Optimisation of sampling parameters for standardised exhaled breath sampling

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Abstract
The lack of standardisation of breath sampling is a major contributing factor to the poor repeatability of results and hence represents a barrier to the adoption of breath tests in clinical practice. On-line and bag breath sampling have advantages but do not suit multicentre clinical studies whereas storage and robust transport are essential for the conduct of wide-scale studies. Several devices have been developed to control sampling parameters and to concentrate volatile organic compounds (VOCs) onto thermal desorption (TD) tubes and subsequently transport those tubes for laboratory analysis. We conducted three experiments to investigate (i) the fraction of breath sampled (whole versus lower expiratory exhaled breath); (ii) breath sample volume (125, 250, 500 and 1000 ml); and (iii) breath sample flow rate (400, 200, 100 and 50 ml min$^{-1}$). The target VOCs were acetone and potential volatile biomarkers for oesophago-gastric cancer belonging to the aldehyde, fatty acids and phenol chemical classes. We also examined the collection execution time and the impact of environmental contamination. The experiments showed that the use of exhaled breath-sampling devices requires the selection of optimum sampling parameters. The increase in sample volume has improved the levels of VOCs detected. However, the influence of the fraction of exhaled breath and the flow rate depends on the target VOCs measured. The concentration of potential volatile biomarkers for oesophago-gastric cancer was not significantly different between the whole and lower airway exhaled breath. While the recovery of phenols and acetone from TD tubes was lower when breath sampling was performed at a higher flow rate, other VOCs were not affected. A dedicated ‘clean air supply’ reduces the contamination from ambient air, but the breath collection device itself can be a source of contaminants. In clinical studies using VOCs to elicit potential biomarkers of gastro-oesophageal cancer, the optimum parameters are 500 mls sample volume of whole breath with a flow rate of 200 ml min$^{-1}$.

Introduction

There has been a growing research interest in the analysis of volatile organic compounds (VOCs) in exhaled breath for disease diagnosis and therapeutic monitoring, yet breath testing remains an under-utilised diagnostic tool in clinical practice. Recent publications have shown that VOCs in exhaled breath are altered in a range of diseases including oesophageal and gastric cancer [1], colorectal cancer [2], lung cancer [3–6], breast cancer [7, 8], liver disease [9, 10], asthma [11], chronic obstructive pulmonary disease [12–14], and inflammatory bowel disease [15, 16]. However, there has been a paucity of external validation studies where researchers have validated the findings in an independent population [17]. Currently, VOCs that are in routine clinical applications include exhaled nitric oxide in asthma [13, 18, 19], C urea breath testing for H. pylori [20] and hydrogen/methane testing for small bowel intestinal overgrowth [21].

The lack of standardisation of breath sampling is a major contributing factor to the poor repeatability of results [22, 23] and hence represents a major barrier to the adoption of breath tests in clinical practice. There is a lesson to be learned from the study of exhaled
nitric oxide, as a biomarker for pulmonary inflammation. A turning point for the use of nitric oxide in the management of asthma was the development of international consensus guidelines (American Thoracic and European Respiratory Societies, 2005) [24] for the standardised measurement of exhaled nitric oxide that ultimately led to its utility as a diagnostic tool in clinical practice.

The critical importance of standardisation of breath analysis techniques for the identification and quantification of VOCs has been acknowledged and investigated in recent years [22, 23, 25]. Respiratory manoeuvres have been shown to influence VOC measurements [26]. The method of collecting breath samples also affects the level and profile of the VOCs measured. On-line sampling using direct injection methods such as proton transfer reaction-mass spectrometry (PTR-MS) [27, 28] and selected ion flow tube-mass spectrometry (SIFT-MS) [29] reduce the effect of environmental contamination and loss of VOCs due to storage and transport. However, on-line measurements using PTR-MS and SIFT-MS are challenging in a clinical environment. The utility of direct measurement has practical challenges as direct sampling on a wide-scale necessitates dedicated breath analysis laboratories/clinics with significant influence on work flow and economic consequences. Nalophan, Tedlar and inertised aluminium bags have been frequently used in clinical profiling studies due to their simplicity and low cost. However, on-line and bag sampling does not suit wide-scale multicentre clinical studies where storage and robust transport methods are essential for the conduct of those studies. Several devices and techniques have been developed to concentrate VOCs onto thermal desorption (TD) tubes and subsequently transport those tubes to the laboratory for analysis. Such an approach allows the control of breath sample volume, sample flow rate and the fraction of breath sampled (whole breath including mouth air versus lower respiratory exhaled breath). Those parameters were the subject of the current investigation with the aim to determine the optimum parameters for use in clinical studies.

Methods

Three experiments were conducted to investigate (i) the fraction of breath sampled (whole breath including mouth air versus lower respiratory exhaled breath); (ii) breath sample volume (125, 250, 500 and 1000 ml); and (iii) breath sample flow rate (400, 200, 100 and 50 ml min\(^{-1}\)).

Breath-sampling device

Exhaled breath samples were collected using a standardised breath-sampling device, ‘Respiration Collector for In Vitro Analysis’ (ReCIVA\(^{TM}\)) (Owlstone Medical, Cambridge, UK) in combination with a dedicated clean air supply ‘Clean Air Supply Pump for ReCIVA’ (CASPER) (Owlstone Medical, Cambridge, UK). For every sampling episode, the ReCIVA allows exhaled breath from the subject to be concentrated onto four Tenax/Carbograph-5TD TD tubes (Markes International Ltd, Llantrisant, UK). The device permits specific fractions of exhaled breath to be collected onto TD tubes through continuous monitoring of pressure and CO\(_2\) levels within the mask during respiration with the pumps within the device being turned on in response to the appropriate phase of the respiratory cycle to allow a specific fraction of exhaled breath within the mask to be pumped onto the TD tube. The CASPER provides a continuous supply of room air at a flow rate of 40 l min\(^{-1}\) that has been passed through a scrubber containing Airpel\(^{\circ}\) (Desotec Ltd, Roeselare, Belgium) activated carbon to remove VOCs. Prior to sample collection all TD tubes were conditioned for 40 min at 330 °C using a TC-20 tube conditioner (Markes International Ltd, Llantrisant, UK). The TD tubes were stored in an airtight container at room temperature and used for sample collection within one hour of conditioning. The four-piece TD tube assembly was inserted into a clean mask for each study participant and then attached to the ReCIVA device ensuring that the TD tube and mask assembly were seated correctly within the device. The ReCIVA device was connected to the controlling computer.

Participants

Ethical approval was obtained (REC 14/LO/1136). In the first experiment, 20 patients undergoing upper gastrointestinal endoscopy at Imperial College Healthcare NHS Trust were recruited. In the second and third experiments, healthy volunteers were invited in order to be able to cope with the demands of high sample volumes and flow rates. Academic staff of the Department of Surgery and Cancer, Imperial College London participated in those experiments. All subjects were required to be non-smokers above the age of 18 and without a history of systemic or metabolic disease.

Sampling process

Prior to participation in the study volunteers were required to be fasted for a minimum of 4 h and rested for 15 min. Participants were asked to hold the ReCIVA device whilst the head strap was attached to ensure a seal is formed between the ReCIVA mask and face. On commencing exhaled breath collection, the participant was asked to perform normal tidal respiration whilst seated at rest. Standard collection parameters as specified by the manufacturers were used during exhaled breath sample collection with the ReCIVA device unless otherwise specified as part of the experimental protocol. Following sample collection the mask was disposed of and the TD tubes were capped and prepared for analysis in the VOC laboratory, Division of Surgery, St Mary’s Hospital, Imperial
Prior to analysis TD tubes were stored in an airtight container at room temperature and all TD tubes were analysed within 6 h of breath sample collection.

**Control for environmental contamination**

Prior to collecting exhaled breath samples, reference samples were collected to assess VOC contamination from the exhaled breath collection system including CASPER air supply, and the ReCIVA mask and tubing. This was performed by connecting the ReCIVA device to a glass head (AMP3 Ltd, Aldershot, UK) and setting the device sampling parameters to ‘always on’ to collect a 250 ml gas sample at a flow rate of 200 ml min$^{-1}$ onto a single TD tube (with three blank tubes in the ReCIVA TD assembly) (figure 1). A clean mask was used for each reference sample collection and comparison was made with a 250 ml room air sample simultaneously collected onto a TD tube using a hand pump (SKC Ltd, Dorset, UK) at a flow rate of 200 ml min$^{-1}$. Comparison was made between TD tube samples collected from the ReCIVA and CASPER attached to a glass head and TD tube samples collected simultaneously from the room air (with identical sample volume and flow rate for each TD tube). This process was repeated 10 times (with a new clean mask used on each occasion).

**Experimental variables**

- **Experiment 1: Fraction of breath sampled**
  Pump ‘A’ within the ReCIVA device was set to collect two TD tubes of whole breath (including mouth air) and pump ‘B’ was simultaneously set to collect two TD tubes of lower airways exhaled breath. Each participant provided a breath sample at a volume set to 250 ml per TD tube and a sample flow rate of 400 ml min$^{-1}$.

- **Experiment 2: Breath sample volume**
  Each participant was asked to provide four sequential exhaled breath samples with the sample volume per tube being set at 125, 250, 500 and 1000 ml. Pumps A and B of the ReCIVA device were set to collect whole breath air (including mouth air) onto four TD tubes with the flow rate being set at 400 ml min$^{-1}$.

- **Experiment 3: Breath sample flow rate**
  Each participant was asked to provide four sequential exhaled breath samples and the sample flow rate per tube was sequentially decreased (400, 200, 100 and 50 ml min$^{-1}$). Pumps A and B of the ReCIVA device were set to collect whole breath (including mouth air) onto four TD tubes and the sample volume was fixed to 500 ml.

**Analysis with gas chromatography mass spectrometry (GC-MS)**

Exhaled breath samples concentrated onto TD tubes were analysed using GC-MS. The TD tubes were desorbed using a Markes TD-100 TD unit (Markes International Ltd, Llantrisant, UK) using a two stage desorption programme, applying a constant flow of helium at 50 ml min$^{-1}$. In the primary desorption stage, TD tubes were dry-purged for 3 min and heated at 280 °C for 10 min. In the secondary desorption...
stage, the cold trap (U-T12ME-2S, Markes International Ltd, Llantrisant, UK) was rapidly (99 °C min⁻¹) heated to from 10 °C to 290 °C. VOCs were transferred from the TD unit to the GC by means of a capillary line heated at 140 °C. GC-MS analysis was performed using an Agilent 7890B GC with 5977A MSD (Agilent Technologies Ltd, Santa Clara, USA) equipped with a ZB-642 capillary column (60 m × 0.25 mm ID × 1.40 μm df; Phenomenex Inc., Torrance, USA) with helium used as the carrier gas (1.0 ml min⁻¹ flow rate). The GC column temperature programme was set as follows: 4 min at 40 °C, ramp to 100 °C at 5 °C min⁻¹ with a 1 min hold, ramp to 110 °C at 5 °C min⁻¹ with a 1 min hold, ramp to 200 °C at 5 °C min⁻¹ with a 1 min hold and finally ramp to 240 °C at 10 °C min⁻¹ with a 4 min hold. The MS transfer line temperature was 240 °C and El source conditions were 70 eV at 230 °C. Mass acquisition was carried out in the range 20–250 m/z with a rate of approximately 6 scans s⁻¹.

Data analysis
VOC analysis was completed using the Agilent Mass Hunter Qualitative Analysis software (Agilent Technologies Ltd, Santa Clara, USA) and VOC identification was completed using the National Institute of Standards and Technology (NIST) Mass Spectral Database version 2.0 (NIST, Boulder, USA). Targeted analysis of abundant compounds (acetone) and representative VOCs of potential volatile biomarkers for oesophago-gastric cancer [1] from the aldehyde, fatty acid and phenol groups was performed.

A two-sided non-parametric Mann–Whitney U-test or Wilcoxon signed–rank test with a Bonferroni correction was applied for analysis. For the sample volume and sample flow rate experiments Friedman’s two-way analysis of variance was used with a post-hoc Wilcoxon signed–rank test to compare the minimum and maximum concentrations for each VOC. For all tests a two-sided p value of 0.05 was deemed to be significant. All statistical analysis was conducted on SPSS Statistics software (IBM, version 24.0).

Results

Experiment 1: Fraction of breath sampled
Ten of the participants were male with a median age of 58.5 years and ten of the participants were female with a median age of 57 years. None of the target VOCs from the aldehydes, fatty acids and phenols groups or acetone were significantly different between lower airways expiratory breath and whole expiratory breath (table 1).

Seven VOCs were present in significantly different concentrations in lower airways expiratory breath compared to whole expiratory breath (supplementary table 1 is available online at stacks.iop.org/JBR/12/016007/mmedia). Methyl formate; 1,4-pentadiene; carbonic acid, dimethyl ester; sulphide, allyl methyl; and 2,3-butanediol were significantly higher in lower airways while methylene chloride and pentane, 3-ethyl were higher in whole breath.

Experiment 2: Breath sample volume
The participants were seven males with a median age of 32.0 years and three females with a median age of 30.0 years. The median time to collect a 125, 250, 500 and 1000 ml volume breath sample was 60.5; 99.5; 173.0 and 332.5 s respectively. Sixteen VOCs belonging to the chemical classes of interest (acetone, aldehydes, fatty acids and phenols) were detected. The concentrations of propanal, acetone, propanoic acid, pentanoic acid, undecanal and dodecanal were elevated with increasing volumes (table 2).

Experiment 3: Breath sample flow rate
The subjects who participated in experiment 2 also took part in this experiment. The median time to collect a breath sample at a flow rate of 50, 100, 200, 400 ml min⁻¹ was 1140.5, 625.0, 302.0 and 169 s respectively. Targeted analysis of 17 of the VOCs of interest (acetone, aldehydes, fatty acids and phenols) was performed. Acetone and phenol decreased in concentration with increasing flow rates while other did not show a statistically significant change (table 3).

Contamination from the collecting system
GC–MS analysis identified eight VOCs that were present in higher concentrations in the samples collected from the ReCIVA and CASPER collection system compared to the room air samples (table 4). Of the eight VOC contaminants associated with the breath collection system, cyclopentane was elevated in whole breath samples compared to lower airway breath samples and the remaining seven VOCs were unchanged (supplementary table 2). There were no significant changes observed across the sample volumes in the concentration of the previously listed eight VOC contaminants associated with the breath collection system (supplementary table 3). Six of the eight VOC contaminants associated with the breath collection system were changed in concentration with altering the flow rate per tube (supplementary table 4).

Discussion
This study indicates that the use of exhaled breath-sampling devices requires the selection of optimum sampling parameters. The increase in sample volume has improved the levels of VOCs detected. However, the influence of the fraction of exhaled breath and the flow rate depends on the target VOCs measured. While the concentration of potential volatile biomarkers for oesophago-gastric cancer was not significantly different between the whole and lower airway exhaled
Table 1. VOCs of interest in lower airways expiratory breath compared to whole expiratory breath.

| VOC              | Whole breath sample, peak area \((\text{peak area}^a)\) | Lower airway sample, peak area \((\text{peak area}^a)\) | Wilcoxon signed-rank test |
|------------------|--------------------------------------------------------|--------------------------------------------------------|---------------------------|
|                  | Median \((\text{fl}^a)\) | IQR \((\text{fl})\) | Median \((\text{fl}^a)\) | IQR \((\text{fl})\) | p-value |
| Formaldehyde     | 527 173                                                | 402 948–791 033                                         | 813 863                   | 619 143–1429 669 | 0.560    |
| Acetaldehyde     | 125 107                                                | 104 402–137 501                                         | 127 108                   | 106 167–141 423 | 1.000    |
| 2-Propenal       | 83 327                                                 | 69 821–96 544                                           | 75 896                    | 65 196–97 183 | 1.000    |
| Propanal         | 39 996                                                 | 34 340–50 739                                           | 40 403                    | 32 780–53 499 | 1.000    |
| Acetone          | 6899 682                                               | 5218 648–10 295 029                                     | 7935 949                  | 5830 200–13 560 797 | 0.280    |
| Acetic acid      | 365 642                                                | 252 766–494 171                                         | 366 524                   | 241 387–428 980 | 1.000    |
| Propanoic acid   | 24 542                                                 | 12 597–47 958                                           | 18 259                    | 13 969–30 386 | 1.000    |
| Hexanal          | 111 335                                                | 80 867–137 495                                         | 101 800                   | 83 450–131 637 | 1.000    |
| Pentanoic acid   | 16 262                                                 | 10 644–23 538                                           | 13 902                    | 9565–22 457 | 1.000    |
| Heptanal         | 105 843                                                | 76 090–116 402                                         | 93 077                    | 72 890–114 313 | 1.000    |
| Benzaldehyde     | 368 196                                                | 329 467–485 275                                        | 418 139                   | 328 131–455 847 | 1.000    |
| Octanal          | 551 223                                                | 298 281–603 765                                        | 476 171                   | 341 987–552 494 | 1.000    |
| Nonanal          | 1388 863                                               | 761 691–1711 686                                      | 1270 318                  | 836 723–1393 758 | 1.000    |
| Decanal          | 1455 705                                               | 792 856–2084 630                                      | 1367 987                  | 934 564–1722 434 | 1.000    |
| Undecanal        | 234 051                                                | 179 397–300 009                                        | 237 929                   | 175 552–295 314 | 1.000    |

\(^{a}\) Mass area units IQR (inter-quartile range).

breath, the level of other VOCs varied. Also, the recovery of some VOCs such as phenols and acetone from TD tubes was lower when breath sampling performed at higher flow rate but the majority of other VOCs were not affected.

It is clearly established that an important consideration in exhaled breath collection is contamination from the ambient air [30–33]. This issue has been approached by using a dedicated ‘clean air supply’ where the inspired air has been passed through a carbon based scrubber to minimise the impact of environmental contamination on the exhaled breath sample. This will minimise the effect of environmental contamination from volatile compounds with rapid wash-out rates in the body but will not be the case for volatiles with longer retention time and from long-term environmental exposure [34, 35]. The effect of environmental exposure on endogenous VOCs should be considered despite using a dedicated ‘clean air supply’ [35]. In addition, the breath collection device itself can be a source of some contaminants. Eight VOCs were present in higher concentrations in breath samples collected from the sampling equipment alone compared to paired room air samples suggesting that these VOCs are contaminants from the sampling equipment. Five VOCs out of the eight detected belonged to the chemical class of siloxanes, and thus are most likely originating from silicone-based tubing and mask materials of the ReCIVA and CASPER assembly.

In order to collect the lower airway exhaled breath, the exhaled breath collection system utilises CO₂ and pressure sensors with the expectation that VOCs transported via blood would be at higher concentrations compared to whole breath samples [36–38]. The experiments showed no significant difference in acetone or potential volatile biomarkers for oesophago-gastric cancer between whole and lower airway exhaled breath. Other VOCs significantly varied between fractions of exhaled breath suggesting an influence of the oral cavity on VOC production.

This study has demonstrated that VOC concentrations are dependent on the exhaled breath collection volumes. Within examined volumes, there was no threshold at which no further changes in VOC concentration were observed. Moreover, using higher collection volumes did not introduce greater environmental contamination into the sample from the breath collection system. Consequently, the largest possible collection volume should be used in clinical studies with careful consideration given to the clinical condition and the time that it will take to collect the samples.

In terms of the flow rates used in this study to pump exhaled breath samples onto TD tubes, the majority of the VOCs investigated showed no reproducible relationship observed between flow rate and VOC concentration. However, recoveries of acetone and phenol were lower when breath sampling was performed at higher flow rates. This may be explained by VOC breakthrough at high flow rates of these VOCs. It is clear that the lowest flow rates require the greatest length of time to collect the breath sample (1140.5 s for a flow rate of 50 ml min⁻¹ compared to 169 s for a flow rate of 400 ml min⁻¹). In addition six of the VOCs associated with contamination from the collection system were present in high concentrations at the lowest flow rate investigated. The selection of a midrange flow rate (e.g. 200 ml min⁻¹) would enable optimum collection of VOCs whilst minimising sample collection time and the impact of environmental contamination.

The study has limitations. The experiments have focused on VOCs relevant to oesophago-gastric cancer and therefore researchers are encouraged to examine target VOCs for specific diseases in future studies. We did not assess the impact of impact of additional sampling parameters such as breathing pattern, body position, and oral versus nasal respiration in this study and these
Table 2. Impact of sample volume per TD tube for VOCs of interest.

| VOC               | Sample volume, ml | Median a | IQR a | Median a | IQR a | Median a | IQR a | Friedman test | Wilcoxon signed-rank test |
|-------------------|-------------------|----------|-------|----------|-------|----------|-------|---------------|--------------------------|
|                   | 125               | 250      | 500   | 1000     |       |          |       |               |                          |
| Acetaldehyde      | 112 363           | 106 353  | 125 702 |          |       |          |       | 6.8           | 0.077                    | 0.059                   |
| Propanal          | 74 842–131 670    | 100 580–172 335 | 86 166–162 932 | 115 046–253 994 |          |          |       | 17            | 0.001 b                  | 0.007 c                |
| Acetone           | 21 961            | 30 488   | 23 636–112 073 | 38 873–103 342 |          |          |       | 30.0          | <0.001 b                 | 0.005 c                |
| Butanal           | 418 691           | 6712 743 | 11 459 756 | 15 619 327 |          |          |       |               |                          |                        |
| Propanal          | 2085 733–5831 696 | 4351 575–11 288 384 | 7419 278–16 880 679 | 10 581 387–24 400 039 |          |          |       |               |                          |                        |
| Acetone           | 28 511            | 17 413–110 369 | 17 364–101 793 |          |       |          |       | 4.4           | 0.218                    | 0.241                   |
| Acetic acid       | 14 858–65 951     | 17 413–110 369 | 17 364–101 793 |          |       |          |       | 5.9           | 0.118                    | 0.114                   |
| Propanoic acid    | 96 505            | 110 809  | 128 210 |          |       |          |       | 4.1           | 0.253                    | 0.047 c                |
| Hexanal           | 76 326–150 560    | 80 253–155 991 | 79 214–199 988 |          |       |          |       | 4.9           | 0.178                    | 0.575                   |
| Octanal           | 77 371            | 88 320  | 90 513  |          |       |          |       | 5.4           | 0.145                    | 0.445                   |
| Pentanoic acid    | 330 624           | 382 157  | 423 678 |          |       |          |       | 4.3           | 0.229                    | 0.386                   |
| Phenol            | 310 326.535      | 391 734  | 284 020.289 |          |       |          |       | 7.2           | 0.066                    | 0.037 c                |
| Nonanal           | 164 287–524 177   | 140 258–633 299 | 202 662–1032 641 |          |       |          |       | 1.4           | 0.696                    | 0.646                   |
| Decanal           | 669 111           | 663 550  | 726 121 |          |       |          |       | 0.4           | 0.948                    | 0.878                   |
| Undecanal         | 401 956–998 650   | 332 495–861 328 | 362 344–800 380 |          |       |          |       | 12            | 0.006 b                  | 0.009 c                |
| Dodecanal         | 216 983–225 469   | 233 142–235 783 | 278 510–295 615 |          |       |          |       | 8.8           | 0.033 b                  | 0.028 c                |
Table 2. (Continued.)

| Sample volume, ml | VOC | Median$^a$ | IQR$^b$ | Median$^c$ | IQR$^d$ | Median$^e$ | IQR$^f$ | Friedman test | Wilcoxon signed-rank test |
|-------------------|-----|------------|---------|------------|---------|------------|---------|---------------|------------------------|
|                   | 125 | 250        | 500     | 1000       |         |             |         |               |                        |
|                   |     |            |         |            |         |             |         | $X^2$         | $p$                    |
|                   | 226079–429 506 | 294 287–473 146 | 320 168–489 184 | 333 597–516 649 |         |             |         |               |                        |

$^a$ Mass area units IQR (inter-quartile range).

$^b$ Denotes statistical significance at <0.05 level.
Table 3. Impact of sample flow rate per TD tube for VOCs of interest.

| VOC          | Flow rate, ml min⁻¹ |
|--------------|---------------------|
|              | 50                  | 100                  | 200                  | 400                  |
|              | Median¹              | Median¹              | Median¹              | Median¹              |
|              | IQR²                 | IQR²                 | IQR²                 | IQR²                 |
| Propanal     | 97 832               | 107 690              | 111 049              | 82 082               |
|              | 72 197–186 872       | 89 651–137 598       | 70 287–169 875       | 73 081–157 092       |
| Acetone      | 25 010 712           | 27 468 955           | 14 278 464           | 6186 127             |
|              | 1620 945–45 696 886  | 17 081 594–37 286 694| 10 546 225–21 689 755| 4370 175–10 644 123  |
| Acetic acid  | 46 376               | 34 391               | 83 509               | 225 641              |
|              | 14 359–122 621       | 12 164–115 307       | 11 261–73 915        | 18 510–1740 438      |
| Butanoic acid| 21 157               | 25 794               | 10 665–52 521        | 7.1 006              |
|              | 20 854–543 325       | 16 745–464 727       | 12 281–418 713       | 0.069 025            |
| Hexanal      | 51 742               | 207 545              | 157 863              | 0.799 799            |
| Heptanal     | 167 621              | 136 794              | 115 180              | 0.285                |
| Benzaldehyde | 683 013              | 461 066              | 932 151              | 0.979 979            |
| Octanal      | 98 201–1414 038      | 123 361–1533 524     | 116 666–1482 761     | 0.575                |
| Phenol       | 713 734              | 603 515              | 668 384              | 0.169                |
| Nonanal      | 269 631–1069 165     | 357 492–1272 999     | 17 787–1048 896      | 0.047                |
| Octanoic acid| 409 728–4537 112     | 411 429–5143 492     | 104 483–3034 270     | 0.721                |
| Decanal      | 62 712               | 88 058               | 86 335               | 0.799                |
| Dodecanoic acid| 32 970–104 732      | 31 450–204 904       | 23 998–206 489       | 0.799                |
| Undecanal    | 93 895–2205 963      | 80 775–4125 535      | 63 867–3365 269      | 0.386                |
| Dodecanal    | 128 484              | 133 796              | 131 410              | 0.285                |

Friedman test: \(\chi^2\) and \(p\) values.
Wilcoxon signed-rank test: \(p\) values.
Table 3. (Continued.)

| VOC   | Flow rate, ml min⁻¹ | Friedman test | Wilcoxon signed-rank test |
|-------|---------------------|---------------|---------------------------|
|       | 50                  |               |                           |
|       | 100                 |               |                           |
|       | 200                 |               |                           |
|       | 400                 |               |                           |
| Tridecanal | Median¹ | Median¹ | Median¹ | Median¹ | X² | p  | p   |
|        | IQR¹                 | IQR¹          | IQR¹                      | IQR¹                  |    |    |     |
|        | 1213 477–2360 380   | 1245 833–2396 100 | 1045 710–1718 316 | 928 745–1272 234 | 0.1 | 0.989 | 0.799 |
|        | 415 191             | 528 554       | 531 838                   | 405 472               |    |    |     |
|        | 344 987–498 596     | 294 163–579 872 | 256 678–726 122          | 327 901–512 779       |    |    |     |

¹ Mass area units IQR (inter-quartile range).

² Denotes statistical significance at <0.05 level.
factors need to be considered to ensure that optimal and reproducible exhaled breath samples are collected in future studies [39–41]. We chose TD-GC-MS as the analytical method in this study as it represents the reference standard of VOC analysis, including exhaled breath. The type of chromatographic column and TD sorbent employed were chosen to investigate a wide spectrum of different compounds. However there is no single sampling or analytical method capable of capturing the whole spectrum of VOCs in breath and this should be considered in study design.

Conclusion

When an exhaled breath collection system is employed there is a significant effect of the sampling parameters on the measured VOCs. Also, some contaminants are produced from the breath-sampling device itself. The largest sample volume is recommended given careful attention to the clinical condition and the practicalities of the largest sample volume is recommended given careful attention to the clinical condition and the practicalities of reproducibility and analytes produced from the breath-sampling device itself. The type of chromatographic column and TD sorbent employed were chosen to investigate a wide spectrum of different compounds. However there is no single sampling or analytical method capable of capturing the whole spectrum of VOCs in breath and this should be considered in study design.

Table 4. VOCs elevated in exhaled breath collection system (ReCIVA and CASPER) compared to room air samples.

| VOC                              | Room air sample, peak area | Mask sample, peak area | Mann–Whitney U-test |
|----------------------------------|-----------------------------|------------------------|---------------------|
| Cyclopentane                     | 12 051                      | 138 112                | <0.001a             |
| Disiloxane, hexamethyl-          | 10 420                      | 19 556 638             | <0.001a             |
| 2-Oxa-1,3,5-trisiloxane, 1,1,3,3-| 14 338                      | 68 011                 | <0.001a             |
| 1,3-bis(2Z)-Hex-2-en-1-yl)-1,1,3,3-| 20 755                      | 343 046                | <0.001b             |
| Trisiloxane, octamethyl-         | 13 430                      | 17 527                 | <0.001b             |
| Heptane, 2,2,6,6-pentamethyl-    | 17 660                      | 367 240                | <0.001b             |
| Cyclotrisiloxane, hexamethyl-    | 20 804                      | 53 059                 | <0.001b             |
| 2-Propenamide                    | 15 866                      | 39 700                 | <0.001b             |

Note. Mask sample: TD tube sample collected from ReCIVA and CASPER attached to the glass head.

a Mass area units IQR (inter-quartile range).

b Denotes statistical significance at <0.05 level.

ORCID iDs

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References

[1] Kumar S, Huang J, Abbassi-Ghadil, Mackenzie H, Veselkova K A, Hoare J M, Lovat L B, Spann L P, Smith D and Hanna G B 2015 Mass spectrometric analysis of exhaled breath for the identification of volatile organic compound biomarkers in esophageal and gastric adenocarcinoma. Ann. Surg. 262 981–90

[2] Altomare D F, Di Lena M, Procetti F, Trizio L, Travaglio E, Tutino M, Dragonieri S, Meneo V and De Gennaro G 2013 Exhaled volatile organic compounds identify patients with colorectal cancer Br. J. Surg. 100 144–50

[3] Pohl D, Goldoni M, Corradi M, Acampa O, Carbognani P, Internullo E, Casalini A and Mutti A 2010 Determination of aldehydes in exhaled breath of patients with lung cancer by means of on-fiber-derivatisation SPME-GC/MS J. Chromatogr. B 878 2643–51

[4] Bajtarevic A et al 2009 Noninvasive detection of lung cancer by analysis of exhaled breath BMC Cancer 9 348

[5] Westhoff M, Litterst P, Freitag L, Uffer W, Bader S and Baumbach J 2009 Ion mobility spectrometry for the detection of volatile organic compounds in exhaled breath of patients with lung cancer: results of a pilot study Thorax 64 744–8

[6] Horvath I, Lazar Z, Gyulai N, Kolli M and Losonczy G 2009 Exhaled biomarkers in lung cancer Eur. Respir. J. 34 261–75

[7] Phillips M, Cataneo R N, Ditkoff B, Fisher P, Greenberg J, Gunawardena R, Kwon C S, Rahbavi-Oskouf and Wong C 2003 Volatile markers of breast cancer in the breath Breast J. 9 184–91

[8] Phillips M, Cataneo R N, Ditkoff B, Fisher P, Greenberg J, Gunawardena R, Kwon C S, Tietje O and Wong C 2006 Prediction of breast cancer using volatile biomarkers in the breath Breast Cancer Res. Treat. 99 19–21

[9] Alkhouiri N, Singh T, Alsabbagh E, Guirgis J, Chami T, Hanouni E, Grove D, Lopez R and Dweik R 2015 Isoprene in the exhaled breath is a novel biomarker for advanced fibrosis in patients with chronic liver disease: a pilot study Clin. Transl. Gastroenterol. 6 e112

[10] Fernandez Del Rio R, O’Hara M E, Holt A, Pemberton P, Shah T, Whitehouse T and Mayhew C A 2015 Volatile biomarkers in breath associated with liver cirrhosis—comparisons of pre- and post-liver transplant breath samples ElifeMedicine 2 1243–50
[11] Rufo J C, Madureira J, Fernandes E O and Moreira A 2016 Volatile organic compounds in asthma diagnosis: a systematic review and meta-analysis Allergy 71 175–88
[12] Phillips C, Mac Pardalain N, Syed Y, Deganello D, Claypole T and Lewis K 2014 Short-term intra-subject variation in exhaled volatile organic compounds (VOCs) in COPD patients and healthy controls and its effect on disease classification Metabolomics 4 395–408
[13] Besa V, Teschner H, Kurth J, Khan A M, Zarogoulidis P, Baumbach J I, Sommerwerck U, Freitag L and Darwiche K 2015 Exhaled volatile organic compounds discriminate patients with chronic obstructive pulmonary disease from healthy subjects Int. J. Chron. Obstruct. Pulmon. Dis. 10 399–406
[14] Allens M, Langejuaen J, Gaida A, Holz O, Schuchardt S, Hohlfeld J M and Zimmermann S 2016 Measurement of exhaled volatile organic compounds from patients with chronic obstructive pulmonary disease (COPD) using closed gas loop GC–IMS and GC–APCI–MS J. Breath Res. 10 026004
[15] Hicks L C, Huang J, Kumar S, Powles S T, Orchard T R, Hanna G B and Williams H R 2015 Analysis of exhaled breath volatile organic compounds in inflammatory bowel disease: a pilot study J. Crohns Colitis 9 731–7
[16] Rieder F et al 2016 A distinct colon–derived breath metabolome is associated with inflammatory bowel disease, but not its complications Clin. Transl. Gastroenterol. 7 e201
[17] Phillips M, Bauer T L, Cataneo R N, Lebauer C, Mundada M, Pass H I, Ramakrishna N, Rom W N and Vallières E 2015 Blinded validation of breath biomarkers of lung cancer, a potential ancillary to chest CT screening PLoS One 10 e0142484
[18] Galkéimer F, Gualar-Hoyas C, Beardmore C S, Pandya H C and Thomas C P 2013 Metabolomics pilot study to identify volatile organic compound markers of childhood asthma in exhaled breath Bioanalysis 5 2339–47
[19] Robroek C M, van Berkel J J, Jobisz Q, van Schooten F J, Dallinga J W, Wouters E F and Dompeling E 2013 Exhaled volatile organic compounds predict exacerbations of childhood asthma in a 1 year prospective study Eur. Respir. J. 42 98–106
[20] Atreja A, Fu A Z, Sanaka M R and Vargh J 2010 Non-invasive testing for Helicobacter pylori in patients hospitalized with peptic ulcer haemorrhage: a cost-effectiveness analysis Dig. Dis. Sci. 55 1356–63
[21] Petrone P, Sarkiyan G, Fernandez M, Coloma E, Akopian G, Ortega A and Kaufman H S 2011 Small intestinal bacterial overgrowth in patients with lower gastrointestinal symptoms and a history of previous abdominal surgery Arch. Surg. 146 444–7
[22] Herbig J and Beauchamp J 2014 Towards standardization in the analysis of breath gas volatiles J. Breath Res. 8 037101
[23] Risby T H 2008 Critical issues for breath analysis J. Breath Res. 2 030302
[24] American Thoracic Society; European Respiratory Society 2005 ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower expiratory nitric oxide and nasal nitric oxide Am. J. Respir. Crit. Care Med. 171 912–30
[25] Beauchamp J D and Pleil J D 2013 Simply breath-taking? Developing a strategy for consistent breath sampling J. Breath Res. 7 042001
[26] Boshier P R, Priest O H, Hanna G B and Marczin N 2011 Influence of respiratory variables on the on-line detection of exhaled trace gases by PTR-MS Thorax 66 919–20
[27] Schwarz K, Filipiak W and Anman A 2009 Determining concentration patterns of volatile compounds in exhaled breath by PTR-MS J. Breath Res. 3 27002
[28] Ellis A M and Mayhew C A 2014 Proton Transfer Reaction Mass Spectrometry: Principles and Applications (New York: Wiley)
[29] Smith D and Spanel P 2005 Selected ion flow tube mass spectrometry (SIFT–MS) for on-line trace gas analysis Mass Spectrom. Rev. 24 661–709
[30] Phillips M, Greenberg J and Sabas M 1994 Alveolar gradient of carbon dioxide in normal human subjects J. Appl. Physiol. 78 1364–74
[31] Schubert J K, Miekiisch W, Birken T, Geiger K and Nöldge-Schomburg G F 2003 Impact of inspired substance concentrations on the results of breath analysis in mechanically ventilated patients Biomarkers 10 138–52
[32] Mauerer F, Wolf A, Fink T, Rittershofer B, Heim N, Volk T, Baumbach J I and Kreuer S 2014 Wash-out of ambient air concentrations for breath measurements J. Breath Res. 8 027107
[33] Spanel P, Dryahnia K and Smith D 2013 A quantitative study of the influence of inhaled compounds on their concentration in exhaled breath J. Breath Res. 7 017106
[34] Pleil J D, Stiegel M A and Sobus U R 2011 Breath biomarkers in environmental health science: exploring patterns in the human exposome J. Breath Res. 5 046005
[35] Pleil J D, Stiegel M A and Risby T H 2013 Clinical breath analysis: discriminating between human endogenous compounds and exogenous (environmental) chemical confounders J. Breath Res. 7 017107
[36] Birken T, Schubert J, Miekiisch W and Nöldge-Schomburg G F 2006 A novel visually CO2-controlled alveolar breath sampling technique Technol. Health Care 14 499–506
[37] Cope K A, Watson M T, Foster W M, Sehnert S S and Risby T H 2004 Effects of ventilation on the collection of exhaled breath in humans J. Appl. Physiol. 98 1371–9
[38] Schubert J K, Spittler K H, Braun G, Geiger K and Guttmann J 2001 CO2 controlled sampling of alveolar gas in mechanically ventilated patients J. Appl. Physiol. 90 486–92
[39] Sukul P, Trefz P, Schubert J K and Miekiisch W 2014 Immediate effects of breath holding maneuvers onto composition of exhaled breath J. Breath Res. 8 037102
[40] Sukul P, Trefz P, Kamysek S, Schubert J K and Miekiisch W 2015 Instant effects of changing body positions on compositions of exhaled breath J. Breath Res. 9 047105
[41] Sukul P, Oertel P, Kamysek S and Trefz P 2017 Oral or nasal breathing? Real-time effects of switching sampling route onto exhaled VOC concentrations J. Breath Res. 11 027101