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PAPER

Optimisation of sampling parameters for standardised exhaled breath sampling

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Supplementary material for this article is available online

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

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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® (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

Figure 1. Collection system used to assess environmental contamination Glasshead (AMP3 Ltd, Aldershot, UK) and ReCIVA device (Owlstone Medical, Cambridge, UK).

College London. 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 EI 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 conduced 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-butanedione 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.

	Whole breath sample, peak area[a]		Lower airway sample, peak area[a]		
VOC	Median	IQR	Median	IQR	Wilcoxon signed-rank test
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	13 969–50 386	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 CO_2 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.

	Sample volume, ml						
	125	250	500	1000	Friedman test		Wilcoxon signed-rank test
VOC	Median[a] IQR[a]	Median[a] IQR[a]	Median[a] IQR[a]	Median[a] IQR[a]	X^2	p	p
Acetaldehyde	112 363	112 205	106 353	125 702	6.8	0.077	0.059
	74 842–131 670	100 580–172 335	86 166–162 932	115 046–253 994			
Propanal	21 961	30 488	37 722	52 408	17	0.001[b]	0.007[b]
	19 106–38 700	21 896–61 047	23 636–112 073	38 873–103 342			
Acetone	4181 697	6712 743	11 459 756	15 619 327	30.0	<0.001[b]	0.005[b]
	2085 733–5831 696	4351 575–11 288 384	7419 278–16 880 679	10 581 387–24 400 039			
Butanal	18 403	28 511	29 136	25 397	4.4	0.218	0.241
	12 148–47 818	14 858–65 951	17 413–110 369	17 364–101 793			
Acetic acid	99 971	28 959	51 347	18 010	5.9	0.118	0.114
	60 907–123 462	14 170–100 357	9013–126 411	9763–100 638			
Propanoic acid	12 393	15 512	21 846	33 557	17	0.001[b]	0.007[b]
	9817–19 322	9876–20 908	13 317–28 401	22 199–46 483			
Hexanal	89 493	96 505	110 809	128 210	4.1	0.253	0.047[b]
	51 543–131 797	76 326–150 560	80 253–155 991	79 214–199 988			
Heptanal	61 404	77 371	88 320	90 513	4.9	0.178	0.575
	99 693–153 102	110 429–142 786	112 198–211 096	131 957–208 433			
Benzaldehyde	329 084	330 624	382 157	423 678	5.4	0.145	0.445
	240 578–446 623	314 027–480 982	305 492–576 337	407 860–499 462			
Octanal	206 025	222 015.171	245 106	2652 767	4.3	0.229	0.386
	152 153–313 775	142 023–266 589	166 482–361 376	184 472–361 114			
Pentanoic acid	10 947	14 420	16 775	19 951	3.0	0.015[b]	0.009[b]
	9410–19 452	12 910–19 975	11 959–26 918	13 325–33 289			
Phenol	175 031	310 326.5536	391 734	284 020.2898	7.2	0.066	0.037[b]
	107 489–492 964	164 287–524 177	140 258–633 299	202 662–1032 641			
Nonanal	654 871	669 111	663 550	726 121	1.4	0.696	0.646
	643 950–654 871	762 951–762 951	748 952–748 952	738 994–738 994			
Decanal	521 150	531 145	529 396	589 733	0.4	0.948	0.878
	375 229–893 885	401 956–998 650	332 495–861 328	362 344–800 380			
Undecanal	169 577	199 771	216 331	262 930	12	0.006[b]	0.009[b]
	177 323–179 496	216 983–225 469	233 142–255 783	278 510–295 615			
Dodecanal	267 502	350 764	351 817	382 130	8.8	0.033[b]	0.028[b]

Table 2. (Continued.)

	Sample volume, ml						
	125	250	500	1000	Friedman test		Wilcoxon signed-rank test
VOC	Median[a] IQR[a]	Median[a] IQR[a]	Median[a] IQR[a]	Median[a] IQR[a]	X^2	p	p
	226 079–429 506	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.

	Flow rate, ml min^{-1}						
	50	100	200	400	Friedman test		Wilcoxon signed-rank test
VOC	Median[a] IQR[a]	Median[a] IQR[a]	Median[a] IQR[a]	Median[a] IQR[a]	X^2	p	p
Propanal	97 832	107 690	111 049	82 082	0.2	0.985	0.799
	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	12.1	0.007[b]	0.013[b]
	1620 9495–45 696 886	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	7.3	0.062	0.139
	14 359–122 621	12 164–115 507	11 297–1177 281	18 510–1740 438			
Propanoic acid	21 157	25 794	39 834	23 646	7.1	0.069	0.285
	12 172–105 000	12 050–155 180	11 261–73 915	10 665–52 521			
Hexanal	151 742	207 545	247 792	157 863	1.4	0.696	0.799
	20 854–543 325	16 745–464 727	10 522–581 214	12 281–418 713			
Butanoic acid	35 074	31 077	38 343	26 355	7.3	0.062	0.093
	29 937–66 724	24 085–66 416	18 011–82 691	13 131–91 042			
Heptanal	167 621	136 794	319 630	200 771	2.3	0.516	0.799
	35 347–365 155	20 142–583 640	15 053–694 298	9321–505 861			
Benzaldehyde	683 013	461 066	768 721	668 384	0.6	0.896	0.575
	98 201–1414 038	123 361–1533 524	69 517–1486 736	116 666–1482 761			
Octanal	290 884	265 468	770 238	376 711	5.4	0.145	0.169
	14 004–954 755	14 968–1722 049	10 535–1710 698	17 787–1048 896			
Phenol	719 734	603 515	545 877	292 988	15.8	0.001[b]	0.047[b]
	269 631–1069 165	357 492–1727 999	191 962–1525 045	65 786–864 256			
Nonanal	2014 266	2464 816	2704 687	1299 570	3.0	0.392	0.721
	409 728–4537 112	411 429–5143 492	208 730–4439 231	104 485–3034 270			
Octanoic acid	62 712	88 058	86 335	90 195	1.1	0.782	0.799
	32 970–104 732	31 450–204 904	21 346–202 641	23 998–206 489			
Decanal	575 901	877 940	1699 521	1083 701	2.6	0.451	0.799
	93 895–2205 963	80 775–4125 535	61 456–6264 396	63 867–3365 269			
Dodecanoic acid	128 484	133 796	131 410	149 499	0.4	0.948	0.386
	106 103–162 528	102 196–222 663	107 682–264 472	124 613–188 765			
Undecanal	1031 658	849 706	1030 516	921 688	0.8	0.840	0.285
	581 556–1170 463	626 400–1445 157	713 625–1682 390	716 795–1643 863			
Dodecanal	1726 776	14 875 51	1604 698	1040 048	3.7	0.293	0.241

Table 3. (Continued.)

	Flow rate, ml min^{-1}						
	50	100	200	400	Friedman test		Wilcoxon signed-rank test
VOC	Median[a] IQR[a]	Median[a] IQR[a]	Median[a] IQR[a]	Median[a] IQR[a]	X^2	p	p
Tridecanal	1213 477–2360 380 415 191 344 987–498 596	1245 833–2396 100 528 554 294 163–579 872	1045 710–1718 316 531 838 256 678–726 122	928 745–1272 234 405 472 327 901–512 779	0.1	0.989	0.799

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

[b] Denotes statistical significance at <0.05 level.

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

	Room air sample, peak area[a]		Mask sample, peak area[a]		
VOC	Median	IQR	Median	IQR	Mann–Whitney *U*-test
Cyclopentane	12 051	11 153–13 941	138 112	80 145–367 963	<0.001[b]
Disiloxane, hexamethyl-	10 420	9022–11 983	19 556 638	880 170–25 499 373	<0.001[b]
2-Oxa-1,3-disilacyclohexane, 1,1,3,3-tetramethyl-	14 338	12 755–16 536	68 011	24 101–1201 545	<0.001[b]
1,3-bis[(2Z)-Hex-2-en-1-yloxy]-1,1,3,3-tetramethyldisiloxane	20 755	17 645–29 569	343 046	165 585–1069 703	<0.001[b]
Trisiloxane, octamethyl-	13 430	10 753–17 306	1752 287	815 687–6127 763	<0.001[b]
Heptane, 2,2,4,6,6-pentamethyl-	17 660	13 278–25 093	367 240	166 605–677 080	<0.001[b]
Cyclotrisiloxane, hexamethyl-	20 804	18 057–25 539	53 059	41 728–116 568	<0.001[b]
2-Propenamide	15 866	12 790–17 465	39 700	27 199–52 062	<0.001[b]

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.

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 collection times during a busy clinical practice. The influence of the fraction of exhaled breath and the flow rate depends on the target VOCs measured. While in profiling studies it is acceptable to use a midrange flow rate and either whole or lower airway exhaled breath, it is important to examine the effect of sampling parameters on target VOCs before embarking on validation experiments or large-scale clinical studies. For instance, for our clinical studies on the use of VOCs for the diagnosis of gastro-oesophageal cancer, we investigated our target VOCs in this study and consequently we intend to use 500 mls sample volume of whole breath with a flow rate of 200 ml min^{-1}.

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Disclosures

The authors declare no conflict of interest.

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