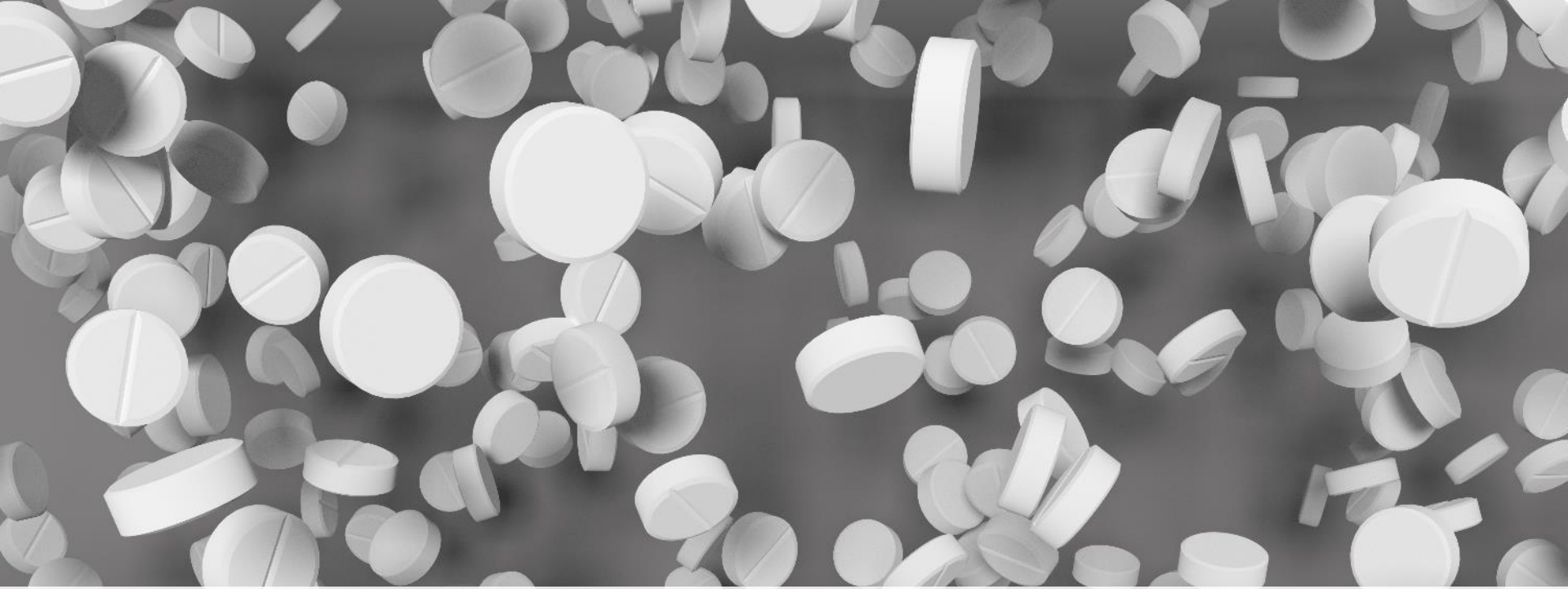

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Pharmaceutical Intermediate Impurity Profiling

Pharma Tours | 2016

Overview

- **Demonstrate new features and capabilities that enable confident identification and quantification of unknown impurities**

Overview

- **Present new GC-MS technology**
- **Demonstrate new features and capabilities that enable confident identification and quantification of unknown impurities**

Overview

- **Present new GC-MS technology**
- **Demonstrate new features and capabilities that enable confident identification and quantification of unknown impurities**
- **Demonstrate pharmaceutical intermediate impurity analysis with real-world samples analyzed in partnership with AstraZeneca**

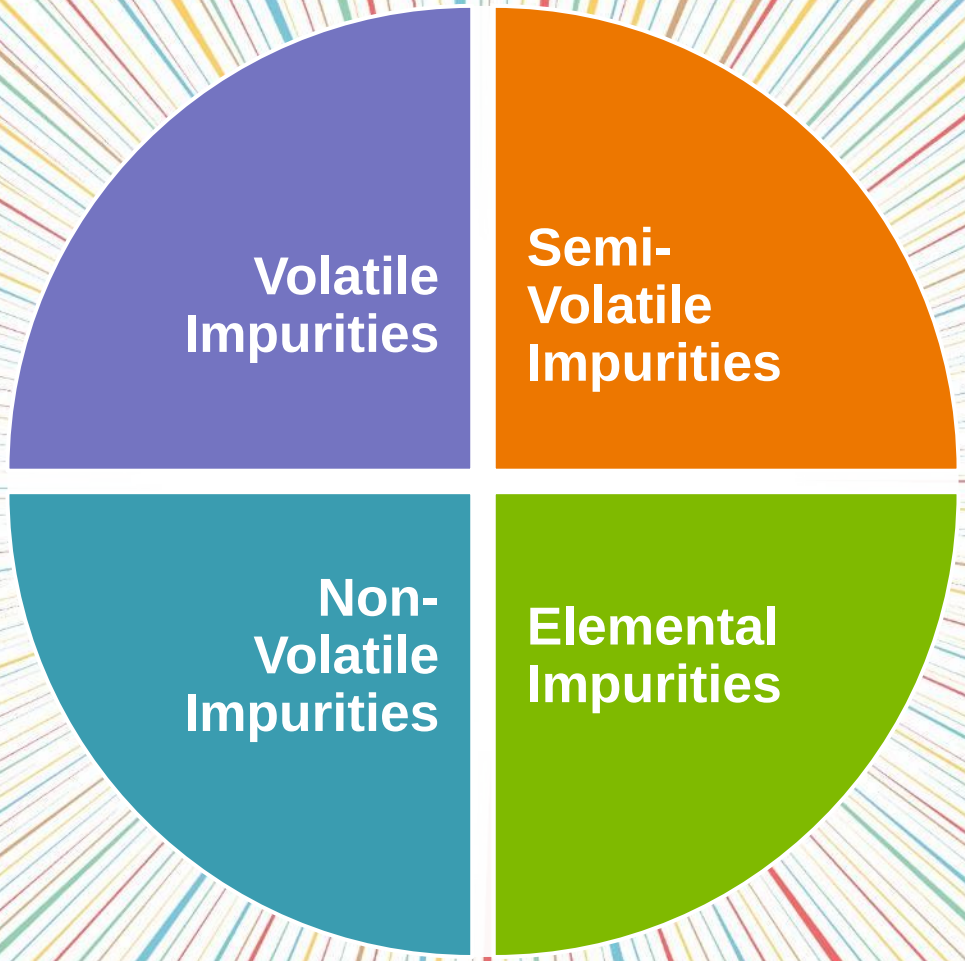
Acknowledgements

bit.ly/AZImpurityWebinar

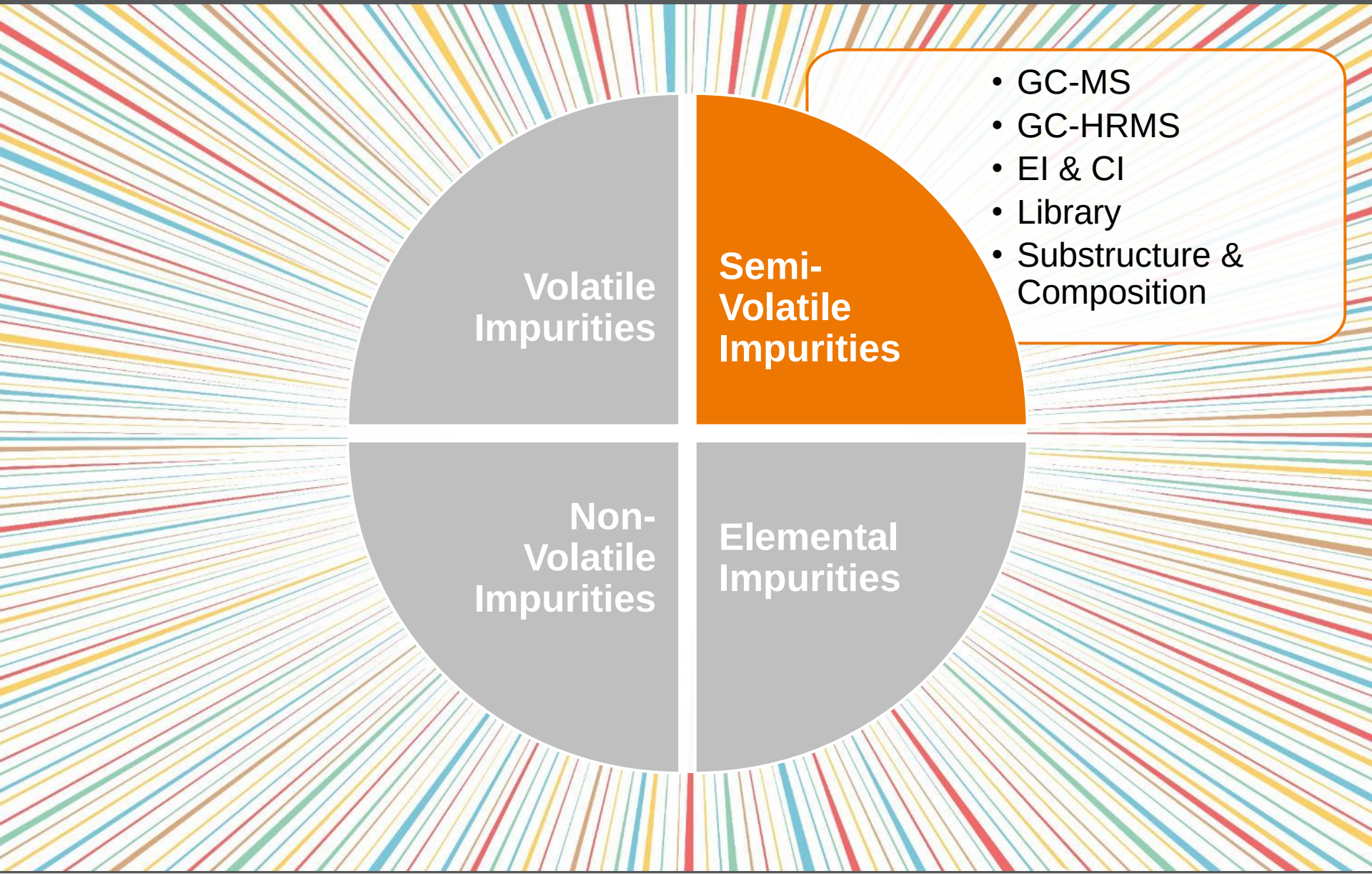
AstraZeneca

ThermoFisher SCIENTIFIC

Analysis of pharmaceutical intermediate impurities

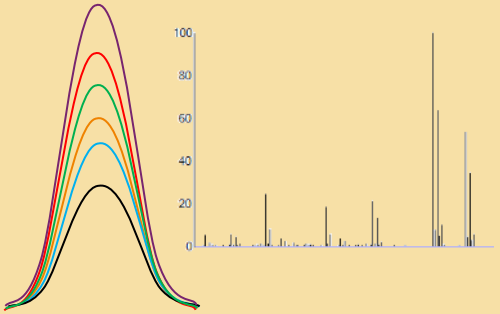


Analysis of pharmaceutical intermediate impurities



Requirements for Identification of Unknowns

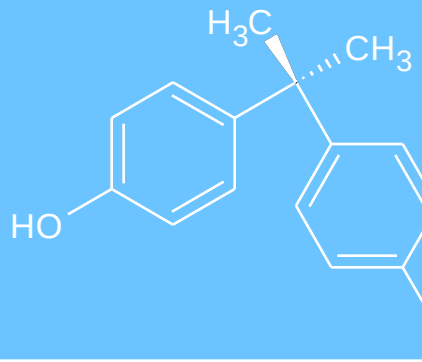
RT & m/z



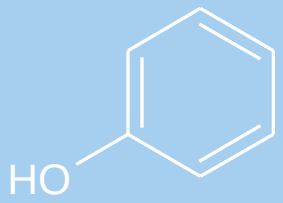
Empirical Formulae

$C_{15}H_{16}O_2$

Structure



Substructure



Name & CAS

4,4'-(propane-2,2-diyl)diphenol

Bisphenol-A

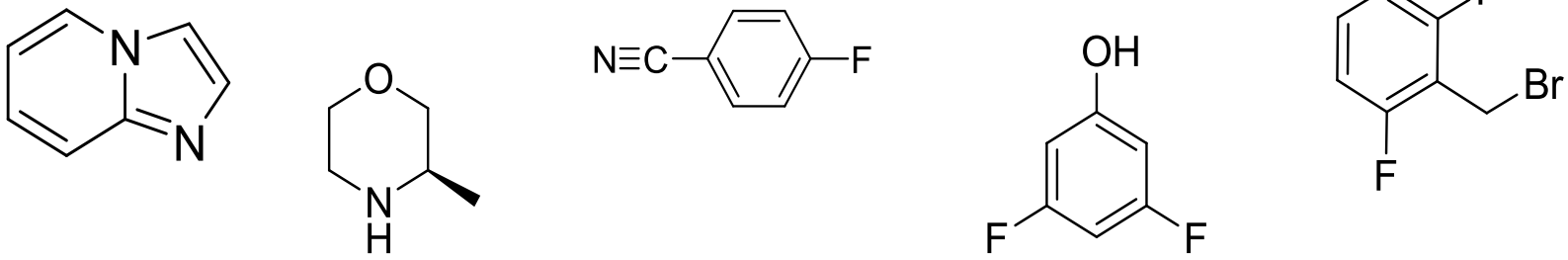
80-05-7

Amount

AstraZeneca study objectives

"High mass accuracy is an advantage when it come to looking at identifying compounds. This increases the confidence in structural characterisation."

- Aim: Analyze low molecular weight compounds, typical of starting materials
  - Analyze at relevant concentrations (1% to 0.0001% (v/v) for liquid samples)
  - Unambiguously confirm structure of intermediate and impurities through accurate mass measurement and sub-structural analysis.
  - Asses performance across concentration ranges.

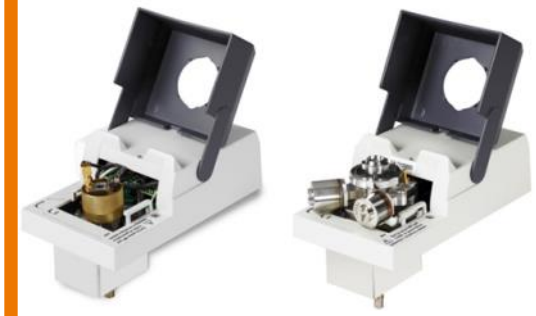


Introducing Thermo Scientific™ Q Exactive™ GC

Quadrupole-Orbitrap GC-MS/MS system

ThermoFisher SCIENTIFIC

Integration of 3 highly successful technologies



Thermo Scientific™ TRACE™ 1310 GC

rapid heat cycling

unique modular injector and detector design

ThermoFisher SCIENTIFIC

Integration of 3 highly successful technologies

Thermo Scientific™ ExtractaBrite™ ion source technology

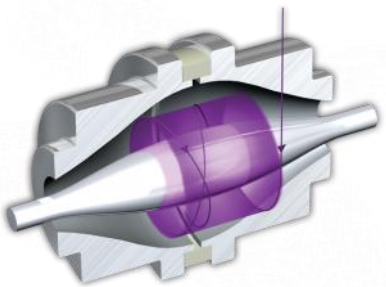
Routine grade robustness

Removable without breaking vacuum

Patented RF lens protects ion guide and quads

ThermoFisher SCIENTIFIC

Integration of 3 highly successful technologies

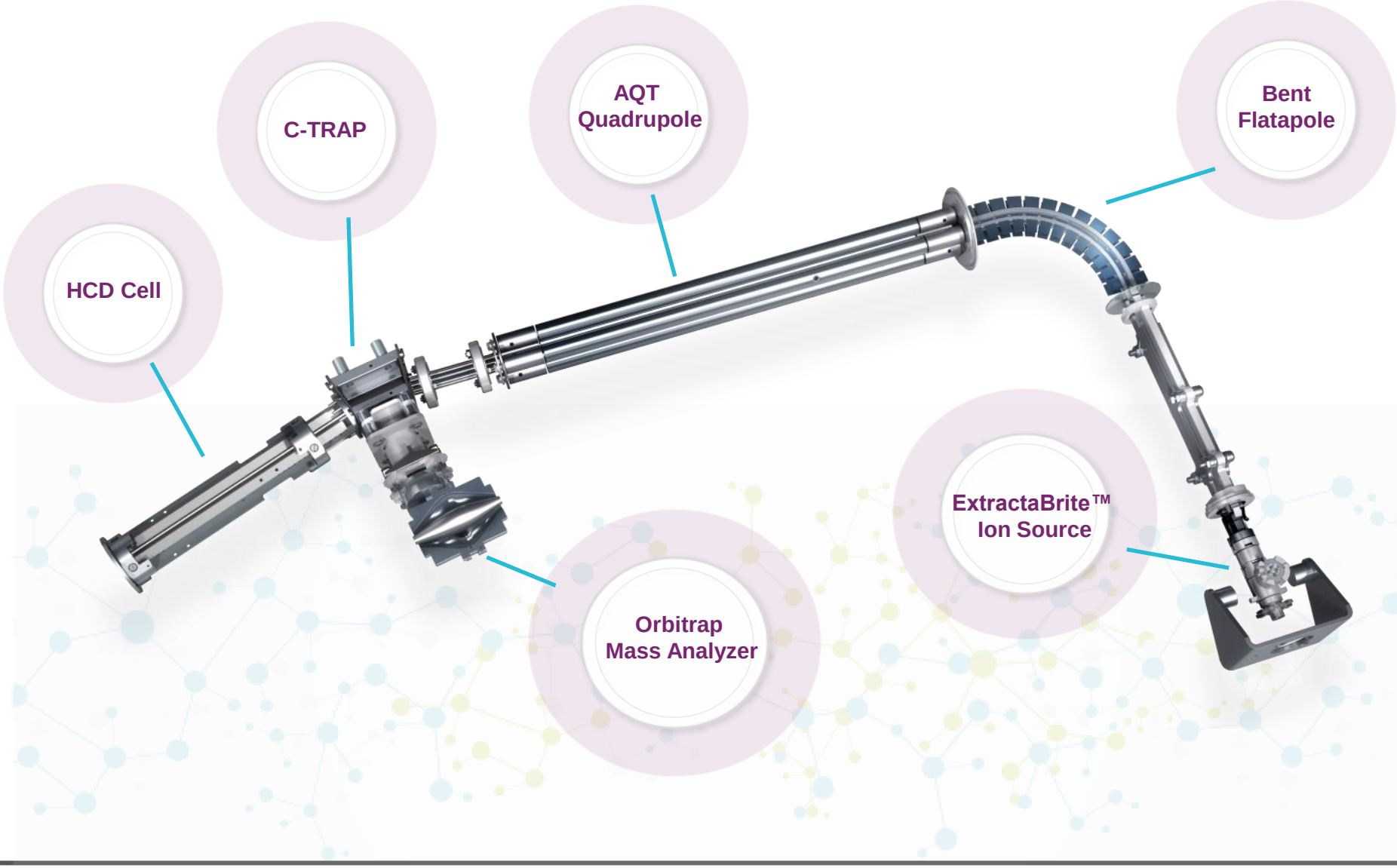


Thermo Scientific™ Orbitrap™ mass analyzer technology

Incredible HR/AM performance

Proven Thermo Scientific™ Q Exactive™ platform

Bringing GC and Orbitrap Technology Together



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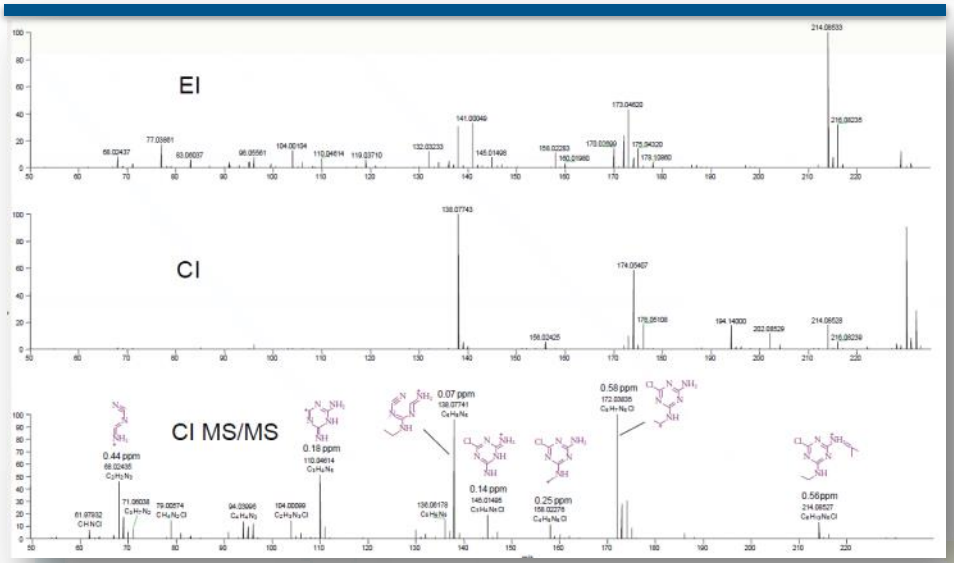
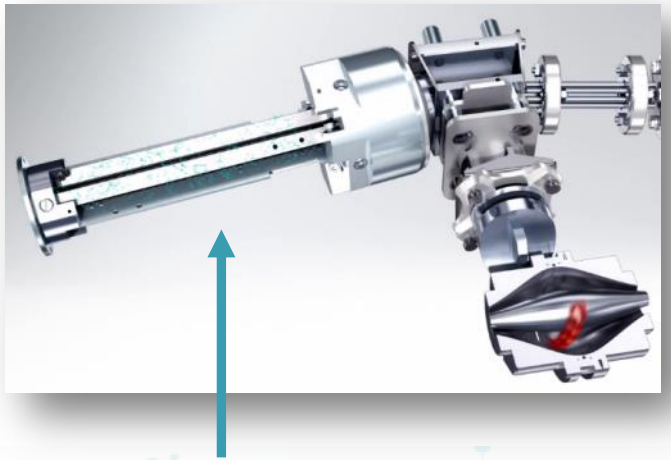
Chemical ionization for molecular ion

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

Q Exactive GC: Compound Discovery and Identification

MS/MS for structural information





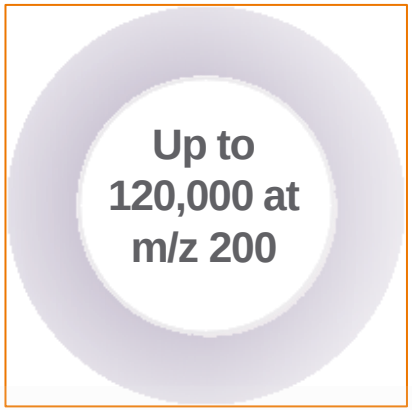
Fragmentation in HCD Cell

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

Breakthrough in GC-MS capability

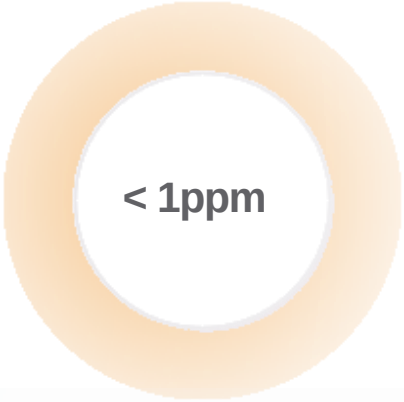
The Power of Q Exactive GC

Resolution



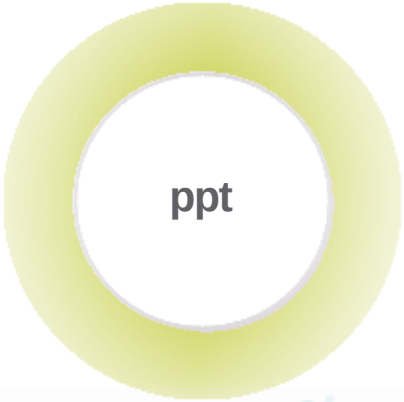
- Highest available
- Maximum selectivity
- Fast enough for GC!

Mass Accuracy



- Every scan
- All concentrations
- In complex matrix
- Across the mass range from 30 – 3000 m/z
- Everyday!

Sensitivity

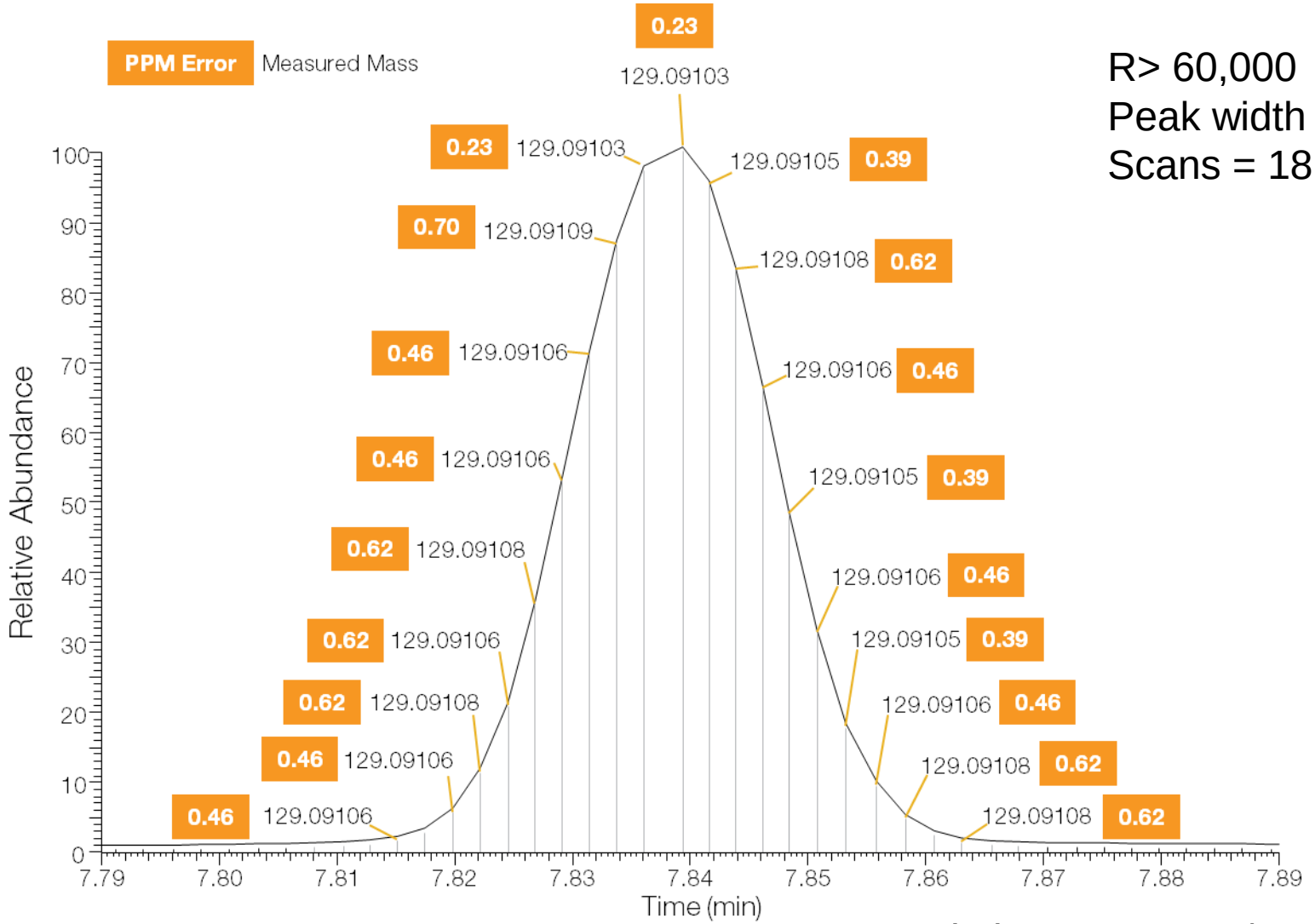


- In full scan
- High selectivity
- High spectral fidelity

Dynamic Range

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

Scan speed and accurate mass error across a peak



R> 60,000
Peak width = 3s
Scans = 18

Ethyl octanoate *m/z* 129.0910

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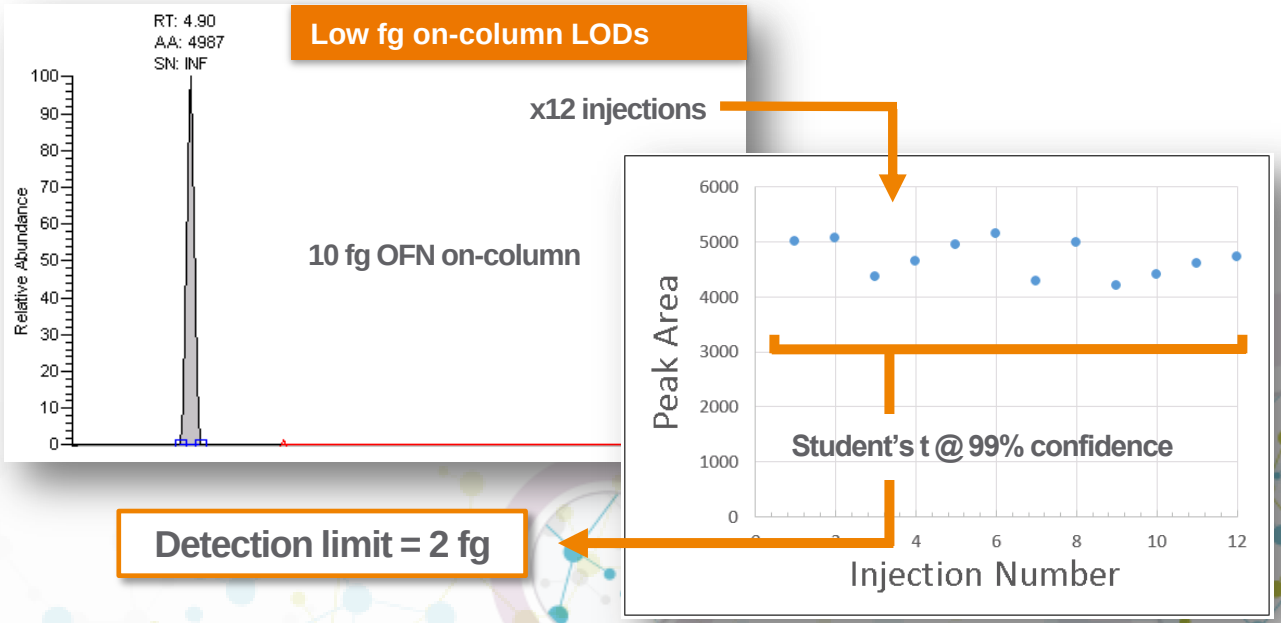
Unprecedented quantitation power for HR/AM GC-MS

PPT Sensitivity

Huge linear range

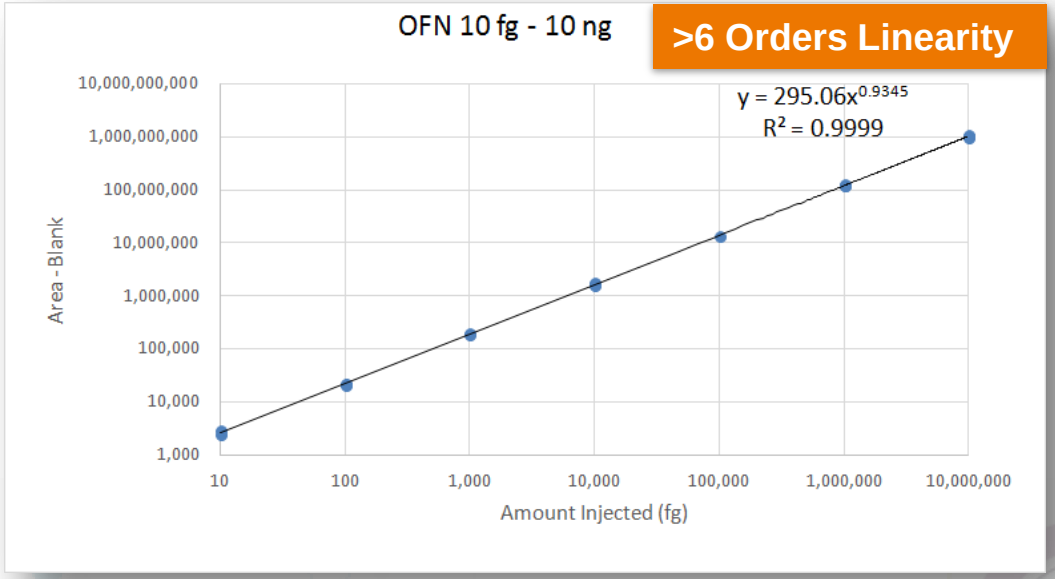
- Low fg on-column detection limits (~best triple quads)
- > 6 Orders linear range*
- Excellent precision
- Highest selectivity
- "triple quad grade" quantitation

*application specific



Breakthrough in GC-MS Performance

Unprecedented quantitation power for HR/AM GC-MS



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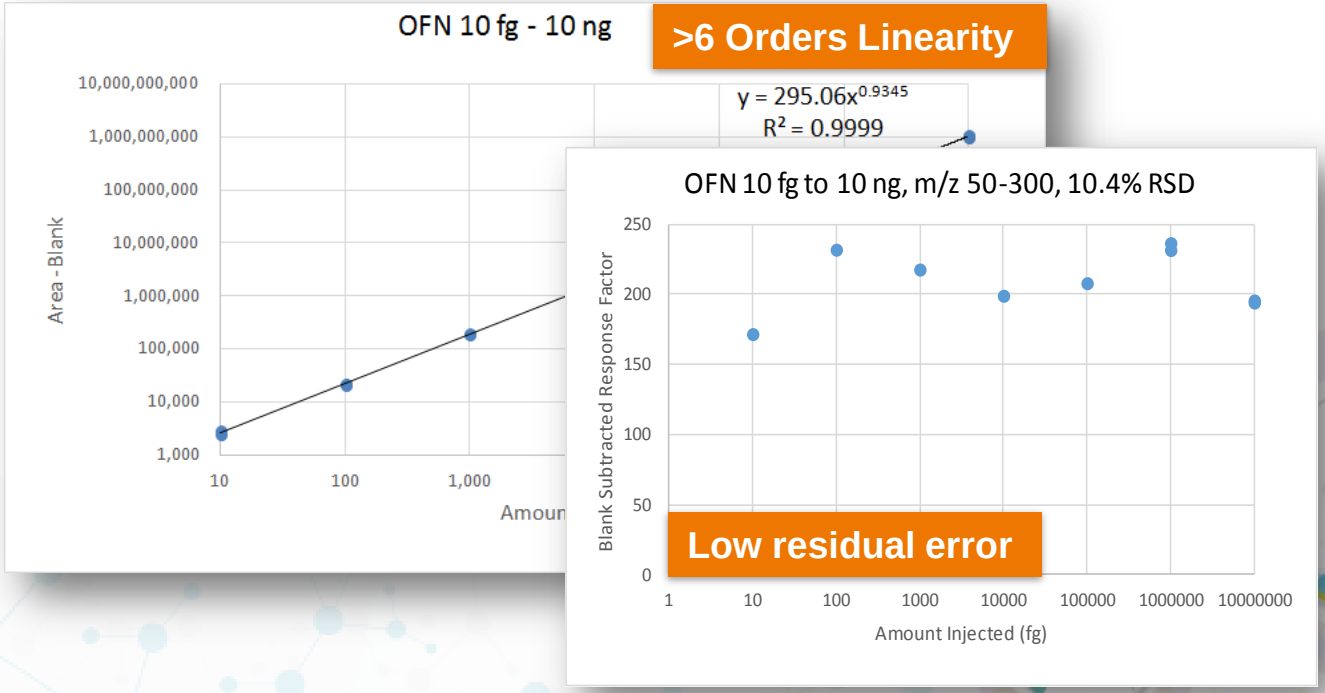
Breakthrough in GC-MS Performance

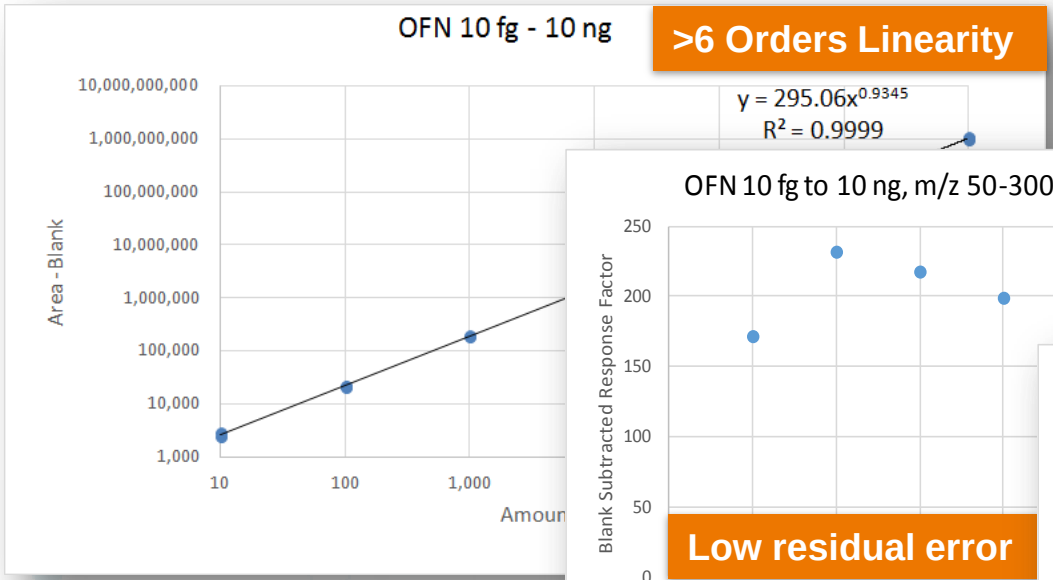
Unprecedented quantitation power for HR/AM GC-MS

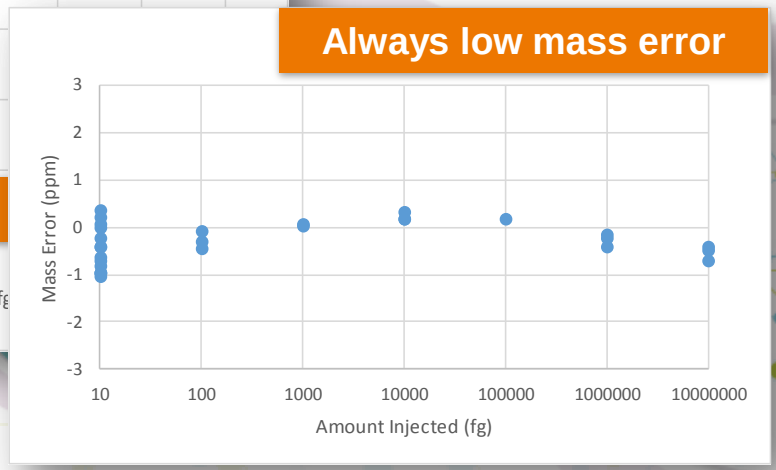


ThermoFisher SCIENTIFIC

Breakthrough in GC-MS Performance

Unprecedented quantitation power for HR/AM GC-MS





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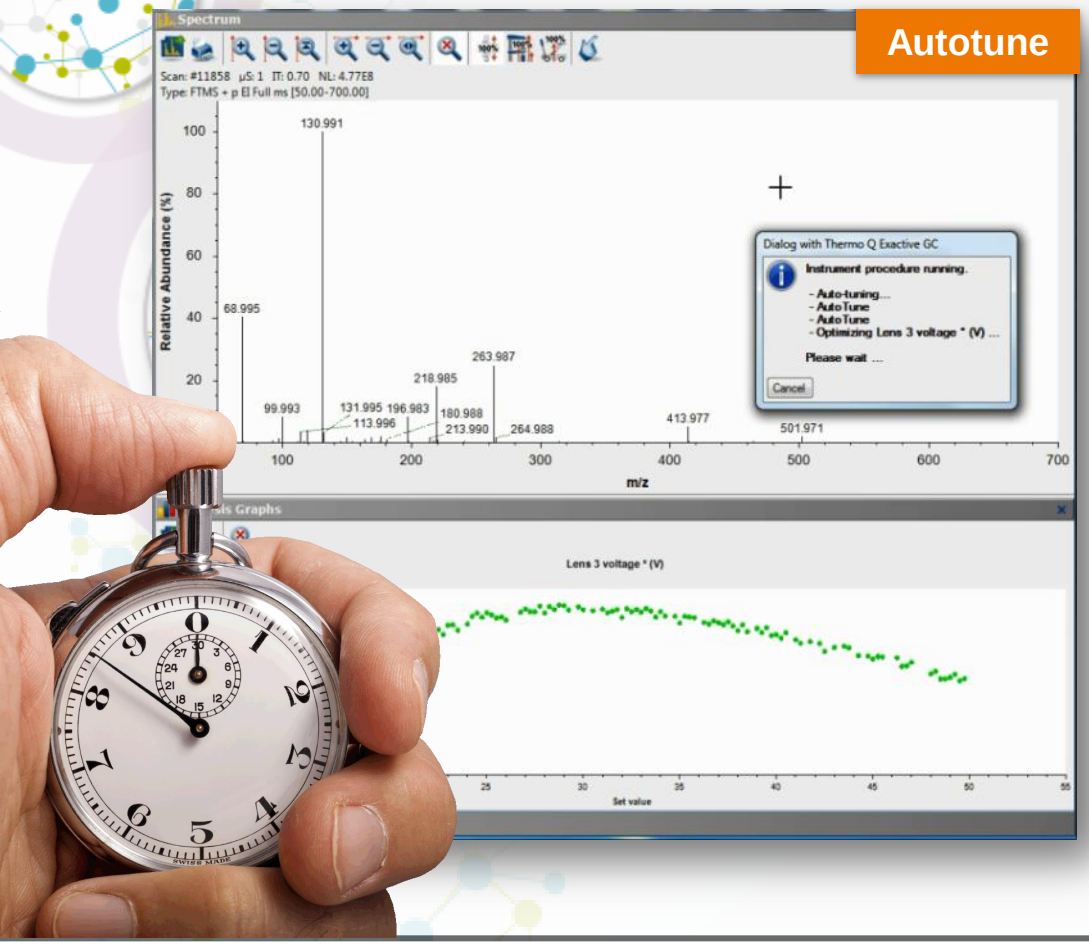
Breakthrough in GC-MS Performance

To get data this good, it's really fast and simple…



Easy set-up

- Familiar Q Exactive environment
- Simple status
- Automated leak checking
- Automated tuning & calibration
- Source and lens tuning
- Mass calibration
- **Ready to go < 5 minutes**

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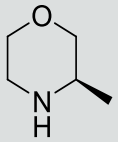
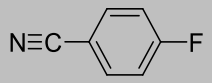
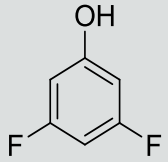
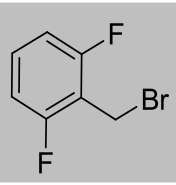
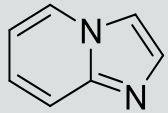
Results

“Impurity detection is important within the pharmaceutical industry. ***High mass accuracy is an advantage*** *when it come to looking at unknown sample impurities.”*

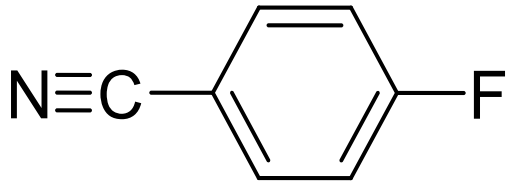
Mass Accuracy

- Aim: demonstrate mass accuracy across a range of test compounds:
  - *"All five compounds were identified by their elemental formula."*
  - *"Each was identified with a mass accuracy of less than **0.9 ppm**"*

Compound Analysed	Mass Accuracy ppm
	**0.6**
	**0.0**
	**0.9**
	**0.7**
	**0.1**

Scan Speed

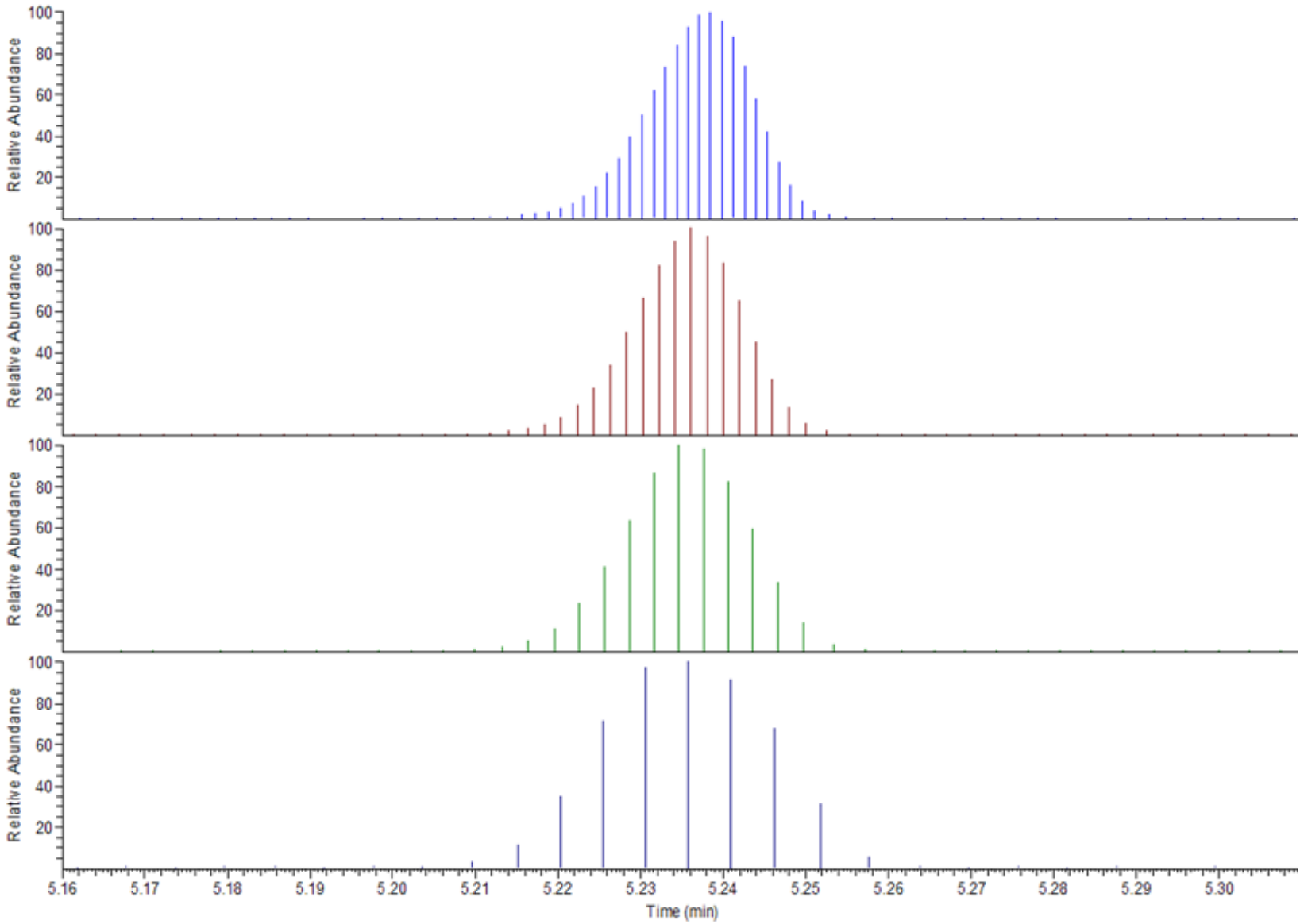
*"For quantitative analysis in GC/MS, a **minimum of 10 scans** is needed for an idealized peak shape."*



- 4-fluorobenzonitrile (2.5 µg/mL (w/v) in methanol) was analysed in CI mode.

- The main component peak has a **peak width of 3.6 seconds**.

Scan speeds & data points



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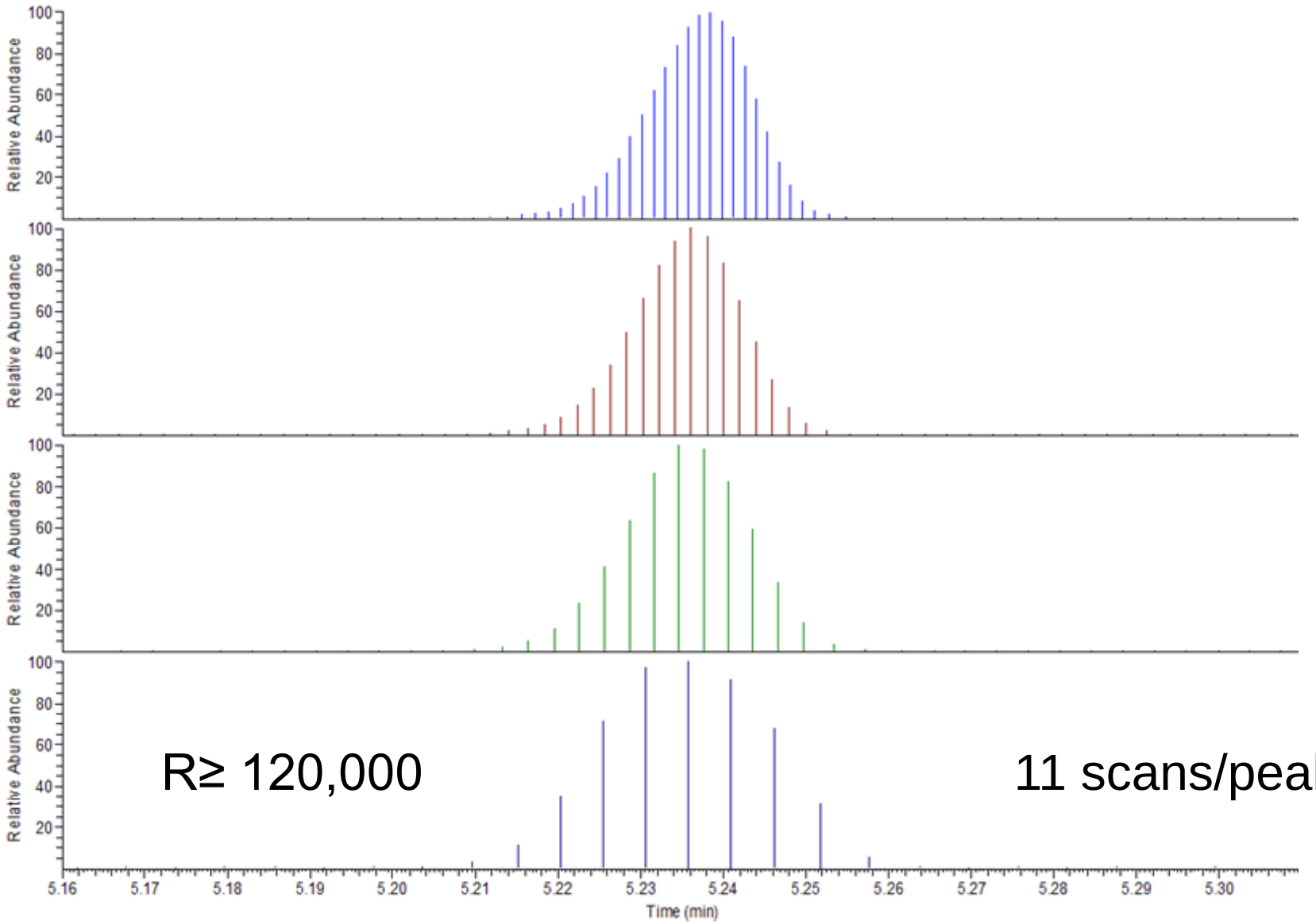
Scan speeds & data points

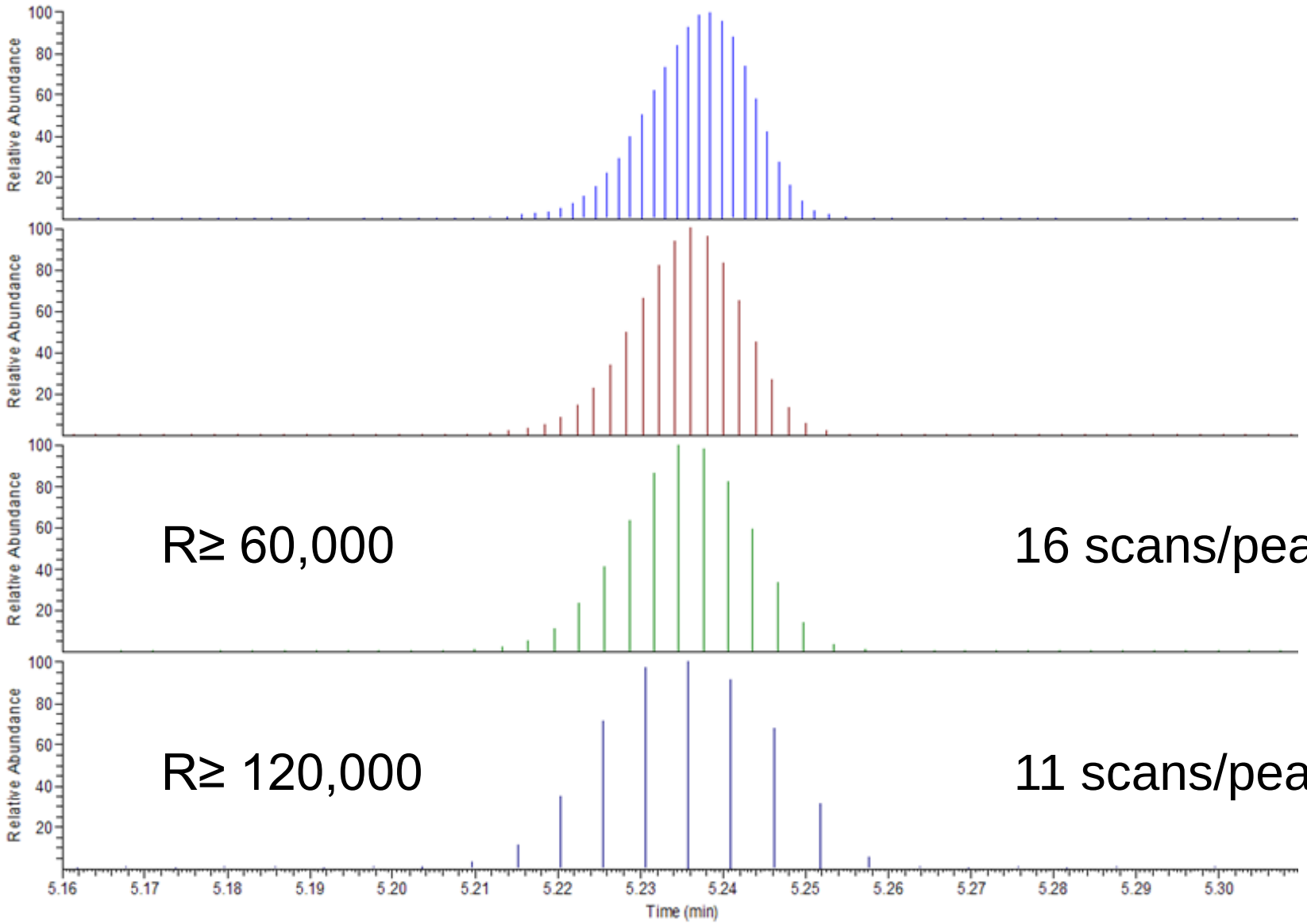


R≥ 120,000

11 scans/peak

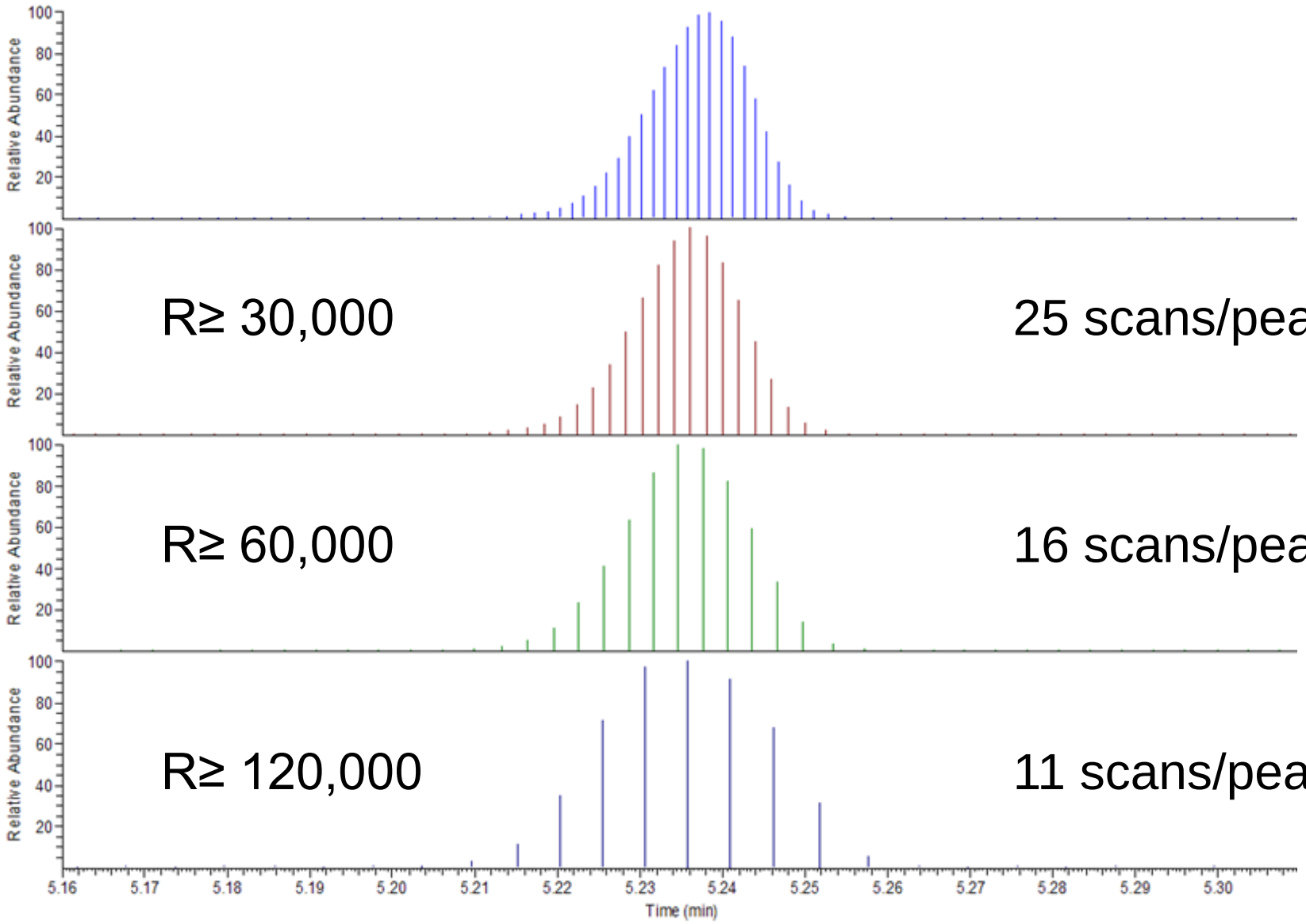
ThermoFisher SCIENTIFIC

Scan speeds & data points



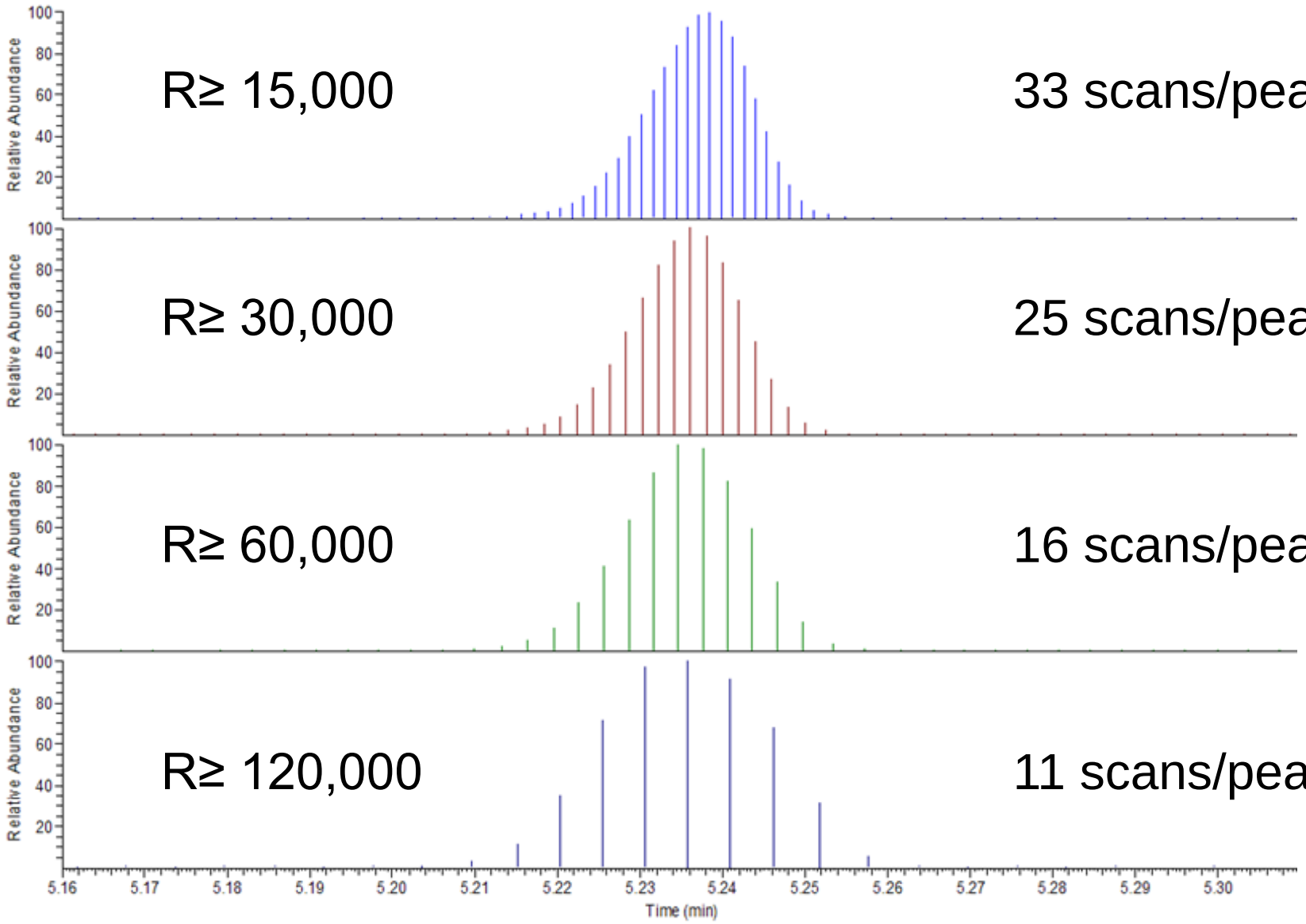
ThermoFisher SCIENTIFIC

Scan speeds & data points



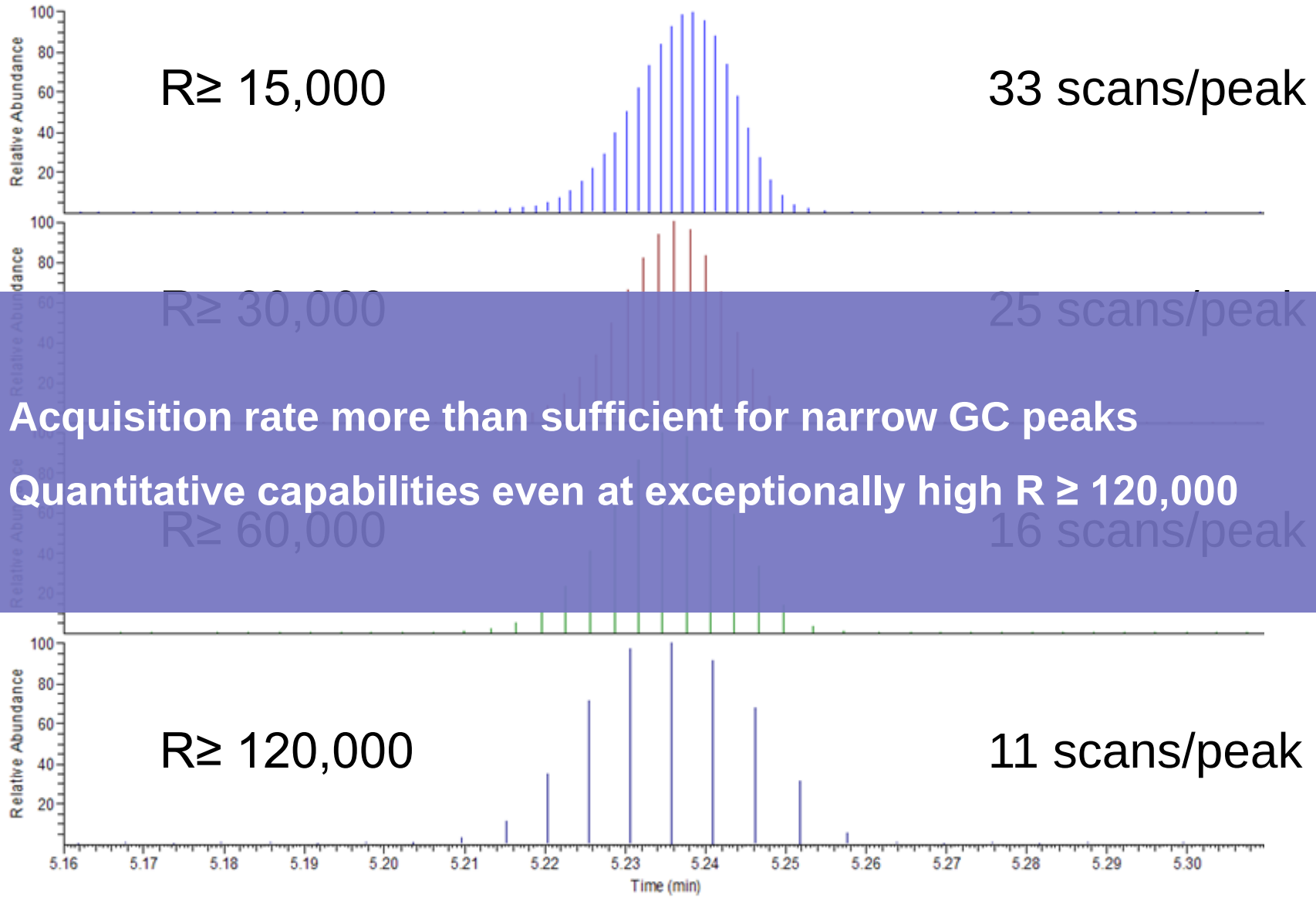
ThermoFisher SCIENTIFIC

Scan speeds & data points



ThermoFisher SCIENTIFIC

Scan speeds & data points



- **Acquisition rate more than sufficient for narrow GC peaks**
- **Quantitative capabilities even at exceptionally high R ≥ 120,000**

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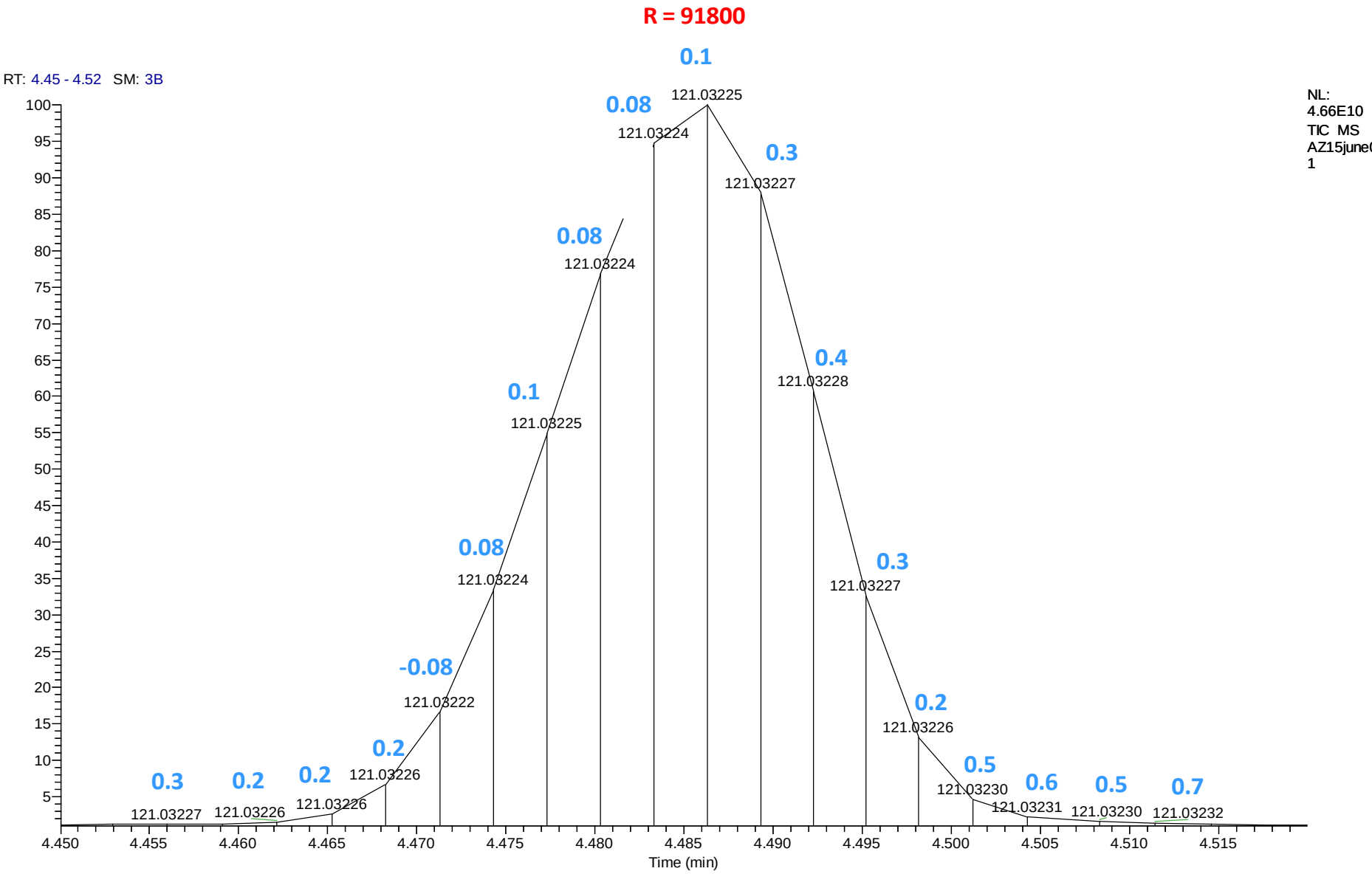
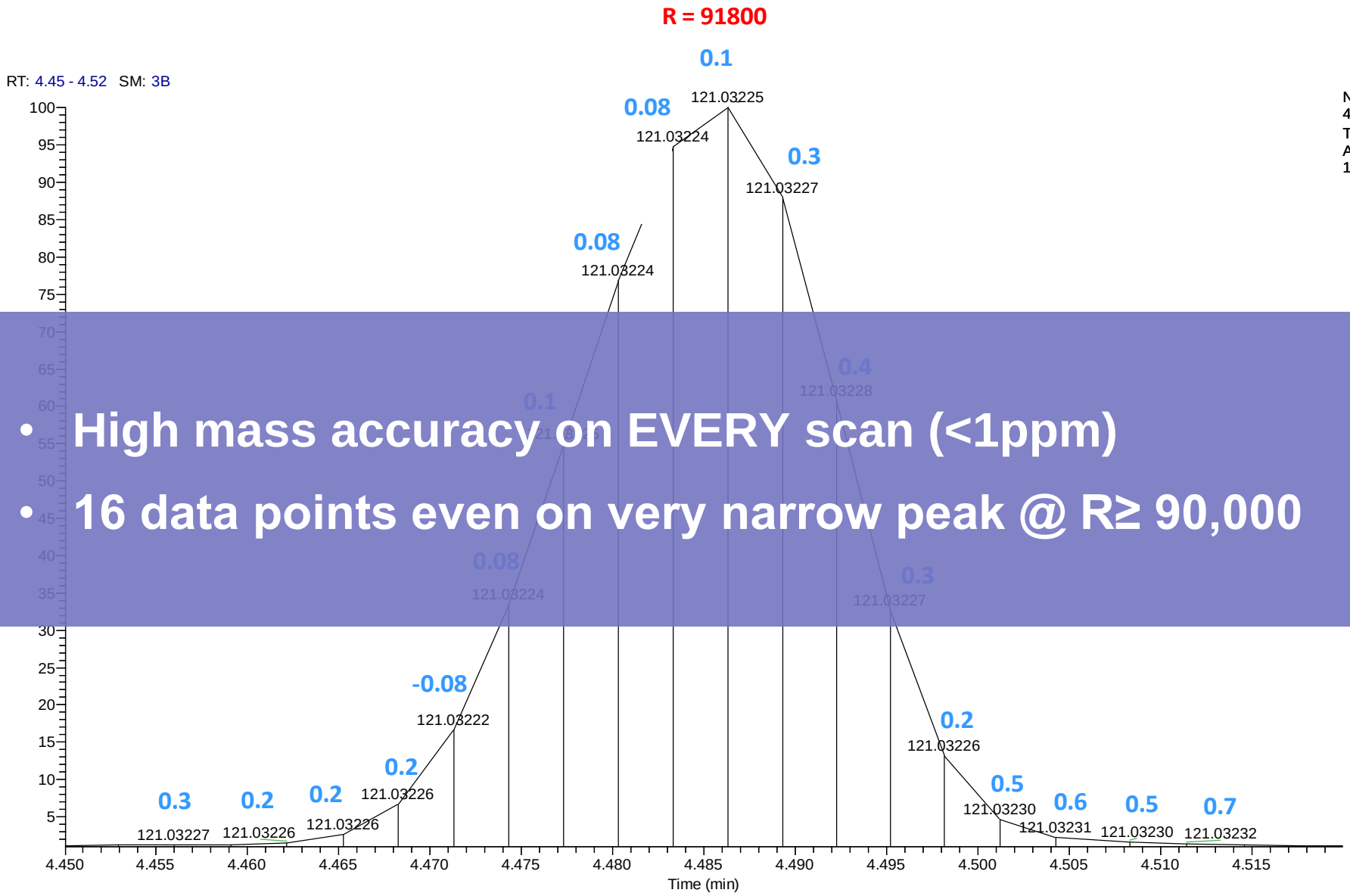
Mass Accuracy Across the Peak



ThermoFisher SCIENTIFIC

Mass Accuracy Across the Peak



- **High mass accuracy on EVERY scan (<1ppm)**
- **16 data points even on very narrow peak @ R≥ 90,000**

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Linearity

- Aim: show linearity across relevant concentration range
  - Linearity of **N,N,N'-trimethylethylenediamine** across a concentration range of 0.0001 to 0.1250 % (v/v in DMSO).
  - Analysed at a resolution of **60,000 RP** (at *m/z* 200) using EI
  - Extracted ion chromatogram of m/z 102.11515 (mass window **2 ± ppm**) and corresponding peak area were used in calibration curve.

Linearity Results

Data Review - diamine01_cal

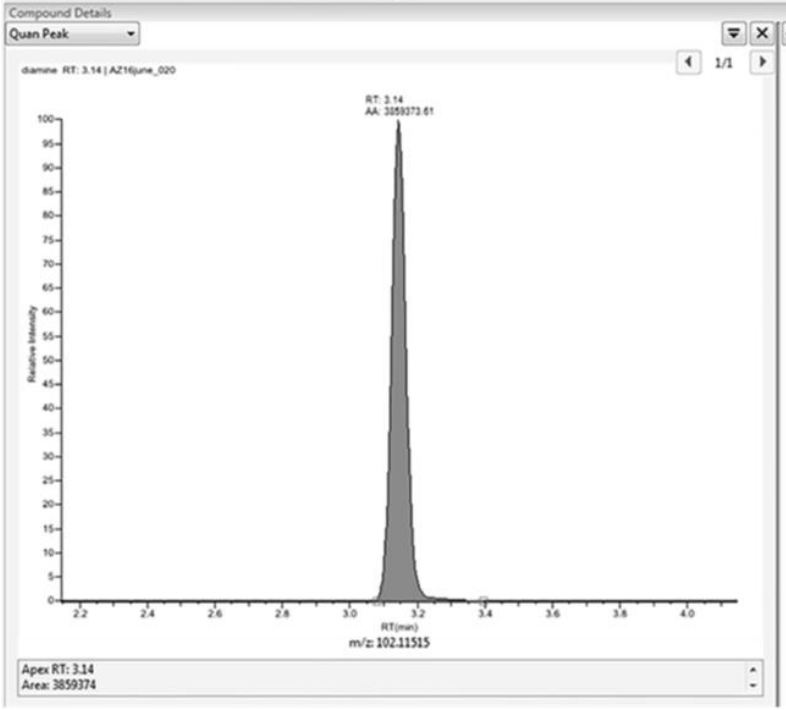
Compounds

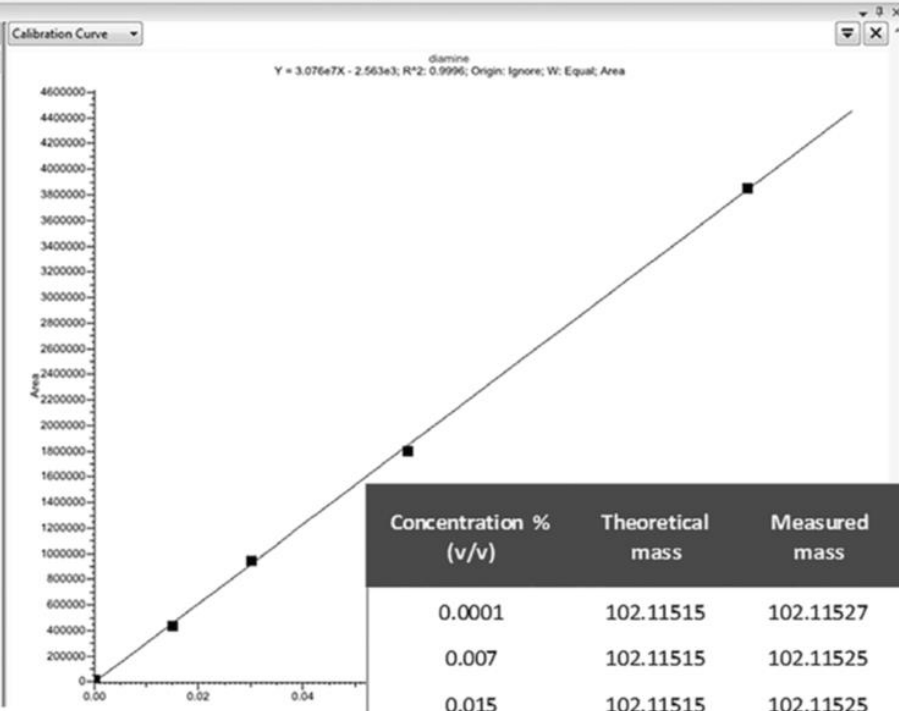
	Compound	Expected RT	Compound Type
1	diamine	3.19	Target Compound

Sample Results

	Filename	Level	Height	Area	Actual RT	m/z (Expected)	m/z (Apex)	m/z (Delta(ppm))	ISTD Amt	ISTD Response	IR	Fragment 1	Fragment 1 (mass delta)
1	AZ16june_020	0.125	1233778	3859374	3.14	102.11515	102.11530	1.42398	N/A	N/A		N/S	N/
2	AZ16june_019	0.06	620520	1801138	3.14	102.11515	102.11528	1.27455	N/A	N/A		N/S	N/
3	AZ16june_018	0.03	338565	945272	3.15	102.11515	102.11530	1.42398	N/A	N/A		N/S	N/
4	AZ16june_017_150617130520	0.015	149369	438038	3.14	102.11515	102.11522	0.67685	N/A	N/A		N/S	N/
5	AZ16june_015	0.0001	3243	18670	3.19	102.11515	102.11526	1.05041	N/A	N/A		N/S	N/
6	AZ16june_014		N/F	N/F	N/F	102.11515	N/F	N/F	N/A	N/A		N/S	N/

Compound Details





Concentration % (v/v)	Theoretical mass	Measured mass	Mass difference [ppm]
0.0001	102.11515	102.11527	1.1
0.007	102.11515	102.11525	0.9
0.015	102.11515	102.11525	0.9
0.03	102.11515	102.11526	1.0
0.06	102.11515	102.11525	0.9
0.125	102.11515	102.11524	0.9

ThermoFisher SCIENTIFIC

Linearity Results

Data Review - diamine01_cal

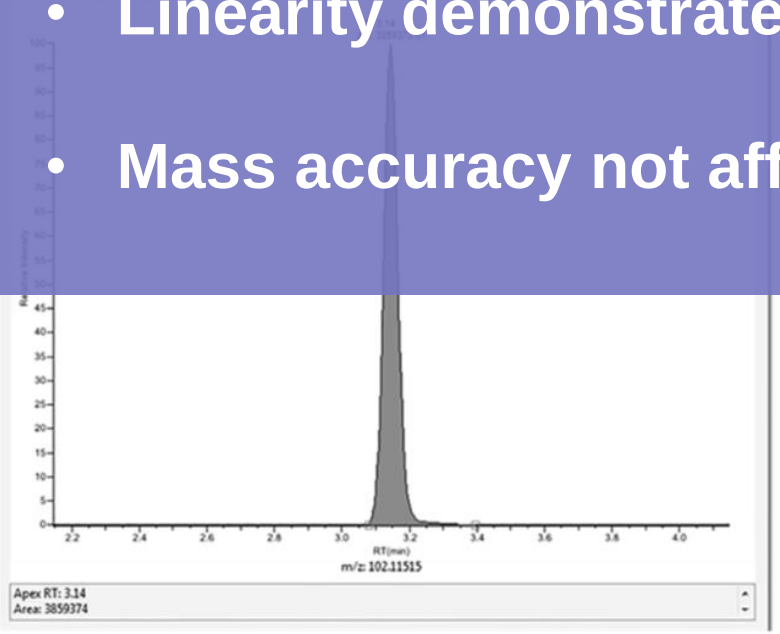
Compounds

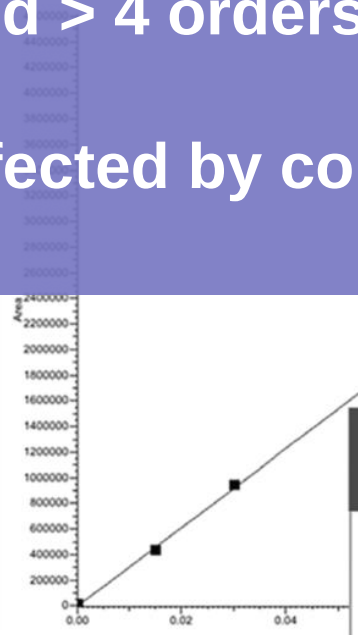
	Compound	Expected RT	Compound Type
1	diamine	3.19	Target Compound

Sample Results

	Filename	Level	Height	Area	Actual RT	m/z (Expected)	m/z (Apex)	m/z (Delta(ppm))	ISTD Amt	ISTD Response	IR	Fragment 1	Fragment 1 (mass delta)
1	AZ16june_020	0.125	1233778	3859374	3.14	102.11515	102.11530	1.42398	N/A	N/A		N/S	N/
2	AZ16june_019	0.06	620520	1801138	3.14	102.11515	102.11528	1.27455	N/A	N/A		N/S	N/
3	AZ16june_018	0.03	338565	945272	3.15	102.11515	102.11530	1.42398	N/A	N/A		N/S	N/
4	AZ16june_017_150617130520	0.015	149369	438038	3.14	102.11515	102.11522	0.67685	N/A	N/A		N/S	N/
5	AZ16june_015	0.0001	3243	18670	3.19	102.11515	102.11526	1.05041	N/A	N/A		N/S	N/

- **Linearity demonstrated > 4 orders of magnitude**
- **Mass accuracy not affected by concentration**





Concentration % (v/v)	Theoretical mass	Measured mass	Mass difference [ppm]
0.0001	102.11515	102.11527	1.1
0.007	102.11515	102.11525	0.9
0.015	102.11515	102.11525	0.9
0.03	102.11515	102.11526	1.0
0.06	102.11515	102.11525	0.9
0.125	102.11515	102.11524	0.9

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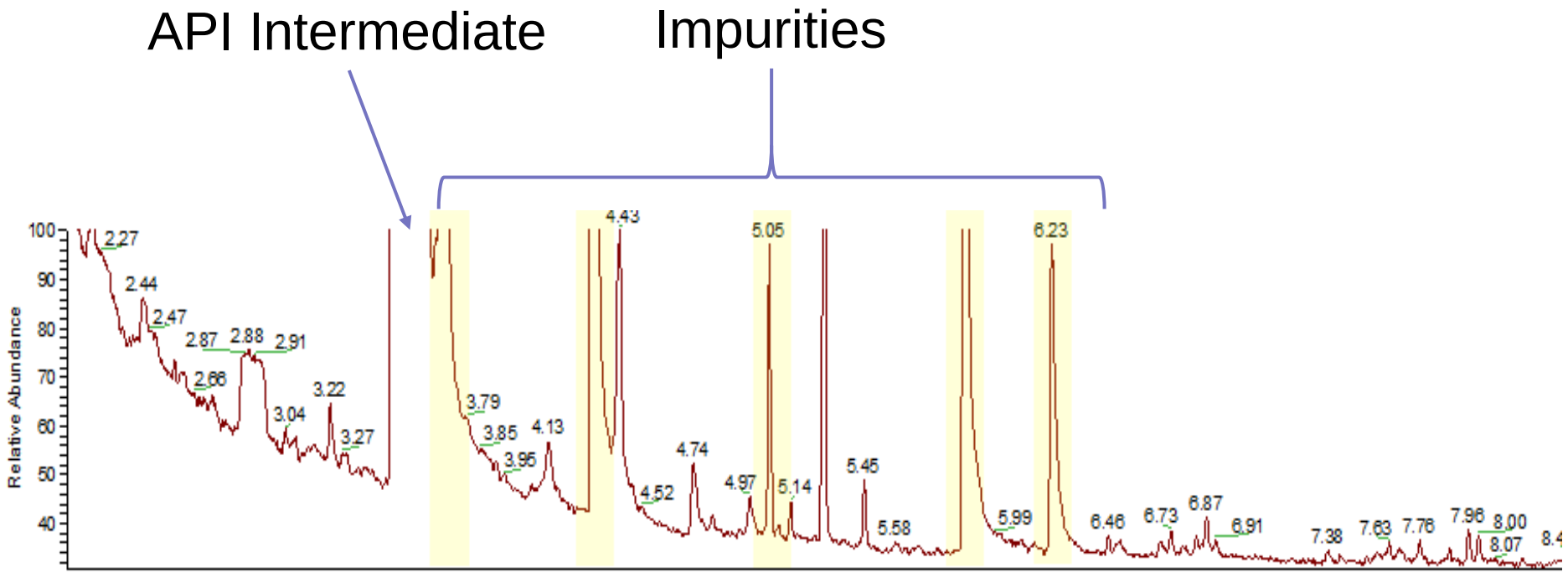
Impurity Detection

- Aim: analyse **(3S)-3-methylmorpholine** and to identify the impurities within the sample.



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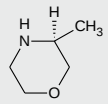
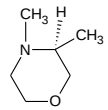
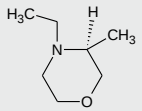
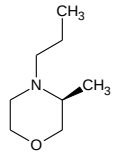
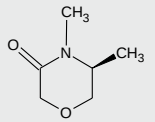
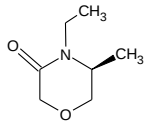
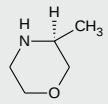
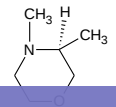
Retention (min)	Compound ID	Exact mass (EI)	Measured mass (EI)	Δppm	Exact mass (PCI)	Measured mass (PCI)	Δppm
3.55	 (3*S*)-3-methylmorpholine Molecular Formula = $C_5H_{11}NO$	101.08352	101.08358	0.6	102.09134	102.09136	0.2
3.75	 Molecular Formula = $C_6H_{13}NO$	115.09917	115.09925	0.7	116.10699	116.10703	0.4
4.32	 Molecular Formula = $C_7H_{15}NO$	129.11482	129.11486	0.4	130.12264	130.12268	0.3
5.06	 Molecular Formula = $C_8H_{17}NO$	155.13047	155.13048	0.1	156.13829	156.13825	0.3
5.87	 Molecular Formula = $C_6H_{11}NO_2$	129.07843	129.07843	0.0	130.08626	130.08634	0.6
6.23	 Molecular Formula = $C_7H_{13}NO_2$	143.09408	143.09414	0.4	144.10193	144.10193	0.2

Retention (min)	Compound ID	Exact mass (EI)	Measured mass (EI)	Δppm	Exact mass (PCI)	Measured mass (PCI)	Δppm
3.55	 (3*S*)-3-methylmorpholine Molecular Formula = $C_5H_{11}NO$	101.08352	101.08358	0.6	102.09134	102.09136	0.2
3.75	 Molecular Formula = $C_6H_{13}NO$	115.09917	115.09925	0.7	116.10699	116.10703	0.4
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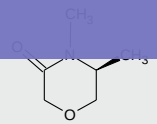
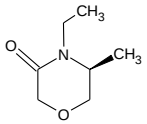
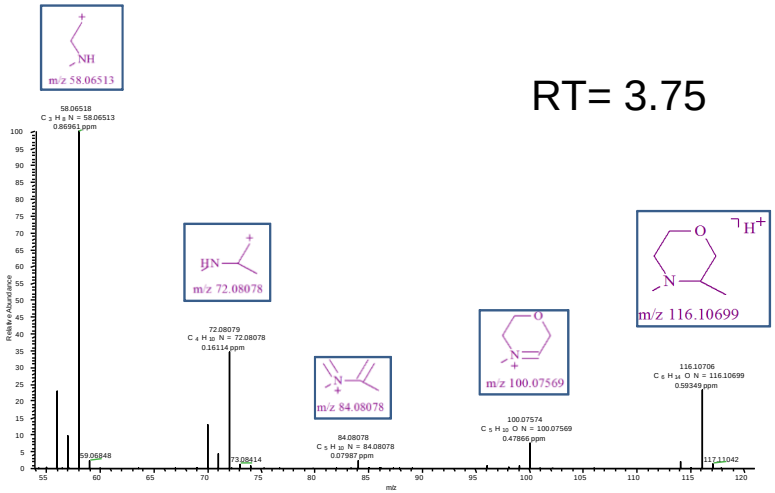
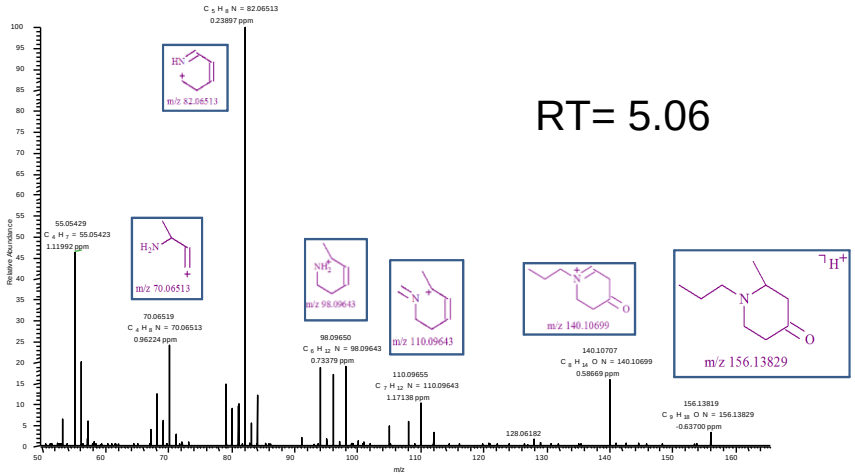
5.87	 Molecular Formula = $C_6H_{11}NO_2$	129.07843	129.07843	0.0	130.08626	130.08634	0.6
6.23	 Molecular Formula = $C_7H_{13}NO_2$	143.09408	143.09414	0.4	144.10193	144.10193	0.2

- **Change from EI to CI in minutes**
- **Mass Accuracy maintained in CI – all <1ppm**
- ***"Delivers unambiguous identification"***

PCI-MS/MS allows full unambiguous structural elucidation





- MS/MS can be performed on any ion
- Full sub-structural composition can be performed
- Thermo Scientific™ Mass Frontier™ software can be used for comprehensive sub structural peak identification

More confidence in search

- Spectra can be identified through proprietary **nominal mass** library search:
  - NIST
  - WILEY
  - Pfleger / Maurer / Weber
  - Existing in-house libraries

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  - NIST
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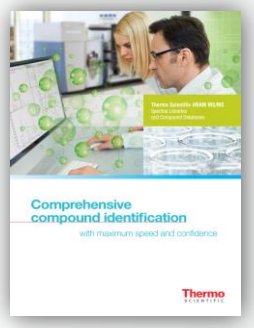
  - Pfleger / Maurer / Weber
  - Existing in-house libraries

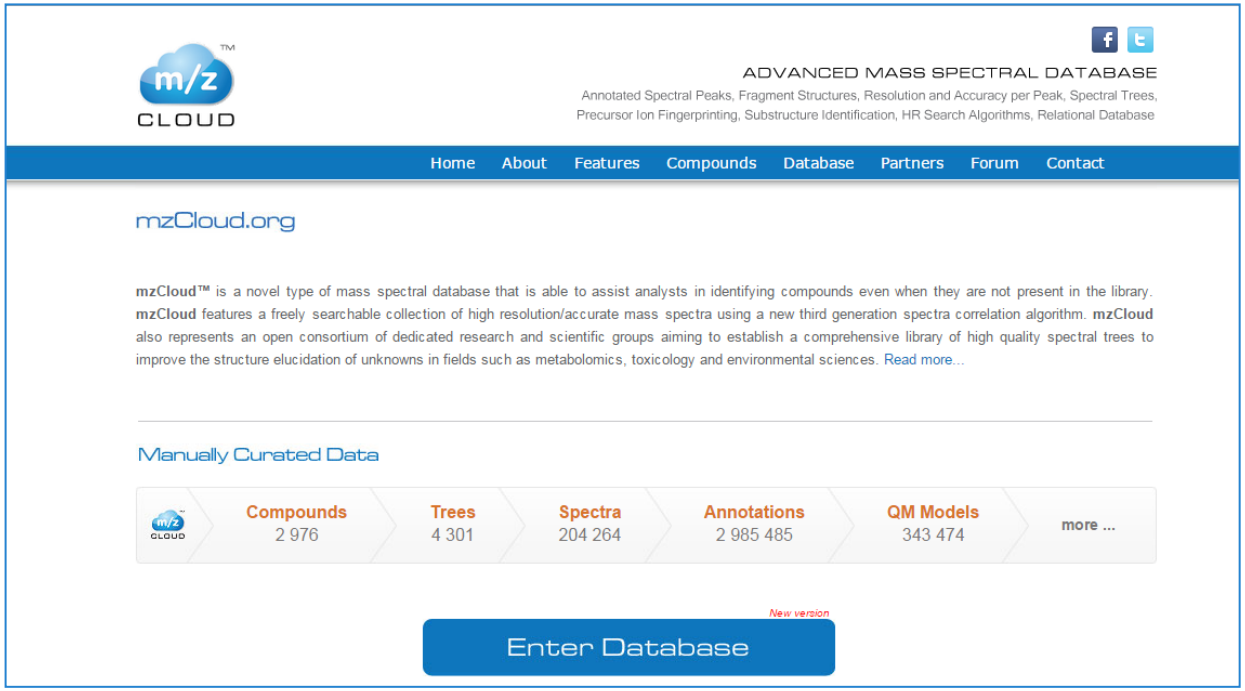
- Or **high resolution accurate mass** MS/MS libraries
  - Thermo Scientific™ High-Resolution Accurate-Mass MS/MS Spectral Libraries

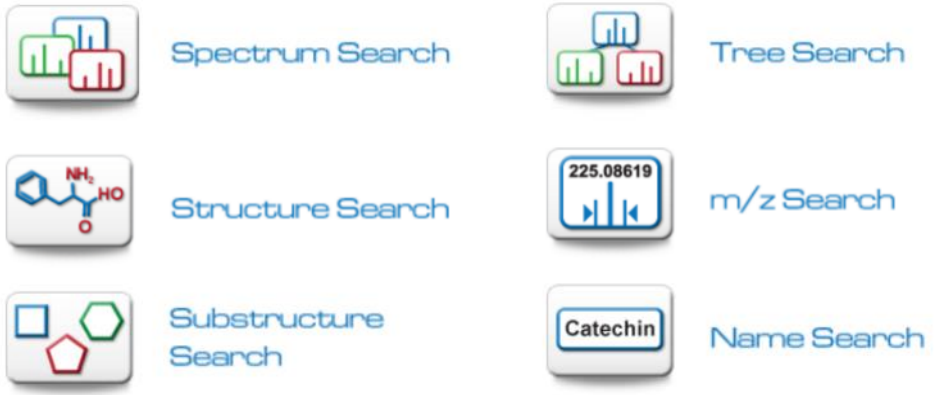


  - mzCloud.org freely available to search online

www.mzcloud.org

- mzCloud™
  - Free
  - Advanced high resolution mass spectral database
  - Search spectrum, name, structure, substructure, and m/z
  - Identify compounds even when they are not present in the library through substructure search





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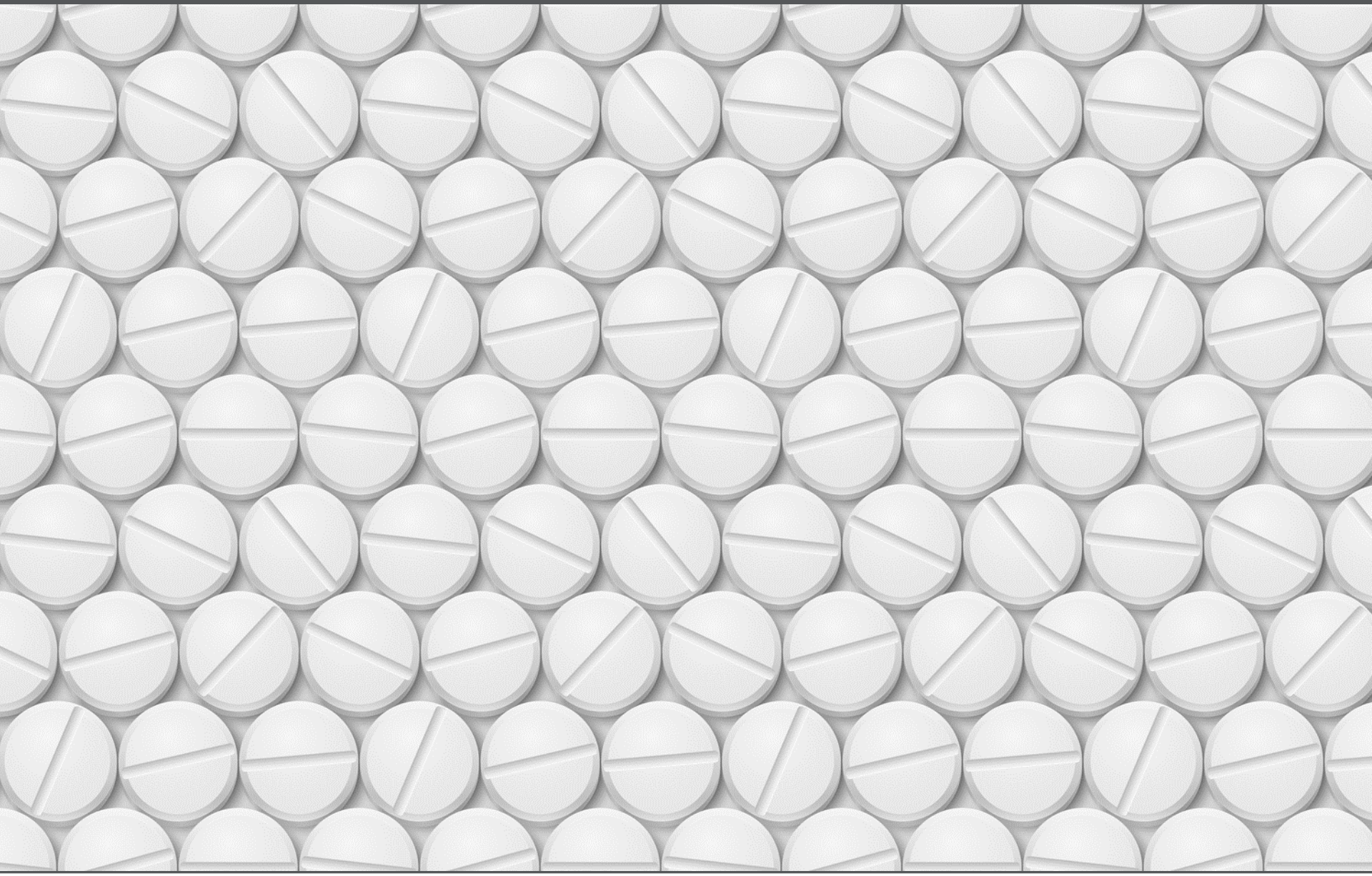
AstraZeneca Conclusions



AstraZeneca Conclusions

The Q Exactive GC MS has been evaluated for both qualitative and quantitative analysis in support of pharmaceutical uses.

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"The accurate mass measurement across a dynamic range, linearity and the ability to identify impurities in both EI & CI have shown that ***this instrument is a powerful tool****."*

AstraZeneca Conclusions

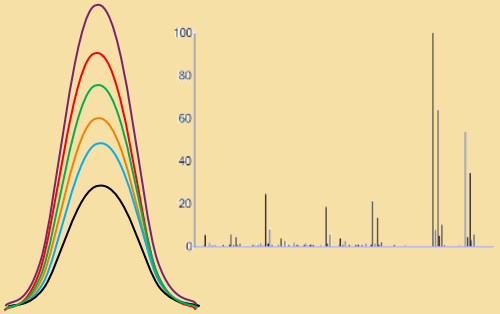
The Q Exactive GC MS has been evaluated for both qualitative and quantitative analysis in support of pharmaceutical uses.

*"The accurate mass measurement across a dynamic range, linearity and the ability to identify impurities in both EI & CI have shown that **this instrument is a powerful tool**."*

*"The speed and efficiency of the **Q Exactive GC gives confidence in impurity identification** with only one elemental formula being identified."*

Requirements for Identification of Unknowns

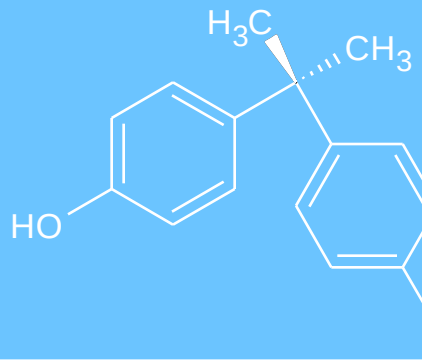
RT & m/z



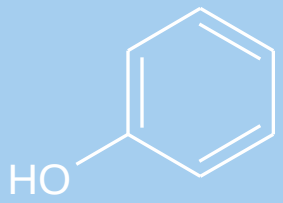
Empirical Formulae

$C_{15}H_{16}O_2$

Structure



Substructure



Name & CAS

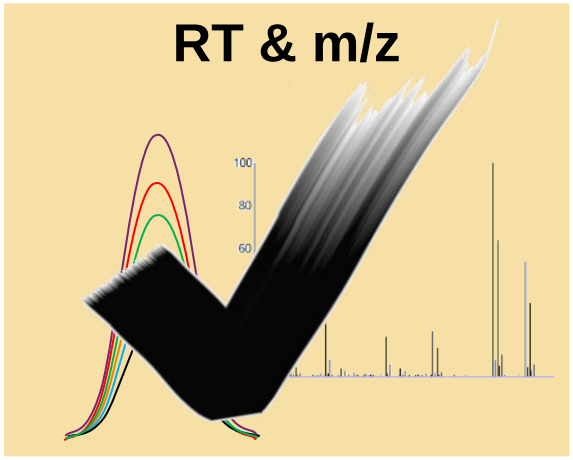
4,4'-(propane-2,2-diyl)diphenol

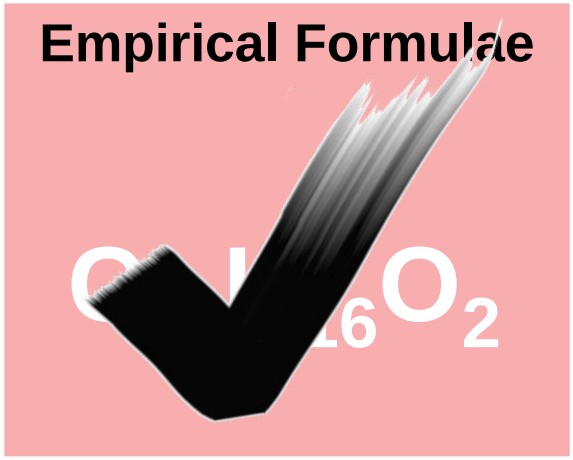
Bisphenol-A

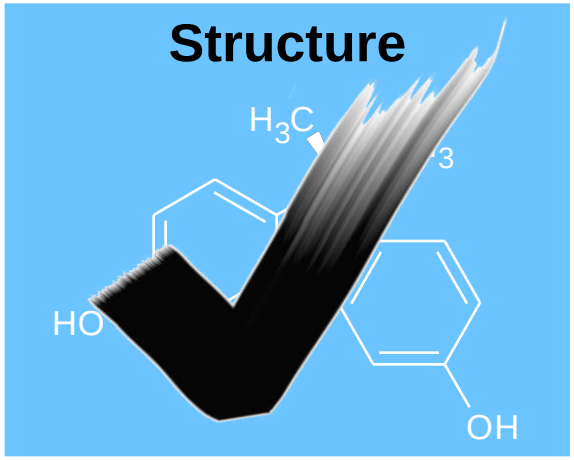
80-05-7

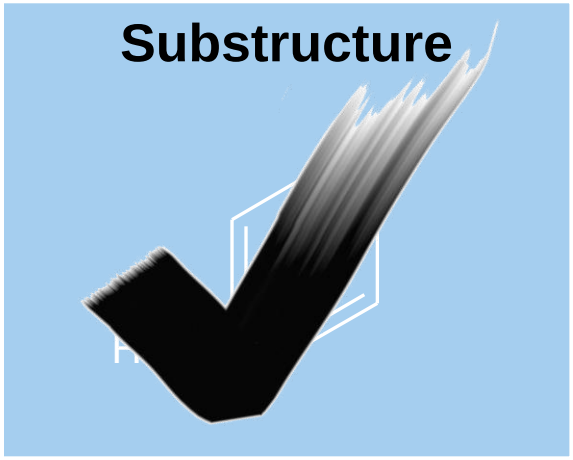
Amount

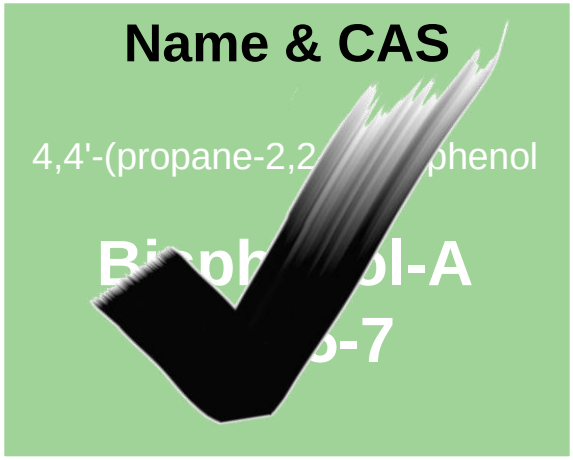
Requirements for Identification of Unknowns











Find out more

www.thermoscientific.com/QExactiveGC

Data in press:

Rapid Communications in Mass Spectrometry, circa April 2016

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