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Detection of acetone and isoprene in human breath using a combination of thermal desorption and selected ion flow tube mass spectrometry

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ABSTRACT

The measurement of volatile chemicals in human exhalant (breath analysis) has recently emerged as a non-invasive technique with the potential for the early diagnosis of disease. A common method of volatile chemical collection is to capture gases onto a solid phase sorbent followed, at a later time, by thermal release and analysis. This technique, termed thermal desorption (TD), may be a useful means to collect and store breath volatiles in a clinical setting prior to analysis. TD is, however, normally used in conjunction with gas chromatography (TD–GC) which results in slow analysis times and the required use of chemical standards. The new technique of selected ion flow tube mass spectrometry (SIFT-MS) offers a more rapid analysis process without the need for standards. SIFT-MS is normally used to analyze gas concentration in real-time and it is unclear whether combined TD and SIFT-MS can be successfully employed for breath analysis. We found that there was an approximate 1 to 1 concordance between levels of isoprene or acetone in the breath of 12 healthy volunteers measured either using real-time SIFT-MS or offline using a combination of SIFT-MS and TD (TD–SIFT-MS). The use of higher volumes of human breath did impact TD–SIFT-MS measurements of isoprene (but not acetone) with an apparent ceiling effect being observed. Nevertheless our findings demonstrate the potential for breath analysis using a combination of TD and SIFT-MS, an approach which may find utility in a clinical setting which does not allow online analysis of breath.

1. Introduction

The early diagnosis of disease plays an important role in reducing mortality and morbidity [1]. For example, treatment plans including lifestyle changes can be implemented earlier which leads to improved patient outcomes [1]. Testing for the presence of disease at the presymptomatic stage requires, however, that any procedure be safe, accurate and inexpensive. Breath analysis has recently emerged as a non-invasive method of detection of disease markers which may offer new and additional diagnostic approaches for clinicians [2]. The technique is based on the assumption that there is an equilibrium of compounds established between the pulmonary blood supply and the air in the lungs [2]. Many applications using the breath analysis technique are at various stages of development including the detection and monitoring of renal failure, early diagnosis of cancer, bacterial infection and the assessment of airway inflammation [3–6]. Despite the undoubted appeal of breath analysis, many problems remain to be overcome including those associated with the collection and analysis processes [2]. There are two types of gaseous analysis: online and offline. Online analysis is characterized by real-time gas analysis recording the concentration of the desired compound continuously without significant delay, whereas offline analysis does not give instant or continuous results and can be quite time consuming [2]. While online analysis has several advantages, one being the near instantaneous results, the difficulty is that it may not be feasible in all clinical settings as not everyone will have access to direct online sampling, therefore samples will need to be collected and then analyzed. A common method of breath collection utilizes plastic bags [2]. Although a useful collection method, the bags are cumbersome to transport, fragile and, due to surface adsorbtion or gassing off, can alter the chemical makeup of the sample [2]. A commonly used alternative to bag sampling is thermal desorption (TD).

TD was primarily developed for workplace and environmental monitoring but has recently been applied to the analysis of human breath [7–11]. TD involves collecting volatile compounds onto a TD tube filled with sorbent such as Tenax, by either diffusion or

Abbreviations: TD, thermal desorption; SIFT-MS, selected ion flow tube mass spectrometry.

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by pumping gas or breath through the tube [12]. The tubes are physically resilient which makes them ideal for transport between the collection and analysis sites and also allows for storage of the absorbed compounds for several days [12]. The volatile chemicals trapped in the tube are then desorbed into a carrier gas such as helium by heating the tube. Desorption of volatiles with subsequent analysis are performed using either a single- or double-stage TD [7]. In double-stage TD the volatile compounds are released from the tubes onto a cold trap and then desorbed a second time before the analysis [7]. Typically the analytical procedure paired with the double-stage TD process is gas chromatography (GC). There are two major disadvantages to the TD–GC combination despite the excellent chemical resolution. The first is the slow analysis times, typically between 20 and 40 min, and the second is that calibration of the GC detection system is needed using standards of the desired compounds [12,13]. As such it can take several hours to determine the concentration of a trace volatile in a single breath sample. A novel analysis technique, selected ion flow tube mass spectrometry (SIFT-MS), can overcome the difficulties associated with the TD–GC combination [7]. SIFT-MS is a rapid analysis system that uses real-time detection. The technique can detect trace volatile compound in the presence of an abundance of much higher atmospheric gases by means of chemical ionization of the gases with charged precursor ions, normally H_3O^+, NO^+ and O_2^+ [14,15]. The precursor ions are created in a microwave discharge source, selected using a quadrupole mass filter, and then injected into a fast flowing helium gas stream where they react with the trace gases introduced at a known flow rate. The reaction produces specific product ions which are sampled by a downstream quadrupole mass spectrometer and quantified by an ion counting system. A mass spectra results and is recorded by an online computer for analysis and storage [14,15]. By knowing the reaction rate constant for the reaction between the precursor ion and the compound of interest, absolute quantification of the volatile compound can be analyzed in real-time without the need for calibration standards [14,15]. It has been previously shown that the TD/SIFT-MS combination provides accurate analysis for both gas standards in a helium mixture and environmental gaseous samples [7]. We have investigated whether TD can be combined with SIFT-MS to produce accurate sampling and quantification of volatiles in high humidity breath (acetone and isoprene) compared with direct sampling with the online SIFT-MS system.

2. Methods

2.1. Thermal desorption

Breath samples collected in TD tubes were introduced into a Markes International (Llantrisant, UK) Unity TD instrument for analysis. The tubes were desorbed at 300 °C for 5 min at a flow rate of 20 ml/min onto a cold trap held at −10 °C containing the sorbents Tenax GR backed by Carbopack B. The cold trap was then heated at 12 °C/min to a holding temperature of 300 °C. After the sample was desorbed the TD instrument was cooled down quickly by running extra dry air through the system. The instrument had a head pressure of helium set to 10 psi which resulted in a flow rate of 0.45 ml/s within the transfer line between the TD instrument and the SIFT-MS [7]. Prior to sampling the tubes were conditioned for 90 min at 300 °C in a stream of nitrogen passed through a hydrocarbon trap (Supelco, US) using a Markes International (Llantrisant, UK) tube conditioner.

2.2. SIFT-MS

The SIFT-MS analysis was run using a Profile 3 instrument from Instrument Science (Crewe, UK). Water was analyzed using the H_3O^+ precursor, with a total precursor count rate of approximately 1 million counts/s. Acetone and isoprene were analyzed using the NO^+ precursor, with a total precursor count rate of approximately 1 million counts/s. The kinetic library was used to quantify levels of all gases by monitoring the H_3O^+ and NO^+ precursors and their specific product ions in the multi-ion monitoring mode as previously reported [14]. The kinetic library contains the precursor and product ions of acetone and isoprene to be used to quantify each compound with their respective rate constants for the reaction. For acetone the reaction produced a product ion of m/z 88 with the kinetic constant for the reaction with m/z 30 being 1.8×10^{-9} cm^3/s [16]. For isoprene the reaction produced a product of m/z 68 with the kinetic constant for the reaction with m/z 30 being 1.7×10^{-9} cm^3/s [17]. The absolute concentrations of the compounds were calculated using the software supplied with the SIFT-MS with ionic diffusion being accounted for by using an approximation instead of the measured values [18]. Quantification of absolute gas quantities was carried out as previously described [7]. Briefly, product ions were monitored over time using the instruments multi-ion monitoring mode. The gas elutes over a period of approximately 20 s and the total quantity of the compound of interest is summed over that period using a combination of the instrument software and a visual identification of the elution 'peak'. The total elution time is noted and the same length of time of background levels contained in the helium gas stream quantified to calculate the 'blank' value, which for isoprene and acetone is 3–4 order of magnitude lower than the measured gas concentration. The blank is then subtracted from that of the level in the sample. By varying the product ions monitored different compounds can be quantified in the same thermal desorption run. The downstream mass spectrometer timing parameters used were flyback: 0.02 s, wait: 0.02 s, precursor: 0.1 s, product: 0.1 s.

3. Sample collection

Sampling was conducted in two ways. The first was a direct breath sampling into the SIFT-MS. Participants were instructed to take a regular inhale and then fully exhale into a disposable mouthpiece attached to the heated sampling line. Sampling was repeated three times for both acetone and isoprene. The second collection method introduced the breath sample onto TD tubes purchased from Markes International (Llantrisant, UK). Participants inhaled normally and then fully exhaled via a disposable mouthpiece into a BioVOC Breath Sampler (Markes International, Llantrisant, UK) which resulted in the capturing of the terminal 130 ml (the volume of the sampler) of exhaled breath [8–11]. A TD tube was then attached to the sampler and the breath sample forced onto the tube by means of a plunger attached to the sampler. For sample volumes greater than 130 ml the procedure was repeated with subsequent exhalations until the required volume had been collected.

4. Results

We first investigated the relationship between sample volume and the quantities of acetone and isoprene in the breath of two subjects using a combination of TD and SIFT-MS. As illustrated in Fig. 1, the quantity of detected acetone increased linearly in proportion to the volume of breath sampled over the range tested, while breath volumes in excess of approximately 400 ml did not result in proportionally larger quantities of isoprene being detected. To determine whether the apparent maximum quantity of isoprene which could be measured was due to the co-desorption of large quantities of water vapour present in breath, we investigated whether purging the TD tubes before desorption, thus reducing the water content of the TD sorbent, would increase the apparent detected quan-

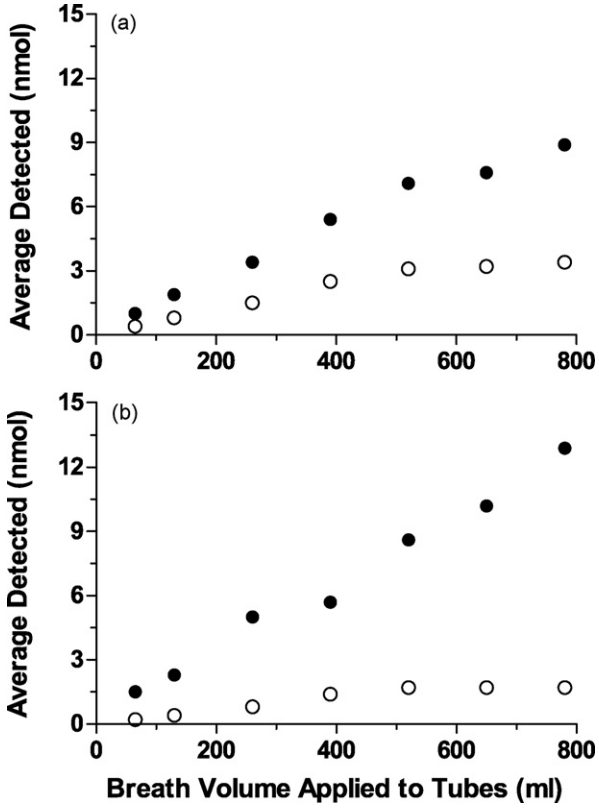

Fig. 1. The relationship between sample volume and measured quantities of acetone (closed circles) and isoprene (open circles) in human breath. Increasing volumes of human breath from two different subjects (a and b) were applied to TD tubes and quantities of isoprene and acetone determined using a combination of TD and SIFT-MS as described in Section 2. The values shown are the mean of duplicate measurements.

tity of acetone and isoprene. Three breath samples of 650 ml of end expired air were applied to three different TD tubes followed by purging with helium for 0, 1, or 10 min at room temperature. Although purging indeed reduced the water content of the desorbed gas flow (Fig. 2), it did not increase the detection of either acetone or isoprene. Purging of TD tubes onto which 130 ml of breath had been applied yielded similar results (data not shown). Based on these results we utilized 130 ml breath sample volumes with no prepurging of the TD tubes in subsequent experiments.

To compare the concentration of breath isoprene and acetone measured by SIFT-MS analysis and TD–SIFT analysis, we obtained

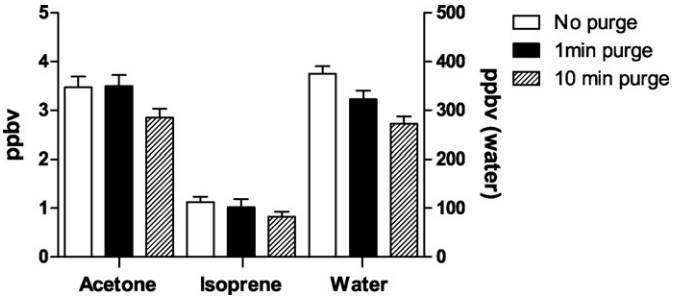

Fig. 2. The effect of purging of TD tubes on the measured quantities of water, acetone and isoprene in human breath. 650 ml of human breath was applied to TD tubes followed by purging the tubes with helium for 0, 1 or 10 min at room temperature. The tubes were then desorbed and the quantities of water, acetone and isoprene measured using SIFT-MS using either the H_3O^+ (water) or NO^+ (acetone and isoprene) as described in Section 2. Note that the abundance of water is plotted using the right axis. The values shown are the mean of duplicate measurements. Bars indicate the SEM.

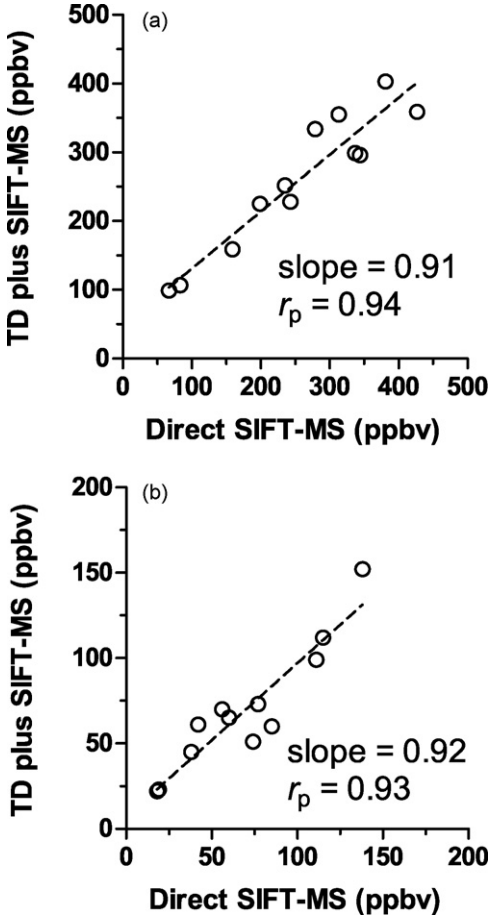

Fig. 3. Comparison of human breath acetone (a) and isoprene (b) levels measured using either direct SIFT-MS or a combination of TD and SIFT-MS analysis methods. Values obtained using direct SIFT-MS analysis are the mean of three consecutive breaths, while values for TD and SIFT-MS are the mean of duplicate TD tubes onto which 130 ml of breath had been applied. The line represents the 'best-fit' linear regression line. The average ratio between the two measurement methods, i.e., the slope of the regression line was 0.91 for acetone and 0.92 for isoprene. The correlation coefficients were 0.94 for acetone and 0.93 for isoprene.

samples for both analysis methods from 12 subjects. There was a linear correlation between direct SIFT-MS and TD–SIFT-MS measured concentrations with an average ratio between measurements being approximately 1 to 1 (Fig. 3). The intra-assay variability of the SIFT-MS and TD were determined by measuring 10 consecutive breaths directly for acetone and isoprene and compared to 10 TD tubes of 130 ml end expired air each collected consecutively. These values were found to be 17% and 25% respectively (mean/SD × 100%). The inter-assay variability was also calculated by repeat measurements ($n = 5$) for acetone and isoprene and was found to be approximately 8% for both methods.

5. Discussion

Our major finding is that offline TD and SIFT-MS analysis can be effectively combined to measure trace volatiles in expired air such as acetone and isoprene, yielding very similar chemical concentrations to that obtained using direct online SIFT-MS analysis. Indeed, an excellent correlation was observed between the direct and indirect methods with the ratio between the gas concentrations measured using the two techniques being close to the ideal 1 to 1 ratio. The advantages of this combination over online SIFT-MS analysis are that breath samples can be taken offline, stored and transported prior to analysis. This is of importance in some clinical

applications when access to the SIFT-MS instrument is not feasible at the point of care. Compared to conventional TD-gas chromatographic analysis, TD–SIFT-MS is significantly faster with sample analysis times of approximately 5 min or less being readily achievable. One technical problem to be overcome is that the oven used to heat the TD tube during desorption is not actively cooled. This is appropriate for GC analysis which takes 20–40 min to perform and allows ample time for the oven to cool to ambient temperature. It is, however, too slow for the much more rapid SIFT-MS analysis. We have utilized a cold-gas stream to speed the cooling process, however a Peltier type cooling device would offer a simpler and likely more effective solution.

Another offline methodology was recently reported which used SIFT-MS to measure the headspace of exhaled breath condensates (EBC) [19]. This technique offers the same advantage as TD–SIFT-MS in that samples can be collected offline, stored and analyzed at a later time. The investigators noted however that the correlation between condensate headspace levels and directly analyzed breath levels was differed between the compounds analyzed with significant correlations observed for acetone, ammonia, methanol and ethanol but not acetaldehyde, formaldehyde, hydrogen cyanide, propanol, and, notably, isoprene. In the case of isoprene this may be due to the low solubility of the compound, making TD–SIFT-MS a better choice for the offline analysis of isoprene [19]. Furthermore, TD–SIFT-MS can analyze air from a single exhalation while EBC is collected from multiple exhalations, a difference which makes EBC less useful for the measurement of rapidly changing analyte levels. However, given that we have only assessed the suitability of TD–SIFT-MS for two compounds further direct comparison between the two methods would be of interest.

Ideally, the use of TD should allow the sensitivity of the SIFT-MS analysis to be increased by sampling increasing volumes of breath. Our results suggest that caution should be exercised when using larger volumes of breath however. For isoprene at least, increasing breath volumes did not result in the expected continuing increase in measured isoprene quantity, thereby limiting the sensitivity of the procedure. We have previously shown that isoprene applied to TD tubes in the absence of water vapour shows a linear relationship with respect to applied gas volume hence ruling out that our observations are a general feature of isoprene analysis using TD [7]. Furthermore, although acetone did not show the same effect over the breath volumes tested, it cannot be ruled out that higher volumes of breath would have resulted in a similar 'ceiling' effect being observed. We initially supposed that this observation was due to water interfering with the release and/or measurement of isoprene when the TD tubes were desorbed. Purging is a technique used to remove the major components of air, in particular water vapour, which may have interfered with the measurement process [20]. Purging can be particularly useful for measurement techniques that are sensitive to this type of contamination such as gas chromatography mass spectrometry. Purging did not, however, increase the measured abundance of either acetone or isoprene although the water content of the desorbed gas was reduced as expected. As such it is more likely that the large quantity of water vapour in breath interferes with the initial absorption of isoprene onto the sorbent. Although we utilized an appropriate sorbent for the analysis of isoprene [21] it remains a possibility that other sorbents would have performed better in this specific application, although we did not test this possibility. Presently, the plateau effect observed with isoprene limits the sensitivity of TD–SIFT-MS since gas volumes cannot be increased past a rather low amount. If this effect is observed with other low abundance breath markers the sensitivity may be too low to detect them, especially as compared with GC-MS. The sensitivity of SIFT-MS is, however, increasing and this limitation may soon be overcome [22].

Even though acetone and isoprene did not exhibit the same relationship between sample volume and measured chemical abundance, the use of lower sample volumes demonstrated an excellent correspondence between direct and indirect TD analysis methods. Indeed, there is an approximately 1:1 correlation observed for both acetone and isoprene. The two methods did, however, differ in terms of within-analysis variance with the TD–SIFT-MS technique being more variable. This is expected given that while both methods are expected to have similar breath-to-breath variance of exhaled gas levels, given that the fundamental variation is predicted for each single breath by the SIFT-MS software [23], the TD process adds further errors derived from the sampling, absorbance and desorption processes.

6. Conclusion

In summary, we have shown that the combination of TD and SIFT-MS results in very similar breath concentrations of acetone and isoprene being measured compared to direct analysis method. If the sampling volume is carefully controlled, the technique is straightforward to perform and may be usefully employed in clinical situations for which direct sampling and analysis is not feasible, thereby extending the clinical situations into which breath analysis could be employed.

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