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Abstract

Breath analysis holds great promise for real-time and non-invasive medical diagnosis. Thus, there is a considerable need for simple-in-use and portable analyzers for rapid detection of breath indicators for different diseases in their early stages. Sensor technology meets all of these demands. However, miniaturized breath analyzers require adequate breath sampling methods. In this context, we propose non-contact sampling; namely the collection of breath samples by exhalation from a distance into a miniaturized collector without bringing the mouth into direct contact with the analyzing device. To evaluate this approach different breathing maneuvers have been tested in a real-time regime on a cohort of 23 volunteers using proton transfer reaction mass spectrometry. The breathing maneuvers embraced distinct depths of respiration, exhalation manners, size of the mouth opening and different sampling distances. Two inhalation modes (normal, relaxed breathing and deep breathing) and two exhalation manners (via smaller and wider lips opening) forming four sampling scenarios were selected. A sampling distance of approximately 2 cm was found to be a reasonable trade-off between sample dilution and requirement of no physical contact of the subject with the analyzer. All four scenarios exhibited comparable measurement reproducibility spread of around 10%. For normal, relaxed inspiration both dead-space and end-tidal phases of exhalation lasted approximately 1.5 s for both expiration protocols. Deep inhalation prolongs the end-tidal phase to about 3 s in the case of blowing via a small lips opening, and by 50% when the air is exhaled via a wide one. In conclusion, non-contact breath sampling can be considered as a promising alternative to the existing breath sampling methods, being relatively close to natural spontaneous breathing.

1. Introduction

Volatile organic compounds (VOCs) produced by a human organism mirror normal and abnormal biochemical processes occurring in the body [1–5]. Their analysis in exhaled air, or other bodily fluids such as urine, sweat, or saliva has, therefore, a great potential for medical diagnosis and therapy monitoring [3]. These compounds are commonly defined as the human volatilome [6], and they can have a systemic origin or stem from exogenous sources such as diet, environmental exposure, or microbiota activity. The disease state influences the normal VOCs profile by generating new species or altering the concentrations of the VOCs that are produced by the organism [3–5]. The detection of this alteration, via e.g. breath analysis, provides a unique and non-invasive tool for tracking disease development, microbiota activity, metabolic

processes or exposure to environmental toxins [3, 7, 8].

Reliability and reproducibility of sampling is probably one of the most demanding challenges in breath gas analysis. A number of different factors related to this phase of analysis can affect the levels of breath species. These embrace factors such as body posture [9, 10], exercise/body movement [10, 11], breathing mode (e.g. breath holding, depth of respiration) [12], exhalation routes (nose versus mouth) [13], or exposure to variable environmental, or dietary contaminants. Moreover, analysis-related phenomena, e.g. pollutants, losses, dilution and interactions with instrument materials, can irreversibly modify the original sample composition and consequently distort the disease-related chemical fingerprint. Ultra-low concentrations of volatile breath constituents (from ppb to low ppt), the presence of highly reactive species and the high humidity of breath samples inducing wet chemistry pose additional challenges and problems.

Currently, there is no commonly accepted standard for breath sampling and numerous approaches are proposed in the literature. Typically, the end-tidal phase of exhalation is targeted as it shows the highest concentrations of systemic species and the lowest levels of exogenous compounds (produced e.g. in the oral cavity) [14]. The end-tidal sampling is usually accomplished by the measurement of expired CO_2 concentrations, measurement of exhaled airflow, or use of buffer volumes collecting the last phase of exhalation [10, 15–19]. Breath sampling can be also performed for a single breath or for multiple breath cycles. Usually, breath samplers impose the use of disposable mouthpieces assisting the flow of the breath stream into a sampler leading into the analytical instrument or the use of a storage container [15–17]. These standardize some sampling conditions (e.g. resistance during exhalation, diameter of breath stream) and isolates the sample from the room air contaminants, but they also cause issues. For example, breath samplers have to be periodically cleaned and sterilized. Moreover, a mouthpiece can also be a source of contaminants if it is not inert it can promote losses of VOCs from the breath sample or can add VOCs coming from the materials of the samplers. Furthermore, breath samplers are usually relatively large-in-size and energy-consuming (heating systems, flow/pressure/CO_2 sensors) and are, therefore, of limited use in miniaturized devices.

When it comes to sensors used for breath analysis, the main technological challenge is the requirement for trace-amount (at least ppb) detection of marker compounds in the presence of real world confounding factors. Factors such as the chemical or physical instability of both breath samples and sensing elements as well as the variation of VOC background and humidity impose numerous problems for stable analysis of real-world breath samples. The process of sampling exhaled breath and preparing it for delivery to the sensors through a sampling inlet system can introduce contamination or lead to the loss of target VOCs. On the other hand, the currently available real-time breath collection methods are rather bulky and heavy [15, 19], and are thus unsuitable for breath collection with sensor-based miniaturized analyzers.

Within this study a different sampling approach has been investigated: a collection of breath samples by exhalation from a distance without bringing the mouth into direct contact with a sample inlet of the breath analyzer. This concept can simplify the design of the analyzer and eliminate the use of disposable mouthpieces, while preserving high patient safety and comfort. To investigate this approach, proton transfer reaction mass spectrometry (PTR-MS) has been applied. Within this study PTR-MS mimicked miniaturized breath analyser, while providing the real-time response, selectivity and sensitivity necessary to monitor prototypic breath VOCs with a breath-to-breath resolution [20].

2. Methods

2.1. Human subjects and sampling protocol

A cohort of 23 healthy subjects (10 males and 13 females, age range 22–59 years, body mass index (BMI) range 18.5–29.8 (median 23.1), 5 of whom were smokers) was recruited. An effort was made to enroll volunteers unfamiliar with breath sampling. All volunteers gave written informed consent to participate and completed a questionnaire describing their basic personal data and smoking status. The measurements were performed under fasting conditions (minimum of 12 h). Moreover, the subjects were asked to refrain from alcohol consumption for 12 h prior to the experiments, smoking for 2 h and from using cosmetics/fragrances and breath mints/chewing gums on the day of the test. Furthermore, the subjects were asked to sit quietly for 10 min prior the test to avoid any temporal changes in levels of breath VOCs related to body movement, as occurs for example with isoprene [10, 11]. The sample collection was approved by the Ethics Commission of Innsbruck Medical University.

2.2. Breath sampling setup

Volunteers were advised to place his/her chin on a special head holder firmly fixed on the table, as shown in figure 1. The holder stabilized the position of the subject head and mouth and facilitated precise and reproducible positioning of the breath collector. The height of the holder could be adjusted to provide the most comfortable position for the subject. The inlet of the PTR-MS instrument was mounted on another adjustable holder, so its position and distance from the volunteer's lips could be smoothly adjusted in response to the test requirements. The subjects were asked to perform 5 sequential exhalations for every

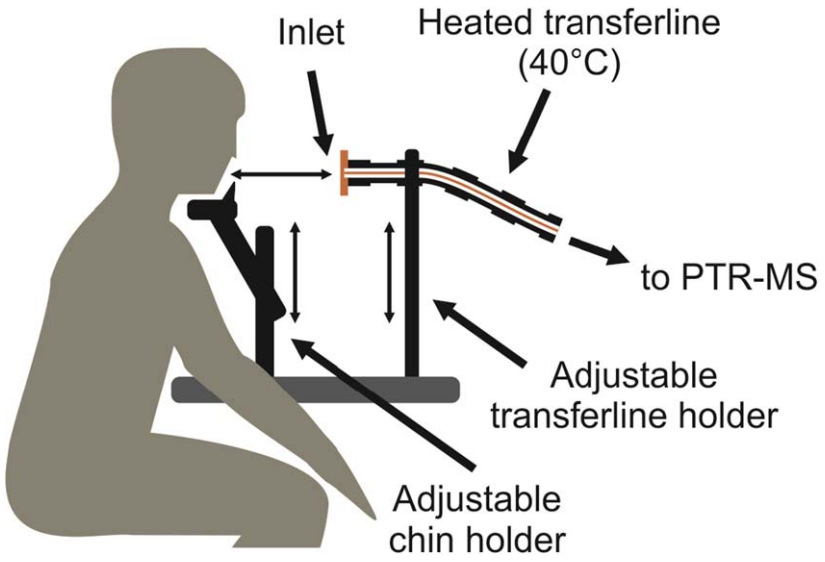

Figure 1. Experimental setup for non-contact sampling tests.

breathing scenario being tested. Four compounds commonly found in human breath were monitored: acetone, isoprene, methanol and dimethyl sulfide (DMS). These compounds were selected because this set of species embraces representatives of hydrophilic (acetone, methanol), hydrophobic (isoprene) and reactive (dimethyl sulfide) compounds.

Two inhalation and two exhalation maneuvers were pre-selected for the tests. The selected inhalation modes embrace (i) normal, relaxed breathing involving only the tidal volume of the lungs and (ii) deep breathing without breath holding involving also inspiratory reserve volume (denoted later in the text as N and D modes, respectively). For the deep breathing mode, the subjects were advised to inhale as much air as they could and immediately blow towards the inlet. Regarding exhalation scenarios, individuals were asked to form with their lips a circular opening and keep this shape during blowing. For the first exhalation mode, subjects blew using an opening of approximately 1 cm in diameter; whereas the second exhalation required the lips forming a wider opening (comparable to lips' shape during breathing on a cold mirror to make water vapor condense). These exhalation modes will be denoted later in the text as S and W, respectively. Subjects practiced several times the formation of opening and subsequent blowing before any measurements were taken. Moreover, the subjects were asked to exhale quietly to avoid rapid exhalation hindering the time of sample collection. Effectively, two inhalation modes and two exhalation modes formed four breathing scenarios denoted later in this paper as NS, NW, DS and DW. The aforementioned breathing maneuvers were selected from the ease of understanding of the sampling protocol and their perception by the volunteers.

To evaluate the influence of the sampling distance the subjects were asked to blow at the sampling inlet from four preselected distances of approximately 2, 5, 7.5 and 10 cm, while having their lips positioned in the central axis of the transfer line inlet. The sampling distance was adjusted exclusively by moving the holder stabilizing the inlet.

2.3. PTR-TOF-MS analysis

An Ionicon Analytik GmbH (Innsbruck, Austria) proton transfer reaction time of flight (TOF) mass spectrometer (PTR-TOF-MS) instrument (PTR TOF 8000) was used for real-time analyses. Proton-transfer reaction mass spectrometry is a well-established sensitive analytical technique for the quantification of molecular species down to the ppt range [21, 22]. Its success stems from its versatility, excellent sensitivity and rapid real-time response. In particular, the last feature opens up a new fascinating opportunity of tracking rapid short-time changes in breath VOC concentrations with a breath-by-breath resolution [10, 11]. In brief, PTR-MS offers a quantification of volatile molecular species at ultra-low levels on the basis of chemical ionization within a drift reactor tube. More specifically, it exploits the proton transfer reaction of precursor hydronium ions (H_3O^+), originating from a hollow cathode discharge, with molecules of interest M:

$$H_3O^+ + M \rightarrow MH^+ + H_2O.$$

The aforementioned reaction process is facile for VOCs with proton affinities higher than water (166.5 kcal mol^{-1}). A favorable consequence of the employment of this ionization mechanism is the fact that the bulk components of breath gas O_2, N_2, and CO_2 do not react with H_3O^+. The only issue with breath is its high humidity, which changes the

humidity in the reaction chamber leading to higher concentrations of protonated water clusters. However, at the high reduced electric fields generally used (~130 Td), this is not a significant problem. The count rates of the nascent product ions MH^+ can subsequently be converted into absolute concentrations of M. A major hallmark of the PTR-MS technique is its near real-time response (100 ms) allowing for concentration measurements of VOCs with breath-by-breath resolution. Moreover, the real-time analysis improves the quality and reliability of the results, since sample collection, storage and pre-concentration that can result in contamination and losses of trace breath VOCs are avoided. The application of a TOF mass analyser in PTR-MS instruments provides increased mass resolving power (4000 m/Δm or better) compared to quadrupole mass spectrometer analysers, and thereby provides the discrimination between many isobaric compounds.

The breath samples were collected using a plain disc-shaped inlet (OD = 25 mm) made from polyetheretherketone (PEEK) and equipped with a 1 mm orifice located in the center of the disc. From this inlet, the breath samples were transferred into the drift tube of the PTR-TOF 8000 via a sampling line comprising of a heated (60 °C) PEEK tube (1.4 m long, ID = 1 mm). The inlet of the tube was equipped with a Luer-lock port. The transfer line facilitated rapid transport of the breath sample to the MS. The pumping rate via the transfer line was set at 4 ml s^{-1}.

The PTR-TOF 8000 settings during experiments were as follows: ion source current 4 mA, source voltage 120 V, source-out voltage 50 V, and source valve opening 55%. With these settings the levels of the major impurity ions relative to that of the H_3O^+ precursor ion were $H_3O^+.H_2O$ (3%), O_2^+ (2.7%), and NO^+ (0.3%). The H_3O^+/VOCs reactions occurred in the drift tube at a total pressure of 2.4 mbar and a gas temperature of 60 °C. The voltage along the drift section was set to 600 V leading to a reduced electric field of approximately 130 Td. The spectral scans of the TOF analyzer ranged from approximately *m/z* 2.7 up to *m/z* 500, and were acquired in a time of 250 ms by co-adding 6250 single 40 μs TOF-MS extractions recorded at a sampling frequency of 10 GHz. The mass resolution obtained from the detected peaks (full width at half maximum (FWHM)) was measured to be approximately 4100 at *m/z* 100. Mass calibration was based on three mass spectral peaks always present in the spectra: $H_3^{18}O^+$ (21.0221), O_2^+ (31.9893), and protonated acetone $C_3H_7O^+$ (59.0491).

The compounds of interest were quantified using the protonated parents of the prototypic VOCs.

2.4. Buffered end-tidal (BET) sampling

In the absence of a gold standard, it is very difficult to validate the non-contact breath sampling developed within this study. Nevertheless, an effort was made to compare the results obtained during non-contact sampling with another well-established breath sampling method. For this purpose, a BET on-line sampler (Ionicon Analytik GmbH) [15] was selected as it is specifically designed for breath analysis with the PTR-MS. Therefore the same analytical instrument (and its settings) could be used for both sampling methods. In short, the BET sampler consists of a heated (60 °C) sampling buffer Teflon tube (30 cm long, 1.27 cm ID (½ inch), and an approximate volume of 40 ml). During sampling, a subject provides only one complete exhalation via a disposable non re-breathing mouthpiece into the buffer tube, in which the end-tidal fraction of the exhalation is buffered. The tube content is continuously drawn from the middle of the buffer tube by the PTR-MS via a heated PEEK transfer line (60 °C, 2 m long, ID = 1 mm). Such an approach increases the measurement time of a single exhalation to 8–12 s, while preserving the advantages of the real-time analysis. Effectively, subjects were requested to provide 5 exhalations via the BET sampler and subsequently 5 exhalations using the DW distant sampling protocol, and the average signal resulting from the individual compounds of interest was used for the statistical analysis. The DW scenario was chosen as it provided longer end-tidal phases and effectively more measurement points for the PTR-MS as it will be shown below. Moreover, to reduce the differences between the compared sampling protocols, individuals were asked to undertake a deep inhalation without breath holding for the BET sampling.

3. Results

Exemplary PTR-MS profiles of acetone, methanol, isoprene and DMS obtained for the five subsequent DW exhalations and a blowing distance of approximately 2 cm are shown in figure 2. It should be mentioned here, that the applied PTR-MS sampling resolution assisted the identification of the dead-space and end-tidal exhalation phases.

Exemplary dependences of breath levels of acetone, isoprene, DMS and methanol on sampling distance obtained for 2 subjects and DS scenario are shown in figure 3. Not unexpectedly, considerable losses were observed for more distant sampling points for all tested scenarios. For instance, for the DS scenario at a distance of 5 cm the concentrations of species of interest dropped by approximately 30% as compared to the levels obtained for 2 cm. At distances of 7.5 cm and 10 cm from the volunteers' lips the observed losses were between 40% and 50% of the values observed for the closest sampling point. It is also worth mentioning that for the larger distances (7.5–10 cm) volunteers tended to exhale faster and stronger trying to reach/target the inlet. This behavior shortened the length of the exhalation and, thereby, the time available for sampling.

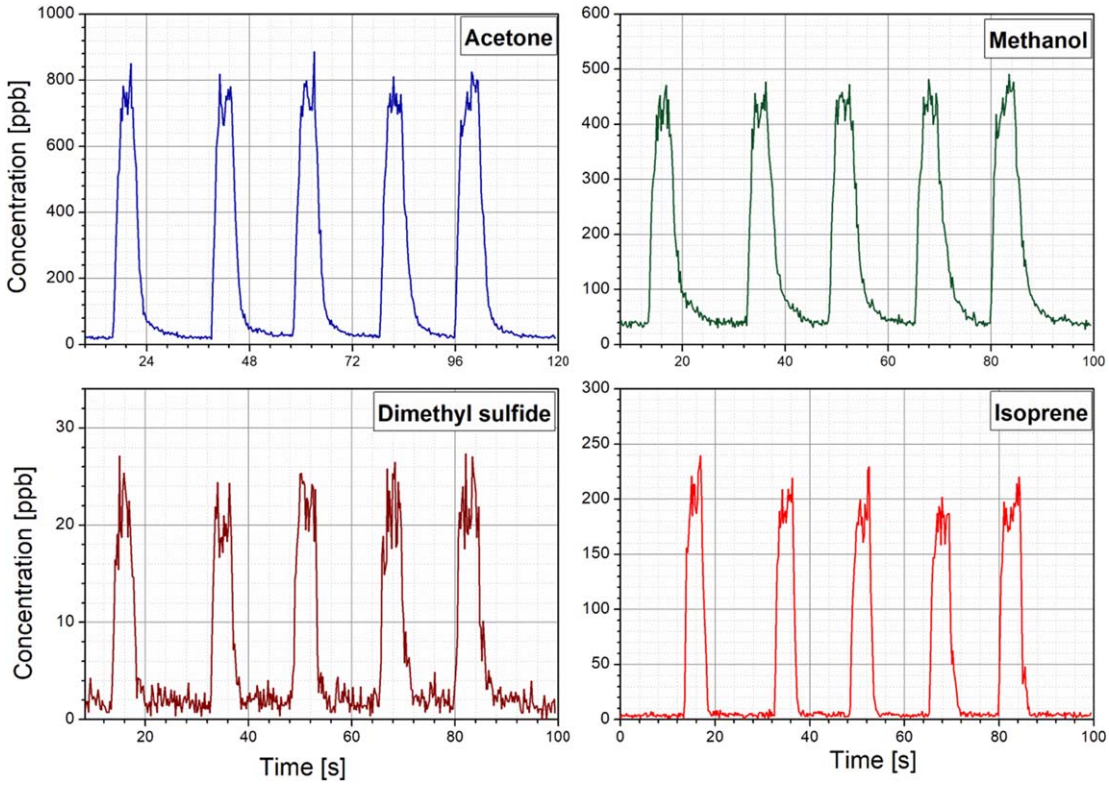

Figure 2. Exemplary breath profiles of acetone, methanol, isoprene and DMS obtained for a distance of 2.0 cm.

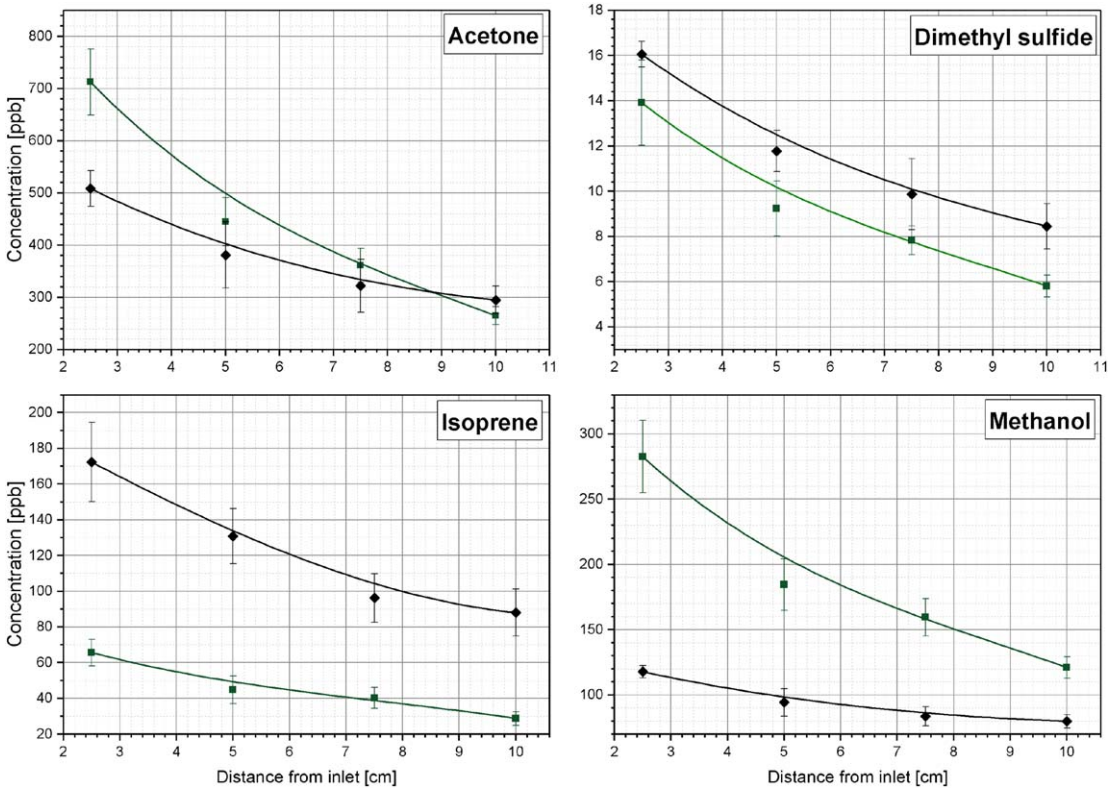

Figure 3. Exemplary dependences of breath levels of acetone, isoprene, DMS and methanol on sampling distance obtained for 2 subjects.

As stated above, four sampling scenarios were compared within this study. Comparisons were performed for a sampling distance of approximately 2.0 cm, which ensured the lowest sample dilution by the ambient air. Four parameters were taken into account to compare the sampling scenarios of interest:

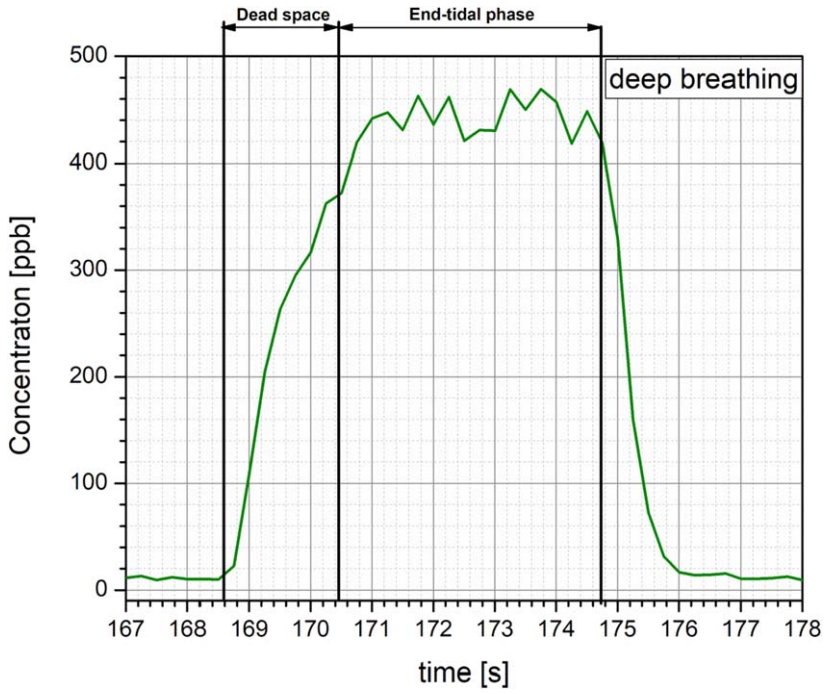

Figure 4. Typical phases of the exhalation profile (acetone) obtained for DS breathing mode.

(i) concentrations of preselected VOCs, (ii) reproducibility of measurements, and (iii) lengths of the dead-space and end-tidal phases of exhalation. An exemplary exhalation profile obtained for acetone and the DS breathing scenario is shown in figure 4. The typical exhalation profile consists of three phases; (i) dead-space phase, (ii) end-tidal phase and (iii) residual phase. The dead-space phase corresponds to the exhalation of air filling the upper air-ways and oral cavity. It is characterized by a steady increase of concentrations of blood-borne VOCs following the increasing contribution of alveolar air. The end-tidal phase contains air that has participated in the blood-air gas exchange in the alveoli and, therefore, exhibits the highest levels of blood-borne volatile markers. Typically, VOC concentration levels during this phase exhibit a pseudo-plateau showing a mild increase [14, 23]. The residual phase in turn consists of a mixture of ambient air and remaining breath gas, which stayed in the proximity of an inlet after the exhalations have been finished. The boundaries between these phases are not very evident and are therefore not easy to define. For this study, the beginning of the dead-space phase was determined by the PTR-MS measurement point preceding the rapid increase of a VOC level; whereas, its end was indicated by the measurement point exhibiting the concentration of an analyte corresponding to the 70% of its maximum level during the end-tidal phase. This threshold of 70%, which is arbitrarily chosen, is used also to determine the end of the end-tidal phase of exhalation. Table 1 summarizes the concentrations, signal reproducibility and durations of dead space and end-tidal phases of exhalation determined for the prototypic VOCs and the 4 tested breathing scenarios.

The results of the BET and DW sampling scenario comparison are presented in table 3.

4. Discussion

Within this study, valuable pieces of information were extracted with regards to the behavior of the subjects during non-contact sampling. First, a number of individuals appeared to be distressed and experienced a kind of anxiety during sampling, which was manifested by distorted breathing patterns and a tendency to shorten or slow down the exhalation and/or inability to keep stable the direction of the breath stream. Consequently, the resulting exhalation profiles of compounds of interest were distorted. This is clearly illustrated in figure 5. These initial problems are attributed to the lack of familiarity with breath tests. However, this is dramatically reduced after 3–4 trial exhalations. Therefore, some practice of non-contact sampling is necessary before real samples are taken for analysis to give the subjects time to get use to and comfortable with the breath sampling protocol. Following this finding, all participants were advised to perform several trial exhalations before the measurements to help relax them. Next, the subjects tended to interpret instructions concerning the inhalation mode, blowing/exhalation mode, or size/shape of the mouth opening in slightly different ways; thereby, affecting the sampling. This problem seemed to stem from the lack of unambiguously understandable terms related to breathing. Interestingly, sampling protocols

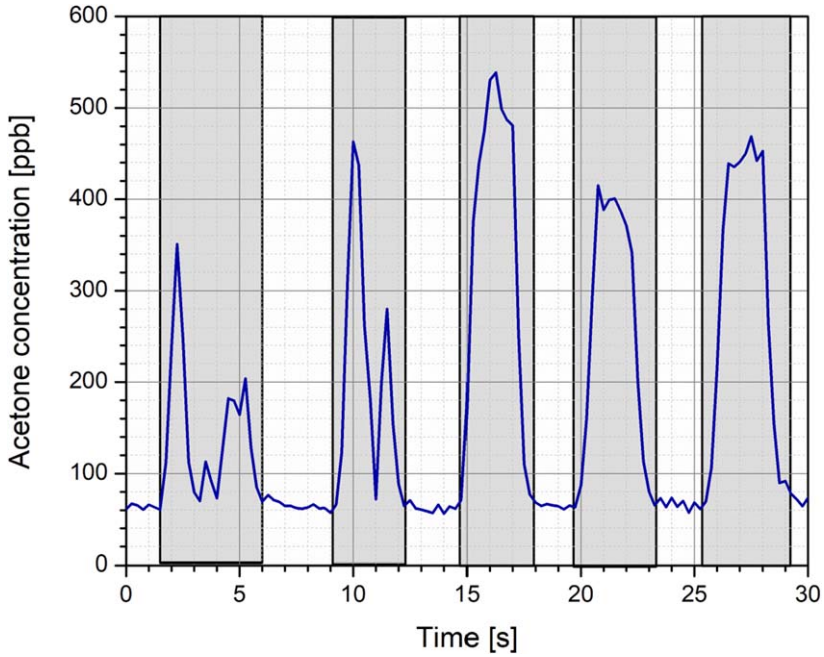

Figure 5. Exemplary acetone profiles obtained during a practice measurement. The first two exhalation profiles are distorted due to the irregular breathing caused by stress.

Table 1. Concentrations, durations of the dead space and end-tidal phases of the exhalation and signal reproducibility levels ($n = 5$) for the volatile breath compounds under study obtained for tested breathing scenarios N–normal inhalation, D–deep inhalation, S–blowing via a small mouth opening, and W–blowing via a wide mouth opening.

			Concentration mean (range) (ppb)	Reproducibility (RSD) mean (range) (%)	Dead space phase mean (range) (s)	Alveolar phase mean (range) (s)
Methanol	S	N	244 (122–515)	8.4 (0.9–20)	1.3 (1.1–2.0)	2.0 (0.7–3.9)
		D	286 (139–530)	9.7 (4.5–17.5)	1.6(1.0–2.5)	3.6 (1.1–8.6)
	W	N	272 (99–498)	5.6 (3.4–10.5)	1.37 (1.0–1.8)	1.8 (1.3–3.5)
		D	285 (106–524)	7.8 (1.7–15.8)	1.7 (1.1–2.3)	2.6 (1.8–7.8)
Acetone	S	N	414 (201–710)	9.5(2.9–25.0)	1.6 (1.1–2.6)	1.5 (0.7–3.5)
		D	527 (240–916)	9.6 (4.5–17)	2,0 (1.2–3.4)	3.0 (1.1–6.0)
	W	N	493 (156–932)	7.0 (1.5–10.5)	1.54 (1.0–2.0)	1.6 (1.1–3.0)
		D	547 (177–997)	7.5 (1.7–19)	1.8 (1.3–2.4)	2.4 (1.5–7.3)
DMS	S	N	14.0 (2.6–42)	13.2 (1.7–25)	1.7 (1.0–2.5)	1.3 (0.4–3.0)
		D	16.6 (2.5–54)	11.7 (5–25)	2.0 (1.0–3.3)	3.0 (0.6–6.7)
	W	N	18.4 (2.0–61)	12.3 (2.6–24.3)	1.5 (1.0–2.0)	1.3 (0.7–2.2)
		D	17.4 (1.8–60)	8.5 (4.1–24)	1.75 (1.3–2.4)	2.0 (1.4–6.7)
Isoprene	S	N	149 (28–150)	12.0 (3.0–30)	1.3 (0.9–2.2)	1.6 (0.5–3.3)
		D	143 (40–360)	13.4 (11–28)	1.7 (0.8–3.5)	2.5 (0.6–6.7)
	W	N	171 (37–383)	11.8 (4.6–24)	1.3 (0.8–1.5)	1.5 (0.9–2.9)
		D	136 (32–348)	10.5 (3.1–19.5)	1.6 (1.0–2.4)	2.1 (1.3–6.3)
Average		S	N	**10.9**	**1.4**	**1.5**
			D	**11.1**	**1.8**	**2.9**
		W	N	**9.2**	**1.4**	**1.5**
			D	**8.5**	**1.7**	**2.3**

were perceived by individual subjects differently. For instance, blowing via a wide opening was described by some subjects as being unpleasant. This blowing mode required also more practice with respect to size and shape of the mouth opening during blowing. Several volunteers reported that deep inhalation was uncomfortable when performed consecutively.

To ensure the highest levels of breath VOCs and to reduce the dilution of the breath gas with room air, samples should be taken as close as possible from the subject's lips. Effectively, the distance of approximately 2 cm can be considered to be a reasonable trade-off between the effect of breath dilution related to distant sampling and the risk of the physical contact

Table 2. The outcome of a Wilcoxon signed rank test and the concentration differences in (%) for tested sampling scenarios. N–normal breathing, D–deep breathing, S–blowing via a small mouth opening, and W–blowing via a wide mouth opening.

(%)	DW versus NS	DW versus DS	DW versus NW	DS versus NS	DS versus NW	NS versus NW
Methanol	13	n.s.	7	15	n.s.	n.s.
Acetone	17	n.s.	10.5	19	n.s.	n.s.
DMS	n.s.	n.s.	n.s.	n.s.	n.s.	−16
Isoprene	n.s.	n.s.	22	n.s.	n.s.	n.s.

Table 3. Comparison of the BET sampler and the DW distant sampling. D-deep inhalation and W–blowing via a wide mouth opening.

	BET RSD (%)	Distant sampling RSD (%)	Relative concentration difference $(C_{BET}-C_{DW})/C_{BET}{}^{*}100$ (%)
Methanol	1.7	5.5	8.0
Acetone	4.3	5.0	1.0
DMS	6.7	15.2	11.0
Isoprene	11.3	13.0	12.5

of the subject lips with the inlet material. Moreover, some volunteers had difficulties in providing a reliable sample for sampling distances larger than 5 cm owing to problems related to a proper targeting of the breath stream towards the inlet orifice. This finding implies that sampling distances longer than 5 cm are of limited use for the non-contact breath sampling protocol.

A review of table 1 reveals that the measured concentrations of the VOCs under study agree reasonably well with the literature values [24–34]. A Wilcoxon signed rank test was used to compare the VOCs' concentrations obtained for different sampling scenarios and a p value of <0.05 was considered as significant (see table 2). Concentrations of the two hydrophilic compounds, i.e. methanol and acetone, were significantly higher when deep inhalation was performed during the breathing scenario. The observed differences amounted to 15% and 19% for methanol and acetone, respectively, for exhalation via a smaller mouth opening and 7% and 10.5% for blowing via a wide one. This difference is not surprising as blowing through a smaller lip opening is longer and therefore causes a breath holding effect. Such an effect was not observed for the hydrophobic species, for which levels were not significantly altered by deep inhalation. The average reproducibility (RSDs) of measurements was spread around 10% for all tested scenarios. Taking into consideration numerous confounding factors, which can affect distant sampling, this number can be considered to be very promising. It is worth mentioning that blowing via a wider lip opening provides better reproducibility (approximately 2%). This improvement is attributed to the facilitated aiming at the inlet while blowing with a larger breath stream. The RSDs for DMS and isoprene were higher as compared to acetone and methanol values. This difference can stem from a higher reactivity in the case of DMS, and for isoprene in terms of the known concentration dependence on even small body movements. The length of the dead-space phase of exhalation was influenced mainly by the breathing mode. In the case of normal inhalation this phase lasted on average for 1.4 s, whereas, deep breathing extended it to 0.3–0.4 s. However, this difference can stem from the applied algorithm of the dead-space phase identification. More pronounced differences were recorded for the end-tidal phase. Normal relaxed inhalation provided 1.5 s long end-tidal phases in case of both blowing modes; while, deep breathing prolonged this phase to approximately 3 s when the subject blew via a small opening, and 2.3 s when the exhalation was performed via a wider one. The shorter end-tidal phase for the latter scenario is not surprising as wider mouth opening increases the breath stream diameter and shortens the exhalation.

The comparison of the BET and non-contact samplings was performed for a DW scenario as it provides longer end-tidal phases of exhalation. The levels of methanol, DMS and isoprene were found to be significantly higher when samples were taken via the BET sampler (Wilcoxon signed-rank test, $p < 0.05$). This difference was spread around 10% resulting most probably from a minor dilution of breath by ambient air during distant sampling. Both sampling methods showed similar measurement reproducibility (RSDs) varying from 3% to 15%. The only exception was DMS, which exhibited twice the RSD in the case of distant sampling. This discrepancy can be attributed to low breath concentrations of this species and 4–5 times shorter time to sample end-tidal phase of exhalation during the DW distant sampling. In particular, the latter affected strongly the quantification of this species by the PTR-MS instrument. Relatively high variability of isoprene levels reflects susceptibility of its levels to even small body movements [10, 11].

5. Conclusions

In the context of rapid advances in breath gas analysis, there is a need for adequate breath sampling methods fulfilling the requirements of the new generation of the miniaturized sensor-based breath analysers. This

study has illustrated that a promising method is non-contact sampling, i.e. a collection of breath samples by exhalation from a distance without bringing the mouth into direct contact with the device as a potential breath sampling method. This is easier for the person and reduces contamination issues. Within this study two inhalation modes (normal, relaxed breathing and deep breathing) and two exhalation types (quiet blowing via small and wide lips opening without breath holding) forming four breathing scenarios were compared. All tested breathing scenarios exhibit comparable measurement reproducibility spread around 10%. In scenarios involving normal and relaxed inspiration, both the dead-space and end-tidal phases last around 1.5 s. Deep inhalation prolongs the end-tidal phase by about a factor of 2 in the case of blowing via a small lips opening, and by approximately 50% when the air is exhaled via a wide one. Moreover, deep breathing increases the concentrations of hydrophilic species by 9%–17%. Regarding sampling distance, a distance of 2 cm was found to be a reasonable trade-off between the necessity of the reduction of the sample dilution and requirement of no physical contact of the subject with the instrument inlet. Effectively, the non-contact sampling from a distance of 2 cm causes 10% sample dilution as compared to the BET sampling.

The main issue is the sampling system of the sensor device, which should be able to provide adequate amounts of the breath sample for the gas sensors. This is in turn is hindered by the time available for sampling. In this study, a 3 s alveolar phase provided approximately 12 ml of breath gas (sampling rate of 4 ml s^{-1}). This amount can be increased by using more efficient micro pumps. Nevertheless, the final selection on the optimal distant breath sampling protocol is determined by the technical specifications of the miniaturized breath analyzer.

In practical terms, distant sampling offers distinct advantages. Firstly, such a concept contributes to the miniaturization of the breath analyzer and removes the need for disposable mouthpieces. Secondly, breath can be sampled during a breathing scenario, which is relatively close to natural spontaneous breathing. Finally, the sampling protocol is simple, convenient, safe and easy to understand/learn for the user. In summary, distant non-contact breath sampling can be considered as an interesting alternative to existing breath sampling methods for use with miniaturized breath analyzers.

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