SRA INSTRUMENTS
ANALYTICAL SOLUTIONS

Research of optimized conditions of Thermal Desorption - GC/MS to diagnose diseases identifying and quantifying VOCs in breath

The study of volatile chemicals from humans has recently attracted increasing attention, particularly regarding the potential of breath volatiles to rapidly and non-invasively diagnose diseases.

Biological monitoring is well-established for assessing personal exposure to chemicals, but recent research is now showing its potential to assist understanding of medical conditions more generally.

However, traditional blood and urine monitoring is invasive, and often requires complex sample preparation prior to GC analysis. Using breath monitoring instead solves these problems, and has the potential to allow rapid point-of-care highlighting of a range of medical conditions.

The literature around breath-based disease diagnosis is extensive, with recent papers on various cancers, asthma, pulmonary disease, respiratory conditions, liver disease and kidney disease, to name but a few.

In addition, an important aspect of research in this area involves investigating natural variability in breath profiles, and the effect of external factors such as diet and environment.

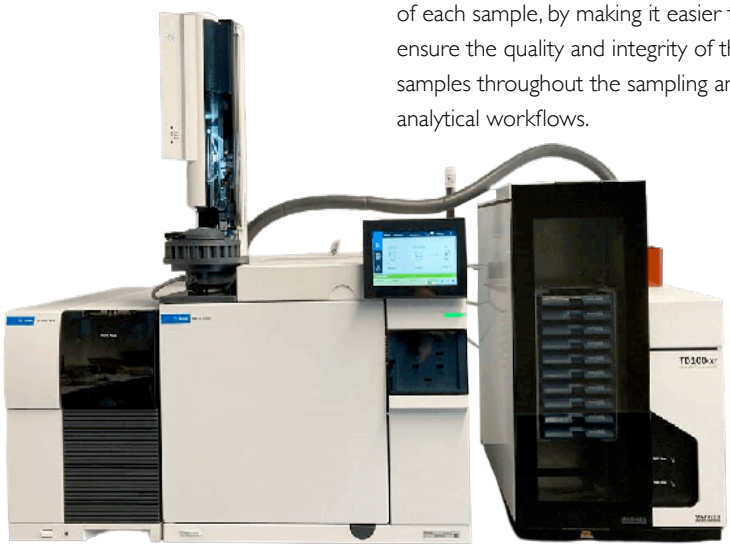
Overview of thermal desorption

Thermal desorption (TD) arose out of the need to improve upon conventional sample preparation techniques such as solvent extraction, solid-phase microextraction, purge-and-trap and static headspace.
It gives greater sensitivity than these techniques and can be used for a wider range of compound classes (from C3–C44). TD is applicable to a wide range of sample types – solids (using dynamic headspace, headspace sorptive extraction or direct desorption), liquids (using immersive sorptive extraction) and gases (using pumped sampling, passive sampling, on-line sampling or canisters).
It is safer and more environmentally friendly than solvent extraction, is easily automated, easy to validate and complies with key standard methods.

The benefits of thermal desorption for breath monitoring

Capturing breath volatiles directly onto sorbent tubes, with analysis by thermal desorption, completely circumvents laborious sample preparation (and consequent potential bias) common with techniques such as solvent extraction of aqueous samples.
In addition, modern thermal desorbers allow multi-bed tubes and traps to be used, widening the range of compounds that can be reliably monitored from a single sample.

Many breath analysis studies target the broadest possible range of vapour-phase compounds, particularly during biomarker discovery phases, and rely on the use of thermal desorption tubes coupled with gas chromatography–mass spectrometry (GC–MS), widely accepted as the 'gold standard' analytical tool. This approach can help to tackle challenges such as high water content and a wide analyte concentration range.
The use of TD-based techniques can also address issues around the irreplaceability of each sample, by making it easier to ensure the quality and integrity of the samples throughout the sampling and analytical workflows.



TD GC/MS SRA Breath profiling solution

Breath analysis workflow

Each sorbent tube deployed in a breath analysis campaign will pass through multiple workflow stages for every sample. Careful control of each stage is critical for generating high-quality data and protecting sample integrity.

Preparation

Before sending sorbent tubes out for sample collection, it is important to ensure that they are free from contamination.

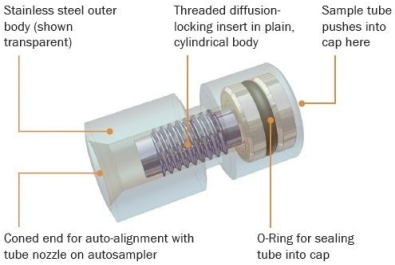
- Conditioning every tube using heat and a flow of inert gas is the most efficient method. Off-line conditioners such as Markes' 20-tube conditioner, **TC-20™**, greatly improve conditioning efficiency.
- Running a blank analysis on a representative number from a batch of conditioned tubes ensures tubes are free from contamination when they leave the laboratory.
- Sealing tubes using robust screw-cap seals with PTFE ferrules during transportation and storage prevents ingress of ambient air and preserves the integrity of the blank tubes.
- Surrogate addition: Automated thermal desorbers can add a precise aliquot of a gas-phase standard to every tube before shipping. This surrogate remains on the tube throughout the remainder of the workflow, and provides validation of the sample tube integrity from the moment the tube leaves the laboratory through to analysis.

Sampling

Many methods have been employed for collecting and preconcentrating low-level VOCs in breath samples.

Storage

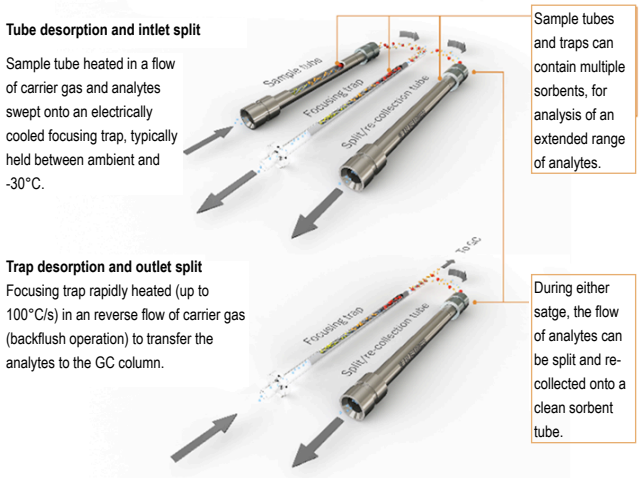
For enhanced sample security, diffusion- locking technology can be incorporated into sorbent tubes, protecting samples from analyte loss or contamination ingress in the event of failure of caps during storage or transportation. The same technology is used to seal sample tubes in the TD autosampler before, during and after analysis.



Thermal desorption analysis

Automated two-stage TD provides a second stage of preconcentration for enhanced sensitivity. In addition, once tubes are loaded into the thermal desorber, they are subject to several automated checks and procedures before desorption to eliminate interferences and test the integrity of the gas flow path. Key steps include:

- Pressurising and leak-testing tubes to ensure the integrity of tube seals
- Dry-purging tubes in the sampling direction to selectively eliminate water
- Automated addition of internal standard prior to desorption, which provides a quality control measure for the full analytical system.



Quality control checks

Breath samples are often taken as part of lengthy clinical trials. The sample tubes are frequently stored prior to analysis. In addition, there is often transportation of the samples between the lab and clinic. To ensure the integrity of the sample, field blanks are used to simulate the whole process and to ensure no contamination has occurred.

Internal standard can be added to field blank and sample tubes prior to sampling (i.e. surrogates) and/or to the tube or focusing trap pre-desorption, providing validation of the sample tube integrity from the moment the tube leaves the laboratory through to analysis.

Archiving valuable samples

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 vital to be able to archive such samples, and quantitative re-collection of split flows in TD analysis offers the ideal solution. Using this method, critical samples can be archived for later re-analysis under the same (or different) analytical conditions.

Studying potential disease biomarkers in breath

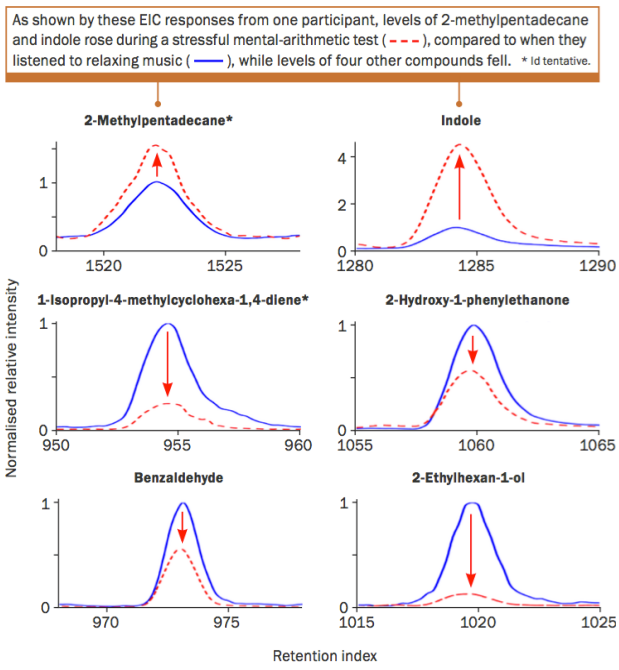
Breath sampling of VOCs has gained prominence as a potentially useful approach to the early diagnosis of many diseases. As well as studies of VOCs with direct links to disease, an important aspect of research in this area involves investigating how other factors such as diet and environment may affect breath volatiles.

As part of this effort, UK researchers have been using TD–GC–MS to study the effect of stress on breath VOCs.

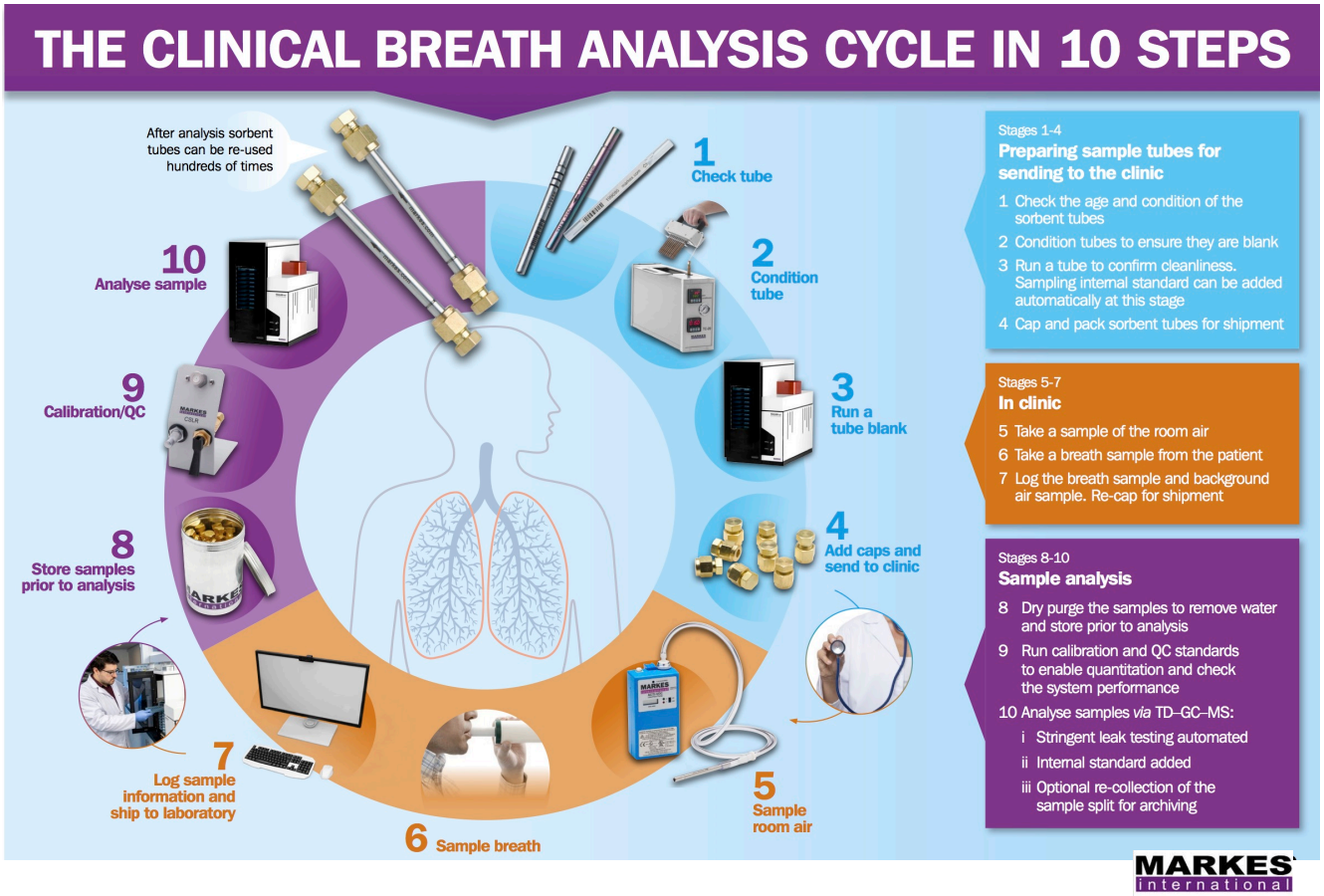
The backflush capability of Markes' TD instruments allows multi-bed tubes and traps to be used, widening the range of compounds that can be reliably monitored from a single sample. An added advantage of sorbent tubes for this study was that samples could be stored until there were sufficient to analyse a large batch in one run, making it easier to schedule GC–MS instrument time.

Typical analytical conditions:

- Sample: 4 L end-exhaled breath, collected onto a sorbent tube using a sampling mask.
- TD (UNITY or TD100)
- Tube (Hydrophobic) - Desorbed at 300°C (5 min)
- Trap (General-purpose hydrophobic)
- Analytes trapped at 10°C, desorbed at 300°C (5 min)
- Split ratio: Splitless.
- Analysis: GC–MS.



Guaranteeing the integrity of clinical breath samples throughout every stage of an extended and multi-disciplinary workflow is crucial to advancing breath analysis towards routine clinical screening. Thermal desorption provides the tools to safeguard samples and ensure analytical quality in a robust, routine workflow that meets the needs of every stage from research through to scaling up to widespread routine screening.



Markes TD100-xr automated thermal desorber for GC and GC–MS – a high-performance, high-throughput platform for the analysis of sub-ppt to percent levels of volatile and semi-volatile organic compounds (VOCs and SVOCs)

Unique combination of advantages:

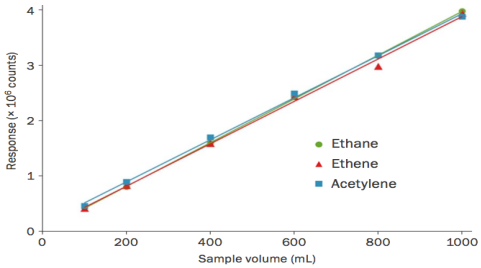
- ✦Productivity and reliability: Mechanically simple automation, 100-tube capacity, sample overlap and cryogen-free focusing deliver robust operation and optimum sample throughput.
- ✦Future-proof: Multi-Gas enabled TD100-xr systems are independently certified for use with helium, nitrogen and hydrogen carrier gases, offering enhanced throughput.
- ✦Versatility: TD100-xr allows simultaneous analysis of VOCs and SVOCs, and accommodates every tube-based TD application on a single platform – from C3 to n-C44 plus reactive compounds. TD100-xr can also be installed onto any make of GC or GC–MS running with hydrogen, helium or nitrogen carrier gas.

MULTI-GAS ENABLED

- ✦Peace of mind:
  - •Automated and quantitative sample re-collection for repeat analysis overcomes the 'one-shot' limitation and simplifies TD method validation.
  - •Enhanced tube traceability with RFID tag and barcode options for tubes.
- ✦Superior data quality:
  - •Quantitation of the widest possible concentration range, through flexible splitting options.
- Full compliance with standard methods, with features such as leak testing, tube sealing, re-collection and internal standard addition.
- Simple method and data validation through use of automated repeat analysis.
- Electrical trap cooling means that the cost and inconvenience of cryogen – and the associated risk of ice blockages – are completely avoided, while fast trap cool-down means short cycle times and optimum productivity

Exceptional trapping functionality

- The focusing trap of TD100-xr allows simultaneous analysis of VOCs and SVOCs, and typically provides over 12 months of continuous use. It's also simple to change (right), and does not require any tools or special training.
- The short flow path, valve and capillary column interface are all ultra-inert and uniformly heated, meaning that the most challenging trace organic chemicals pass through the system without degradation or deposition.

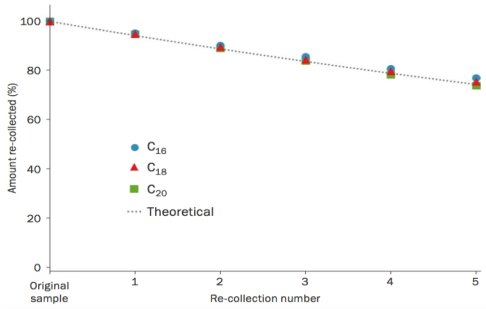


The excellent performance of the focusing trap at the heart of all Markes' TD is demonstrated by the linearity obtained for high volumes of ultra-volatile C2 hydrocarbons. (Such compounds should only be sampled on-line or using canisters, but we show this data here to demonstrate the power of the trap

Quantitative sample re-collection for automated repeat analysis
Simple method validation and extended dynamic range

- Automated re-collection* of split flow during trap desorption/GC injection (onto the same or a fresh sorbent tube) allows samples to be archived or re-analysed in a completely unattended fashion. For example, 'High/Low' analysis allows quantitative measurement of trace and major sample components in the same sample.
- Manual re-collection of both inlet and outlet split flows allows validation of double-split TD methods.

** Patent number GB 2395785. Automated re-collection is only available with TD100-xr Advanced models.*



Complete, reproducible transfer of compounds through TD100-xr is validated by the close agreement between theoretical and actual results for a series of re-collections – a level of performance that underpins the value of re-collection for easy method validation.

SRA INSTRUMENTS ANALYTICAL SOLUTIONS

SRA Instruments S.p.A
20063 Cernusco S/N (MI)
Tel +39 02 9214 3258
www.srainstruments.com
info@srainstruments.com

SRA Instruments SAS
69280 Marcy l'Etoile Lyon
Tel +33 04 7844 2947
www.srainstruments.com
info@sra-instruments.com