

Biomarker discovery

Essential guide to analysing VOCs in breath and other biological samples



Contents



Introduction 3

Breath sampling: The gold-standard approach	5
Analytical workflow for biomarker discovery	6



Breath 7

Breath profiling	8
Biomarker discovery	9
Archiving valuable samples	10
Simulating metabolic changes	11
Identifying changes in breath profiles	12
Biomarker confidence	13
Disease diagnosis	14



Biological fluids 15

Urine analysis	16
Blood volatiles	17



Skin volatiles..... 18

Skin volatiles	19
Mosquito-attracting chemicals	20



Relevant analytical products..... 21

BioVOC-2	22
TD100-xr	23
Centri	24
HiSorb	25
Thin-film SPME	26
INSIGHT	27
BenchTOF2	28
ChromSpace 1D	29
ChromSpace	30
ChromCompare+	31



About SepSolve Analytical..... 32

For more information on any of the applications described, or to discuss how our biomarker discovery technologies could benefit you, please contact our applications specialists at hello@sepsolve.com, or by telephoning any of our regional offices (see back cover for details).

The applications in this e-book are for research use only and not for use in diagnostic procedures.

Introduction

The accurate identification and measurement of biomarkers in biological samples – such as breath and biofluids – has the potential to provide rapid, minimally-invasive diagnosis of a range of physiological and pathological conditions.

In this Applications Guide, we describe how flexible sampling coupled with high-performance GC-MS or GC×GC-MS enables the maximum possible number of compounds to be identified in a realistic run time.



The rise of biomarker discovery

Volatile organic compounds (VOCs) emitted by the human body have great potential in **disease diagnosis**. This is largely due to the discovery of so-called 'biomarkers', which provide indicators of normal or abnormal states.



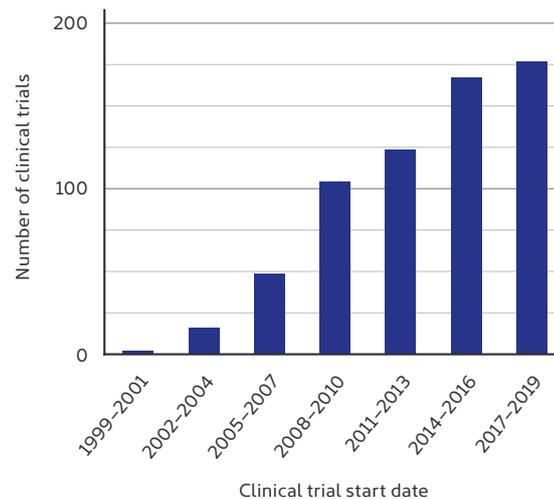
Recently, research has focused on **non-invasive approaches to biomarker discovery** – most commonly breath and urine. This has also been accompanied by an increasing interest from the clinical community, as witnessed by the recent rise in the number of clinical trials involving breath analysis.

>800
VOCs
published as
biomarkers in
the literature

Such developments open up the possibility of breath analysis making the leap from research to clinical practice. This would allow the screening of large populations, **improving patient survival** and reducing costs for health services.

However, a successful transition from benchtop to bedside depends on a **valid basis for reproducible and standardised VOC collection**. This is where thermal desorption coupled with GC-MS plays an important role, for preconcentration, separation, and ultimately confident identification, of potential biomarkers.

Breath analysis dates back to 1971 when Linus Pauling and co-workers detected around 250 compounds in human exhaled air.



The number of clinical trials involving breath analysis has increased rapidly in the last 20 years. (Source: ClinicalTrials.org)

Breath sampling: The gold-standard approach

In large-scale clinical trials, breath samples may be collected across multiple sites (e.g. at clinics and hospitals, or by primary care physicians), meaning secure transport of samples to the analytical laboratory is imperative.

Thermal desorption (TD) coupled with GC-MS is known as the 'gold standard' for breath analysis, due to its ability to provide a comprehensive breath profile with high confidence and sensitivity.



The **BioVOC-2™** breath sampler from Markes International is a simple, affordable device for collecting breath VOCs and transferring them to TD tubes. See page 22 for more information.



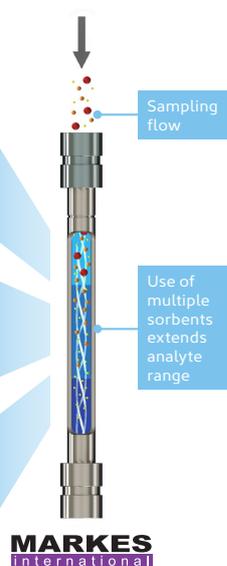
Alternatively, breath samples can be collected onto a **sampling bag**, before transfer to TD tubes using a **low-flow pump** (e.g. ACTI-VOC PLUS™, Markes International)



The **ReCIVA®** breath sampler from Owlstone Medical allows exhaled breath to be directly transferred onto up to four TD tubes.

Delivering quality data in breath analysis

Markes International's world-leading TD instruments deliver improved data quality, sample security and – when combined with SepSolve's advanced separation and detection techniques – the ability to discover more from your breath samples.



Advantages of Markes' TD systems

- ▶ Tubes are compact and can be sealed for secure transport and storage.
- ▶ Patented DiffLok™ caps prevent both analyte loss and artefact ingress throughout automated analysis.
- ▶ Secure re-collection allows repeat analysis of invaluable clinical samples.
- ▶ Backflush desorption allows multi-bed sorbents to be used, for compatibility with a wide analyte range.
- ▶ Dry-purging of the tubes and focusing trap allows selective elimination of water.
- ▶ Cryogen-free trap focusing results in enhanced sensitivity with low running costs.
- ▶ Quality control is achieved by adding internal standards to the tubes or the focusing trap.
- ▶ Pre-desorption leak test of every sample ensures data quality.
- ▶ Barcoding and/or RFID tags ensures sample and data traceability.

Analytical workflow for biomarker discovery

SAMPLE INTRODUCTION

Breath



Automated thermal desorption using TD100-xr™: [See page 23](#)

Breath & biological fluids



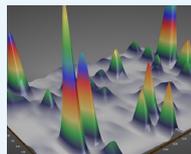
Automated, unattended analysis of liquids, solids and semi-solids, and up to 50 TD tubes, using Centri®: [See page 24](#)

ENRICHMENT

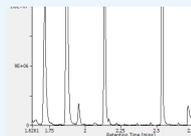


At the heart of both approaches is the electrically-cooled focusing trap, which transfers analytes to the GC-MS in a narrow band for optimal chromatography.

SEPARATION



Enhanced separation by GCxGC for biomarker discovery: [See page 27](#)



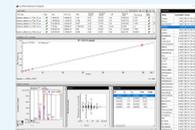
Once target biomarkers are known, high-throughput fast GC can be adopted.

DETECTION



Confident targeted and untargeted screening using BenchTOF2™ with unique Tandem Ionisation®: [See page 28](#)

DATA ANALYSIS



Data analysis (both qualitative and quantitative) in ChromSpace® with ChromCompare+ to automatically identify correlations: [See pages 29-31](#)

Breath

Breath monitoring has attracted much attention for its potential to non-invasively diagnose a range of physiological and pathological conditions.

In this section, we show how breath sampling, performed with sorbent tubes at point-of-care, followed by prompt, sensitive TD-GC-MS analysis, offers the prospect of rapid diagnosis of medical conditions.



Breath profiling

Finding 'hidden' compounds with deconvolution

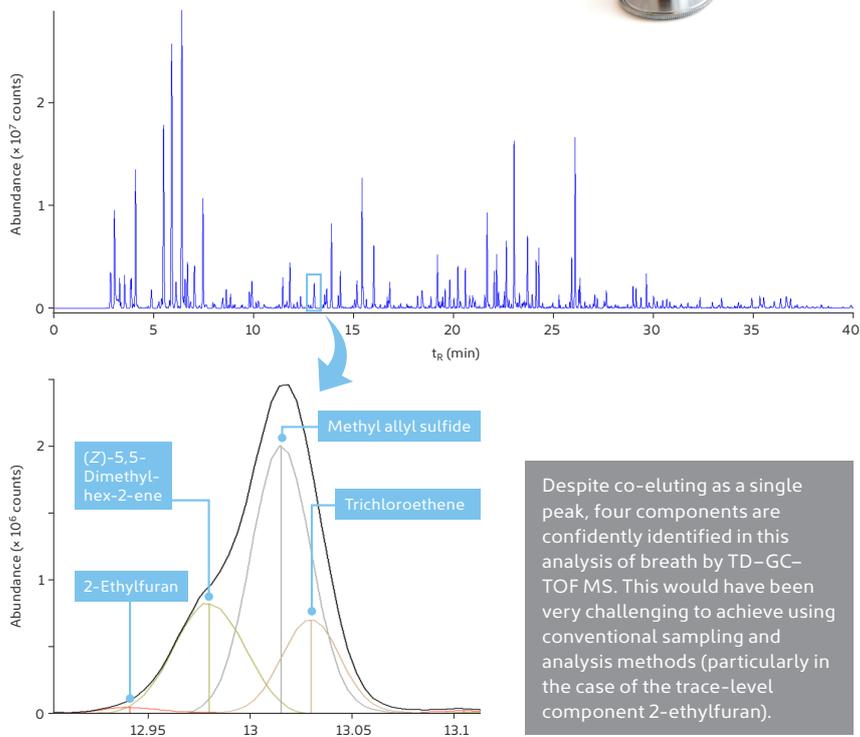
Breath analysis is a challenging application, with hundreds of different components, some of which are present at part-per-trillion levels.

TD-GC-TOF MS addresses this challenge by providing a complete chemical fingerprint, with full spectral information for reliable identification of targets and non-targets, and enhanced sensitivity.

Note that, unlike quadrupole MS instruments, TOF MS systems do not exhibit spectral skew across peaks. This provides greater confidence when applying deconvolution algorithms and a lower chance of false positives/negatives, helping to ensure information of diagnostic value is not missed.

Analytical details

► **Sampling:** Two successive 129 mL samples of end-tidal breath, collected onto the same sorbent tube using a disposable Bio-VOC™ sampler. ► **TD:** TD100-xr™. ► **TOF MS:** BenchTOF™; m/z 40–400. ► **Software:** ChromSpace®.



Despite co-eluting as a single peak, four components are confidently identified in this analysis of breath by TD-GC-TOF MS. This would have been very challenging to achieve using conventional sampling and analysis methods (particularly in the case of the trace-level component 2-ethylfuran).



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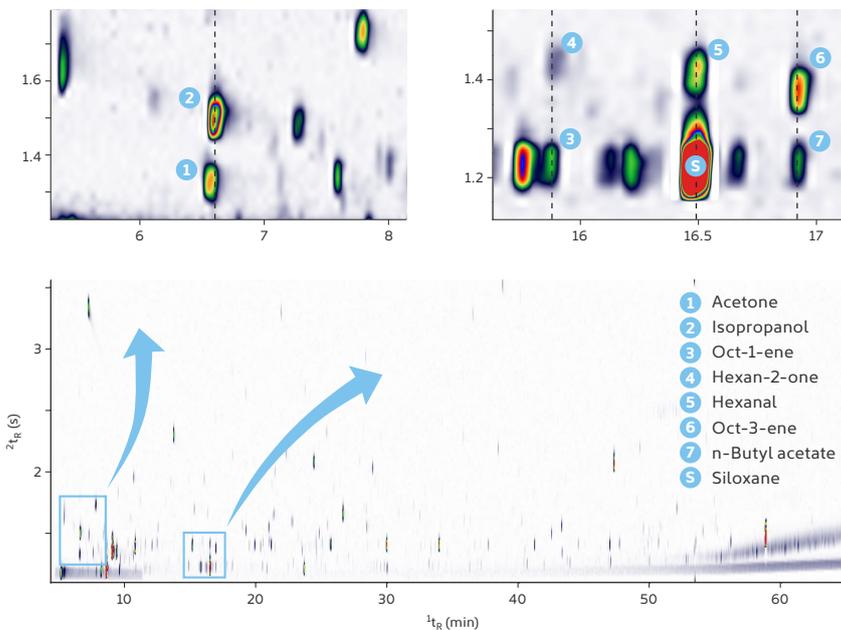
Improved separation by GC×GC

One of the key challenges in breath analysis is that hundreds of different VOCs can be present, often at trace levels – making it difficult to isolate and identify biomarkers of disease.

TD coupled with GC×GC and TOF MS has been proposed as a powerful technique for the analysis of such complex samples. The high sensitivity and enhanced separation of the system ensures that trace metabolites are not masked or overlooked, and provides cleaner spectra for confident identification of potential biomarkers.

Analytical details

► **Sampling:** 1 L breath, collected using sampling bags and transferred to 'Biomonitoring' sorbent tubes using an ACTI-VOC™ low-flow pump. ► **TD:** Centri®.
► **GC×GC:** INSIGHT® flow modulator; P_M 2.5 s.
► **TOF MS:** BenchTOF™; m/z 35–450. ► **Software:** ChromSpace®.



Four pairs of components would have co-eluted in a 1D GC separation of this breath sample – but using GC×GC with TOF MS all eight compounds can be confidently identified.



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Archiving valuable samples

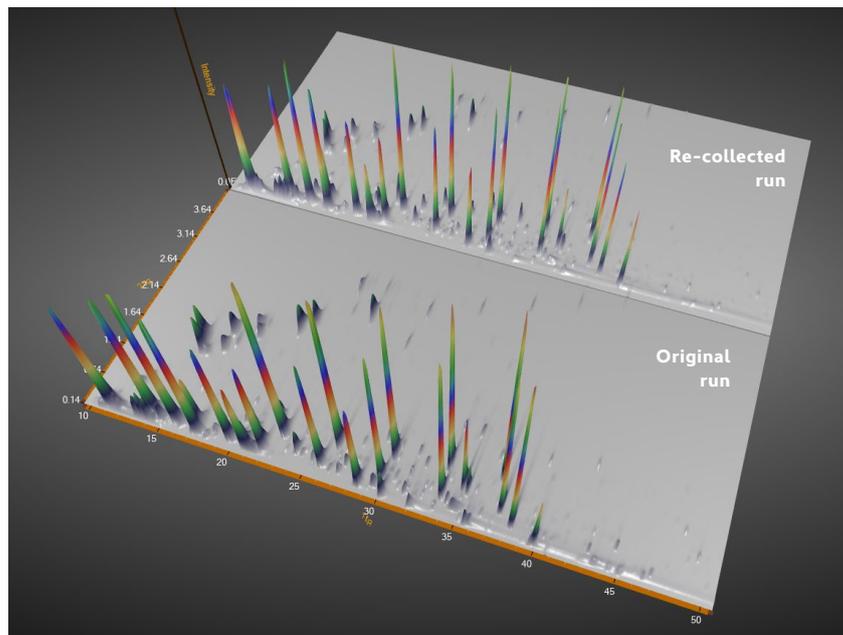
Secure sample re-collection

Breath samples taken as part of a clinical study are often a unique snapshot of the patient's clinical journey, and are therefore not repeatable. It is therefore vital to be able to archive such samples, and quantitative re-collection of split flows by Markes' thermal desorbers offers the ideal solution.

Using this method, critical samples can be stored and re-analysed (using the same or different analytical conditions) to confirm compound identity, without having to repeat sample collection – a feature that is particularly useful when trying to identify biomarker patterns.

Analytical details

▶ **Sampling:** 1 L breath, collected using sampling bags and transferred immediately to 'Biomonitoring' sorbent tubes. ▶ **TD:** Instrument: TD100-xr™; Overall split ratio: 4:1. ▶ **GC×GC:** INSIGHT® flow modulator (SepSolve Analytical); P_M 2.5 s. ▶ **TOF MS:** Instrument: BenchTOF™; m/z 35–450. ▶ **Software:** ChromSpace®.



TD–GC×GC–TOF MS surface charts for a 1 L breath sample and the re-collected portion show excellent agreement in relative responses across the volatility range.

Simulating metabolic changes

Standardisation using peppermint oil

With breath analysis techniques constantly evolving, the 'peppermint trial' was developed by researchers as a way to benchmark methods. To simulate a real-world scenario, participants' breath was measured before and after ingestion of a peppermint capsule to produce a controlled, artificial change in the breath profile and mimic a change in metabolism due to disease.

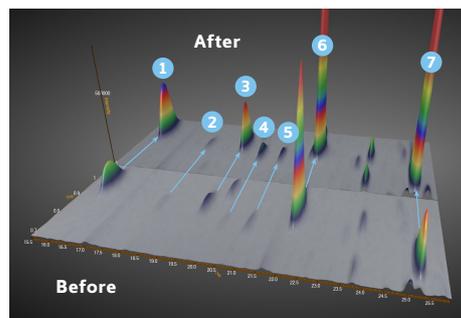
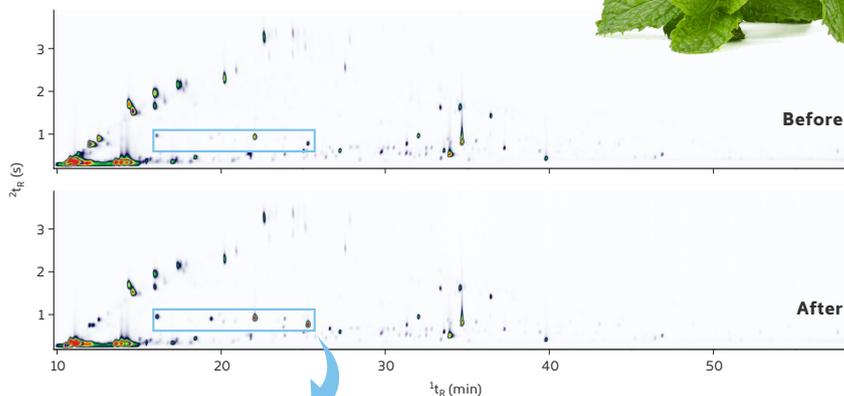
A marked increase was observed for a number of terpenes – which are plant metabolites commonly found in essential oils, such as peppermint oil. In this study, the enhanced separation of GC×GC ensured that trace metabolites were not masked or overlooked, and provided cleaner spectra for confident identification of the pseudo-biomarkers against existing commercial libraries, such as NIST.

Analytical details

► **Sampling:** 1 L breath, collected using sampling bags and transferred immediately to 'Biomonitoring' sorbent tubes. ► **TD:** TD100-xr™. ► **GC×GC:** INSIGHT® flow modulator; P_M 4.0 s. ► **TOF MS:** BenchTOF™; m/z 35–600. ► **Software:** ChromSpace®.



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- | | |
|------------------|--------------|
| 1 α-Pinene | 5 β-Myrcene |
| 2 Camphene | 6 Limonene |
| 3 β-Ginene | 7 Eucalyptol |
| 4 β-Phellandrene | |

Numerous terpenes show an increase 30 min after ingestion of a peppermint oil capsule, as shown in the highlighted region of this TD-GC×GC-TOF MS analysis of breath.

Identifying changes in breath profiles

Uncovering hidden differences with easy-to-use chemometrics

Incorrect identification of compounds during the discovery phase of a biomarker investigation can compromise the validity of an entire trial. To avoid this, the analytical techniques and data-mining algorithms must be robust and reliable.

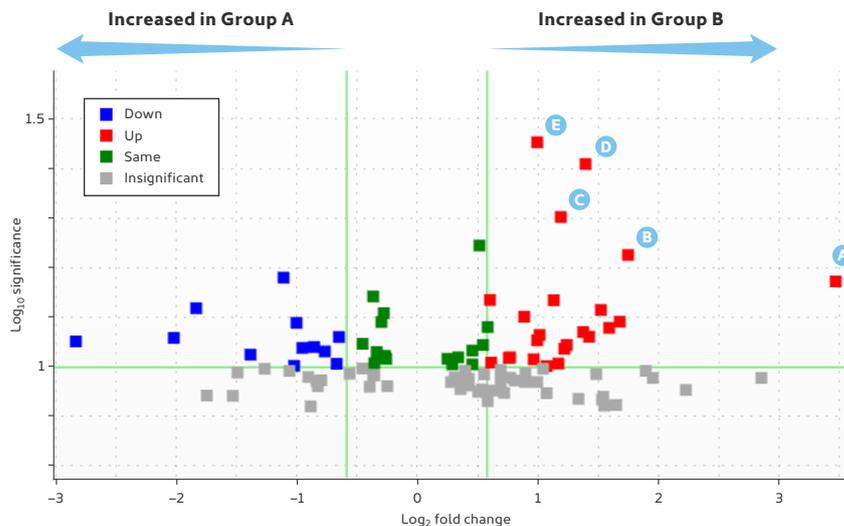
In this example, TD–GC×GC with BenchTOF MS was used for exploratory profiling of biomarkers in breath, coupled with Feature Discovery in ChromCompare+ to uncover hidden compositional changes, as part of an automated untargeted workflow. ChromCompare+ also allows the raw data to be imported directly, minimising manual steps and reducing the risk of operator error.

Analytical details

▶ **Sampling:** Nine 1 L breath samples, collected from two groups of participants (A & B) using sampling bags, and transferred to 'Biomonitoring' sorbent tubes.

▶ **TD:** Centri®. ▶ **GC×GC:** INSIGHT® flow modulator; P_M 2.5 s. ▶ **TOF MS:** BenchTOF™; m/z 45–450.

▶ **Software:** ChromCompare+™



As shown by this 'volcano' plot of 100 chromatographic features, five compounds (A–E) were present in greater abundance (and with a high statistical significance) in breath samples from Group B. Such changes are typical of those that could indicate altered metabolism caused by disease.

- A (Z)-1-(Methylthio)prop-1-ene
- B Allyl methyl sulfide
- C 1-(Methylthio)propane
- D Dimethyl disulfide
- E (E)-1-(Methylthio)prop-1-ene



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Biomarker confidence

Tandem Ionisation for confirmation of biomarker identity

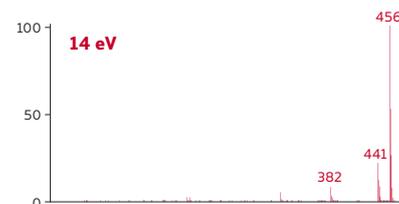
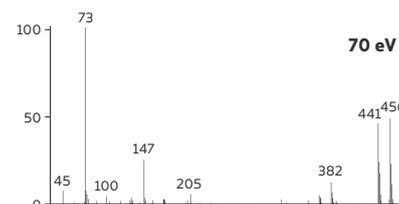
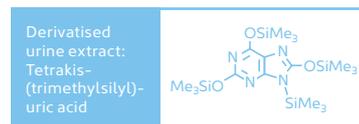
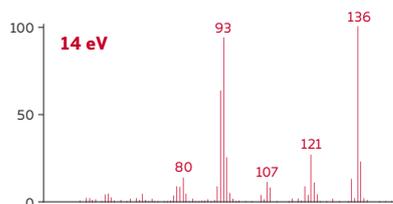
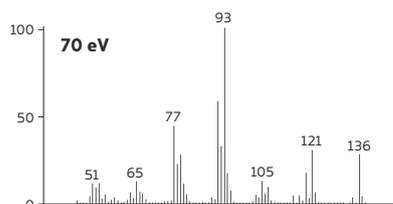
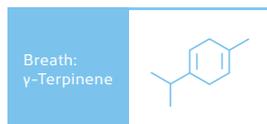
Even with the benefits offered by GC×GC, identification of some analytes can be challenging, due to their similar spectra and/or weak molecular ions. 'Soft' electron ionisation (at 10–20 eV) can address this, by delivering complementary spectra with enhanced structurally-significant ions, for added confidence in analyte identification.

This soft EI capability is available with Tandem Ionisation® for BenchTOF2™ mass spectrometers, allowing conventional and soft mass spectra to be collected simultaneously in a single analytical run.

The soft EI spectra are repeatable and contain a sufficient degree of fragmentation for libraries to be created, allowing fast and automated screening of both datasets and enhanced confidence in identification.

Analytical details

- ▶ **GC×GC:** INSIGHT® flow modulator; P_M 2.5 s.
- ▶ **TOF MS:** BenchTOF™; m/z 35–450; Tandem Ionisation®: 70/14 eV. ▶ **Software:** ChromSpace®.



A reduction in fragment ions and an increase in molecular ion intensity is evident in the 14 eV spectra of two compounds from a breath sample and a derivatised urine extract, obtained by Tandem Ionisation. Note in particular the reduction in the intensity of the trimethylsilyl fragment (m/z 73) in the derivatised uric acid.



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Disease diagnosis

Using TD-GC-MS to rapidly diagnose malaria

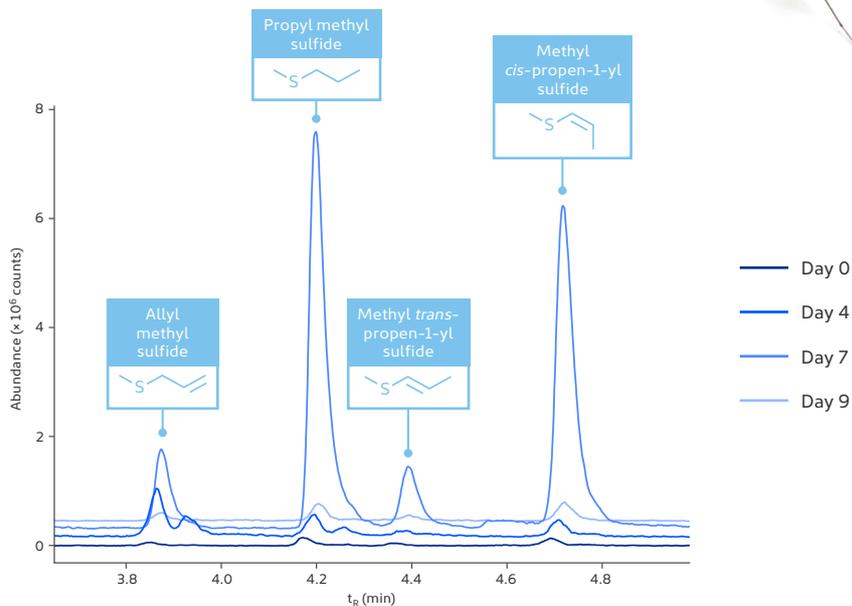
In 2017, over 400,000 deaths were attributed to malaria worldwide, and there remains a need for a simple, inexpensive and reliable diagnostic test for the disease.

To examine the potential of breath monitoring to fulfill this role, a controlled human malaria infection study was conducted by researchers from Washington University in St. Louis. Volunteers were inoculated with red blood cells infected with the malaria parasite *Plasmodium falciparum*, and daily breath samples were analysed by TD-GC-MS.

As a result, four breath thioethers were highlighted as putative malaria biomarkers. The use of inert sorbent tubes and an inert TD flow path ensured that chemically labile species such as these sulfur compounds were preserved in the breath sample.

Analytical details

► **Sampling:** 1 L breath, collected using sampling bags, and pumped onto 'Odour/Sulfur' sorbent tubes. ► **TD:** UNITY™ 2; tube desorb 280°C (10 min); trap low 30°C; trap high 300°C; outlet split 6:1. ► **GC-MS** analysis.



In this TD-GC-MS study of breath, the abundance of four thioethers was highest 7 days after inoculation with *Plasmodium falciparum* – when levels of the parasite were also at a maximum. Following drug treatment (day 9), thioether concentrations rapidly decreased.

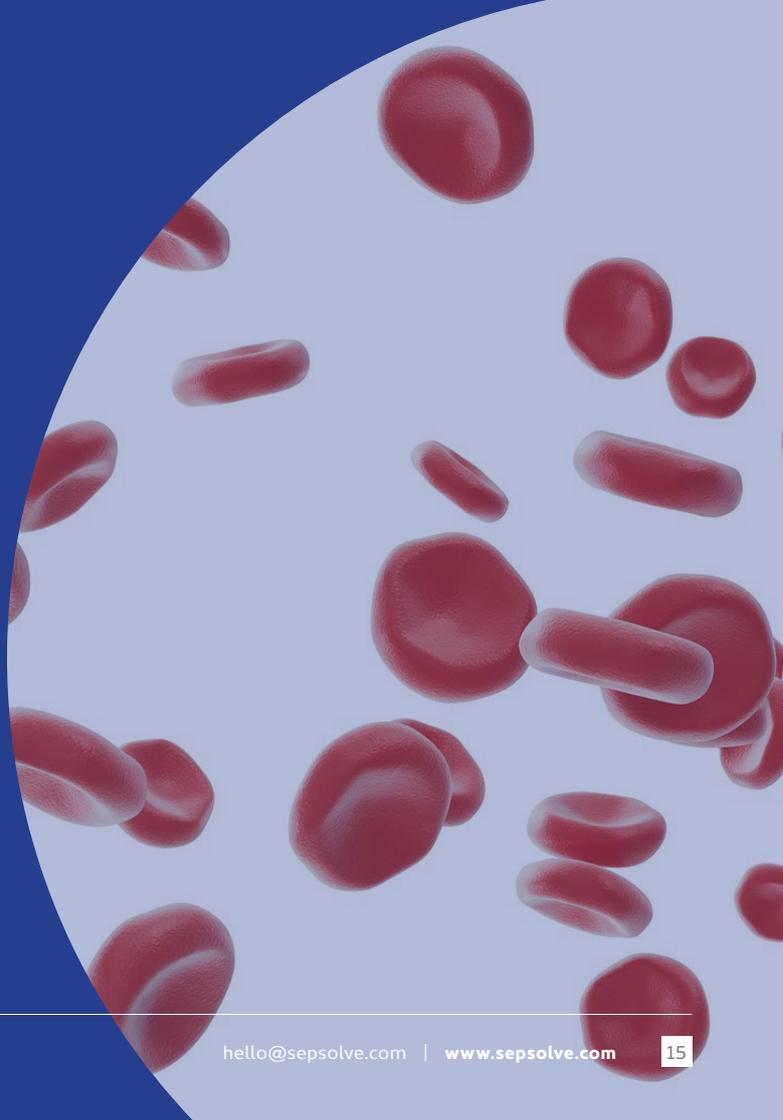


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Application Note

Biological fluids

Volatile organic compounds (VOCs) are continuously emitted by the human body and released not only through breath, but also through biological fluids such as blood, saliva and urine.

This section looks at how TD and GC-MS workflows can be extended to look beyond breath, for a more holistic approach to biomarker investigation.



Urine analysis

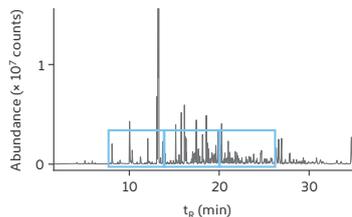
High-capacity sorptive extraction of VOCs for oesophago-gastric cancer

Early-stage oesophago-gastric cancer (OGC) is asymptomatic or shows non-specific symptoms. Many patients present at an advanced stage, reducing the prospect of successful treatment. Urine VOC analysis has the potential to become a simple, inexpensive and reliable screening test to highlight those patients requiring more invasive procedures (e.g. endoscopy).

Researchers from Imperial College London have carried out a pilot study involving HiSorb high-capacity sorptive extraction of urine coupled with TD-GC-MS analysis. Results obtained are promising, with 90% correct discrimination between cancer and control. HiSorb is useful for such large-scale studies, thanks to its convenience, low cost, ruggedness and sensitivity.

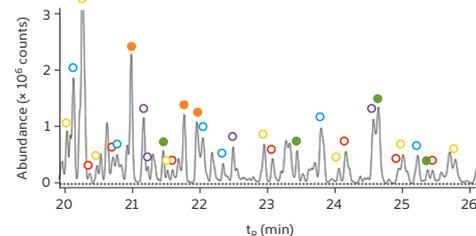
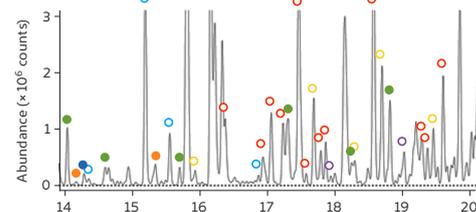
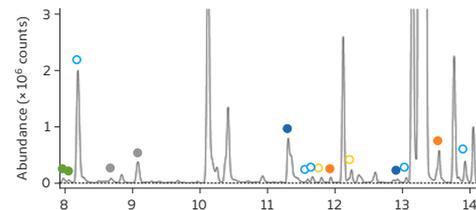
Analytical details

► **Sampling:** Urine headspace, extracted using a HiSorb™ probe for 1 h at 60°C and 300 rpm. ► **TD:** TD100-xr™; tube desorb 280°C (10 min); trap high 290°C (4 min); splitless. ► **GC-MS** analysis.



- Hydrocarbon
- Heterocycle
- Alcohol/phenol
- Aldehyde
- Ketone
- Carboxylic acid
- Ester/lactone
- Ether/nitrile

This urine headspace profile – obtained using headspace HiSorb and TD-GC-MS – shows a comprehensive VOC profile, which includes carboxylic acids, ketones, alcohols, aldehydes, heterocycles and alkanes. The data was acquired as part of a study of 50 cancer patients and 50 control subjects, carried out by S.-T. Chin, Q. Wen and G. Hanna at the Department of Surgery & Cancer, Imperial College London.



Blood volatiles

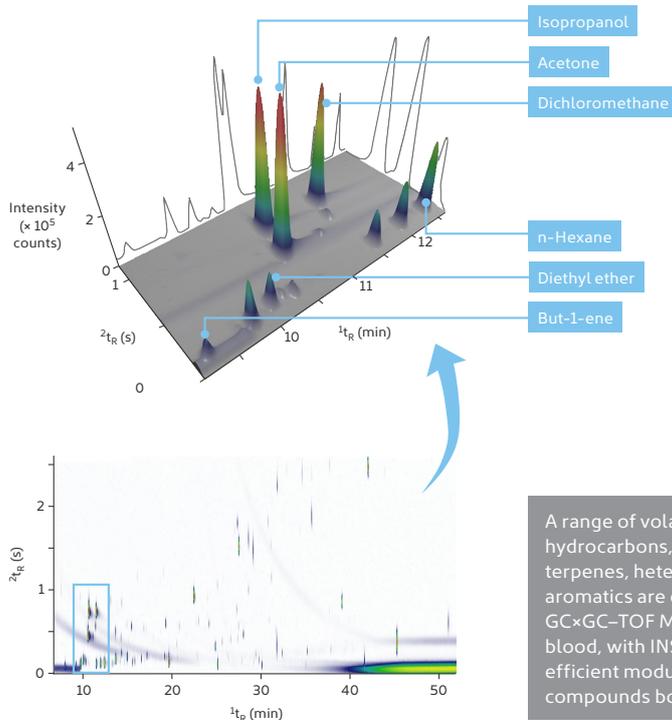
Using scents to discriminate between fresh and aged blood

VOCs in blood have been used to monitor occupational exposure to hazardous chemicals, to identify biomarkers of disease and to even improve the training of search-and-rescue dogs during disasters.

For the latter, analysts need to be able to reliably profile the diversity of compounds present in blood, and also monitor how this profile changes over time. This challenge is easily addressed by GC×GC-TOF MS, with the INSIGHT® flow modulator enabling efficient modulation of VOCs in this complex matrix – a task that is impossible using thermal modulators.

Analytical details

► **Sampling:** Headspace of fresh blood injected onto a Tenax® GR-Carbograph™ B sorbent tube. ► **TD:** TD100-xr™. ► **GC×GC:** INSIGHT® flow modulator; P_M 2.5 s. ► **TOF MS:** BenchTOF™; m/z 35–400. Tandem Ionisation®: 70/14 eV. ► **Software:** ChromSpace®.



A range of volatiles including hydrocarbons, ketones, terpenes, heterocyclics and aromatics are detected in this GC×GC-TOF MS analysis of blood, with INSIGHT providing efficient modulation of compounds boiling below C_5 .



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Ref.: L.M. Dubois et al., *Journal of Chromatography A*, 2017, 1501: 117–127.

Skin volatiles

Understanding the volatile organic compounds (VOCs) emitted from human skin is important for a range of applications, including for training search-and-rescue dogs, assessing their effect on fragrances, and for unlocking their potential for disease diagnosis.

In this section, we highlight two innovative approaches for enhancing the discovery of skin volatiles.



Skin volatiles

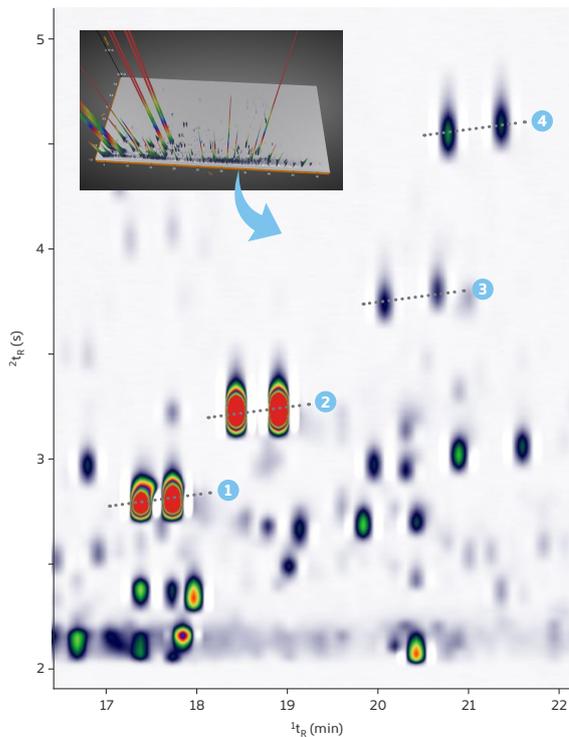
Robust sampling with thin-film SPME

The sampling of skin volatiles can be challenging because of difficulties in reproducibly transferring chemicals to the analytical instrument. One approach that avoids the need for transferring media (such as gauze swabs) is SPME, but the fibers are fragile, and have limited sensitivity because of the small amount of sorptive phase (~0.5 μL).

Here, we show the use of an improved approach – a thin film of SPME sorptive phase, supported on a robust carbon mesh. The result is improved sensitivity and detection of skin volatiles, when used in combination with TD-GC \times GC-TOF MS.

Analytical details

► **Sampling:** TF-SPME membrane (DVB-PDMS phase) held in contact with the skin for 1 h using a gauze, followed by insertion into an empty TD tube. ► **TD:** Centri[®]; tube desorb 270°C (10 min). ► **GC \times GC:** INSIGHT[®] flow modulator; P_M : 7 s. ► **TOF MS:** BenchTOF[™]; m/z 45–450. ► **Software:** ChromSpace[®].



1 Ethyl styrenes



2 Vinyl styrenes



3 Ethyl benzaldehydes



4 Cinnamaldehydes



Over 500 volatiles were detected in this sample of skin, using in-situ sampling with TF-SPME, followed by preconcentration, separation and detection with TD-GC \times GC-TOF MS.

Mosquito-attracting chemicals



Development of new malaria prevention strategies

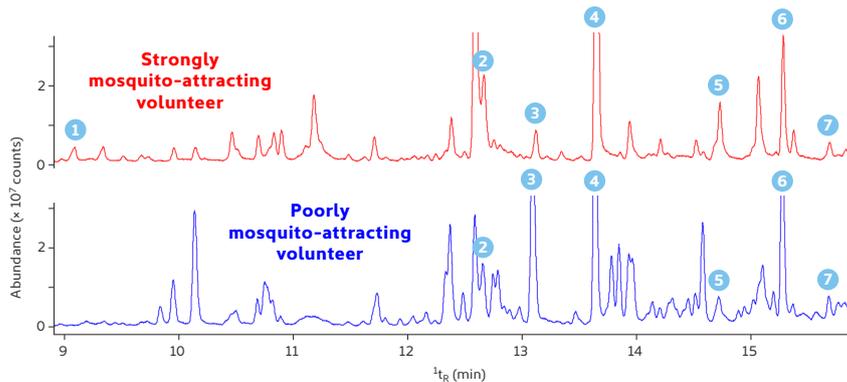
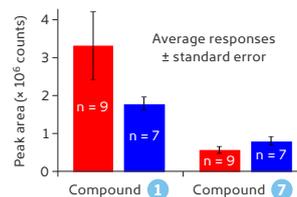
Skin volatiles are a major factor determining the attractiveness of humans to mosquitoes, and hence to the risk of exposure to diseases such as yellow fever and malaria.

Researchers at Wageningen University collected skin emanations using glass beads, analysis of which eliminates the effect of confounding factors like skin temperature or humidity. Subsequent direct desorption of the beads in a TD instrument allows a pre-desorption dry-purge to remove residual water and oxygen, which would otherwise interfere with the GC analysis. The resulting confident identification of mosquito-repelling chemicals holds promise for assisting the development of new malaria prevention strategies.

Analytical details

► **Sampling:** Volunteers were asked to rub the sole of their foot over 100 glass beads (4 mm diameter) on a PTFE tray for 10 min. Twenty beads were then placed in an empty TD tube. ► **TD:** UNITY-xr or TD100-xr; tube desorb 150°C (10 min); trap low -10°C; trap high 250°C (3 min); outlet split 6:1. ► **GC-MS** analysis.

- 1 3-Methylbutanoic acid
- 2 6-Methylhept-5-en-2-one
- 3 Octanal
- 4 2-Ethylhexan-1-ol
- 5 Acetophenone
- 6 Nonanal
- 7 2-Phenylethanol



Desorbing skin volatiles from glass beads, with TD-GC-MS analysis, proved to be a straightforward approach to identifying compounds that attracted (#1) and repelled (#7) mosquitoes (*Anopheles gambiae*) across a group of volunteers. Data courtesy of Niels Verhulst, Wageningen University and Research Centre, The Netherlands.



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Ref.: N.O. Verhulst et al.,
*Infection, Genetics &
Evolution*, 2013, 18: 87–93.

Relevant analytical products

The following pages describe the key products used in the applications shown in this guide. At SepSolve, we can combine these products into single packages that precisely fit your analytical needs.



BioVOC-2

Breath sampling for clinical research

BioVOC-2™ from Markes International is a simple breath sampler, comprising a cylindrical polymer body fitted with an inlet cap and a plunger for expelling the breath sample on to a sorbent-packed TD tube.



The participant simply blows into the mouthpiece, and BioVOC-2 captures the last 129 mL of breath. This is the portion that has been in close contact with the blood vessels in the lungs, and therefore provides the most representative picture of VOCs in the body, without risking contamination with mouth air.

Exhaling into BioVOC-2



Transferring a sample from BioVOC-2 into a sorbent tube



Key features of BioVOC-2

- ▶ Low cost and easy-to-use – no need for medically-qualified staff.
- ▶ Restricted opening prevents sample contamination during insertion of plunger and transfer of sample to sorbent tube
- ▶ Low-emission body for minimal risk of analytical artefacts.
- ▶ Compatible with TD sampling tubes for 'gold standard' breath analysis.
- ▶ Sample collection can be repeated multiple times using the same BioVOC-2 and sorbent tube, for a larger total sample volume and enhanced sensitivity for improved detection of trace-level VOCs.

BioVOC-2 provides a simple workflow for breath sampling.

TD100-xr

World-leading thermal desorption

SepSolve offers the entire range of thermal desorbers from Markes International for analysis of samples collected onto sorbent-packed TD tubes.

Markes' TD systems use advanced two-stage desorption technology (below) to deliver maximum sensitivity for the widest range of breath constituents – including volatiles, semi-volatiles, polar compounds and reactive species.



Key features of TD100-xr

- ▶ Cryogen-free trapping reduces running costs.
- ▶ Re-collection of split flows onto clean sorbent tubes overcomes the 'one-shot' limitation of other TD systems, and allows samples to be re-analysed for simple method/data validation.
- ▶ Automated options allow selective elimination of water and air to minimise GC-MS interference.
- ▶ Integrates with all major makes of GC and GC-MS.
- ▶ DiffLok™ caps remain in place on tubes through the entire automated TD sequence on the TD100-xr, preserving sample integrity while overcoming the need to uncap and recap tubes.
- ▶ Leak-testing, water management and internal standard addition ensure highest data quality.
- ▶ Compatible with the TubeTAG™ informatics system, which ensures chain of custody through electronic sample tracking, and recording tube history.



Step 1

Samples are desorbed onto the cryogen-free focusing trap.

Step 2

The focusing trap is rapidly heated in a reverse flow of carrier gas, to transfer analytes to the GC.

Efficient trap operation (shown here) is central to the performance of Markes' thermal desorbers, while also enabling samples to be split and re-collected for repeat analysis.

Centri

Automated sample extraction and enrichment

Common sample introduction techniques such as headspace and SPME are popular due to their simplicity and ease of operation. However, they generally lack sensitivity, and are 'one-shot' techniques requiring duplicate sampling for replicate analysis.

The Centri® platform from Markes International is the first system to offer fully automated, unattended sample extraction and enrichment of VOCs and SVOCs in liquid, solid and semi-solid samples, as well as tube-based analysis of gaseous samples. Using Markes' advanced cryogen-free trapping technology, Centri enhances the sensitivity and extends the performance of several popular sample introduction techniques, including headspace and SPME.



Key features of Centri

- ▶ A single platform for analysis of VOCs and SVOCs from breath and biological fluids (e.g. urine and saliva).
- ▶ Analyte trapping provides high sensitivity by delivering a sharp, concentrated band of vapour to the GC.
- ▶ Enrichment by 'sample stacking' improves sensitivity for trace analyses.
- ▶ Selective elimination of water and other interferences improves data confidence for analysis of biological fluids.
- ▶ Increased productivity with prep-ahead mode and automatic tool-change.
- ▶ Sample splitting and re-collection allows repeat analysis or storage of VOCs/SVOCs released by perishable liquids.
- ▶ Extended rail provides liquid injection on the same system, e.g. to extend analysis for liquid extracts and/or derivatised samples.



HiSorb
high-capacity
sorptive extraction



Headspace and
headspace-trap



Thermal
desorption



SPME and
SPME-trap

All sampling modes on Centri can benefit from analyte refocusing using Markes' trapping technology.

HiSorb

High-capacity sorptive extraction

HiSorb™ probes use high-capacity sorbent polymer to extract and concentrate a wide range of organic compounds from liquid and solid samples.

As well as being compatible with Centri® automation, probes can be used to sample manually prior to automated TD–GC–MS analysis, using systems like the TD100-xr™.



Key features of HiSorb

- ▶ In combination with Centri, HiSorb offers fully automated sorptive extraction for the first time.
- ▶ Robust, easy-to-use probes allow unattended sample preparation and maximum productivity.
- ▶ Detection limits are lower than for SPME because of the larger capacity of the sorbent.
- ▶ Probes are compatible with both headspace and immersive sampling.
- ▶ Re-usable probes and tubes minimise the cost per sample.
- ▶ The cost of solvent consumption and disposal is eliminated.
- ▶ Multiple sorptive extraction phases for compatibility with a wide analyte range.

HiSorb probes have a metal core that supports a section of PDMS for sample extraction. They work on the same principles as SPME, but on a much larger scale, giving greatly improved detection limits.

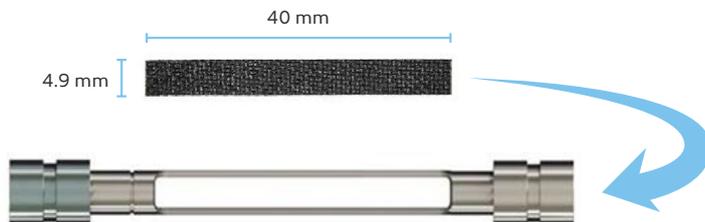
Thin-film SPME

Improved sensitivity and robustness

Thin-film solid-phase microextraction (TF-SPME) is a sampling technique that uses carbon mesh sheets impregnated with sorbent phase(s).

It works on the same principles as conventional fiber-based SPME, but the planar geometry and greater sorbent thickness (>40 μm) result in a significantly greater volume of extraction phase and ratio of surface area to volume.

Like conventional SPME, TF-SPME can be used to extract volatile and semi-volatile analytes from solids and liquids, using immersive or headspace techniques, often with higher sensitivity – making it an ideal sampling tool for ultra-trace level analysis. In biomarker discovery, this enhanced sensitivity coupled with its planar geometry makes TF-SPME well-suited for sampling of skin volatiles.



Key features of TF-SPME

- ▶ An extension of SPME, but with improved capacity:
 - In pre-equilibrium: 26× greater surface area
 - At equilibrium: 98× greater sorbent volume.
- ▶ Improved sensitivity for challenging applications.
- ▶ Robust design overcomes the fragility of conventional SPME fibers.
- ▶ Various sorbent phases available, including PDMS, DVB/PDMS, HLB/PDMS and CAR/PDMS.
- ▶ Solvent-free approach for 'greener' sample preparation.
- ▶ Reusable >100 times.
- ▶ Desorption using Markes' TD allows users to benefit from world-leading trapping technology, including optional re-collection.

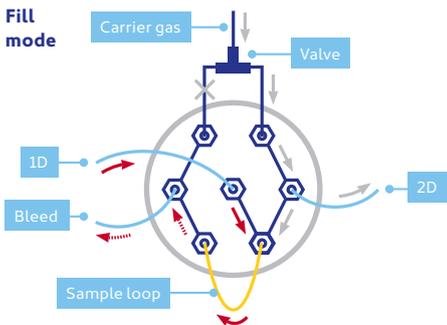
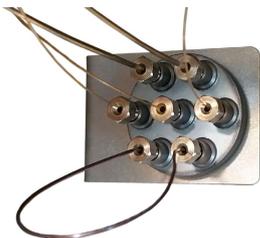
TF-SPME membranes are placed inside empty thermal desorption tubes for analysis, where they benefit from trap-based focusing and optional re-collection using Markes' TD systems.

INSIGHT

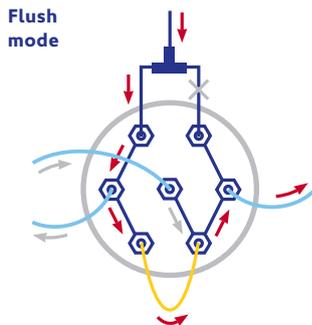
Flow-modulated GC×GC

The modulator is the most critical part of a GC×GC system – ineffective modulation can result in broad, tailing peaks in the second dimension, which limits peak capacity.

SepSolve's INSIGHT® flow modulator overcomes the practical and performance difficulties experienced with other modulator designs – for example, the volatility restrictions associated with thermal modulators.



The primary column eluate enters the sample loop (with any overflow directed to a bleed line), while the modulation valve directs auxiliary carrier gas to the secondary column.



The valve switches, and the contents of the sample loop are flushed rapidly onto the secondary column as a narrow band.

Key features of INSIGHT

- ▶ Flow-modulation technology allows efficient modulation of volatiles – unlike thermal modulators, which struggle with analytes boiling below C₅ because of their reliance on a cold jet.
- ▶ Cryogen-free operation lowers running costs for routine GC×GC.
- ▶ Excellent repeatability for routine applications – peak area RSDs are typically <5%.
- ▶ Flexible configuration options:
 - GC×GC
 - Heart-cut GC–GC
 - Backflushing
 - Parallel detection, e.g. TOF MS and FID.
- ▶ Retrofittable to all popular GCs.
- ▶ A compact design allows two INSIGHT devices to be configured in a single GC oven for enhanced productivity through dual-channel acquisition.

Differential flows in the INSIGHT modulator are used to 'fill' and 'flush' a sample loop – meaning low running costs for routine GC×GC and none of the logistical issues associated with liquid cryogen.

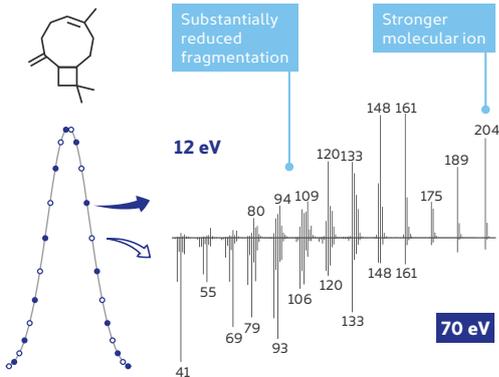
BenchTOF2

High-definition time-of-flight mass spectrometers

BenchTOF2™ mass spectrometers are the next generation of the successful BenchTOF series of time-of-flight (TOF) mass spectrometers, allowing labs to adapt to increased workloads and new challenges, while improving data confidence and reducing costs.

Like all TOF instruments, BenchTOF2 simultaneously analyses all ions, maximising sensitivity and providing full-range spectra for both target compounds and unknowns in a single run.

Unlike other mass spectrometers, BenchTOF2 can further enhance confidence by simultaneously acquiring hard and soft EI spectra using patented Tandem Ionisation® technology, and so confidently identify challenging compounds (e.g. isomers) in streamlined workflows.



Key features of BenchTOF2

- ▶ Identify analytes confidently through an unbeatable combination of SIM-level sensitivity, high-quality spectra, excellent selectivity and smart software tools.
- ▶ Improve detection limits with full spectral information, to detect a wide range of trace-level species in a single analysis, with the option for retrospective searching.
- ▶ Extend dynamic range across five orders of magnitude to accurately quantify high-concentration compounds while maintaining low detection limits.
- ▶ Compatible with fast GC and GC×GC thanks to BenchTOF2's fast acquisition speeds.
- ▶ Increase productivity through use of H₂ carrier gas, for lower cost of ownership, fast return on investment, and faster chromatographic separations.
- ▶ Tandem Ionisation provides enhanced confidence in identification, especially for isomers.

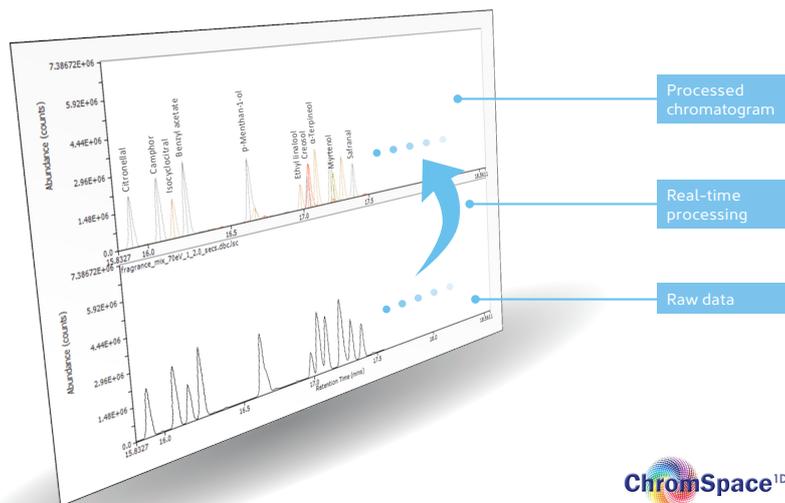
By enabling two sets of complementary spectra to be generated from a single run (as shown here for caryophyllene), Tandem Ionisation allows complex samples containing structurally similar compounds to be comprehensively characterised.

ChromSpace 1D

Intuitive data-mining for GC and GC-MS

ChromSpace® 1D provides streamlined qualitative and quantitative analysis of GC and GC-MS data across a range of file types, with an icon-driven interface making it quick and easy to find the tools you need.

Additionally, real-time capability (below) for BenchTOF2 mass spectrometers means that automated processing can take place while the sample is still running – speeding up method development and providing rapid turnaround on urgent samples.



Key features of ChromSpace 1D

- ▶ Intuitive processing of GC(-MS) data for a range of file types (including .lsc, .d, .raw, .rsd and .cdf).
- ▶ Easy-to-learn workflows with fast method creation, batch processing, quantitation, and reporting/exporting.
- ▶ Automated background subtraction of interferences, and uncovering of 'hidden' compounds with easy-to-use deconvolution.
- ▶ Time-saving features such as one-click library hits and saved EIC sets.
- ▶ Fast screening for compounds (or classes) of interest using the Compound Explorer toolkit.
- ▶ Integrated control of BenchTOF2, as well as a range of GCs and sample introduction platforms, for real-time data-processing capability.
- ▶ Automatic alignment of chromatograms and discovery of significant differences between samples with the addition of the ChromCompare+ chemometrics module.

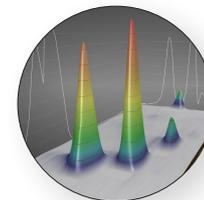
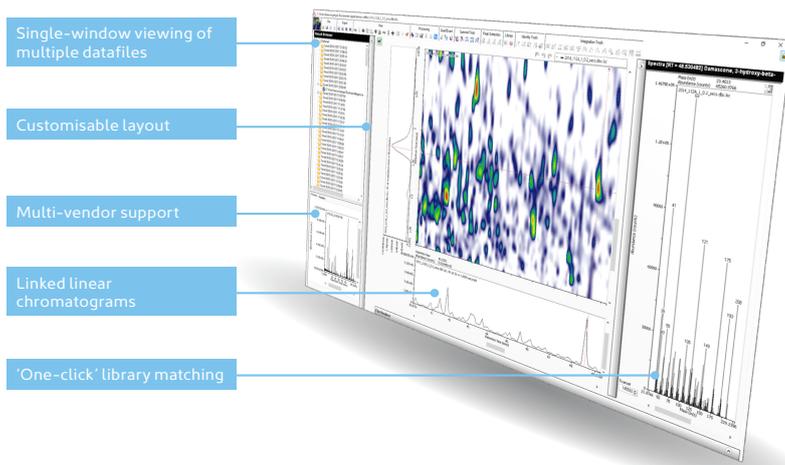
The real-time processing capability of ChromSpace 1D allows data to be analysed as it is acquired, with no user intervention.

ChromSpace

Powerful software for GC×GC and GC×GC–MS

Specialist software is needed to visualise GC×GC data sets in a way that makes them easy to interpret, but many packages are difficult to use, and tend to neglect the 'true' linear chromatography, which presents barriers to peak-shape review and integration.

Designed using feedback from experts in industry and academia, ChromSpace® software solves all these problems by providing an easy-to-learn interface with powerful graphical functionality that maintains a link to the underlying linear data sets.



Key features of ChromSpace

- ▶ All of the powerful tools of ChromSpace 1D but with extended compatibility for GC×GC(-MS) data.
- ▶ Designed for ease of use:
 - Multiple GC×GC chromatograms can be viewed in a single window
 - Flexible options for peak merging, with simple review of peak integration
 - Interactive 3D surface charts can be overlaid, stacked and inverted.
- ▶ Fast group-type analysis with easy-to-use stencils and reporting.
- ▶ Network licensing to easily unify lab processing.
- ▶ Integrated control of BenchTOF2, as well as a range of GCs, INSIGHT® modulators and sample introduction platforms.
- ▶ Real-time display of 1D and 2D chromatograms means data can be explored while the sample is still running.
- ▶ Compatible with a range of file formats.

Uniquely among GC×GC software platforms, ChromSpace incorporates powerful, versatile processing within an easy-to-learn interface.

ChromCompare+

Easy-to-use chemometrics platform for GC, GC-MS and GCxGC

ChromCompare+™ is a powerful, easy-to-use chemometrics platform for comparing multiple chromatograms and extracting useful insights into the constituents present.

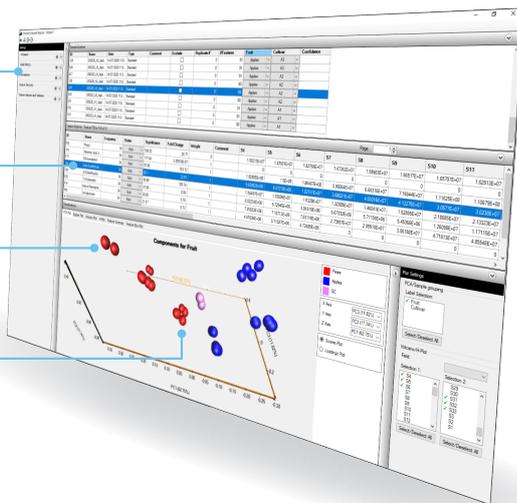
ChromCompare+ offers the flexibility to process a range of file formats using various data-mining strategies. The innovative untargeted approach used by ChromCompare+ allows maximum analytical information to be extracted from the raw data when you don't know what you are looking for.

Flexibility to import peak lists or raw data in a range of file formats

Automatic discovery of the most significant differences

Easy visualisation of patterns and trends

Automatic classification of samples using prediction models



Key features of ChromCompare+

- ▶ An easy-to-use chemometrics platform to transform complex chromatograms into meaningful results.
- ▶ Account for retention time drift using automated alignment of chromatograms.
- ▶ Uncover significant differences between sample classes
- ▶ Accelerate data analysis using automated untargeted workflows.
- ▶ Process a range of GC and GC-MS file formats for both 1D and 2D data.
- ▶ Quickly classify unknown samples using prediction models.
- ▶ Benefit from a host of advanced visualisation tools to display the key trends and relationships between your samples.

With ChromCompare+ you don't have to be a statistician to gain greater insight into your complex datasets, and you don't have to know what compounds are important.

