Breath of life

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From breathalysers to detection of diseases, there is much chemistry behind an action we perform over 23000 times a day, typically without thinking about it

Exam links

This article links to the following topics in the **AQA**, **Edexcel**, **OCR**, **WJEC**, **CCEA**, **SQA** and **IB Diploma** exam specifications:

- volatile organic compounds (VOCs)
- gas chromatography-mass spectrometry (GC-MS)
- biomarkers
- structures of organic compounds

Breathing is the process of moving air into and out of our lungs, through our mouth or nose. We need the oxygen in air for cell respiration (to break down molecules from foods for energy), which produces carbon dioxide as a waste product that is expelled when we exhale. But there is much more to our breath than just carbon dioxide.

Human breath is mainly composed of nitrogen (~74%), followed by oxygen (~15%), water vapour (~5%) and then carbon dioxide at around 5%. The missing 1% includes argon and small amounts of hydrogen, carbon monoxide and ammonia, followed by even smaller amounts of **volatile organic compounds** (**VOCs**, see Chemistry Review, Vol. 26, No. 1, pp. 18–22).

In the same breath

The minute amounts of VOCs were first discovered by Linus Pauling in 1971. A typical collection of breath samples might contain around 3000 different VOCs in total, mostly at extremely low concentrations ranging from parts per trillion (ppt) to parts per billion (ppb) by volume — around the level of one drop of water in a swimming pool.

Most VOCs in our breath are believed to originate from chemical processes within tissues in the body (*endogenous*), such as inflammation. Exposure to carcinogens (such as cigarette smoke) will lead to the detection of VOCs derived from outside the body (*exogenous*). We can divide the majority of VOCs into five categories:

- *Hydrocarbons*: produced mainly from polyunsaturated fatty acids.
- *Alcohols*: absorbed through the digestive tract and metabolised (i.e. broken down), mainly in the liver.
- *Aldehydes*: formed from metabolised alcohols, lipid peroxidation, tobacco smoke and dietary sources.
- *Ketones*: produced from fatty acids by the liver and influenced by increased fat or protein intake in the diet.
- *Aromatics and nitriles*: typically thought to be due to exogenous pollutants, such as tobacco smoke and pollution.

Testing the breath for traces of alcohol is a familiar concept (Box 1), but what else can we discover from our breath?

In 1974, small-chain hydrocarbons in breath were first identified and quantified. The researchers proposed that the concentrations of these volatile hydrocarbons could be used as a measurable indicator, called a **biomarker**, of *oxidative stress*.

Box 1 A nose for crime

The most well-known area of breath analysis relates to breathalysers (see CHEMISTRY REVIEW, Vol. 8, No. 2, pp. 2–7). In 1967 the UK Road Safety Act introduced the first legally enforceable maximum alcohol (ethanol) blood level for drivers, above which it became an offence to be in charge of a motor vehicle. It also made the roadside breathalyser available for use by the police. Lion Laboratories' version of the breathalyser, which won the Queen's Award for Technological Achievement, was called the Alcolyser. It included crystal-filled tubes that changed colour above a certain level of alcohol in the breath.

Passing ethanol vapour over potassium dichromate(VI) crystals, moistened with dilute sulfuric acid, leads to the crystals changing from orange to green, as the chromium(VI) is reduced to chromium(III). The flow of electrons in this redox reaction produces an electric current, which can be used to determine the amount of ethanol. These are the two half equations for the reaction of ethanol with dichromate(VI):

$CH_3CH_2OH(l) + H_2O(l) \rightarrow CH_3CO_2H(aq) + 4H^+(aq) + 4e^-$

$Cr_2O_7^{2-}(aq) + 14H^+(aq) + 6e^- \rightarrow 2Cr^{3+}(aq) + 7H_2O(l)$

Later models used a fuel cell alcohol sensor, in which a platinum electrode oxidises the ethanol, providing a more reliable kerbside test and removing the need for blood or urine samples to be taken at a police station. Other types of breathalysers use infrared spectroscopy (see CHEMISTRY REVIEW, Vol. 21, No. 2, pp. 2–6) to detect alcohol on the breath.

Some breathalysers use platinum electrodes to oxidise ethanol

Oxidative stress is an imbalance between the production of **free radicals** and the ability of the body to counteract or detoxify their harmful effects through reaction with antioxidants (see CHEMISTRY REVIEW, Vol. 23, No. 4, p. 34). This was the first study to propose the mechanism for the production of breath molecules (hydrocarbons).

The researchers proved their hypothesis by demonstrating that the concentrations of products of fatty acid oxidation, formed in oxidative stress, could be reduced by administering antioxidants. The fact that analysis of breath could distinguish between when a person is healthy and ill, at an early stage, using a technique that is inexpensive, non-invasive and painless (much more straightforward than having a blood test), has spawned research devoted to the detection and diagnosis of disease through breath sensing, called **breathomics**.

Breath-taking

So, how do we detect tiny amounts of hundreds of VOCs? We need an analytical method that is highly sensitive, precise and has high-resolution. Most of the fundamental studies used **gas chromatography-mass spectrometry (GC-MS)** (see Figure 1 and CHEMISTRY REVIEW, Vol. 21, No. 4, p. 5). First, exhaled breath is collected and temporarily stored in designated containers (such as in inert poly(tetrafluoroethene), PTFE, bags). Then, a helium stream is used to carry the sample though a long, heated capillary column. The VOCs are separated based on their chemical properties, consecutively ionised and separated by their mass/charge (m/z) ratio and then identified by comparison with a spectral library using computer software.

A standard method of sampling is needed for reliable analysis of breath biomarkers, because different methods can greatly

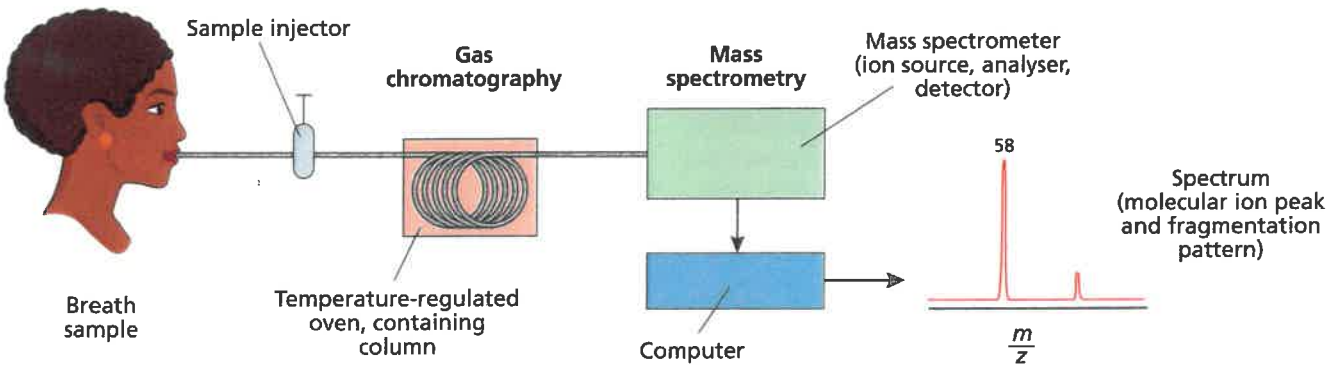


Figure 1 The main features of a GC-MS system used in breath analysis

Glossary

Biomarker A detectable substance that exhibits features indicative of biological processes.

Breathomics Breath research, i.e. the study of the set of metabolites present within exhaled air.

Free radical An atom or group of atoms with an unpaired valence electron. This unpaired electron tends to make radicals highly reactive.

Gas chromatography-mass spectrometry (GC-MS) An analytical method that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. Gas chromatography is a technique that uses an inert gas and a long column lined with a stationary phase to separate components of a mixture. Mass spectrometry is a means of measuring the mass of molecular ions and fragments of molecules, providing information about the identity of the molecules in question.

Partition coefficient A measure of the relative affinity of a substance for two different phases, e.g. the air in the lungs and the bloodstream, or the blood and the tissues.

Volatile organic compounds (VOCs) Organic (carbon-containing) chemicals that vaporise easily at room temperature and are present as gases at trace levels in the atmosphere.

Box 2 VOCs in the breath

The levels of VOCs in the breath of 30 healthy volunteers over a 6-month period (reported by Claire Turner et al. in 2006) are displayed in Figure 2. Ammonia was shown to be a major breath metabolite, with a geometric mean of 833 ppb, followed by propanone (477 ppb), methanol (461 ppb), ethanol (112 ppb), isoprene (106 ppb), ethanal (22 ppb) and propanol (18 ppb).

Structurally, ethanal is CH_3CHO, isoprene is $H_2C{=}C(CH_3){-}CH{=}CH_2$ (see Chemistry Review, Vol. 26, No. 1, pp. 2–7), ethanol is CH_3CH_2OH, methanol is CH_3OH, propanone is CH_3COCH_3 and ammonia is NH_3. In the body, propanol is assumed to be mostly propan-2-ol ($CH_3CH(OH)CH_3$) but in this study the structural isomers were not distinguished. The structures of these VOCs are shown in Figure 3.

affect the results. For example, the body posture of the person, the flow or volume of the breath during collection, sampling via the nose or mouth, and the number of breath samples taken, can all have an effect. Recently, a gum has been designed that absorbs VOCs. Once a patient has chewed the gum for 15 minutes it can be sent away for analysis. Although there are no accepted standardised methods for VOC breath-gas sampling and analysis, most agree that direct sampling is preferable to storage of breath for later analysis, as decomposition of samples or loss of compounds by diffusion is avoided.

Sniff out the truth

Once a sample is analysed, it is far from straightforward to identify the specific markers for a particular disease. For example, research has probed the profiles of ammonia, propanone (acetone), methanol, ethanol, propanol, acetaldehyde (ethanal) and isoprene (2-methyl-1,3-butadiene) in the breath of 30 healthy volunteers over a 6-month period (Box 2). But the VOC

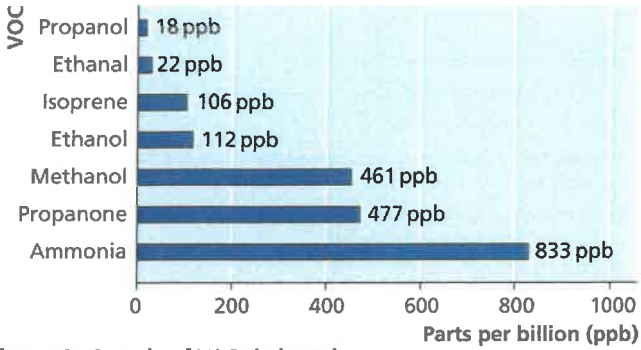


Figure 2 Sample of VOCs in breath

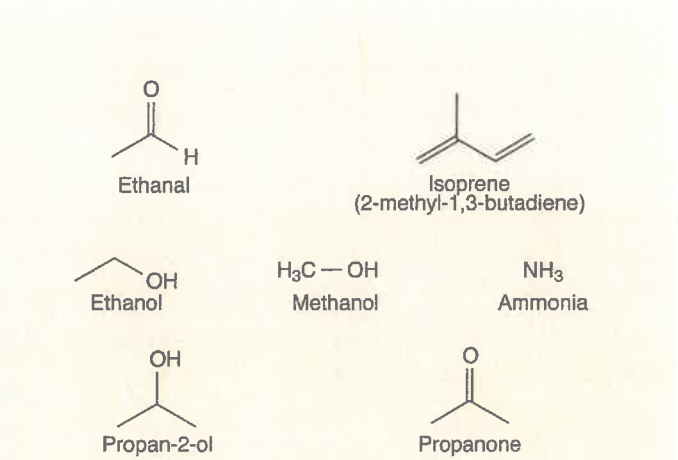


Figure 3 Structures of VOCs found in breath

levels detected in breath will depend on many factors, including the concentration in ambient air, the duration of exposure, the solubility and **partition coefficient** into tissues, and the age, gender, mass and fat content of the individual.

Also, many VOCs may be produced in the airways, in the oral cavity by bacterial infections, by bacteria in the gut, or emitted from mucus, saliva and aerosols created in the respiratory tract. For example, very low concentrations of propan-2-ol and ethanal in exhaled breath appear to be derived from both the digestive system and the mouth. In comparison, propanone, methanol and isoprene showed similar profiles for mouth- or nose-exhaled breath, indicating that these compounds originate from the digestive system.

Consuming food and drink may also affect VOCs, for example, methanol is present in foods such as apples. Garlic, onion, mint, banana and coffee (Figure 4) are also known to emit volatiles at

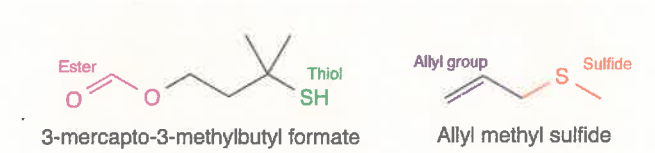
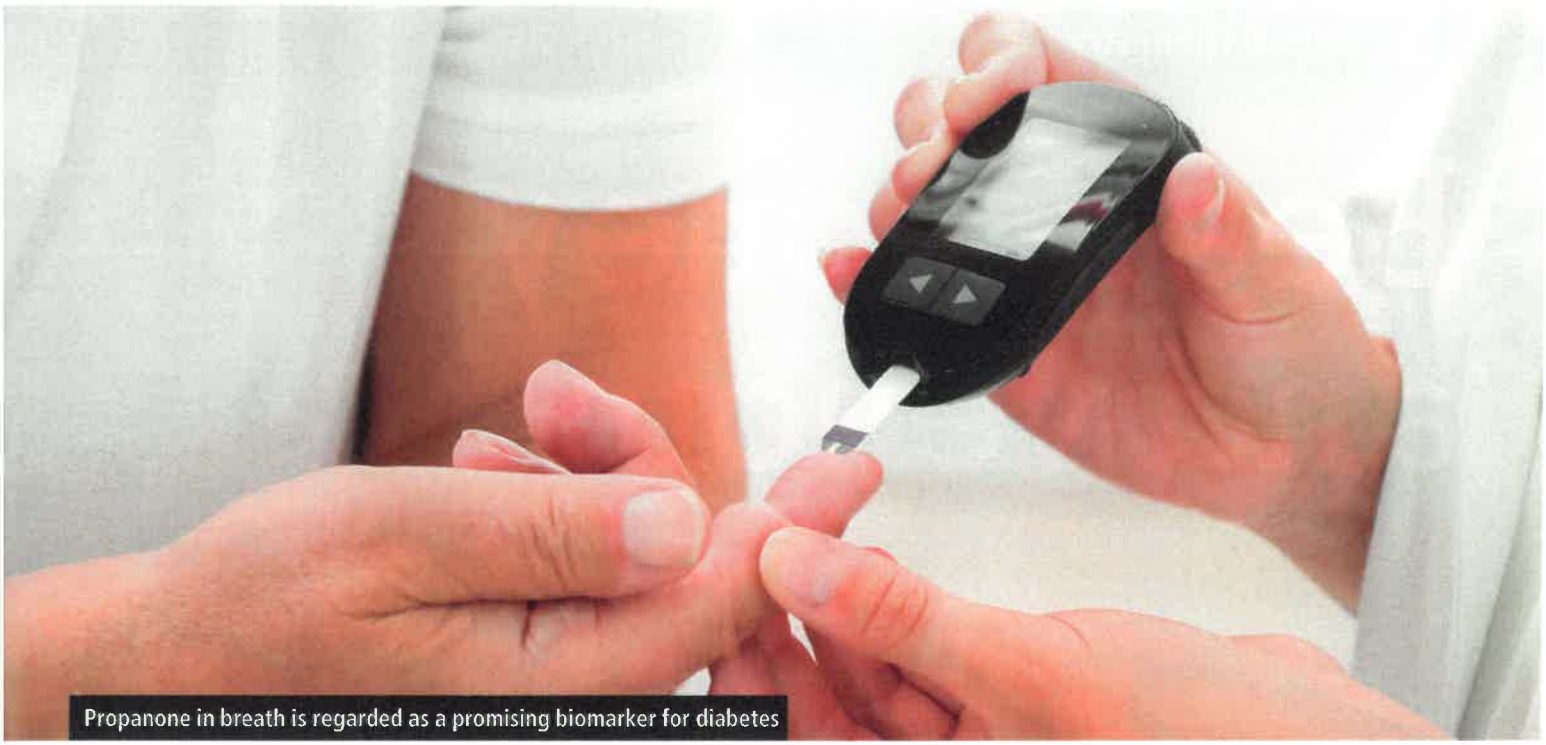


Figure 4 'Coffee breath' has been linked to 3-mercapto-3-methylbutyl formate, which has a 'catty' odour, while 'garlic breath' is attributed to allyl methyl sulfide, a product of the breakdown of garlic



Propanone in breath is regarded as a promising biomarker for diabetes

trace concentrations, which have been detected, after ingestion, in mouth-exhaled breath. There are also issues with exposure to pollution and indoor contaminants (see CHEMISTRY REVIEW, Vol. 17, No. 4, pp. 8–10). For example, compounds present in cigarette smoke, such as ethanenitrile (acetonitrile), benzene, toluene (methylbenzene) and styrene (ethenylbenzene) have been identified in both smokers' and passive smokers' breath.

Detection of diseases

The challenges associated with identifying unique biomarkers, coupled with using different sampling methods, means that the results from different studies can be contradictory. A notable exception is analysis of breath propanone, often described as having a 'fruity smell', which is widely regarded as a promising biomarker for diabetes.

Practice exam questions

1 Combine the two half equations in Box 1 to give an overall reaction. (2 marks)

2 Which of the breath volatiles shown in Box 2 is responsible for a peak at *m/z* 58 in a mass spectrum? (2 marks)

3 What are the IUPAC names of the compounds shown in Figure 5? (3 marks)

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Useful CHEMISTRY REVIEW articles on breath analysis and electronic noses:

'Analysing food flavours', Vol. 6, No. 5, pp. 2–7.

'Don't hold your breath: the diagnostic potential of breath analysis', Vol. 18, No. 2, pp. 13–16.

'The smell of success', Vol. 21, No. 4, p. 34.

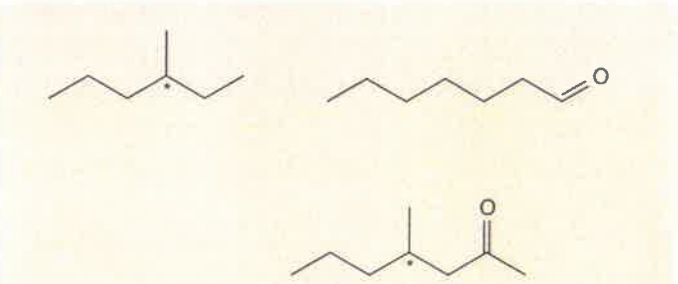

Figure 5 Examples of VOCs reported to be useful for diagnosis of breast cancer. The stereochemistry of the chiral centres (*) has not been deduced

For a given disease, as studies indicate changes in levels of multiple VOCs (Figure 5), researchers are adopting a strategy of identifying patterns in the complex mixture. The technique uses an electronic nose ('e-nose'), a device that detects various VOCs using multiple sensors and transforms the data to identify trends, leading to a 'breath-print'. Recognising a specific disease from a breath-print of exhaled substances could provide a suitable and reliable method for discriminating between healthy and diseased states, and it could soon be done using a simple tool when we visit the doctor's surgery.

Key points

- Human breath contains traces of ~3000 different volatile organic compounds (VOCs).
- Endogenous VOCs arise from body processes. Exogenous VOCs originate from outside the body.
- Breathomics is the detection and diagnosis of disease by analysing breath.
- VOCs are detected using gas chromatography-mass spectrometry (GC-MS).

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