SepSolve Analytical

# Delivering quality data in biomarker discovery

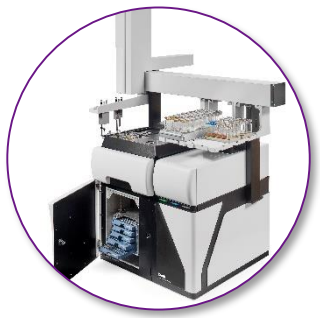
Laura McGregor
Product Marketing Manager

# Who are SepSolve Analytical?

Experts in analytical chemistry

SCHAUENBURG Analytics

MARKES international



Sample preparation

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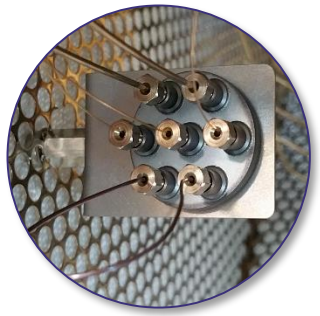
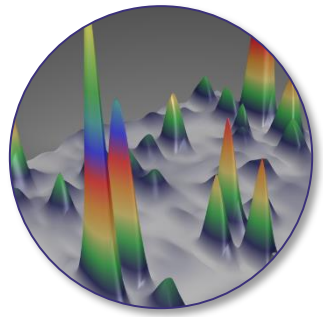


Separation

Identification



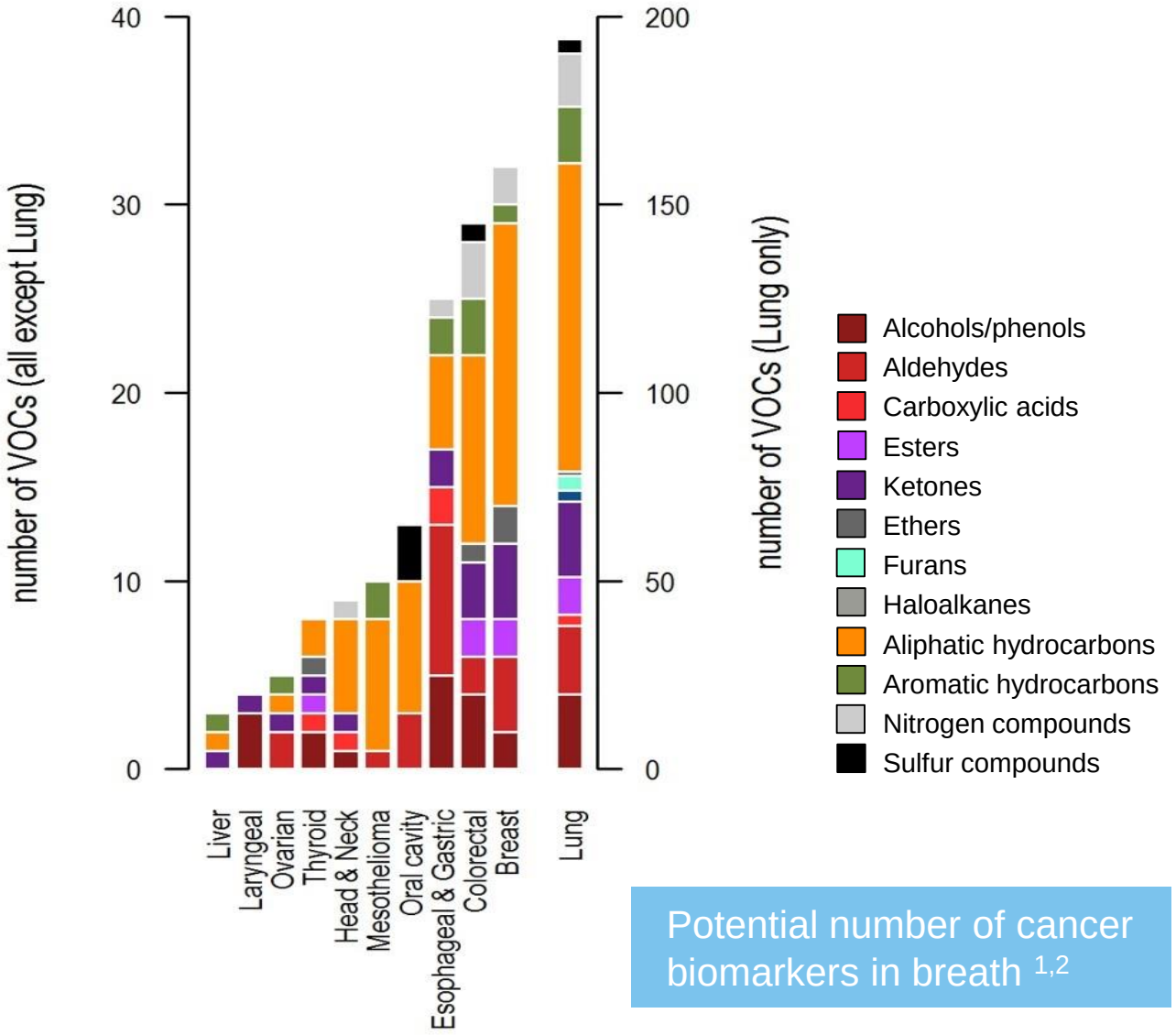
Data analysis

# Outline

- Biomarker analysis & the challenges involved
- Breath analysis workflow using thermal desorption (TD)-GC-MS
- Typical errors and ways to overcome them
- Beyond breath – investigating other sources of biomarkers

# The diversity of the breath volatilome

- >800 VOCs have previously been reported from breath[1, 2]
  - Many different types of compounds
  - Need to quantify at trace levels (sub-ppb)
  - Are these all ‘real’ biomarkers?
  - The problem of lack of consensus

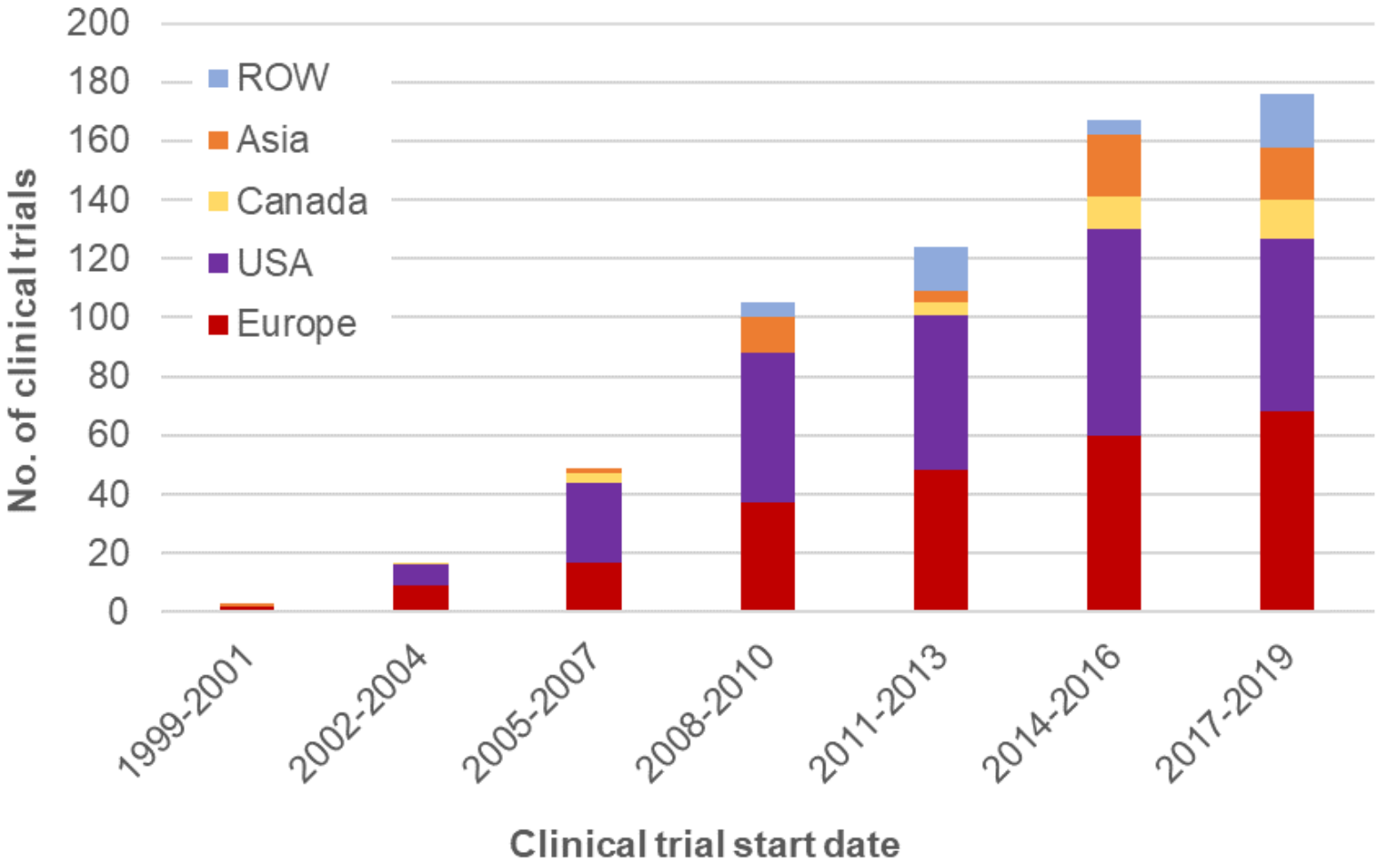



Potential number of cancer biomarkers in breath [1,2]



*1. Journal of Breath Research* 8, no. January 13, 2014
*2. JAMA Oncology*, August 16, 2018, e182815

# Breath VOC analysis in clinical research




Clinical trials involving breath analysis

(based on start date)

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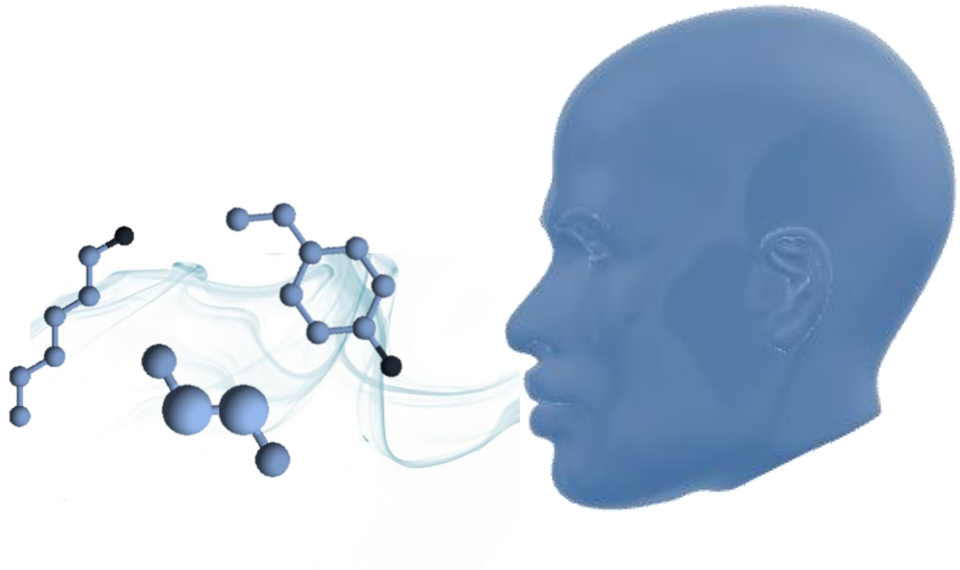
Data source: clinicaltrials.gov (using search terms “breath” and “biomarker”)

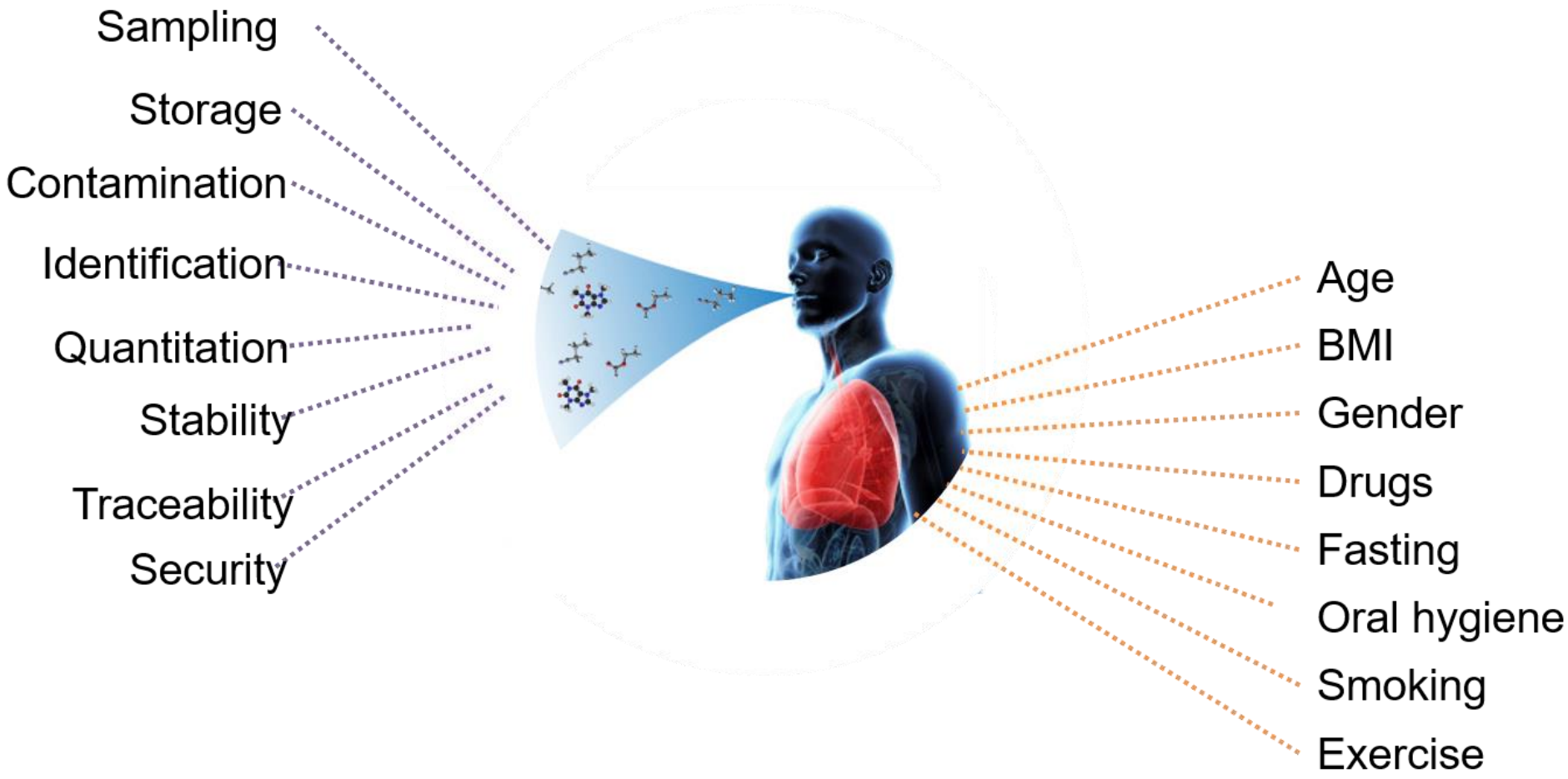
# Analytical challenges in breath analysis

- Collection vessels:
  - Bags, tubes, Bio-VOC, ReCIVA
- Sampling alveolar air:
  - Low volumes, high humidity…
- High sample complexity:
  - Low concentration of volatiles (ppbv to sub-pptv)
  - Requires highly sensitive detection
- System reproducibility:
  - Sampling device
  - TD-GC(×GC)-MS
- Minimal chemical background of all devices and apparatus used



# Breath VOCs: sources of variability

## Application of breath analysis in clinical practice is limited

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Source: https://doi.org/10.1016/j.jtho.2016.02.017

# Why thermal desorption?

## Breath VOC sampling, transport and concentration

### Problem

1. Collect breath from multiple clinical sites, transport and store samples
2. Breath is saturated with water vapour
3. Untargeted analysis, biomarkers present at trace levels
4. Clinical samples are invaluable
5. Empty tubes and samples transported between lab and clinic. Lengthy clinical studies.

### Solution

- ✓ TD tubes are compact, they can be sealed and conveniently transported
- ✓ Dry-purging of the tubes and focusing trap
- ✓ Cryogen-free trap focusing
- ✓ Re-collection
- ✓ Internal standard addition to tubes or focusing trap

# Sample security

## Breath analysis using thermal desorption (TD)

- Sorbent tubes are:
  - ✓ Sealed at point of use
  - ✓ Customised for breath analysis
  - ✓ Compatible with RFID tags for sample tracking and integrity
  - ✓ Compatible with fixed and variable-volume samplers

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Brass caps for secure transport

*Transport to lab*


DiffLok™ caps for secure analysis

# Standardisation

## Type of QC samples in ‘breathomics’ studies and how to prepare them

| Sample type | Description | Preparation |
|---|---|---|
| Extraction/process blank | What is not of biological origin/carryover | Run a blank of the whole system under the same conditions (e.g. empty tube) |
| System suitability sample | System check BEFORE precious biological samples | Load a clean TD tube with a standard mixture |
| Internal standards | Given sample complexity this is not applicable to all metabolites | Add ISTD to every sample. ISTD (or ISTDs) is similar to analytes but not overlapping |
| Within-laboratory inter-study sample/standard reference materials (SRMs) | Long-term reference material for use across research community | Standards are added to a clean tube under standardised conditions, typically by a third party/authority (e.g. National Physical Laboratory). |

Abridged from: D. Broadhurst, R. Goodacre, S. N. Reinke, et al. 2018. “Guidelines and Considerations for the Use of System Suitability and Quality Control Samples in Mass Spectrometry Assays Applied in Untargeted Clinical Metabolomic Studies.” *Metabolomics* 14 (6).

# Standardisation

## Type of standards in ‘breathomics’ studies and how to prepare them

- Automated standard addition to **blank tubes** prior to sampling
  - Intra-study ‘pooled’ sample
  - System check standard

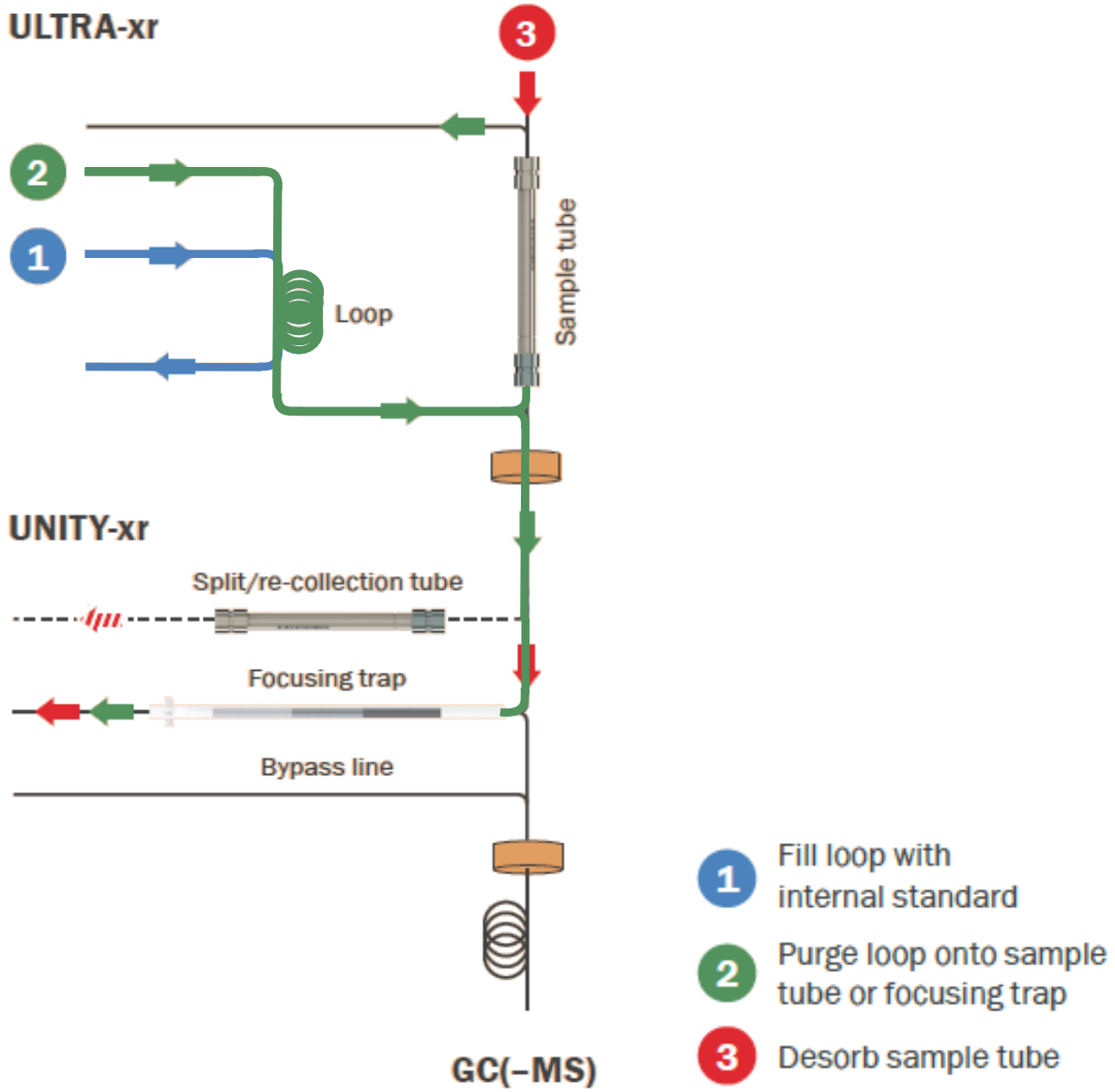
- Automated standard addition to **sampled tubes** prior to analysis
  - Internal standard

- Automated standard addition to **focusing trap**
  - System suitability sample

# Automated standard addition

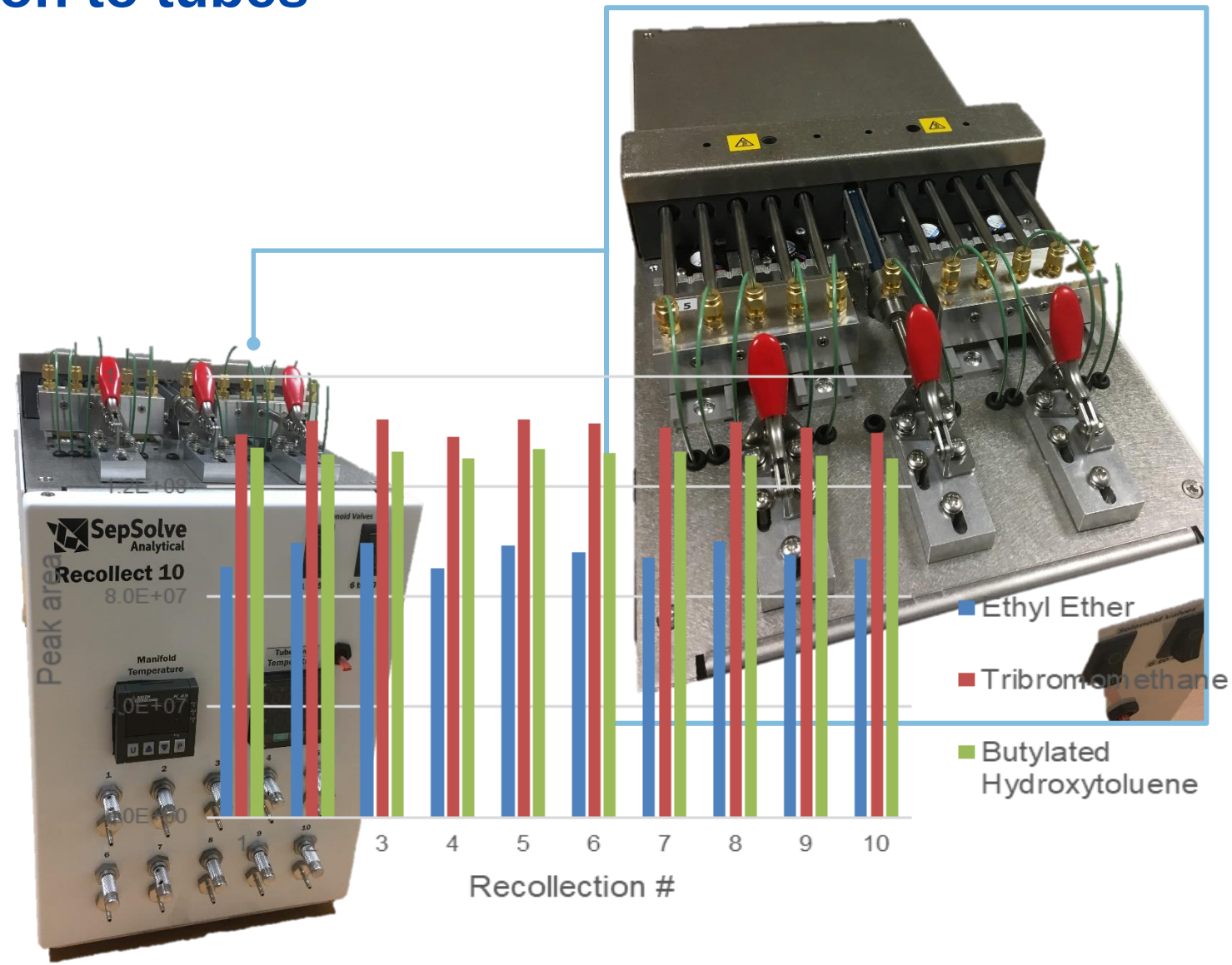
## Gas standards

- Automated standard addition to **tubes** or **focusing trap**



# Automated standard addition to tubes

- Split high-loading samples between multiple tubes
- For replicate analysis and statistical comparison
- Stand-alone, and compatible with industry-standard TD tubes



# Automated standard addition to tubes

## Liquid standards

**1.** TD tubes loaded on to custom trays

**2.** Liquid syringe loads standards on to tubes

**3.** Robotic tool change to headspace syringe

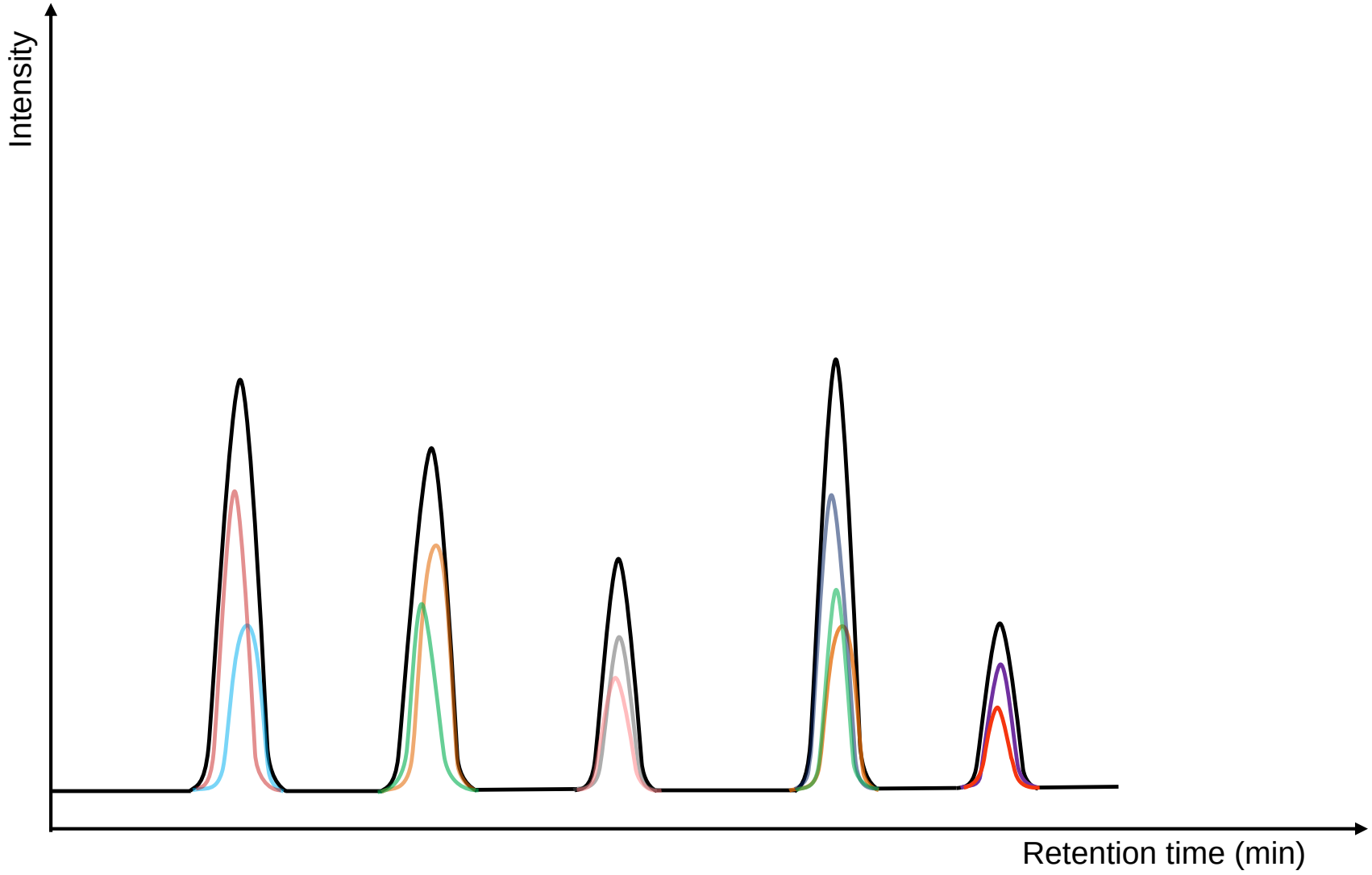

**4.** Purge tube with $N_2$ to eliminate solvent

- Bespoke tray design to accommodate 15 thermal desorption tubes
- Workflow for fully automated spiking of sorbent tubes with liquid standards using a bespoke SPR

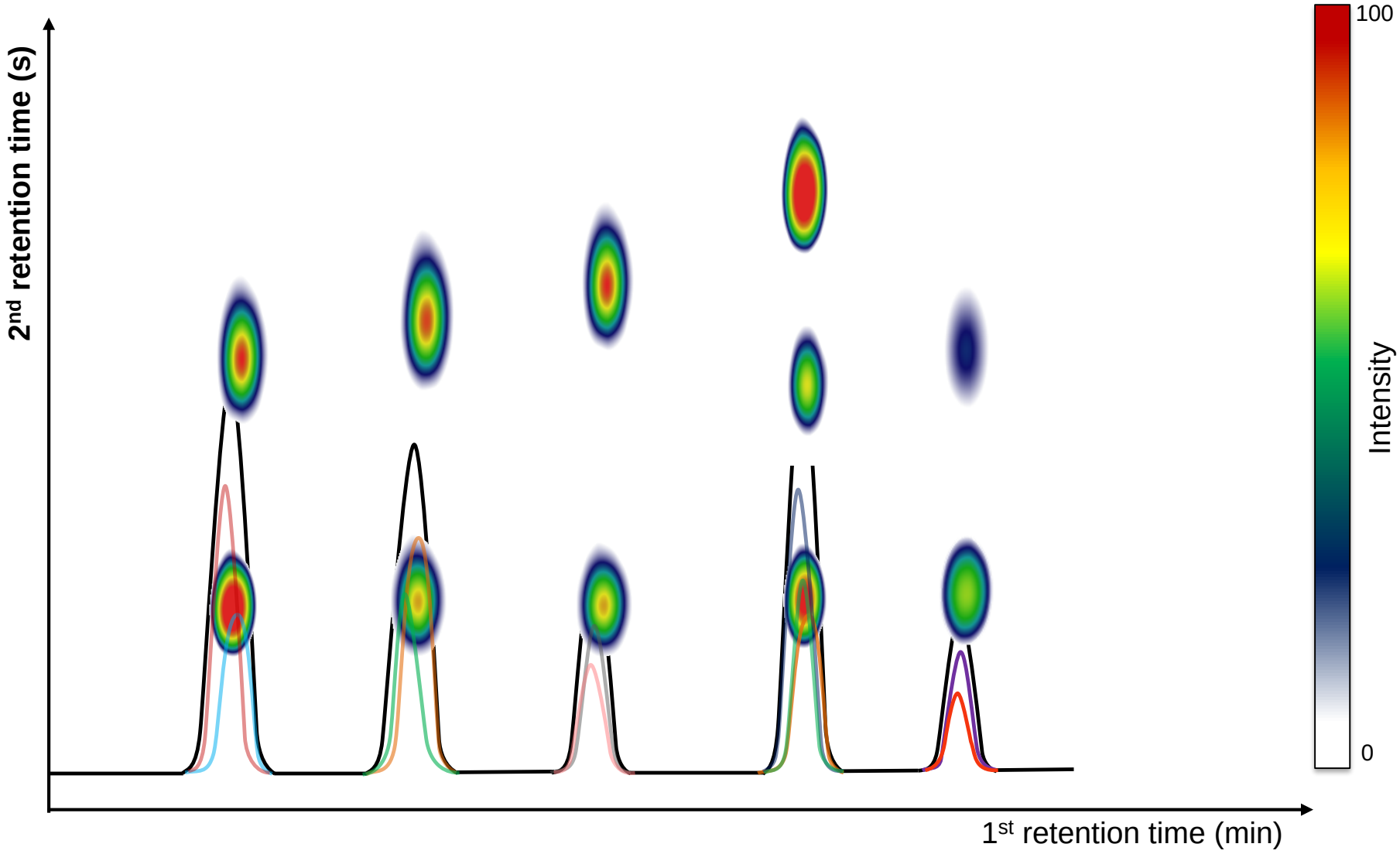
# Increasing confidence in biomarker identification

- Are you **sure** it's the compound you say it is?

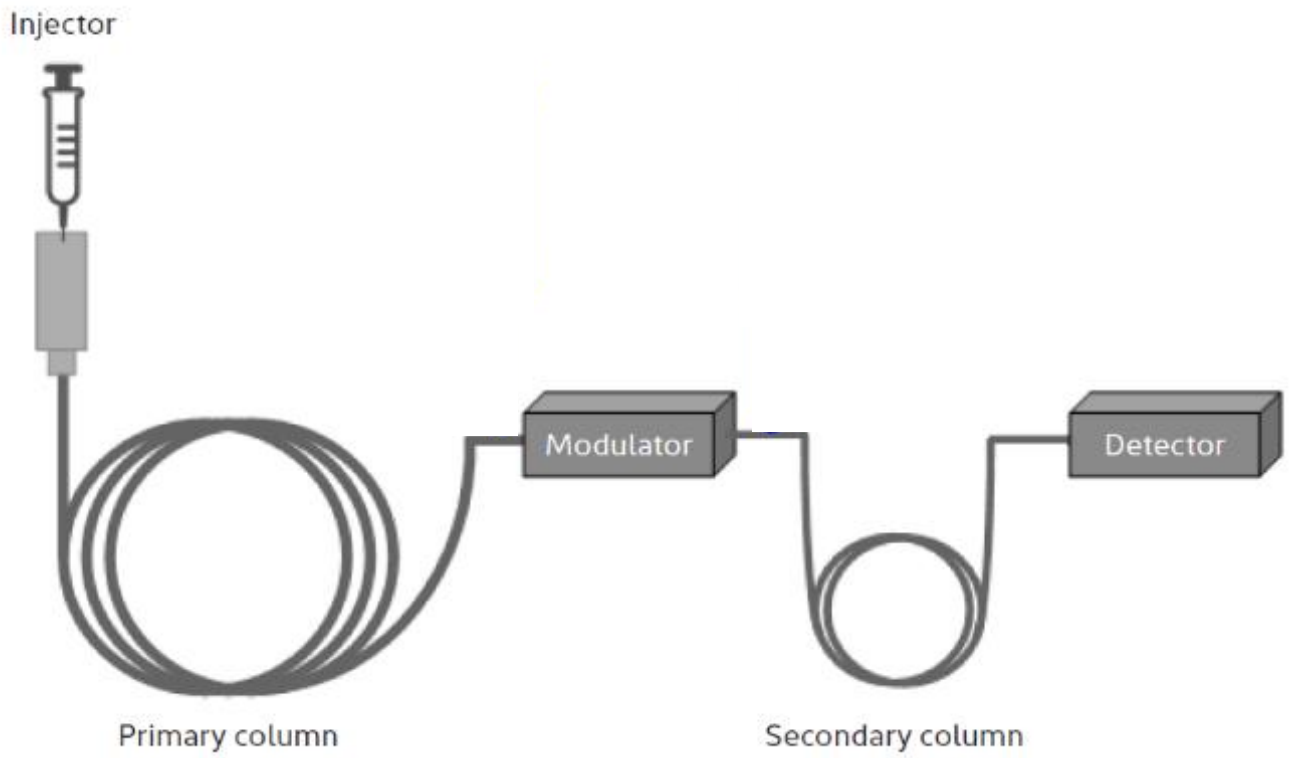
# The challenge…

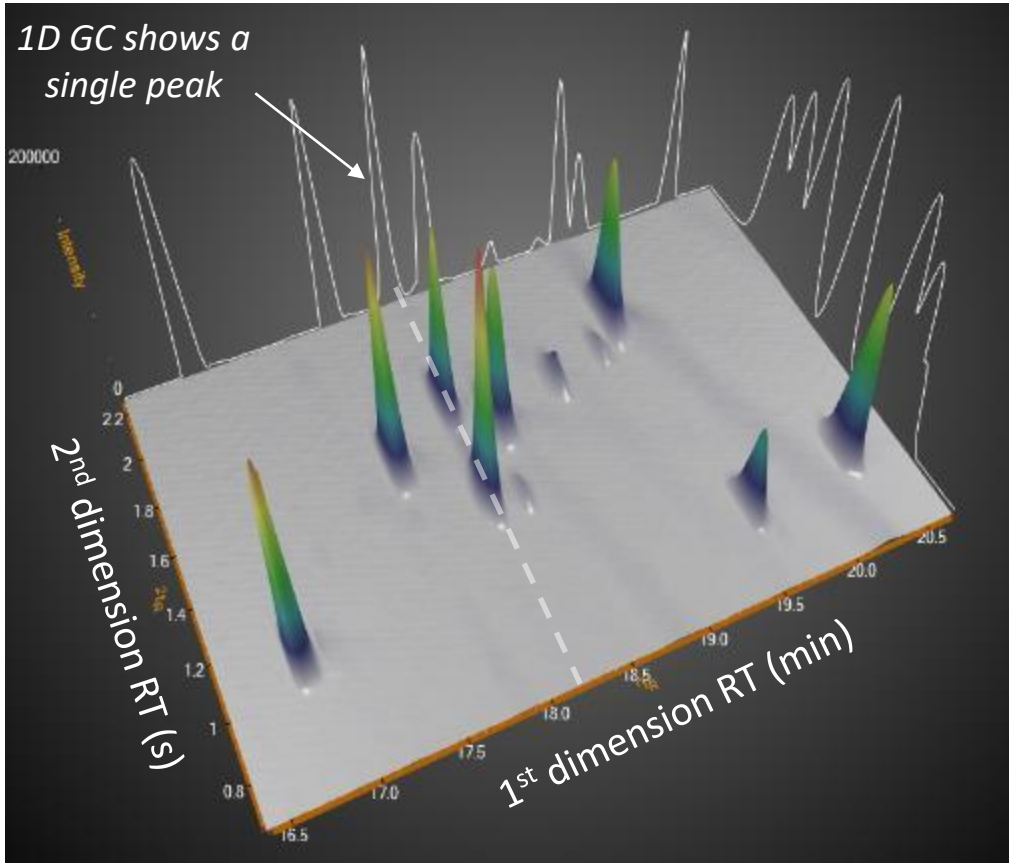


# What is GC×GC?

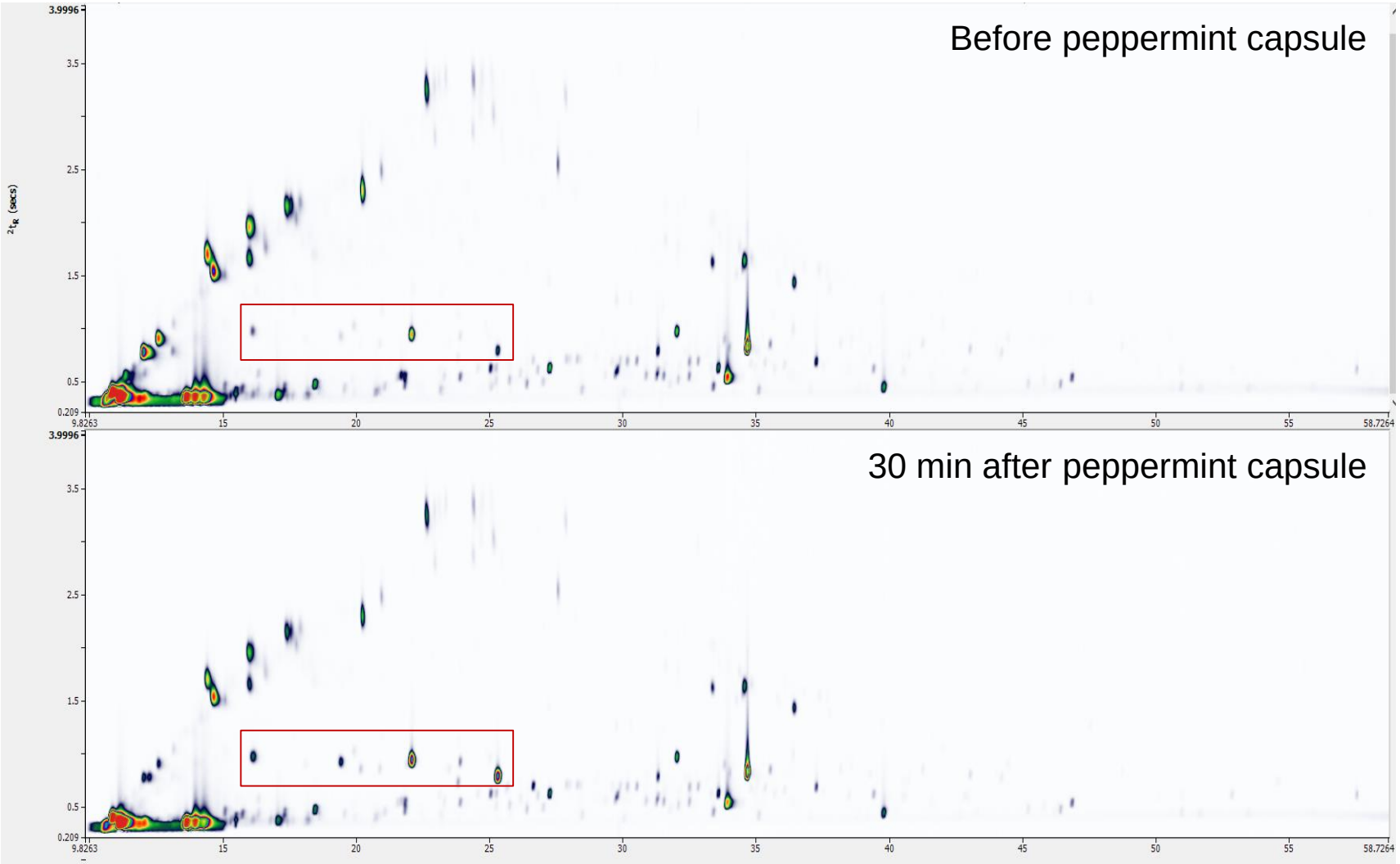


# What is GC×GC?


Injector
Modulator
Detector
Primary column
Secondary column

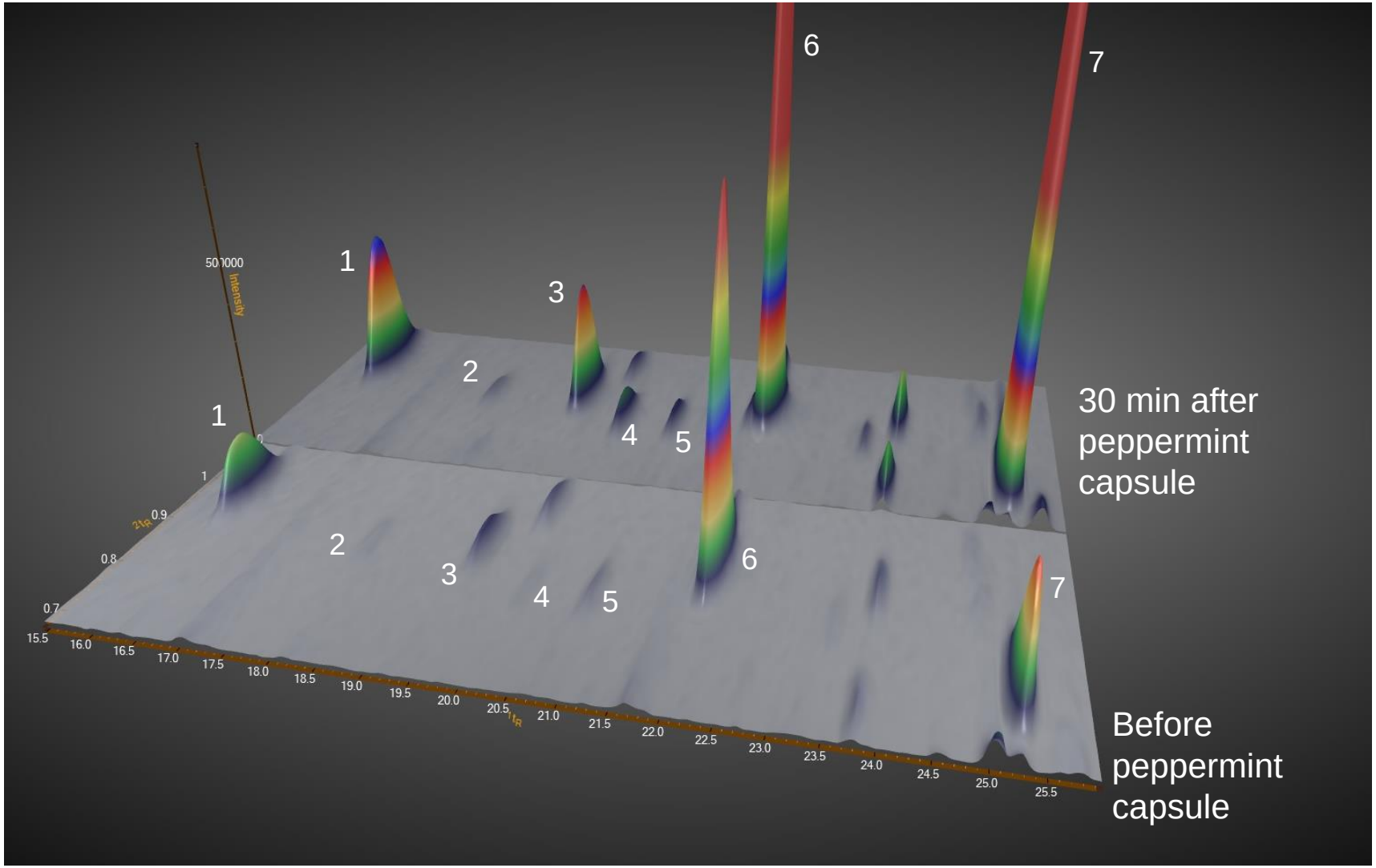

1D GC shows a single peak
200000
Intensity
0
2.2
2
1.8
1.6
1.4
1.2
1
0.8
2nd dimension RT (s)
16.5
17.0
17.5
18.0
18.5
19.0
19.5
20.0
20.5
1st dimension RT (min)

# Improved separation by GC×GC




- Breath VOCs were measured before & after consumption of a peppermint capsule

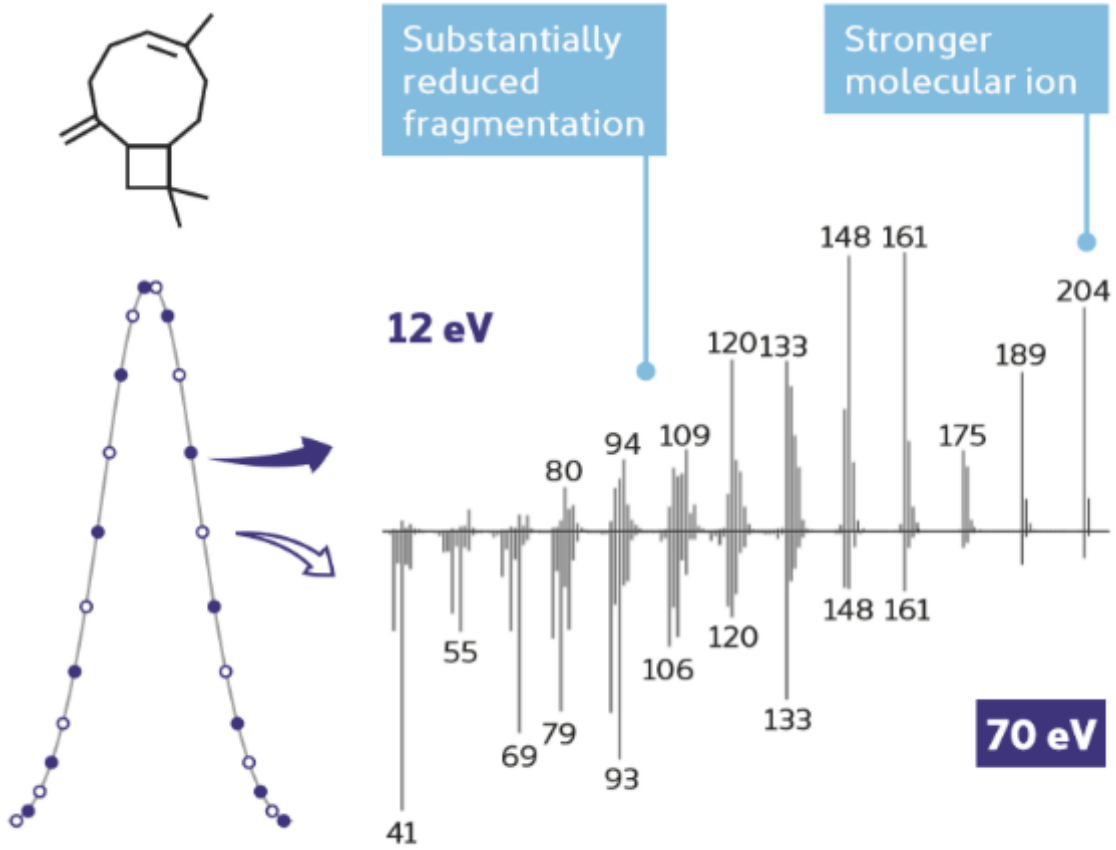
# Improved separation by GC×GC




1 α-Pinene
2 Camphene
3 β-Pinene
4 δ-3-Carene
5 α-Phellandrene
6 β-Mycene
7 Limonene
8 Eucalyptol

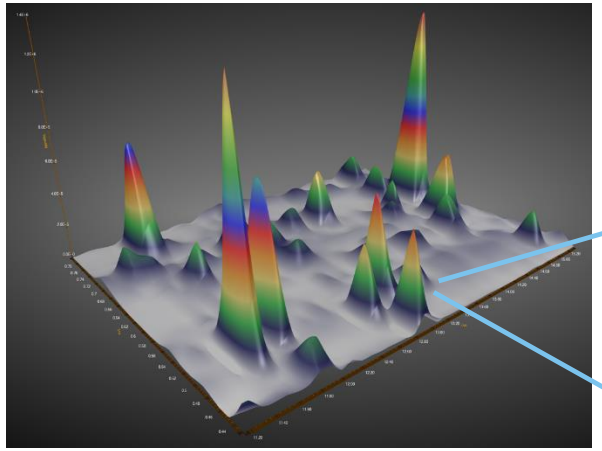
# Increased confidence with Tandem Ionisation®

- Acquire hard and soft EI simultaneously
- Additional confirmation of analyte identity
- No added analysis time
- Patented technology exclusive to BenchTOF

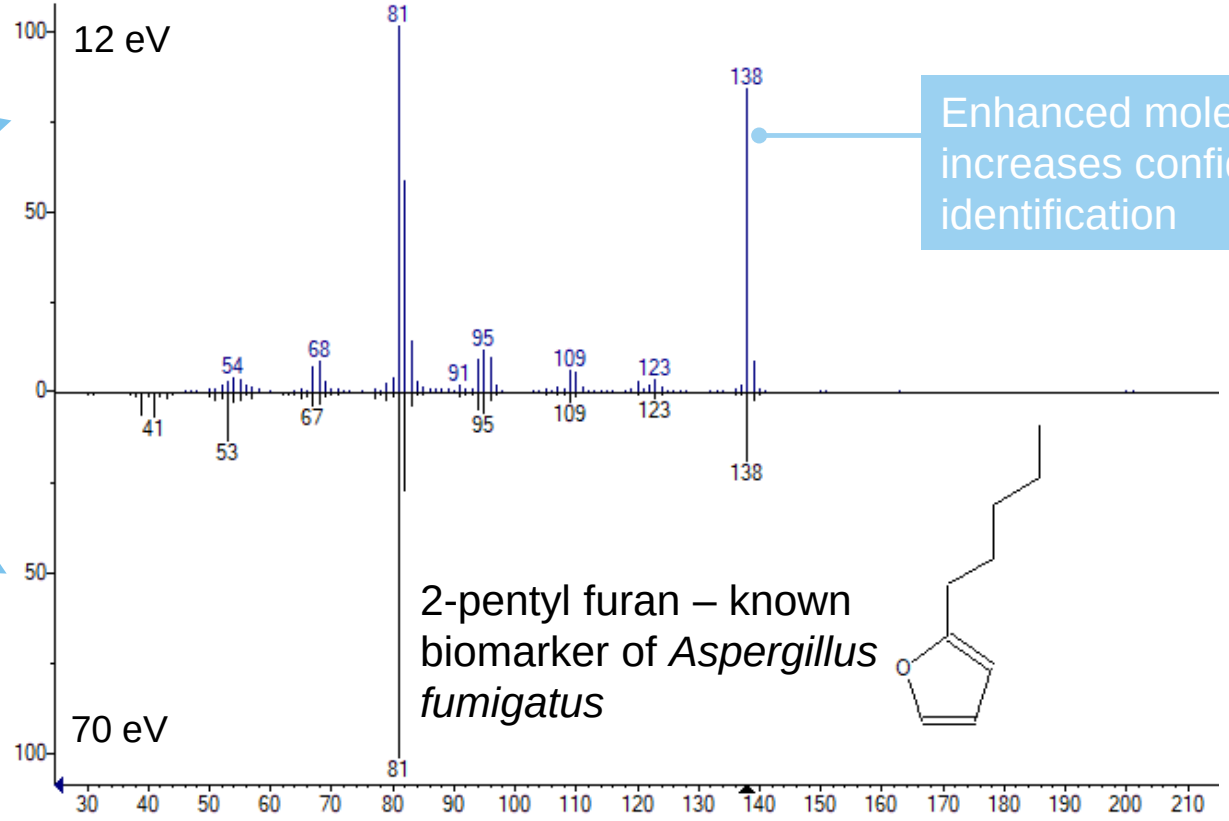


# Delivering increased confidence in identification

## Tandem Ionisation®



One analytical run delivers two complementary sets of data

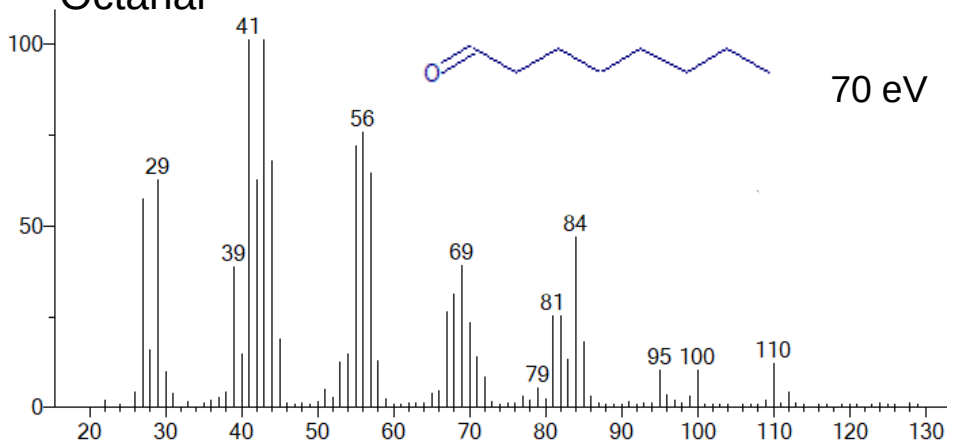



Enhanced molecular ion increases confidence in identification

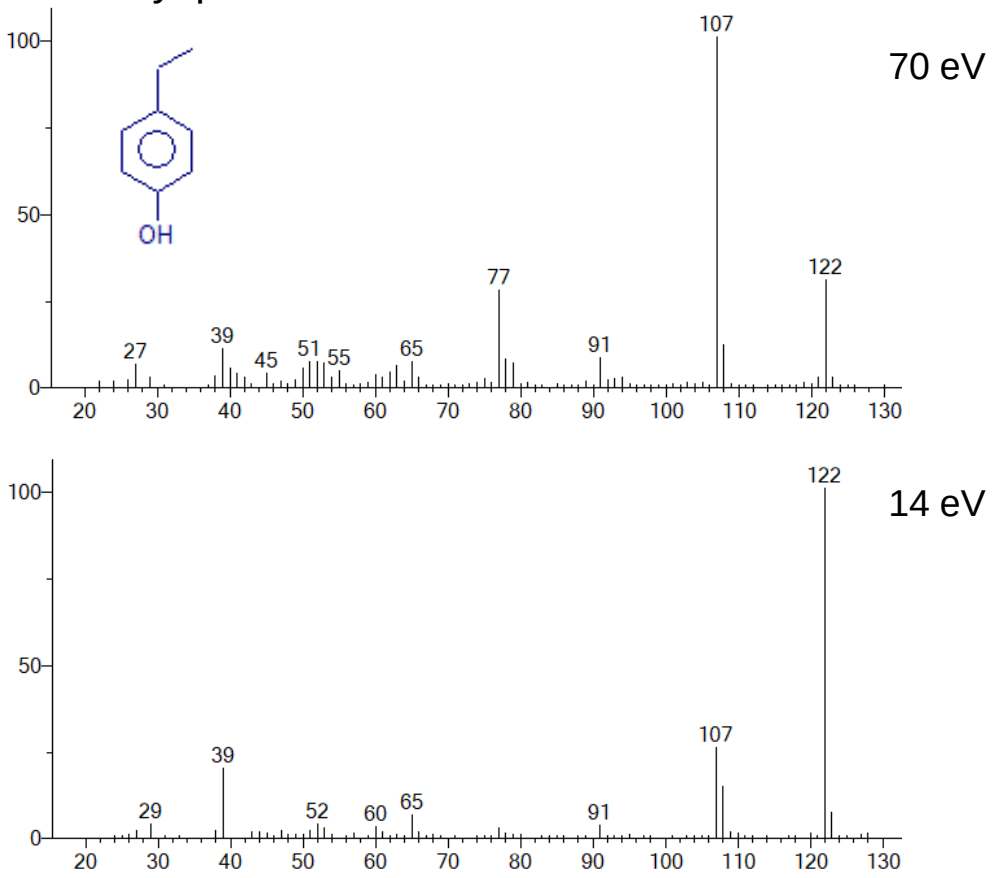
2-pentyl furan – known biomarker of *Aspergillus fumigatus*

# Other examples of TI for known breath biomarkers

Octanal

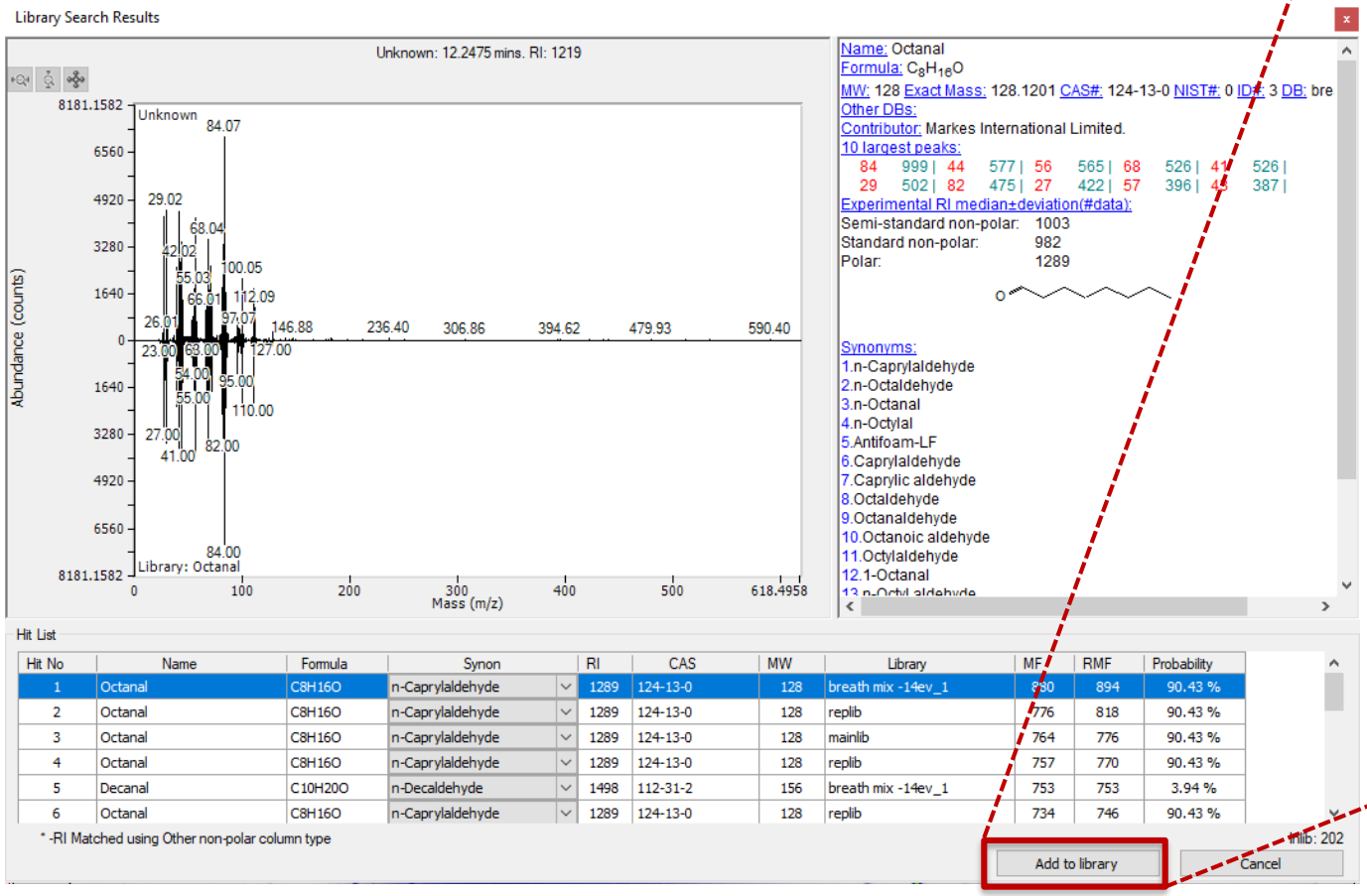


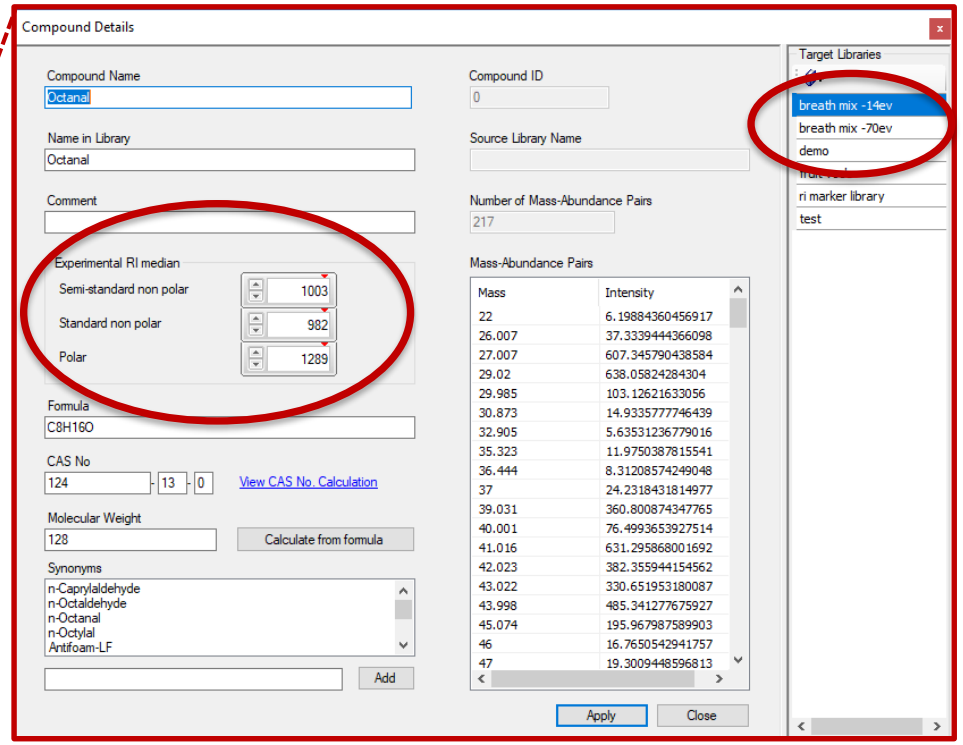

4-Ethyl phenol



# Triple confirmation of identity

## 70 eV and 14 eV plus retention indices (RI)



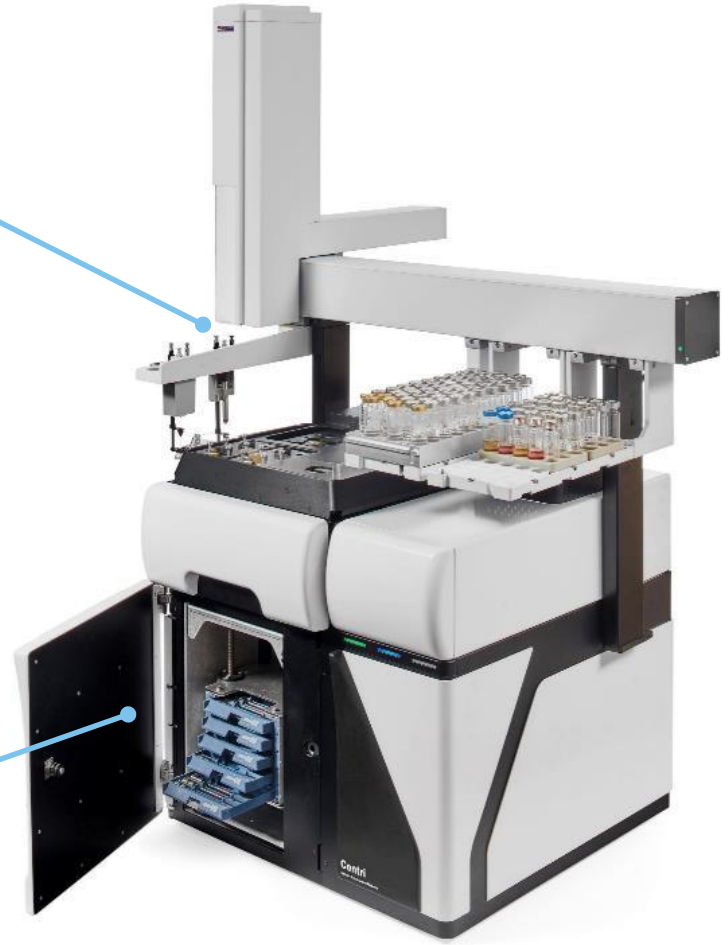

- ✓ RI confirmation
- ✓ 14 eV confirmation
- ✓ 70 eV confirmation

# Beyond breath…

## A single platform for the analysis of VOCs in breath and biological fluids

- **Liquids, solids and semi-solids:**
  - Urine, exhaled breath condensate, saliva, sputum as alternatives to breath
  - Skin, sweat: monitoring of physical or psychological stress, hydration state
  - Stool: GI infections, microbiome studies

- **Gases:** Analysis of breath biomarkers

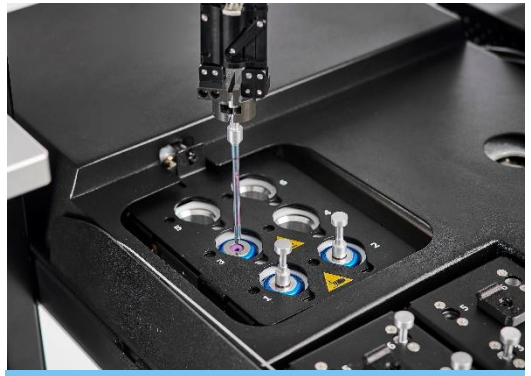


# What does Centri® do?


Headspace-trap
(& classical headspace)


Tube-based
thermal desorption


SPME-trap
(& classical SPME)



HiSorb high-capacity
sorptive extraction

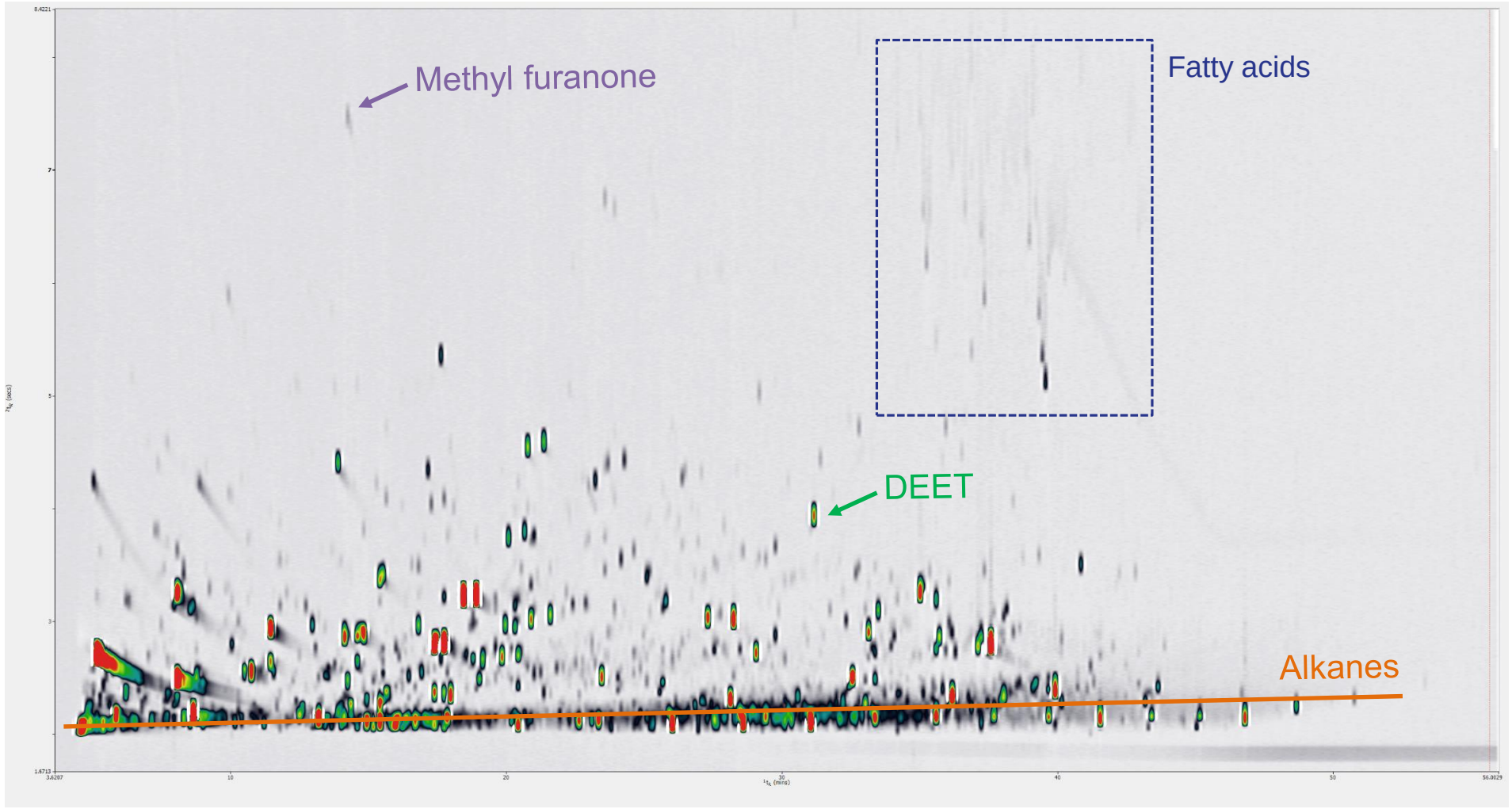
# Innovation at the heart of Centri

## Why is a trap important?

- Secure sample recollection
- Improved chromatography
- Selectivity
- Sample enrichment
- Flexibility

# Beyond breath…

## Thin Film-SPME-GC×GC-TOF MS of skin VOCs



# Beyond breath…

## HiSorb sorptive extraction

- Analysis of a range of biological samples (e.g. breath, urine, sputum…) using a single analytical platform

- HiSorb probes are ideal for high sensitivity immersive sampling of urine

- log $K_{O/W}$ < 2 = low recovery by PDMS
  e.g. Propanoic Acid $K_{O/W}$ = 0.33
- 2< log $K_{O/W}$ < 4 partial extraction by PDMS
  e.g. Octanal $K_{O/W}$ = 2.78
- log $K_{O/W}$ > 4 excellent recovery by PDMS
  e.g. Decane $K_{O/W}$ = 5.25

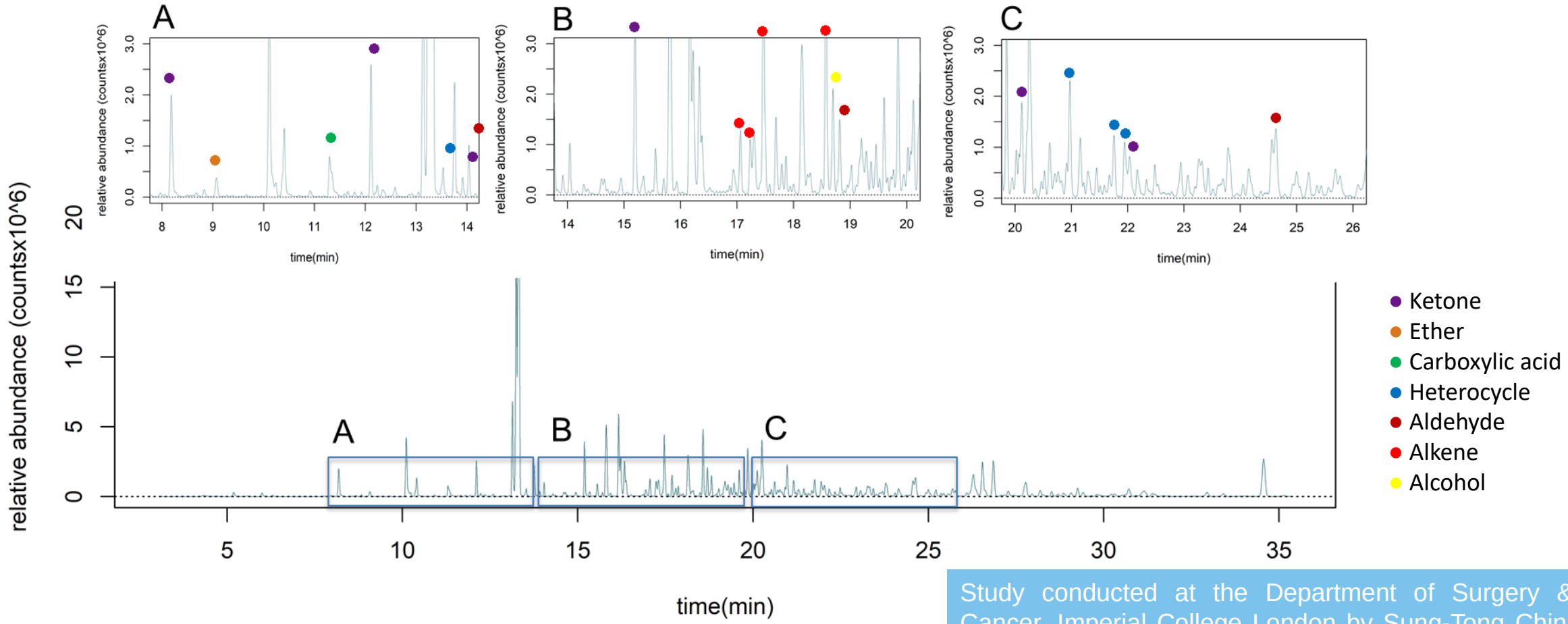
# HiSorb high-capacity sorptive extraction

## Fully automated workflow


- Suitable for immersive sampling of liquids and headspace sampling of solids/liquids
- Urinary biomarkers have potential as a simple, inexpensive and non-invasive test for early cancer detection

# Case study for HiSorb extraction

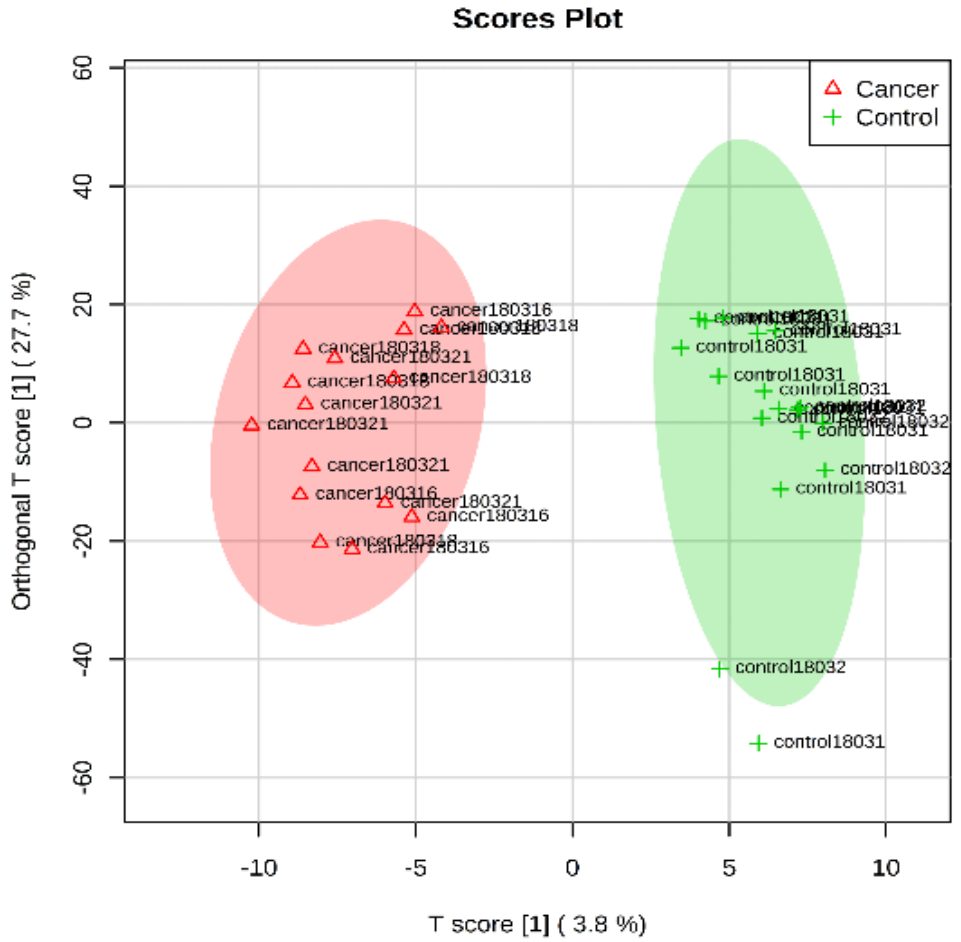
## Urinary VOC analysis in oesophago-gastric cancer detection




Study conducted at the Department of Surgery & Cancer, Imperial College London by Sung-Tong Chin, Qing Wen and George Hanna

# Case study for HiSorb extraction

- Urinary VOC analysis in oesophago-gastric cancer detection
  - Good linearity $R^2$= 0.991 - 0.994
  - Limits of quantitation: 30 - 70 ng/g
  - Urine is sampled from 50 cancer patients and 50 controls
  - 90% of correct discrimination between cancer and control
  - Applicable to large-scale clinical studies: convenient, low cost, rugged, sensitive




Study conducted at the Department of Surgery & Cancer, Imperial College London by Sung-Tong Chin, Qing Wen and George Hanna

# The workflow of breath analysis

## TD-GC-MS is the “golden standard” technique

**Automated thermal desorbers**

Pre-concentration, automation, water management, leak testing…

**Automation & concentration platform**

Biofluids (urine, blood…), tissues, culture media

**GC×GC-TOF MS**

Biomarker ‘discovery’ platform

# Summary

- TD-GC(×GC)-MS is the gold-standard technique for breath
- All metabolomics studies must ensure data quality
- When data are not properly QC checked, this leads to wrong data interpretations
- TD can make breath samples more amenable to QC sample preparation and QC checks
- Confident identification is possible with improved separation and/or additional checks (soft ionisation, retention indices…)

# Thanks for listening! Any questions?

## Contact SepSolve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