Redefining GC-MS in the Laboratory with GC Orbitrap

Michal Godula, Ph.D.
Thermo Fisher Scientific
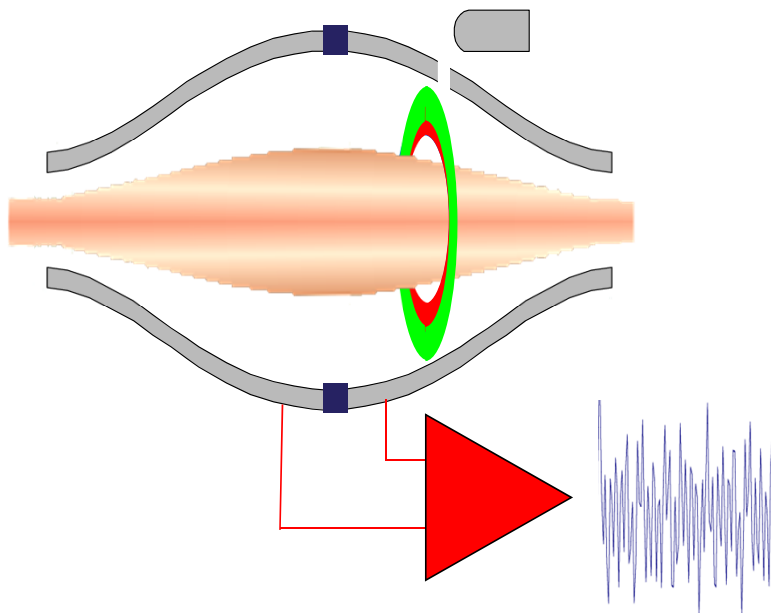michal.godula@thermofisher.com

Thermo Scientific™ Q Exactive™ GC Hybrid Quadrupole-Orbitrap™ GC-MS/MS System

Offering Unprecedented Depth in GC-MS Analysis

- Hugely powerful HR/AM performance for GC-MS
- Uncompromised quantitative and qualitative capability
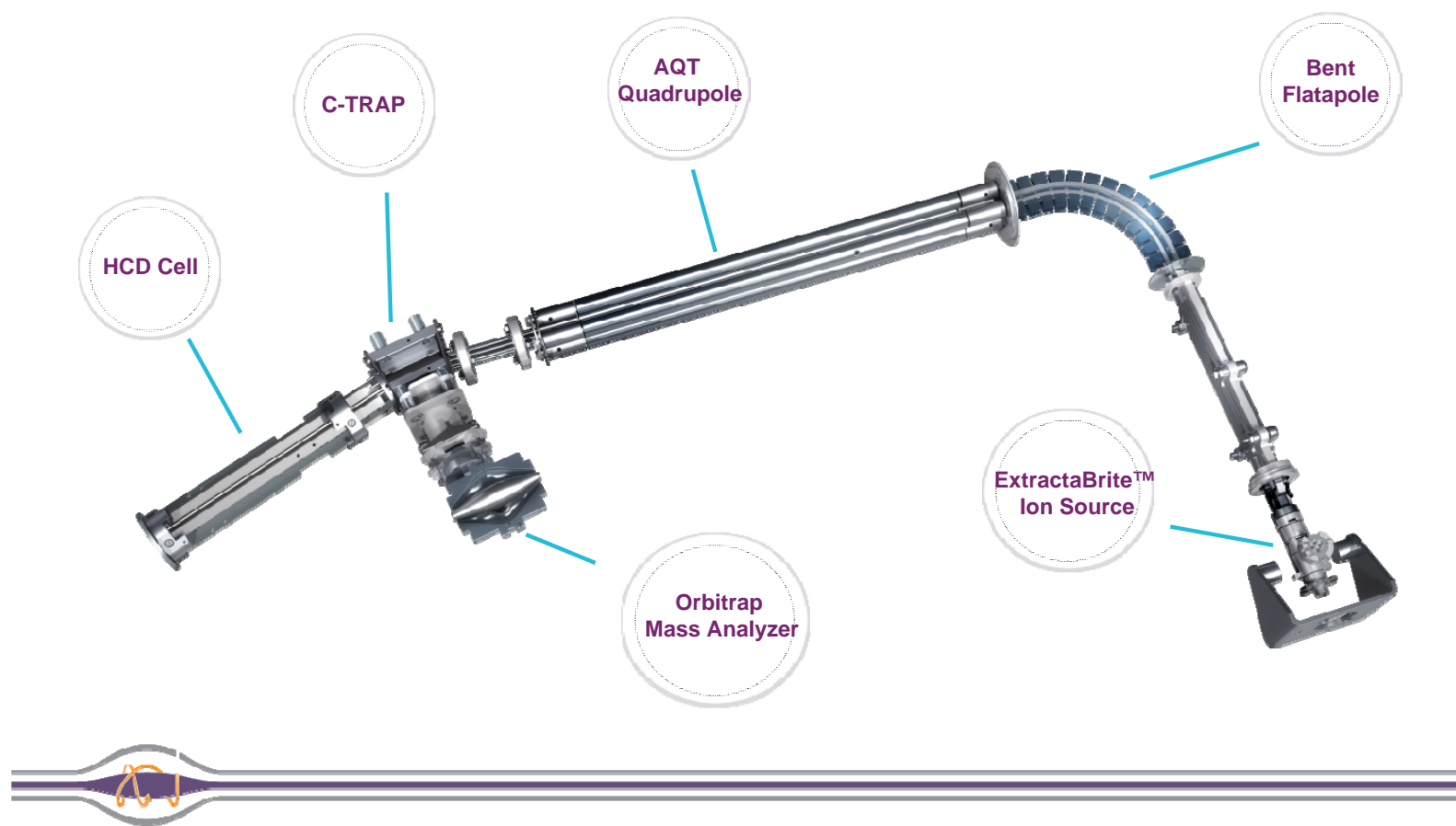- Reliable, robust and easy to operate

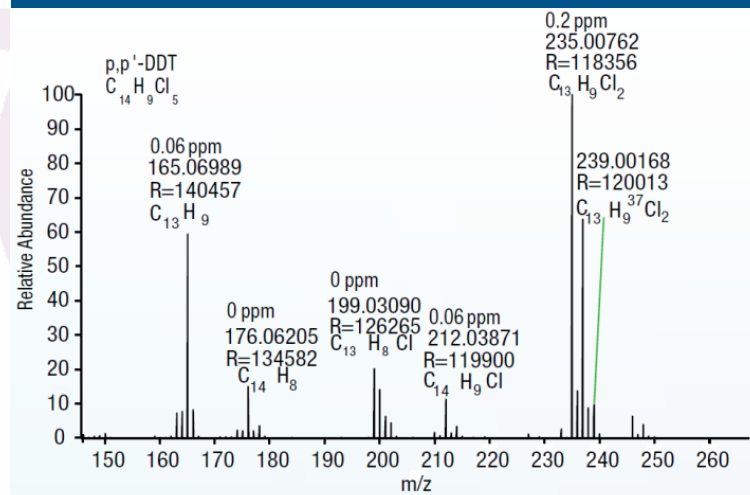Makarov A. *Anal. Chem.* 2000, *72*, 1156-1162.

Bringing GC and Orbitrap Technology Together

C-TRAP

AQT Quadrupole

Bent Flatapole

HCD Cell

ExtractaBrite™ Ion Source

Orbitrap Mass Analyzer

ThermoFisher
SCIENTIFIC

Highest selectivity and confidence with high resolving power

Unrivalled Resolving Power

- Up to 120k @ m/z 200

- Allows excellent selectivity in complex matrix, maintaining confidence in detection and identification

- Drives highly resolved data sets and high capacity peak detection

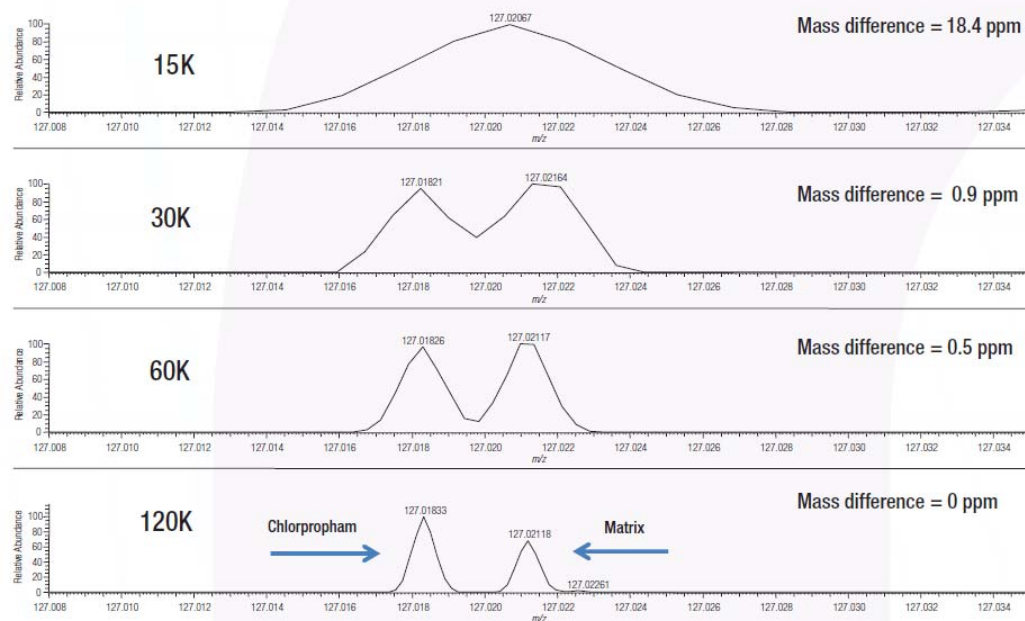- Fast scanning Orbitrap for well defined, reproducible GC peaks
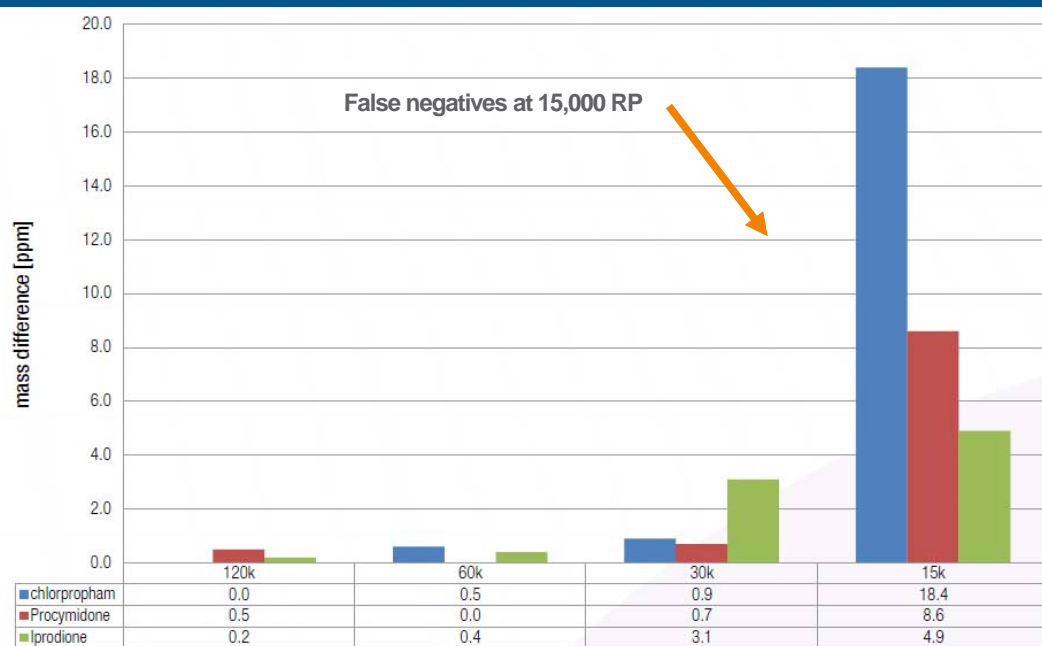
Thermo Fisher
SCIENTIFIC

Highest selectivity and confidence with high resolving power

Chlorprophram

- 10 ng/g QuEChERS extract of leek

- Full scan with resolving power of >30k (FWHM @ m/z 200) provides interference free detection

- Excellent mass accuracy for confident identification

Thermo Fisher
SCIENTIFIC

High resolving power crucial to avoid false negatives

False negatives at 15,000 RP

mass difference [ppm]	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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Highest selectivity and confidence with high resolving power

Fast acquisitions

- Important for accurate profiling of narrow GC peaks

- Full scan with resolving power of 60 (FWHM @ m/z 200) generates **17 scans**

- Fast enough for GC!

XIC of dieldrin in baby food (*m/z* 262.85642) 100ppb

60,000 RP (m/z 200)
2.4 s peak

17 scans

Thermo Fisher
S C I E N T I F I C
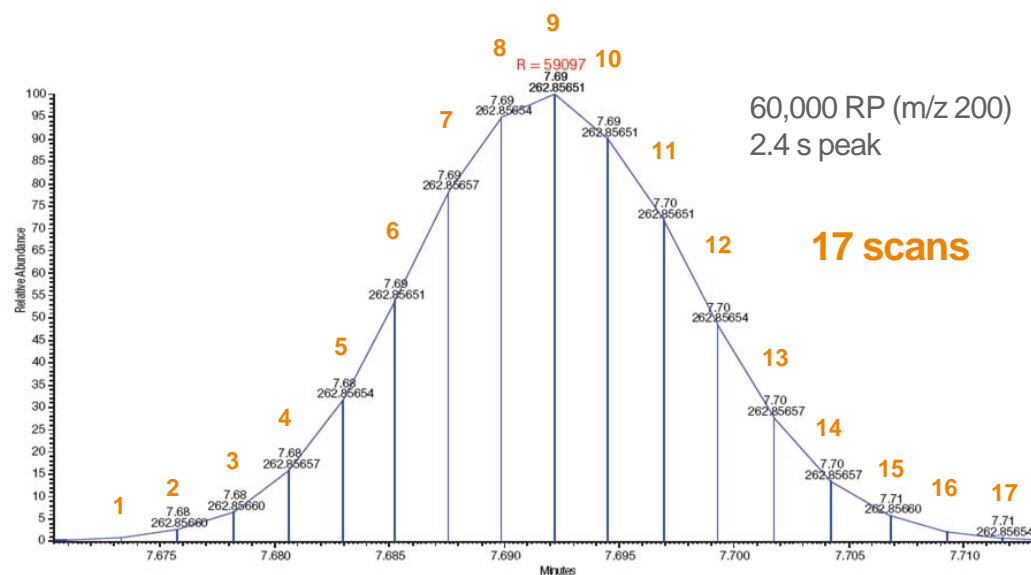
Breakthrough in GC-MS Performance

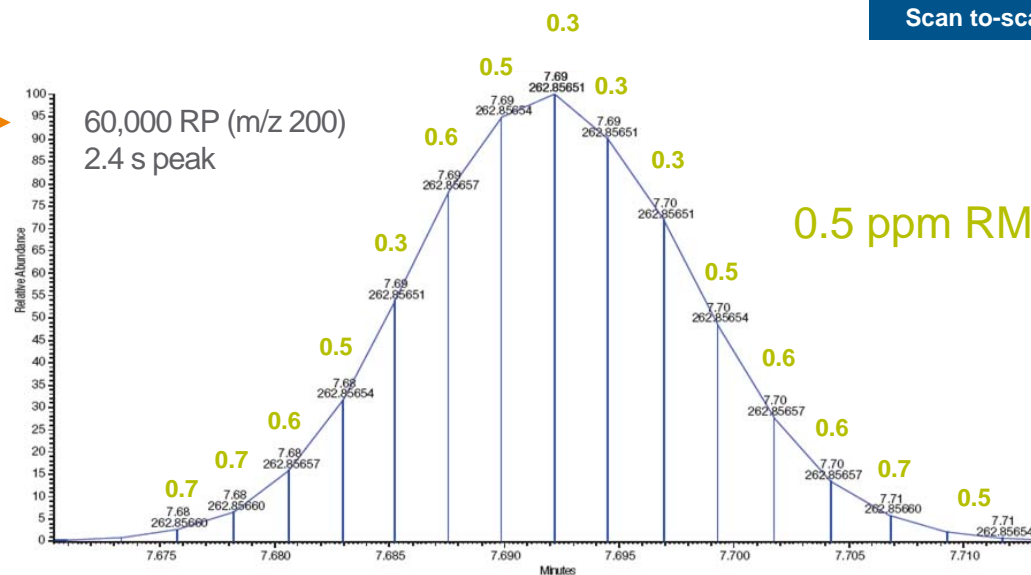## Consistently excellent mass accuracy

< 1ppm (internal)

< 3ppm (external)

- Scan-to-scan
- Low level in matrix
- Over full mass range
- Over full concentration range
- No need for continuously calibrating in sequence

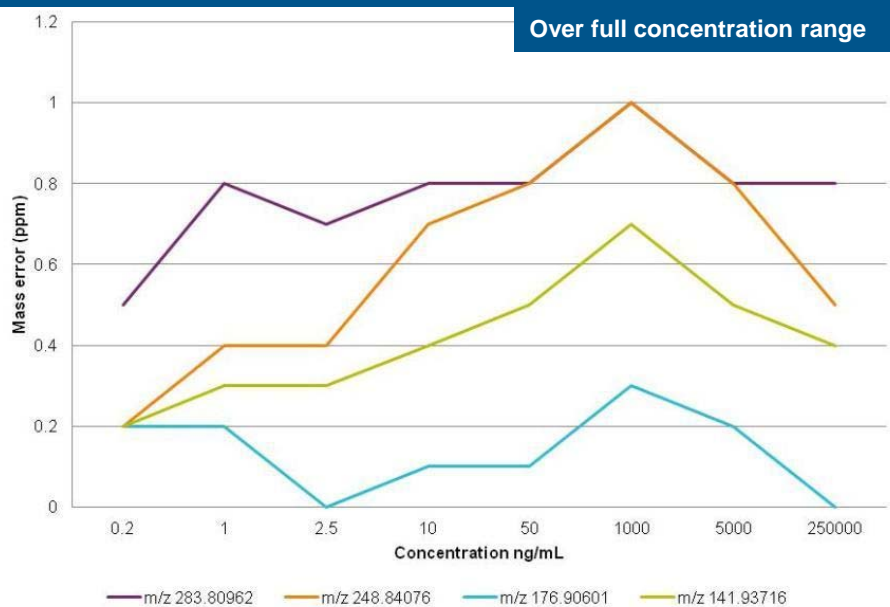XIC of dieldrin in baby food (*m/z* 262.85642) 100ppb

Scan to-scan

60,000 RP (m/z 200)
2.4 s peak

0.5 ppm RMS

Breakthrough in GC-MS Performance

Consistently excellent mass accuracy

Over full concentration range

m/z 283.80962 · m/z 248.84076 · m/z 176.90601 · m/z 141.93716

Mass accuracy over >6 orders

- Hexachlorobenzene
- 0.2 – 250,000 ng/mL
- Mass accuracy within 1 ppm
- Orbitrap spectral fidelity independent of concentration

Data courtesy of Hans Mol and Marc Tienstra, RIKILT

Q Exactive GC system

Unprecedented Depth in Analysis

RP 120,000 (FWHM @ m/z 200)

EI/CI; Full-scan, Timed-SIM

MS/MS capability

Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS System

Unprecedented Depth in Analysis

RP 120,000 (FWHM @ m/z 200)

EI/CI; Full-scan, Timed-SIM

MS/MS capability

Redefining Routine GC-MS

RP 60,000 (FWHM @ m/z 200)

EI/CI; Full-scan; Timed-SIM

Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS System

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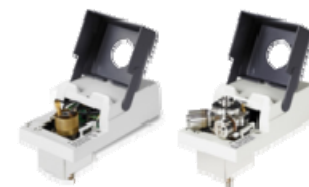
Thermo Fisher[TM] TRACE[TM] 1310 GC system

Unique modular injector and detector design

Short cycle time

Insight into the Orbitrap GC-MS Systems
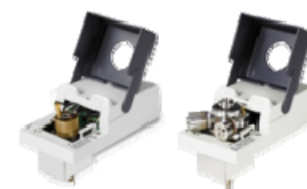
TRACE 1310 GC system

Unique modular injector and detector design

Short cycle time

Thermo Scientific™ ExtractaBrite™ ion source technology

Routine grade robustness

Patented RF lens

Removable without breaking vacuum through VPI

Vacuum-free column replacement through VPI

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Insight into the Orbitrap GC-MS Systems

Orbitrap mass analyzer

Incredible HRAM performance

Extended Dynamic Range

TRACE 1310 GC system

Unique modular injector and detector design

Short cycle time

Thermo Scientific™ ExtractaBrite™ ion source technology

Routine grade robustness

Patented RF lens

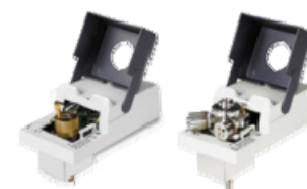**Removable without breaking vacuum through VPI**

Vacuum-free column replacement through VPI

Thermo Fisher
SCIENTIFIC

Resolving Power

Up to 120,000 at *m/z* 200

- Maximum selectivity
- Fast enough for GC

Mass Accuracy

< 1ppm
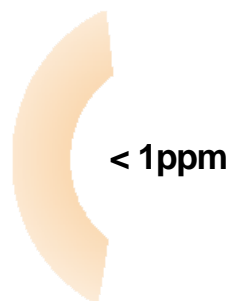
- Every scan
- All concentrations
- In complex matrix
- Across the mass range
- Everyday!

Sensitivity

ppt

- In full-scan
- High selectivity
- High spectral fidelity

Dynamic Range

>6 orders

- Excellent coverage in sample profiling
- "Triple quad grade" quantitation in full-scan

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Non- targeted full scan acquisition

1x → Full-scan →

Targeted quantitation

"the things you expect"
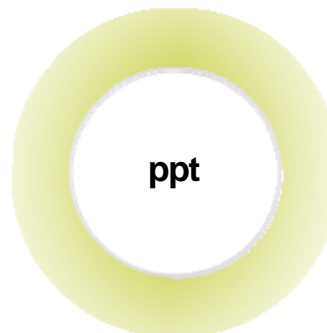
i.e. Commonly used pesticides for a particular commodity

+

Targeted screening

"the things you may suspect"

i.e. Unusual, banned or alternate pesticides for particular commodity

+

Non-targeted screening

"the things you don't expect"

i.e. Other contaminants or compounds, could be known or unknown

Known unknown ID

Unknown unknown ID

Thermo Fisher SCIENTIFIC
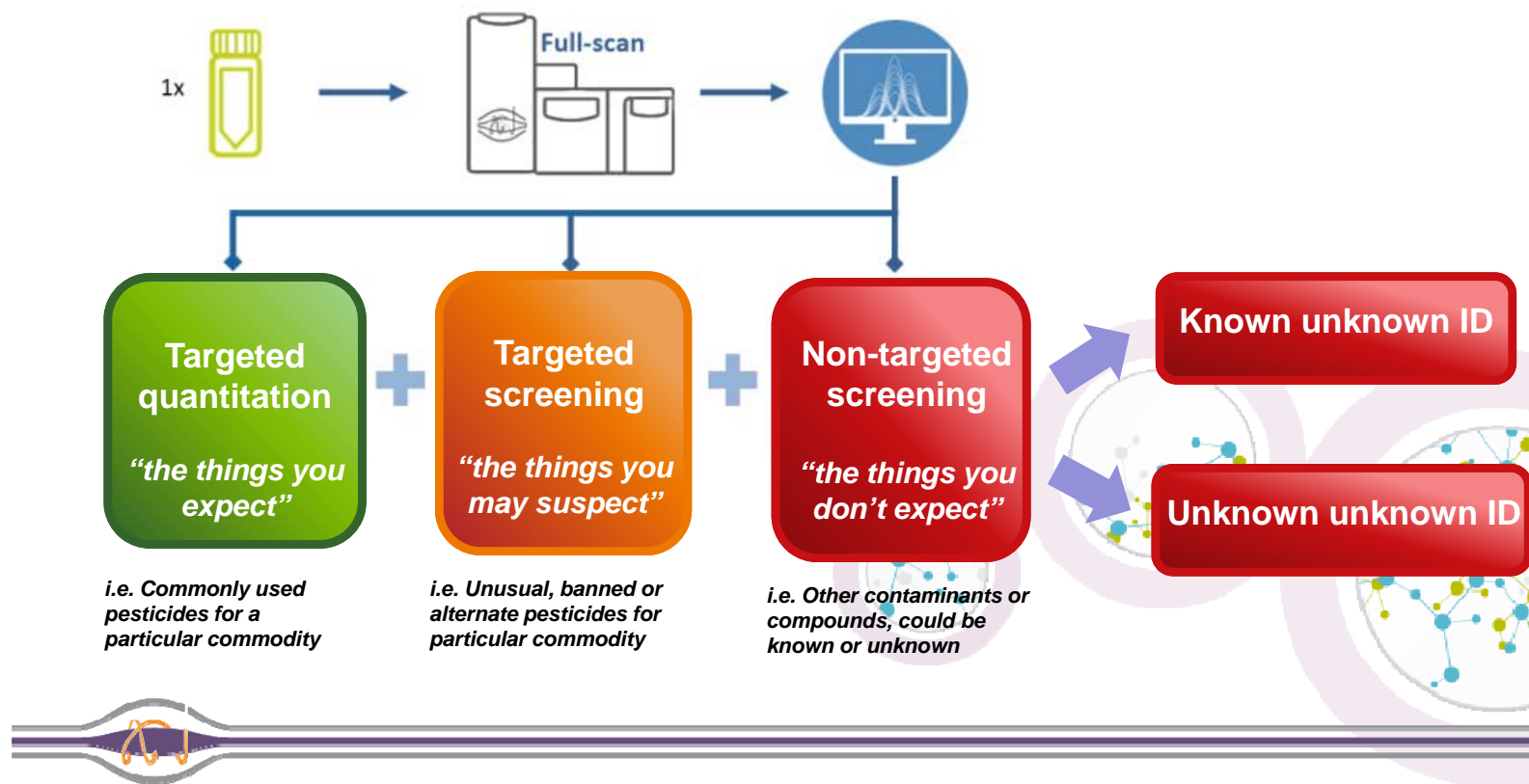
- 4 O-ring samples
  - **A** Red
  - **B** Brown
  - **C** White
  - **D** Black
  - **Blank** (control)

- Solvents
  - **Water**
  - **5M NaCl**
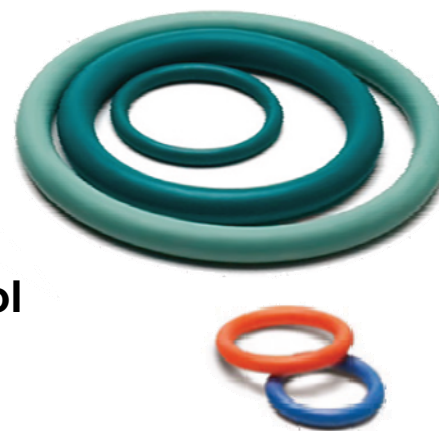 - **50% Ethanol**
  - **100% Ethanol**
  - 1% PS-80
  - 0.5N NaOH
  - 0.1M Phosphoric Acid
- **40 ° C for 30 days**

SMITHERS
R A P R A

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GC-MS Instrument Conditions

Thermo Scientific™ Q Exactive™ GC
Hybrid Quadrupole-Orbitrap GC-MS/MS

Thermo Scientific™ Trace™ 1310 GC

Thermo Scientific™ TraceFinder™

TRACE 1310 GC Parameters

Injection Volume (mL):	1
Liner	Single gooseneck
Inlet (°C):	280
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program:	
Temperature 1 (°C):	40
Hold Time (min):	1
Temperature 2 (°C):	320
Rate (°C/min)	15
Hold Time (min):	10

Q Exactive GC MS Parameters

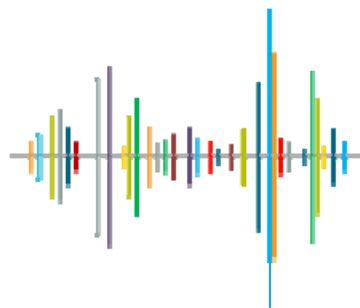|	
Transfer line (°C):	280
Ionization type:	EI
Ion source(°C):	230
Electron energy (eV):	70
Acquisition mode:	Full scan
Mass range (Da)	50-650
Mass resolution (FWHM):	**60k**
Lockmass (m/z):	207.03235

TraceFinder Non-targeted Screening Overview

1. Detect and refine
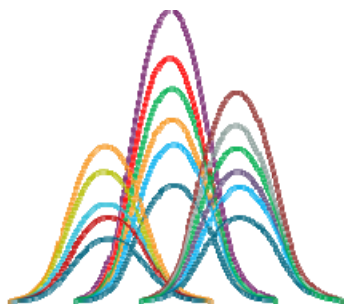
- Sensitive and selective peak detection
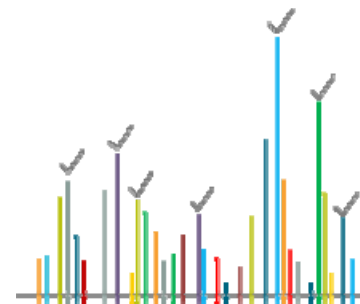- High resolution spectral deconvolution
- Clean spectrum

2. Generate candidates

- Search spectra against spectral libraries
- HRAM o low resolution spectral libraries (NIST, Wiley…)
- Candidates list generated

3. Filter and identify

- High resolution filtering of candidates
- Putative identifications made

Automatic compound identification

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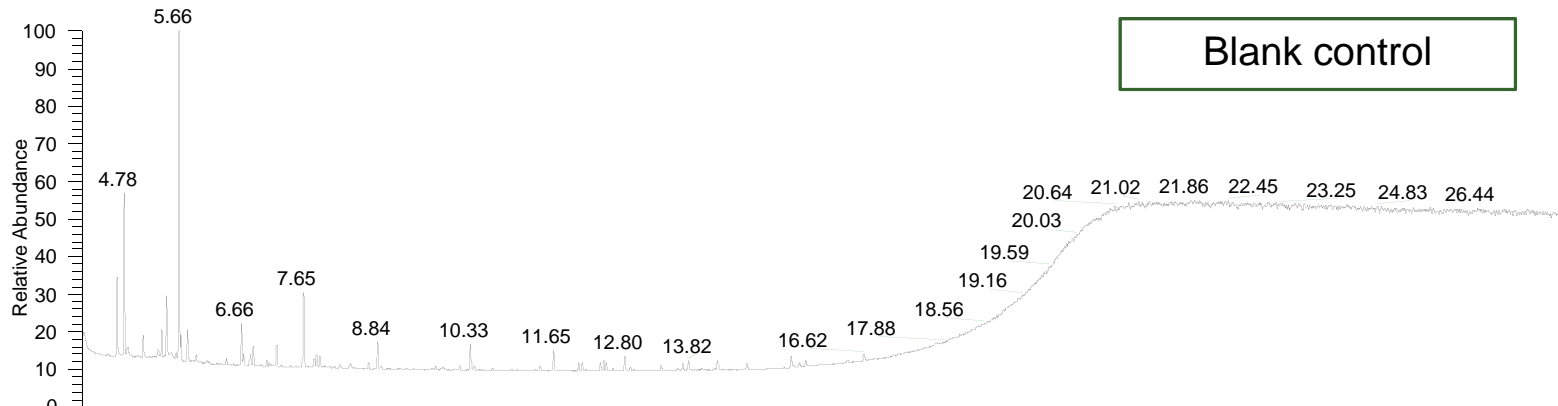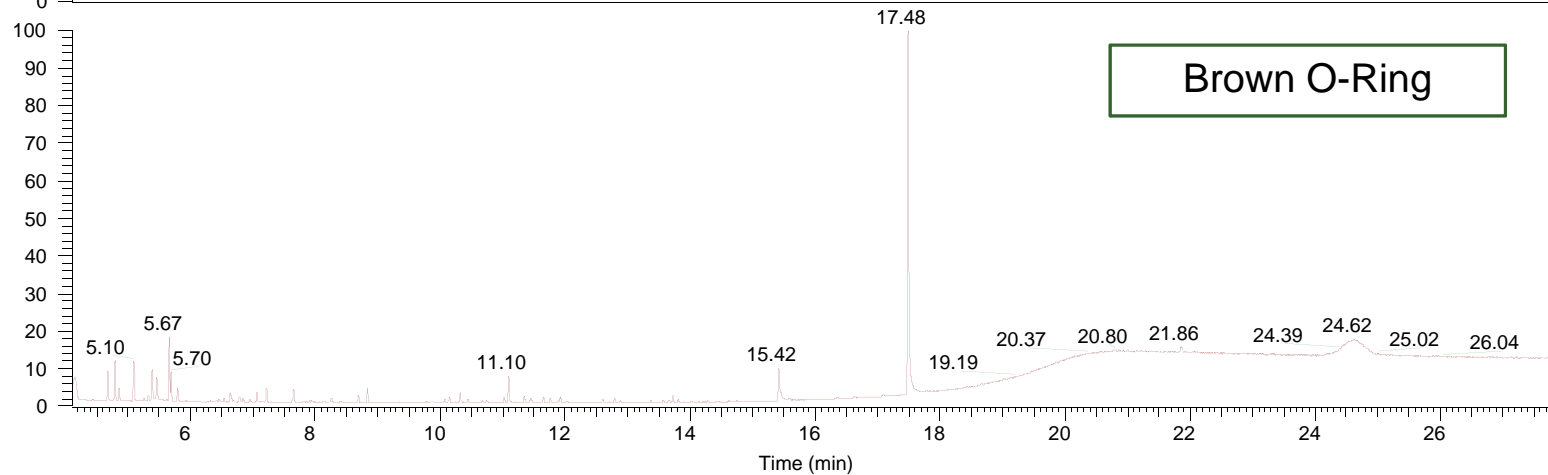
- TraceFinder first performs an **accurate mass** deconvolution of the data.
- Extracts all of the peaks in the chromatogram.
- Provides a cleaned spectrum for library matching.
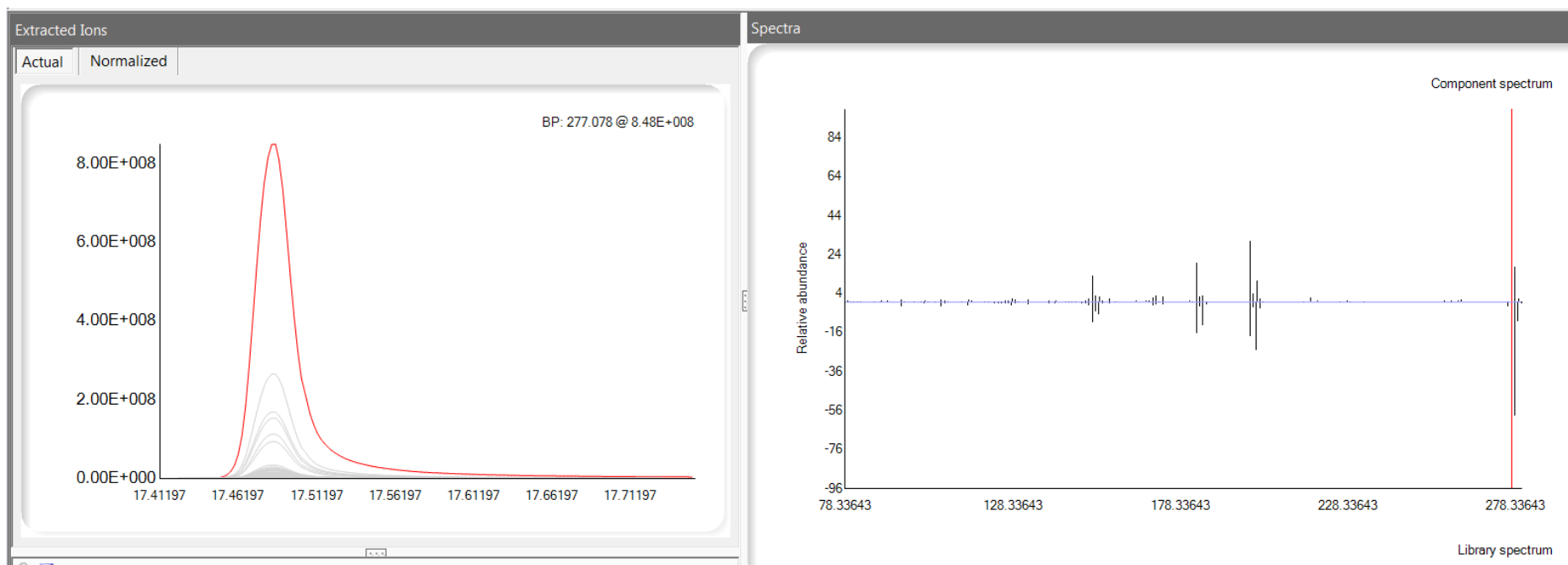
- 2051 peaks were extracted from the brown O-ring sample

Heat Map indicates the peaks elevated in the sample.

Thermo Fisher SCIENTIFIC
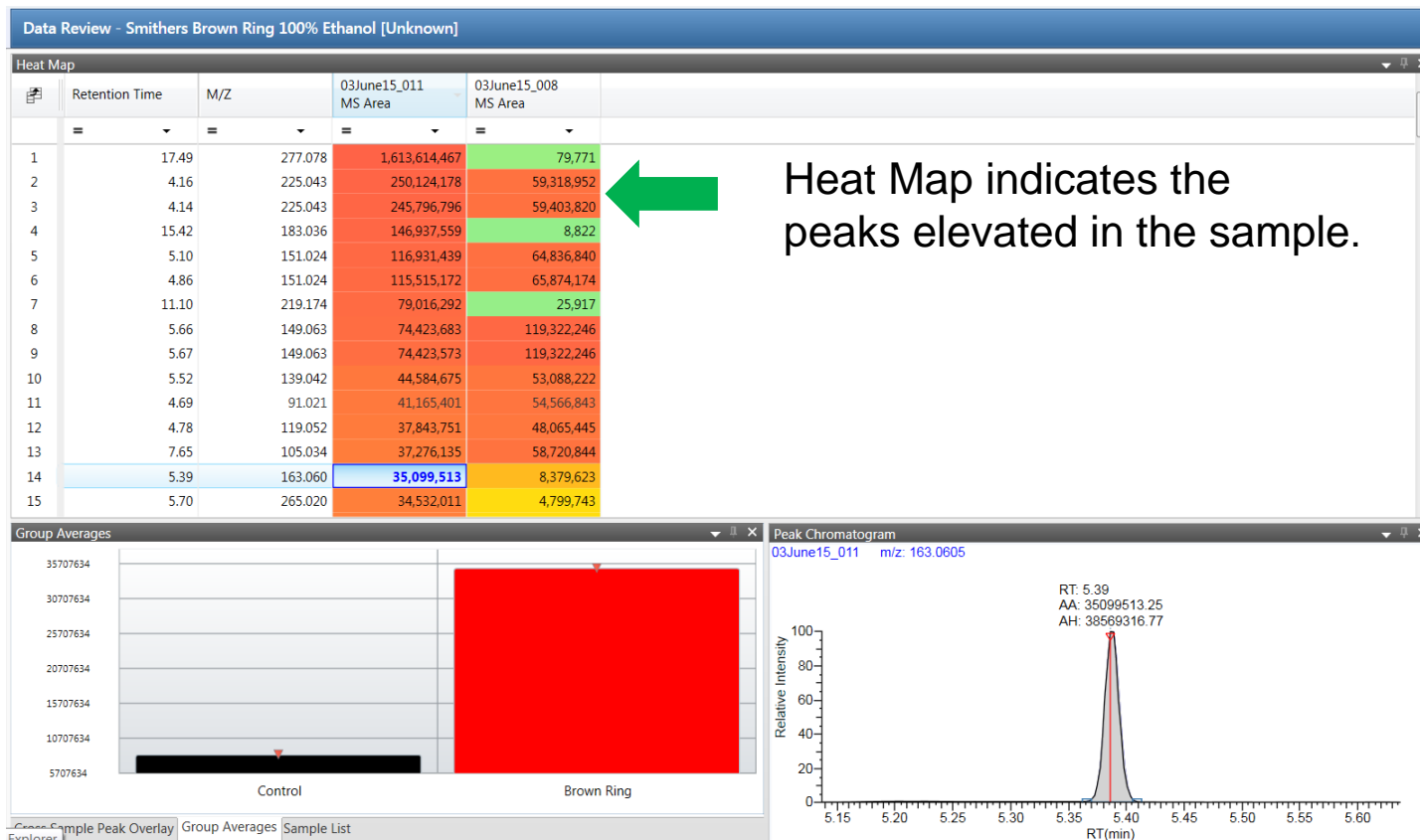
Cross Sample Peak List

	Retention Time	M/Z	Control Average Area	Brown Ring Average Area	Brown Ring Fold
	= ▼	= ▼	= ▼	= ▼	= ▼
1	11.93	263.201	1	15,395,04[	15,395,046
2	16.33	260.075	1	2,782,29[	2,782,295
3	12.89	219.037	1	2,770,68[	2,770,686
4	17.49	277.078	79,771	1,613,614,46[	20,228
5	15.42	183.036	8,822	146,937,55[	16,655
6	13.57	185.042	1,249	5,963,24[	4,773
7	11.10	219.174	25,917	79,016,29[	3,049
8	11.02	221.154	2,586	4,183,05[	1,617
9	11.46	185.042	6,077	6,840,82[	1,126
10	18.01	183.036	4,685	4,161,11[	888

Peaks list can also be sorted by <u>fold difference</u> compared with control to isolate the differential peaks that could be low or high intensity.

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Peak Identification

Score	Matched Compound	Formula	CAS	SI	HRF Score	M+ m/z	M+	M+ Lib	% Elements
94.4	1,4-Dihydrophenacetic acid,...	C18H30O2		728	99.4959	278.22403	Yes	Yes	100
70.4	1,5-Dioxaspiro[5.6]dodeca-7,...	C18H32O2Si2		524	99.8259	336.19353	No	Yes	100
57.7	Benzoic acid, 3,5-bis(1,1-dim...	C17H26O3	1620-64-0	706	58.9058	278.18764	No	Yes	100
56.8	3,5-di-tert-Butyl-4-hydroxyph...	C17H26O3	20170-32-5	659	58.9058	278.18764	No	Yes	100
55.4	Benzenemethanol, 3,5-bis(1,1...	C17H26O3	14387-17-8	591	58.9058	278.18764	No	Yes	100
51.3	Monoallyl phthalate, TBDMS ...	C17H24O4Si		517	52.4488	320.14383	No	No	100
44.5	2,6-Bis(tert-butyl)phenol, TMS...	C17H30OSi	10416-73-6	514	35.6312	278.20604	No	Yes	100
42.4	12-Cyclohex-3-enyl-3-methyl...	C23H24N2O		533	29.2285	344.18831	No	Yes	100
41.6	6-Oxo-5-phenyl-2,3,5,6-tetrah...	C16H13N3O	87365-22-8	525	27.7606	263.10531	No	Yes	100

Spectra

26 Hits from NIST are sorted based on:

- Spectral matching
- High Resolution Filtering (HRF) score.

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Combined SI and HRF values give an overall score (%) to quickly and confidently identify the compound. Eliminates other hits that would be valid if only SI used.

Peak Identification

Score	Matched Compound	Formula	CAS	SI	HRF Score	M+ m/z	M+	M+ Lib	% Elements
94.4	1,4-Dihydrophenacetic acid,...	C18H30O2		728	99.4959	218.22403	Yes	Yes	100
70.4	1,5-Dioxaspiro[5.6]dodeca-7,...	C18H32O2Si2		524	99.8259	336.19353	No	Yes	100
57.7	Benzoic acid, 3,5-bis(1,1-dim...	C17H26O3	1620-64-0	706	58.9058	218.18764	No	Yes	100
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51.3	Monoallyl phthalate, TBDMS ...	C17H24O4Si		517	52.4488	320.14383	No	No	100
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42.4	12-Cyclohex-3-enyl-3-methyl...	C23H24N2O		533	29.2285	344.18831	No	Yes	100
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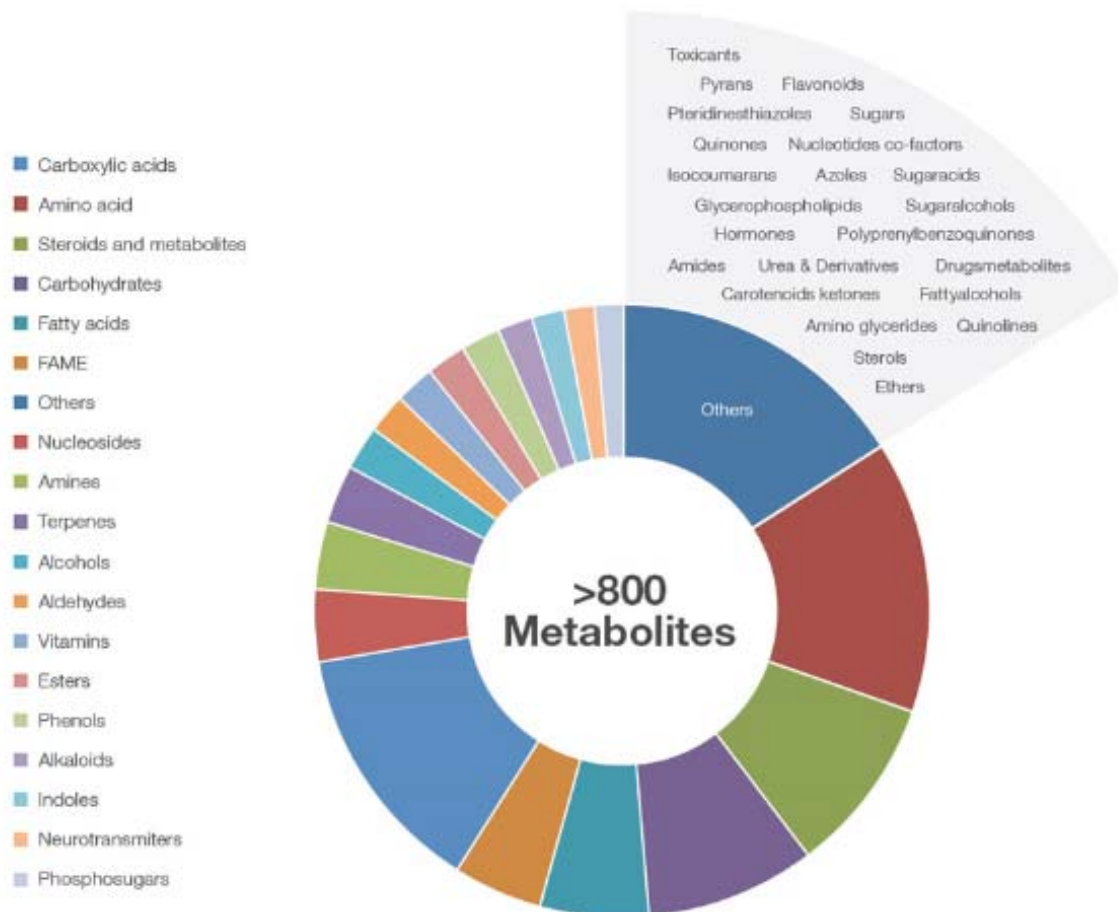| Measured m/z	Area	Fragment ID	Theo m/z	Mass error (ppm)
278.22412	956521	C(12)18 H30O2	278.22403	0.32348
264.20401	2825159	C(12)16 C(13)1 H27O2	264.2039	0.39818
263.20071	15464145	C(12)17 H27O2	263.20055	0.6079
249.18506	17789	C(12)16 H25O2	249.1849	0.64209
236.17262	717539	C(12)14 C(13)1 H23O2	236.1726	0.06436
235.16931	4502672	C(12)15 H23O2	235.16925	0.25514
234.19318	420047	C(12)15 C(13)1 H25O	234.19334	0.70369
233.19005	2618908	C(12)16 H25O	233.18999	0.2573
233.15364	179702	C(12)15 H21O2	233.1536	0.17156
232.18231	164562	C(12)16 H24O	232.18216	0.64604
222.15691	15338	C(12)13 C(13)1 H21O2	222.15695	0.20166
221.15359	145557	C(12)14 H21O2	221.1536	0.04522
220.17767	117435	C(12)14 C(13)1 H23O	220.17769	0.11264
220.14131	70817	C(12)13 C(13)1 H19O2	220.1413	0.02362
219.17430	717976	C(12)15 H23O	219.17434	0.1825
219.13797	551886	C(12)14 H19O2	219.13795	0.09127
218.16208	227603	C(12)14 C(13)1 H21O	218.16204	0.16135
217.15871	1435532	C(12)15 H21O	217.15869	0.0921
207.13797	43932	C(12)13 H19O2	207.13795	0.09655
205.15869	28947	C(12)14 H21O	205.15869	0
205.12231	94843	C(12)13 H17O2	205.1223	0.04875
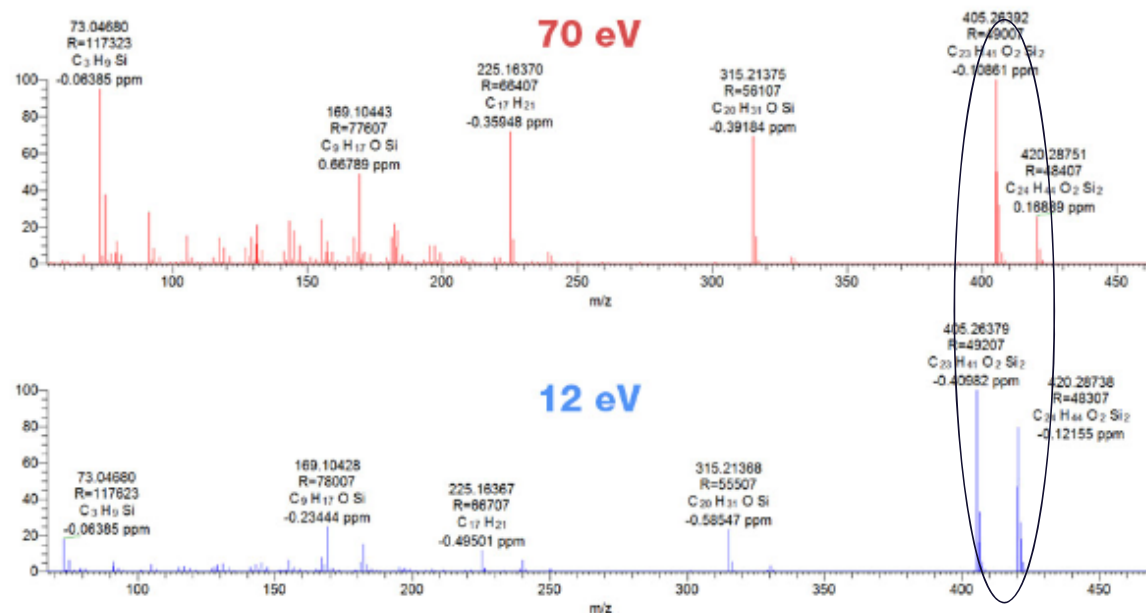
• Top: spectrum acquired with classic 70eV
• Bottom: spectrum acuquired with low 12eV, showing enhancement of molecular ion

The key benefits of VeV are:
Fully automated for optimum performance: VeV set-up is very simple and easy with fast, fully automated tuning.
Increased confidence in identification: VeV promotes molecular ion and diagnostic high mass signals, important information for compound identification and confirmation.

- Carboxylic acids
- Amino acid
- Steroids and metabolites
- Carbohydrates
- Fatty acids
- FAME
- Others
- Nucleosides
- Amines
- Terpenes
- Alcohols
- Aldehydes
- Vitamins
- Esters
- Phenols
- Alkaloids
- Indoles
- Neurotransmiters
- Phosphosugars

>800 Metabolites

Toxicants, Pyrans, Flavonoids, Pteridinesthiazoles, Sugars, Quinones, Nucleotides co-factors, Isocoumarans, Azoles, Sugaracids, Glycerophospholipids, Sugaralcohols, Hormones, Polyprenylbenzoquinones, Amides, Urea & Derivatives, Drugsmetabolites, Carotenoids ketones, Fattyalcohols, Amino glycerides, Quinolines, Sterols, Ethers, Others

- High resolution, high mass accuracy spectra acquired at 60,000 RP (m/z 200)
- Spectra refined and curated with elemental composition of each EI fragment verified
- Kovats retention index for each entry
- PubChem ID for unique metabolites
- Can be used in combination with existing unit mass libraries

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Chemical ionization for molecular ion

Remove entire ion source or change to CI source in under 2 minutes without venting
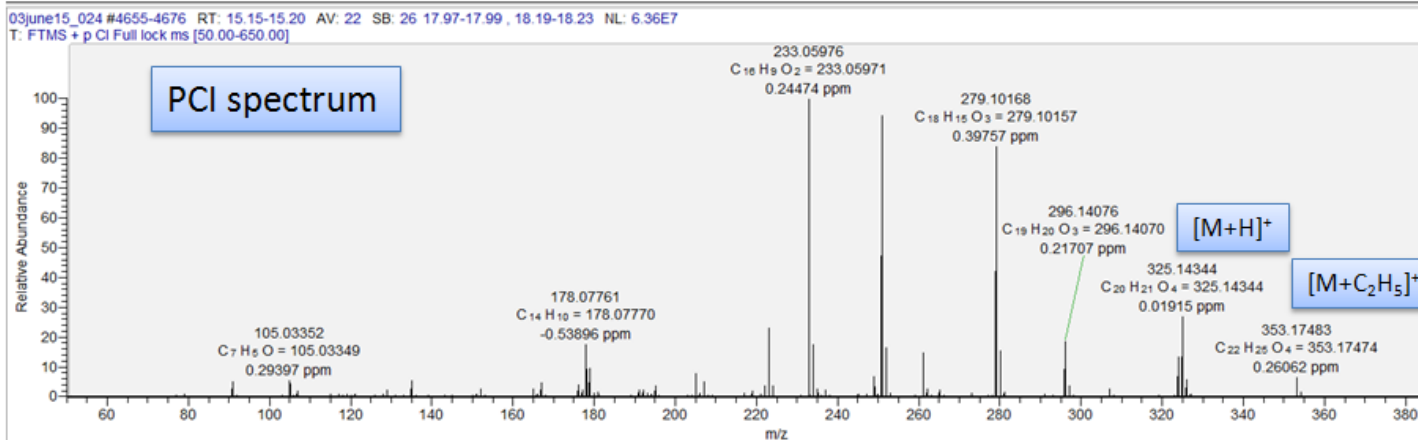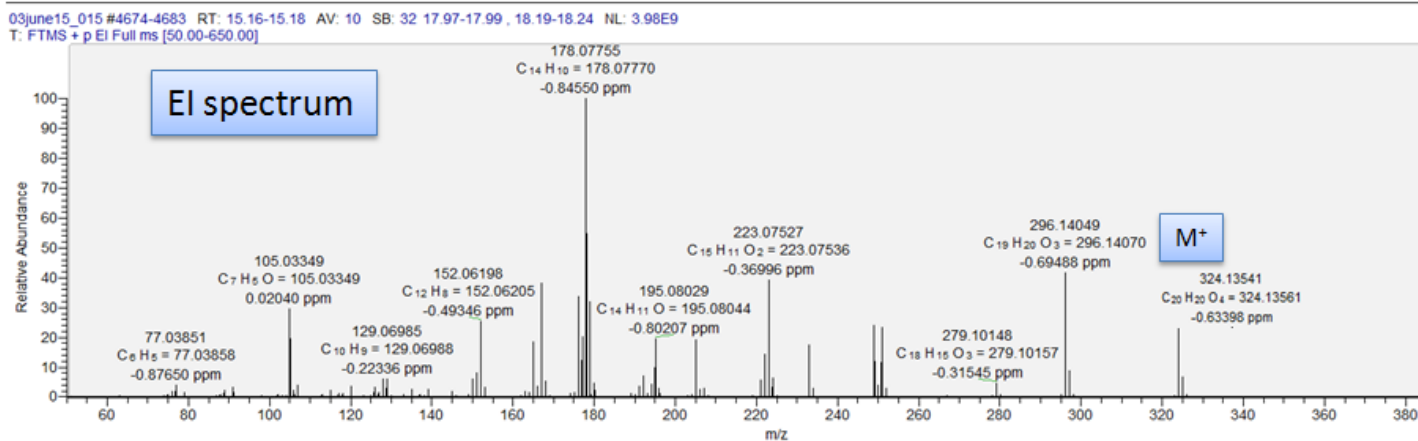
…

MS/MS for structural information
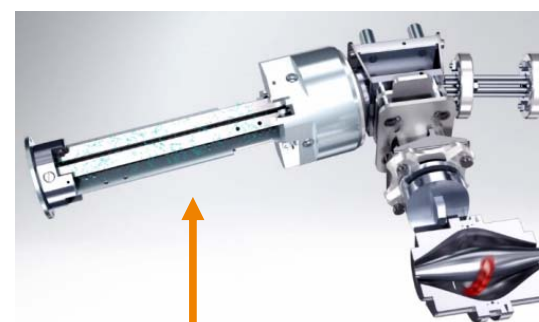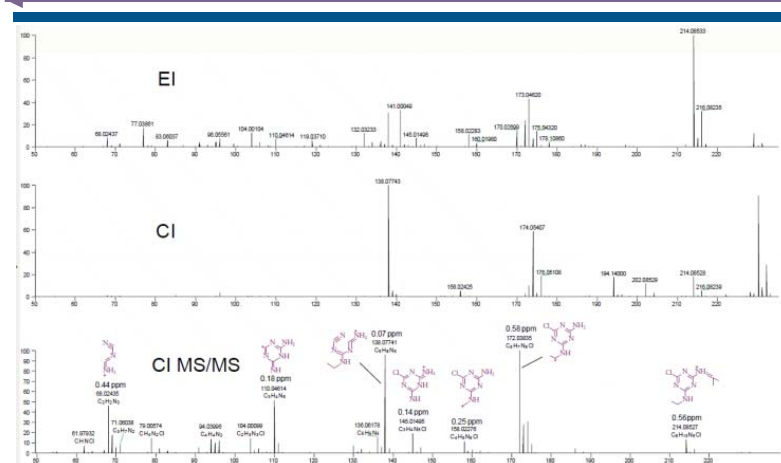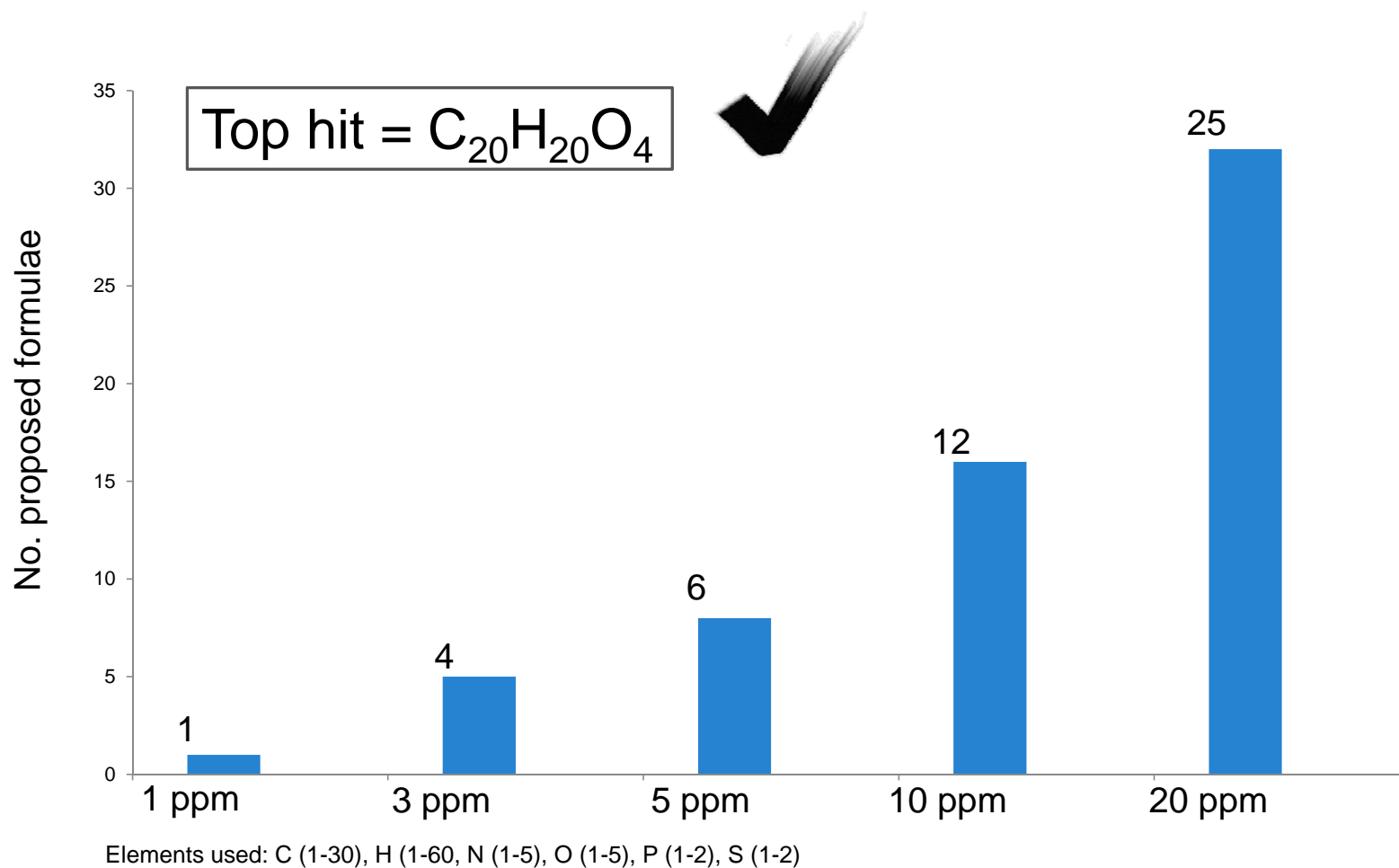

Fragmentation in HCD Cell

- Thermo Scientific™ Mass Frontier™ software can be used for sub-structural interpretation
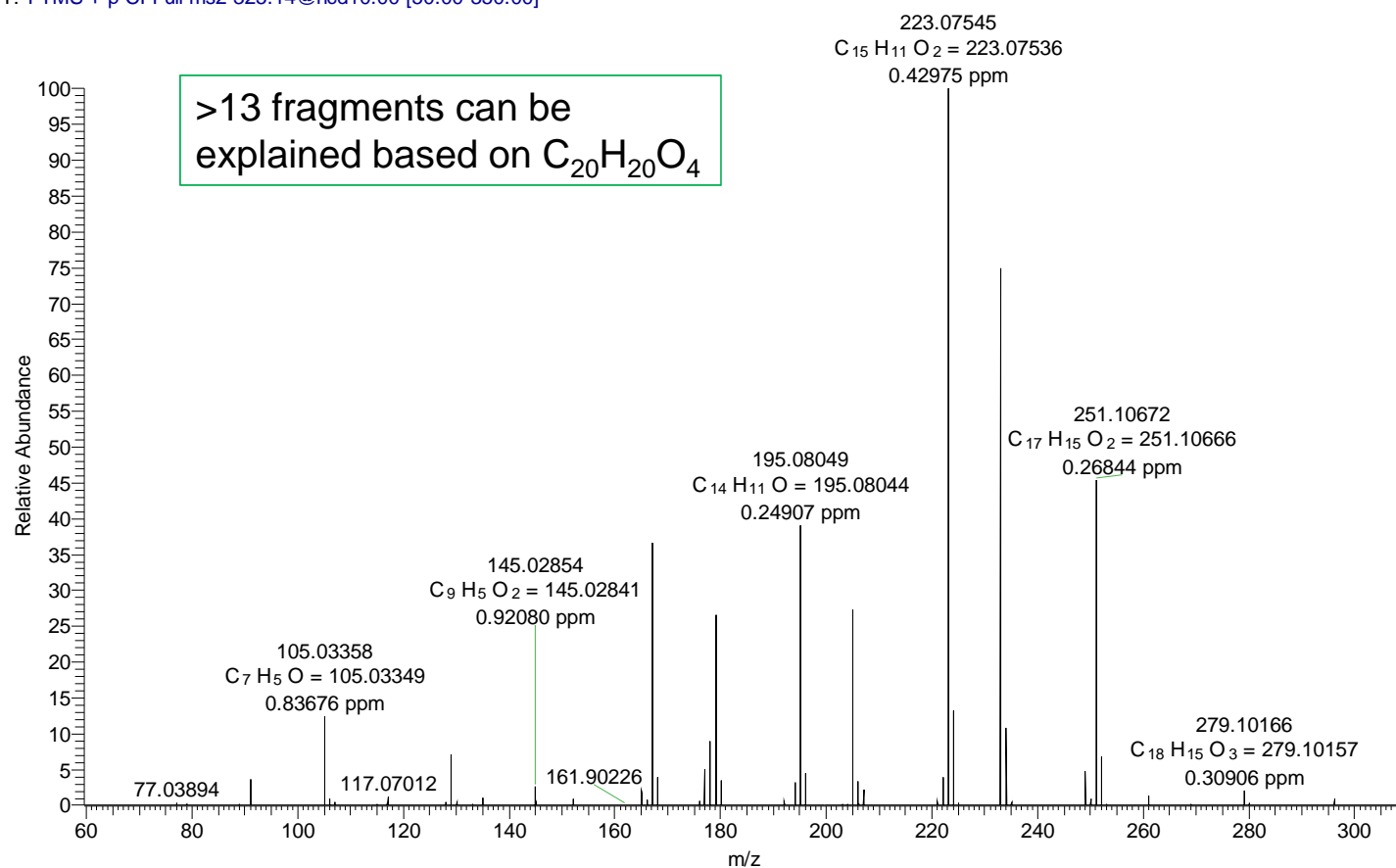- Sub ppm accurate mass allows for higher confidence in fragment IDs

Top hit = $C_{20}H_{20}O_4$

Elements used: C (1-30), H (1-60, N (1-5), O (1-5), P (1-2), S (1-2)

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Conclusion

- Thermo Scientific Q Exactive and Exactive GC systems are **easy-to-use**, dedicated HRAM GC-MS platforms that provides **highly sensitive**, **routine grade performance** in both targeted and untargeted screening experiments

- Thanks to the **superior resolving power**, **accurate mass** measurements and extended **linear dynamic range**, the Orbtrap GC-MS systems provide a **greater confidence** in the discovery, identification and quantification of compounds for the **ultimate sample analysis workflow**

Find out more about our GC Orbitrap GC-MS and GC-MS/MS

http://info1.thermoscientific.com/OrbitrapGCMS

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