

A portable device for measuring breath acetone based on sample preconcentration and cavity enhanced spectroscopy

Thomas P. J. Blaikie[†], John Couper, Gus Hancock, Philip L. Hurst, Robert Peverall, Graham Richmond, Grant A. D. Ritchie, David Taylor[†], Kevin Valentine*

Department of Chemistry, Physical and Theoretical Chemistry Laboratory, University of Oxford,
South Parks Road. Oxford OX1 3QZ, UK

[†] Oxford Medical Diagnostics Ltd, Centre for Innovation and Enterprise, Begbroke Science
Park, Begbroke Hill, Begbroke OX5 1PF, UK

KEYWORDS: breath acetone; laser detection; cavity enhanced spectroscopy; preconcentrator

ABSTRACT

A portable and compact device is demonstrated for measuring acetone in breath samples. The device features a 7 cm long high finesse optical cavity as an optical sensor that is coupled to a miniature adsorption preconcentrator containing 0.5 g of polymer material. Acetone is trapped out of breath and released into the optical cavity where it is probed by a near-infrared diode laser operating at ~1670 nm. With an optical cavity mirror reflectivity of 99.994 % a limit of detection of 159 ppbv (1σ) is demonstrated on samples from breath bags. Initial results on direct breath

sampling are presented with a precision of 100 ppbv. The method is validated with measurements made using an ion-molecule reaction mass spectrometer. Data are presented on elevated breath acetone from two individuals following an overnight fast and exercise, and from a third individual during several days of routine behaviour.

Introduction

An abundant volatile organic compound (VOC) found in human breath, acetone has strong links with diabetes and diet.¹⁻¹⁸ Produced from the decarboxylation of acetoacetate (a so-called ketone body resulting from the β -oxidation of fatty acids, acetyl co-enzyme A production and ketogenesis in the liver), it is readily miscible in blood and easily escapes the bloodstream into the lungs. Periods of restricted calorific intake (e.g. fasting or starvation), extreme exercise, metabolic disorders (e.g. diabetes – which can lead to diabetic ketoacidosis), and type of diet (e.g. a ketogenic diet) can all lead to elevated blood ketones and breath acetone.¹⁹ Undiagnosed patients with diabetes often present with ketoacidosis²⁰⁻²² and would therefore be expected to exhale much higher concentrations of acetone than normally found in breath.

Numerous studies have been undertaken on breath acetone, measuring the normal variations in exhaled breath, monitoring the response to diet or fasting and also with a view to probing the metabolism of sufferers of diabetes or the relationship of breath acetone to their glycemic state.^{8,9,14,16,17,23-26} Several recent studies have sought to relate levels of breath acetone to the presence of type-2 diabetes.²⁵⁻²⁷ On the whole, mass spectrometry has been the analytical mainstay of many of these studies because of its sensitivity, specificity and analyte bandwidth. Recently however, in an attempt to maintain some of these desirable properties but for robust single analyte detection, several groups have developed optical strategies attempting to focus on

a compact and practical solution.^{9,27-35} Notably, Wang *et al.*^{9,27,33-35} have developed an easily transportable UV-laser cavity ring-down apparatus, which has been used recently to gather clinical data.²⁷ Here, we demonstrate a breath acetone sensor instrument that consists of a compact laser-diode based cavity enhanced absorption cell coupled to a miniature preconcentrator; this coupled technology allows for a scale of physical dimensions commensurate with a hand-held device. The device's analytical performance is assessed and is demonstrated on the relatively complex breath matrix. The use of narrow band absorption spectroscopy contributes significantly to the specificity of the technique; in addition, the nature of the preconcentrator is surmised to be important in mitigating interferences from different breath species such as water CO₂ and methane.

Acetone detection strategy

In principle the spectroscopic acetone detection method is similar that described previously,³⁰ but at a slightly different wavelength. In this device we chose to detect acetone at a wavelength around 1672 nm on the $|10\rangle\pm|1\rangle$ mode within the $|v_1v_2\rangle\pm|v_3\rangle$ combination band (notation attributed to Kjaergaard et al.³⁶, where v_1 and v_2 are out-of-plane CH oscillators and v_3 is the in-plane CH oscillator; and where \pm refers to the symmetry of the two equivalent out-of-plane oscillator wavefunctions with respect to reflection in the skeletal plane). As before, a specific wavelength is selected to be as free from interference as possible, especially from those species that are in relatively large abundance in breath, such as CO₂, water and methane, however, for this device, this is not as critical since the material within the preconcentrator does not preferentially adsorb any of these. No contribution from the water continuum (as discussed in [28]) was observed to contribute to the absorption but such is the quantity of water in breath that diligence was still

required to make certain that the laser wavelength was not coincident with nearby water absorption peaks.

The acetone spectrum at this wavelength and at room temperature and atmospheric pressure is relatively featureless on the scale of $< 1\text{cm}^{-1}$ and so rather than detecting the specific spectral signature of acetone, we are probing a signal level change due to the presence of acetone, which is more prone to noise and system instability. This strategy has clearly worked before (as it works here), but it is a factor that undoubtedly contributes to the limitations of the device. The acetone absorption cross section within a small spectral window of width 0.5 nm centred at 1669.73 nm is $1.0 \times 10^{-21} \text{ cm}^2$.^[31]

Preconcentrator and optical cavity designs

The preconcentrator is tubular in design with an internal volume of 1.6 cm^3 . It is of aluminium construction with a total mass of 7 g, and is of similar concept to that shown in [37]. Inside the preconcentrator there is a nickel foam insert (Recemat BV, NL; relative density 5%) that serves two purposes: first to aid in thermal transmission throughout the adsorbing polymer material, and second to help maintain a gas flow path through the device. The preconcentrator is wrapped in a silicone rubber thin film heater (Minco Sa. Type HR5584R4; 25.4 38.1 mm; 28 W) and is shown in figure 1. The internal volume of the preconcentrator is such that it accepts approximately 0.5 g of the polymer material Porapak QS (Waters Corp.; 80-100 mesh), with which it is loaded prior to being preconditioned at 220 °C for three hours with a 10 sccm flow of nitrogen passing through it (for this operation it is heated in an oven). Tests for the breakthrough of 10 ppm acetone in a 1 litre total volume of synthetic air mixture show that at a flow of 1.2 l/min there is less than 1.5 % transmission with the preconcentrator held at 35 °C. Porapak QS has been

specifically chosen because of its hydrophobic nature and inability to trap water, CO₂ and methane.

The optical cavity required for cavity enhanced absorption spectroscopy,^{38,39} consists of a solid aluminium tube of internal diameter 9 mm and length 7 cm, with 12.5 mm diameter plano-concave mirrors (Layertec GmbH) held firm at either end. The internal volume of the cavity is therefore approximately 4.5 cm³. The mirror reflectivity is nominally 99.995 % between 1600 and 1700 nm, and the radius of curvature of the concave reflective surface of each mirror is 25 cm. There is a small sintered micro-particle filter at the sample inlet of the optical cavity to keep the interior of the cavity clean (pore size 0.5 microns; Swagelok). The laser used for this device is a fibre-coupled distributed feedback device (NEL NLK1U5EAAA, centre wavelength 1669 nm, coupled via single mode PM fibre) of quoted maximum power 10 mW. A current noise source is capacitively coupled to the laser diode current supply in an attempt to reduce the mode quality of the laser, so that residual cavity mode noise is reduced.^{40,41} The laser temperature is controlled via a PI feedback system coupled to an on-board (i.e. on the laser) TEC. The laser light is delivered via a fibre decoupler/collimator and an adjustable right-angle turning mirror, to the cavity. At the detection end of the cavity there is an interference filter (bk Interferenzoptik Elektronik GmbH bk-1670-037-B; centred at 1670 ± 10 nm) to help reduce unwanted signal from reaching the detector (including ASE: amplified spontaneous emission from the laser), a focusing lens (f=20 mm) and an InGaAs photodiode (Hamamatsu G12181-010K, diameter 1 mm, TO-18 package). The signal from the photodiode is amplified (gain = 10⁸ A/W) before being recorded.

Valves in the device are of a miniature solenoid type (Parker Hannifin X-valve and ten-X valve models) and the pump, a miniature diaphragm type (Parker Hannifin CTS series). Pressures are

monitored with miniature gauges (Sensortech HCA-baro), positioned as in figure 1. For sample analysis from breath bags there is a sample inlet for connecting the bags; in addition to this there is also a dry/clean air inlet which passes air through molecular sieve (2 g of Hydranal, 3Å: Sigma Aldrich), removing water, VOCs, and impurities, and providing a clean air source for purging the device. All the valves and the pump are controlled via the microprocessor (Microchip Technology Inc. PIC16F877A), as is the laser and the preconcentrator. The microprocessor also has several integrated ADC inputs, which are used to record the absorption, pressure and temperature signals.

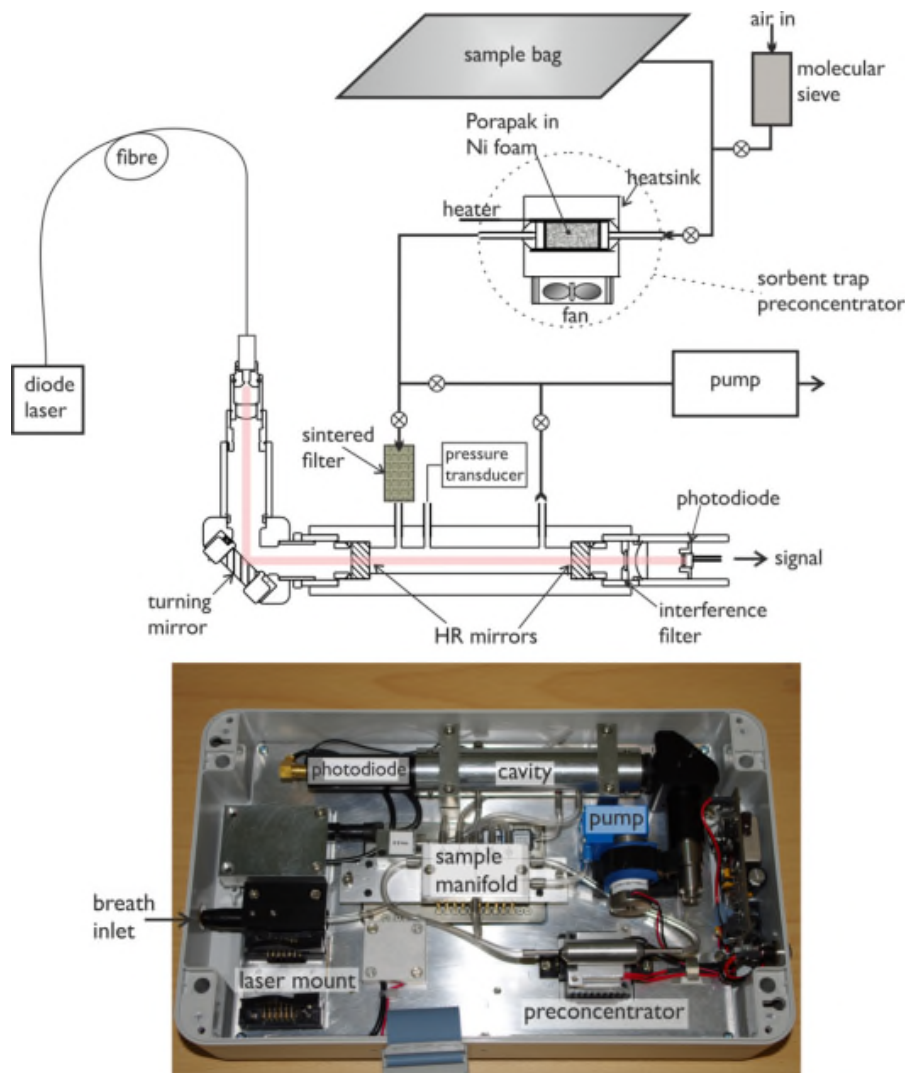


Figure 1. Schematic of the experimental arrangement (top). Breath sample is drawn through the sorbent trap preconcentrator (35 °C) by the miniature diaphragm pump and then heated to desorb acetone (130 °C). The acetone sample is then flushed into the optical cavity where it is measured spectroscopically. Bottom: a photograph of the device with principle components labelled. Note: all the main circuitry is in the lid of the box, which includes an LCD screen. The circuit board pictured to the right of the image controls an optional Peltier unit for heating/cooling the preconcentrator. Also, during operation there is an insulating sleeve covering the preconcentrator. The entire device weighs ~ 1 kg, and measures 0.28×0.17×0.06 m (w×d×h).

Method

In this study breath is either collected in 1.1 l aluminised breath bags (FAN GmbH) which are then connected to the device, or sampled directly using a side-stream method. Upon the analysis initiation, the device first self-flushes by drawing in air through the clean-air inlet for several seconds, having previously stabilised the temperature of the preconcentrator to 35 °C. A background sample of clean air is admitted to the optical cavity at this moment, and the cavity is isolated. The gas handling path is then evacuated and the sample drawn through the preconcentrator (held at a constant 35 °C) at a flow rate of approximately 1200 cm³/min for approximately 15 s: hence the sample size is 300 cm³. To help remove any residual interfering species, particularly water, the system is flushed again with clean air for a few seconds. The preconcentrator is then isolated and begins its heating sequence, taking about 90 seconds to reach 130 °C. At this point, the background sample in the cavity is analysed, after which the cavity is evacuated and then filled with the sample from the preconcentrator. The clean-air inlet is also opened in order to assist in the sample transfer and equalise the pressure within the cavity at one atmosphere. The cavity is isolated and a measurement taken. Following the measurement, the

system is evacuated and flushed with clean air, and the preconcentrator allowed to cool (fan assisted), ready for the next sample.

During both the measurements of the background and absorption, the laser is scanned repetitively at a rate of 100 Hz over a range of about 0.1 cm^{-1} centred at 5989 cm^{-1} . An average is taken of the centre 70% of the scan (avoiding the turning points of the applied current ramp), accumulated over 1000 scans. Data are recorded with the microprocessor, via an internal 10 bit ADC.

The quantity of acetone is calculated using the equation³⁹

$$\frac{I_0 - I}{I} = \Gamma \frac{\alpha L}{1 - R} \quad \text{equation 1}$$

where L is the physical length of the optical cavity, R is the geometric-mean mirror reflectivity and α is the absorption coefficient (the product of the absorption cross-section and absorber number density). I and I_0 are the measured signals with and without acetone. The absolute concentration is converted into relative concentration (ppmv) by taking into account the absolute pressure measured within the filled cavity. Clearly the preconcentrator facilitates a volumetric enhancement factor given by the combination of the temperature-dependant efficiency of the preconcentrator in trapping and re-releasing acetone, the relative sample size compared to the cavity volume, and the transfer efficiency. We term this the amplification factor, ‘ Γ ’, which can be found by calibrating the device against a mass spectrometer (in this case) with synthetic acetone/air samples. Our experience with the calibration is that it need be performed only once for a given preconcentrator, and the same amplification factor used for several hundred preconcentrator cycles. Furthermore, similar designs of preconcentrator yield similar

amplification factors (in the same device), and so it would seem that consistency in preconcentrator manufacture would yield consistent Γ -factors, although, as we shall present later, there is a slight deviation from the calibration when measuring breath.

A typical calibration curve is shown in figure 2 (as used in this study) generated using samples of 100 ppmv acetone/synthetic air (BOC Speciality Gases), diluted to varying extents in N_2 (BOC, N4.8). The amplification factor derived from this is 18.1 ± 0.2 , and also noticeable is a small offset (6.2 ± 0.9 ppmv) that must be taken account of in the analysis. The offset is as consistent as the amplification factor and our investigations have led us to conclude that it is probably due to acetone released by one particular material during the heating cycle (possibly from the PVC tubing attached to the preconcentrator inlet/outlet: acetyl acetone is a stabiliser used in PVC production). Following their measurement with the device, samples used for the determination of the calibration and during the device testing were immediately measured using an ion-molecule reaction mass spectrometer (V&F Analysen und Messtechnik GmbH, Austria) calibrated against standard gas mixtures (BOC Ltd). The mass spectrometer uses Hg^+ reactant ions, so as to minimise molecular fragmentation, thus avoiding complicated mass fingerprints. (More details can be found in references [16,28]). Also shown in figure 2 (inset) are measurements used in the determination of the mirror reflectivity and the analytical performance of the optical cavity alone. In this case, calibrated samples (1, 10, 25, 100 ppmv acetone in synthetic air; BOC Ltd: Speciality gases UK) are repeatedly introduced directly into the optical cavity (four measurements for each concentration, total pressure 1 atm) and the absorption measured. The mirror reflectivity (geometric mean), deduced from the gradient of the linear fit and equation 1 (where $\Gamma = 1$), is equal to 99.994 %. The limit of detection for the optical cavity derived from measuring these synthetic samples is 580 ppbv (1σ), or expressed as a minimum detectable

optical absorption coefficient $\alpha_{\min} = 2.9 \times 10^{-8} \text{ cm}^{-1}$.

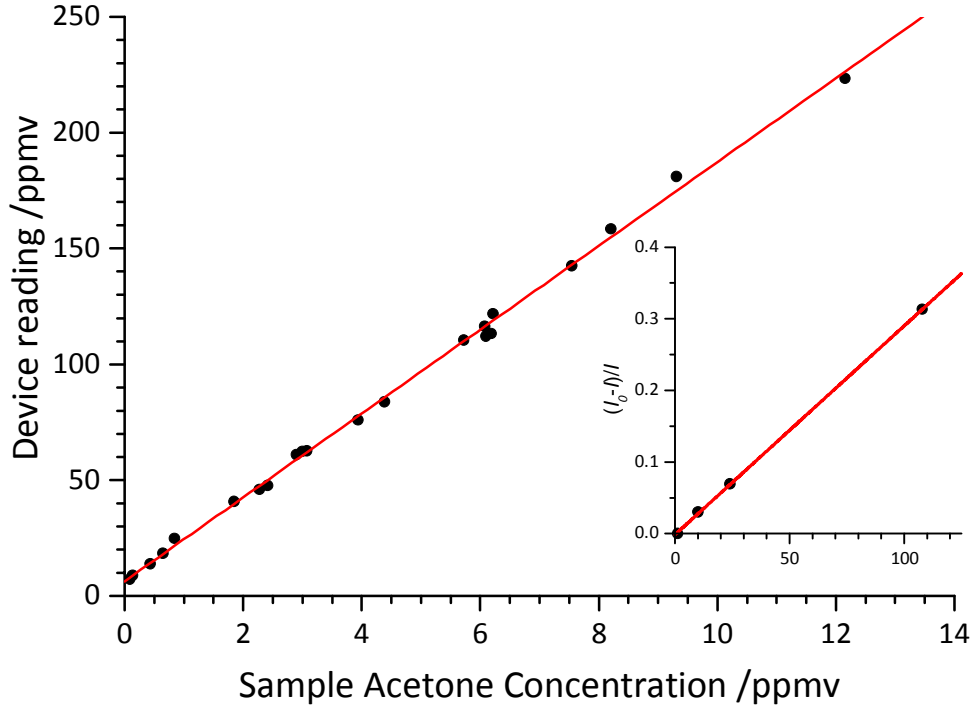


Figure 2. Calibration of the optical cavity (inset) and entire preconcentration measurement procedure (main graph). Samples are generated from premixed air/acetone mixtures and come either straight from the cylinder (inset) or are diluted in sample bags (main graph).

In addition to breath bag (indirect) sampling the device was configured (see figure 3) with the capability to sample directly from breath using a side-stream sampling technique. In this mode, an additional existing pressure sensor in the device was coupled to a tube connected via a T-piece to the breath inlet (i.e. where the sample bag is normally connected). An individual gives a breath sample by breathing through the tube via an HME filter (Intersurgical, Humidity and Moisture Exchange filter, present to restrict moisture ingress). The breath passes through the tube and out into the atmosphere with a flow that is restricted by a throttle valve. When a 5 mBar

increase in tube pressure is detected by the additional pressure sensor, the device is triggered into action and waits for 5 seconds before drawing some of the breath in through the preconcentrator for 15 s, while breath is continuously being delivered. Following this, the device undergoes the normal analysis procedure. For result validation the exhaled breath that passes directly out of the tube is collected during the 15 s sampling period by opening an additional valve to a breath bag, and then analysed by mass spectrometry.

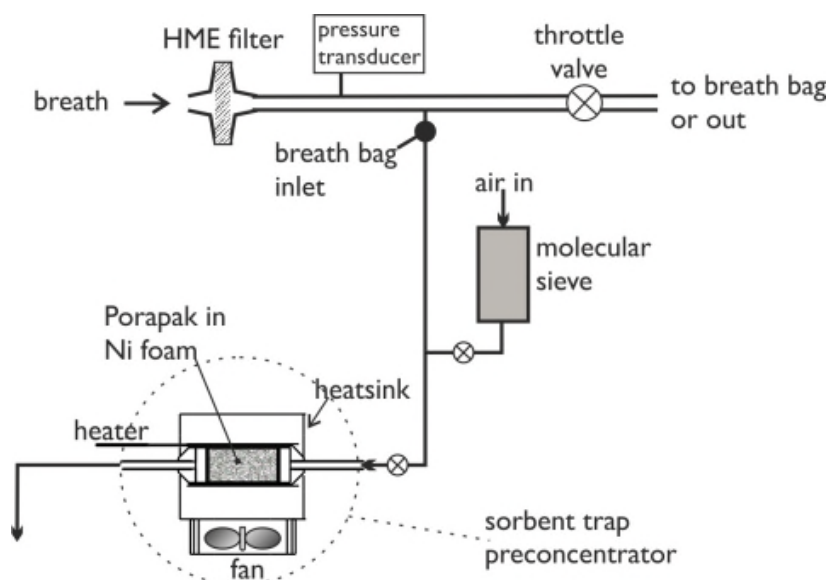


Figure 3. Changes to the device configuration for direct breath sampling (compare with figure 1). Breath is delivered straight from the mouth via a humidity and moisture exchange filter and sampled ‘side-stream’ into the device.

Results and discussion

To test the device over a range of breath acetone levels, breath samples were analysed from two individuals (males aged 25 and 44 yrs) following and during various phases of fasting and/or exercise over a period of several days. To obtain samples, the subjects were asked to take a deep

breath and hold their breath for about five seconds, and then expel perhaps half their breath before filling a breath bag (1.1 l, FAN GmbH, as before) with the remainder. This ensured end-tidal breath conditions, but was not necessary for testing the device, as the concentrations within the breath bags were measured independently using the mass spectrometer. The bag samples were measured for acetone using the portable device and then straightaway with the mass spectrometer, just as for the calibration measurements. The device and mass spectrometer acetone readings are plotted in figure 4(a) (the optical measurements are adjusted by 106 ppbv to compensate for isoprene content: see further discussion, this section). One individual undertook two studies involving an overnight fast and a 10 km run (the following morning) before giving breath samples every half hour for the next 6 hours (continuing the fast) and is responsible for all of the data shown below 6 ppmv. The other subject gave breath samples at various occasions during a more prolonged (38 hours) fast (but no exercise), reaching much higher breath acetone levels (up to 23 ppmv). Figure 4(b) shows the evolution of the breath from a single study following exercise and continued fast, right up to the first food intake, 7 hours later. These individuals' responses to fasting are not unexpected. (They are nevertheless individual responses and we have not sought to control exactly the pre-fasting and exercise behaviour.) When an individual fasts for long enough (or exacerbates the fasting through exercise), sources of glucose begin to be exhausted, and the body mobilizes triacylglycerol reserves as fatty acids which are oxidized and used as a respiratory fuel, which is associated with an increase in the rate of production of ketone bodies.

Previously we identified isoprene as a potential interference to acetone measurements at slightly longer wavelengths (1690 nm)²⁸ and we still expect spectral interference at ~1670 nm. However, tests done on trapping/preconcentrating isoprene from synthetic samples with the device suggest

that the enhancement factor is lower for isoprene than acetone (by a factor of ~ 0.7), thus, it has less significance than before (previously it was deduced that where adjusted for, the typical population distribution of breath isoprene levels effectively contributes an additional 20 ppbv to the precision of the acetone measurement). The breath data in figure 4(a) have been adjusted for isoprene content by subtracting an equivalent acetone absorption corresponding to 106 ppbv (calculated from average isoprene concentration (118 ppbv) from the dataset in reference [42] and adjusted for differences between acetone and isoprene in the preconcentrator efficiency and optical absorption coefficient).

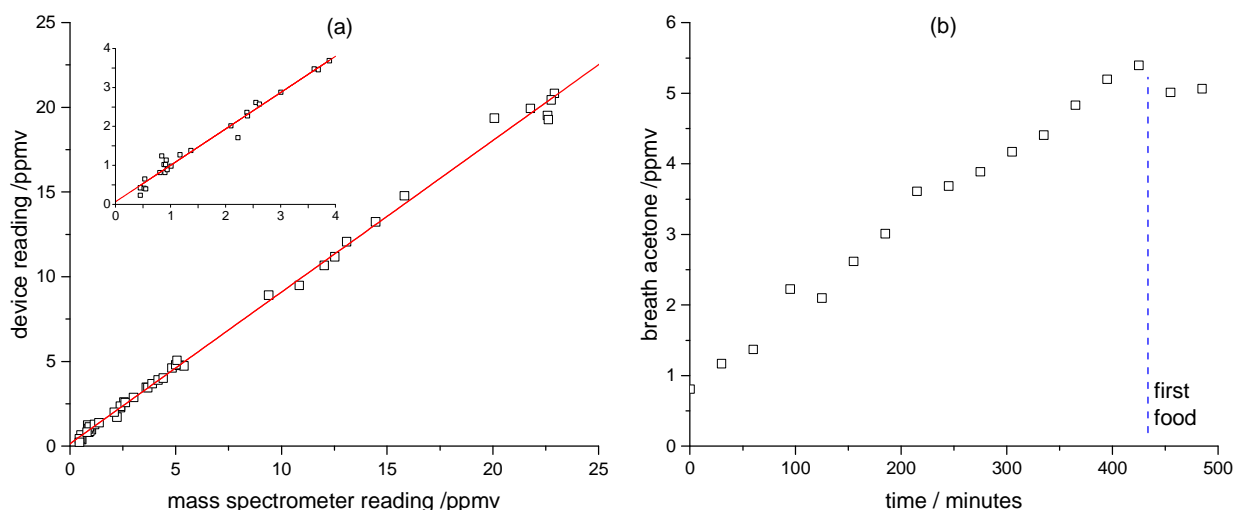


Figure 4. (a) Measurements of breath acetone from two volunteers on three different occasions after an overnight fast and following exercise (10 km run) (see text for details). Inset: a linear fit to the lowest 25 data points. The mass spectrometer was calibrated against standard cylinder samples of either 1 ppmv or 5 ppmv in air. (b) The temporal progression of the breath acetone of

one volunteer following overnight fast and exercise, during a continued fast over the next 7 hours.

The limit of detection of the device can be deduced from the linear fit to the data for lower breath acetone levels (figure 4(a), inset) and is 159 ppbv (1σ). This is consistent with the analytical performance of the device for synthetic test samples as derived from the data in figure 3 where the limit of detection (1σ) derived from the calibration data is found to be 148 ppbv (adjusting for the amplification factor). From figure 2 (inset), the effective contribution from the optical measurement is about 32 ppbv, which is very much a minor contribution. From our experience we estimate that the mass spectrometer measurement has an uncertainty of $< \sim 50$ ppbv for breath samples (in the mode which we operate, which is optimized for convenience and speed), and so it appears from all these data that the dominant source of uncertainty in the measurement is from the sample handling and/or preconcentration procedure. (Note that there is no contribution to the error in the optical-only measurement from the uncertainty in the mass spectrometer measurements, as there is in the other measurements.) We also note that there is a small deviation from equivalence in the relationship between mass spectrometer data and device data in figure 4(a). For the data below 4 ppm the slope is 0.94 and for the entire dataset it is 0.9, indicating some non-linearity across this range and some deviation from the calibration. This could indicate that the calibration is not entirely valid for breath samples, perhaps because of their relatively high humidity compared to the gas mixtures used for calibration, but could also reflect an excessive accumulated loading of the device with high acetone levels.

In a different experiment another individual tested the device in direct sampling mode with the results shown in figure 5 (to avoid confusion we refer to direct sampling here to mean that the sample was taken directly from the mouth into the device as depicted in figure 3 and described in

the method section; this avoids the bag collection stage, but many aspects of the method, e.g. preconcentration, remain the same). These data represent normal variations in breath acetone for this individual over four days of usual activity (i.e. no fasting, and occasional exercise). Intriguingly, the precision achieved with this sampling technique is 100 ppbv (1σ), which is clearly better than that from the indirect breath bag sampling. The positive zero-offset could reflect a larger than average breath isoprene for this individual, although we cannot rule out a change in calibration-offset perhaps as a consequence of the slightly different sampling dynamics. Other than being the result of sample standardization, the precise reason for the increase in performance is not clear, although we suspect that it may be due to better regulation of the relative amounts of water in the samples. (Note also that the slope of the straight line best fit in figure 5 is 0.96.)

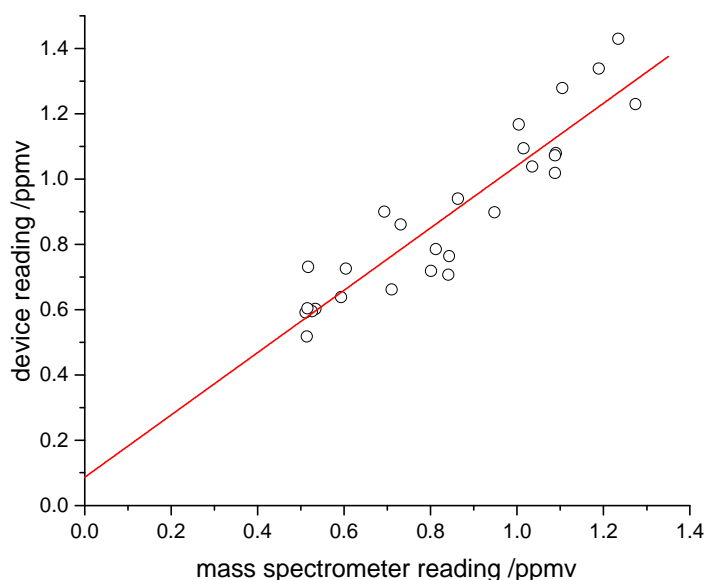


Figure 5. Results from direct breath sampling using a side-stream technique. The calibration, offset and isoprene correction are the same as for previous measurements.

From simple considerations of the volume of sampled breath and the volume of the spectroscopic cell, preconcentrator and tubing (300 cm^3 and $\sim 5.5\text{ cm}^3$, respectively), it would seem that a large volumetric enhancement factor is possible. Clearly, working at a preconcentrator desorption temperature of $130\text{ }^\circ\text{C}$ means that the equilibrium concentration of acetone around the preconcentrator material is sub-optimal for maximum signal size. However, while greater amplification factors can be achieved at higher release temperatures, we have found that for this device, heating to higher temperatures does not decrease the uncertainty in the acetone measurement. One possible contributor to this seems to be associated with a notable increase in the zero-concentration offset (see comments in method section), which increases rapidly from $140\text{ }^\circ\text{C}$ with an associated increase in its uncertainty. Further work is required to see if this can be mitigated by a more judicious selection of materials, or, for example, whether there is a significant contribution to the increase in uncertainty merely because it takes longer to heat to higher temperatures. (The current preconcentrator design requires 90 s to attain $130\text{ }^\circ\text{C}$.)

Conclusions

We have developed a self-contained compact breath acetone device that is truly portable. The method is based on the combination of a miniature preconcentrator and a sensitive but short optical cavity, a combination that is tested and proven in this paper. The preconcentrator not only aids in the detection of acetone through trapping acetone from a relatively large breath volume (several hundred cm^3), but also lets common interferences through: in this case, water, CO_2 and methane. Despite containing only a small amount of analyte (0.5 g), the preconcentrator has a lifetime of several hundred measurements before the analyte needs replacing or reconditioning. Measurements on individuals under fasting, exercise and normal conditions have been presented, indicating the utility of the device across a wide dynamic range. Initial measurements on side-

stream direct breath sampling have been presented that result in a precision of 100 ppbv of acetone. Currently, the device is powered via a 12 V power supply, but has a sufficiently small power requirement that battery operation should be possible for a reasonable number of measurements.

AUTHOR INFORMATION

Corresponding Author

* email: Robert.Peverall@chem.ox.ac.uk

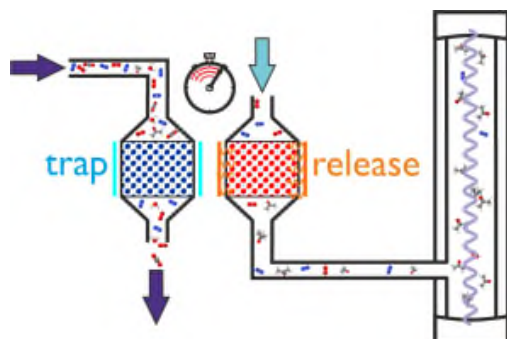
Author Contributions

The work reported here is comprised of contributions from all authors. All authors have given approval to the final version of the manuscript.

Acknowledgment

The authors would like to thank the participants in this research who gave up their food for the sake of science.

FOR TABLE OF CONTENTS ONLY



-
- ¹ Tassopoulos CN, Barnett D, Russel Fraser T. The Lancet **1969**, 293, 1282-1286.
- ² Goschke H, Lauffenburger T. Res. Experimen. Med. **1975**, 165, 233-244.
- ³ Crofford OB, Mallard RE, Winton RE, Rogers NL, Jackson JC, Keller U, Trans. Am. Clin. Climatol. Assoc. **1977**, 88, 128-139.
- ⁴ Jones AW. J. Anal. Toxic. **1987**, 11, 67-69.
- ⁵ Kinoyama M, Nitta H, Watanabe A, Ueda H, J. Health Sci. **2008**, 54, 471-477.
- ⁶ Teshima N, Li J, Toda K, Dasgupta PK. Anal. Chim. Acta **2005**, 535, 189-199.
- ⁷ Minh TDC, Blake DR, Galassetti PR, Diab. Res. Clin. Pract. **2012**, 97, 195-205.
- ⁸ Turner C, Walton C, Hoashi S, Evans M, J. Breath Res. **2009**, 3, 046004
- ⁹ Wang C, Mbi A, Shepherd M, IEEE Sensors J. **2010**, 10, 54-63.
- ¹⁰ Righettoni M, Tricoli A, J. Breath Res. **2011**, 5, 037109.
- ¹¹ Deng CH, Zhang J, Yu XF, Zhang W, Zhang X, J. Chrom. B **2004**, 810, 269-275.
- ¹² Musa-Veloso K, Likhodii S, Cunnane SC, Am J Clin Nutr **2002**, 76, 65-70.

-
- ¹³ Nelson N, Lagesson V, Nosratabadi AR, Lugvisson J, Tagesson C, Paediatric Research **1998**, 44, 363-367.
- ¹⁴ Spanel P, Dryahina K, Rejskova A, Chippendale TWE, Smith D, Physiol. Meas. **2011**, 32, N23-N31.
- ¹⁵ Smith D, Spanel P, Davies S, J. Appl. Physiol. **1999**, 87, 1584-1588.
- ¹⁶ Blaikie TPJ, Edge JA, Hancock G, Lunn D, Megson C, Peverall R, Richmond G, Ritchie GAD, Taylor D, J. Breath Res. **2014**, 8(4), Art. 046010.
- ¹⁷ Walton C, Patel M, Pitts D, Knight P, Hoashi S, Evans M, Turner C. J. Breath Res. **2014**, 8(3), Art. 037108
- ¹⁸ Reyes-Reyes A, Horsten RC, Urbach HP, Bhattacharya N. Anal. Chem. **2015**, 87(1), 507-512
- ¹⁹ Laffel L, Diabetes Metab. Res. and Revs. **1999**, 15, 412-426.
- ²⁰ Klocker AA, Phelan H, Twigg SM, Craig ME, Diabetes Med. **2013**, 10, 818-824.
- ²¹ Vanelli M, Chiari G, Capuano C, Iovane B, Bernardini A, Giacalone T, Diabetes Nutr. and metab. **2003**, 16, 312-316.
- ²² Ali Z, Levine B, Ripple M, Fowler DR, Am J Forens Med and Pathol **2012**, 33, 189-193.
- ²³ Minh TDC, Oliver SR, Ngo J, Flores R, Midyett J, Meinardi S, Carlson MK, Rowland FS, Blake DR, Galassetti PR, Am. J. Physiol. Endocrinol. Metab. **2011**, 300, E1166-E1175.
- ²⁴ Rydosz A, J. Diabet. Sci. Tech. **2015**, 9(4), 881-884.
- ²⁵ Chen ZY, Sun MX, Gong ZY, Yuan Y, Zhao XM, Liu WC, Jiang CY, Wang ZN, Li YX,. Prog. Biochem. Biophys. **2015**, 42(5), 483-490.
- ²⁶ Li WW, Liu Y, Lu XY, Huang Y, Liu Y, Cheng S, Duan Y, J. Breath Res. **2015**, 9(1), Art. 016005.

-
- ²⁷ Sun MX, Chen ZY, Gong, ZY, Zhao X, Jiang C, Yuan Y, Wang Z, Li Y, Wang C, Anal. Bioanal. Chem. **2015**, 407(6), 1641-1650.
- ²⁸ Lewicki R, Wysocki G, Kosterev AA, Tittel FK, Opt. Express **2007**, 15, 7357-7366.
- ²⁹ McCurdy MR, Bakhirkin Y, Wysocki G, Lewicki R, Tittel FK, J. Breath Res. **2007**, 1, Art. 014001.
- ³⁰ Hancock G, Langley CE, Peverall R, Ritchie GAD, Taylor D. Anal. Chem. **2014** 86(12), 5838-5843.
- ³¹ Denzer W, Hancock G, Islam M, Langley CE, Peverall R, Ritchie GAD, Taylor D, Analyst **2011**, 136, 801-806. And *adden*. Analyst **2011**, 136 5308-5308
- ³² Ciaffoni L, Hancock G, Harrison JJ, Van Helden J-PH, Langley CE, Peverall R, Ritchie GAD, Wood S, Anal. Chem. **2013**, 85, 846-850.
- ³³ Wang C and Mbi A, Measure. Sci. and Technol. **2007**, 18, 2731-2741.
- ³⁴ Wang C, Scherrer ST and Hossain D. Appl. Spectrosc. **2004**, 58, 784-791.
- ³⁵ Sun MX, Jiang CY, Gong ZY, Zhao X, Chen Z, Wang Z, Kang M, Li Y, Wang C, Rev. Sci. Instrum. **2015** 86(9) Art. 095003.
- ³⁶ H. Kjaergaard and B. Henry, J. Chem. Phys. **1991**, 94, 5844-5854.
- ³⁷ Grate JW, Anheier NC, Baldwin DL, Anal. Chem. **2005**, 77, p1867-1875.
- ³⁸ Berden G, Peeters R, Meijer G, Int. Rev. Phys. Chem. **2000**, 19(4), p565-607.
- ³⁹ Mazurenka M, Orr-Ewing AJ, Peverall R, Ritchie GAD, Annu. Rep. Prog. Chem., Sect. C, **2005**, 101, 100.
- ⁴⁰ Ciaffoni L, Couper J, Hancock G, Peverall R, Robbins PA, Ritchie GAD, Opt. Express **2014**, 22(14), 17030-17038.

⁴¹ Manfred KM, Kirkbride JMR, Ciaffoni L, Peverall R, Ritchie GAD, Opt. Letts **2014**, 39(24), 6811-6814.

⁴² Turner C, Spanel P, Smith D, Physiol. Meas. **2006**, 27, 13-22.