

ACCEPTED MANUSCRIPT • OPEN ACCESS

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

To cite this article before publication: Maxim Wilkinson *et al* 2020 *J. Breath Res.* in press <https://doi.org/10.1088/1752-7163/ab7e17>

Manuscript version: Accepted Manuscript

Accepted Manuscript is “the version of the article accepted for publication including all changes made as a result of the peer review process, and which may also include the addition to the article by IOP Publishing of a header, an article ID, a cover sheet and/or an ‘Accepted Manuscript’ watermark, but excluding any other editing, typesetting or other changes made by IOP Publishing and/or its licensors”

This Accepted Manuscript is © 2020 The Author(s). Published by IOP Publishing Ltd.

As the Version of Record of this article is going to be / has been published on a gold open access basis under a CC BY 3.0 licence, this Accepted Manuscript is available for reuse under a CC BY 3.0 licence immediately.

Everyone is permitted to use all or part of the original content in this article, provided that they adhere to all the terms of the licence <https://creativecommons.org/licenses/by/3.0>

Although reasonable endeavours have been taken to obtain all necessary permissions from third parties to include their copyrighted content within this article, their full citation and copyright line may not be present in this Accepted Manuscript version. Before using any content from this article, please refer to the Version of Record on IOPscience once published for full citation and copyright details, as permissions may be required. All third party content is fully copyright protected and is not published on a gold open access basis under a CC BY licence, unless that is specifically stated in the figure caption in the Version of Record.

View the [article online](#) for updates and enhancements.

Effects of High Relative Humidity and Dry Purging on VOCs Obtained During Breath Sampling on Common Sorbent Tubes

Maxim Wilkinson^a, Iain R. White^b, Royston Goodacre^c, Tamara Nijssen^d, Stephen J. Fowler^{a,e*}

^a Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

^b Laboratory for Environmental and Life Sciences, University of Nova Gorica, Vipavska 13, SI-5000 Nova Gorica, Slovenia

^c Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Biosciences Building, Crown Street, Liverpool L69 7ZB, UK

^d Philips Research, Philips, Eindhoven, Netherlands

^e Manchester Academic Health Science Centre and NIHR Biomedical Research Centre, Manchester University Hospitals NHS Foundation Trust, UK

Accepted Manuscript

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 H₂O min⁻¹ for TenaxGR, TenaxTA/Carbograph1TD and TenaxTA/Carbograph5TD respectively when flushed with 50 ml min⁻¹ of N₂. 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.

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 (Rattray *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 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 (Dewulf and Van Langenhove 1999; Rothweiler *et al.* 1991; Woolfenden 1997). These have described that the breakthrough of VOCs is affected

1
2
3 by the relative humidity (RH) of the carrier gas (Ho *et al.* 2017; Peng and Batterman 2000;
4 Trabue *et al.* 2008). If sampled directly from the mouth, exhaled breath has close to 100% RH
5 and, as such, the retention of VOCs on the tubes is likely to be affected. While all of the
6 commonly used sorbents in breath sampling tend to retain less water than the molecular
7 sieves that are prevalent in air quality monitoring, there are differences in hydrophobicity
8 between them that may affect their suitability for pre-concentrating exhaled VOCs.
9

10
11
12
13
14
15 Dry purging wet samples is important to ensure reproducible analysis of tubes sampled in
16 high RH (Gawlowski *et al.* 2000). As little as 1 mg of water remaining on the sorbent tube can
17 affect the TD-GC-MS process in several ways. Firstly the excess water may cause the cold trap
18 in the thermal desorption unit to double fire as ice forms during trapping. Secondly, the rapid
19 expansion of water on the trap that occurs during firing can also lead to an irreproducible split
20 during injection into the GC. Finally, further problems occur during the trap desorption onto
21 the column as temperature ramps often start well below 100 °C, allowing the water to
22 condense onto the stationary phase. Once condensed, water can inhibit the interaction of
23 analytes and the stationary phase leading to poor peak shape and irreproducible peak
24 retention and sensitivity, or may react with the stationary phase increasing the column bleed.
25
26
27
28
29
30
31
32
33

34
35 This study, aims to assess how high RH affects VOC recovery on three commonly used and
36 commercially available sorbent tubes, TenaxGR (GR), TenaxTA/ Carbotrap1TD (TA1TD) and
37 TenaxTA/Carbotrap5TD (TA5TD), in order to determine their respective strengths and
38 weaknesses for breath sampling. The repeatability of loading a standard mix onto the
39 sorbents in dry gas is determined and the impact of high RH on the adsorption of VOCs is
40 characterised. Furthermore, the weight of water retained on the sorbent tubes during
41 sampling is described and dry purging times are suggested for each sorbent. Finally, a direct
42 comparison of breath samples taken on the three sorbent materials is made and differences
43 between them are highlighted.
44
45
46
47
48
49
50
51

52 Methods

53 Sorbent tubes

54
55
56
57
58
59
60

1
2
3 All sorbent tubes were purchased new for the study (Markes International, Llantrisant, UK).
4
5 The tubes were packed with a total of 200, 350 and 400 mg of sorbent for TenaxGR, TA1TD
6
7 and TA5TD respectively. The ratio of sorbents in the dual bed tube is undisclosed by the
8
9 manufacturer.

10 11 Standard mix

12
13
14
15 A total of 29 VOCs, representative of common compounds found in breath and an alkane
16
17 ladder were diluted into methanol to form a standard mix, Table S2. Alkane ladders consist of
18
19 a continuous series of straight chain alkanes (i.e. hexane to pentadecane) which allow the
20
21 calculation of generalised retention indices for the compounds in breath. The standard mix
22
23 was loaded onto sorbent tubes using a calibration standards loading rig (Markes
24
25 International) whilst being purged with ultrapure Helium at 50 mL min⁻¹ for 2 min. To assess
26
27 the relative standard deviation of recovery for each compound, seven tubes of each sorbent
28
29 were loaded with 2 µL of the standard mix before analysis by GC-MS.

30 31 Gas loading rig

32
33
34 The gas loading rig (GLR), Figure 1, was designed to allow the simultaneous loading of a VOC
35
36 rich gas onto multiple sorbent tubes. Breath or the standard mix were loaded onto TA5TD
37
38 sorbent tubes which were then placed into an Ultra autosampler (Markes International)
39
40 connected to a modified Unity 2 thermal desorption system (Markes International). Tubes
41
42 were desorbed at 280 °C for 5 mins with the trap not in-line and flow was diverted through
43
44 1.5" Teflon tube connected to a 1/4" PTFE tee piece with the through port blocked off to
45
46 create an elbow joint. This was connected to 1/16" Teflon tubing which split flow into two
47
48 through a 1/16" PFA tee piece. Carrier flow was constricted by the Teflon capillary tubing and
49
50 controlled by the pneumatics on a 6890 Agilent GC (Agilent, SantaClara, CA, USA). Desorbed
51
52 VOCs were dynamically diluted in two 1/4" PTFE tee-pieces (Swagelok, Solon, OH, USA) where
53
54 they mixed with dry or humid N₂ before being purged onto target sorbent tubes for analysis
55
56 and comparison.
57
58
59
60

1
2
3 Dry and humid diluent was generated by flowing N₂ (OFN, BOC gases, UK) through a Big
4 universal trap (Agilent) to a stainless steel tee-piece (Swagelok) and, once split, through 250
5 mL glass Dreschel flasks which were either empty or contained 200ml of water. The flows of
6 humidified and dry N₂ were both maintained at 200 mL min⁻¹ using needle valves (Swagelok).
7
8 humidified and dry N₂ were both maintained at 200 mL min⁻¹ using needle valves (Swagelok).
9
10 Two loading volumes, 500 and 1000 mL were used to match the collections volumes of the
11 ReCIVA. The flasks were placed on a heater plate (Cole-Parmer, Staffordshire, UK) set to 60
12 °C and were wrapped in heater tape (Briskheat, Columbus, US) set to 34 °C. All transfer lines
13 from the flasks and thermal desorption system to the target sorbent tubes were also heated
14 with the same tape.
15
16 from the flasks and thermal desorption system to the target sorbent tubes were also heated
17 with the same tape.
18
19
20

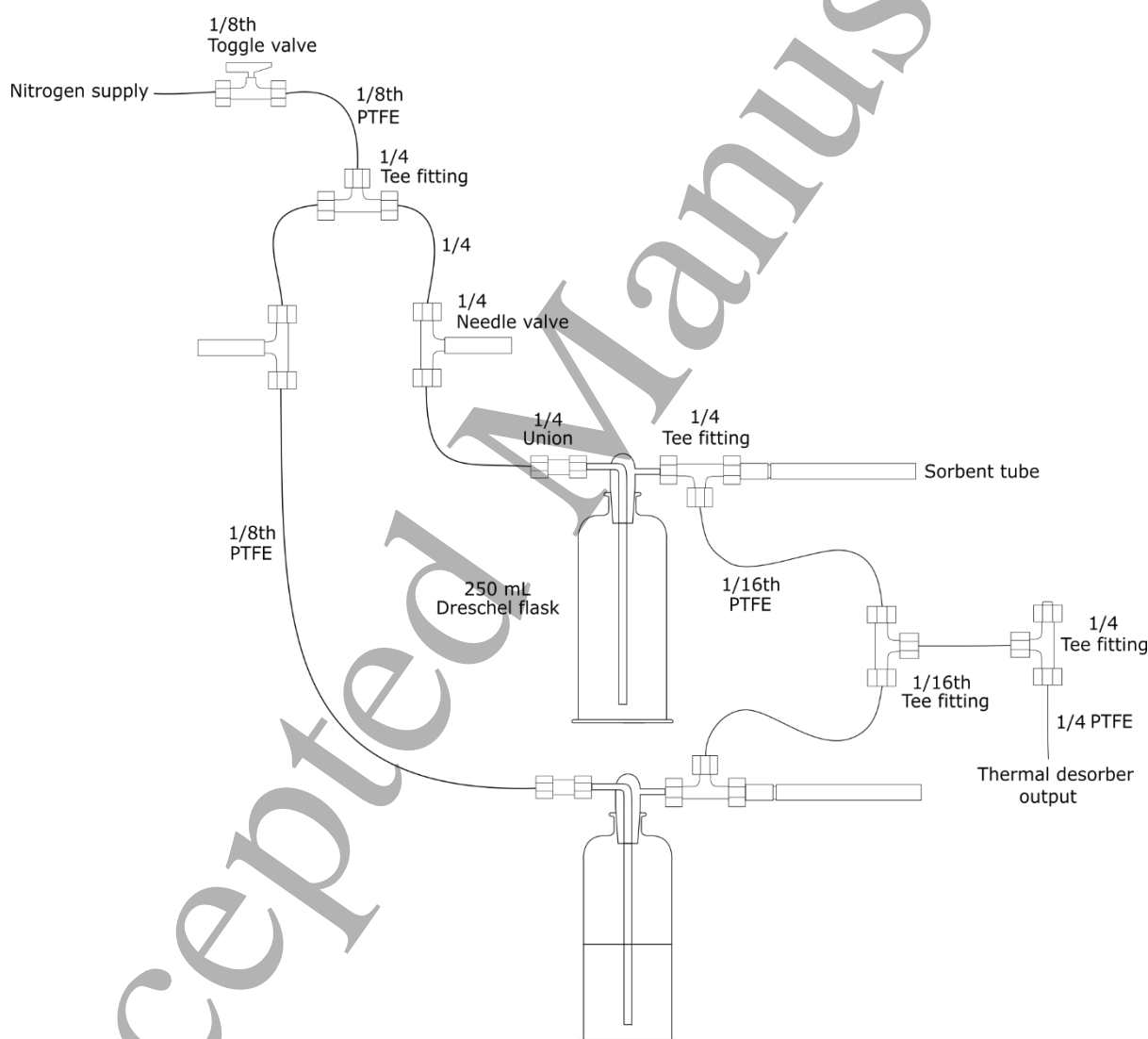


Figure 1 - Design of the gas loading rig, all fractions are in inches.

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_2 , resulting in a per tube flow of 50 mL min^{-1} . The tubes were not heated during purging. All purging was carried out in the direction of sampling to minimize breakthrough of VOCs.

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^{-1} 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^{-1} . 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 $180 \text{ }^\circ\text{C}$ before re-use to remove siloxane contaminants. A disposable filter disk (Philips

1
2
3 Respironics, Eindhoven, Netherlands) was used between the mask and the ReCIVA to prevent
4 contamination.
5
6

7 8 Thermal desorption-gas chromatography-mass spectrometry 9

10
11 For analysis, 100 μL of a gaseous calibration standard (1 ppmv, 4-Bromofluorobenzene in
12 nitrogen, Thames Restek, UK) was loaded onto all tubes as an internal standard. Before
13 desorption, tubes were pre-purged for 2 minutes in 50 mL min^{-1} He carrier gas to ensure air
14 and any remaining moisture was removed from the tubes. Sorbent tubes were then desorbed
15 at 320 $^{\circ}\text{C}$ in 50 mL min^{-1} He carrier gas using a TD-100 (Markes International) onto a general
16 purpose hydrophobic trap (Markes International) for cryo-focussing at 0 $^{\circ}\text{C}$. VOCs were
17 desorbed at 330 $^{\circ}\text{C}$ into the GC column capillary gas chromatograph (7890B GC, Agilent,
18 SantaClara, CA, USA) fitted with a DB-5 ms Ultra Inert column (length 30 m \times internal diameter
19 0.25 mm, film thickness 25 μm , (5%-Phenyl)-methylpolysiloxane, Agilent) using the following
20 temperature ramp: 40 $^{\circ}\text{C}$ hold 0 min, ramp 6 $^{\circ}\text{C min}^{-1}$ to 170 $^{\circ}\text{C}$, hold 0 min, ramp 15 $^{\circ}\text{C min}^{-1}$
21 to 190 $^{\circ}\text{C}$ to a total time of 23 min with an He carrier gas flow of 1.3 mL min^{-1} . A triple-
22 quadrupole mass spectrometer (7010, Agilent) was used in full scan mode (MS1) with a scan
23 range of m/z 40–500 Da at 4 Hz using electron ionization at 70 eV.
24
25
26
27
28
29
30
31
32
33
34
35

36 Data analysis 37

38
39 All files were converted to the open mzXML format prior to pre-processing. Chromatograms
40 were screened for inclusion in the final dataset by manual appraisal and all samples
41 deconvolved and aligned using eRah (Domingo-Almenara *et al.* 2016) for untargeted work
42 and Masshunter Quantitative analysis (Agilent) for targeted analysis. For the eRah
43 deconvolution abundance was calculated as the peak area for selected ions whilst the peak
44 area for a single extracted ion was used during the targeted analysis. All peak areas were
45 normalised to the internal standard on a run by run basis. Data processing was performed in
46 R. Univariate comparisons were made with Wilcoxon tests and p -values adjusted for multiple
47 comparisons by Bonferroni correction. Comparison of the gradients was by analysis of
48 covariance (ANCOVA). Multivariate data analysis was performed by principal component
49 discriminant function analysis (PC-DFA). Limits of detection were calculated as the mean + 3.3
50 \times the standard deviation of the signal in the background samples (Armbruster and Pry 2008).
51
52
53
54
55
56
57
58
59
60

Results and discussion

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.

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.

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.

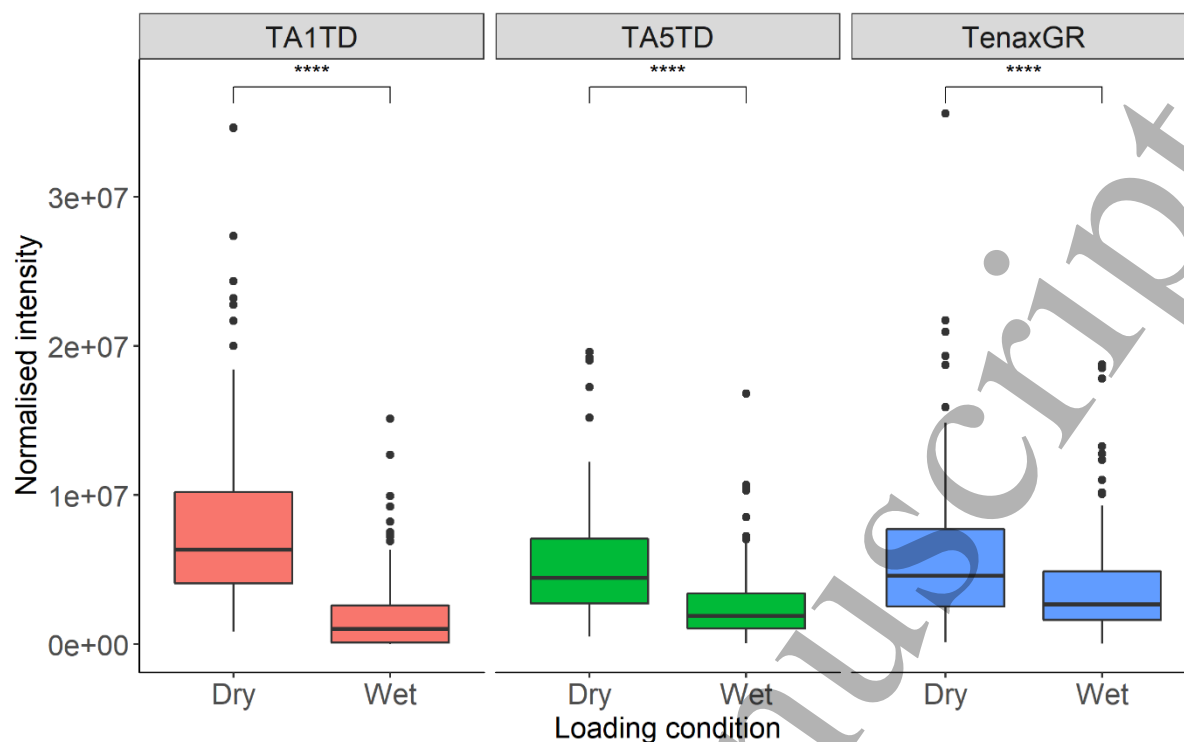


Figure 2– Box and whisker plot of the impact of loading VOCs onto sorbent tubes in wet and dry gas for all 29 compounds in the standard mix. Significance levels are shown as $p < 0.0001$ - **** based on a Wilcoxon test. The whiskers here represent the quartiles $\pm 1.5 \times$ the interquartile range.

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 (LogK_{ow}) can be observed for the majority of the compounds, Figure 3, Table S4. LogK_{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

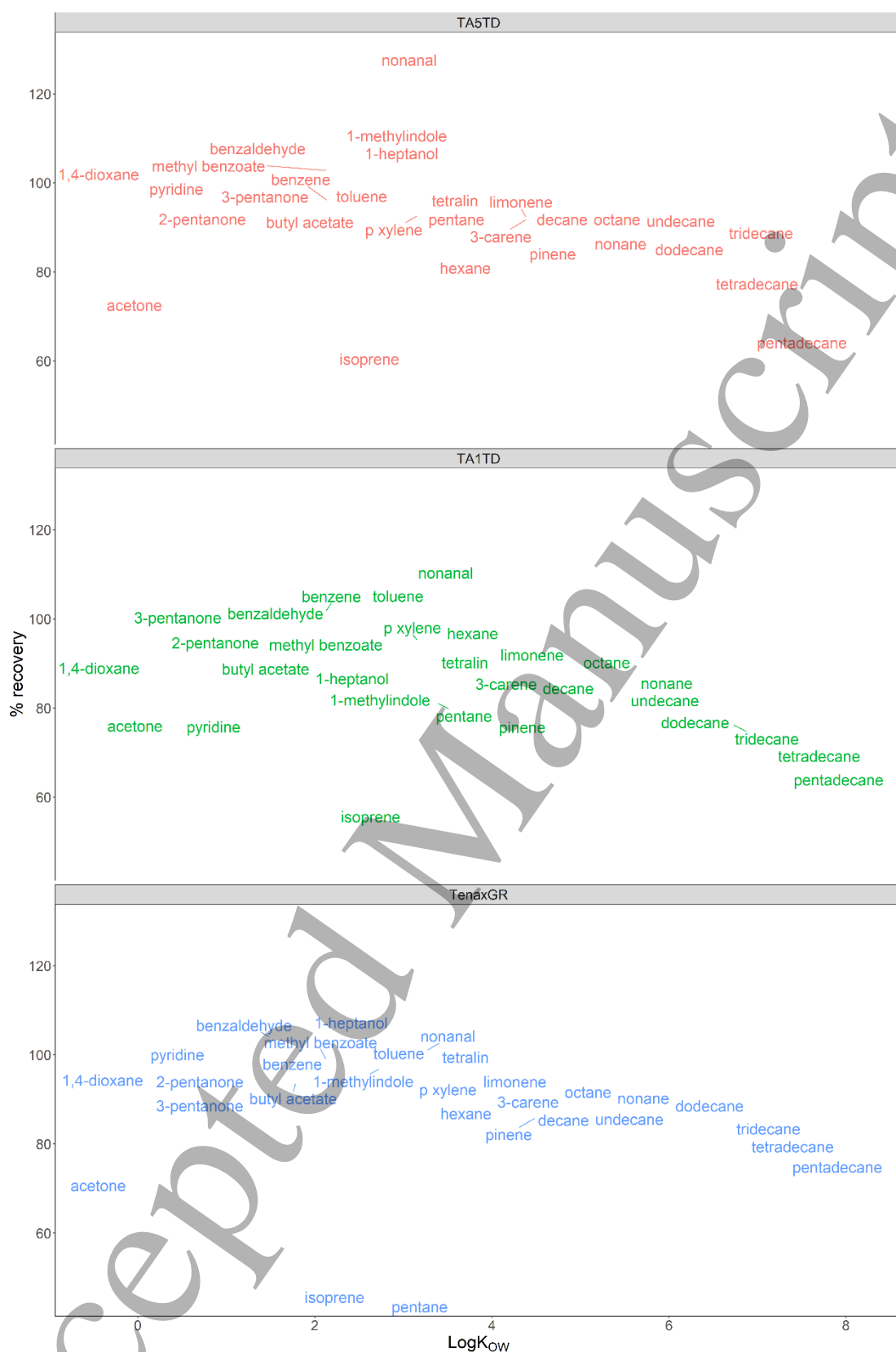


Figure 3 - Interaction between the hydrophobicity of a compound and its percentage recovery when loaded onto tubes packed with each sorbent in wet versus dry gas. Where LogK_{ow} is the logarithm of the octanol-water partition coefficient.

1
2
3 very low in TenaxGR when considering its logK_{ow} value; this can be attributed to poor
4 quantification of the compound due to breakthrough. For all three sorbents acetone and
5 isoprene were recovered at lower levels than expected for their logK_{ow} values, likely due to
6 breakthrough, though further tests would be required to demonstrate this.
7
8
9

10
11 Previous work in air quality analysis has shown that breakthrough volumes are reduced when
12 sampling in a humid environment (Ho *et al.* 2017; Trabue *et al.* 2008). The results presented
13 here also demonstrated a reduction in the intensity of recovered compounds in wet gas and
14 report that compound class and chemical properties affect recovery at high RH. However, the
15 reduced sensitivity does not appear to be due to simple VOC breakthrough as the largest
16 differences between the wet and dry loading were observed for the heaviest compounds
17 which have the highest theoretical breakthrough. Ho *et al.* (2017) posit that as sorbent tubes
18 become loaded with water, access to the binding sites on the sorptive material is thus
19 inhibited. Polar compounds, with low LogK_{ow} values, more readily dissolve and are ultimately
20 able to displace water from the active sites. This trend can be most clearly observed for the
21 alkane ladder in the standard mix where, as the alkane chain gets longer, and therefore less
22 water soluble, the percentage recovery of wet *versus* dry is reduced. This is an important
23 consideration, as it may increase the method detection limit for compounds in breath and
24 prevent highly insoluble trace gases from being detected. It also highlights the importance of
25 rigorous analytical protocols when quantifying breath VOCs. Where possible synthetic breath
26 gas standards should be analysed in moist air and analytical protocol validation should detail
27 the relative humidity used.
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43

44 Water loaded during breath sampling

45
46 The weight of water loaded onto each type of sorbent was assessed for 500 and 1000 mL of
47 breath sampled using the ReCIVA. The mean (standard deviation) collection times were 473 s
48 (± 22) and 899 s (± 43) for 500 and 1000 mL respectively. The dual bed TA5TD tubes retained
49 a greater volume of water ($p < 0.0001$) than either of the other two sorbents at both sampling
50 volumes. TA1TD retained the least water ($p < 0.01$ *versus* TenaxGR) at both sampling volumes,
51 Figure 4.
52
53
54
55
56
57
58
59
60

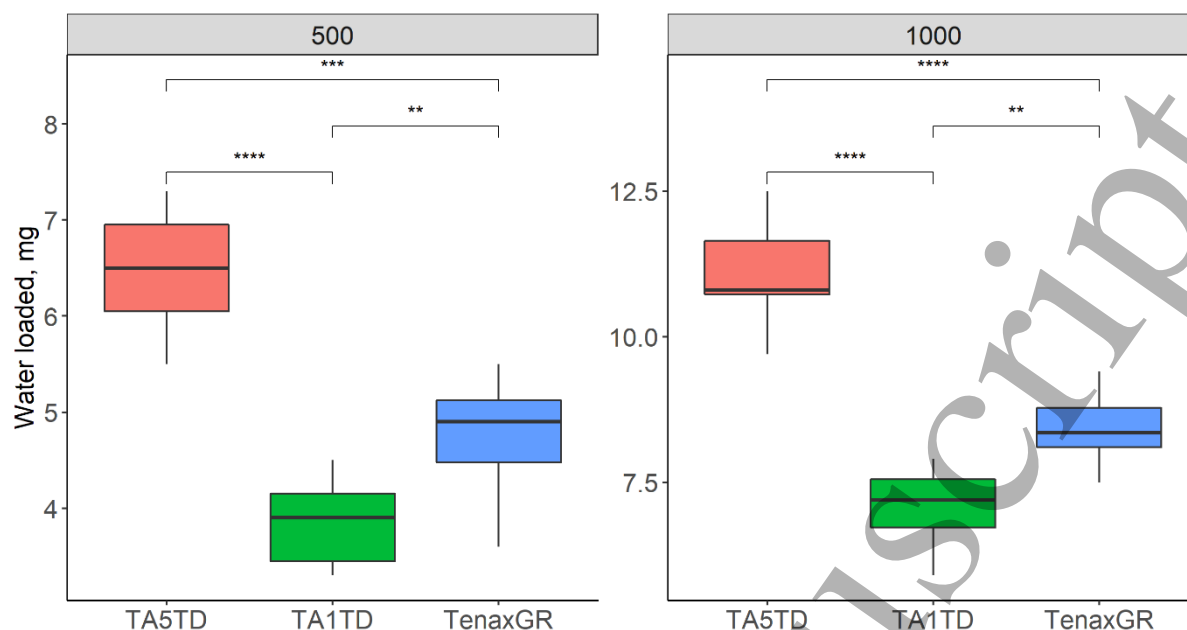


Figure 4 – Box and whisker plot of the weight of water loaded onto the different sorbent materials while using the ReCIVA sampling device to collect either 500 or 1000 mL of breath. Significance levels are shown as; $p < 0.01$ - **; $p < 0.001$ - ***; $p < 0.0001$ - **** based on a Wilcoxon test. The whiskers here represent the quartiles ± 1.5 x the interquartile range.

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 the levels observed during ReCIVA sampling. TenaxGR and TA1TD exhibited similar rates of purging, losing 1.14 and 1.13 mg min^{-1} of water respectively ($p > 0.05$). Water was lost more slowly from the TA5TD tubes at a rate of 0.89 mg min^{-1} ($p < 0.001$), Figure S2. Purging times at $50 \text{ mL min}^{-1} \text{ N}_2$ 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.

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 (Wilde *et al.* 2019; Doran *et al.* 2018) but resulted in data that were too low

1
2
3 quality for inclusion. Following deconvolution the aligned and cleaned peak list contained 103
4 compounds. Separation between the different sorbents was observed after applying a PC-
5 DFA to these data, primarily across linear discriminant (LD) 1, Figure 5
6
7
8
9

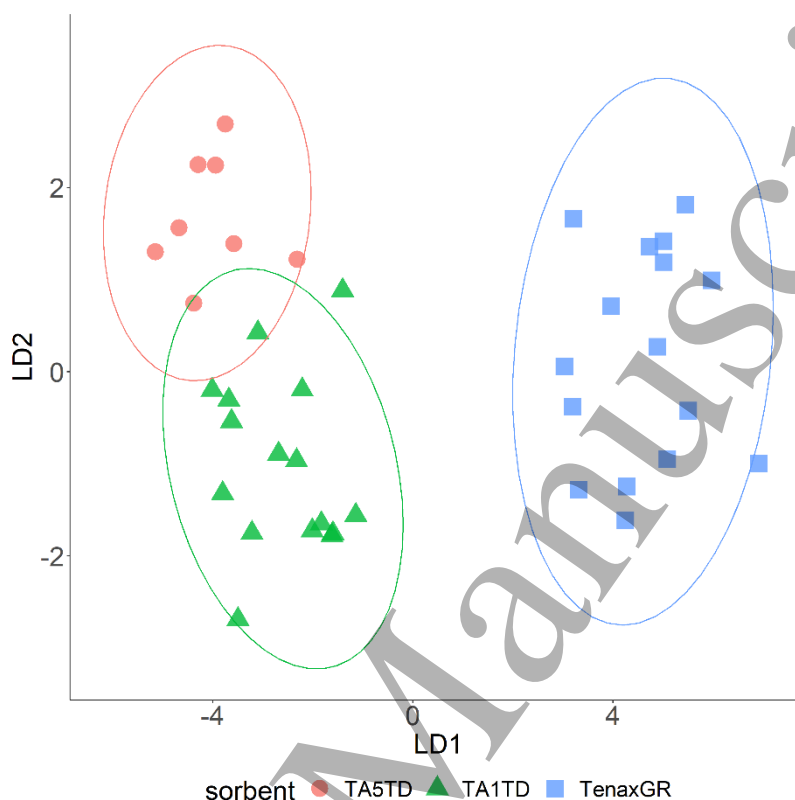


Figure .

Figure 5 – Principal component discriminant function analysis of breath samples from the ReCIVA on three different sorbent materials. The ovals represent the 95% confidence intervals for each of the point clouds.

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.

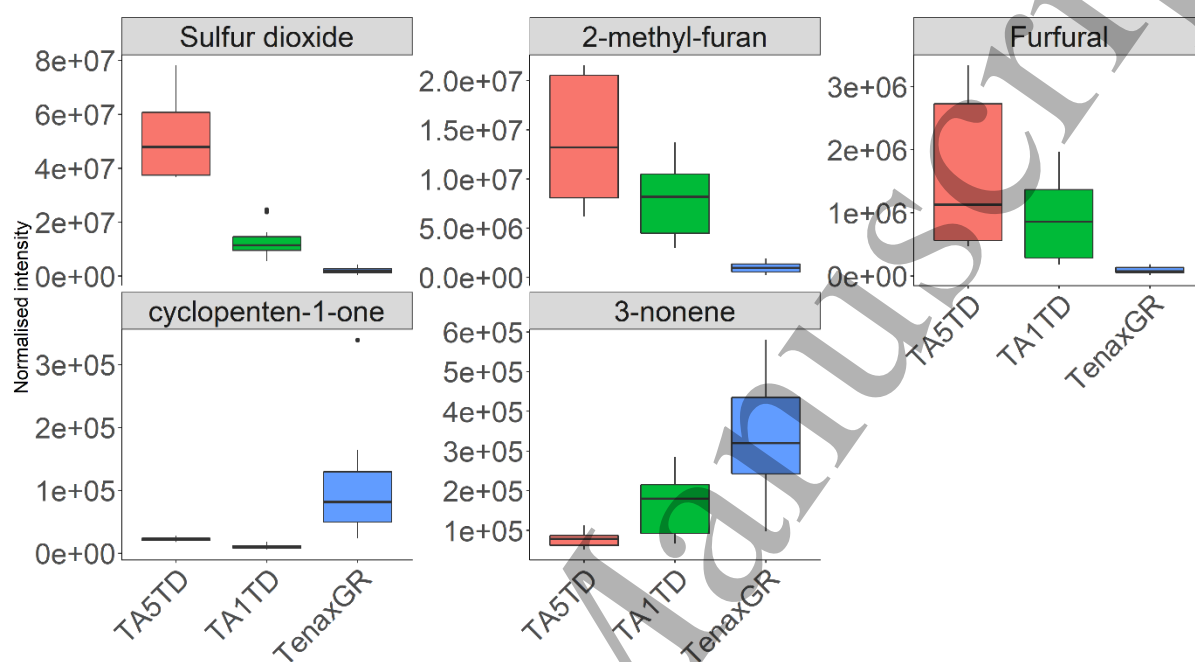


Figure 6 – Box and whisker plot of the compounds selected from the loadings plot of the principal component discriminant function analysis of breath samples from the ReCIVA. The whiskers here represent the quartiles ± 1.5 x the interquartile range.

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

1
2
3 saturated with water, particularly for carbonyl compounds (Lomonaco *et al.* 2018; Sanchez
4 and Sacks 2005). It may be important to reduce the humidity of the gas being drawn over
5 sorbent tubes during sampling in order to improve sampling accuracy and mechanisms for
6 achieving this have been suggested in air quality monitoring studies (Maceira *et al.* 2017).
7 Efficient dry purging of tubes is important to ensure accurate and reliable analysis and
8 optimised dry purge volumes are necessary to prevent analytes of interest from breaking
9 through the sorbent material as water is removed (Gawłowski *et al.* 2000). A method for doing
10 so is presented in this work but further optimisation is likely possible. For example, the dry
11 purge flow rate was not optimised and it is likely that shorter purge times could be achieved
12 with higher flows. Furthermore Ho *et al.* (2017) highlight that tube temperature might be an
13 important factor to consider during dry purging to reduce the loss of volatiles of interest.
14 Alternative methods of trapping VOCs such as needle trap micro extraction (NTME) have been
15 shown to provide reliable results when applied to breath sampling (Filipiak *et al.* 2012; Biagini
16 *et al.*, 2017). Additionally NTME does not retain water during sampling removing the need for
17 a dry purging step and for certain applications this technique could be used instead of sorbent
18 tubes.

19
20 For untargeted studies, choosing the best sorbent material for breath sampling remains
21 challenging. Based on repeat analyses of breath samples, all three sorbent materials
22 performed well, with only five compounds highlighted as significantly different between
23 sorbent types. It should be noted that the same trap was used for the analysis of all the
24 samples rather than attempting to match the trap to the tube. It is possible that this had a
25 dampening effect on the comparison of the sorbents, making it harder to identify subtle
26 differences between them. Of the differences observed, three of the five compounds
27 identified as being of interest from the loadings plot were shown to be sorbent background
28 contaminants. All the sorbent materials used in the study give off contaminant VOCs which
29 can affect the limits of detection that can be achieved for co-eluting compounds. While many
30 of these compounds are found at very low intensities they can include alkanes, benzene and
31 toluene, xylene and several aldehydes in Tenax tubes (MacLeod and Ames 1986; Uhde and
32 Salthammer, 2007). Importantly, these background contaminants are often also endogenous
33 breath VOCs of potential interest. For example, 2-methyl-furan and furfural were found in the
34 samples taken on dual bed sorbent tubes with the highest recovery in TA5TD. Both
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 compounds have been previously found in human breath: furfural has been suggested as a
4 marker to discriminate healthy controls from those with gastric cancer (Xu *et al.* 2013) and 2-
5 methyl-furan was linked to a loss of asthma control (Brinkman *et al.* 2016). Both compounds
6 are also known additives to food products as flavourings and are likely to arise from
7 exogenous sources. The compounds share high levels of structural similarity and, in the
8 present study, it is possible that one of them was ingested and subsequently metabolised to
9 generate the other. It was expected that the tubes packed with TA5TD would show better
10 retention of highly volatile compounds due to the increased sorbent strength of Carbograp
11 5TD. This was borne out by the higher concentrations of 2-methyl furan.

12
13
14
15
16
17
18
19
20
21 This study was mainly focussed on the impact of water retention on the ability of sorbent
22 materials to retain VOCs. There are also other properties that may impact their use for
23 different types of studies. The friability (the tendency of a compound breaking down due to
24 compression) of the sorbent materials is another key factor for consideration in studies that
25 involve shipping tubes between the sampling location and the laboratory. This is due to the
26 higher chance of mechanical impact that may damage the sorbent material. Graphitised
27 carbon blacks such as Carbograp 1TD and Carbograp 5TD are particularly vulnerable to this
28 kind of damage and scanning electron microscopy may provide useful insights into the impact
29 of mechanical shocks to sorbent materials. The number of thermal cycles that each tube can
30 undergo before the artefacts from the thermal desorption start to impact limits of detection
31 was also outside the remit of this study, but is interesting to consider due to the high costs
32 incurred in regularly replacing sorbent tubes.

33 34 35 36 37 38 39 40 41 42 43 44 Recommendations and Conclusion

45
46
47 Our results indicate that all of the sorbent materials under investigation are effected by high
48 RH and that there were few distinguishing compounds observed when comparing breath
49 samples. To help determine which sorbent material is most applicable for future studies the
50 following recommendations are made:
51
52

- 53
54
55
56 • TA5TD tubes are able to trap a wider range of compounds than either TA1TD or
57 TenaxGR. For studies targeting light VOCs (including isoprene and acetone) TA5TD
58
59
60

1
2
3 should be chosen and the sampling volume should be reduced below 500 mL to
4 prevent breakthrough from occurring.

- 5
6
7 • TA1TD and TenaxGR retained significantly less water, therefore requiring less dry
8 purging and potentially offering greater pre-purge storage stability. Therefore, for
9 studies that are sampled at sites where dry purging cannot be performed before
10 shipping TA1TD or TenaxGR are most applicable. Of the two, TA1TD exhibited higher
11 breakthrough volumes than TenaxGR for lighter compounds.
12
13 • Finally, if a study requires sampling at a distant location from analytical laboratory
14 then selecting a non-friable sorbent material could reduce the chance of sorbent
15 breakdown during transport. If durability is a key concern, TenaxGR should be chosen.
16
17
18
19
20
21

22 This work has compared the suitability of three different sorbents for the offline analysis of
23 breath VOCs. All three sorbents performed well suggesting that selection of one of these
24 three sorbent tubes over another is unlikely to be a critical factor in experimental design. Each
25 sorbent material offers certain advantages that render it more suitable to specific studies.
26 However, it is critical that sorbent tubes are dry purged before storage and analysis to prevent
27 the degradation of breath samples and a possible method for this has been described here.
28 Furthermore, the impact of relative humidity during the loading of VOCs onto sorbent
29 materials has been highlighted and should be a consideration in the design and application of
30 future breath sampling devices.
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

- Armbruster, D. A., & Pry, T. (2008). Limit of Blank, Limit of Detection and Limit of Quantitation. *Clinical Biochemist Reviews*, **29**, 49–52.
- Biagini, D., Lomonaco, T., Ghimenti, S., Bellagambi, F. G., Onor, M., Scali, M. C., Barletta, V., Marzilli, M., Salvo, P., Trivella, M. G., Fuoco, R. & Di Francesco, F. (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. *Journal of Breath Research*, **11**, 047110.
- Brinkman, P., Fens, N., van de Pol, M. A., Sterk, P. J., & Sinha, A. (2016). Identification of exhaled volatile organic compounds (VOCs) associated with loss of asthma control. *European Respiratory Journal*, **48**, PA1840.
- Buist, H. E., Wit-Bos, L. de, Bouwman, T., & Vaes, W. H. J. (2012). Predicting blood:Air partition coefficients using basic physicochemical properties. *Regulatory Toxicology and Pharmacology*, **62**, 23–28.
- Comes, P., Gonzalez-Flesca, N., Menard, T., & Grimalt, J. O. (1993). Langmuir-derived equations for the prediction of solid adsorbent breakthrough volumes of volatile organic compounds in atmospheric emission effluents. *Analytical Chemistry*, **65**, 1048–1053.
- Dewulf, J., & 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. *Journal of Chromatography A*, **843**, 163–177.
- Domingo-Almenara, X., Brezmes, J., Vinaixa, M., Samino, S., Ramirez, N., Ramon-Krauel, M., Lerin, C., Díaz, M., Ibáñez, L., Correig, X., Perera-Lluna, A., & Yanes, O. (2016). ERah: A Computational Tool Integrating Spectral Deconvolution and Alignment with Quantification and Identification of Metabolites in GC/MS-Based Metabolomics. *Analytical Chemistry*, **88**, 9821–9829.
- Doran, S. L. F., Romano, A., & Hanna, G. B. (2018). Optimisation of sampling parameters for standardised exhaled breath sampling. *Journal of Breath Research*, **12**, 016007.
- Filipiak, W., Filipiak, A., Ager, C., Wiesenhofer, H., & Amann, A. (2012). Optimization of sampling parameters for collection and preconcentration of alveolar air by needle traps. *Journal of Breath Research*, **6**, 027107.
- Gallego, E., Roca, F. J., Perales, J. F., & Guardino, X. (2011). Comparative study of the adsorption performance of an active multi-sorbent bed tube (Carbotrap, Carbopack X, Carboxen 569) and a Radiello® diffusive sampler for the analysis of VOCs. *Talanta*, **85**, 662–672.
- Gawłowski, J., Gierczak, T., Pietruszyska, E., Gawrys, M., & 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**, 2112–2117.
- Harshman, S. W., Mani, N., Geier, B. A., Kwak, J., Shepard, P., Fan, M., Sudberry, G. L., Mayes, R. S., Ott, D. K., Martin, J. A., & Grigsby, C. C. (2016). Storage stability of exhaled breath on Tenax TA. *Journal of Breath Research*, **10**, 046008.
- Ho, S. S. H., Chow, J. C., Watson, J. G., Wang, L., Qu, L., Dai, W., Huang, Y., & Cao, J. (2017). Influences of relative humidities and temperatures on the collection of C2-C5 aliphatic hydrocarbons with multi-bed (Tenax TA, Carbograph 1TD, Carboxen 1003) sorbent tube method. *Atmospheric Environment*, **151**, 45–51.

- 1
2
3 Kang, S., & Paul Thomas, C. L. (2016). How long may a breath sample be stored for at -80 °C?
4 A study of the stability of volatile organic compounds trapped onto a mixed
5 Tenax:Carbograph trap adsorbent bed from exhaled breath. *Journal of Breath Research*,
6 **10**, 026011.
7
8 Lawal, O., Ahmed, W. M., Nijssen, T. M. E., Goodacre, R., & Fowler, S. J. (2017). Exhaled breath
9 analysis: a review of 'breath-taking' methods for off-line analysis. *Metabolomics*, **13**, 110.
10 Lomonaco, T., Romani, A., Ghimenti, S., Biagini, D., Bellagambi, F. G., Onor, M., Salvo, P.,
11 Fuoco, R., & Di Francesco, F. (2018). Determination of carbonyl compounds in exhaled
12 breath by on-sorbent derivatization coupled with thermal desorption and gas
13 chromatography-tandem mass spectrometry. *Journal of Breath Research*, **12**, 046004.
14
15 Maceira, A., Vallecillos, L., Borrull, F., & Marcé, R. M. (2017). New approach to resolve the
16 humidity problem in VOC determination in outdoor air samples using solid adsorbent tubes
17 followed by TD-GC-MS. *Science of the Total Environment*, **599–600**, 1718–1727.
18
19 MacLeod, G., & Ames, J. M. (1986). Comparative assessment of the artefact background on
20 thermal desorption of tenax GC and tenax TA. *Journal of Chromatography A*, **355**, 393–
21 398.
22
23 Maier, I., & Fieber, M. (1988). Retention Characteristics of Volatile Compounds on Tenax-Ta.
24 *Journal of High Resolution Chromatography*, **11**, 566–576.
25
26 Parsons, H. M., Ekman, D. R., Collette, T. W., & Viant, M. R. (2009). Spectral relative standard
27 deviation: A practical benchmark in metabolomics. *Analyst*, **134**, 478–485.
28
29 Peng, C.-Y., & Batterman, S. (2000). Performance evaluation of a sorbent tube sampling
30 method using short path thermal desorption for volatile organic compounds. *Journal of*
31 *Environmental Monitoring*, **2**, 313–324.
32
33 Rattray, N. J. W., Hamrang, Z., Trivedi, D. K., Goodacre, R., & Fowler, S. J. (2014). Taking your
34 breath away: metabolomics breathes life in to personalized medicine. *Trends in*
35 *Biotechnology*, **32**, 538–548.
36
37 Rothweiler, H., Wäger, P. A., & Schlatter, C. (1991). Comparison of Tenax Ta and Carbotrap
38 for sampling and analysis of volatile organic compounds in air. *Atmospheric Environment.*
39 *Part B, Urban Atmosphere*, **25**, 231–235.
40
41 Sanchez, J. M., & Sacks, R. D. (2005). On-line multi-bed sorption trap for VOC analysis of large-
42 volume vapor samples: Injection plug width, effects of water vapor and sample
43 decomposition. *Journal of Separation Science*, **28**, 22–30.
44
45 Thompson, M., & Ellison, S. L. R. (2012). Dark uncertainty. *Analytical Methods*, **4**, 2609–2612.
46
47 Trabue, S. L., Scoggin, K. D., Li, H., Burns, R., & Xin, H. (2008). Field sampling method for
48 quantifying odorants in humid environments. *Environmental Science and Technology*, **42**,
49 3745–3750.
50
51 Uhde, E., & Salthammer, T. (2007). Impact of reaction products from building materials and
52 furnishings on indoor air quality-A review of recent advances in indoor chemistry.
53 *Atmospheric Environment*, **41**, 3111–3128.
54
55 White, I. R., & Fowler, S. J. (2018). Capturing and Storing Exhaled Breath for Offline Analysis.
56 *Breath Analysis*, Elsevier Inc, 13-31.
57
58 Wilde, M. J., Cordell, R. L., Salman, D., Zhao, B., Ibrahim, W., Bryant, L., Ruskiewicz, D.,
59 Singapuri, A., Free, R., Gaillard, E. A., Beardsmore, C., Thomas, C. L. P, Brightling, C. E.,
60 Siddiqui, S., and Monks, P. (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. *Journal of Chromatography A*, **1594**, 160–
172.

1
2
3 Woolfenden, E. (1997). Monitoring vocs in air using sorbent tubes followed by thermal
4 desorption-capillary gc analysis: Summary of data and practical guidelines. *Journal of the*
5 *Air and Waste Management Association*, **47**, 20–36.

6
7 Woolfenden, E. (2010). Sorbent-based sampling methods for volatile and semi-volatile
8 organic compounds in air. Part 2. Sorbent selection and other aspects of optimizing air
9 monitoring methods. *Journal of Chromatography A*, **1217**, 2685–2694.

10
11 Xu, Z. Q., Broza, Y. Y., Ionsecu, R., Tisch, U., Ding, L., Liu, H., Song, Q., Pan, Y. Y., Xiong, F. X.,
12 Gu, K. S., Sun, G. P., Chen, Z. D., Leja, M., & Haick, H. (2013). A nanomaterial-based breath
13 test for distinguishing gastric cancer from benign gastric conditions. *British Journal of*
14 *Cancer*, **108**, 941–950
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Accepted Manuscript