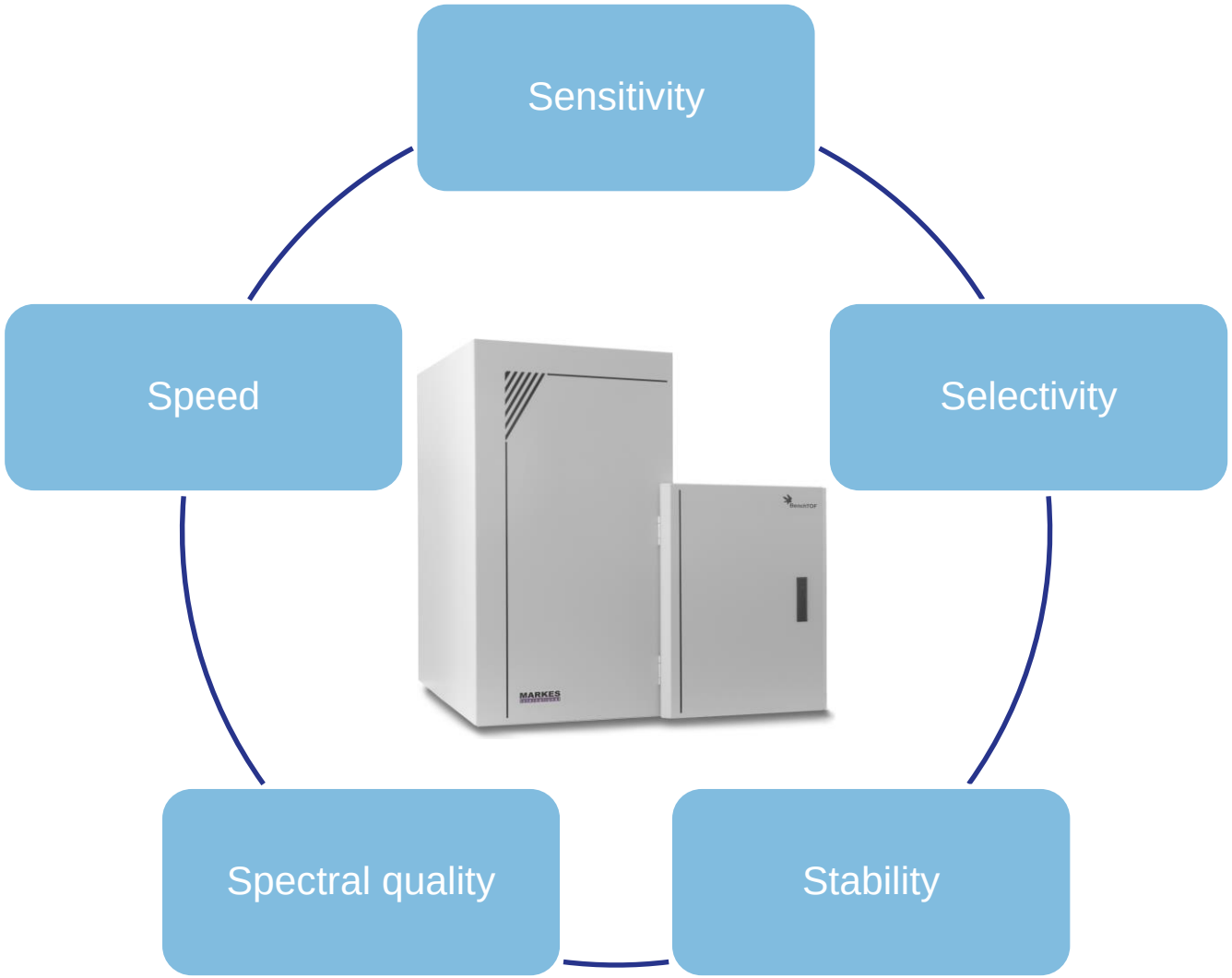
Extra dimensions of information by GC×GC-TOF MS with Tandem Ionisation

Aaron Parker
European Sales Manager

BenchTOF™

Time-of-flight mass spectrometry

- Fast acquisition rates over a wide mass range
- Sensitive detection of targets AND non-targets
- Confident identification with reference-quality spectra
- Robust, long-term performance



BenchTOF technology

Proprietary design

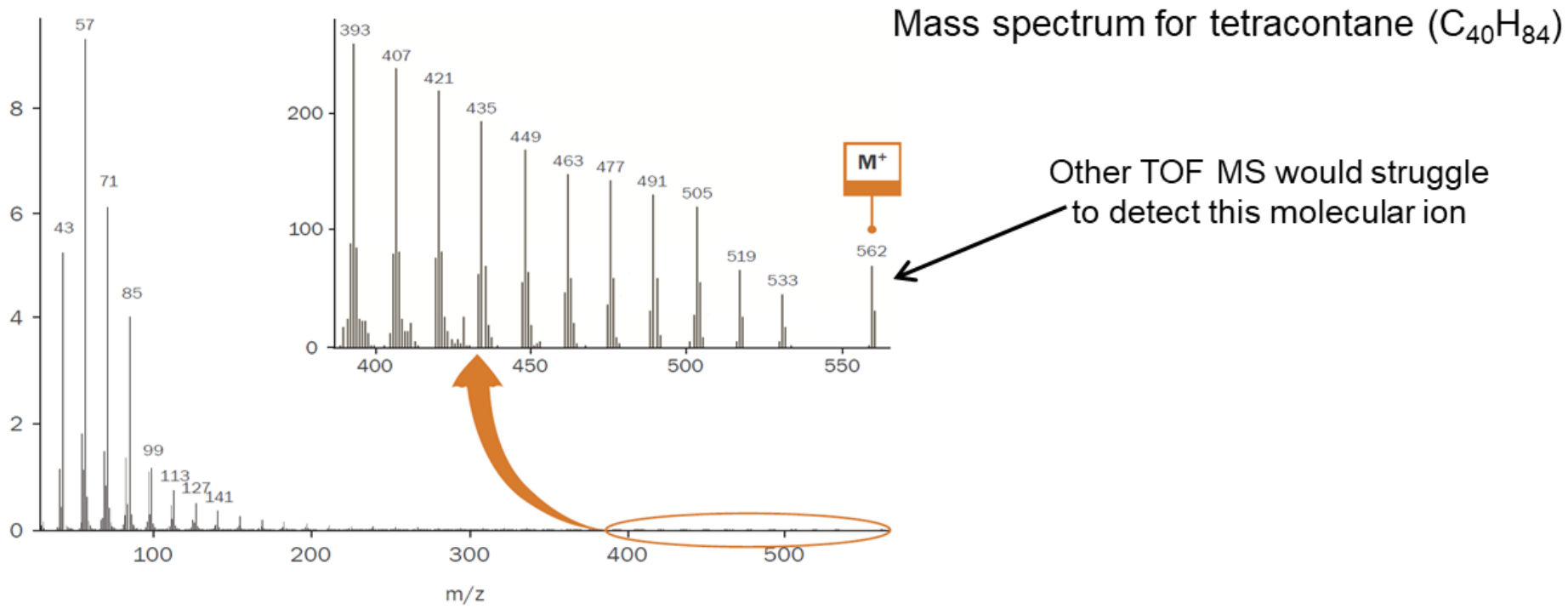
Differentially pumped source and analyser chambers

Direct extraction optimises sensitivity

Multi-stage reflectron optimises resolution

BenchTOF technology

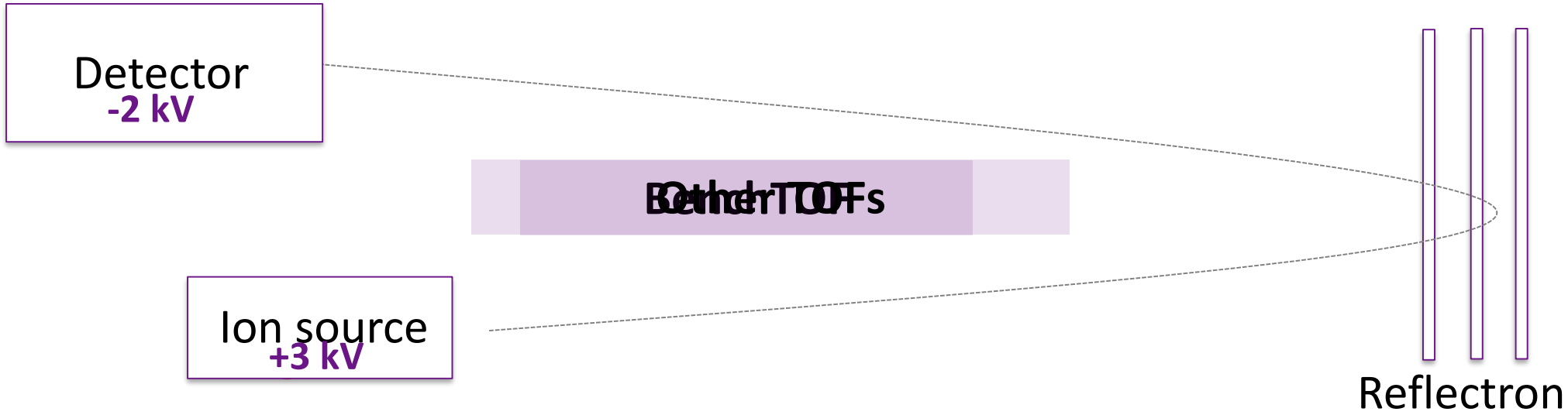
Truly NIST compatible spectra

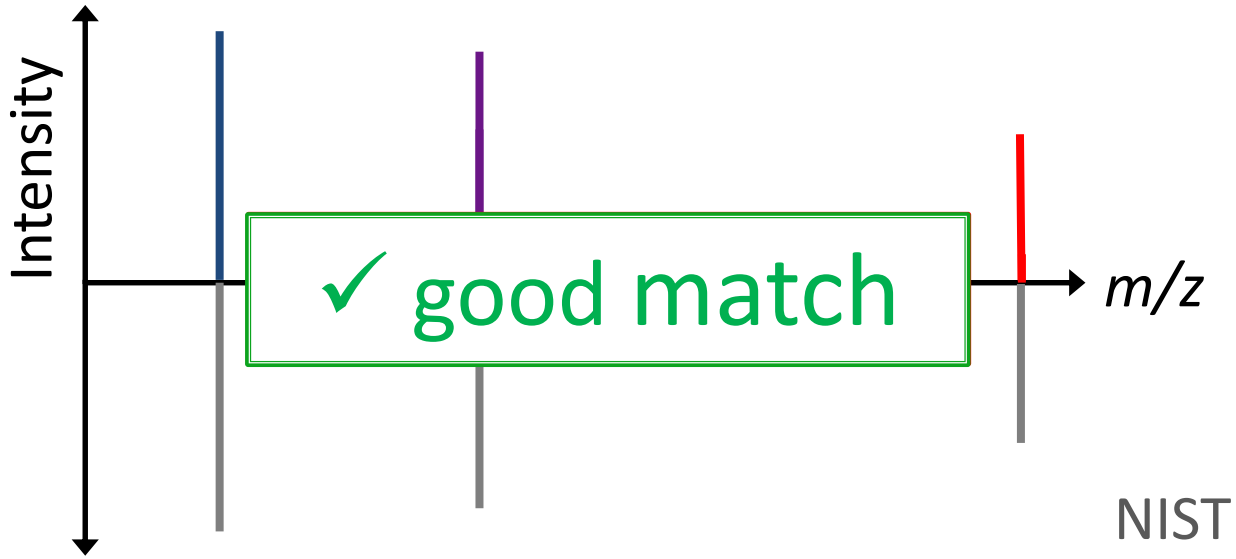


"The impressive sensitivity, speed and spectral quality of the BenchTOF has enabled us to address research questions that literally 'escaped' our previous set-up"

Dr Carsten Müller, School of Biosciences, Cardiff University

What is mass discrimination?

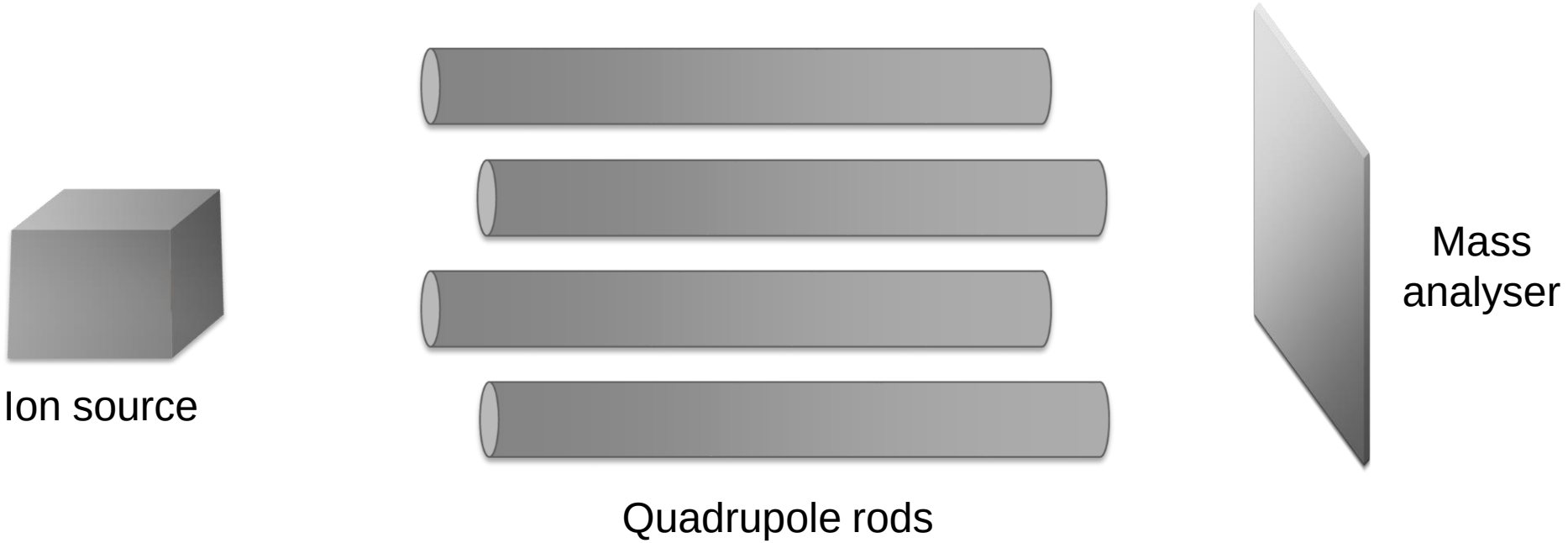




NIST

Quadrupoles are mass filters

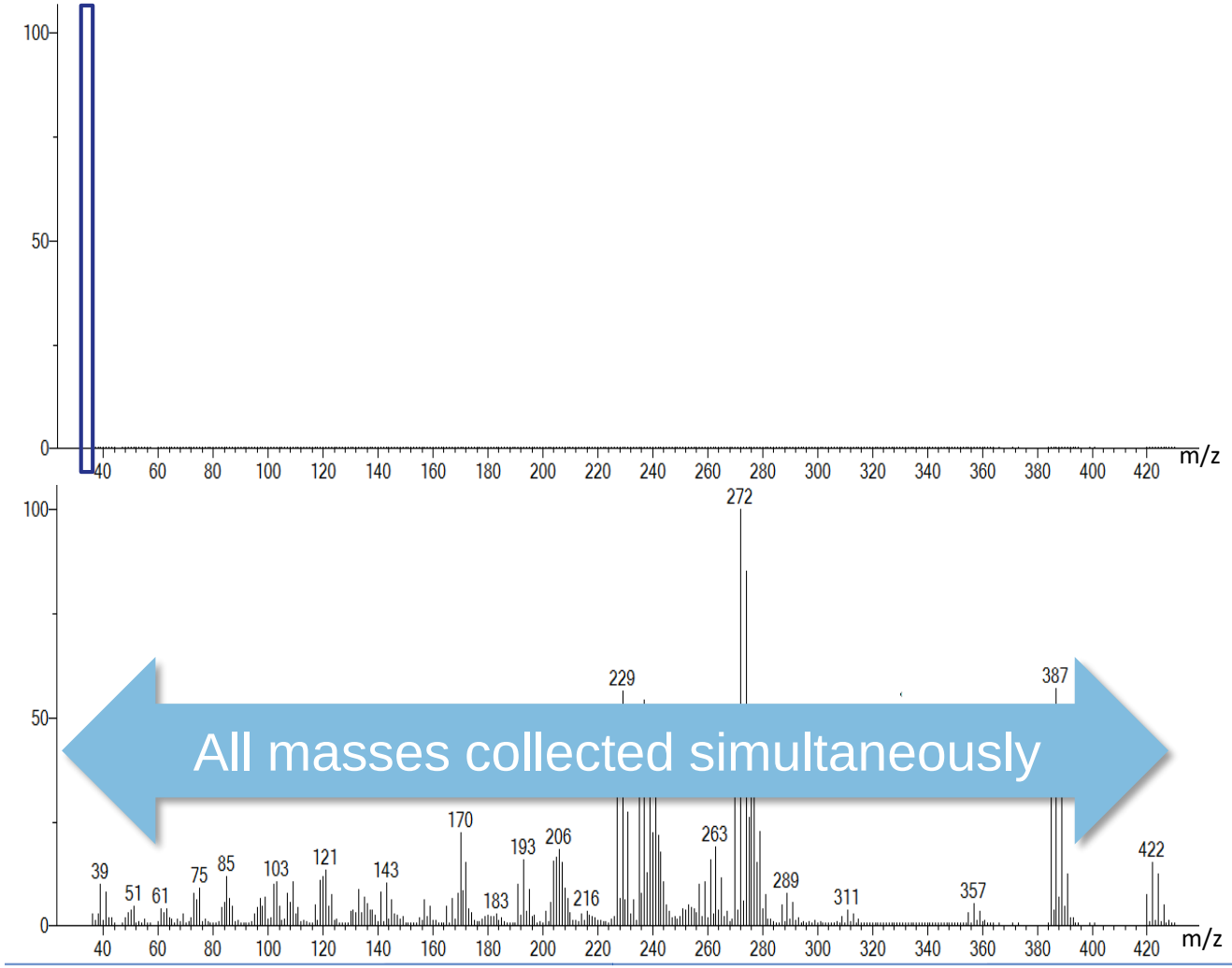
This is wasteful!



- Use SIM mode to improve sensitivity, but this only looks at target compounds

TOF vs. quadrupole MS

TOF technology does not rely on scanning individual masses



Quadrupole MS

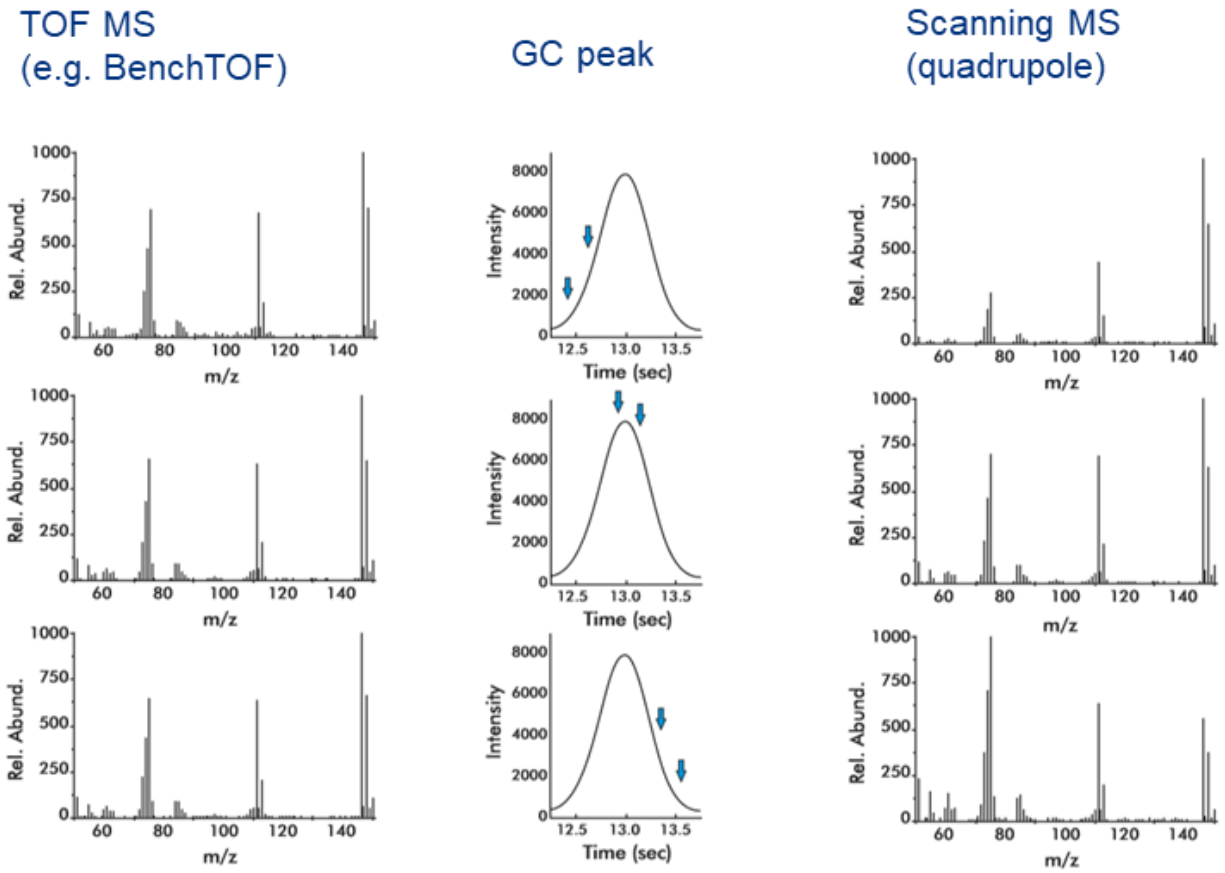
~ 0.4 s per full scan

BenchTOF

~ 4000 **full-range** spectra collected in the same time

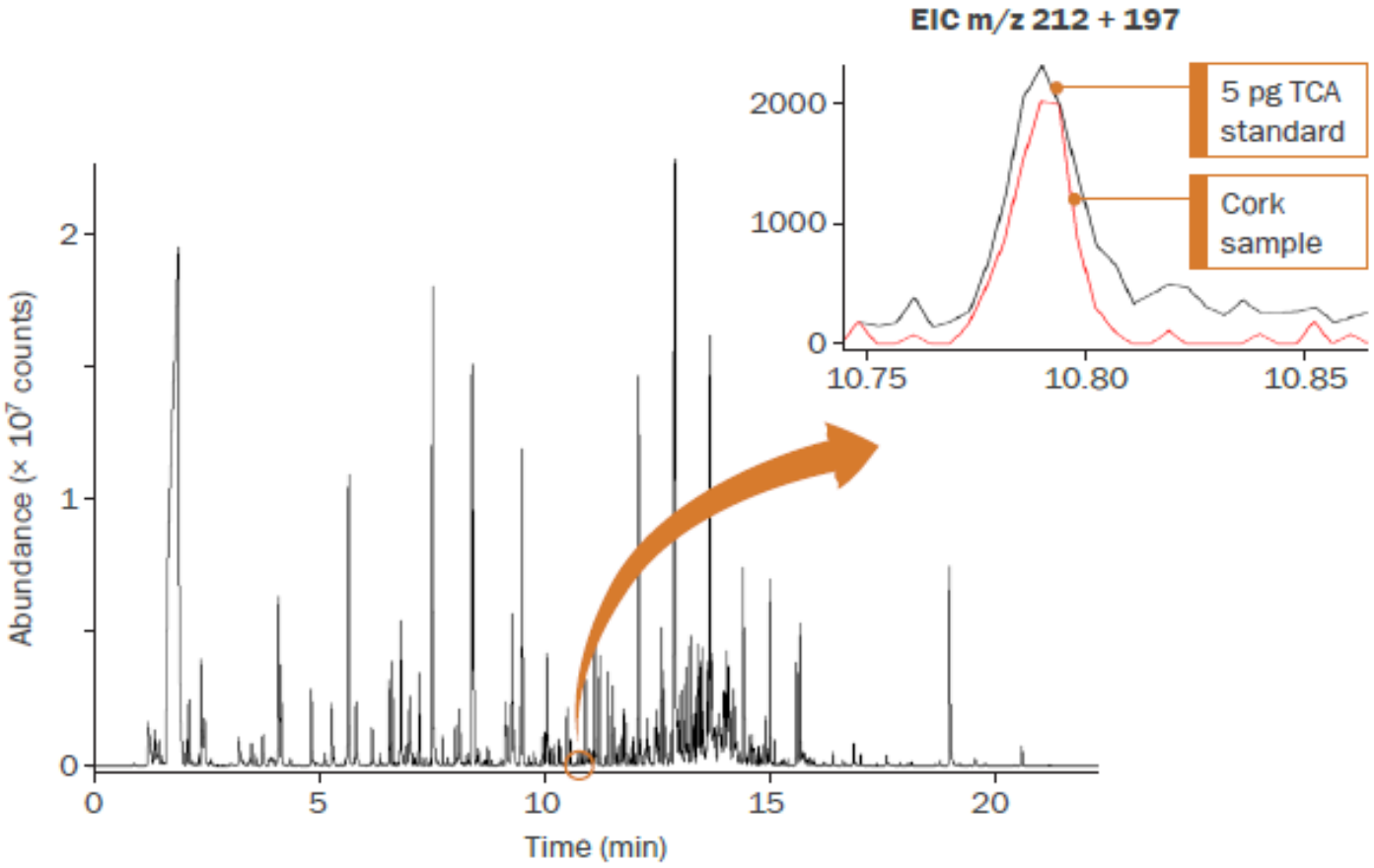
Spectral quality versus quadrupole MS

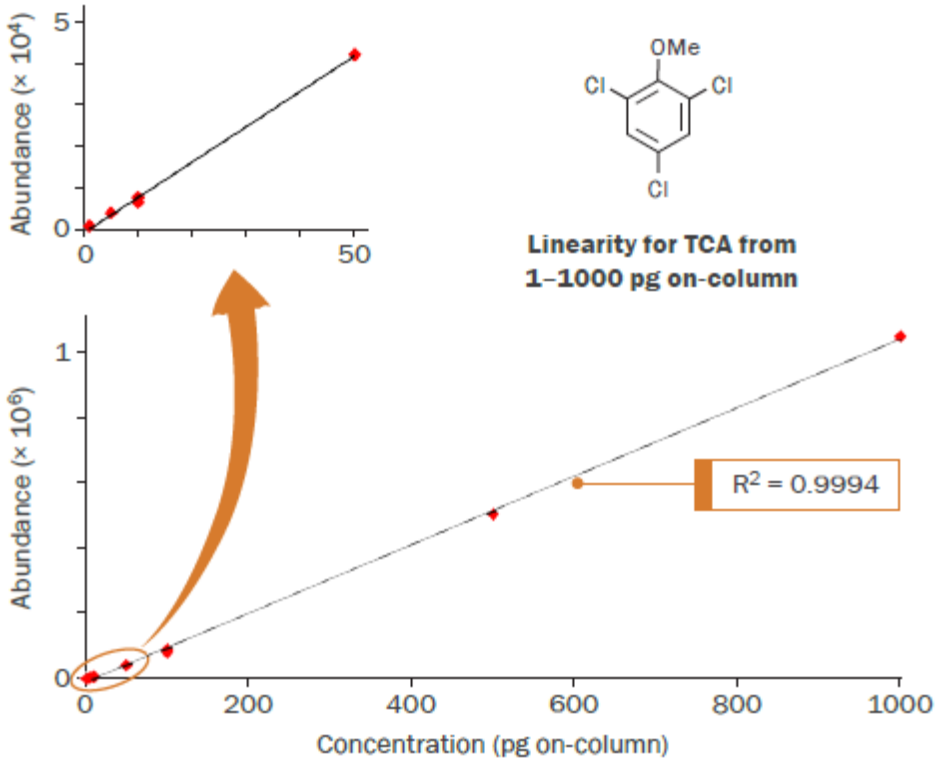
qMS experience spectral skewing



More robust deconvolution is possible with TOF MS due to no spectral skewing – for confident identification of hidden or masked peaks

Excellent sensitivity and linearity

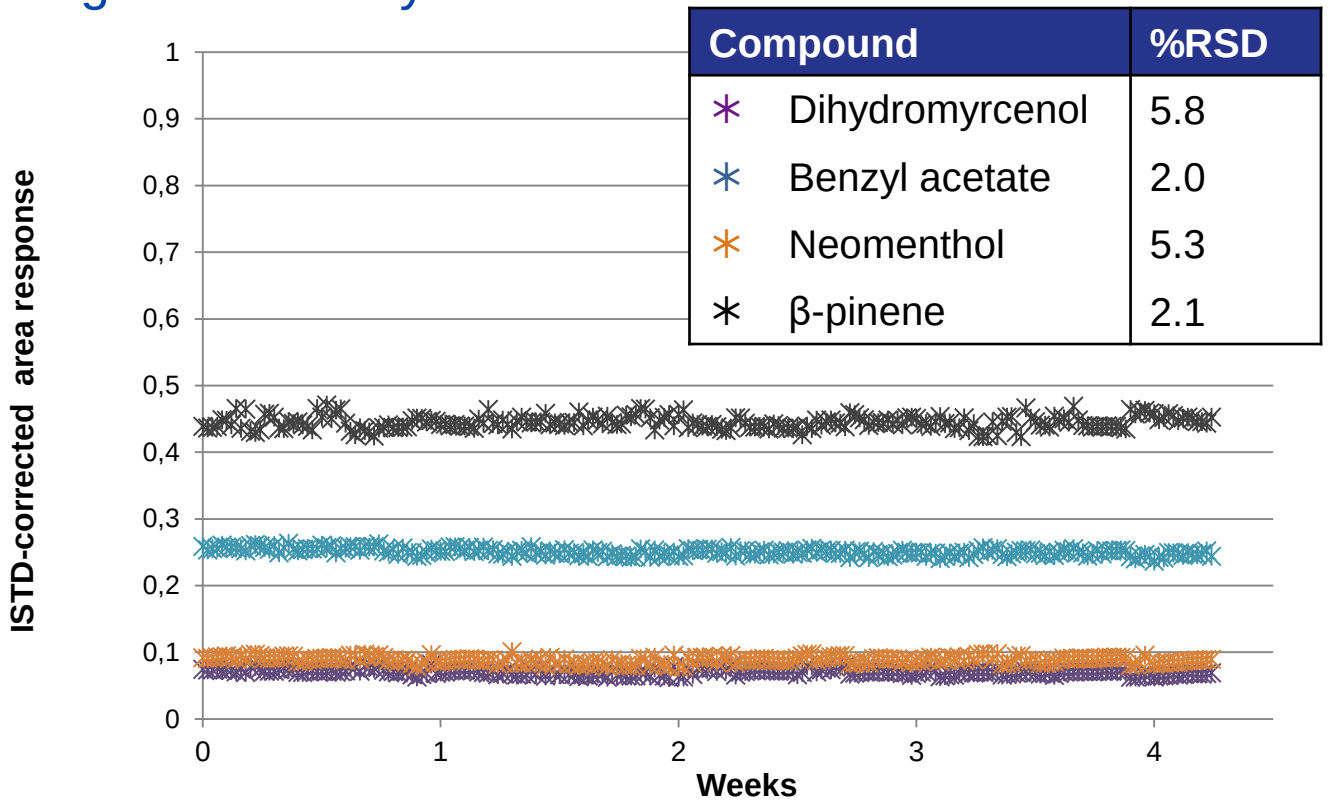




- Identification of ‘cork taint’ from 2,4,6-trichloroanisole

Confident results every time

Long-term stability



Compound	%RSD
Dihydromyrcenol	5.8
Benzyl acetate	2.0
Neomenthol	5.3
β-pinene	2.1

- >200 replicate injections of a complex fragrance mix
- Stable response over 4 weeks, with no additional tuning
- Minimal downtime – ion source cleaning is rarely required outside of annual maintenance visits

"Our two BenchTOF are the ultimate trace gas detectors: highly sensitive, very reliable and convenient to use."

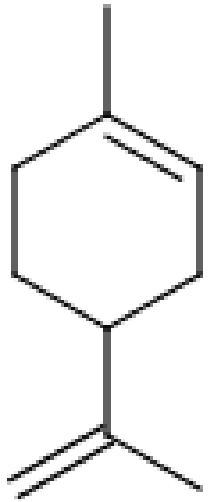
Peter Boeker, University of Bonn

What is soft ionisation?

Hard ionisation

Soft ionisation

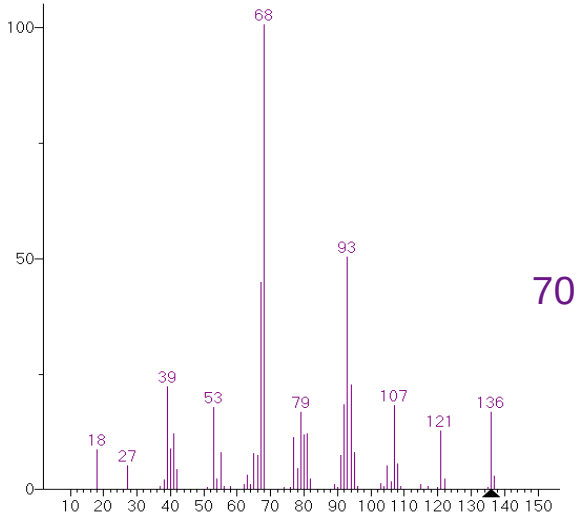
What is soft electron ionisation?



Limonene

Hard ionisation

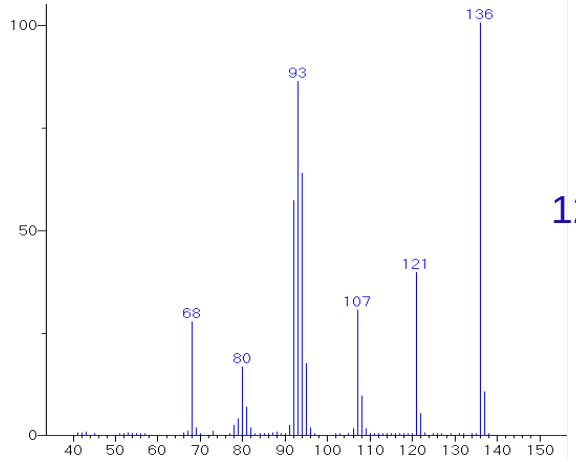
(e.g. 70 eV)



70 eV spectrum

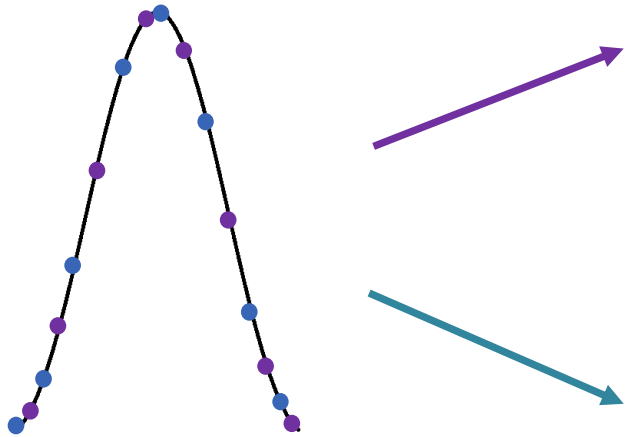
Soft ionisation

(e.g. 10-20 eV)



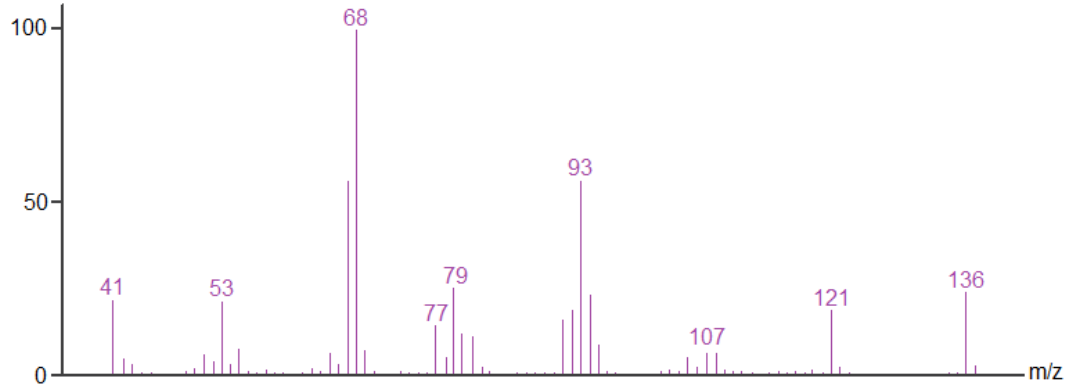
12 eV spectrum

Tandem Ionisation®

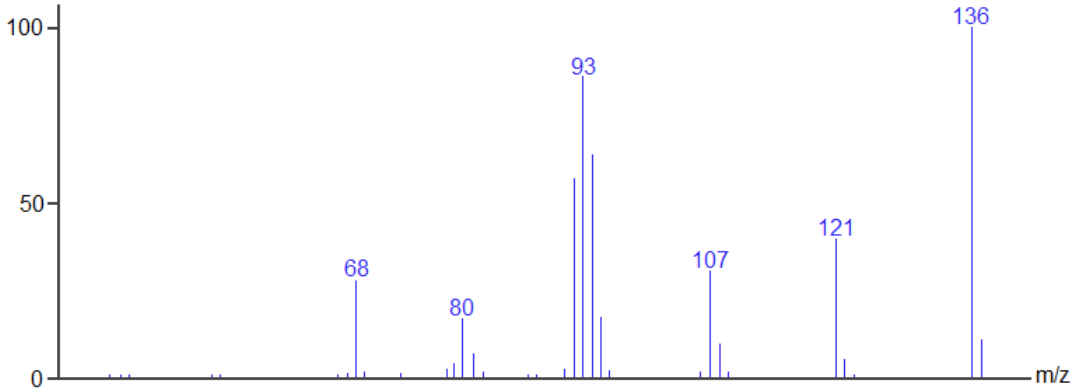


Tandem ionisation
(70 eV /14 eV)
at 200 Hz

70 eV
(100 Hz)



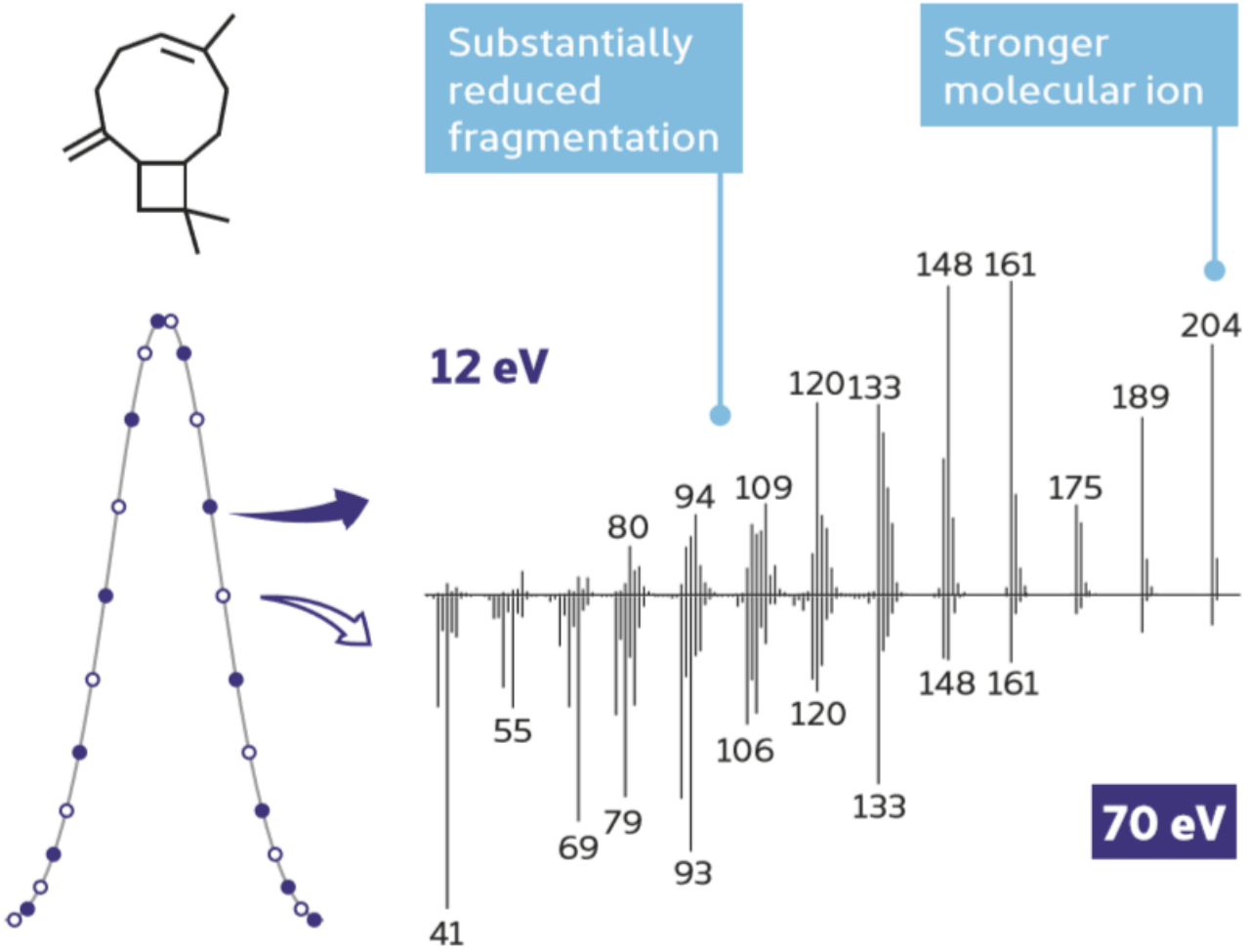
14 eV
(100 Hz)



- Collect NIST- quality 70 eV and soft EI data simultaneously
- Software automatically generates two files from the raw data

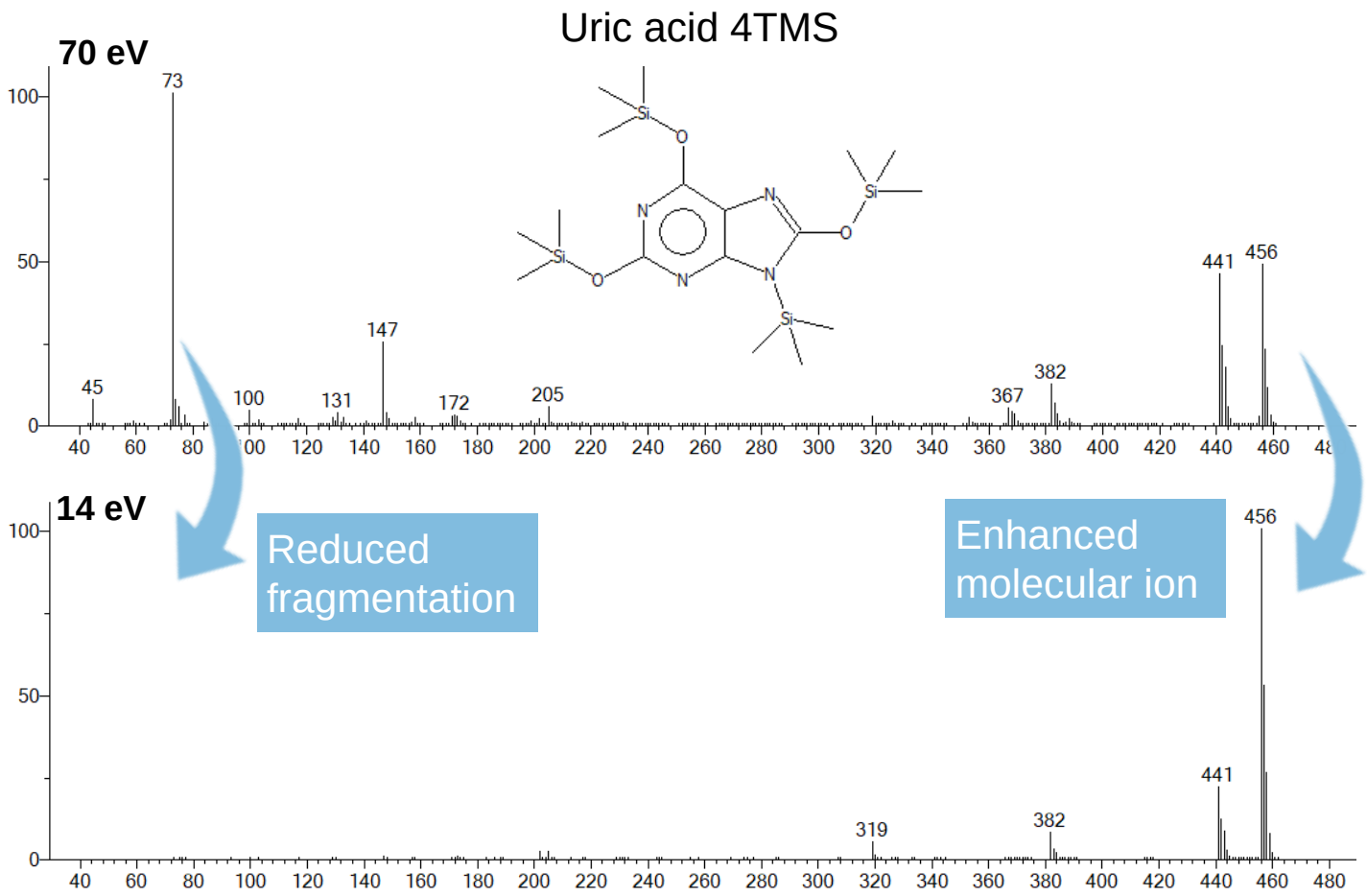
Tandem Ionisation

- Acquire hard and soft EI simultaneously
- Complementary spectra to confirm compound identity
- Confident isomer identification
  - Not possible based on 70 eV data alone
- Patented technology
- **Exclusive to BenchTOF**



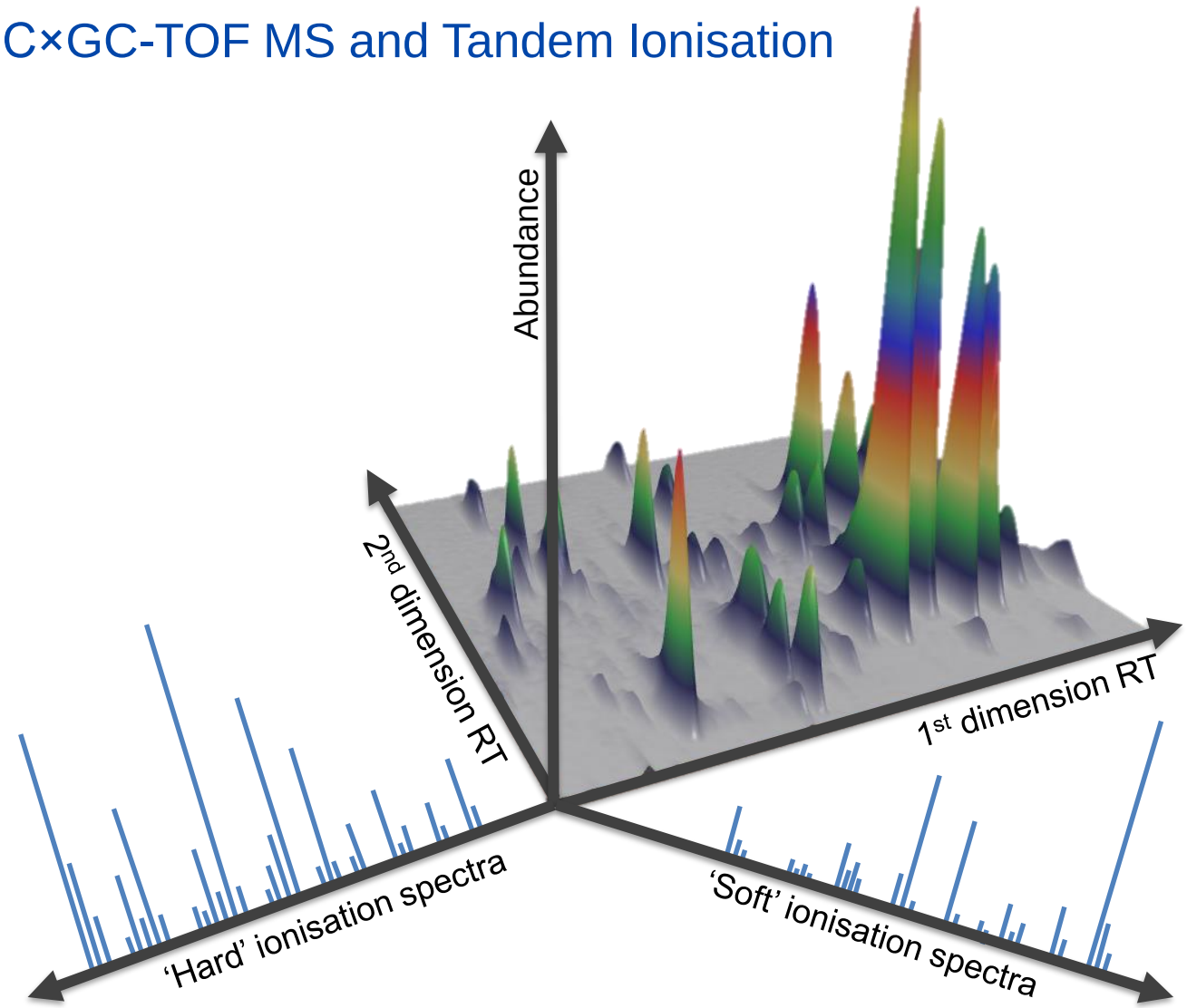
Reduced fragmentation

- Enhanced molecular ion and other structurally-significant ions
- Soft EI reduces the dominant ions associated with derivatisation
- Improved confidence in identification, especially within complex samples.



Gaining extra dimensions of information…

…with GC×GC-TOF MS and Tandem Ionisation



Case studies

Fragrance allergens

Breath and FAME profiling

Allergens

Background information

- Contact allergy following skin contact with a sufficient amount of these ingredients (e.g. in cosmetics)
  - Affects ~5% of population

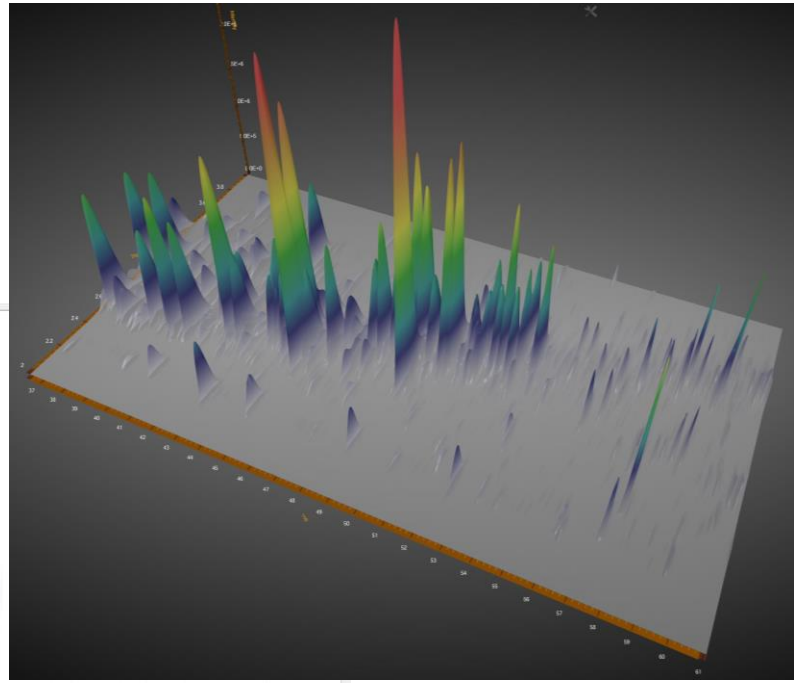
- Can trigger serious health effects…
  - Asthma attacks
  - Headaches/migraines
  - Respiratory irritation

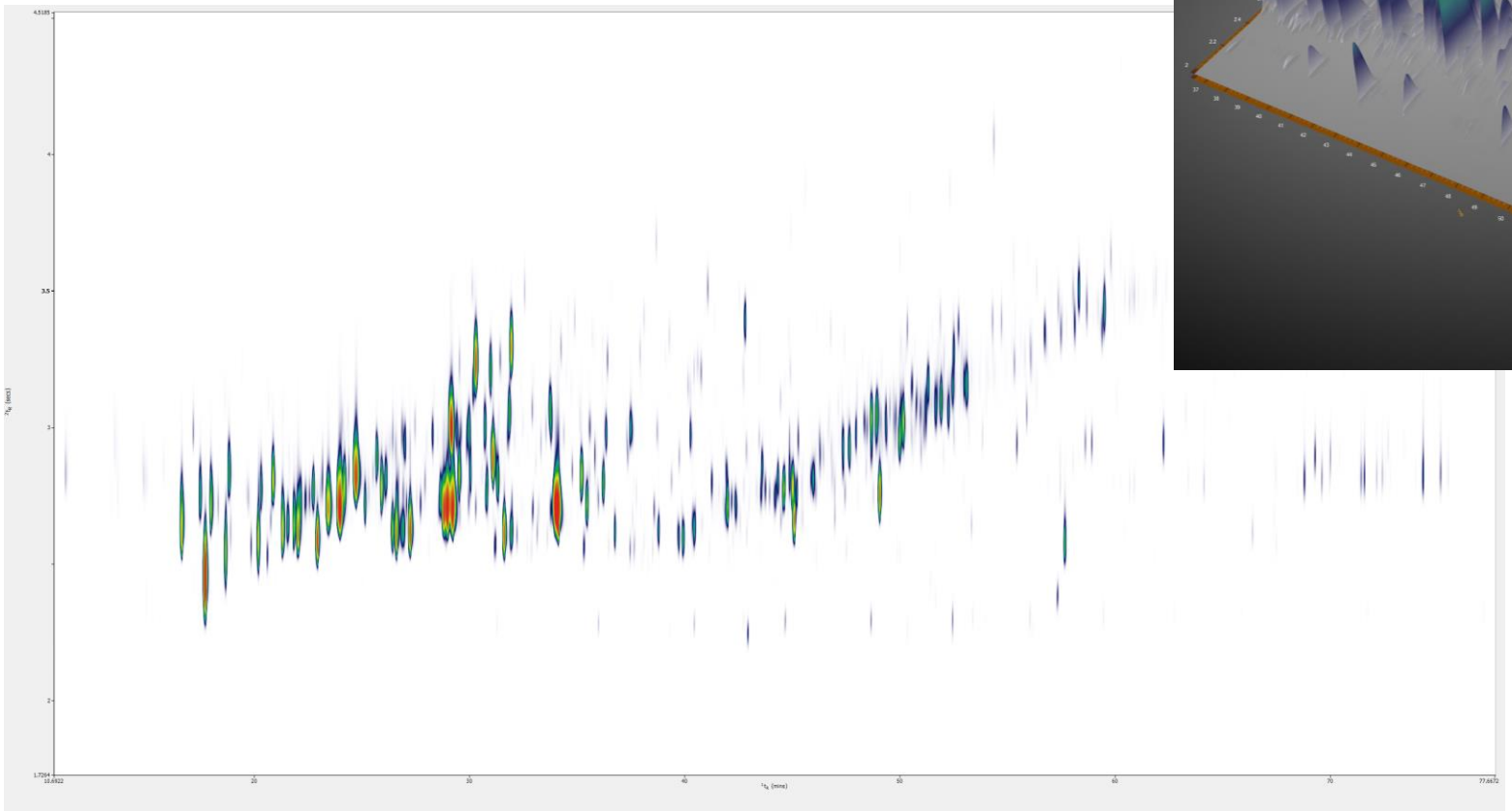
- Can be found in many fragranced products…



Why GC×GC?

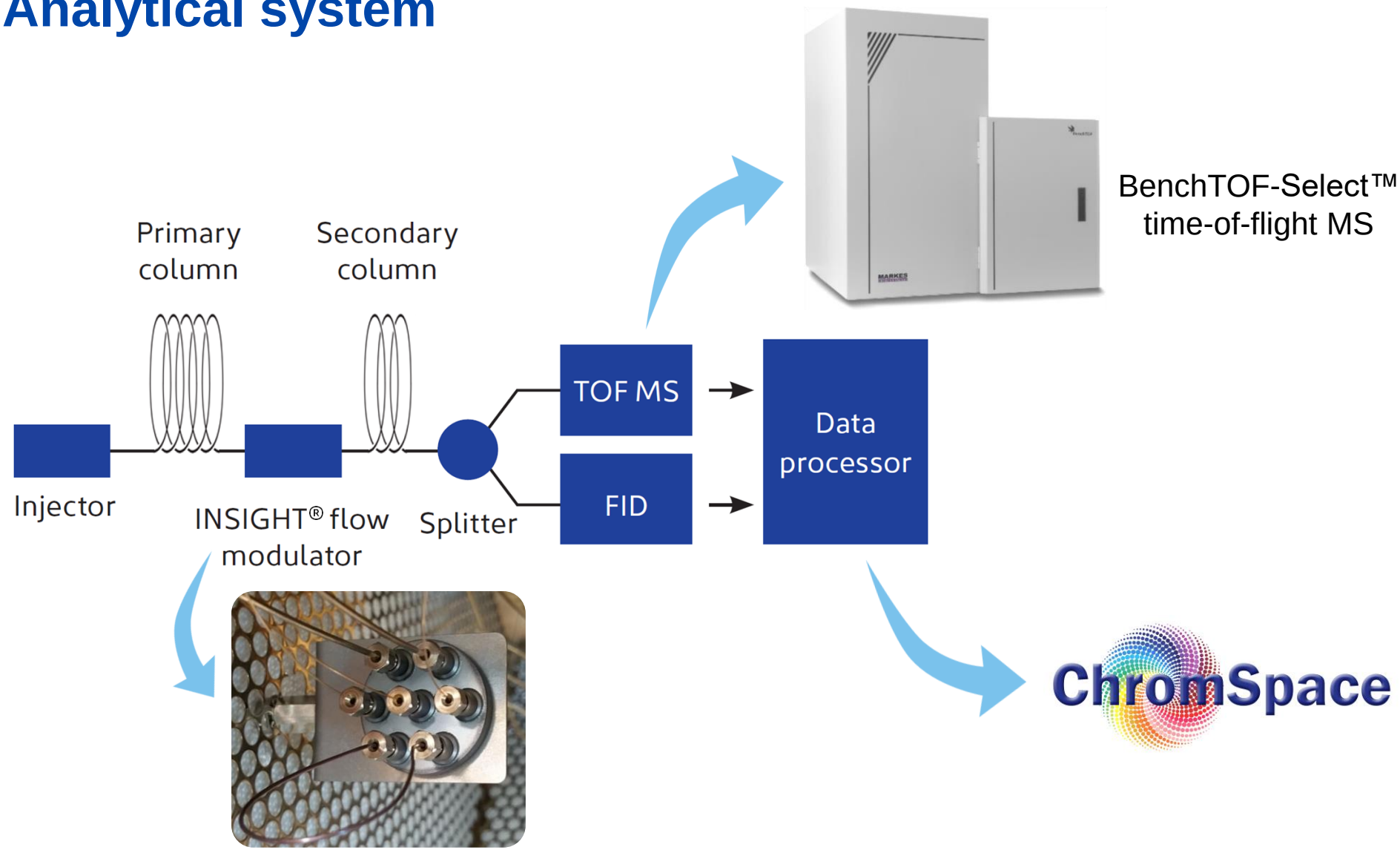
Fragrances and essential oils are complex



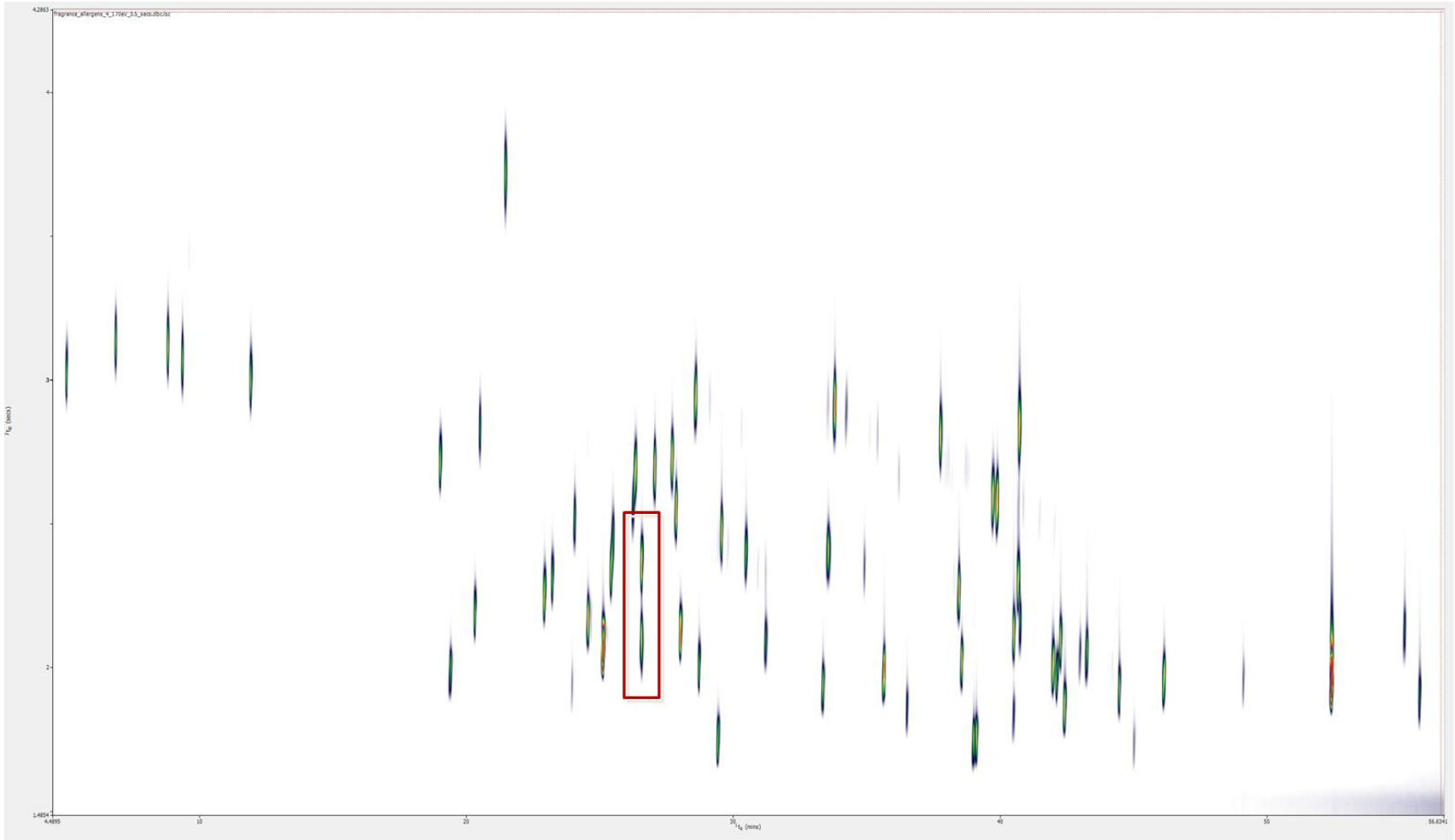


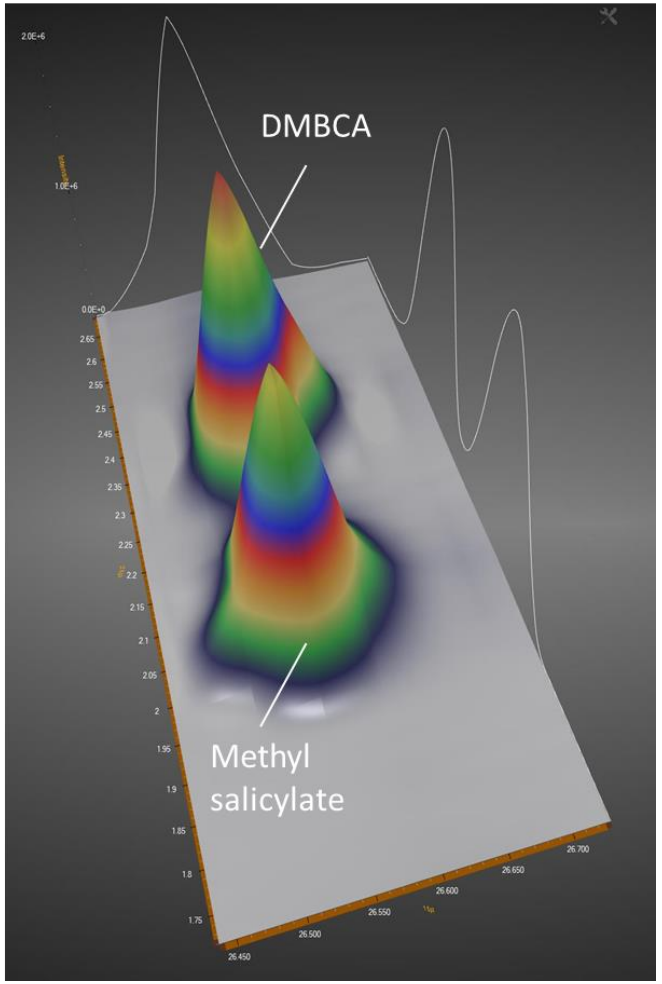
- Chamomile essential oil with > 800 peaks detected

Analytical system



Separation of fragrance allergens

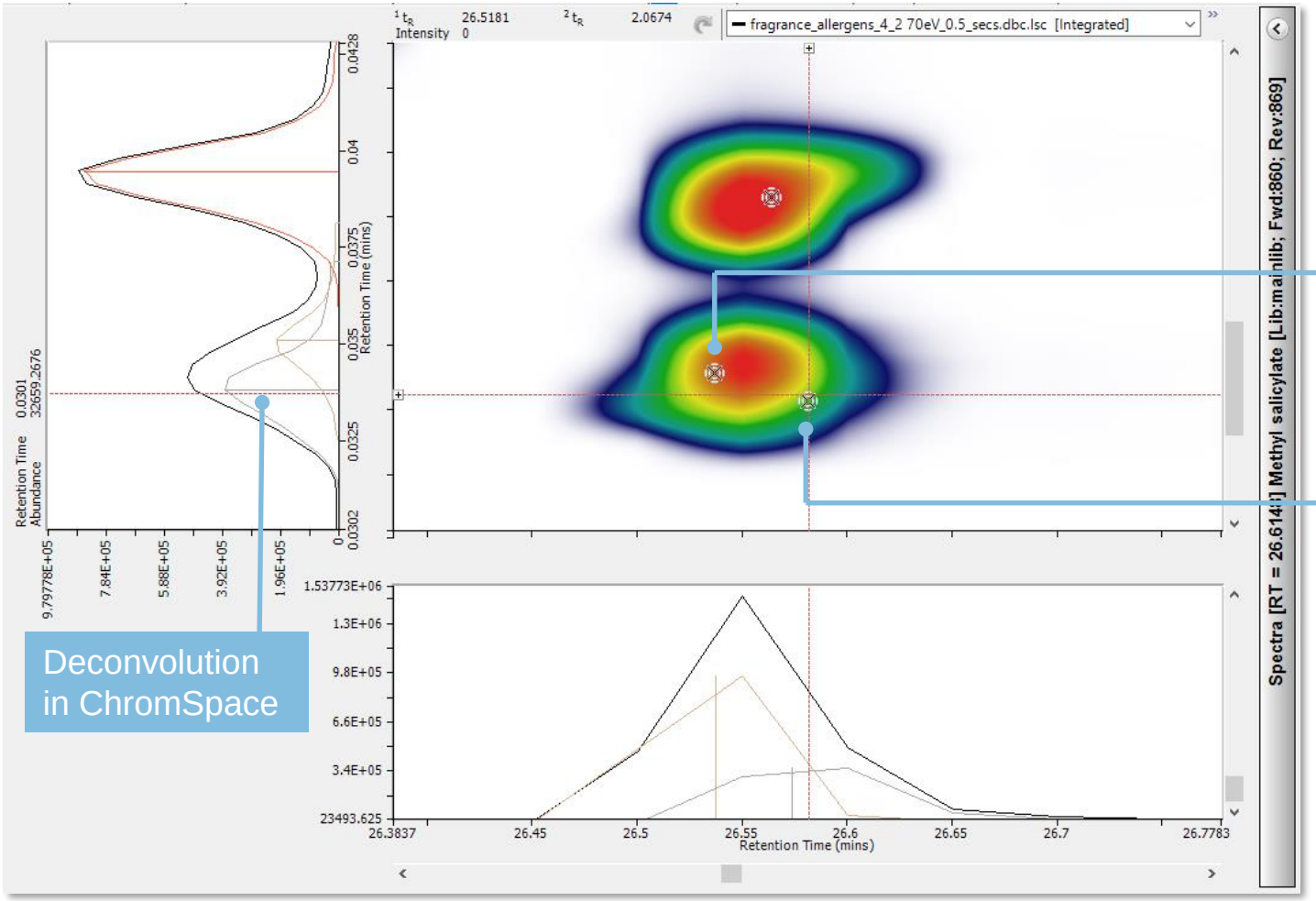




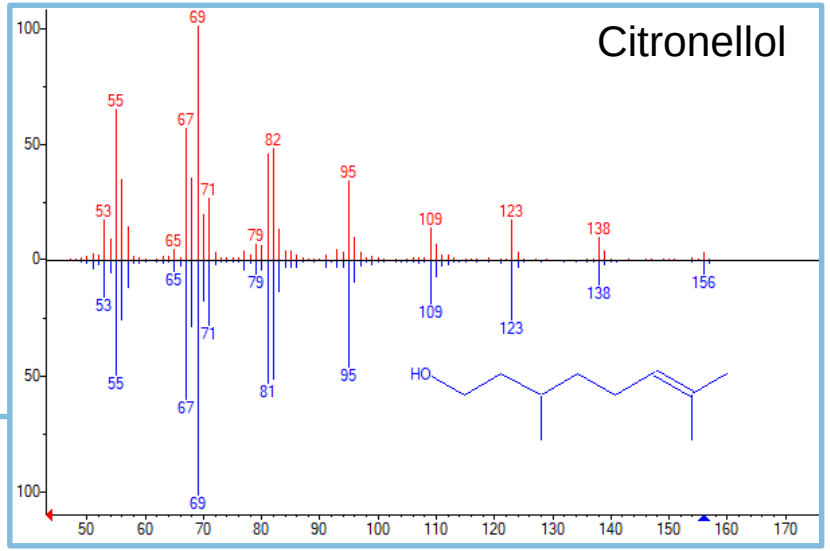
- Flow-modulated GC×GC–TOF MS chromatogram for 64 allergens.

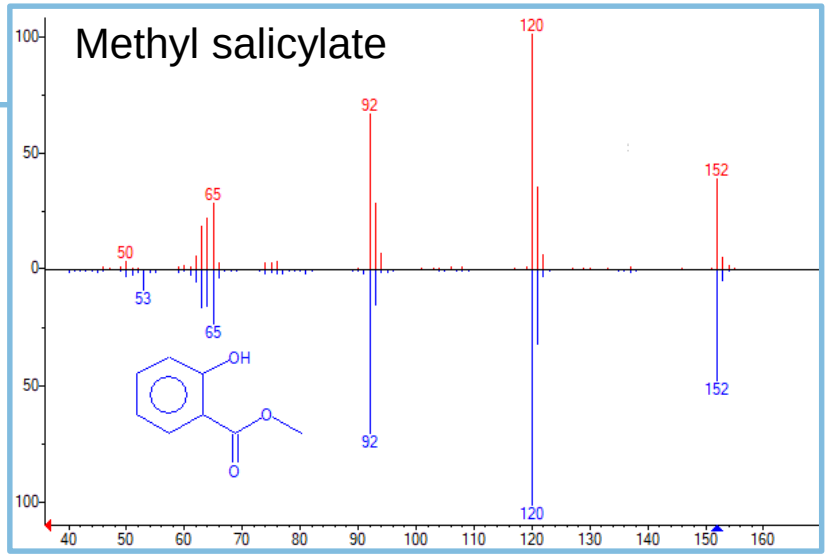
Confident identification with BenchTOF

Confirmation of peak purity

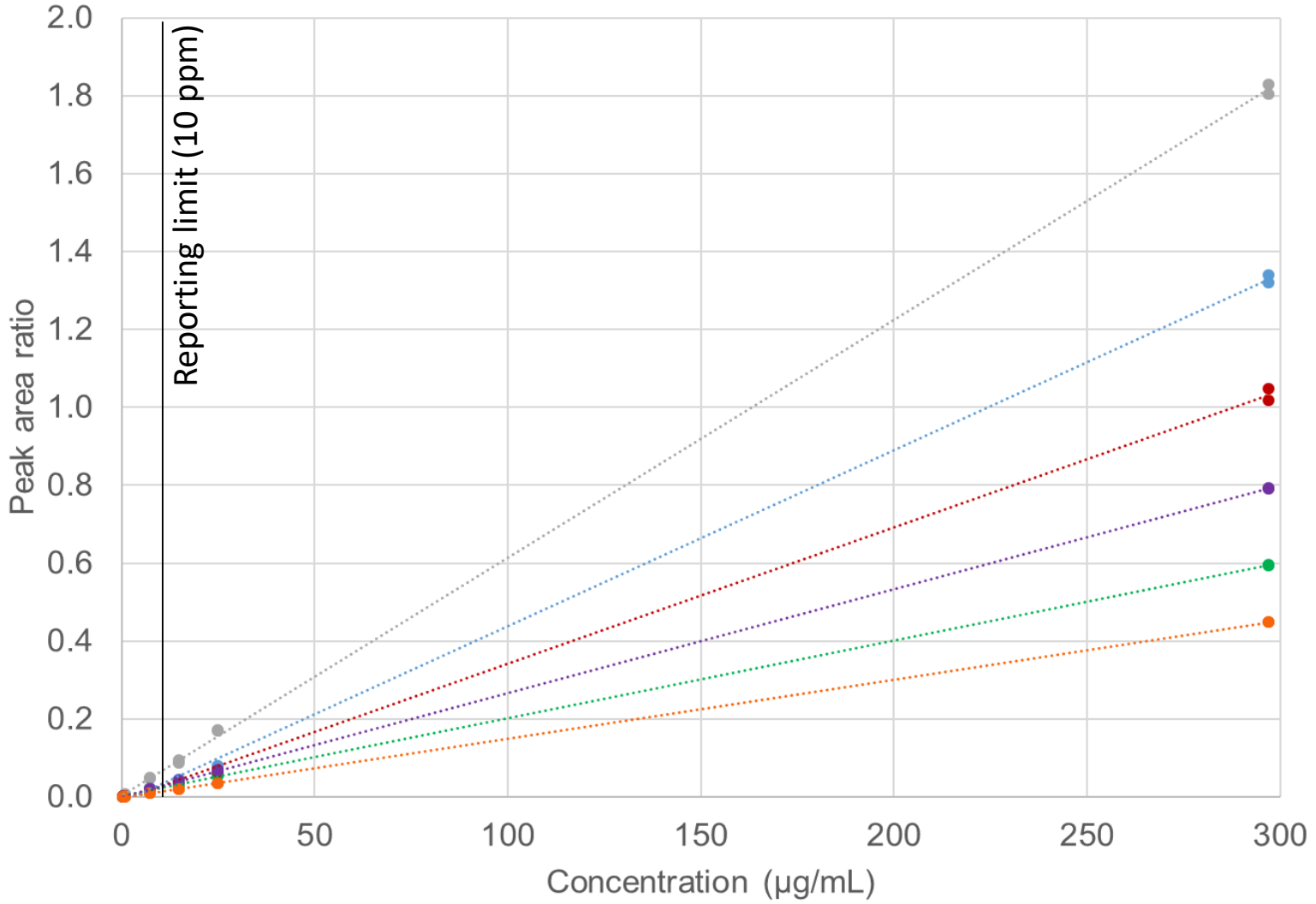


Deconvolution in ChromSpace





GC×GC-TOF MS linearity

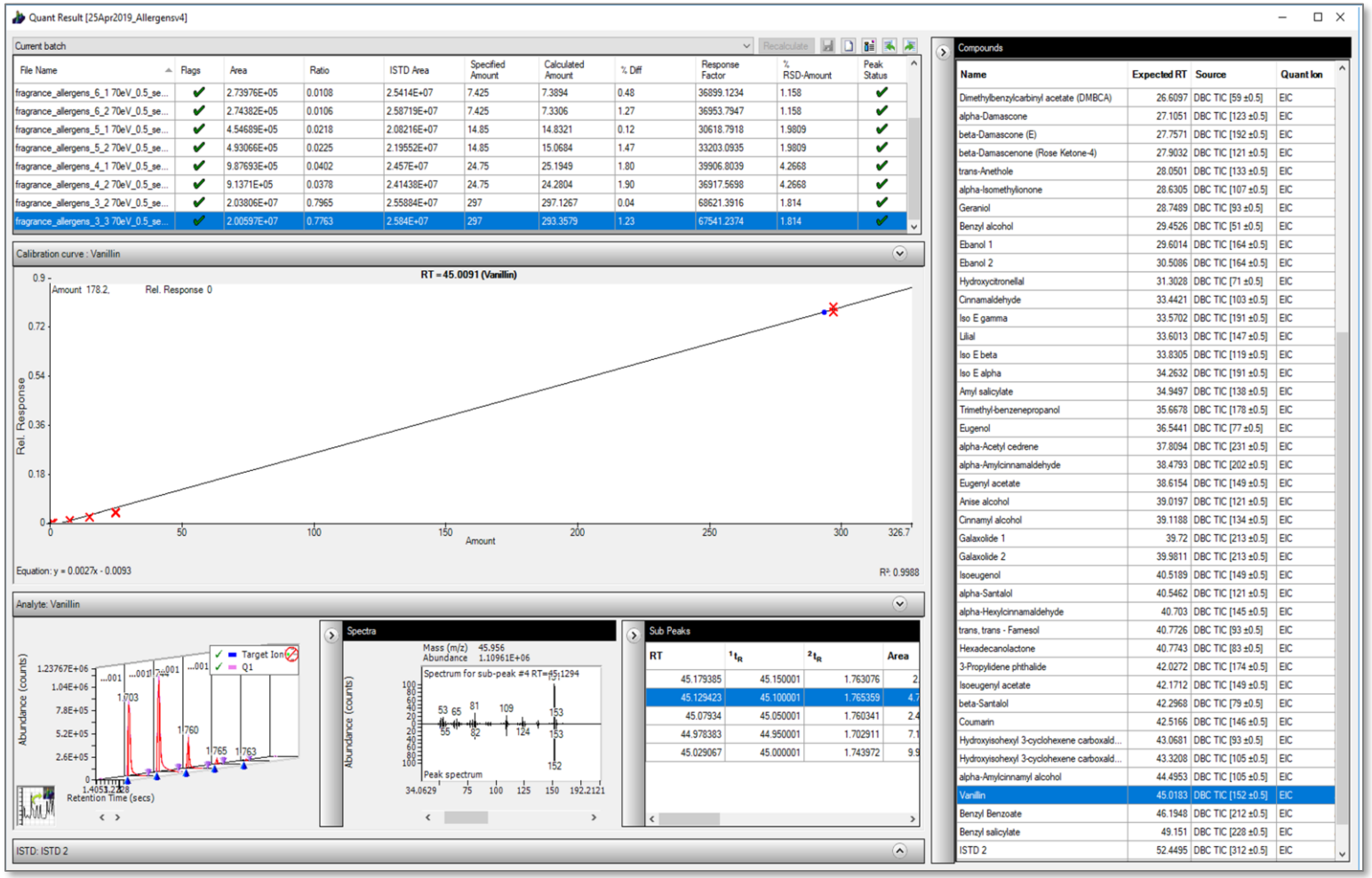


Compound	R^2
Methyl oct-2-ynoate	0.9999
α-Isomethylionone	0.9998
Lilial	0.9994
Benzyl cinnamate	0.9994
3-Propylidene phthalide	1.0000
Neral	0.9996

- Duplicate analysis at six concentration levels (from 0.37–300 µg/mL)
- Average $R^2 > 0.999$

ChromSpace software

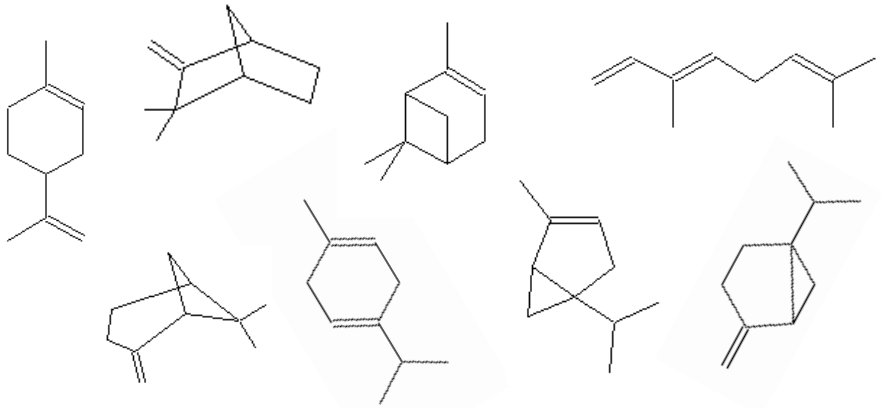
Simple workflows for EIC quantitation



- Flagging system shows any issues in identification or suitability parameters
- Export results as a report, to .csv or to LIMS
- Easy EIC quant: Compatible with various file formats
- Simple review of identification and integration

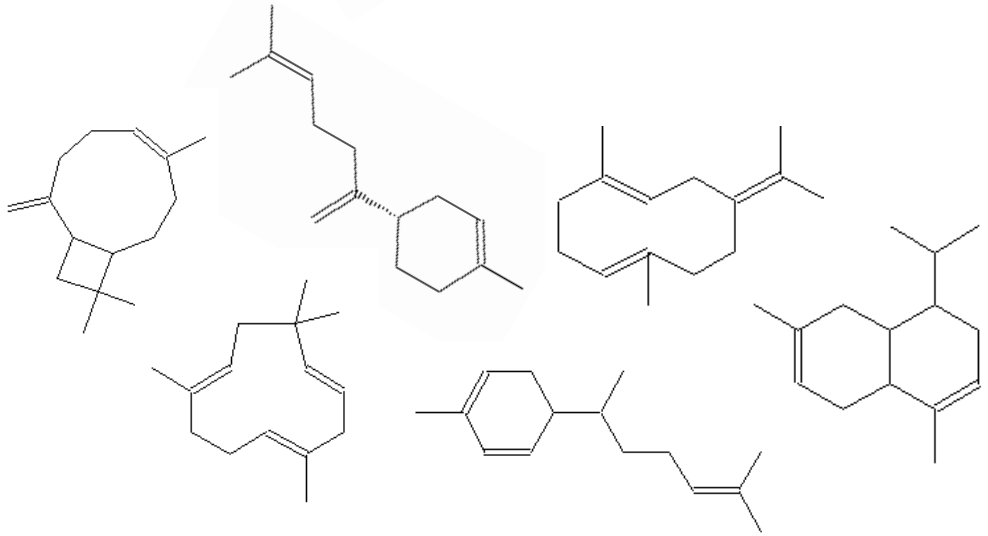
Remaining challenges?

And how we can address them…



Monoterpenes

m/z 136.1252



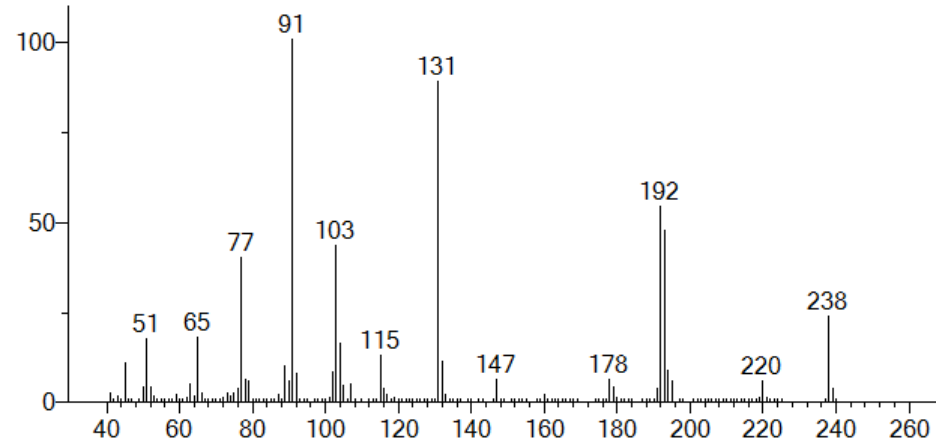
Sesquiterpenes

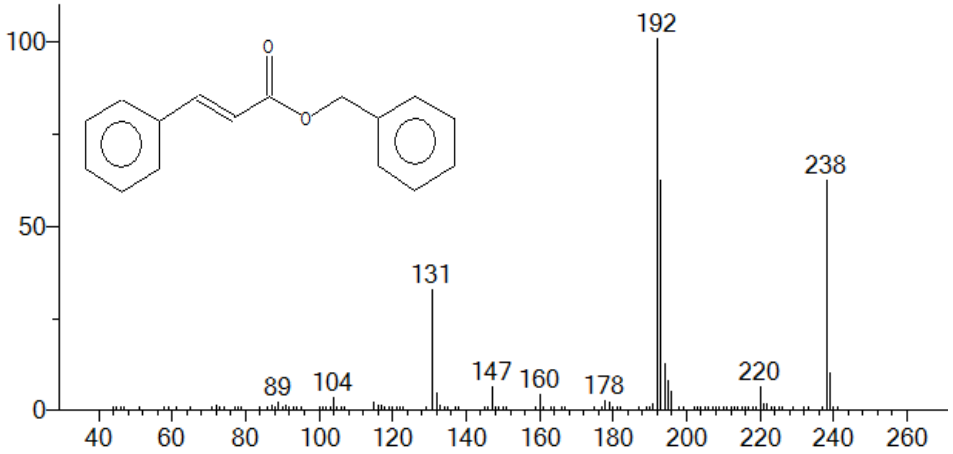
m/z 204.1878

Reduced fragmentation...

…using soft EI

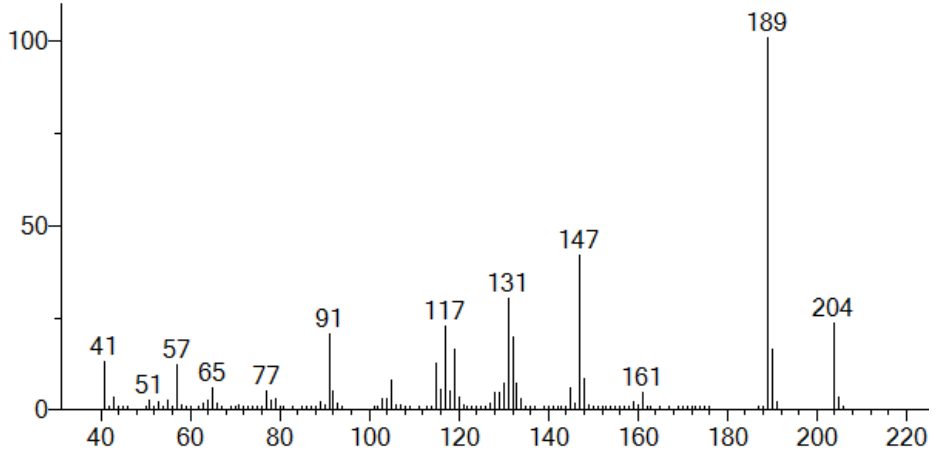
Benzyl cinnamate



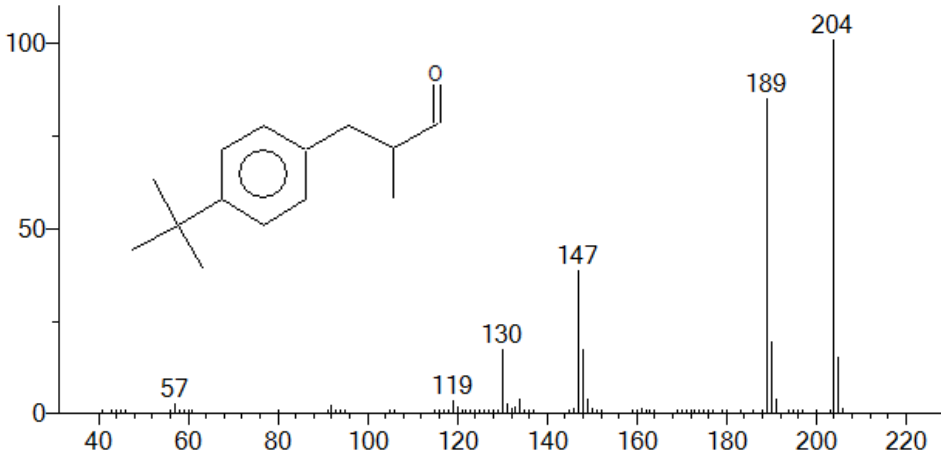


Lilial

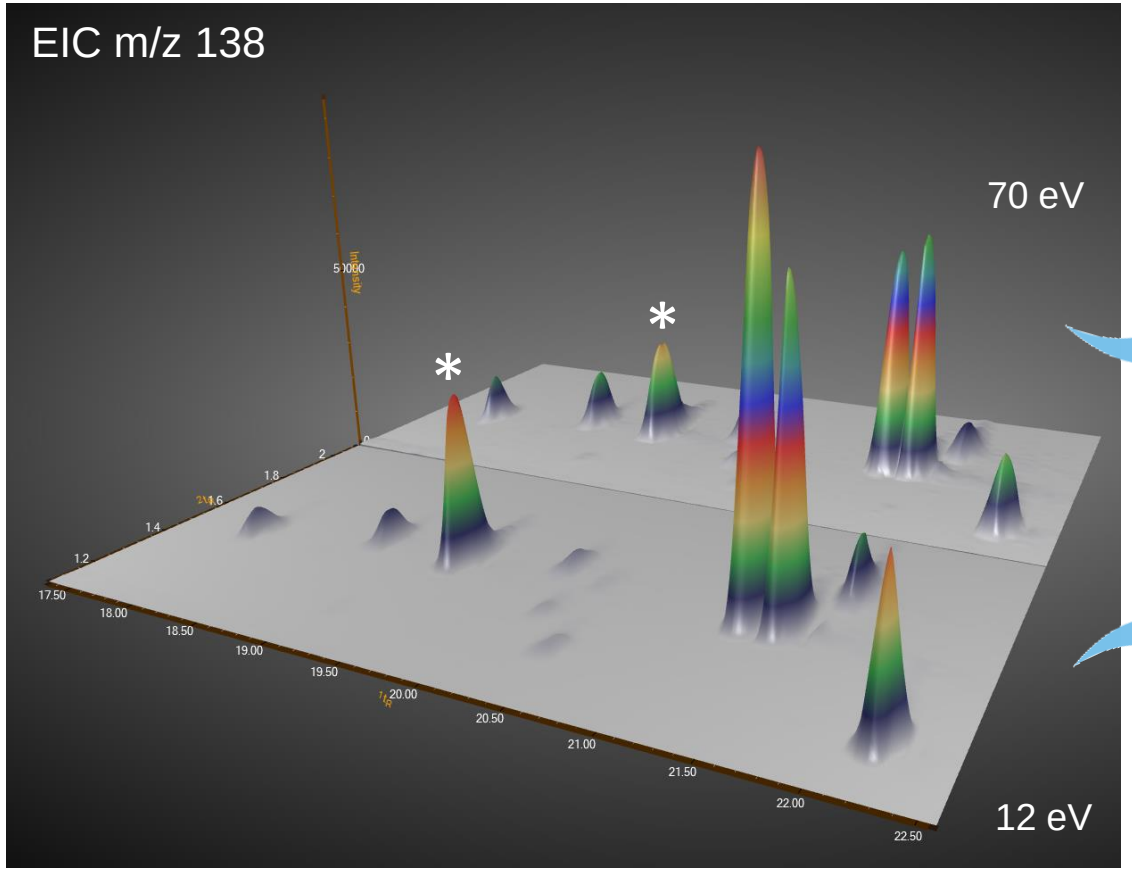
70 eV

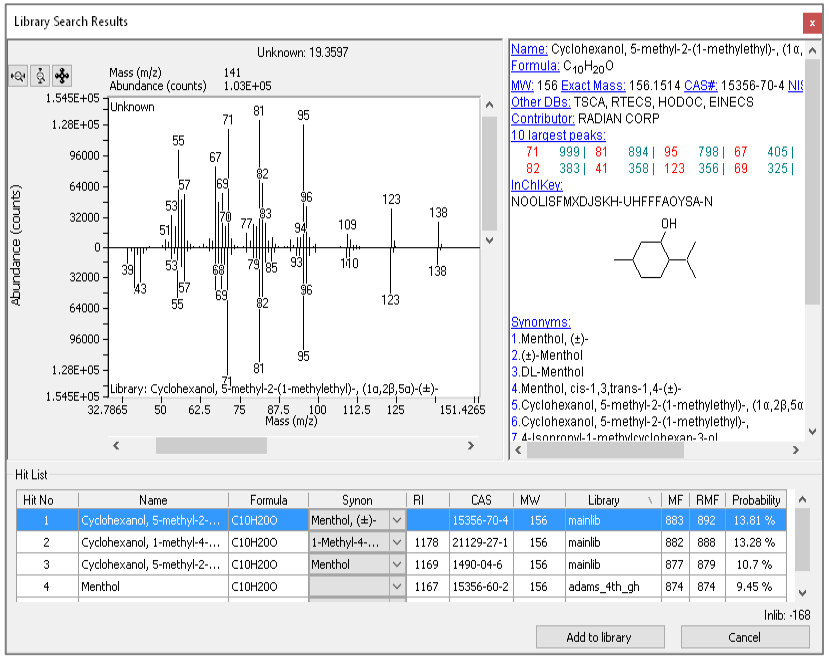


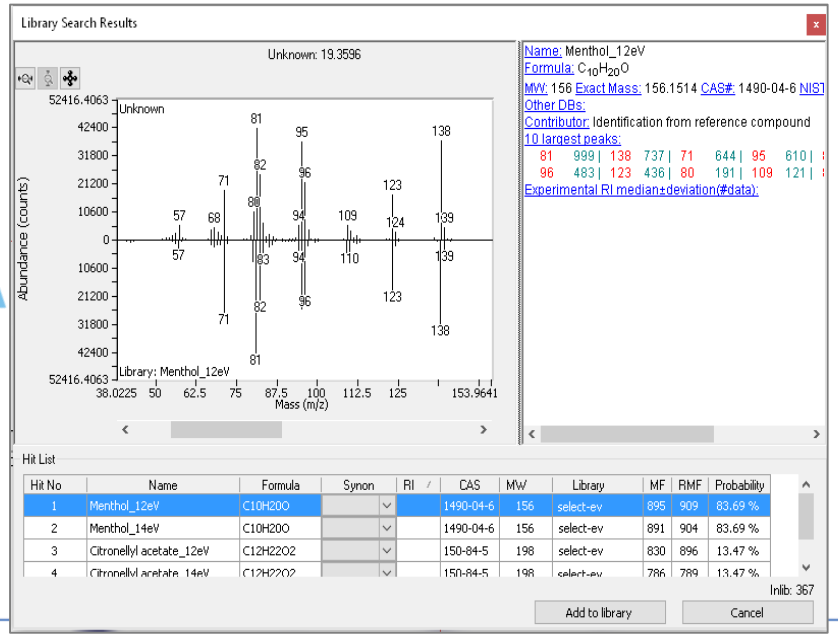
12 eV



An additional level of confidence







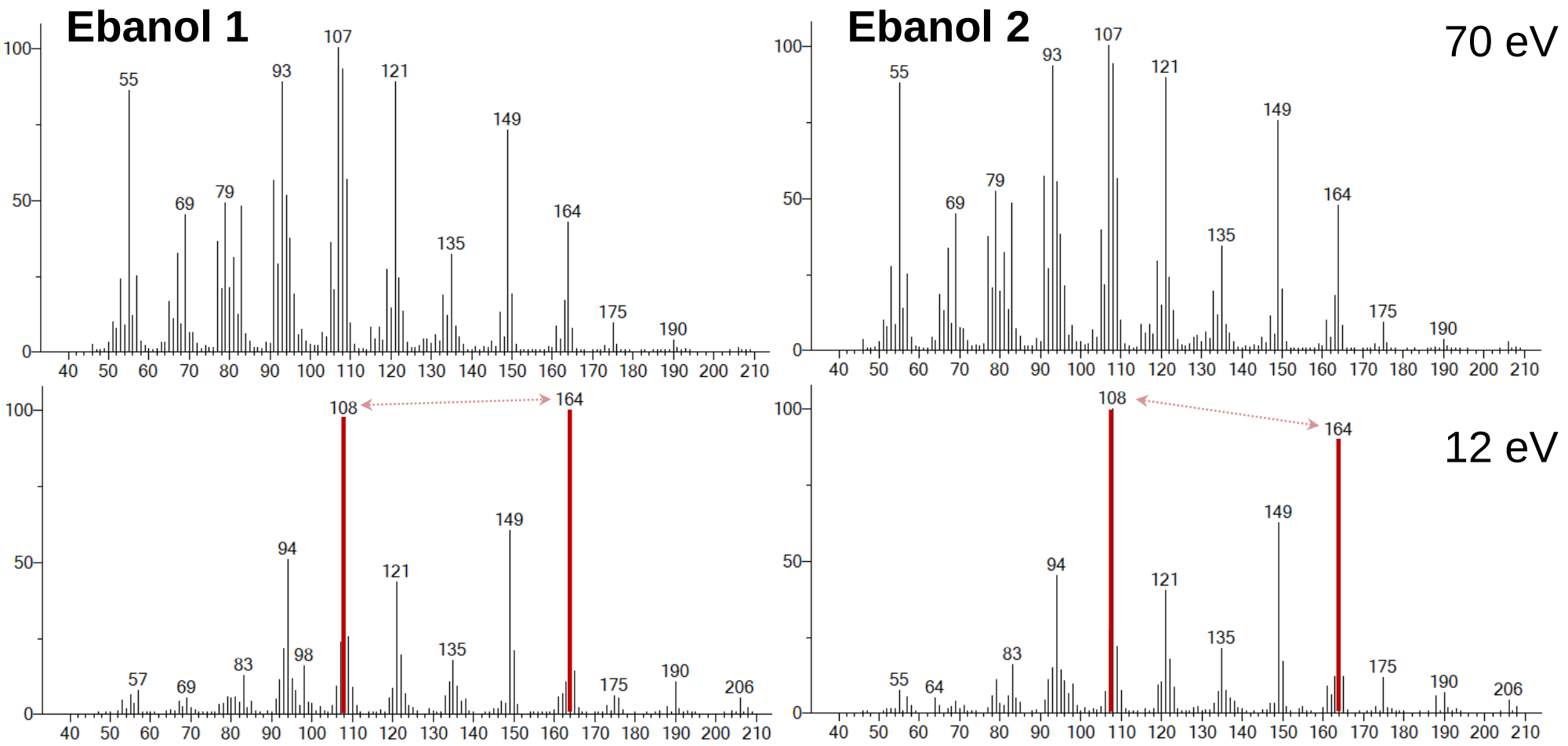
Tandem Ionisation for enhanced QC

Compound	^1t_R (min)	^2t_R (s)	RI *	Ion ratios	70 eV	12 eV	Status
Limonene	9.4015	0.0711	1031	68:93	1.87	0.33	✓
				93:136	0.43	1.71	
Ebanol 1	29.5469	2.3986	1280	108:164	2.12	0.96	✓
				149:164	1.50	0.59	
Ebanol 2	30.4589	2.3284	1290	108:164	2.10	1.11	✓
				149:164	1.56	0.71	
α-Santalol	40.4967	2.1180	1689	93:121	1.62	1.11	✓
				121:202	8.93	3.28	
β-Santalol	42.2505	2.0882	1711	94:122	2.08	1.04	✓
				122:202	15.45	9.47	
Benzyl cinnamate	55.7114	1.8944	2091	91:192	2.28	0.17	✓
				192:238	2.84	2.33	

- Using four unique qualification criteria (^1t_R, ^2t_R, RI and ion ratios at both 70 eV and 12 eV).

Confirmation of isomer identification

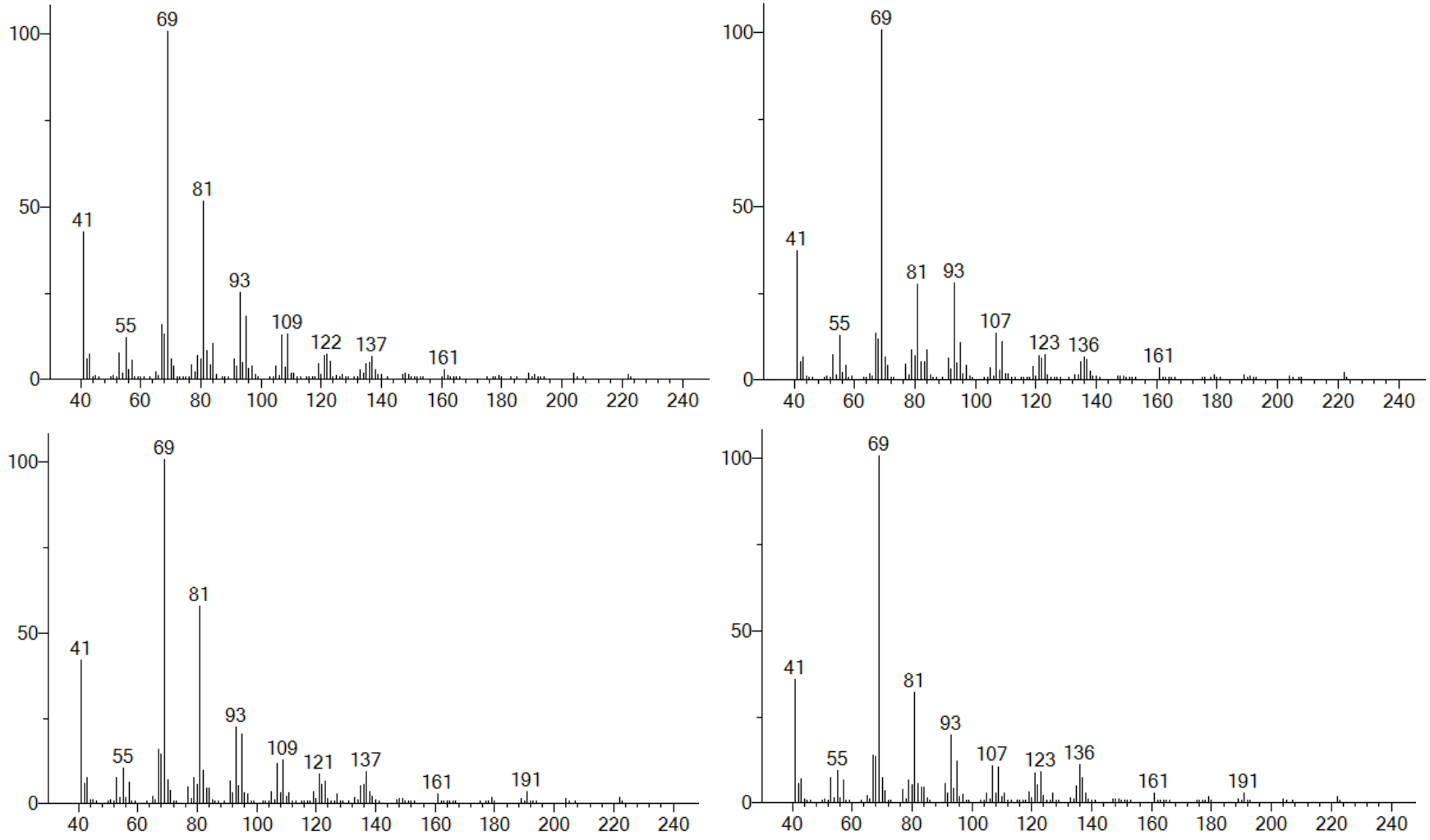
Difficult or impossible to identify by 70 eV alone



Confirmation of isomer identification

Difficult or impossible to identify by 70 eV alone

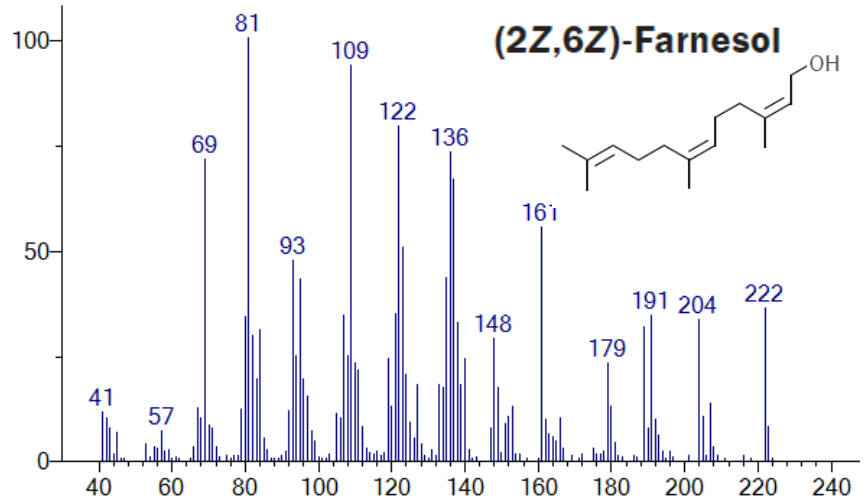
Four isomers of farnesol ($C_{15}H_{26}O$)

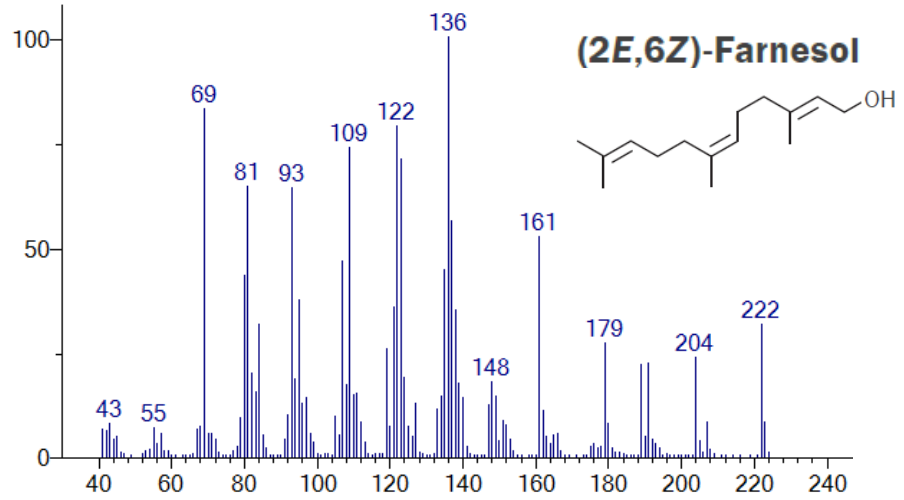


70 eV

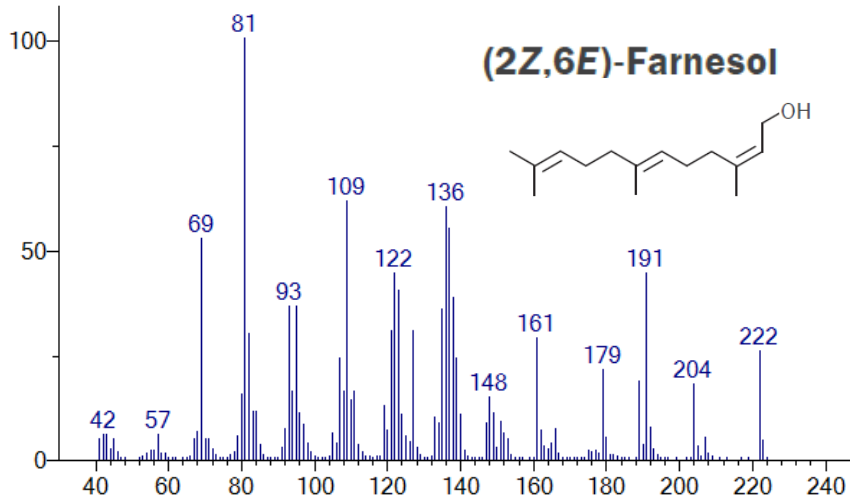
Confirmation of isomer identification

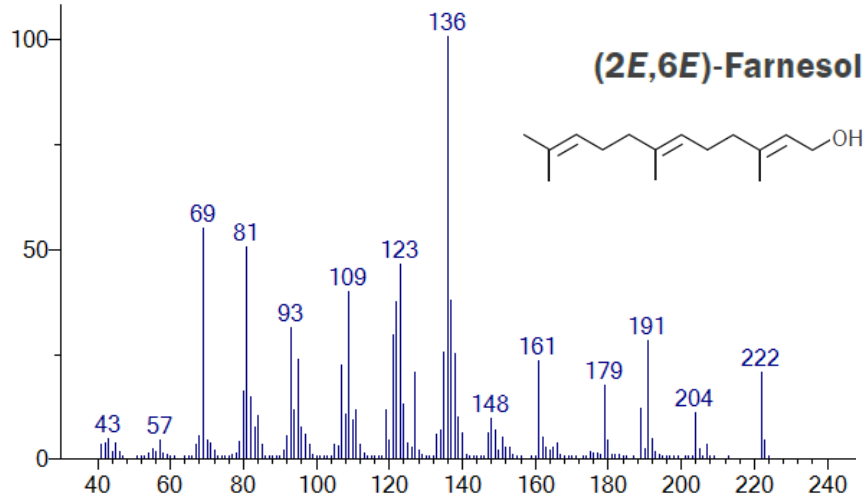
Distinct fragmentation patterns with Tandem Ionisation





12 eV





Case studies

Fragrance allergens

Breath and FAME profiling

Existing customers

In breath analysis

Analytical system

Breath VOCs

1. Sampling using Bio-VOC

2. Transfer breath to sorbent tube

3. Sample analysis by TD-GC-TOF MS

4. Real-time data processing by TOF-DS

- Simple, automated analysis of breath VOCs
- For discovery of biomarkers of disease and precision medicine

Sampling breath for TD analysis

Bio-VOC™ breath sampler

HEALTH & SAFETY LABORATORY

- Designed by UK Health & Safety Laboratory

- Collects **end-tidal air** and transfers it to a sorbent tube

Bio-VOC: The headlines

- Easy to use – no need for medically qualified staff
- Volume of sampler = 129 mL
- Constructed of inert materials – PTFE and similar polymers
- No solvent leaching or ad/absorption of analytes on to sampler from sampler
- Restricted opening prevents sample contamination during insertion of plunger and transfer of sample to sorbent tube
- Can be used multiple times for one participant within a single sampling session

Alternative sampling strategies

ReCIVA® breath sampler

- Comfortable sampling mask for direct collection of breath VOCs on up to 4 sorbent tubes
- Collection of larger sample volumes by extending sampling time
- In use in the world's largest breath-based clinical trials, at over 100 clinical sites around the world

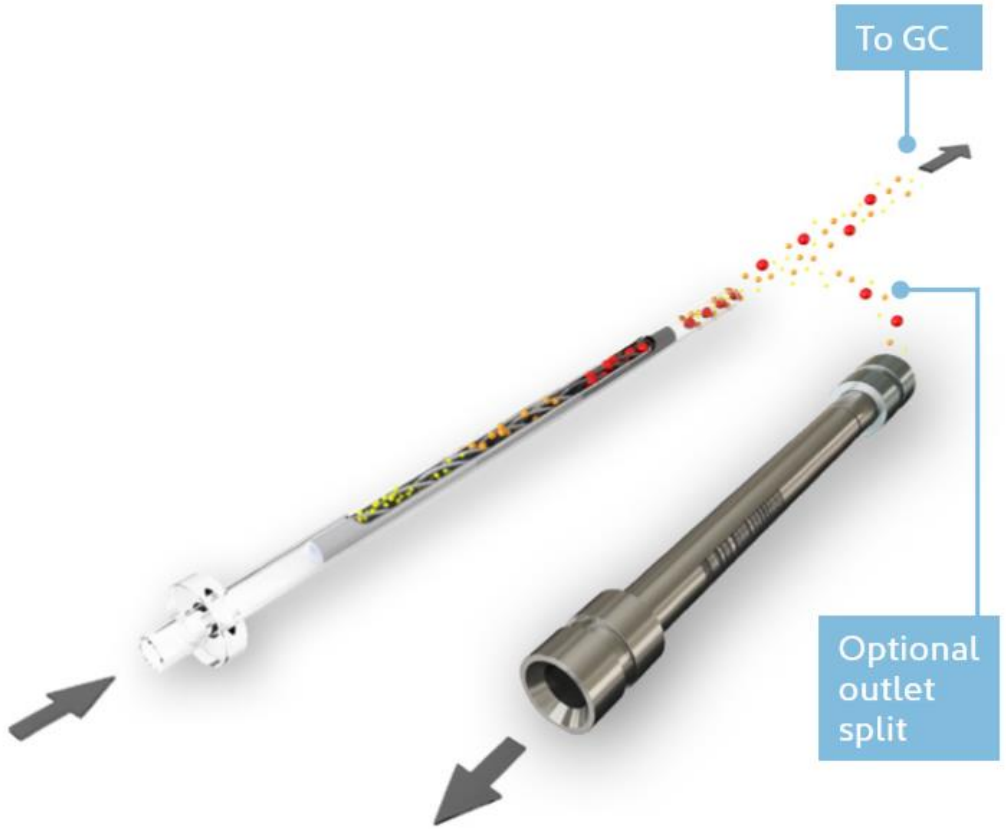
Thermal desorption (TD)

Step 1: Tube desorption and optional inlet split

- Samples are flushed/desorbed onto the focusing trap
- Efficient operation of the trap and cryogen-free cooling reduces costs and ensures high sample throughput
- Secure recollection means that TD need not be a “one-shot” technique.

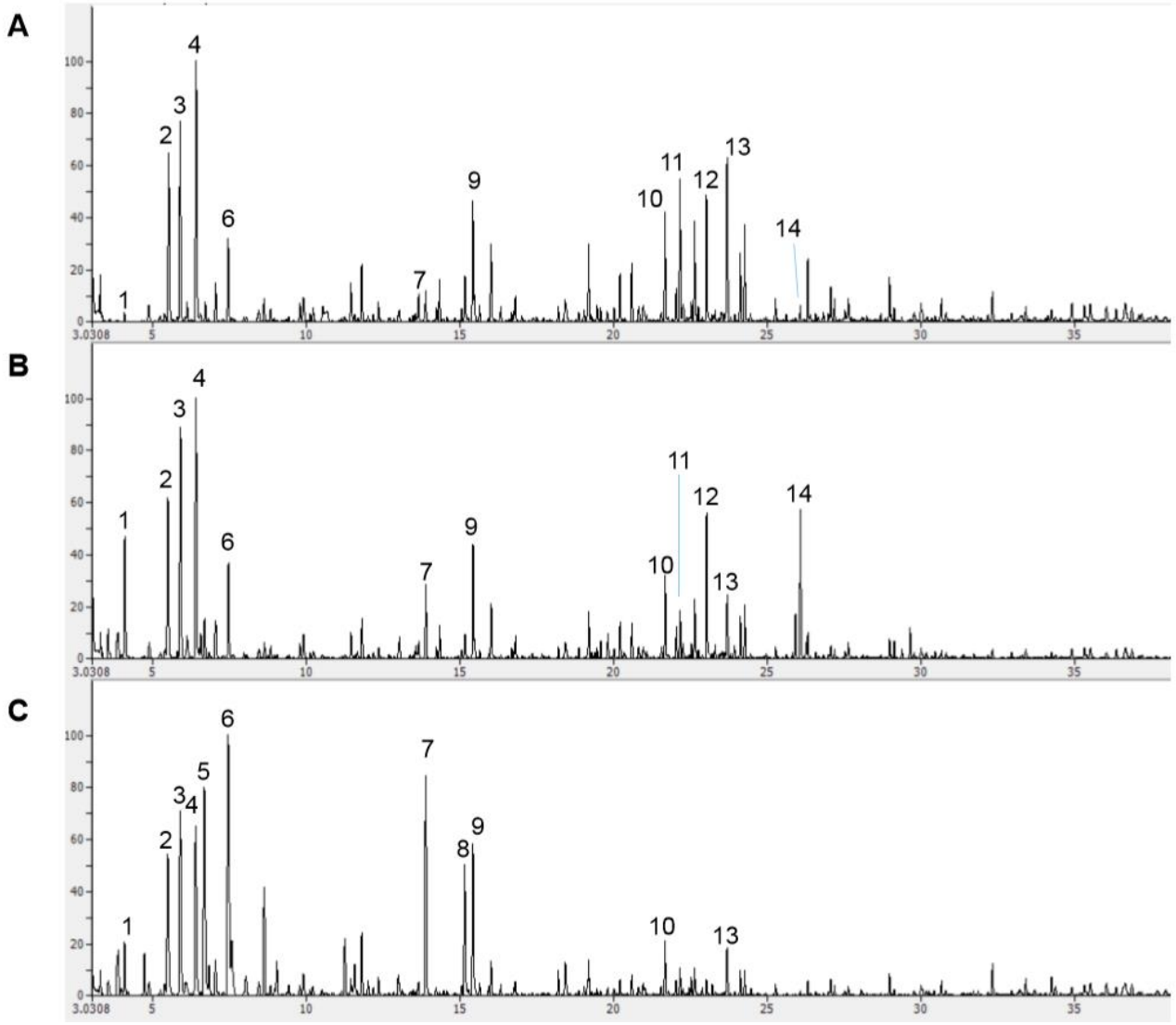
Thermal desorption (TD)

Step 2: Trap desorption and optional outlet split



- Focusing trap rapidly heated (up to 100 ºC/s) in a reverse flow of carrier gas (‘backflush’ operation)
- The analytes are transferred to the GC (with an optional outlet split)

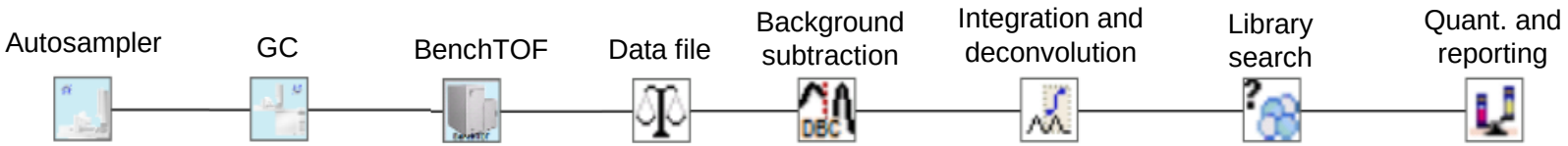
Breath analysis by TD-GC-TOF MS

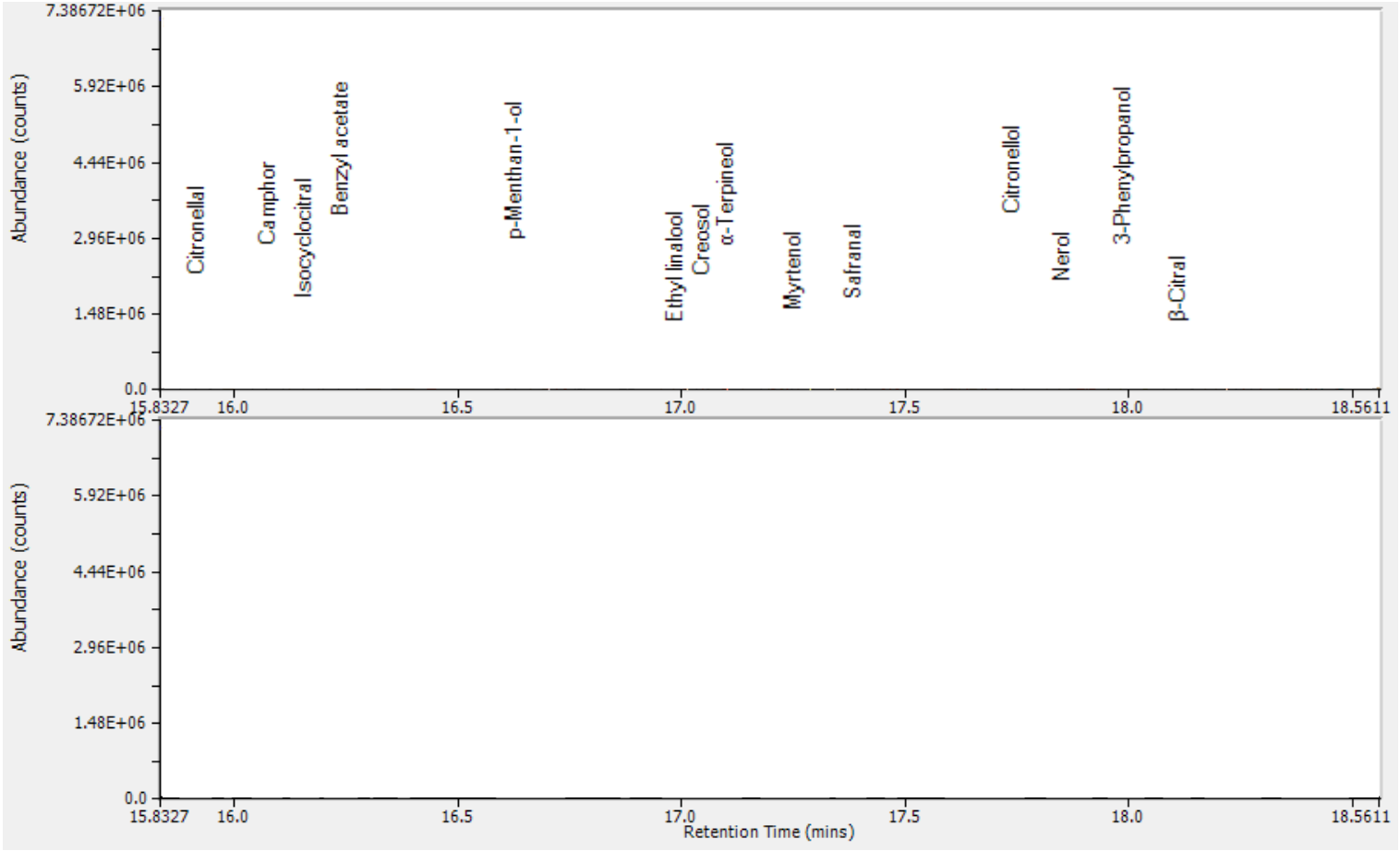


1 Acetaldehyde
2 Ethanol
3 Isoprene
4 Acetone
5 Isopropanol
6 Dichloromethane
7 Methyl pivalate
8 Pyridine
9 Toluene
10 Benzaldehyde
11 Limonene
12 2-Ethylhexan-1-ol
13 Phenol
14 Menthol

- TD–GC–TOF MS chromatograms of end-tidal breath from three participants

Real-time data processing



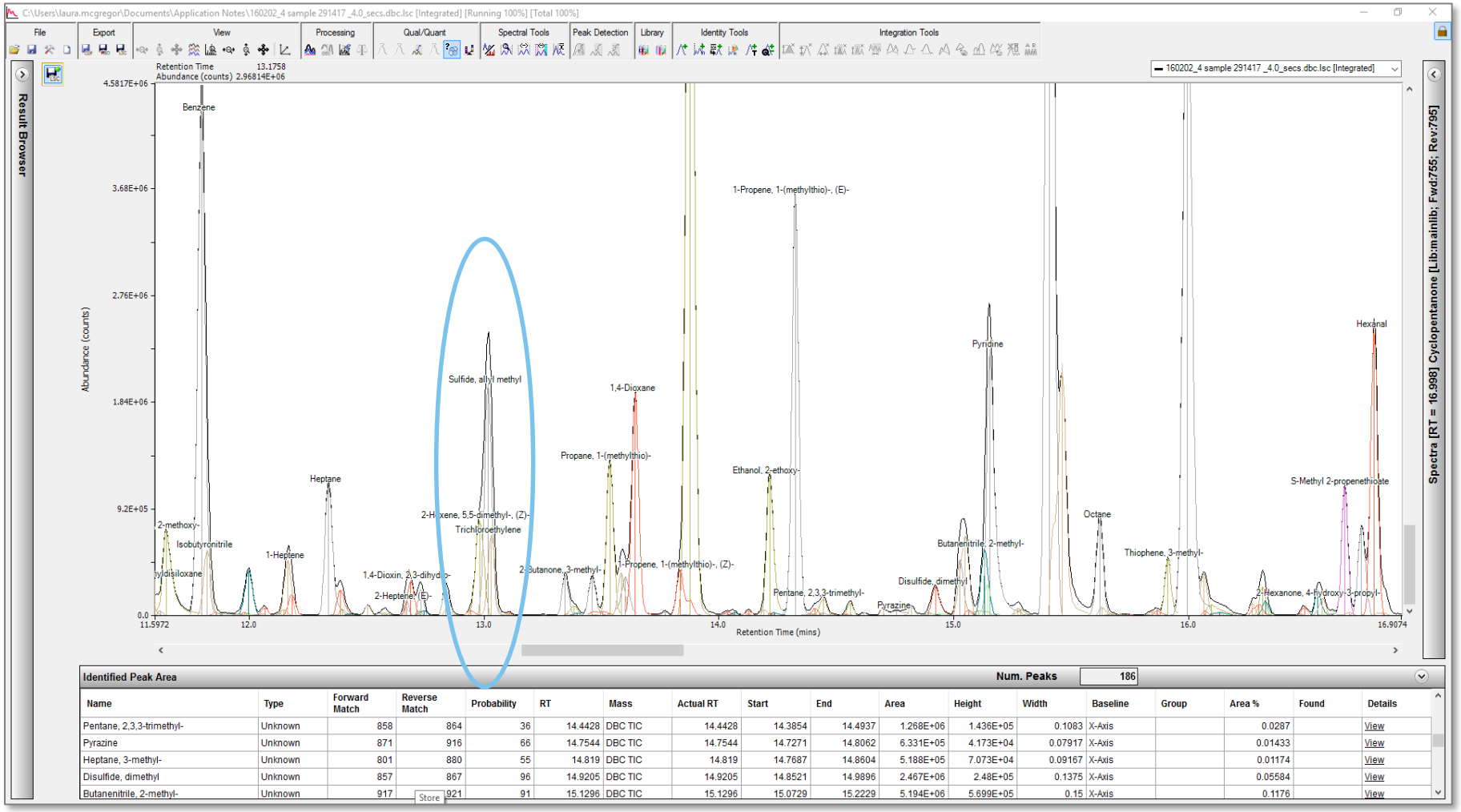


- Background subtraction
- Integration
- Deconvolution
- Library-searching…

…all while the sample is still acquiring!

Uncover hidden peaks…

…with deconvolution in TOF-DS

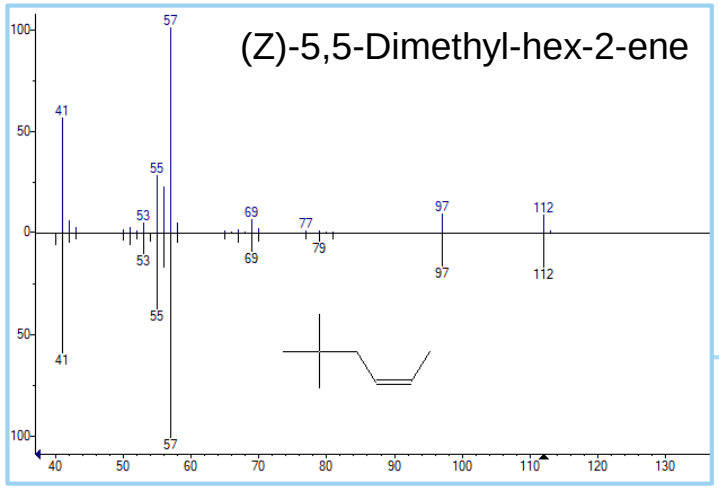


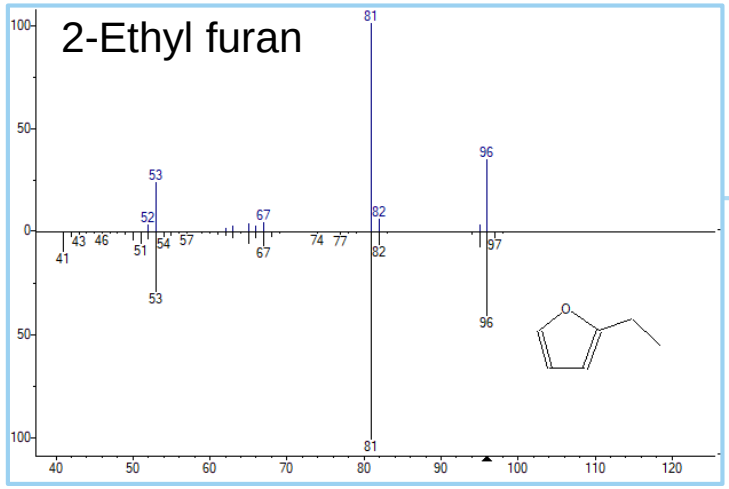
Name	Type	Forward Match	Reverse Match	Probability	RT	Mass	Actual RT	Start	End	Area	Height	Width	Baseline	Group	Area %	Found	Details
Pentane, 2,3,3-trimethyl-	Unknown	858	864	36	14.4428	DBC TIC	14.4428	14.3854	14.4937	1.268E+06	1.436E+05	0.1083	X-Axis		0.0287		View
Pyrazine	Unknown	871	916	66	14.7544	DBC TIC	14.7544	14.7271	14.8062	6.331E+05	4.173E+04	0.07917	X-Axis		0.01433		View
Heptane, 3-methyl-	Unknown	801	880	55	14.819	DBC TIC	14.819	14.7687	14.8604	5.188E+05	7.073E+04	0.09167	X-Axis		0.01174		View
Disulfide, dimethyl	Unknown	857	867	96	14.9205	DBC TIC	14.9205	14.8521	14.9896	2.467E+06	2.48E+05	0.1375	X-Axis		0.05584		View
Butanenitrile, 2-methyl-	Unknown	917	921	91	15.1296	DBC TIC	15.1296	15.0729	15.2229	5.194E+06	5.699E+05	0.15	X-Axis		0.1176		View

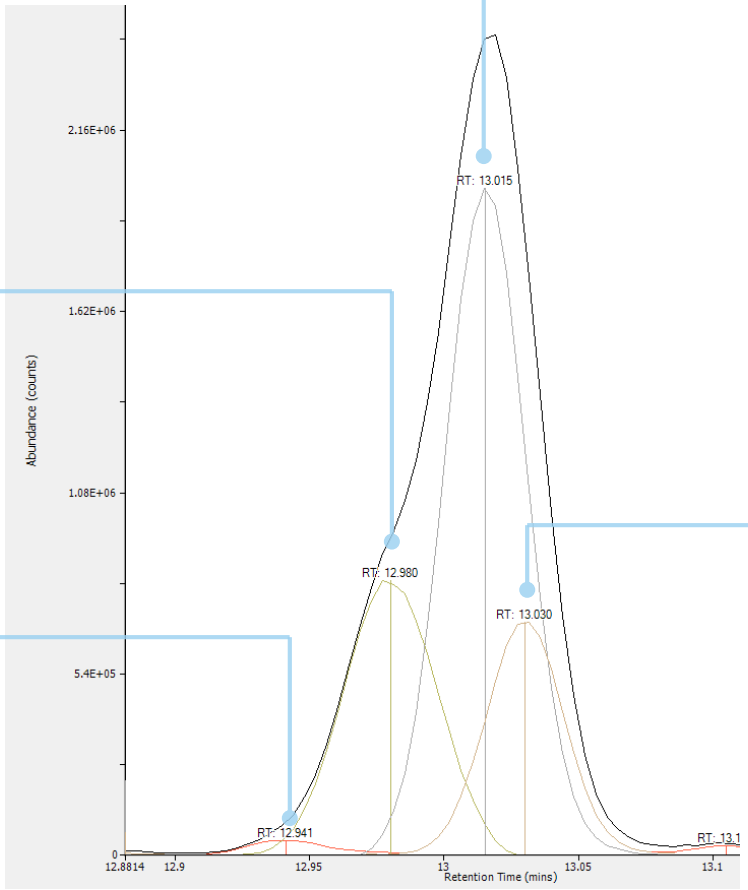
- Deconvolution allows masked or co-eluting peaks to be confidently identified

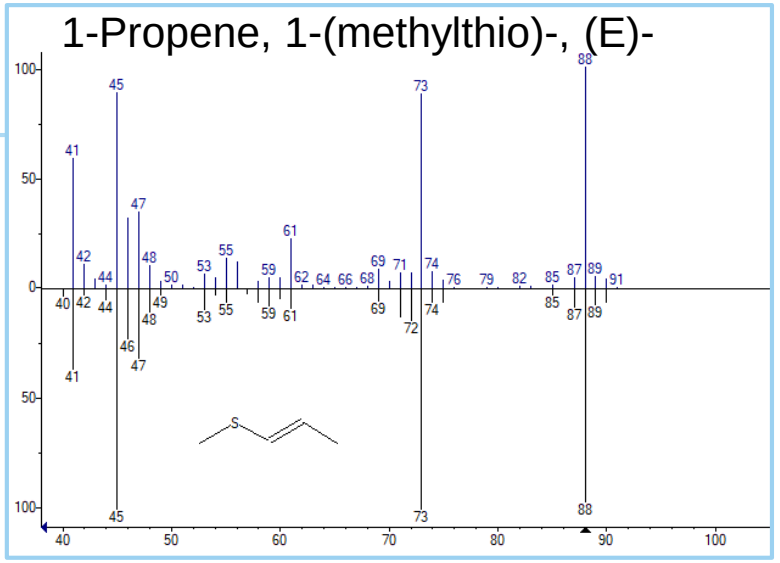
Uncover hidden peaks…

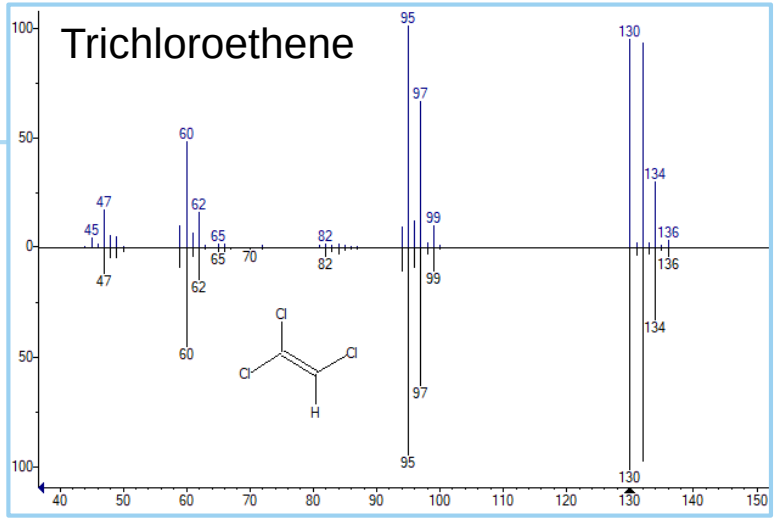
…with deconvolution in TOF-DS





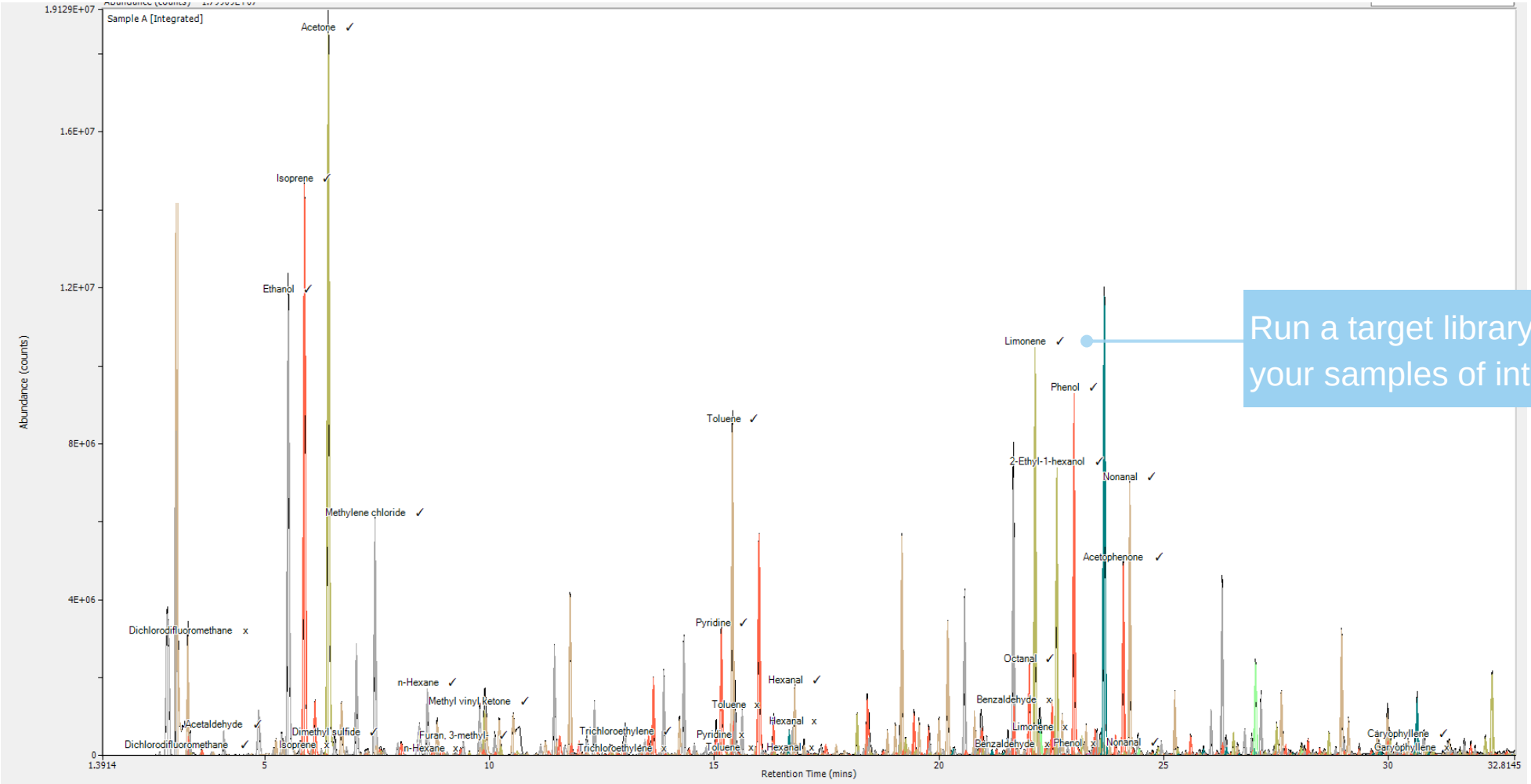






Comparative analysis by ChromCompare®

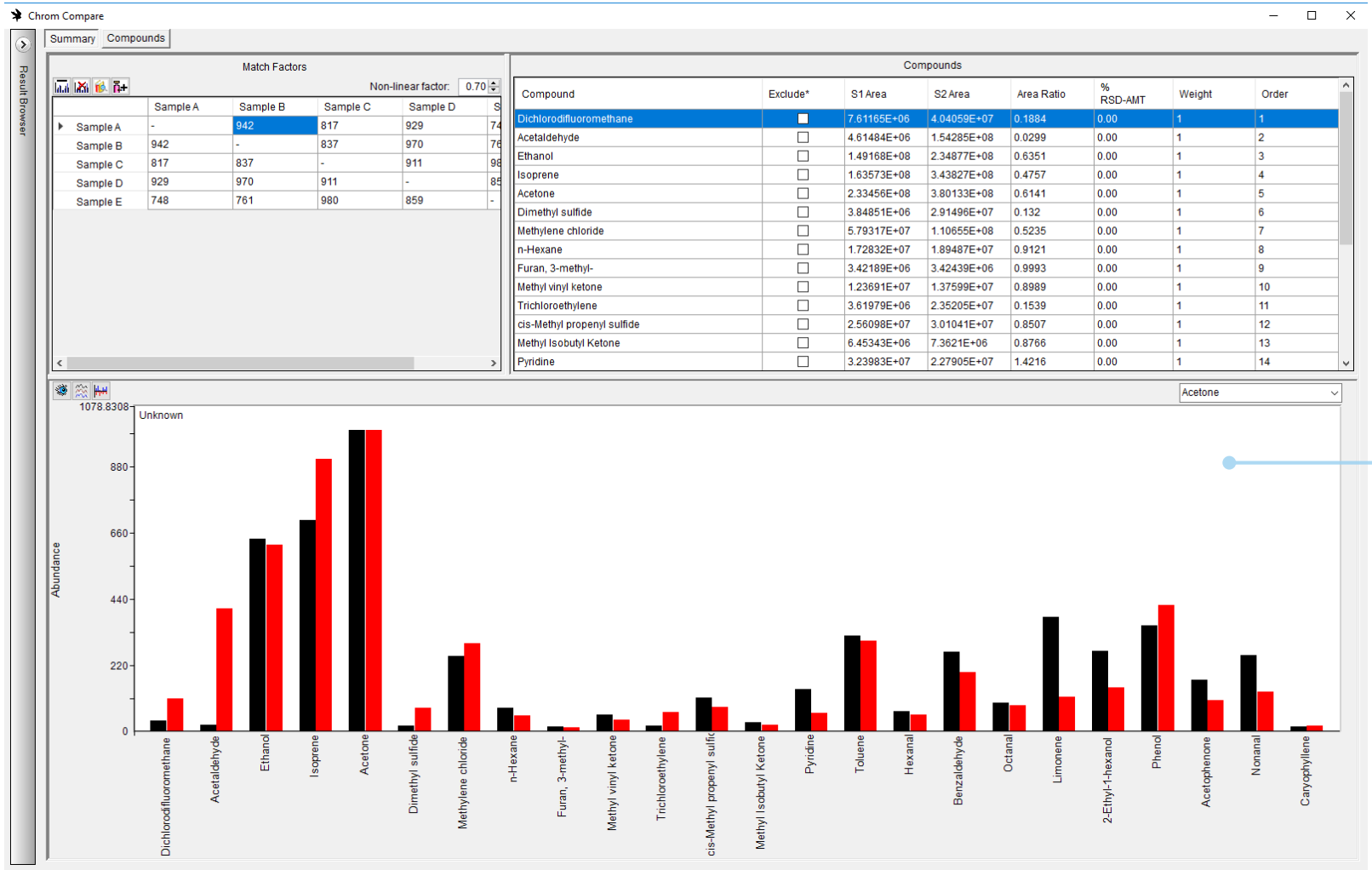
Step 1: Apply a target library



Run a target library search against your samples of interest

Comparative analysis by ChromCompare®

Step 2: Launch file in ChromCompare module

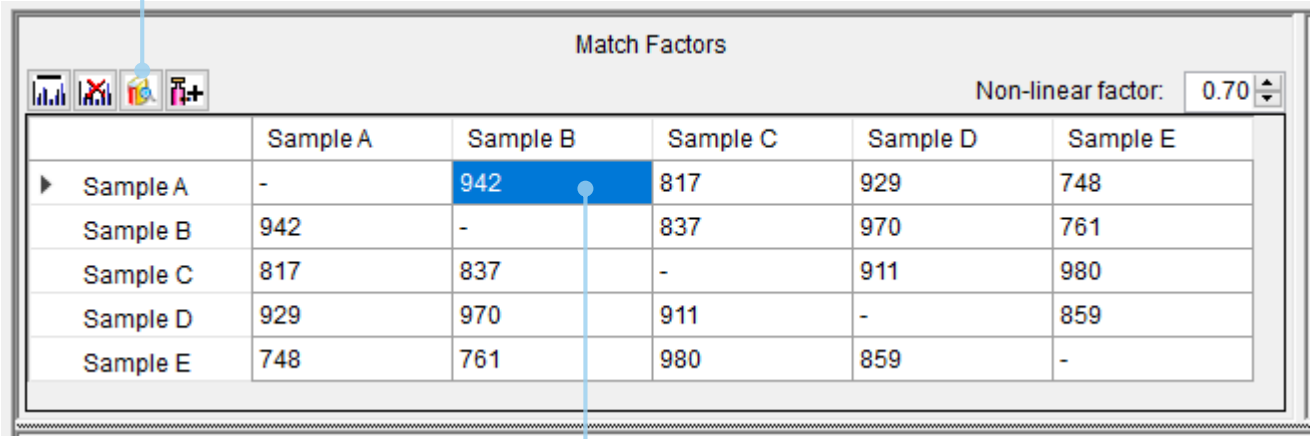


ChromCompare displays the relative abundances of target compounds in a histogram (or H-plot)

Comparative analysis by ChromCompare®

Step 3: View results in match factor matrix

Store H-plots to a database for searching against subsequent samples



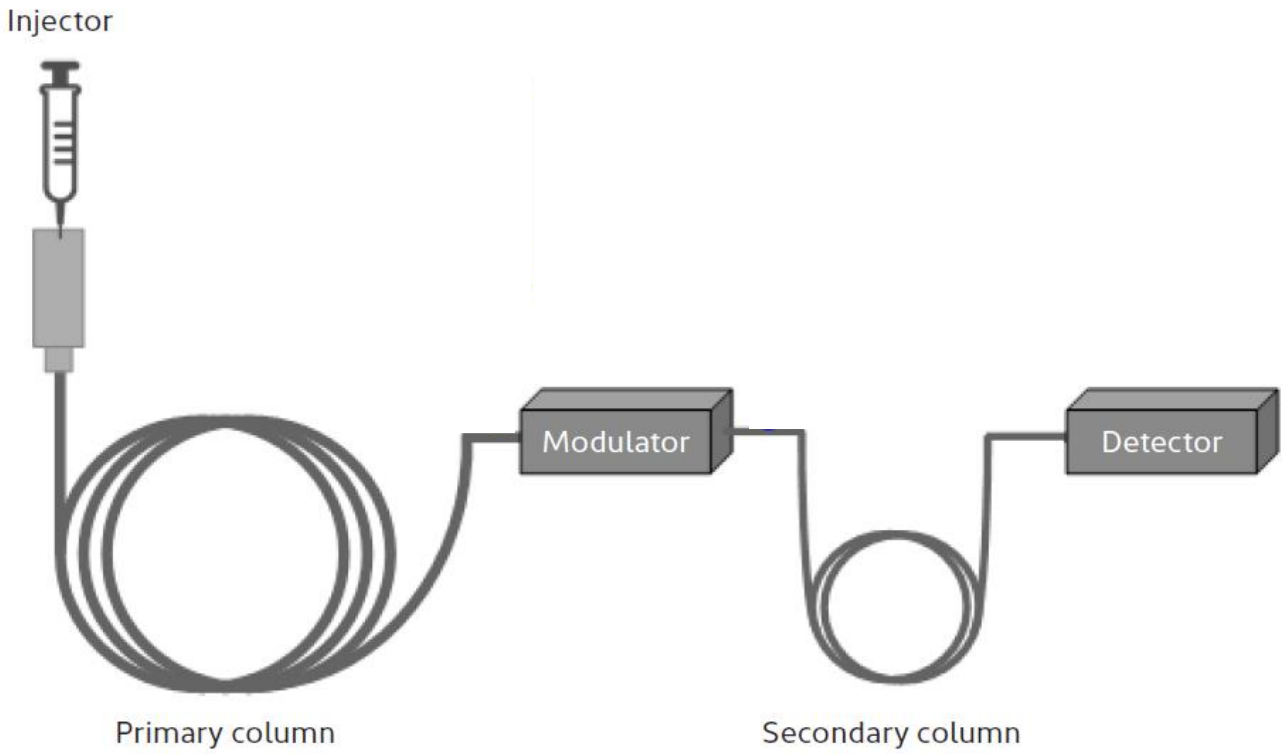
	Sample A	Sample B	Sample C	Sample D	Sample E
Sample A	-	942	817	929	748
Sample B	942	-	837	970	761
Sample C	817	837	-	911	980
Sample D	929	970	911	-	859
Sample E	748	761	980	859	-

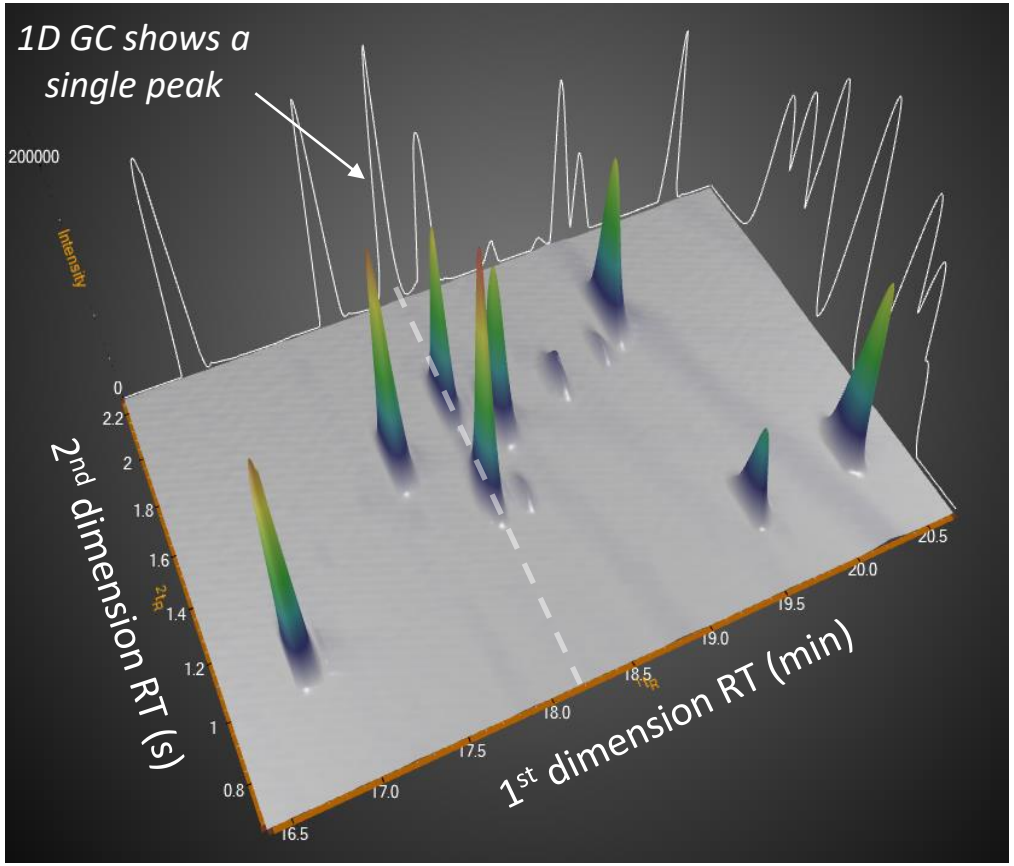
Strong match factor of 942 between breath samples A and B

- Match factor matrix displays results for pairwise comparison of samples
- Match factor (1-1000) indicates how similar/different samples are
  - The higher the match factor, the greater the similarity
- Fast, simple, robust and objective comparisons between samples

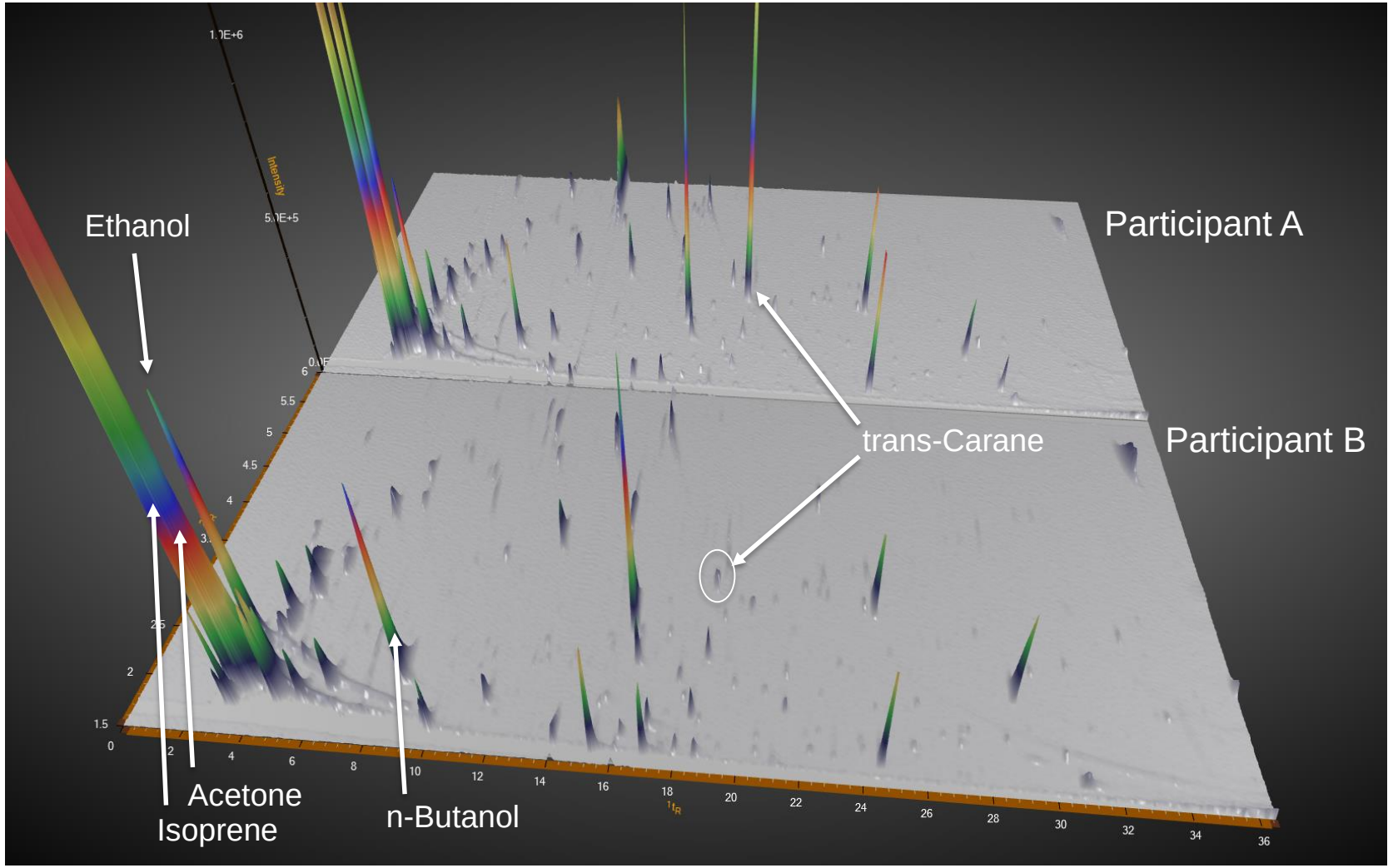
What if we add GC×GC?

Analytical system



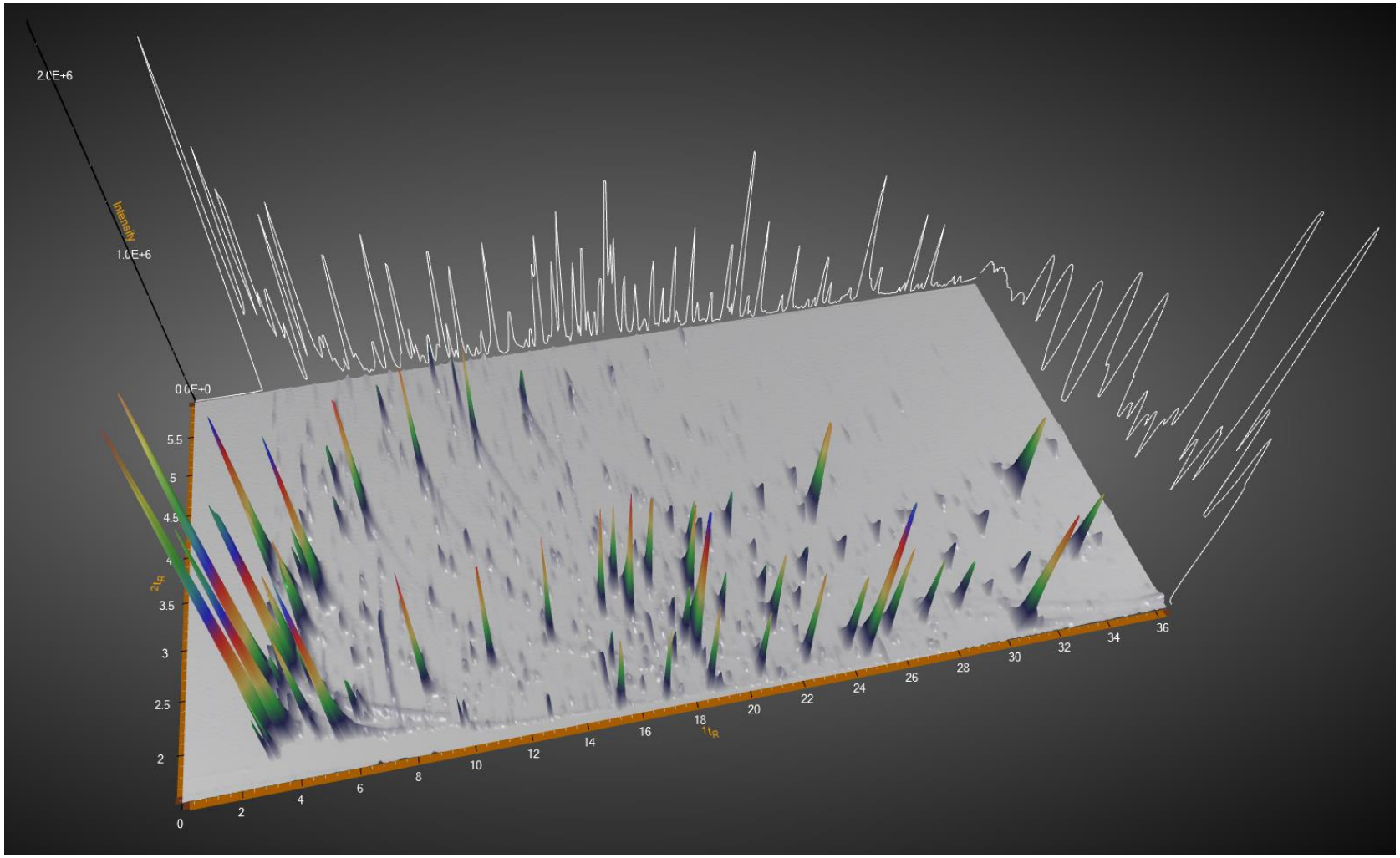


GC×GC-TOF MS as a screening tool for breath biomarkers



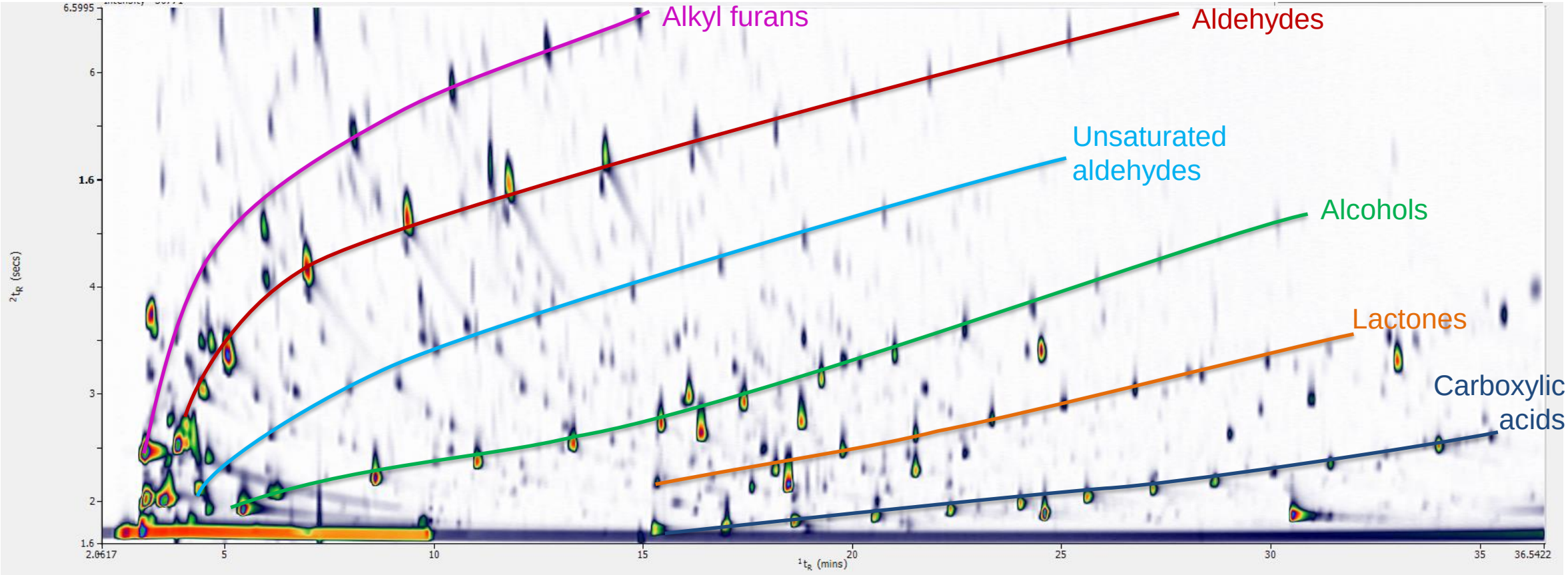
- TD-GC×GC-TOF MS surface charts of breath profiles from two participants
- Enhanced separation for increased confidence in identification of biomarkers of disease

Enhanced separation of chemical classes



- TD-GC×GC-TOF MS surface chart of a complex breath profile after eating
- >800 peaks detected
- Impossible to fully characterise by 1D GC

Enhanced separation of chemical classes

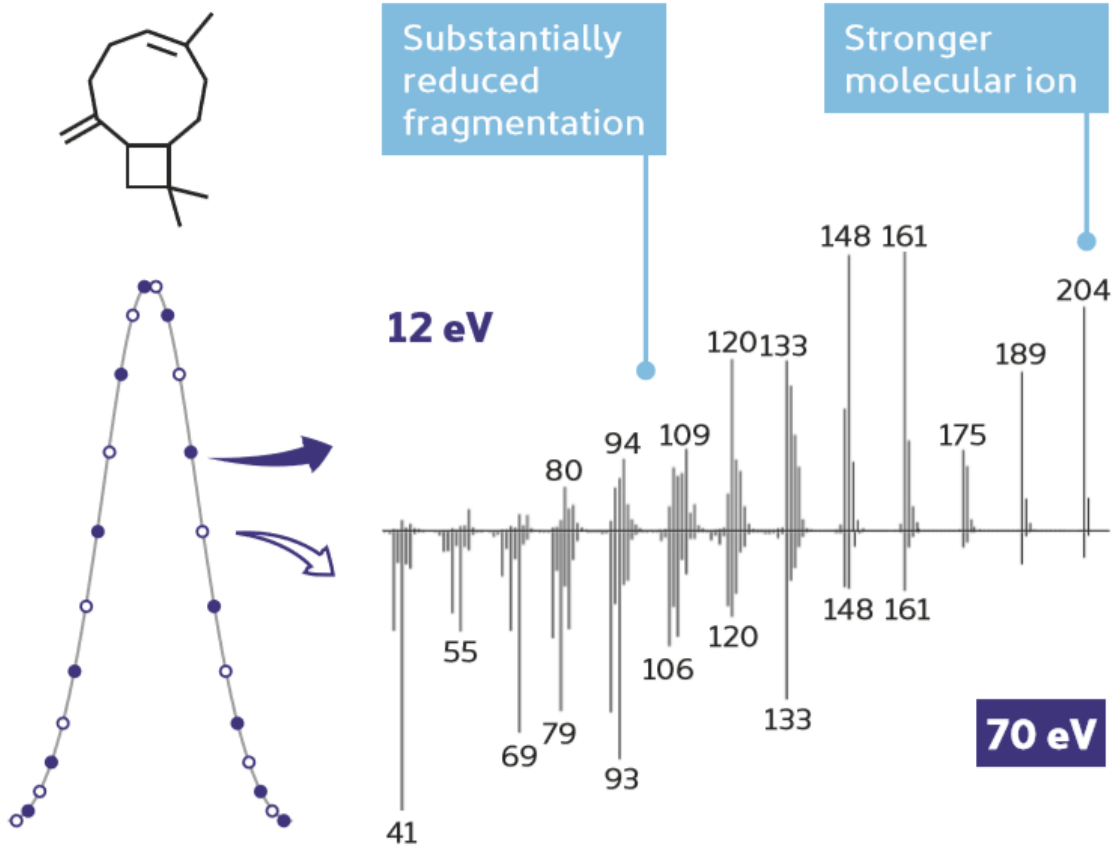


- Structured ordering of GC×GC simplifies the identification process
- Enhanced separation provides cleaner spectra for confident identification

What more can we offer?

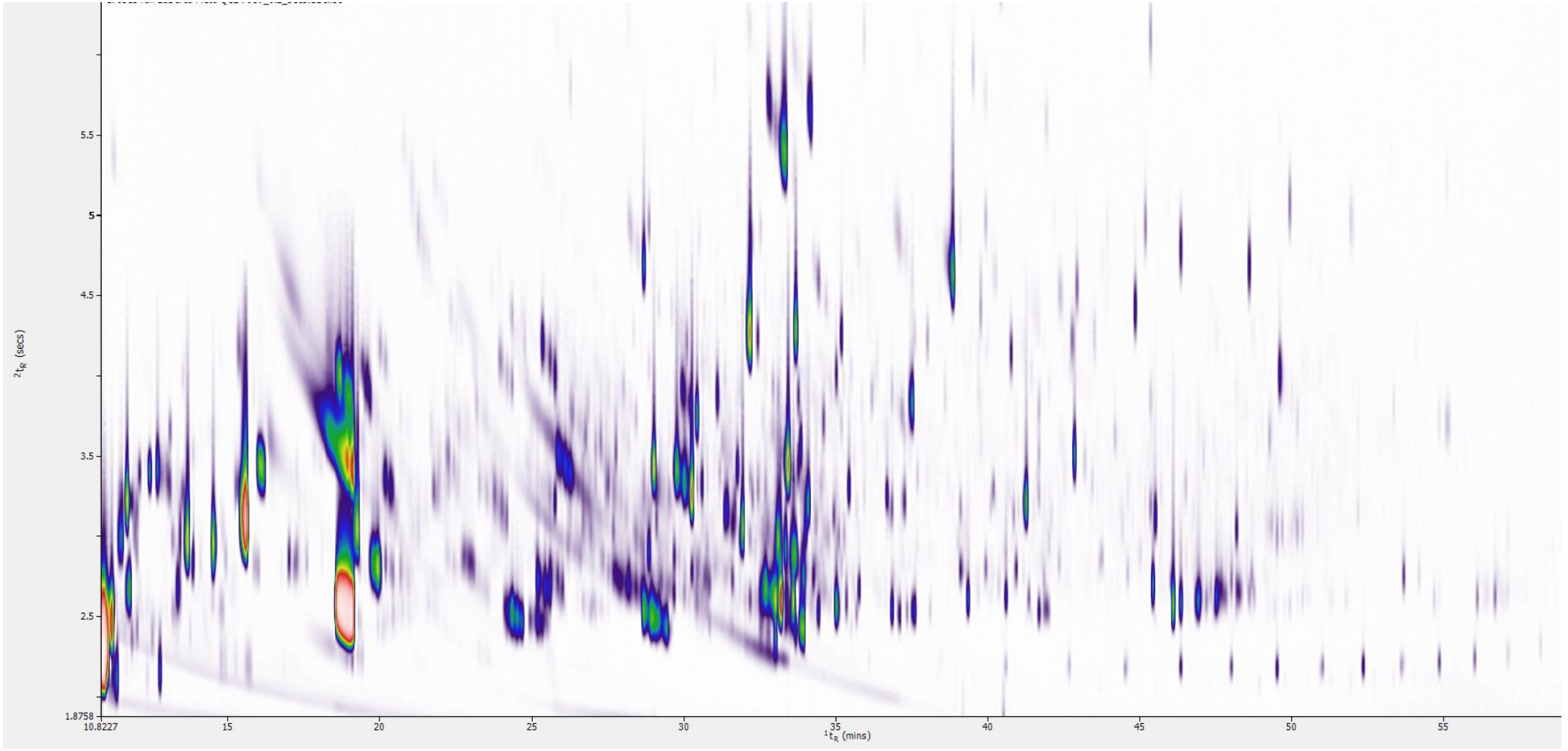
Tandem Ionisation for enhanced confidence in identification

- Acquire hard and soft EI simultaneously
- Complementary spectra to confirm compound identity
- Between-spectra ion ratios as additional qualifiers
- Confident isomer identification
  - Not possible based on 70 eV data alone
- Patented technology



Tandem Ionisation in biological monitoring

Derivatised Urine extract



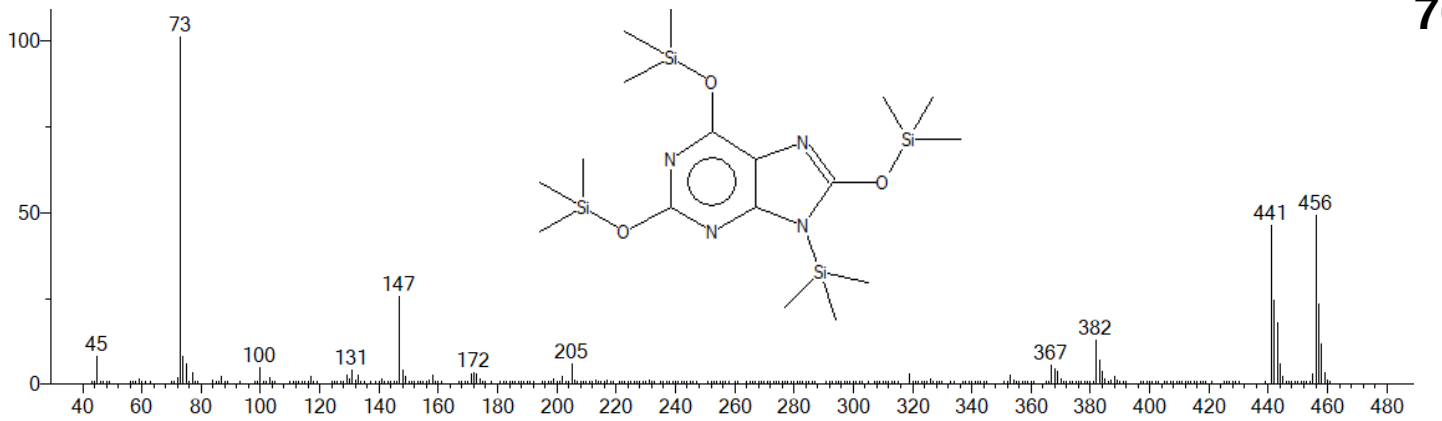
Tandem Ionisation in biological monitoring

Derivatised compounds

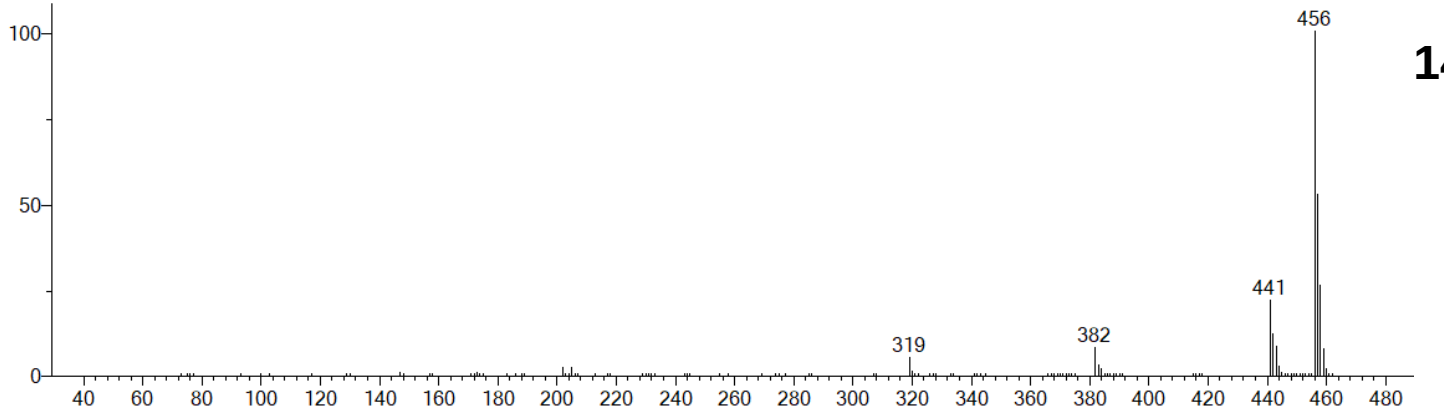
- Reduces the dominant ions associated with derivatisation
- Enhanced molecular ion and other structurally-significant ions
- Improved confidence in identification, especially within complex samples.

Uric acid 4TMS

70 eV



14 eV



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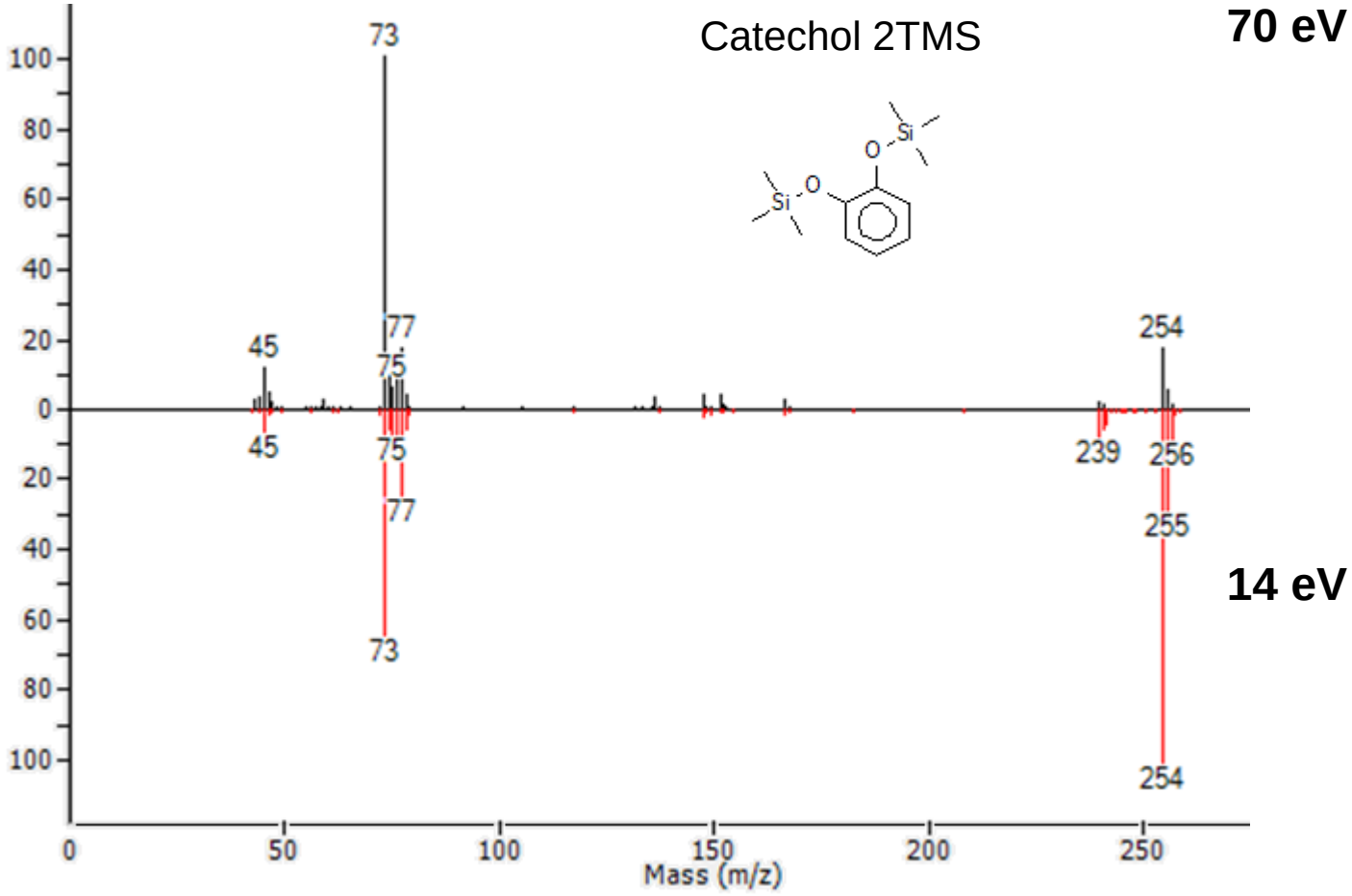
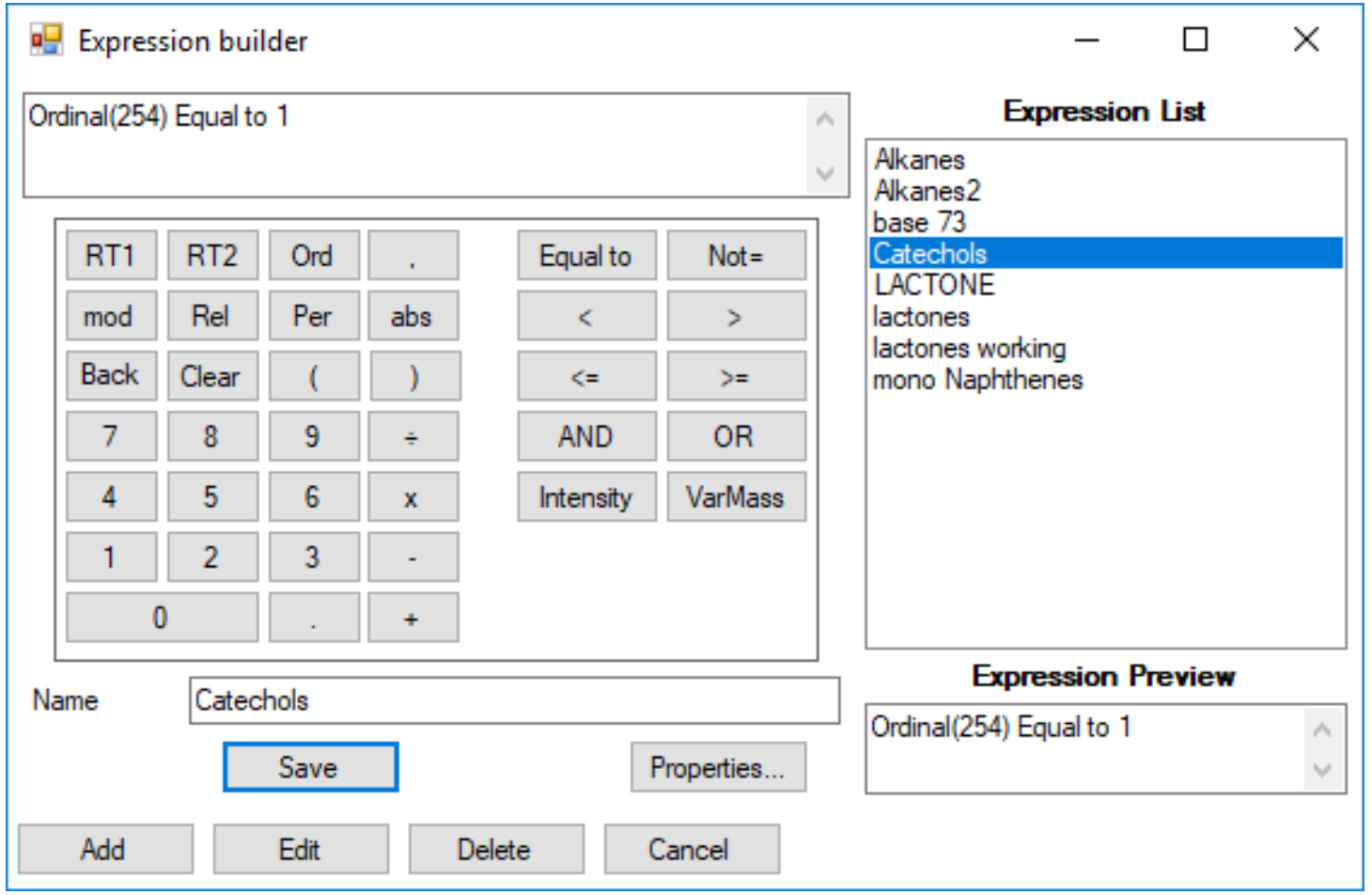


Tandem Ionisation in biological monitoring

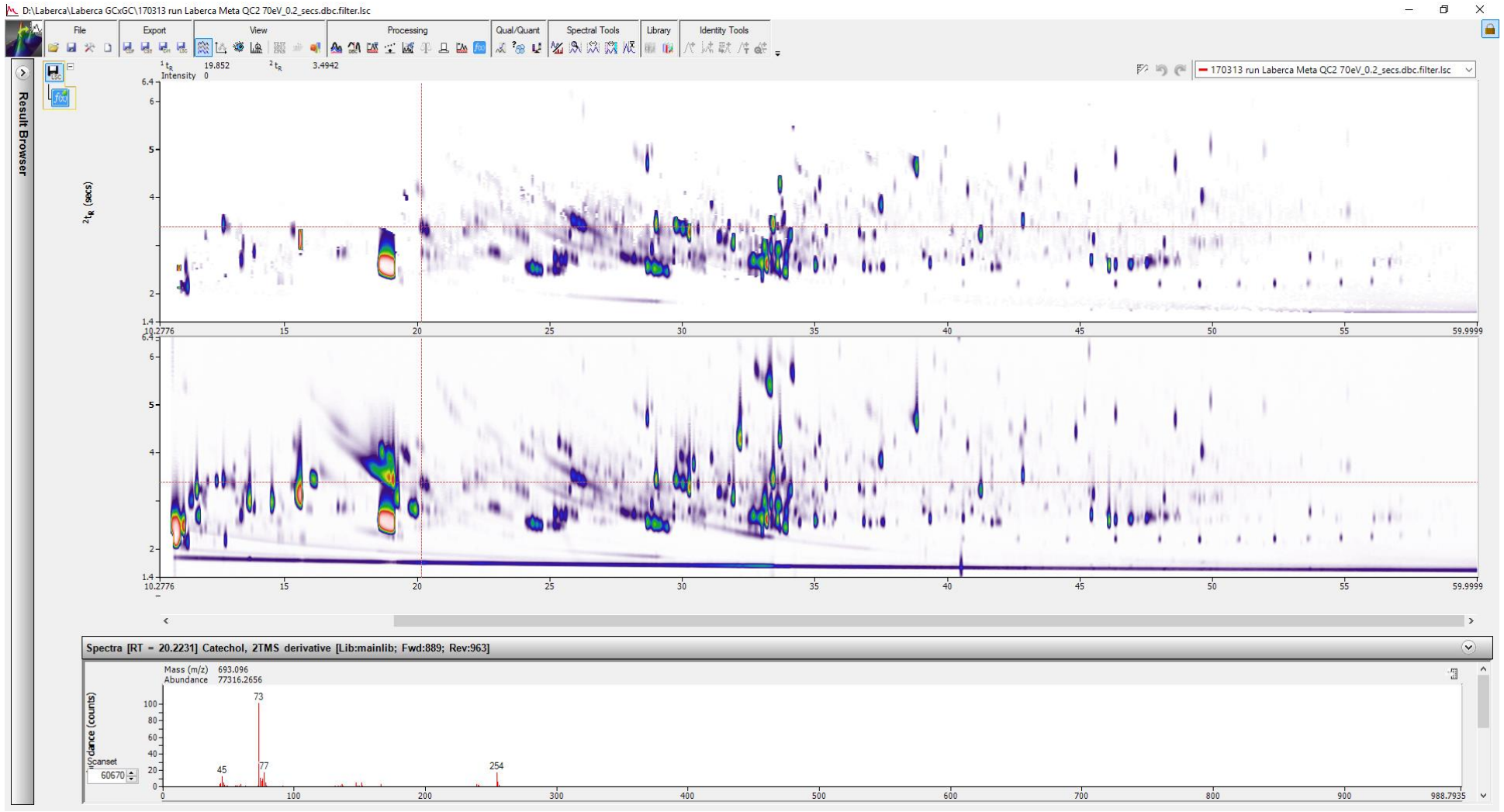
Simplified parametric filtering (scripting) with Soft Ionisation

- Within the software we can write ‘scripts’ or ‘filters’ to search for specific compounds or compound classes
- Much more powerful than extracted ion chromatograms (EICs)



Tandem Ionisation in biological monitoring

Simplified parametric filtering (scripting) with Soft Ionisation

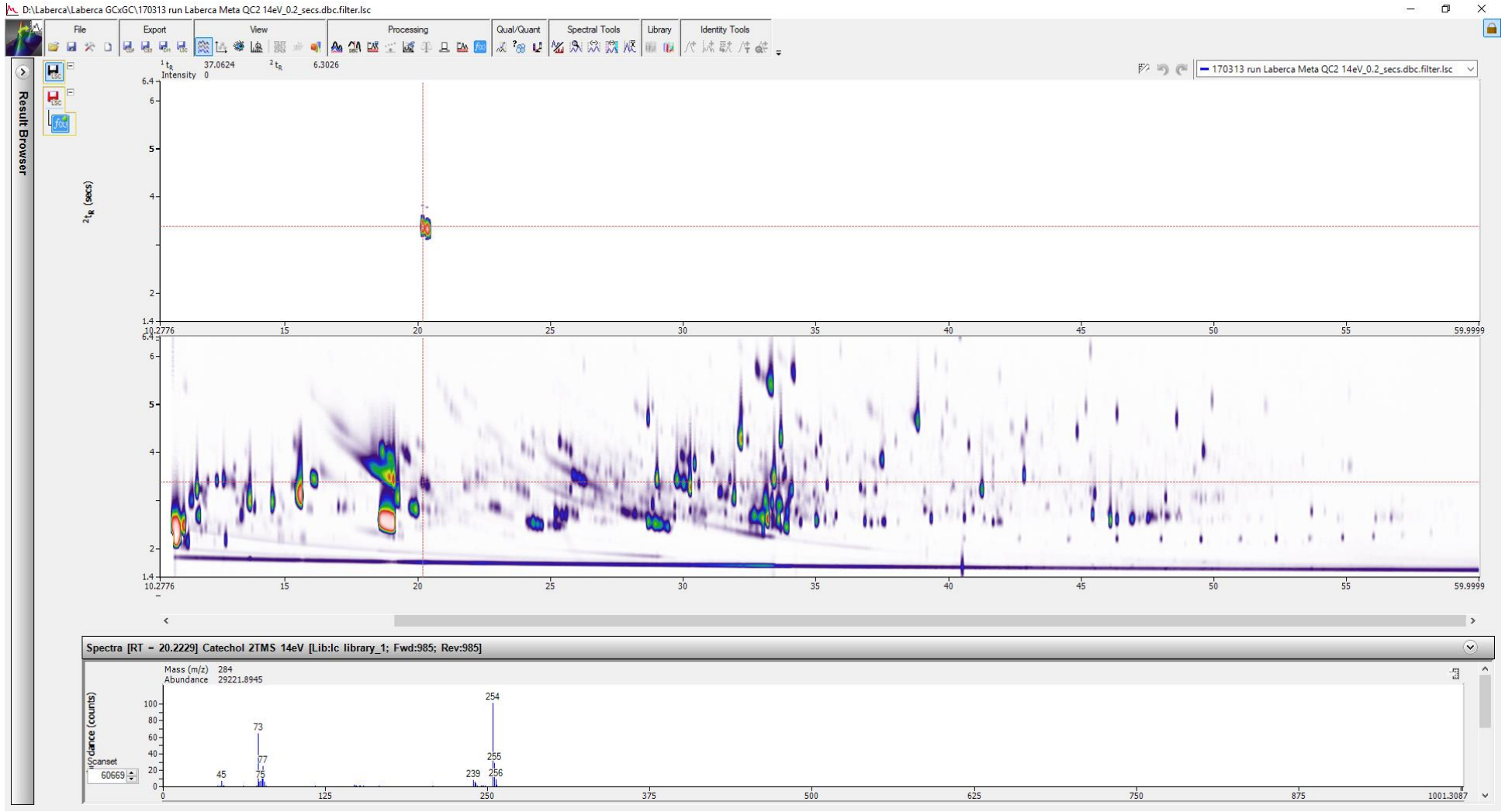


Filtered image searching for Catechol base peak m/z 73

TIC 70eV

Tandem Ionisation in biological monitoring

Simplified parametric filtering (scripting) with Soft Ionisation



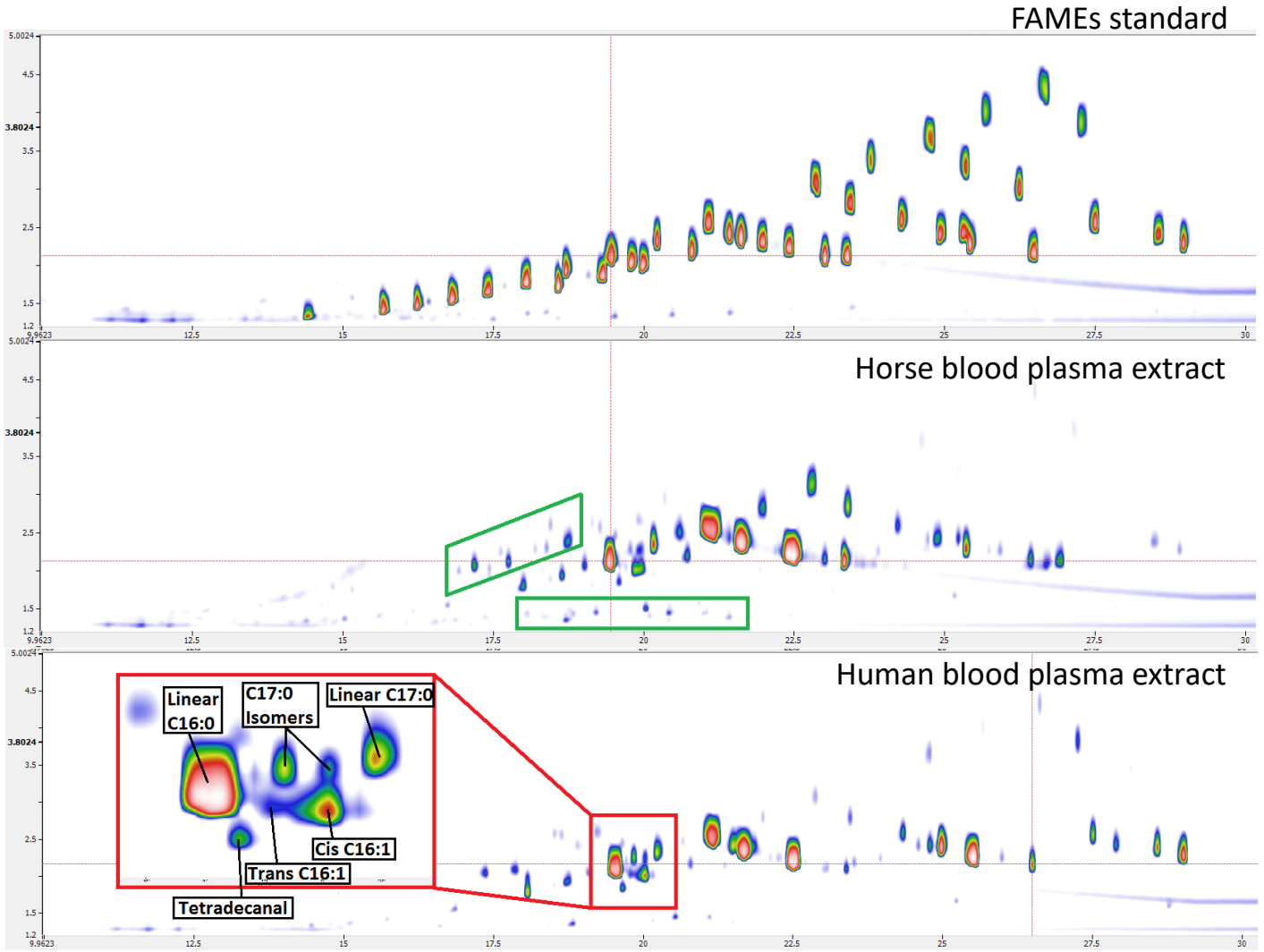
Filtered image searching for Catechol base peak now m/z 254

TIC 14eV

Tandem Ionisation for biological monitoring

FAMEs in blood plasma extracts

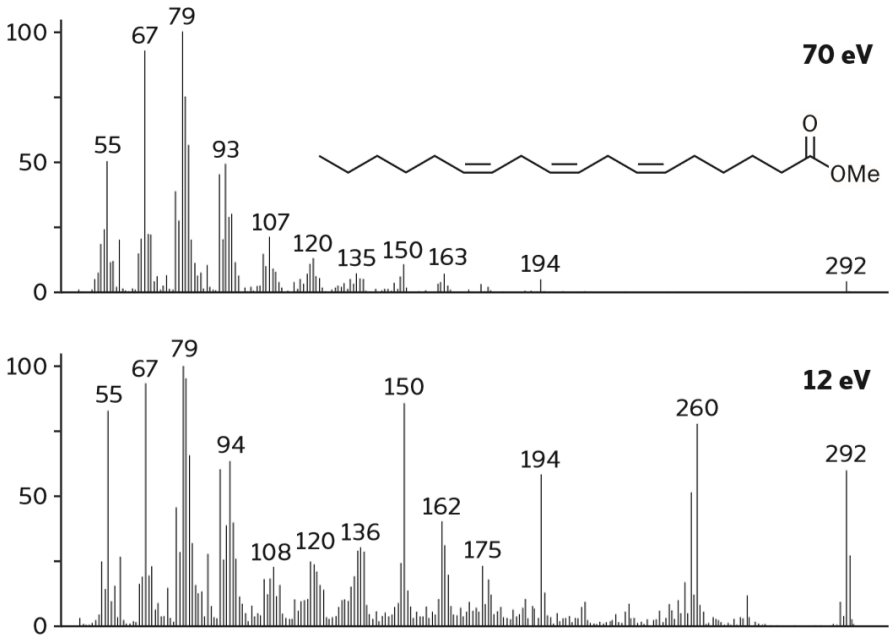
- Expansion in red shows FAMEs of interest in human blood plasma
- Green boxes highlight interferences separated from compounds of interest
- In 1D GC, interferences cause the quantified concentration of FAMEs to be overestimated



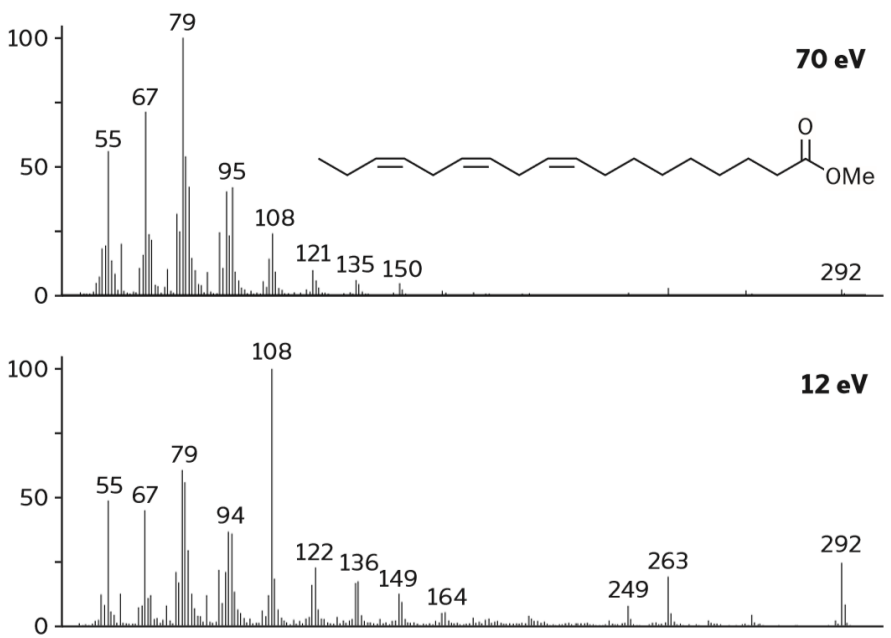
Tandem Ionisation for biological monitoring

FAMEs in blood plasma extracts

C18:3 (ω–6)



C18:3 (ω–3)



- Enhanced confidence in identification of compounds with similar 70 eV spectra

What more can we offer?

Centri® sample automation and concentration platform

- Centri is the ideal platform to tackle the diverse sample matrices involved in biological monitoring
- Multi-purpose, GC sample-introduction for the analysis of solid, liquid and gaseous samples
- World-leading trapping technology with secure sample recollection for added confidence in any sampling mode

SPME and SPME-trap

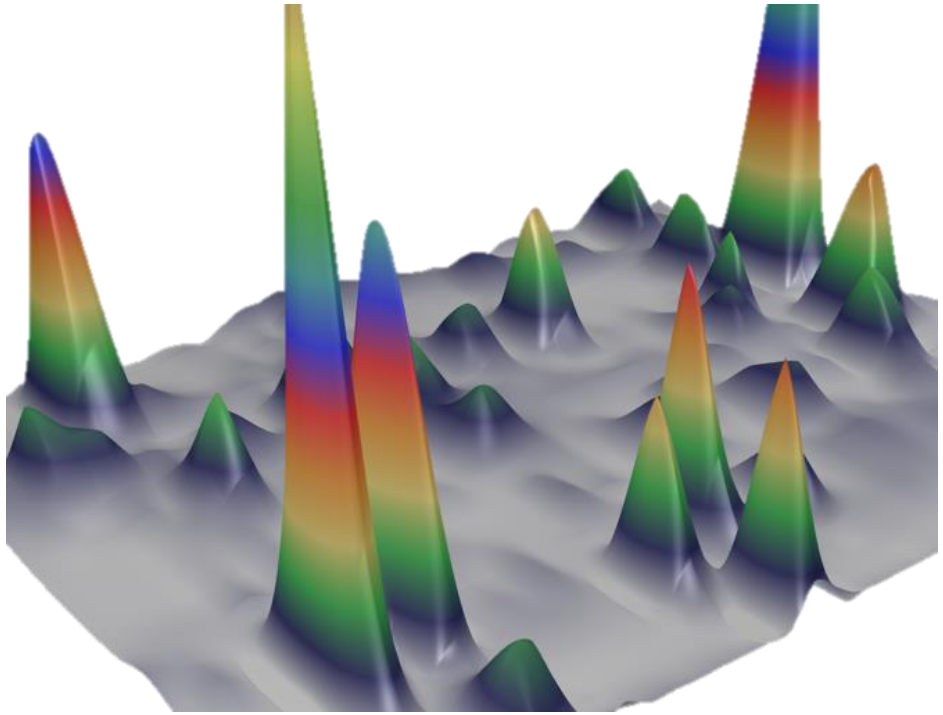
Headspace and headspace-trap

HiSorb high-capacity sorptive extraction

Tube-based thermal desorption

Summary

- Flexible, multi-hyphenated systems to solve analytical challenges
- BenchTOF provides the speed, sensitivity, selectivity, stability and spectral quality required for confident analysis
- Tandem Ionisation and parallel detection provide additional layers of information on sample composition
- Tandem Ionisation provides increased confidence in identification
- Accurate Deconvolution allows you to find the ‘hidden’ peaks
- ChromSpace software provides streamlined workflows to unify lab processing



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