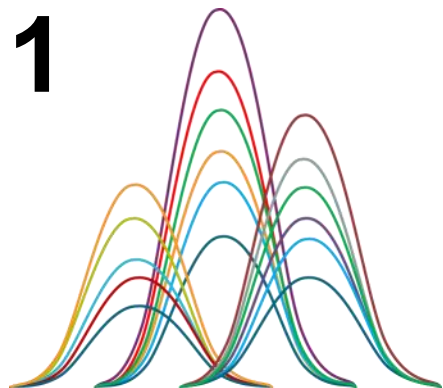
# New: Variable Electron Voltage (VeV)



# Non-targeted Screening with Q Exactive GC

## detect and refine

1



- Sensitive and selective peak detection
- High resolution spectral deconvolution
- Clean spectrum

## generate candidates

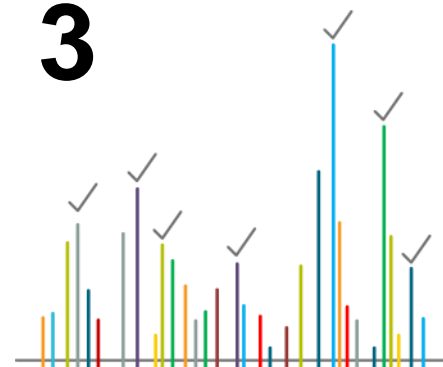
2



- Search spectra against spectral libraries
- HRAM or unit mass
- Candidates list generated

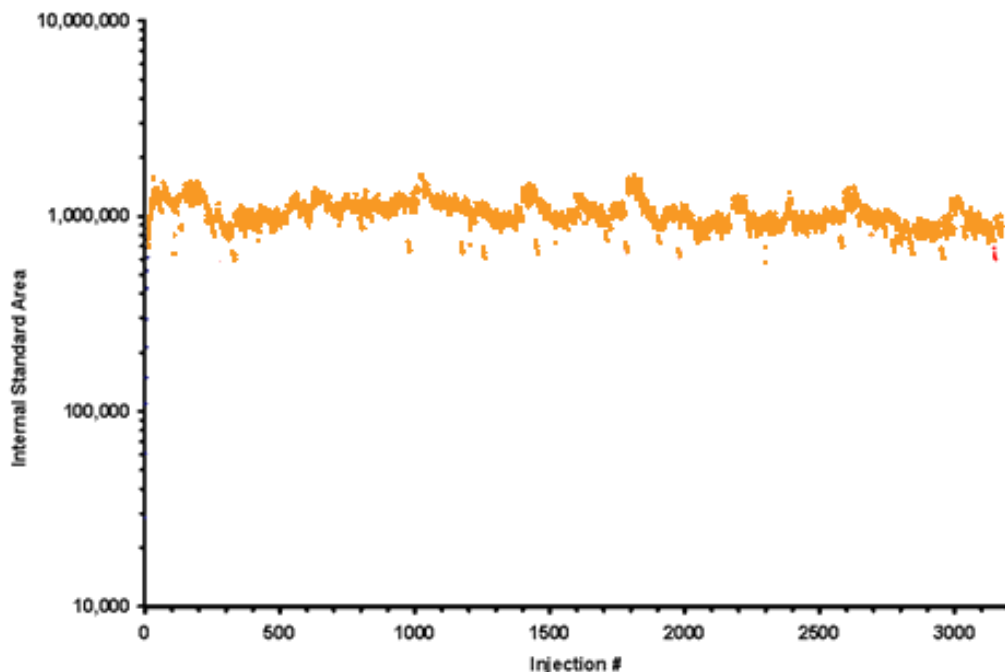
## filter and identify

3



- High resolution filtering of candidates
- Putative identifications made

# Maximum Uptime: Routine-grade Robustness



## ExtractaBrite Ion Source Design

- Dual independent heated zones
- Patented RF lens protects post source ion optics
- Repeller designed to overcome any ion burn

## Source Robustness in Matrix

- >3000 injections ExtractaBrite Ion Source
- BSTFA derivatized urine
- No source maintenance required during study





# “Never Vent” Philosophy

- Patented source plug
  - **GC column change** without venting
- ExtractaBrite Ion Source
  - **Source change** without venting
  - Including all areas where ion burn can form
- Minutes to use the vacuum probe interlock system



# Conclusions

- Analysis with high resolution mass spectrometry is an effective way to increase the scope of the analysis and with simple data acquisition.
- This allows for more compounds to be analysed from a single injection without prior optimization of the acquisition parameters.
- Routine resolving power of 70,000 FWHM eliminates isobaric interferences, increasing confidence in results when screening residues in complex matrices.
- Screening both targeted and non targeted as well as quantitation is possible with Orbitrap

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### Food Additives & Contaminants

Publication details, including instructions for authors, can be found at <http://www.tandfonline.com/loi/tfa>

### Quantitative targeted of relevant pesticides, bakery products by liquid chromatography–mass spectrometry

### Large pesticide multiresidue screening method by liquid chromatography–mass spectrometry



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## Analytica Chimica Acta

journal homepage: [www.elsevier.com/locate/aca](http://www.elsevier.com/locate/aca)



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Anal Bioanal Chem  
DOI 10.1007/s00216-015-8709-z

PAPER IN FOREFRONT

### Liquid chromatography–mass spectrometry with simultaneous selective pesticide

### Targeted analysis of multiple pharmaceuticals, plant toxins and other secondary metabolites in herbal dietary supplements by ultra-high performance liquid chromatography–quadrupole–orbital ion trap mass spectrometry

Lukas Vaclavik\*, Alexander J. Krynitsky, Jeanne I. Rader

María del Mar Gómez-Ramos  
Amadeo R. Fernández-Alba<sup>1</sup>

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Regulatory Science, 5100 Paint Branch Parkway, HFS-717, College Park, MD 20740, USA



### High resolution tandem mass spectrometry method for control of pesticide residues, mycotoxins, and pyrrolizidine alkaloids

Zbynek Dzuman<sup>a</sup>, Milena Zachariasova<sup>a,\*</sup>, Zdenka Veprikova<sup>a</sup>, Michal Godula<sup>b</sup>, Jana Hajslova<sup>a</sup>

<sup>a</sup> University of Chemistry and Technology, Prague, Technická 3, Prague 6, 16628, Czech Republic

<sup>b</sup> Thermo Scientific, Slunecna 27, Prague 10, 10000, Czech Republic

Received: 21 January 2015 / Revised: 8 April 2015 / Accepted: 15 April 2015  
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The image features a dark blue background with vertical light streaks. A horizontal white line with a lens flare effect is positioned across the middle. The word "Thermo" is written in a large, bold, white sans-serif font, with "SCIENTIFIC" in a smaller, spaced-out white sans-serif font below it. A white waveform graphic with several data points is overlaid on the lower part of the logo. The tagline "Transform Your Science" is written in a bold, white sans-serif font at the bottom.

**Thermo**  
SCIENTIFIC

**Transform Your Science**