Thermal desorption: Ensuring data quality in breath analysis

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Introduction

The analysis of VOCs in exhaled breath offers an exciting potential opportunity to diagnose life-threatening diseases in a non-invasive and inexpensive fashion. If accepted into clinical practice, this approach would allow cost-effective screening of large populations, facilitating early diagnosis, improving patient outcomes and reducing health care costs.[1]

Figure 1: Sorbent tubes with long-term storage caps.

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 (TD) tubes (Figure 1) 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. In this poster, we present recent advances in TD technology for optimising data quality in breath analysis.

Breath analysis workflow

Each sorbent tube deployed in a breath analysis campaign will pass through multiple workflow stages for every sample (Figure 2). Careful control of each stage is critical for generating high-quality data and protecting sample integrity.

Figure 2: Typical breath analysis workflow with TD–GC–MS.

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™ (Figure 3), 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.

Figure 3: TC-20 tube conditioner.

- **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 and storage

Many methods have been employed for collecting and preconcentrating low-level VOCs in breath samples. Devices such as the Bio-VOC™ (Figure 4) and the ReCIVA® collect breath samples directly onto sorbent tubes, minimising the risk of contamination from room air and providing a straightforward and quick sampling procedure for clinical staff to administer.

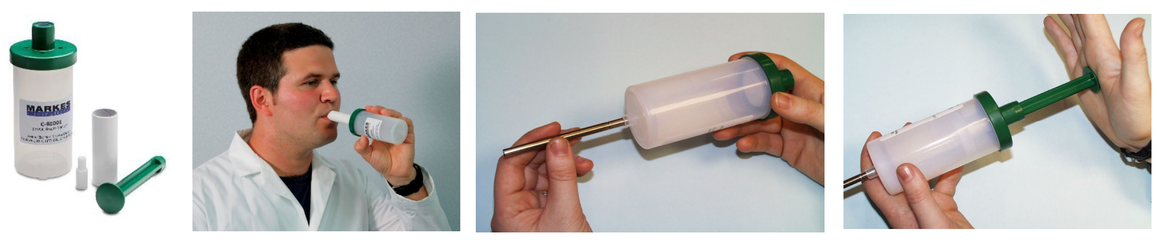

Figure 4: Collecting a breath sample with the Bio-VOC.

As well as being mechanically robust, well-characterised and easy to transport, sorbent tubes also extend the storage stability of breath samples. Many VOCs are stable for several weeks at room temperature or when refrigerated, and extended storage conditions of six weeks at –80°C have been reported in the literature for breath samples using Markes International's TD tubes.[2] This is a major improvement upon breath bags, which can only be stored for a few hours.

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 (Figure 5). The same technology is used to seal sample tubes in the TD autosampler before, during and after analysis.

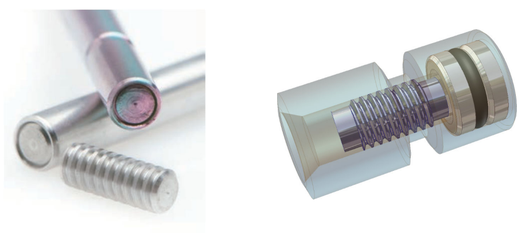

Figure 5: The diffusion-limiting helical flow path incorporated into SafeLok™ sample tubes (left) and DiffLok™ autosampler caps (right).

Thermal desorption analysis

Automated two-stage TD provides a second stage of preconcentration for enhanced sensitivity (Figure 6). 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.

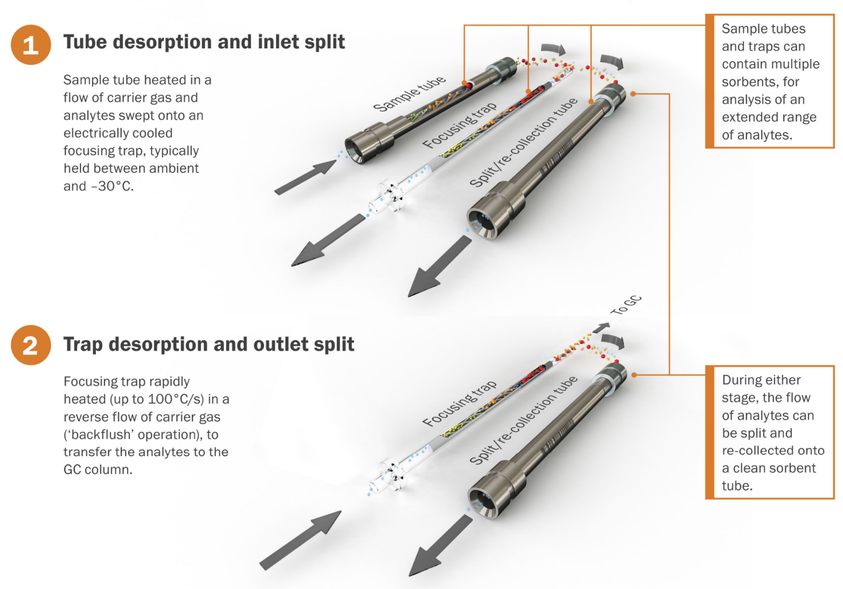

Figure 6: Overview of two-stage thermal desorption (TD).

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 (Figure 7).

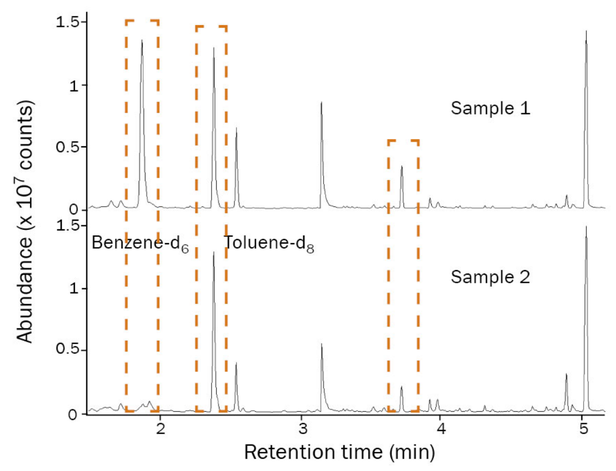

Figure 7: Sorbent tubes were pre-loaded with benzene-d_6 before sampling. Toluene-d_8 was added as a system internal standard automatically during the analysis. Toluene-d_8 is present in both results at a consistent response, proving the analytical system is working correctly. Benzene-d_6 is missing from Sample 2, indicating a problem with the sampling or transportation process.

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 (Figure 6) offers the ideal solution. Using this method, critical samples can be archived for later re-analysis under the same (or different) analytical conditions (Figure 8).

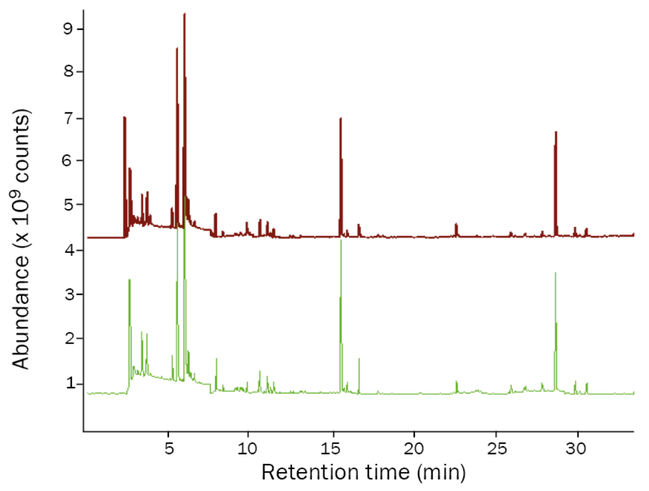

Figure 8: 1 L breath sample (top) and the re-collected portion (bottom) showing excellent agreement in relative responses across the volatility range.

Conclusions

Recent technical developments related to sorbent tube sampling and TD–GC–MS analysis of VOCs in breath samples ensures improved data quality and enhanced sensitivity. Innovations such as dry-purging, diffusion-locking, surrogate and internal standard addition, and sample re-collection ensure sample security and robust analysis.

References

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2. S. Kang and C.L.P. Thomas, How long may a breath sample be stored for at −80 °C? A study of the stability of volatile organic compounds trapped onto a mixed Tenax:Carbograph trap adsorbent bed from exhaled breath, *Journal of Breath Research*, 2016, 10: 026011, https://doi.org/10.1088/1752-7155/10/2/026011.

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