



## PAPER

## OPEN ACCESS

RECEIVED  
7 January 2020REVISED  
4 March 2020ACCEPTED FOR PUBLICATION  
9 March 2020PUBLISHED  
16 July 2020

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# Effects of high relative humidity and dry purging on VOCs obtained during breath sampling on common sorbent tubes

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Keywords: VOCs, Breath sampling, ReCIVA

Supplementary material for this article is available [online](#)

## Abstract

Offline breath analysis by thermal desorption gas chromatography mass spectrometry (TD-GC-MS) requires the use of sorbent traps to concentrate and store volatile compounds. The selection of which sorbent to use and best practices for managing high relative humidity are important considerations to allow for reproducible, untargeted, biomarker discovery in water saturated breath samples. This work aims to assess three commonly used sorbent materials for their use in breath volatile sampling and determine how the high relative humidity inherent in such samples effects the capture of volatile compounds of interest. TenaxGR, TenaxTA/Carbograph1TD and TenaxTA/Carbograph5TD tubes were selected as they are the most commonly used sorbents in the breath sampling literature. The recovery of 29 compounds in a standard mix loaded using high humidity gas was tested for each sorbent and compared to loading in dry gas. Water retention and dry purge rates were determined for each sorbent for 500 ml and 1000 ml breath collections. Finally, breath samples were collected simultaneously on to each sorbent type using the ReCIVA and analysed by TD-GC-MS. All three sorbents exhibited acceptable reproducibility when loaded with the standard mix in dry gas (RSD < 10%). Loading the standard mix in humid gas led to reduced recovery of compounds based on their chemical properties. Dry purging performance for each sorbent material was assessed and was shown to be 1.14, 1.13 and 0.89 mg H<sub>2</sub>O min<sup>-1</sup> for TenaxGR, TenaxTA/Carbograph1TD and TenaxTA/Carbograph5TD respectively when flushed with 50 ml min<sup>-1</sup> of N<sub>2</sub>. A comparison of breath profiles on different sorbents showed differences in background artefacts (sulfur dioxide, cyclopenten-1-one and 3-nonene) and endogenous breath compounds (2-methyl-furan and furfural). This work demonstrates that high relative humidity during sampling reduces the ability of sorbent tubes to capture volatile compounds and could impact method detection limits during breath sampling. Sufficient water to impair accurate analysis was retained on all tubes. Minimal differences were observed between sorbent materials when used to sample breath, however, suggestions are provided for sorbent selection for future studies.

## 1. Introduction

Untargeted analysis of breath is a burgeoning field with the potential to enable early detection of a range of diseases in a non-invasive manner (White

and Fowler 2018). Currently, thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) is the gold standard for untargeted analysis, offering high sensitivity paired with the ability to accurately identify compounds of interest (Ratray *et al* 2014).

Typically, for analysis by GC-MS, volatile organic compounds (VOCs) are captured onto sorbent tubes, allowing them to be transported from the sampling location and stored for several weeks (Harshman *et al* 2016, Kang and Paul Thomas 2016).

A large variety of different sorbent materials are available to researchers (Woolfenden 2010). Sorbent tubes can be purchased ready packed or made in-house and require thorough thermal conditioning before use. A list of different sorbent materials that have been used to pre-concentrate and store VOCs is provided in table S1. As most applications in the breath sampling field involve biomarker discovery, the porous polymer sorbents TenaxTA and TenaxGR have been the most used (Lawal *et al* 2017) due to their wide sampling range (Maier and Fieber 1988). TenaxGR is generally preferred to TenaxTA due to a slightly greater breakthrough volume arising from the addition of graphite to the polymer. The breakthrough volume for a specific compound is defined as the amount of gas that can be passed through a desorption tube before the eluting vapour reaches 5% of the applied test concentration (Comes *et al* 1993).

An increasing number of studies have taken advantage of the larger breakthrough volumes offered by multi-bed tubes that combine TenaxTA with graphitised carbon black sorbents such as the Carbograph mixes (Gallego *et al* 2011). As the sorptive strength of the second sorbent increases, a wider range of VOCs can be captured on to tubes. This must be balanced with the amount of water that is retained and other factors relating to durability and stability of the tubes.

A large volume of work has been carried out to investigate the properties of sorbent materials in the field of air quality analysis (Rothweiler *et al* 1991, Woolfenden 1997, Dewulf and Van Langenhove 1999). These have described that the breakthrough of VOCs is affected by the relative humidity (RH) of the carrier gas (Peng and Batterman 2000, Trabue *et al* 2008, Ho *et al* 2017). If sampled directly from the mouth, exhaled breath has close to 100% RH and, as such, the retention of VOCs on the tubes is likely to be affected. While all of the commonly used sorbents in breath sampling tend to retain less water than the molecular sieves that are prevalent in air quality monitoring, there are differences in hydrophobicity between them that may affect their suitability for pre-concentrating exhaled VOCs.

Dry purging wet samples is important to ensure reproducible analysis of tubes sampled in high RH (Gawłowski *et al* 2000). As little as 1 mg of water remaining on the sorbent tube can affect the TD-GC-MS process in several ways. Firstly the excess water may cause the cold trap in the thermal desorption unit to double fire as ice forms during trapping. Secondly, the rapid expansion of water on the trap that occurs during firing can also lead to an irreproducible split during injection into the GC. Finally,

further problems occur during the trap desorption onto the column as temperature ramps often start well below 100 °C, allowing the water to condense onto the stationary phase. Once condensed, water can inhibit the interaction of analytes and the stationary phase leading to poor peak shape and irreproducible peak retention and sensitivity, or may react with the stationary phase increasing the column bleed.

This study, aims to assess how high RH affects VOC recovery on three commonly used and commercially available sorbent tubes, TenaxGR (GR), TenaxTA/Carbotrap1TD (TA1TD) and TenaxTA/Carbotrap5TD (TA5TD), in order to determine their respective strengths and weaknesses for breath sampling. The repeatability of loading a standard mix onto the sorbents in dry gas is determined and the impact of high RH on the adsorption of VOCs is characterised. Furthermore, the weight of water retained on the sorbent tubes during sampling is described and dry purging times are suggested for each sorbent. Finally, a direct comparison of breath samples taken on the three sorbent materials is made and differences between them are highlighted.

## 2. Methods

### 2.1. Sorbent tubes

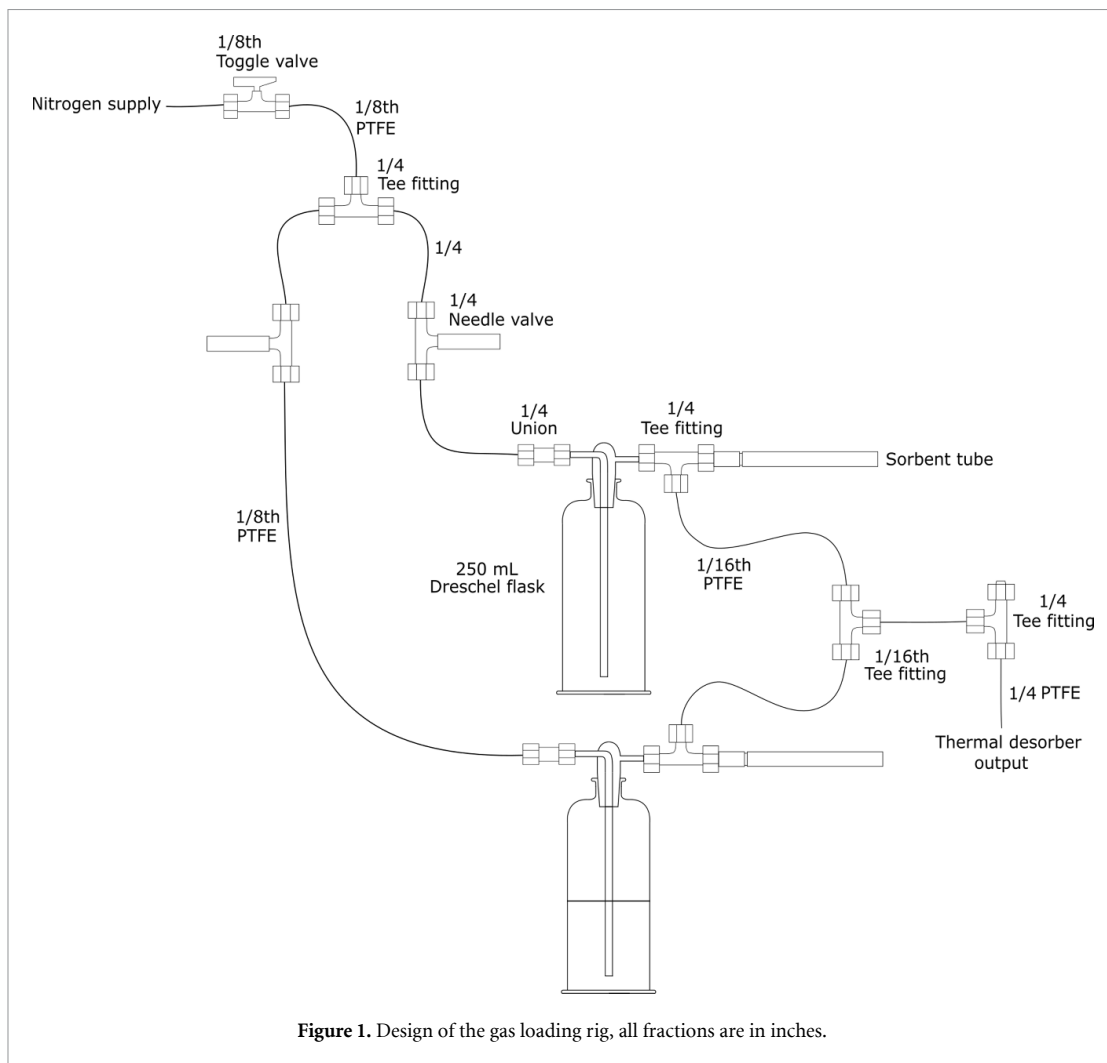
All sorbent tubes were purchased new for the study (Markes International, Llantrisant, UK). The tubes were packed with a total of 200, 350 and 400 mg of sorbent for TenaxGR, TA1TD and TA5TD respectively. The ratio of sorbents in the dual bed tube is undisclosed by the manufacturer.

### 2.2. Standard mix

A total of 29 VOCs, representative of common compounds found in breath and an alkane ladder were diluted into methanol to form a standard mix, table S2. Alkane ladders consist of a continuous series of straight chain alkanes (i.e. hexane to pentadecane) which allow the calculation of generalised retention indices for the compounds in breath. The standard mix was loaded onto sorbent tubes using a calibration standards loading rig (Markes International) whilst being purged with ultrapure Helium at 50 ml min<sup>-1</sup> for 2 min. To assess the relative standard deviation of recovery for each compound, seven tubes of each sorbent were loaded with 2 µl of the standard mix before analysis by GC-MS.

### 2.3. Gas loading rig

The gas loading rig (GLR), figure 1, was designed to allow the simultaneous loading of a VOC rich gas onto multiple sorbent tubes. Breath or the standard mix were loaded onto TA5TD sorbent tubes which were then placed into an Ultra autosampler (Markes International) connected to a modified Unity 2 thermal desorption system (Markes International). Tubes were desorbed at 280 °C for 5 mins with the



trap not in-line and flow was diverted through 1.5'' Teflon tube connected to a 1/4'' PTFE tee piece with the through port blocked off to create an elbow joint. This was connected to 1/16'' Teflon tubing which split flow into two through a 1/16'' PFA tee piece. Carrier flow was constricted by the Teflon capillary tubing and controlled by the pneumatics on a 6890 Agilent GC (Agilent, Santa Clara, CA, USA). Desorbed VOCs were dynamically diluted in two 1/4'' PTFE tee-pieces (Swagelok, Solon, OH, USA) where they mixed with dry or humid N<sub>2</sub> before being purged onto target sorbent tubes for analysis and comparison.

Dry and humid diluent was generated by flowing N<sub>2</sub> (OFN, BOC gases, UK) through a Big universal trap (Agilent) to a stainless steel tee-piece (Swagelok) and, once split, through 250 ml glass Dreschel flasks which were either empty or contained 200 ml of water. The flows of humidified and dry N<sub>2</sub> were both maintained at 200 ml min<sup>-1</sup> using needle valves (Swagelok). Two loading volumes, 500 and 1000 ml were used to match the collections volumes of the ReCIVA. The flasks were placed on a heater plate (Cole-Parmer, Staffordshire, UK) set to 60 °C and were wrapped in heater tape (Briskheat, Columbus, US) set to 34 °C. All transfer lines from the flasks and

thermal desorption system to the target sorbent tubes were also heated with the same tape.

#### 2.4. Dry purging experiments

A total of five tubes of each sorbent type were loaded with water using the GLR. Each tube was purged and weighed at minute intervals to allow the rate of purging to be assessed. The average water weight loaded onto sorbent tubes was calculated from eight breath samples for each sorbent taken on the ReCIVA. The optimal dry purge time was determined based on the volume of gas required to purge the mean mass of water + 3σ, ensuring complete water removal for 99.87% of possible breath samples. Tubes were purged using a TC20 (Markes International), a purging rig designed to bake out sorbent tubes, set to an input of 24 psi (165 kPa) clean N<sub>2</sub>, resulting in a per tube flow of 50 ml min<sup>-1</sup>. The tubes were not heated during purging. All purging was carried out in the direction of sampling to minimize breakthrough of VOCs.

#### 2.5. Breath collection

Breath was collected using a ReCIVA (Owlstone Medical, Cambridge, UK) sampling device connected to

a CASPER air pump (Owlstone medical, Cambridge, UK). In brief, the ReCIVA is a face mounted sampling device that receives 40 l min<sup>-1</sup> of VOC free gas from the CASPER. Participants were instructed to breathe normally for 30 s to train the ReCIVA on their breathing profile. The sampler is capable of collecting specific fractions of exhaled air based on the exhalation pressure profile. Two pumps within the device allow simultaneous collection of different portions of exhaled air for comparison.

In order to compare the sorbent materials, four samples were collected simultaneously, with both pumps set to collect on the lower airway fraction of breath at a flow of 200 ml min<sup>-1</sup>. A 5 min washout period preceded each breath sample to remove light exogenous compounds from the airways. Six separate breath collections were performed. Three collections were completed at 500 ml and three at 1000 ml representing suitable parameters for clinical sampling. For each breath collection all three sorbent tubes were used with the fourth pump inlet filled with a random duplicate.

Following sampling the ReCIVA masks were washed in detergent, rinsed and baked overnight at 180 °C before re-use to remove siloxane contaminants. A disposable filter disk (Philips Respironics, Eindhoven, Netherlands) was used between the mask and the ReCIVA to prevent contamination.

## 2.6. Thermal desorption-gas chromatography-mass spectrometry

For analysis, 100 µl of a gaseous calibration standard (1 ppmv, 4-Bromofluorobenzene in nitrogen, Thames Restek, UK) was loaded onto all tubes as an internal standard. Before desorption, tubes were pre-purged for 2 min in 50 ml min<sup>-1</sup> He carrier gas to ensure air and any remaining moisture was removed from the tubes. Sorbent tubes were then desorbed at 320 °C in 50 ml min<sup>-1</sup> He carrier gas using a TD-100 (Markes International) onto a general purpose hydrophobic trap (Markes International) for cryo-focussing at 0 °C. VOCs were desorbed at 330 °C into the GC column capillary gas chromatograph (7890B GC, Agilent, Santa Clara, CA, USA) fitted with a DB-5 ms Ultra Inert column (length 30 m × internal diameter 0.25 mm, film thickness 25 µm, (5%-Phenyl)-methylpolysiloxane, Agilent) using the following temperature ramp: 40 °C hold 0 min, ramp 6 °C min<sup>-1</sup> to 170 °C, hold 0 min, ramp 15 °C min<sup>-1</sup> to 190 °C to a total time of 23 min with an He carrier gas flow of 1.3 ml min<sup>-1</sup>. A triple-quadrupole mass spectrometer (7010, Agilent) was used in full scan mode (MS1) with a scan range of *m/z* 40–500 Da at 4 Hz using electron ionization at 70 eV.

## 2.7. Data analysis

All files were converted to the open mzXML format prior to pre-processing. Chromatograms were screened for inclusion in the final dataset by manual

appraisal and all samples deconvolved and aligned using eRah (Domingo-Almenara *et al* 2016) for untargeted work and Masshunter Quantitative analysis (Agilent) for targeted analysis. For the eRah deconvolution abundance was calculated as the peak area for selected ions whilst the peak area for a single extracted ion was used during the targeted analysis. All peak areas were normalised to the internal standard on a run by run basis. Data processing was performed in R. Univariate comparisons were made with Wilcoxon tests and *p*-values adjusted for multiple comparisons by Bonferroni correction. Comparison of the gradients was by analysis of covariance (ANCOVA). Multivariate data analysis was performed by principal component discriminant function analysis (PC-DFA). Limits of detection were calculated as the mean + 3.3 x the standard deviation of the signal in the background samples (Armbruster and Pry 2008).

## 3. Results and discussion

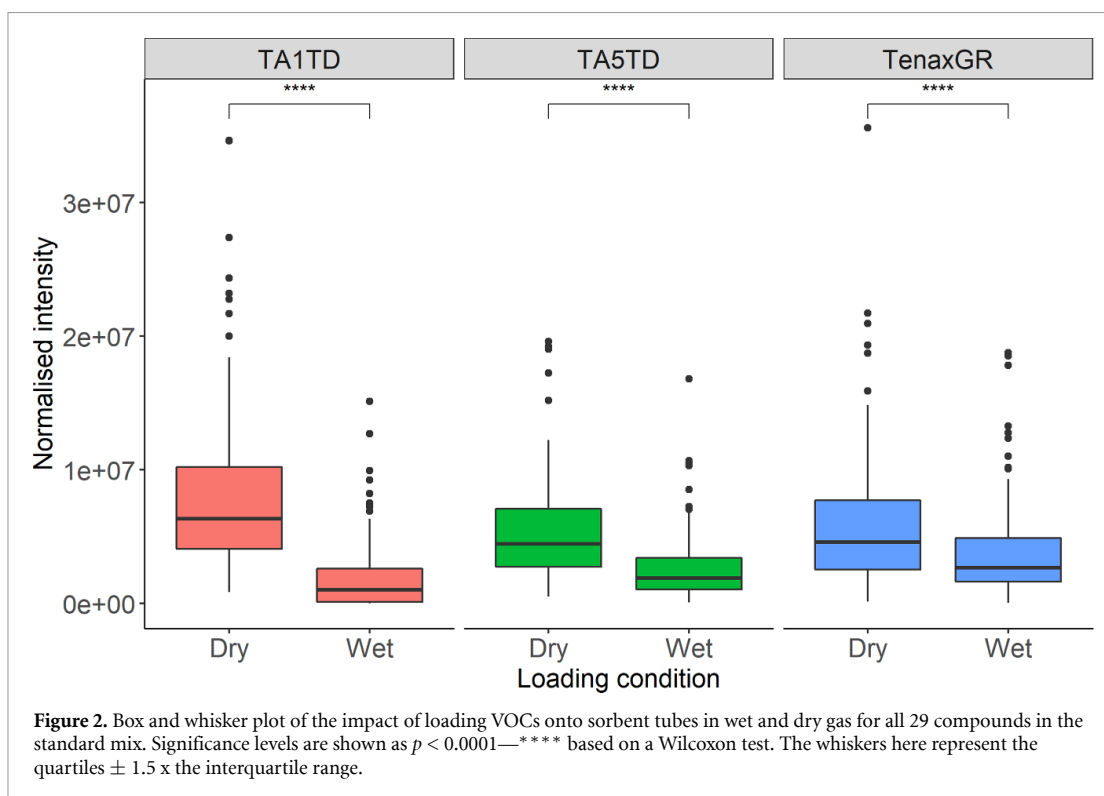
### 3.1. Reproducibility of loading

Seven tubes of each sorbent material were directly injected with the standard mix using the calibration standards loading rig in order to assess the reproducibility of sampling under optimal conditions in dry gas. An arbitrary cut-off of 10% RSD was selected based on previously published values (Parsons *et al* 2009, Thompson and Ellison 2012). High levels of variability were seen for certain compounds in the mix, particularly pyridine and nonanal, for all three sorbents (likely as trap artefacts and contamination from the serial dilution, respectively). Overall, the majority of compounds (86, 76 and 79% for TA5TD, TA1TD and TenaxGR respectively) had RSDs of less than 10% of their peak areas, table S3. No significant overall differences in the reproducibility of measurements were observed between the sorbent materials, figure S1 ([stacks.iop.org/JBR/14/046006/mmedia](https://stacks.iop.org/JBR/14/046006/mmedia)).

The majority of compounds in the standard mix showed good reproducibility (<10% RSD) both within and between sorbent types. Isoprene showed higher levels of variability on TenaxGR (RSD of 19%) than the other sorbent materials, likely due its lower breakthrough volumes resulting in less accurate trapping of the VOC.

### 3.2. Effects of loading VOCs in wet gas

The standard mix was loaded onto each of the sorbent materials using the GLR, allowing simultaneous loading of the same mixture in wet and dry gas. No difference was observed in the sorbent only backgrounds when the wet and dry arms of the GLR were compared. A pronounced reduction in the recovered levels of VOCs was seen on all three of the sorbents when comparing the wet *versus* dry samples, figure 2. No differences (*p* > 0.05) were observed between the average percentage recoveries of the VOCs in wet gas



between the three different sorbent types. The percentage recovery is defined here as 100 times the peak area in the wet gas divided by the peak area in the dry gas.

When the percentage recovery based on loading in wet *versus* dry gas is considered for each VOC individually, an inverse correlation with octanol-water partition coefficient ( $\text{LogK}_{\text{OW}}$ ) can be observed for the majority of the compounds, figure 3, table S4.  $\text{LogK}_{\text{OW}}$  is the ratio of a chemical's concentration in the octanol phase against the concentration in the aqueous phase in a two-phase system. It is an important characteristic in breath analysis as it will directly affect the adsorption, distribution and excretion of VOCs throughout the body and across the capillary-alveolar barrier in the lungs (Buist *et al* 2012).

The alkane ladder in the standard mix shows the clearest difference with the heaviest compounds such as pentadecane and tetradecane being recovered at  $\sim 60\%$  in the wet samples when compared to  $\sim 90\%$  recovery for hexane and octane. Recovery of pentane was

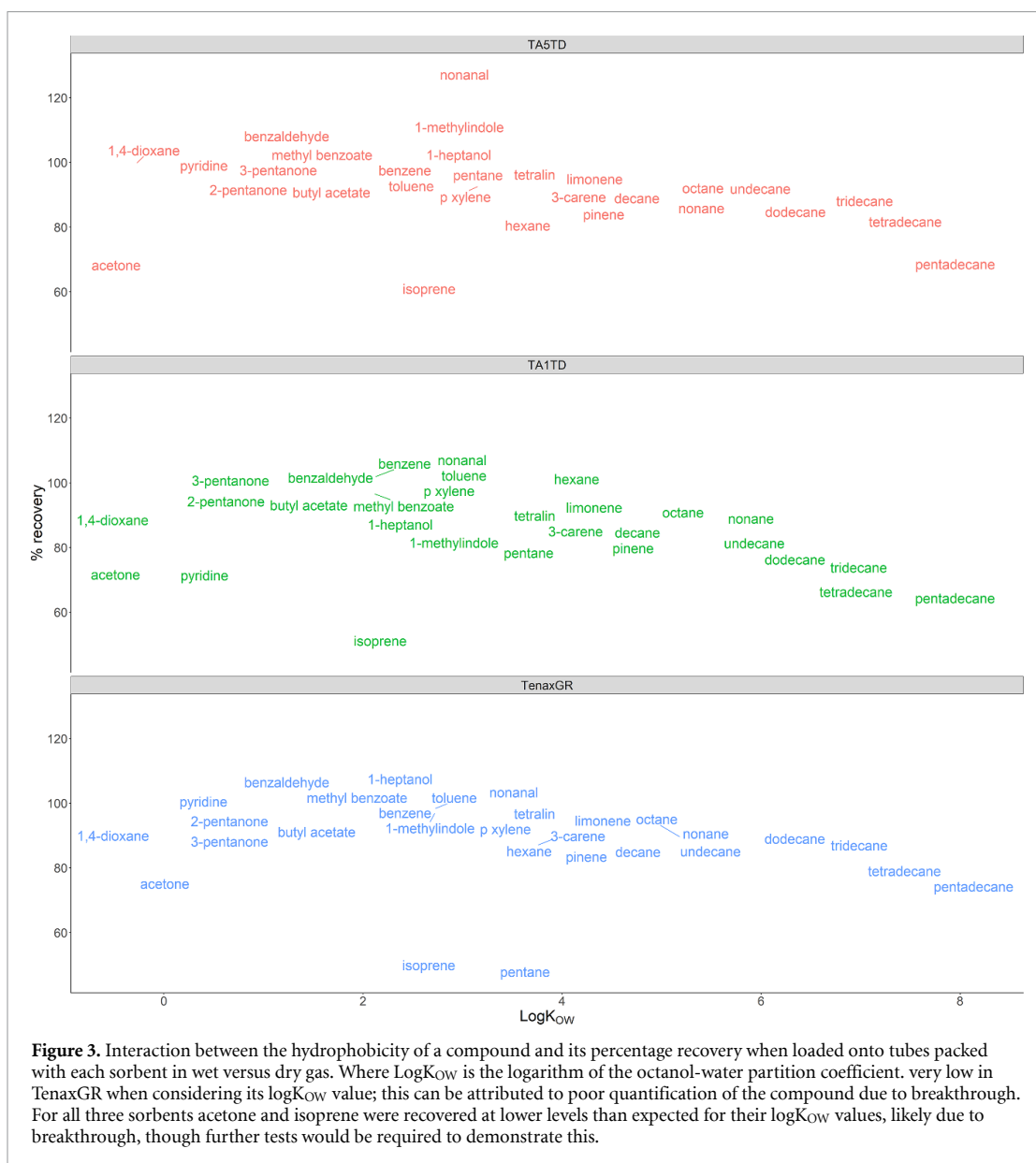
Previous work in air quality analysis has shown that breakthrough volumes are reduced when sampling in a humid environment (Trabue *et al* 2008, Ho *et al* 2017). The results presented here also demonstrated a reduction in the intensity of recovered compounds in wet gas and report that compound class and chemical properties affect recovery at high RH. However, the reduced sensitivity does not appear to be due to simple VOC breakthrough as the largest differences between the wet and dry loading were observed for the heaviest compounds

which have the highest theoretical breakthrough. (Ho *et al* 2017) posit that as sorbent tubes become loaded with water, access to the binding sites on the sorptive material is thus inhibited. Polar compounds, with low  $\text{LogK}_{\text{OW}}$  values, more readily dissolve and are ultimately able to displace water from the active sites. This trend can be most clearly observed for the alkane ladder in the standard mix where, as the alkane chain gets longer, and therefore less water soluble, the percentage recovery of wet *versus* dry is reduced. This is an important consideration, as it may increase the method detection limit for compounds in breath and prevent highly insoluble trace gases from being detected. It also highlights the importance of rigorous analytical protocols when quantifying breath VOCs. Where possible synthetic breath gas standards should be analysed in moist air and analytical protocol validation should detail the relative humidity used.

### 3.3. Water loaded during breath sampling

The weight of water loaded onto each type of sorbent was assessed for 500 and 1000 ml of breath sampled using the ReCIVA. The mean (standard deviation) collection times were 473 s ( $\pm 22$ ) and 899 s ( $\pm 43$ ) for 500 and 1000 ml respectively. The dual bed TA5TD tubes retained a greater volume of water ( $p < 0.0001$ ) than either of the other two sorbents at both sampling volumes. TA1TD retained the least water ( $p < 0.01$  *versus* TenaxGR) at both sampling volumes, figure 4.

The GLR was used to load water onto tubes containing each of the sorbents. Two loading volumes of 500 ml and 1000 ml were used and the GLR was calibrated to load a volume of water equivalent to



**Figure 3.** Interaction between the hydrophobicity of a compound and its percentage recovery when loaded onto tubes packed with each sorbent in wet versus dry gas. Where LogK<sub>OW</sub> is the logarithm of the octanol-water partition coefficient. very low in TenaxGR when considering its logK<sub>OW</sub> value; this can be attributed to poor quantification of the compound due to breakthrough. For all three sorbents acetone and isoprene were recovered at lower levels than expected for their logK<sub>OW</sub> values, likely due to breakthrough, though further tests would be required to demonstrate this.

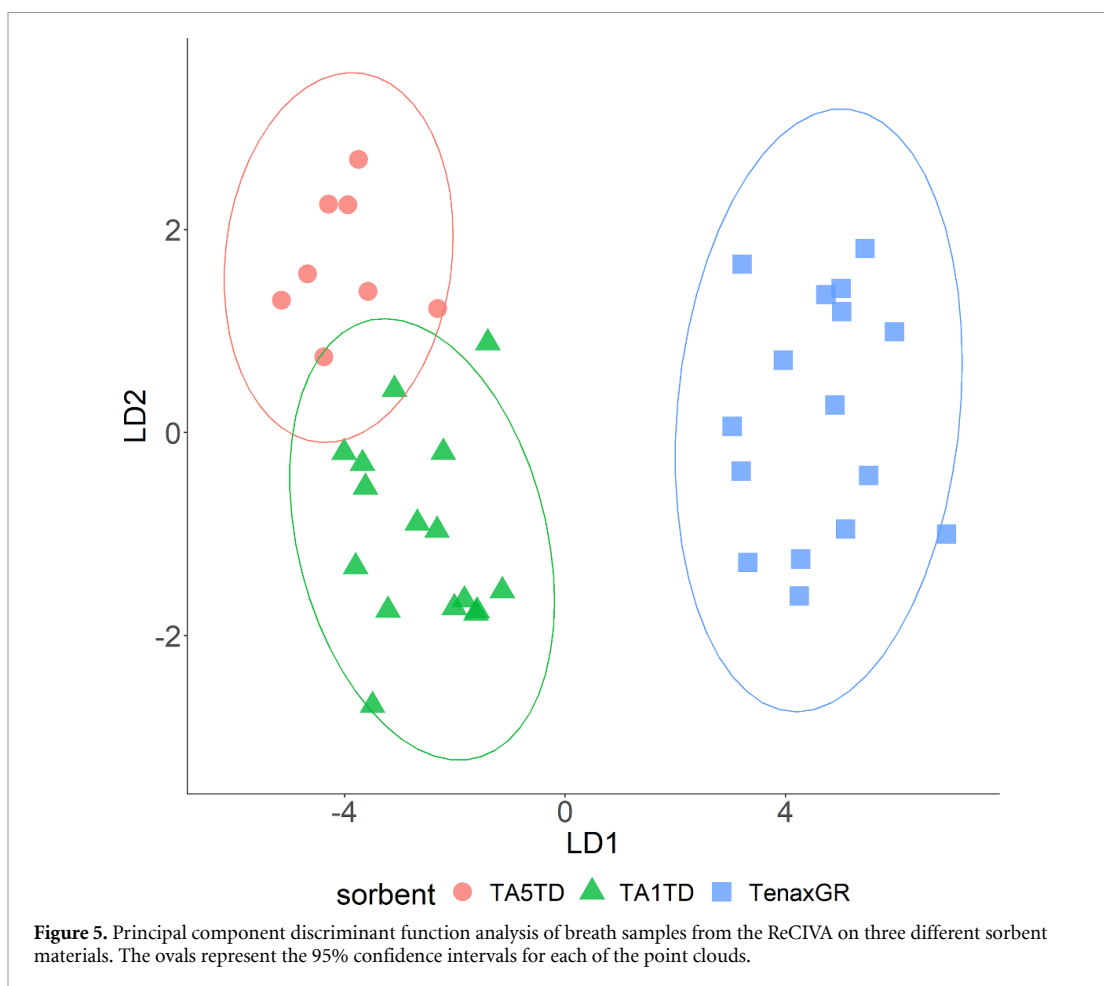
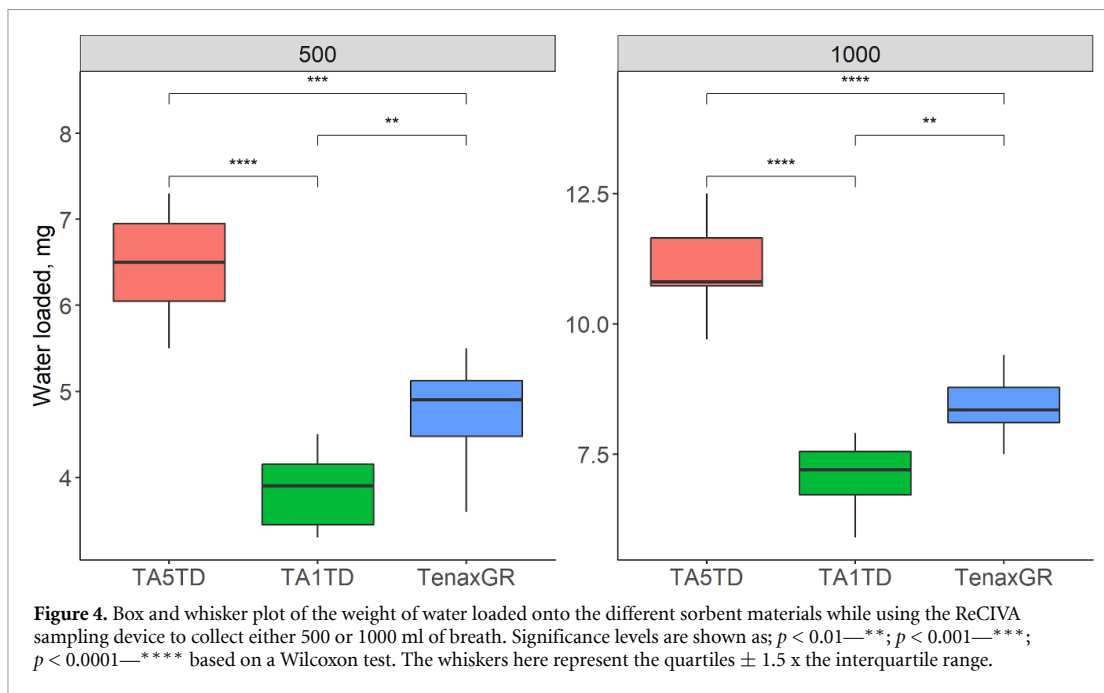
the levels observed during ReCIVA sampling. TenaxGR and TA1TD exhibited similar rates of purging, losing 1.14 and 1.13 mg min<sup>-1</sup> of water respectively ( $p > 0.05$ ). Water was lost more slowly from the TA5TD tubes at a rate of 0.89 mg min<sup>-1</sup> ( $p < 0.001$ ), figure S2. Purging times at 50 ml min<sup>-1</sup> N<sub>2</sub> were determined for 500 ml sampling 9, 4 and 5 min are suggested for TA5TD, TA1TD and TenaxGR respectively. These increase to 14, 8 and 8 min for the same sorbents when 1000 ml of breath is loaded onto the tubes, table S5.

### 3.4. Compound retention during breath sampling

Breath samples were collected using the ReCIVA and analysed in an untargeted manner. Different numbers of samples were included in the data analysis for each sorbent type (8, 16 and 14 for TA5TD, TA1TD and TenaxGR respectively) due to insufficient dry purging being performed. The dry purge volumes

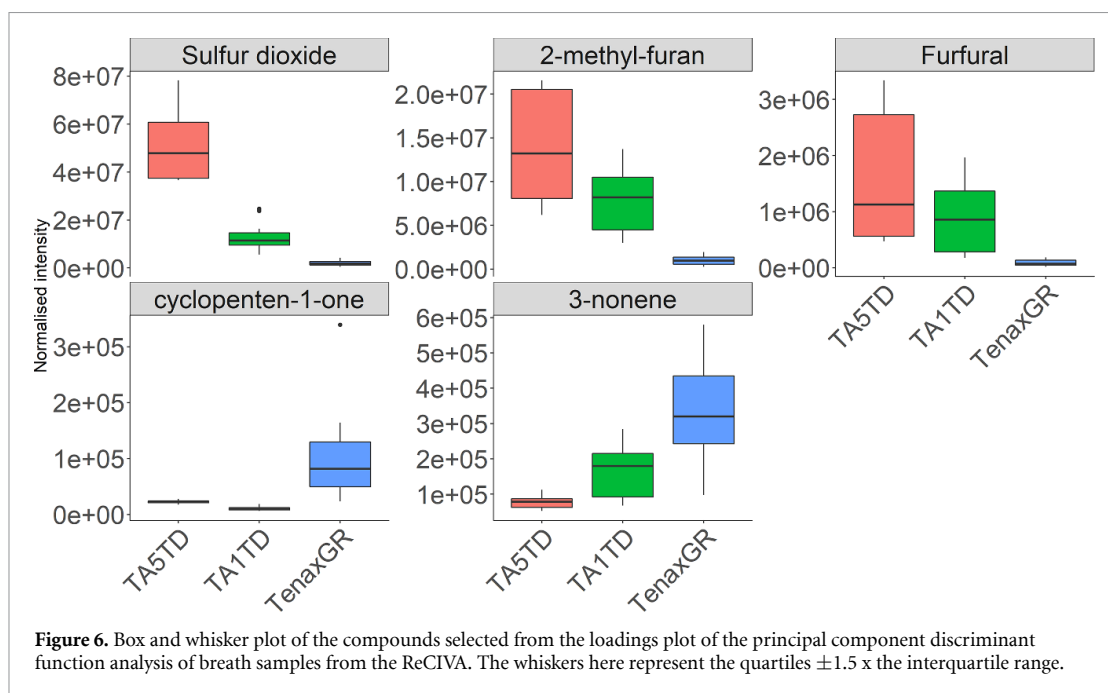
were deliberately chosen to reflect known practices at other institutions (Doran *et al* 2018, Wilde *et al* 2019) but resulted in data that were too low quality for inclusion. Following deconvolution the aligned and cleaned peak list contained 103 compounds. Separation between the different sorbents was observed after applying a PC-DFA to these data, primarily across linear discriminant (LD) 1, figure 5.

Five compounds were identified, figure 6, from the loadings plot of LD1 as being important in separating the different sorbent materials. These compounds were shown to be recovered at different levels using an ANOVA with a *post hoc* Tukey test, table S6. The tubes packed with TA5TD showed the highest recovery of three VOCs; sulfur dioxide, 2-methyl-furan and furfural. These compounds were also recovered on TA1TD tubes but at a lower level. Sulfur dioxide was found to be a background contaminant arising from the sorbent material through



comparison to sorbent blanks run concurrently with breath samples. Tubes packed with TenaxGR did not recover these compounds above the limit of detection. Two other compounds were important

in separating the sorbents in LD1; cyclopenten-1-one and 3-nonene. Concentrations of both of these VOCs were highest in the TenaxGR tubes. Reduced concentrations of 3-pentanone were detected, and



Cyclopenten-1-one was not recovered on TA1TD tubes. Neither compound was recovered in the TA5TD tubes. Both of these compounds were found in the sorbent background samples and appear to be contaminants.

Many breath sampling papers report little or no dry purging based on appraisal of the literature. As manufacturers of breath gas samplers strive to minimize interaction between samples and the surfaces that line the device interior, sample flow path has become shorter. This is particularly evident in the recently designed ReCIVA sampler where the tubes are directly below the participant's mouth. Concomitantly, the likelihood of significant volumes of water being loaded onto sampling tubes has increased. In these tests a 5 min washout period was used to reduce the impact of exogenous VOCs on the results. During this time the thermal desorption tubes were present in the ReCIVA mask and it is possible that water, and VOCs, may passively diffuse onto the tubes. For future work conducting the washout with the tubes capped or with blanking rods in place may reduce variability in the results. It has also been noted that on-sorbent chemical reactions occur, and may be more likely if the tube is saturated with water, particularly for carbonyl compounds (Sanchez and Sacks 2005, Lomonaco *et al* 2018). It may be important to reduce the humidity of the gas being drawn over sorbent tubes during sampling in order to improve sampling accuracy and mechanisms for achieving this have been suggested in air quality monitoring studies (Maceira *et al* 2017). Efficient dry purging of tubes is important to ensure accurate and reliable analysis and optimised dry purge volumes are necessary to prevent analytes of interest from breaking through the sorbent material as water is removed (Gawlowski *et al* 2000).

A method for doing so is presented in this work but further optimisation is likely possible. For example, the dry purge flow rate was not optimised and it is likely that shorter purge times could be achieved with higher flows. Furthermore (Ho *et al* 2017) highlight that tube temperature might be an important factor to consider during dry purging to reduce the loss of volatiles of interest. Alternative methods of trapping VOCs such as needle trap micro extraction (NTME) have been shown to provide reliable results when applied to breath sampling (Filipiak *et al* 2012, Biagini *et al* 2017). Additionally NTME does not retain water during sampling removing the need for a dry purging step and for certain applications this technique could be used instead of sorbent tubes.

For untargeted studies, choosing the best sorbent material for breath sampling remains challenging. Based on repeat analyses of breath samples, all three sorbent materials performed well, with only five compounds highlighted as significantly different between sorbent types. It should be noted that the same trap was used for the analysis of all the samples rather than attempting to match the trap to the tube. It is possible that this had a dampening effect on the comparison of the sorbents, making it harder to identify subtle differences between them. Of the differences observed, three of the five compounds identified as being of interest from the loadings plot were shown to be sorbent background contaminants. All the sorbent materials used in the study give off contaminant VOCs which can affect the limits of detection that can be achieved for co-eluting compounds. While many of these compounds are found at very low intensities they can include alkanes, benzene and toluene, xylene and several aldehydes in Tenax tubes (Macleod and Ames 1986, Uhde and Salthammer



2007). Importantly, these background contaminants are often also endogenous breath VOCs of potential interest. For example, 2-methyl-furan and furfural were found in the samples taken on dual bed sorbent tubes with the highest recovery in TA5TD. Both compounds have been previously found in human breath: furfural has been suggested as a marker to discriminate healthy controls from those with gastric cancer (Xu *et al* 2013) and 2-methyl-furan was linked to a loss of asthma control (Brinkman *et al* 2016). Both compounds are also known additives to food products as flavourings and are likely to arise from exogenous sources. The compounds share high levels of structural similarity and, in the present study, it is possible that one of them was ingested and subsequently metabolised to generate the other. It was expected that the tubes packed with TA5TD would show better retention of highly volatile compounds due to the increased sorbent strength of Carbograph 5TD. This was borne out by the higher concentrations of 2-methyl furan.

This study was mainly focussed on the impact of water retention on the ability of sorbent materials to retain VOCs. There are also other properties that may impact their use for different types of studies. The friability (the tendency of a compound breaking down due to compression) of the sorbent materials is another key factor for consideration in studies that involve shipping tubes between the sampling location and the laboratory. This is due to the higher chance of mechanical impact that may damage the sorbent material. Graphitised carbon blacks such as Carbograph 1TD and Carbograph 5TD are particularly vulnerable to this kind of damage and scanning electron microscopy may provide useful insights into the impact of mechanical shocks to sorbent materials. The number of thermal cycles that each tube can undergo before the artefacts from the thermal desorption start to impact limits of detection was also outside the remit of this study, but is interesting to consider due to the high costs incurred in regularly replacing sorbent tubes.

#### 4. Recommendations and conclusion

Our results indicate that all of the sorbent materials under investigation are effected by high RH and that there were few distinguishing compounds observed when comparing breath samples. To help determine which sorbent material is most applicable for future studies the following recommendations are made:

- TA5TD tubes are able to trap a wider range of compounds than either TA1TD or TenaxGR. For studies targeting light VOCs (including isoprene and acetone) TA5TD should be chosen and the sampling volume should be reduced below 500 ml to prevent breakthrough from occurring.

- TA1TD and TenaxGR retained significantly less water, therefore requiring less dry purging and potentially offering greater pre-purge storage stability. Therefore, for studies that are sampled at sites where dry purging cannot be performed before shipping TA1TD or TenaxGR are most applicable. Of the two, TA1TD exhibited higher breakthrough volumes than TenaxGR for lighter compounds.
- Finally, if a study requires sampling at a distant location from analytical laboratory then selecting a non-friable sorbent material could reduce the chance of sorbent breakdown during transport. If durability is a key concern, TenaxGR should be chosen.

This work has compared the suitability of three different sorbents for the offline analysis of breath VOCs. All three sorbents performed well suggesting that selection of one of these three sorbent tubes over another is unlikely to be a critical factor in experimental design. Each sorbent material offers certain advantages that render it more suitable to specific studies. However, it is critical that sorbent tubes are dry purged before storage and analysis to prevent the degradation of breath samples and a possible method for this has been described here. Furthermore, the impact of relative humidity during the loading of VOCs onto sorbent materials has been highlighted and should be a consideration in the design and application of future breath sampling devices.

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#### References

- Armbruster D A and Pry T 2008 Limit of blank, limit of detection and limit of quantitation *Clin. Biochem. Rev.* **29** 49–52
- Biagini D *et al* 2017 Determination of volatile organic compounds in exhaled breath of heart failure patients by needle trap micro-extraction coupled with gas chromatography-tandem mass spectrometry *J. Breath Res.* **11** 047110
- Brinkman P, Fens N, van de Pol M A, Sterk P J and Sinha A 2016 Identification of exhaled volatile organic compounds (VOCs) associated with loss of asthma control *Eur. Respir. J.* **48** PA1840
- Buist H E, Wit-bos de L, Bouwman T and Vaes W H J 2012 Predicting blood: airpartition coefficients using basic physicochemical properties *Regul. Toxicol. Pharm.* **62** 23–28
- Comes P, Gonzalez-Flesca N, Menard T and Grimalt J O 1993 Langmuir-derived equations for the prediction of solid adsorbent breakthrough volumes of volatile organic compounds in atmospheric emission effluents *Anal. Chem.* **65** 1048–53
- Dewulf J and Van Langenhove H 1999 Anthropogenic volatile organic compounds in ambient air and natural waters: a review on recent developments of analytical methodology, performance and interpretation of field measurements *J. Chromatogr. A* **843** 163–77
- Domingo-Almenara X *et al* 2016 ERah: a computational tool integrating spectral deconvolution and alignment with

- quantification and identification of metabolites in GC/MS-based metabolomics *Anal. Chem.* **88** 9821–9
- Doran S L F, Romano A and Hanna G B 2018 Optimisation of sampling parameters for standardised exhaled breath sampling *J. Breath Res.* **12** 016007
- Filipiak W, Filipiak A, Ager C, Wiesenhofer H and Amann A 2012 Optimization of sampling parameters for collection and preconcentration of alveolar air by needle traps *J. Breath Res.* **6** 027107
- Gallego E, Roca F J, Perales J F and Guardino X 2011 Comparative study of the adsorption performance of an active multi-sorbent bed tube (Carbotrap, Carboxen 569) and a radiello®diffusive sampler for the analysis of VOCs *Talanta* **85** 662–72
- Gawlowski J, Gierczak T, Pietruszynska E, Gawrys M and Niedzielski J 2000 Dry purge for the removal of water from the solid sorbents used to sample volatile organic compounds from the atmospheric air *Analyst* **125** 2112–17
- Harshman S W et al 2016 Storage stability of exhaled breath on Tenax TA *J. Breath Res.* **10** 046008
- Ho S S H, Chow J C, Watson J G, Wang L, Qu L, Dai W, Huang Y and Cao J 2017 Influences of relative humidities and temperatures on the collection of C2–C5 aliphatic hydrocarbons with multi-bed (Tenax TA, Carboxen 1TD, Carboxen 1003) sorbent tube method *Atmos. Environ.* **151** 45–51
- Kang S and Paul Thomas C L 2016 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: carbographtrap adsorbent bed from exhaled breath *J. Breath Res.* **10** 026011
- Lawal O, Ahmed W M, Nijssen T M E, Goodacre R and Fowler S J 2017 Exhaled breath analysis: a review of ‘breath-taking’ methods for off-line analysis *Metabolomics* **13** 110
- Lomonaco T, Romani A, Ghimenti S, Biagini D, Bellagambi F G, Onor M, Salvo P, Fuoco R and Di Francesco F 2018 Determination of carbonyl compounds in exhaled breath by on-sorbent derivatization coupled with thermal desorption and gas chromatography-tandem mass spectrometry *J. Breath Res.* **12** 046004
- Maceira A, Vallecillos L, Borrull F and Marcé R M 2017 New approach to resolve the humidity problem in VOC determination in outdoor air samples using solid adsorbent tubes followed by TD-GC-MS *Sci. Total Environ.* **599–600** 1718–27
- Macleod G and Ames J M 1986 Comparative assessment of the artefact background on thermal desorption of tenax GC and tenax TA *J. Chromatogr. A* **355** 393–8
- Maier I and Fieber M 1988 Retention characteristics of volatile compounds on Tenax-Ta *J. High. Resolut. Chromatogr.* **11** 566–76
- Parsons H M, Ekman D R, Collette T W and Viant M R 2009 Spectral relative standard deviation: A practical benchmark in metabolomics *Analyst* **134** 478–85
- Peng C-Y and Batterman S 2000 Performance evaluation of a sorbent tube sampling method using short path thermal desorption for volatile organic compounds *J. Environ. Monit.* **2** 313–24
- Ratray N J W, Hamrang Z, Trivedi D K, Goodacre R and Fowler S J 2014 Taking your breath away: metabolomics breathes life in to personalized medicine *Trends Biotechnol.* **32** 538–48
- Rothweiler H, Wäger P A and Schlatter C 1991 Comparison of tenax Ta and carbotrap for sampling and analysis of volatile organic compounds in air *Atmos. Environ. B* **25** 231–5
- Sanchez J M and Sacks R D 2005 On-line multi-bed sorption trap for VOC analysis of large-volume vapor samples: injection plug width, effects of water vapor and sample decomposition *J. Sep. Sci.* **28** 22–30
- Thompson M and Ellison S L R 2012 Dark uncertainty *Anal. Methods* **4** 2609–12
- Trabue S L, Scoggin K D, Li H, Burns R and Xin H 2008 Field sampling method for quantifying odorants in humid environments *Environ. Sci. Technol.* **42** 3745–50
- Uhde E and Salthammer T 2007 Impact of reaction products from building materials and furnishings on indoor air quality-A review of recent advances in indoor chemistry *Atmos. Environ.* **41** 3111–28
- White I R and Fowler S J 2018 Capturing and storing exhaled breath for offline analysis *Breath Analysis* (Amsterdam: Elsevier) pp 13–31
- Wilde M J et al 2019 Breath analysis by two-dimensional gas chromatography with dual flame ionisation and mass spectrometric detection—method optimisation and integration within a large-scale clinical study *J. Chromatogr. A* **1594** 160–72
- Woolfenden E 1997 Monitoring vocs in air using sorbent tubes followed by thermal desorption-capillary gc analysis: summary of data and practical guidelines *J. Air Waste Manage. Assoc.* **47** 20–36
- Woolfenden E 2010 Sorbent-based sampling methods for volatile and semi-volatile organic compounds in air. Part 2. Sorbent selection and other aspects of optimizing air monitoring methods *J. Chromatogr. A* **1217** 2685–94
- Xu Z Q et al 2013 A nanomaterial-based breath test for distinguishing gastric cancer from benign gastric conditions *Br. J. Cancer* **108** 941–50