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Application Note 147

Breath sampling for clinical research and occupational health monitoring

Summary

In this Application Note, we describe the design and operation of the new BioVOC-2™ breath sampler, and summarise the applications of this approach, which include clinical diagnostics, occupational health monitoring, and assessing exposure of the public to VOCs.

Introduction

Breath provides a window into the health of the human body, and analysing the volatile organic compounds (VOCs) it contains can provide information on topics as diverse as the diagnosis of disease, the monitoring of metabolic stress, and exposure to toxic chemicals.

Various techniques are available to study these VOCs, but one of the simplest is direct capture of exhaled VOCs onto a sorbent-packed tube, with subsequent analysis by thermal desorption–gas chromatography–mass spectrometry (TD–GC–MS). In this application note, we describe the BioVOC-2™ device from Markes International, which is a low-cost, easy-to-use sampler that uses this approach to capture VOCs in breath.

This application note is split into three parts. We first describe the construction and operation of BioVOC-2, and then compare its performance against the original Bio-VOC™ model. Finally, we summarise the variety of applications to which these sampling devices are being applied.

1. Overview of BioVOC-2

Design

The BioVOC-2 breath sampler from Markes International (Figure 1) comprises a cylindrical polymer body fitted with an inlet cap, a sealing plug for the outlet end, and a plunger for expelling the breath sample.

The original design was developed by the UK Health & Safety Executive, and commercialised by Markes International as Bio-VOC in the late 1990s.[1] BioVOC-2 has the same original design, but uses a different type of non-emitting, non-binding polymer for the body of the sampler. This provides the same high performance as the previous model, while minimising any risk of contaminant interference or adsorption of analytes.

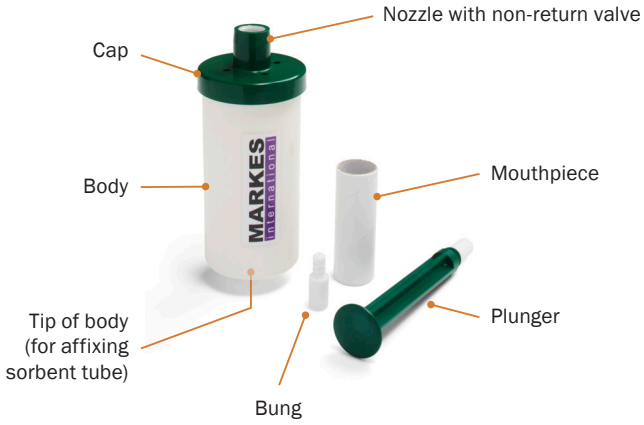

Figure 1: Markes International's BioVOC-2 breath sampler.

Portion of breath sampled

A key challenge for carrying out reproducible studies into breath VOCs is consistently capturing the correct portion of the exhaled breath (the 'tidal volume', which for an adult is typically 400–500 mL[2]). This is because the composition of the exhaled air changes as it moves from the alveoli to the mouth. In particular, mouth air can have quite a different VOC profile depending on food intake, oral hygiene and microbes, and can therefore be a source of contamination.[5]

Consequently, sampling devices that capture all airway fractions (such as Tedlar bags) can suffer from poor reproducibility. In contrast, BioVOC-2 captures just the last 129 mL of each 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.

Sampling procedure

The operation of BioVOC-2 is as follows:

1. The subject breathes into the BioVOC-2 through a disposable cardboard mouthpiece fitted to the nozzle (Figure 2).
2. The mouthpiece is removed from the nozzle and replaced with the plunger. A sorbent tube is attached to the other end of the BioVOC-2.

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Figure 2: Exhaling into the BioVOC-2.

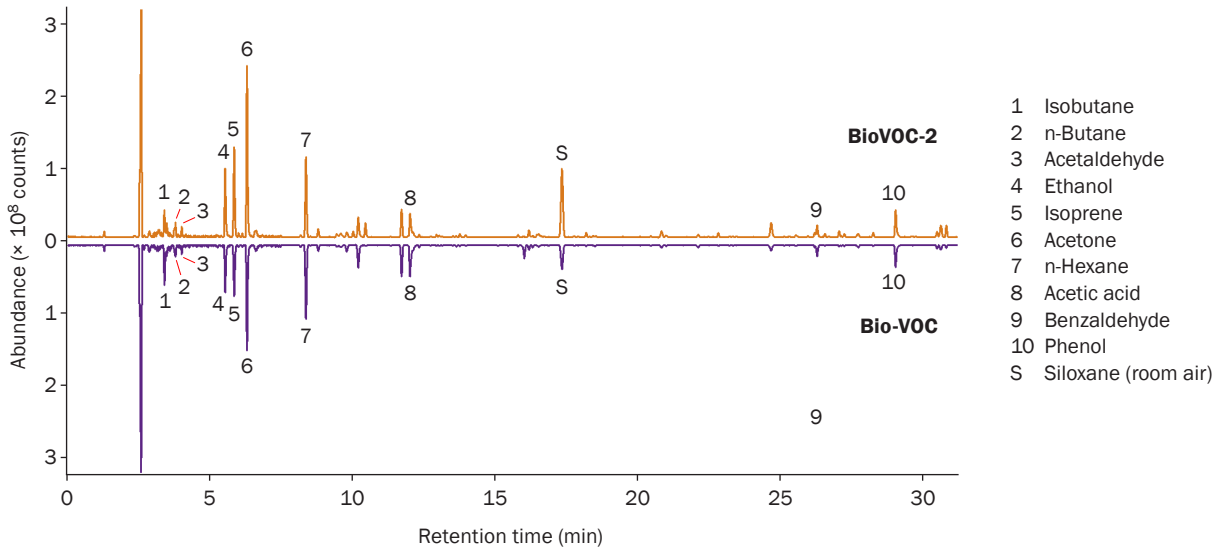
Figure 3: Transferring a sample from BioVOC-2 into a sorbent tube.

3. The plunger is pushed in steadily to transfer the trapped breath sample onto the tube (Figure 3).
4. The tube can be analysed by TD–GC–MS immediately, or sealed with long-term storage caps and tested at a later date (breath samples stored in this way have been shown to be stable for several weeks[4]).

Sample collection (steps 1–3) can be repeated multiple times using the same BioVOC-2 and sorbent tube, resulting in a larger total sample volume and enhanced sensitivity for improved detection of trace-level VOCs.[5]

2. Comparison of BioVOC-2 and Bio-VOC

To demonstrate the results of breath sampling using the new BioVOC-2 sampler, a breath sample from a healthy volunteer was taken, and the results compared with those obtained with the Bio-VOC. The TD–GC–MS chromatograms (Figure 4) show the ability of BioVOC-2 to discriminate between known breath compounds, and that its performance is comparable to the previous Bio-VOC model. Minor differences are ascribable to the inherent variability of breath.



Breath sampling:

Sampler:	Bio-VOC (Markes International) or BioVOC-2 (Markes International part no. C-BIO02-01)

TD:

Instrument:	TD100-xr™ (Markes International)
TD tube:	'Biomonitoring' stainless steel tubes (part no. C2-AAXX-5149)
Tube desorb:	280°C (10 min)
Focusing trap:	'Material emissions' (part no. U-T12ME-2S)
Trap low:	10°C
Trap desorb:	290°C (4 min)

GC:

Column:	ZB-624™ (Phenomenex), 60 m × 0.25 mm × 1.40 µm
Oven:	40°C (4 min), then 5°C/min to 100°C (1 min), then 5°C/min to 110°C (1 min), then 5°C/min to 200°C (1 min), then 10°C/min to 240°C (4 min)
Column flow:	Helium (2 mL/min)

Quadrupole MS:

Scan mode:	m/z 20–350
Source:	240°C
Transfer line:	240°C

Figure 4: TD–GC–MS profiles of breath obtained from a healthy volunteer, sampled using BioVOC-2 (top) and Bio-VOC (bottom) using the experimental conditions shown.

3. Applications of Bio-VOC to breath sampling

The Bio-VOC has been used for a wide variety of applications, and this range is expected to continue to broaden with the release of the new BioVOC-2. Some of these studies are summarised below.

Clinical diagnostics

It has long been known that a fruity smell of acetone on the breath can be an early indication of diabetes, and that the pungent smell of ammonia can be a sign of kidney failure. However, it is now becoming clear that the pattern of chemicals in the breath can provide a reliable diagnosis of a wider range of serious diseases.

Moreover, in contrast to some intrusive and/or expensive medical procedures (e.g. CT scans, tissue biopsies or endoscopies), breath analysis is inexpensive and non-invasive, and several research groups worldwide have used Bio-VOC to pursue this exciting field of study. Some examples are:

- **Lung cancer:** A range of VOCs including fatty acids (propanoic acid and nonanoic acid) and aldehydes (hexanal, heptanal, octanal and nonanal) was found to discriminate between the breath of patients with lung cancer and those of controls.[13]
- **Breast cancer:** Researchers investigated an untargeted analysis approach to assessing breath volatiles, and in doing so demonstrated a significant increase in four aldehydes (hexanal, heptanal, octanal and nonanal) in the exhaled breath of breast cancer patients.[7]
- **Liver disease:** Eight compounds in alveolar breath were found to discriminate well between healthy volunteers and patients with liver cirrhosis.[13] The same group also found that dimethyl sulfide and low-boiling ketones could be used as indicators of 'foetor hepaticus', a musty breath smell associated with liver disease.[9]
- **Inflammatory bowel disease (IBD):** Researchers found that the breath of IBD patients could be discriminated from those of healthy controls,[10] which is significant because IBD can lead to chronic bowel inflammation and increased risk of bowel cancer.
- **Halitosis:** Researchers identified a total of 14 compounds associated with halitosis in both alveolar and mouth air.[13]

Occupational health monitoring

Monitoring VOCs in workplace air has been standard practice in a number of industries for many years. However, VOCs can be absorbed through the skin or ingested, as well as being inhaled. Therefore, a low atmospheric concentration of VOCs does not necessarily mean that personal exposure levels will also be low.

Breath monitoring, being quick and non-invasive, has long been popular for assessing the total VOC exposure by all routes, and is the ultimate exposure test for workers in high-risk industries such as paint manufacture, petrochemical refining and dry-cleaning.

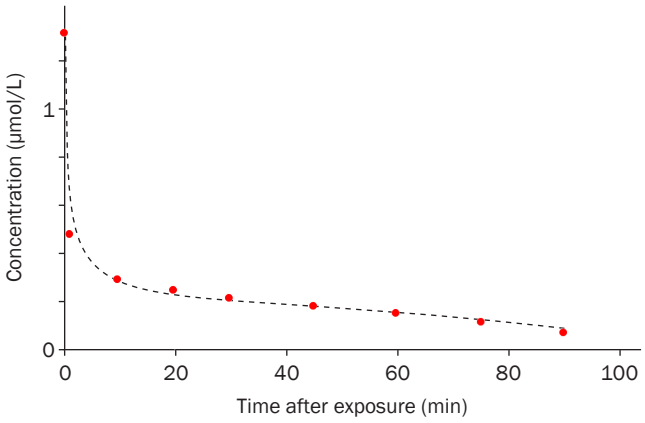


Figure 5: Rapid fall of the concentration of butanone in breath after exposure ends.

The concentration of VOCs in a person's blood (and so their breath) starts to decrease soon after exposure ends (Figure 5). Timing is therefore critical, and ideally the sample should be collected in a clean area 10 minutes after the end of exposure. As a result of this factor, breath monitoring is usually used to assess short-term exposure, but it can also provide an indication of long-term exposure.

Breath monitoring also provides a valuable check on the efficacy of respiratory protective apparatus and protective clothing at work. It can also be used to assess risk, by taking samples before and after the operation of a particular task and recording changes in the breath VOC profile. Guidance notes are available that provide information on interpreting tetrachloroethene, acetone and butan-2-one exposure with respect to occupational limits.[12]

Some examples of Bio-VOC being used for occupational health assessment are described below.

- **Workplace exposure:** Researchers found a significant correlation between the levels of VOCs in ambient air and concentrations in the alveolar air of house painters, varnishing workers, car painters and petrol station workers. They also noticed that benzene derivatives were more strongly absorbed into the body than esters.[13]
- **Fire-fighting:** Researchers found that a number of polycyclic aromatic hydrocarbons (PAHs) were present in the breath of fully-protected firefighters following exposure to a structural fire. Absorption of these chemicals through the skin around the neck (the least well-protected area) appeared to be the most likely route.[14] The same group found that breath sampling was suitable for the detection of less volatile three- and four-ring PAHs, which previously have generally required blood or urine sampling.[15]
- **Dry-cleaning:** Bio-VOC has proven to be very useful for monitoring biological exposure to tetrachloroethene (also known as perchloroethylene) in the dry-cleaning industry. This chemical is fat-soluble, has a long half-life and is poorly metabolised, with only 1–2% being excreted in the urine. This means it can build up in fatty tissue after prolonged exposure even at low levels. Data obtained using Bio-VOC has demonstrated its value for detecting both background levels and short-term exposures (Figure 6).

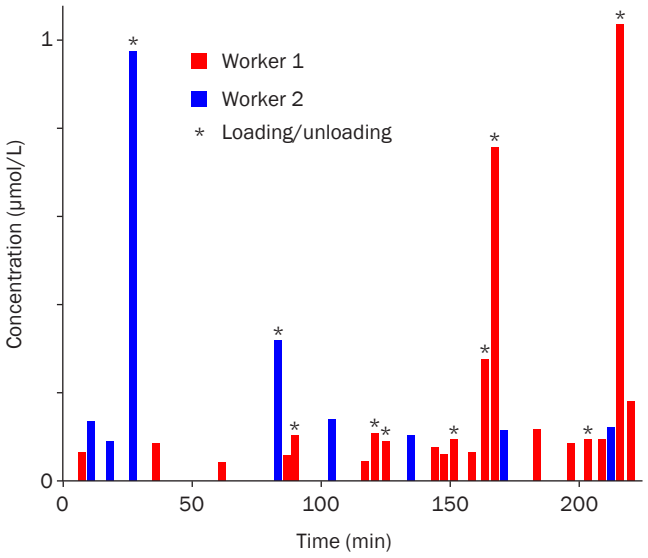


Figure 6: Monitoring of dry-cleaning workers handling tetrachloroethane using Bio-VOC shows performance at both background-level and high exposures. In this case, it is clear that exposure is greatest when loading and unloading machines.

- **Footwear manufacture:** A wide range of solvents are used during footwear manufacture. The greatest potential for dermal exposure occurs when applying a special glue to the soles of the shoe, an operation commonly carried out by hand. Breath sampling showed relatively high biological exposure to a range of solvents (Figure 7).

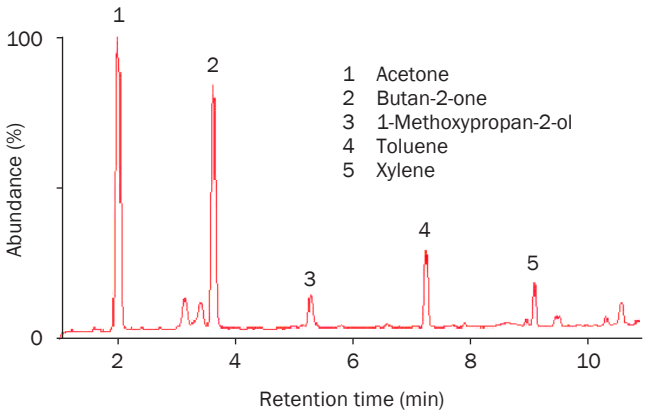


Figure 7: Solvents detected on the breath of shoe industry workers using Bio-VOC.

Assessing exposure of the public to VOCs

Exposure to VOCs is of course not restricted to occupational situations, with smoking and urban pollution being the most prominent examples of everyday activities that are associated with exposure to hazardous chemicals.

However, in recent years there has been an increasing understanding that chemicals in consumer products and construction materials, as well as pollutants in indoor air, can also lead to significant and potentially harmful personal exposures. These are now the focus of considerable attention.

With regard to the use of breath monitoring, the same principles as occupational health monitoring can be applied to this area.

Some examples of Bio-VOC being used for public exposure assessment are described briefly below.

- **Use of personal care products:** Researchers quantified the alveolar-air concentrations of two cyclic siloxanes used in cosmetics, and found for one of them a significant difference in levels between individuals who did not use any personal care products beforehand, and those who did.[16]
- **Exposure to by-products of swimming-pool disinfection:** Two potentially carcinogenic trihalomethanes, chloroform and bromodichloromethane, were found in the breath of swimmers immediately following bathing.[17]
- **Smoking:** Analysis of the breath of tobacco smokers and e-cigarette users showed that the former received a much larger burden of organic compounds (including benzene, toluene, naphthalene and other pollutants of general concern), whereas e-cigarettes led to strong absorptions of propylene glycol and glycerol.[18]

Conclusions

The Bio-VOC breath sampler has been used for breath sampling in many situations, including clinical research, occupational health monitoring, and public exposure scenarios. This popularity has stemmed from its low cost and ease of use, and its compatibility with TD sampling tubes and TD–GC–MS equipment. Samples stored on sorbent tubes are also stable for several weeks.

The release of the new BioVOC-2 represents a continuation of this successful design, which retains the low-emission body for minimal risk of analytical artefacts. In summary, it is an easy-to-use, affordable tool for a range of biological monitoring scenarios, including early disease diagnosis, occupational health and consumer exposure.

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For a video on how to use the BioVOC-2, please visit our website.

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Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.