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Capillary thermal desorption unit for near real-time analysis of VOCs at sub-trace levels. Application to the analysis of environmental air contamination and breath samples

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Abstract

A capillary microtrap thermal desorption module is developed for near real-time analysis of volatile organic compounds (VOCs) at sub-ppbv levels in air samples. The device allows the direct injection of the thermally desorbed VOCs into a chromatographic column. It does not use a second cryotrap to focalize the adsorbed compounds before entering the separation column so reducing the formation of artifacts. The connection of the microtrap to a GC-MS allows the quantitative determination of VOCs in less than 40 minutes with detection limits of between 5-10 pptv (25ºC and 760 mm Hg), which correspond to 19-43 $ng \cdot m^{-3}$, using sampling volumes of 775 cm^3. The microtrap is applied to the analysis of environmental air contamination in different laboratories of our faculty. The results obtained indicate that most volatile compounds are easily diffused through the air and that they also may contaminate the surrounding areas when the habitual safety precautions (e.g., working under fume hoods) are used during the manipulation of solvents. The application of the microtrap to the analysis of VOCs in breath samples suggest that 2,5-dimethylfuran may be a strong indicator of a person's smoking status.

Introduction

The concept that blood, urine, and other body fluids and tissues can be collected and analyzed to yield information for the diagnosis of disease states or to monitor disease progression and/or therapy is an essential concept in modern medicine. Many important developments in medical monitoring technologies and diagnostic methods have focused on blood and urine analysis for clinical diagnosis. Diagnoses based on breath analysis are much less developed and not yet widely used in clinical practice [1], in spite of the

fact that breath measurement has enormous potential, especially given its inherent safety. The only requirement for the collection of a breath sample is that the subject must be breathing. However, despite its great promise and the fact that there have been hundreds of publications on breath analysis there are only a handful of tests that are used clinically and a few others that are used in research [2].

Breath testing challenges scientists in two ways. First, it is technically very difficult to analyze breath volatile organic compounds (VOCs) that are present at picomolar concentrations. Second, the source and biochemical significance of most breath VOCs are still unknown [3,4]. It is important to develop methods capable of trace determination and so a preconcentration technique is required before the introduction of the collected sample into a gas chromatographic column. To meet the need for clinical applications, a relatively inexpensive, portable instrument capable of providing nonintrusive, real-time, sensitive, and accurate analysis of breath gases for medical diagnosis is highly desirable.

In current public health, smoking is one of the most important single risk factors and is related to several pathological conditions, mainly pulmonary diseases. However, so far we have not identified any valid biomarker that serve as proxies for tobacco exposure and their possible association with and predictive capacity of tobacco-related diseases. This highlights the importance and urgency of conducting research in this area [5].

A variety of biomarkers to evaluate environmental tobacco smoke (ETS) exposure have been proposed. Different chemicals present in tobacco smoke, such as carbon monoxide and cyanide (the latter metabolized in the body to thiocyanate), can be analyzed in

biological fluids. However, these chemicals are not specific markers of ETS exposure as there are significant sources of carbon monoxide and cyanide, including the body's own metabolism, other than ETS. The measurement of nicotine metabolites in biological fluids (mainly blood and urine) has attracted more attention from the scientific community. The determination of cotinine, the major proximate metabolite of nicotine, has been widely used by scientist to evaluate ETS exposure given that this substance reflects exposure to nicotine, which is almost specific to tobacco [6]. Nicotine-derived nitrosoamines such as 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) have also been proposed [7]. This nitrosoamine is metabolized to a butanol metabolite (4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol, NNAL), and its glucuronide (NNAL-GLUC). From the different metabolites evaluated, cotinine appears to be the most specific and most sensitive biomarker for exposure to nicotine from ETS. A limitation of using cotinine, however, is that it indicates ongoing exposure but not long-term exposure to ETS. A further limitation to the use of nicotine metabolites in general is that their determination is complex, requiring the taking of urine or blood samples. The possibility of using breath tests to evaluate exposure to ETS is very promising given that it is a procedure that is safe and easy to perform. Preliminary studies have suggested that some VOCs detected in breath samples may be biomarkers (e.g., 1,3-butadiene, benzene, 2,5-dimethylfuran) [8-16].

The aim of this study is to develop a capillary microtrap thermal desorption module without the use of a secondary cryogenic trap for the focusing of the retained compounds before entering the column in the GC system. This in-house device has been specifically designed for analysis of VOCs at ppbv-pptv levels in breath samples. The system allows fast analysis (less than 40 minutes) of air sample volumes below 1 L.

This will help to monitor near real-time environmental contamination and it will be used for the study and evaluation of smoking biomarkers in breath. The concentration device used here is a variation of one previously described [14,17].

Experimental

Apparatus

The experimental system used for these studies is shown in Figure 1. There is currently no commercial instrument able to analyze sub-ppmv VOCs in breath samples on-line. It is therefore interesting to develop an in-house concentrator/injector device specifically designed for this type of analysis. Such a device should be able to perform two main functions: firstly, to retain target VOCs quantitatively from samples and, secondly, to achieve a fast and quantitative desorption of the adsorbed analytes to be sent to a GC column as a small injection band, preventing the formation of broad peaks with tails in the chromatograms.

A previous study [17] describes in detail the design of a similar microtrap configuration as the one used in the present study, and the heating process and control circuitry have also been described. As there is no single sorbent material that meets the full ideal criteria for concentrating all VOCs from an air matrix, a multi-bed trap must be used for this purpose [17,18]. In this study, we used a three-bed trap for general breath monitoring as it has been found to be effective in analyzing common VOCs present in breath samples [14]. Quantities of 2.5±0.2 mg of Carboxen 1000 and Carbopack X

(Supelco, Bellefonte, PA, USA) and 5.5±0.2 mg of Carbopack B (Supelco) were sequentially introduced in an 80 mm long, 1.35 mm ID Ni/Co alloy tube (Accu-Tube Corp., Englewood, CO, USA). The packed tube was preconditioned by heating at 300ºC for 4 h with a continuous nitrogen flow (200 $mL \cdot min^{-1}$). Sampling and desorption flow were in opposite directions to avoid the least volatile compounds entering into contact with the strongest adsorbent (Carboxen 1000). Under the conditions employed, desorption was complete and no memory effects were observed in subsequent heating cycles.

A vacuum pump (Air Cadet Vacuum Station, Barnant Co., Barrington, IL, USA) was used to pull sample gas from left to right through the trap tube shown in Figure 1 at a sample flow rate of 31 $cm^3 \cdot min^{-1}$. A three-way valve V1 (01380-05, Cole Parmer, Vernon Hills, IL, USA) and two-way valves V2 and V3 (LFVA1230113H, Lee Co., Westbrook, CT, USA) were used for flow control of the sample and the carrier gas. For sample collection, V2 and V3 were opened and V1 was set to the vacuum pump direction. After sample collection was complete, valve V2 was closed for 10 s to let all the sample gas remaining in the lines go through the trap. Valve V3 was then closed and valve V1 was switched to allow the carrier gas to move through the trap line. The use of a bypass carrier gas line (valve V3 line) was necessary during sample collection as otherwise part of the sample moves directly to the separation columns due to the strong vacuum pump of the MS detector. In the case of breath samples, this problem is aggravated due to the presence of substantial amounts of water vapor. A previous study [14] shows that this configuration helps to maintain the pneumatic conditions in the separation column during the sampling process and prevent thermal decomposition on the surface of the carbon-based sorbents of the most reactive compounds in the samples.

Monitoring of the desorption temperature was performed with a J-type thermocouple using 0.127 mm ID wire (36 AWG, Omega Engineering, Stamford, CT, USA) attached to the outer wall of the tube. In order to obtain very rapid heating with a minimal overshoot, a current pulse was applied to the trap for 0.9 s increasing the temperature from 25ºC to the desired desorption temperature (~300ºC). The use of a second low current pulse to maintain the trap temperature, as described in previous studies [14,17], was not necessary as all the adsorbed compounds are quantitatively desorbed with the first pulse.

Materials and methods

All analytes were reagent grade or better. Standards were prepared in 10 L Tedlar gas sampling bags (SKC Inc., Eighty Four, PA, USA) by injecting 1 to 2 μL of individual components and diluting them with dry air. Vapor concentrations were in the range of 8–46 ppmv.

Component separation was achieved by the use of a 30 m length of nonpolar 5% phenyl 95% dimethylpolysiloxane column with 0.25 mm ID and 0.25 μm film thickness (DB-5, J&W Scientific, Folsom, CA, USA for FID applications, and ZB-5ms, Zebron, Phenomenex, Torrance, CA, USA for MS applications). For the evaluation of the developed on-line microtrap, a GC 8000 series (Fisons Instruments, Milano, Italy) with an FID at 250ºC was used. For the analysis of the breath and air samples, a Focus GC

(Thermo Scientific, Waltham, MA, USA) with a mass spectrometer detector (DSQ II, Thermo Scientific) was used.

Helium carrier gas was used after purification for water vapor, hydrocarbons, and oxygen. Instrument and data control was provided by a 12-bit A/D board (PCI-1710HG, Advantech, Taiwan). A sampling rate of 10 Hz was selected.

Sample Collection

Breath samples from students and staff of the University of Girona were collected in 1 L Tedlar gas-sampling bags and analyzed immediately after collection. For each sample, 775 cm^3 of breath was pulled through the multibed sorption microtrap at a rate of 31 $cm^3 \cdot min^{-1}$. It is known that significant changes in breath molecule concentrations take place as a result of variations in ventilation parameters during sampling, and hyperventilation may occur when a breath sample is collected from spontaneously breathing subjects [19]. However, this study was not aimed at determining the amounts of compounds from metabolic sources but rather at developing a reliable and quantitative method with low detection limits that allows the determination of adequate breath biomarkers to establish smoking status. For this reason, spontaneously breathing subjects were used. During exhalation, the first 5 s was not collected in the sampling bag in order to reduce the contamination by dead-space air.

For the analysis of environmental air, 1 L of sample was taken with a 1 L gas syringe (SGE JUMBO syringe, SGE Europe, UK), the sample was introduced in a 1 L Tedlar

bag and analyzed with the system developed. The same sampling volume as in the breath analysis was used unless otherwise specified. Smaller sample volumes were used in situations of large contamination, where sampling time was decreased to avoid the breakthrough of sorbents.

Results and Discussion

Microtrap Evaluation

This study aimed to simplify an "in-house" thermal desorption (TD) module to perform environmental air contamination and breath analysis for compounds at sub-ppmv levels in a reduced time using sample volumes smaller than 1 L. Table 1 shows the list of the target compounds, chosen as probable smoking biomarkers, used in the microtrap evaluation process.

Commercial instrumentation is based on TD modules containing two different traps. A preliminary trap is used to retain the compounds of interest. After the adsorption process, the retained compounds are thermally desorbed but they cannot be directly sent to a GC column because the bandwidth of the compounds after this thermal desorption is excessive. Thus, a second cryogenic trap is needed to focalize the compounds. This cryotrap is also heated and compounds can be sent to a GC column. This process has two main disadvantages. First, the use of two traps and two heating processes increases the formation of artifacts and many compounds can be formed during the heating process, which may lead to the detection of compounds that are not present in the original sample [20]. Second, the use of a cryotrap increases the complexity of the

instrumentation and makes it necessary to use cryogenic gases, which complicates the development of portable instruments based on this configuration. It has been demonstrated that it is possible to develop miniaturized TD modules without the use of cryogenic traps, which allow the direct injection of the retained VOCs in the multibed trap into a GC column [17,20,21]. When using this simplified configuration, the formation of artifacts due to thermal oxidation reactions in the surface of the sorbents is reduced and more reproducible results can be obtained [20].

Feng and Mitra [21] showed that the greater the internal diameter of the adsorbent microtrap, the greater the bandwidth of the desorbed compounds. However, the use of small-internal diameter traps has the disadvantage that there is an increase in the pressure drop along the trap, which decreases the sampling flow rate. This represents a problem when large amount of air samples are to be analyzed. In our case, a trap with a 1.35 mm ID was found to be a workable compromise.

When TD modules without the use of a secondary cryogenic trap are developed, the heat transfer through the material of the microtrap support is also important to obtain fast heating of carbon-based sorbents. The use of glass tubes for the design of the trap simplifies its manipulation and construction. Using glass supports, the heating process has to be performed through a heating wire directly connected to the external wall of the glass support [22,23]. The use of small, thick layers of glass is recommended to facilitate the heat transfer to the carbon sorbents. Unfortunately, the use of glass supports results in a moderate heat transfer through the glass wall leading to a slower desorption process of the retained compounds in the carbon-based sorbents. As can be seen in Figure 2a, the use of a glass support for the trap yields broad peaks at the

beginning of the chromatogram making it difficult to separate pairs of compounds 1-2 and 3-4 when the trap is heated at temperatures around 380ºC (solid-line plot). Larger temperatures have to be applied to achieve a faster desorption of the compounds and to obtain narrow peaks in the chromatograms (broken-line plots). However, when temperatures above 400ºC are applied, carbon-based sorbents are unstable and start to decompose, resulting in the detection of highly volatile compounds being released from them (peaks appearing between 30-35 s). The use of metallic supports results in a better heat transfer to the sorbents, which leads to faster desorption of compounds (Figure 2b). It is not necessary to apply desorption temperatures above the stability temperature of the carbon-based sorbents to obtain constant bandwidths for the desorbed compounds. A desorption temperature around 300ºC has been found to be sufficient to obtain adequate bandwidths [17], which also helps to prevent thermal degradation of the most reactive VOCs [20].

The use of carbon molecular sieve (CMS) sorbents in the multibed trap suffers from the disadvantage that this bed determines the water uptake of the system, which is a problem when air samples containing large amounts of water vapour have to be analyzed, as in the case of breath samples. Unfortunately, CMS are required for the quantitative adsorption of compounds that are more volatile than pentane and high polarity low-molecular-weight compounds [24,25]. The evaluation of a multibed trap without the presence of a CMS showed that some of the main components in breath samples (e.g., acetone, methanol, ethanol) cannot be adequately adsorbed when only graphitized carbons are present in the sorbent tube. Given that Carboxen 1000 saturates at larger volumes of water than other CMS [26], it would seem to be the best choice for the analysis of breath samples.

Similar trap configurations to the one used in this study have previously been described using four-bed [17,20] and three-bed configurations [14]. The use of a three-bed trap is adequate for the analysis of breath samples and a previous study has managed to detect more than 250 VOCs using this configuration [14]. However, the cost of a GCxGC-TOF-MS instrument and the complexity of the technique make it unsuitable for routine analysis in which it is necessary to evaluate a large number of samples in short periods of time. The use of conventional GC instruments with simple detectors is preferable for this type of study.

GC with FID as a detector is the most common and simplest instrumentation for the analysis of VOCs. FID is a suitable detector when a synthetic sample with a known composition is analyzed. However, when a complex sample such as breath is evaluated, this detector yields many peaks that cannot be correctly assigned to a specific compound because of the complexity and variability of samples. As can be seen in Figure 3, there are many compounds detected in a breath sample at ppbv-pptv levels. In the case illustrated, only acetone, isoprene (the main components in all breath samples), and hexane (a known environmental air contaminant in this sample) can be adequately assigned due to the lack of specificity of the detector. Unfortunately, the use of an FID only allows screening analysis of breath samples, making it impossible to perform an adequate study of biomarkers using a GC-FID instrument. An MS detector is more suitable for the performance of efficient biomarker studies in breath samples.

Figure 4 shows the chromatograms of the analysis of breath samples from a smoker and a non-smoker using the MS detector in scan mode with an m/z interval from 35 to 200.

The target compounds were quantified using a target ion and identified using qualifier ions and retention times, which are shown in Table 1. The large variation in the concentrations of the compounds in breath samples (acetone at ppmv level and other target VOCs at ppbv and pptv levels) results in a non-smoker chromatogram in which many compounds are seen as minor components that can easily be associated with the noise of the chromatogram when poorly trained staff perform the analysis.

Quantitative Analysis

Calibration data was evaluated for all the target compounds selected. Calibration ranges (5 ng to 150 μg) were chosen to cover a mass range similar to that expected in breath samples for healthy non-smokers. Sampling times similar to those used in breath samples analysis (5-25 minutes) were also used in the calibration analysis. Three replicates were conducted at each sampling time, and five different mass values were used for calibrations. All compounds showed linearity in the range studied and the determination coefficients were above 0.99 in all cases, which can be considered adequate taking into account the small concentrations of the standards and the use of adsorption times of up to 25 minutes.

The quantification limits obtained for all the compounds were in the 20-40 pptv range for 775 cm^3 of sample. The experimental detection limits (determined for peaks with a signal-to-noise ratio of five in the chromatograms) were between 5-10 pptv for the target compounds. The use of larger sample volumes could lead to reduced quantification and detection limits. However, this would also increase the analysis time and require a large amount of sample. The results obtained in the analysis of 31

different breath samples from smokers and non-smokers show that the quantification limits obtained in the analysis of 775 cm^3 of breath sample was enough to determine all the target compounds evaluated. The analysis of sample volumes below 620 cm^3 does not allow the identification of one of the target compounds, 2,5-dimethylfuran, in some samples. In the chosen conditions, 40 minutes is sufficient to perform the complete analysis (adsorption, thermal desorption, and GC analysis).

Environmental Air and Breath Analysis

The objective of the device being developed in this study is not to determine all the compounds present in breath samples but rather to determine the specific target compounds set out in Table 1.

As indicated in the "Trap Evaluation" section, the use of FID detection does not allow the correct identification of the target VOCs in breath samples. However, the use of this detector has been useful to observe the effect of environmental air contamination in the breath of volunteers working in different laboratories. Figure 5 shows the chromatogram of a volunteer non-smoker working in a chemistry laboratory (Fig. 5a). The peaks detected at around 3, 4, and 6 minutes are not usually present at these levels in the breath of healthy volunteers. Posterior analysis of the environmental air of the laboratory where the volunteer was working (Fig. 5b) showed the presence of these compounds in the air of the laboratory, which were also breathed by the volunteer resulting in exogenous contamination of his breath. The analysis of a sample from another volunteer in the same laboratory after the ingestion of a sweet shows the

presence of the same environmental air contamination as well as the exogenous contamination from the flavours in the sweet (Fig. 5c).

The contamination due to exposure in working environments was evaluated during working and non-working periods. Figure 6 shows the evolution of the concentration profiles for four VOCs (acetone, methylene chloride, hexane, and ethyl acetate) in the environmental air of a chemistry laboratory during a working day. These solvents are used daily in an adjacent laboratory where basic safety precautions (e.g., working in safety cabinets with fume hoods) are taken during their manipulation. However, some poor working habits were also revealed (e.g., laboratory doors were usually open and VOCs had the possibility to be diffused into common areas and other laboratories). The routine use of acetone and methylene chloride in this laboratory releases higher contents of these VOCs into the air during working hours. Profiles obtained for hexane and ethyl acetate show some undetermined maximum levels at specific time periods as these solvents were used discontinuously. Note that at the beginning of the working day levels detected for all compounds are minimum because these solvents were not in use during the night. A slight decrease in the contamination was also observed just after 13:00 h due to the lunch break. The analysis of the environmental air of the same laboratory during non-working days was always significantly lower, with levels detected above detection limits but below quantification (concentrations ranging between 10-40 pptv).

These results are very promising as they demonstrate the possibility of the developed system performing near real-time air contamination analyses. Other similar systems, and also common passive sampling systems, only allow the determination of the

average contamination over a period of some hours. The developed system permits one analysis to be made every 40 minutes with detection limits in the low pptv levels ($ng \cdot m^{-3}$). This leads to a more precise knowledge of the concentration profile of contaminants and may help for preventative measures to be taken faster possibly avoiding dangerous conditions.

The analysis of employees' breath samples working in those laboratories where contamination was found showed these contaminants to be present in their breath at higher levels than were detected in volunteers not working near to a laboratory (Figure 7). In the case of methylene chloride, concentrations detected in breath samples of employees working in a chemistry laboratory ranged from 4 to 103 ppbv (n=16, sd=26). Levels detected for those who do not work in or near a chemistry laboratory ranged from 0.9 to 4.5 ppbv (n=15, sd=0.8). The large variability found in the first group is due to the different exposition of the volunteers to the contaminant. 103.1 ppbv were detected in a person who was directly manipulating this solvent (using a fume hood). Other employees who did not manipulate this solvent reached values of between 4 and 49 ppbv, depending on their proximity to the source and the time of day. These results show that the use of conventional safety precautions (conventional fume hoods) when working with solvents containing VOCs is not sufficient to prevent contamination of the environmental air. Moreover, the high diffusivity of the most volatile compounds also results in the contamination of the surrounding areas. This means that more intensive safety precautions should be taken when these solvents are used.

The results obtained in the evaluation of the target compounds chosen as probable smoking biomarkers (Table 2) fit well with the findings of earlier studies [8-

11,13,14,16]. Benzene, toluene, and m-,p-xylene concentrations detected in smokers (n=14) show a significant difference (p<0.001, test U of Mann-Whitney) to those levels found in non-smokers (n=17). The evaluation of the data obtained for *o*-xylene does not show significant difference between the two groups (p=0.082). The main problem observed with benzene, toluene, and *m*-,*p*-xylene is that all these compounds are also present in non-smokers and their variability in non-smokers is sufficiently large as to make it difficult to determine their smoking status unless samples from smokers are analyzed shortly after the last puff of the cigarette. As can be seen in Table 2, the maximum concentration levels found for these compounds in non-smokers were always higher than the minimum levels found in smokers. Previous studies [9,12,13] found that benzene content in breath decreases rapidly to pre-exposure levels in less than 1 hour after smoking.

In order to distinguish between endogenous substances and exogenous contaminants, corrections for background concentrations of volatile compounds in inhaled air is mandatory. For this reason, the most promising results for probable smoking biomarkers seem to be obtained with 2,5-dimethylfuran. This compound has only been detected in one sample from a non-smoker, who was a passive smoker living in a house with smokers and had more than 3 hours of contact with the smoke of cigarettes every day. This suggests that correction for background concentration may not be needed for this compound and also that it might be a strong indicator of passive smoking.

Conclusions

The simplicity and high sensitivity of the capillary TD module developed and the ability to determine the presence of VOCs at sub-ppbv levels in both environmental air and breath samples in a short period of time (less than 40 minutes) introduces new perspectives in this type of analysis. The possibility of obtaining near real-time determination of VOCs has allowed us to demonstrate that contamination by highly volatile compounds takes place very easily. Routine safety precautions for working with solvents containing highly volatile compounds are not sufficient for a full prevention against contamination of the environmental air of the working space and surrounding areas.

The analysis of probable smoking biomarkers in breath samples shows that compounds such as benzene and toluene can be used for the determination of smoking status but only when samples are analyzed shortly after smoking. The results obtained with 2,5-dimethylfuran seem to be very promising as this compound has only been detected in active smokers and one passive smoker.

The possible use of 2,5-dimethylfuran as a smoking biomarker has also been proposed in other previous studies [8,14-16]. However, the reduced number of samples evaluated in these studies makes them insufficient for adequate statistical analysis and, furthermore, there is the need for a clinical study to test this hypothesis and the utility of the compound as a useful smoking status biomarker. This study is currently been performed in our laboratory and the results will be evaluated after a suitable number of samples have been analyzed.

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Figure Captions

Figure 1. Diagram of the capillary multi-bed sorption microtrap / GC system developed. Valves V_1, V_2, and V_3 are used to control the gas flow direction through the trap tube. Inset shows details of the three-bed sorption trap design (see text for details).

Figure 2. Chromatograms of a synthetic mixture containing five highly volatile compounds to evaluate the desorption performance of the microtrap after a rapid heating pulse is applied to the trap for the desorption of the adsorbed VOCs. (a) glass support, (b) metallic support. Desorption temperatures: 380ºC (solid lines) and 500ºC (dashed line).

Figure 3. GC-FID chromatogram of a human breath sample collected from a volunteer. A sample collection time from a 1 L gas sampling bag of 25.0 min at 31 $cm^3 \cdot min^{-1}$ was used (775 cm^3 breath sample).

Figure 4. GC-MS chromatograms of the analysis of breath samples from a smoker after 1 minute of smoking (a) and a non-smoker (b). Detection in scan mode (m/z range 35-200). Peak numbers correspond to the compounds number in Table 1.

Figure 5. GC-FID chromatograms of: a) breath sample from a non-smoker working in a laboratory, b) the environmental contamination of this laboratory, and c) breath sample of a non-smoker working in the same laboratory after eating a flavored sweet.

Keynote: A - Acetone; C - Environmental air contamination; F – Sweet flavors.

Figure 6. Concentration profiles found for acetone, methylene chloride, hexane, and ethyl acetate in the environmental air of a chemistry laboratory during a working day.

Figure 7. Median and quartiles for the methylene chloride levels detected in breath samples from employees working in a chemistry laboratory and those who do not work in or near a chemistry laboratory ($p<0.001$).

Table 1. Target compounds in chromatographic elution order, and their quantifier and qualifier ions. Retention times (t_R) and method detection limits (MDL) are indicated for the GC-MS determination and the analysis of 775 cm^3 of sample.

#	Compound	Quantifier ion	Qualifier ions	t_R (min)	MDL (pptv)[a]	MDL ($ng \cdot m^{-3}$)
1	benzene	78	77, 51, 52	3.24	5	16
2	2,5-dimethylfuran	96	95, 81, 53	3.89	5	20
3	toluene	91	92	5.03	5	19
4	*m*-, *p*-xylene	91	106, 105, 77	6.89	10	43
5	*o*-xylene	91	106, 105, 77	7.07	10	43

a) values determined at 25ºC and 760 mm Hg.

Table 2. Statistics obtained on analysing the target compounds for 31 breath samples. Concentration values expressed in ppbv (25ºC and 760 mm Hg).

Non-smokers (n=17)

	benzene	dimethylfuran	toluene	*m*-, *p*-xylene	*o*-xylene
Mean	1.94	0.03	1.23	0.10	0.44
Median	0.73	0.00	0.97	0.07	0.23
Variance	5.26	0.02	1.45	0.01	0.52
Minimum	0.12	bdl*	0.14	0.03	0.03
Maximum	6.88	0.58	3.63	0.39	2.60

* below detection limit (method detection limit = 5 pptv)

Smokers (n=14)

	benzene	dimethylfuran	toluene	*m*-, *p*-xylene	*o*-xylene
Mean	271.87	25.39	89.27	1.00	1.76
Median	28.09	4.73	12.12	0.28	1.19
Variance	$41x10^4$	$42\ x10^2$	$37\ x10^2$	5.37	1.99
Minimum	5.22	0.37	1.31	0.07	0.44
Maximum	2292.54	246.72	675.76	8.97	5.15

Figure 1

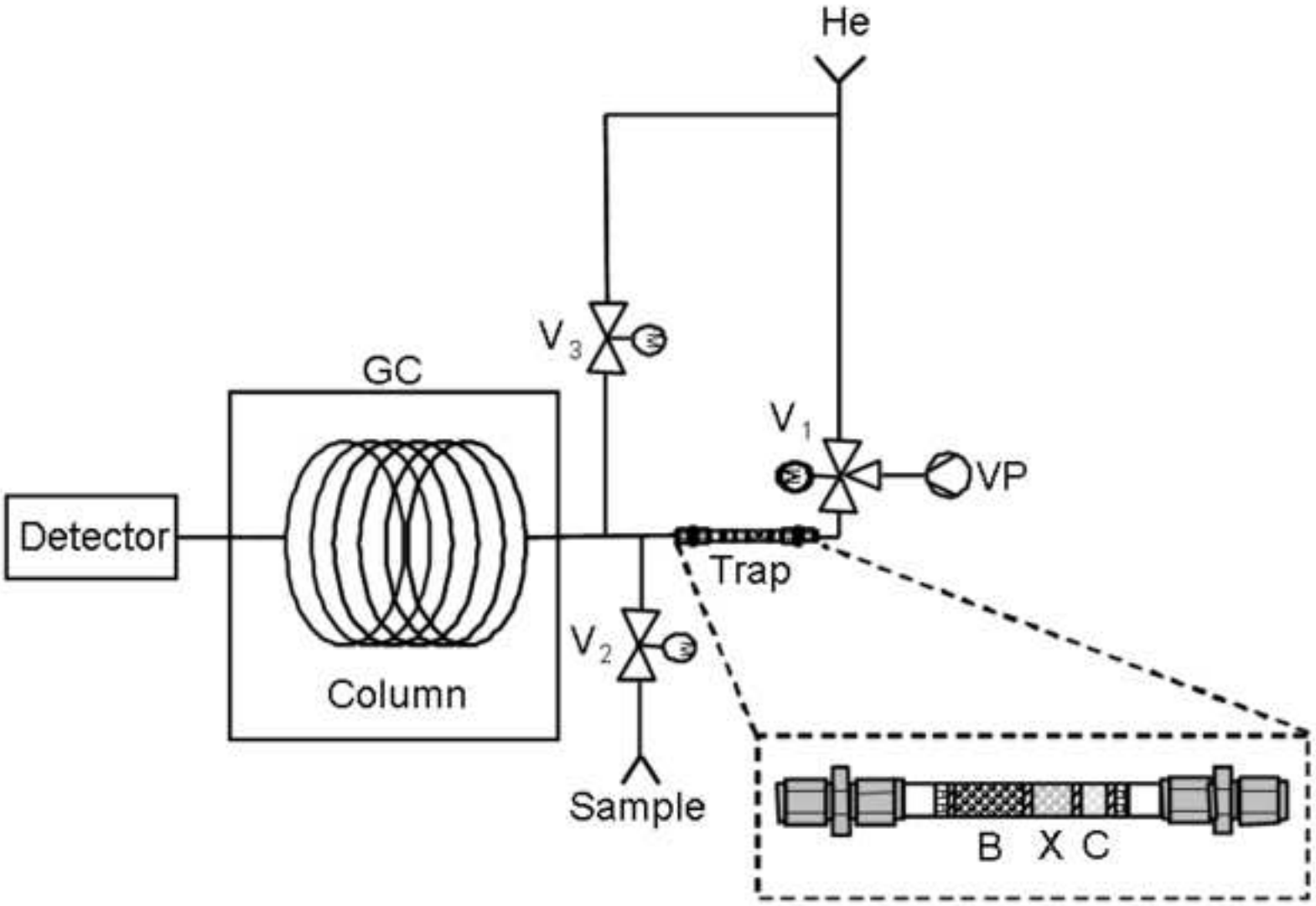


Figure 2

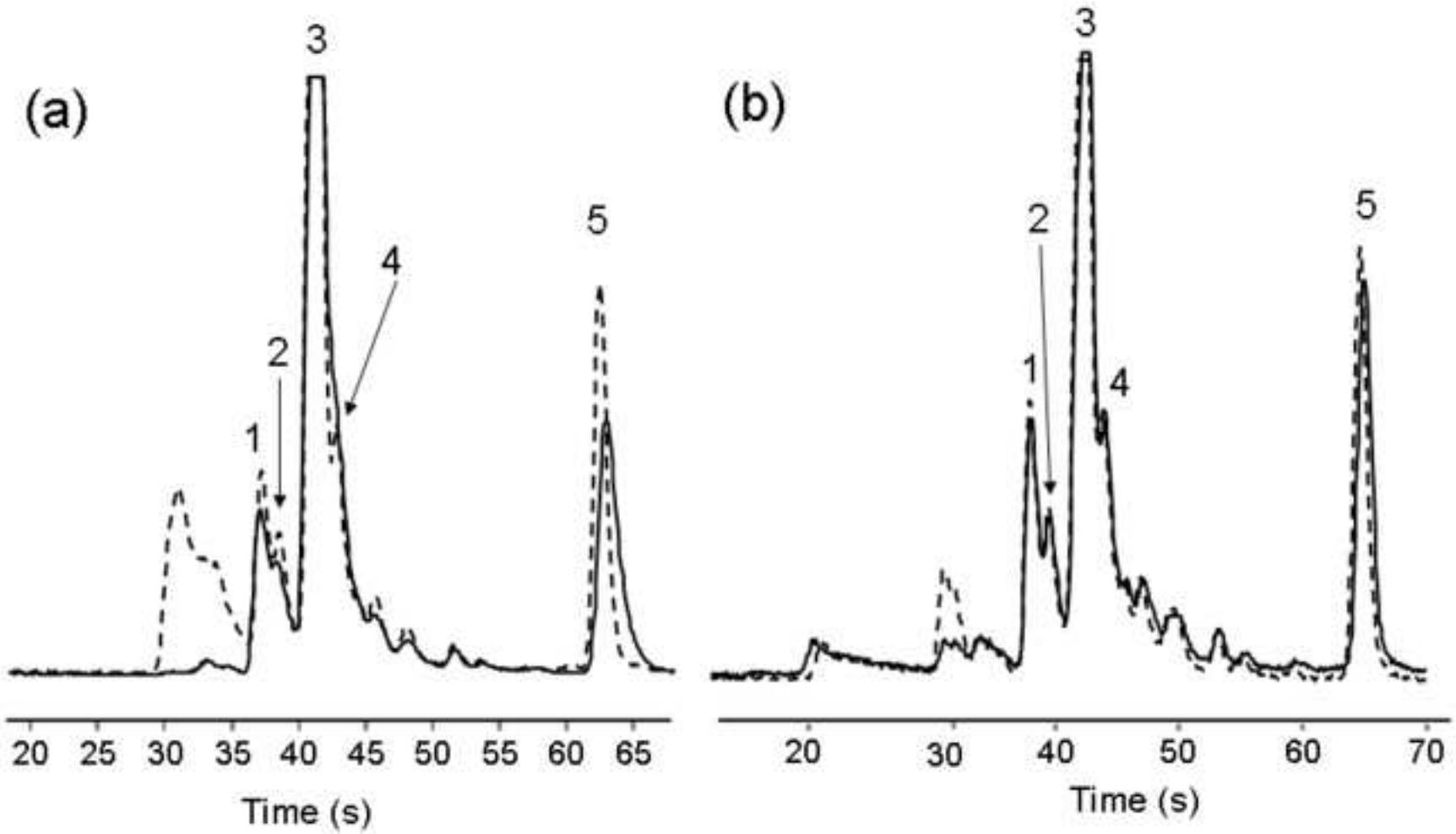

(a)
3
5
4
2
1
20 25 30 35 40 45 50 55 60 65
Time (s)
(b)
3
2
5
1
4
20 30 40 50 60 70
Time (s)

Figure 3

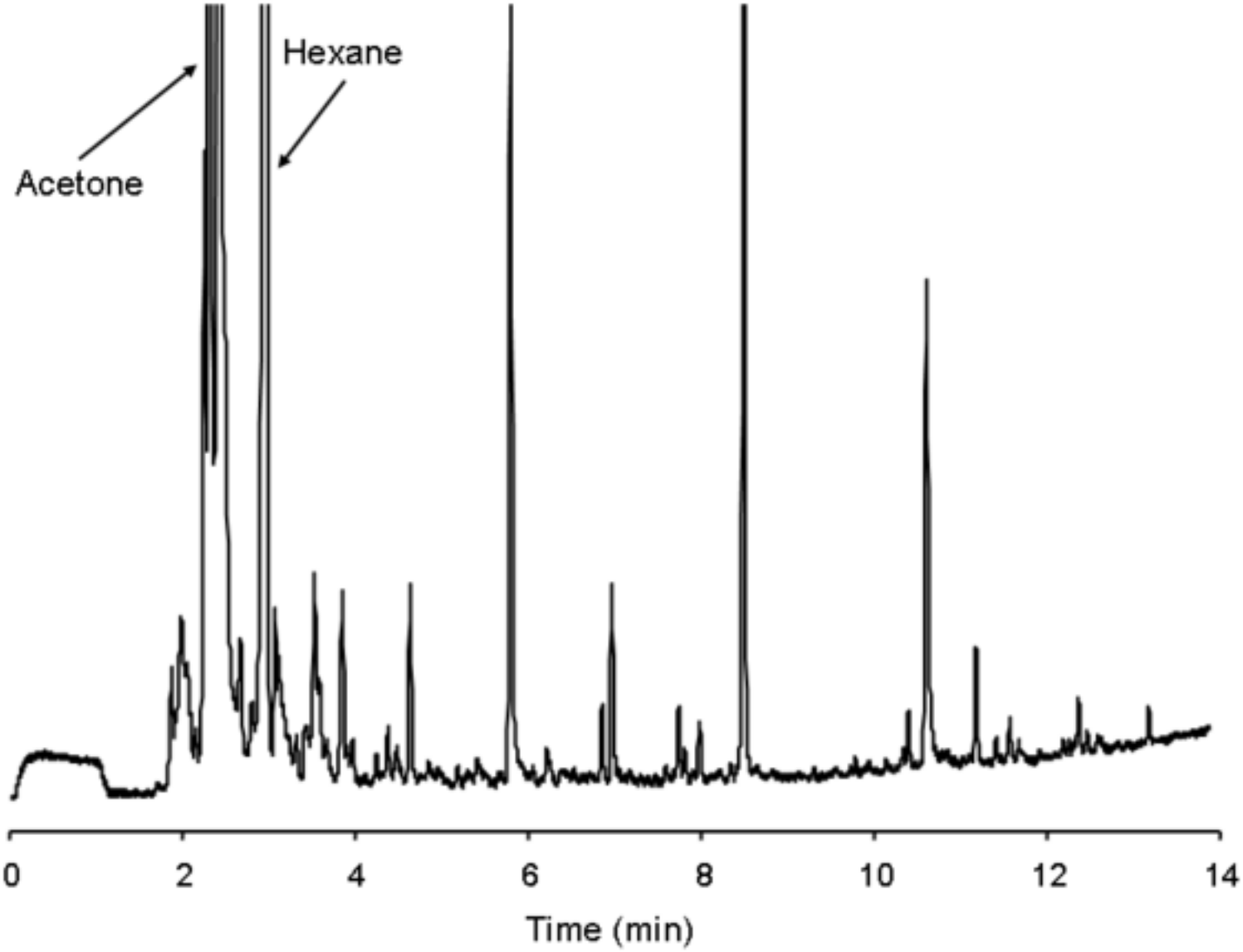
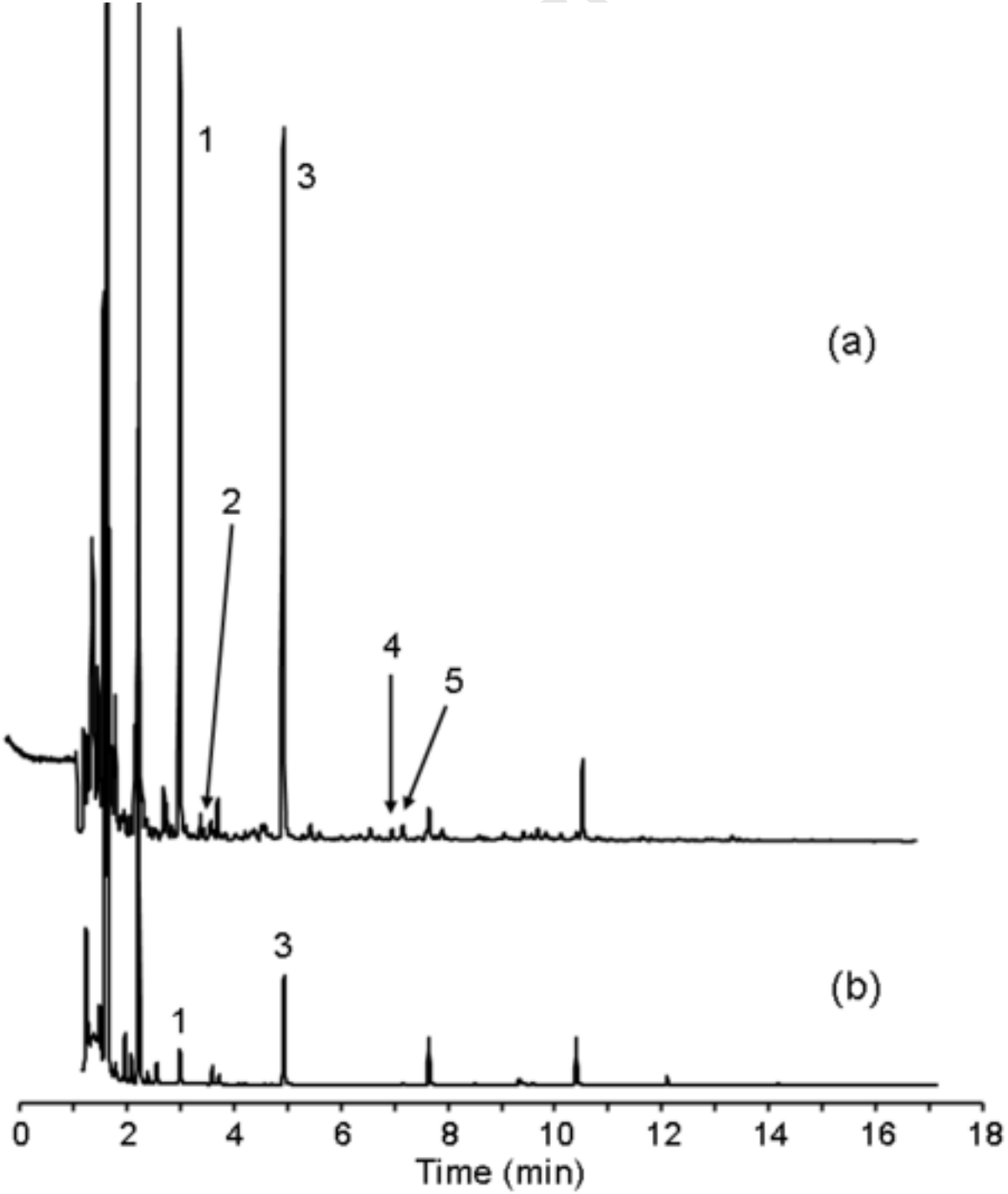


Figure 4



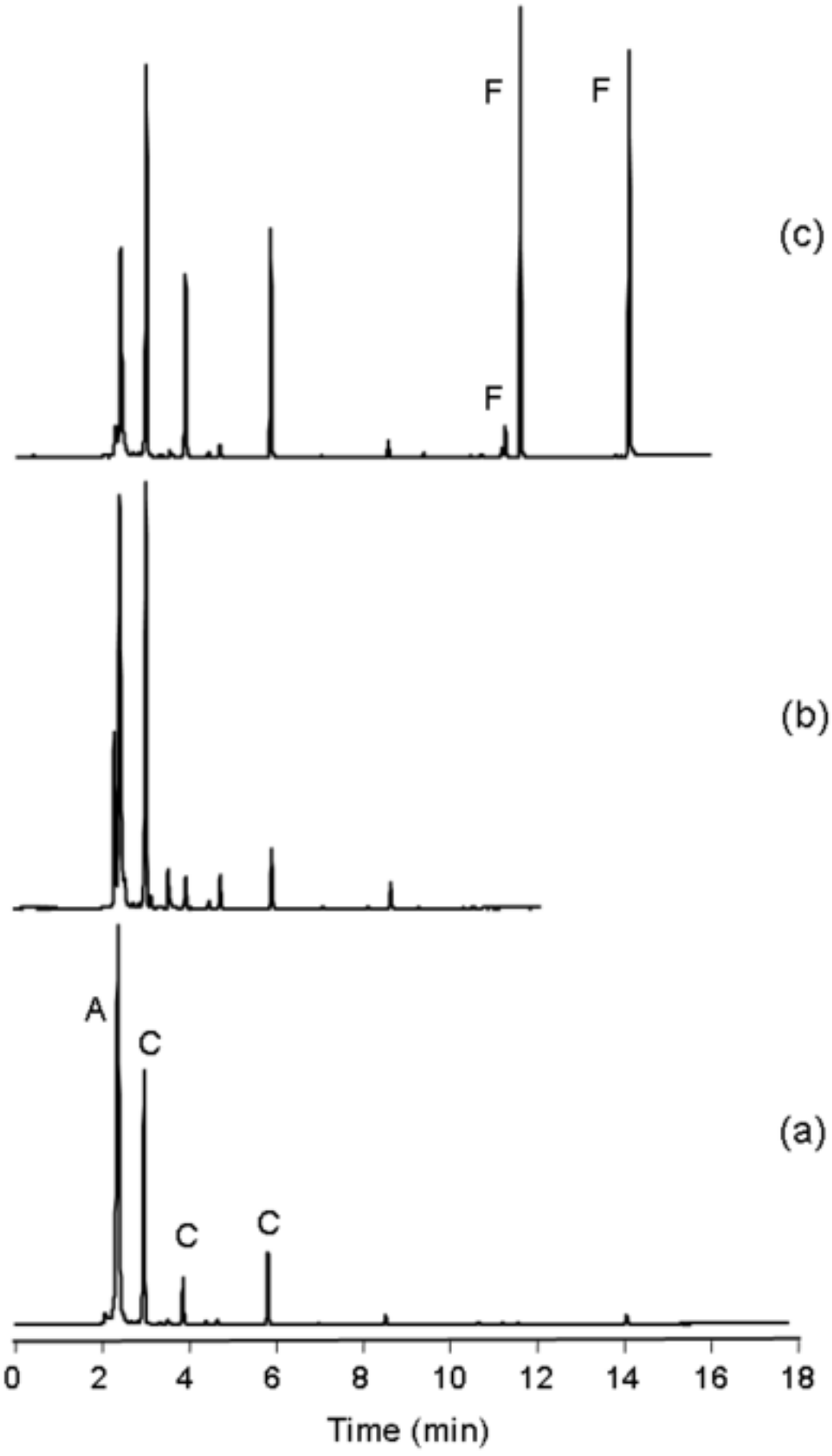


Figure 6

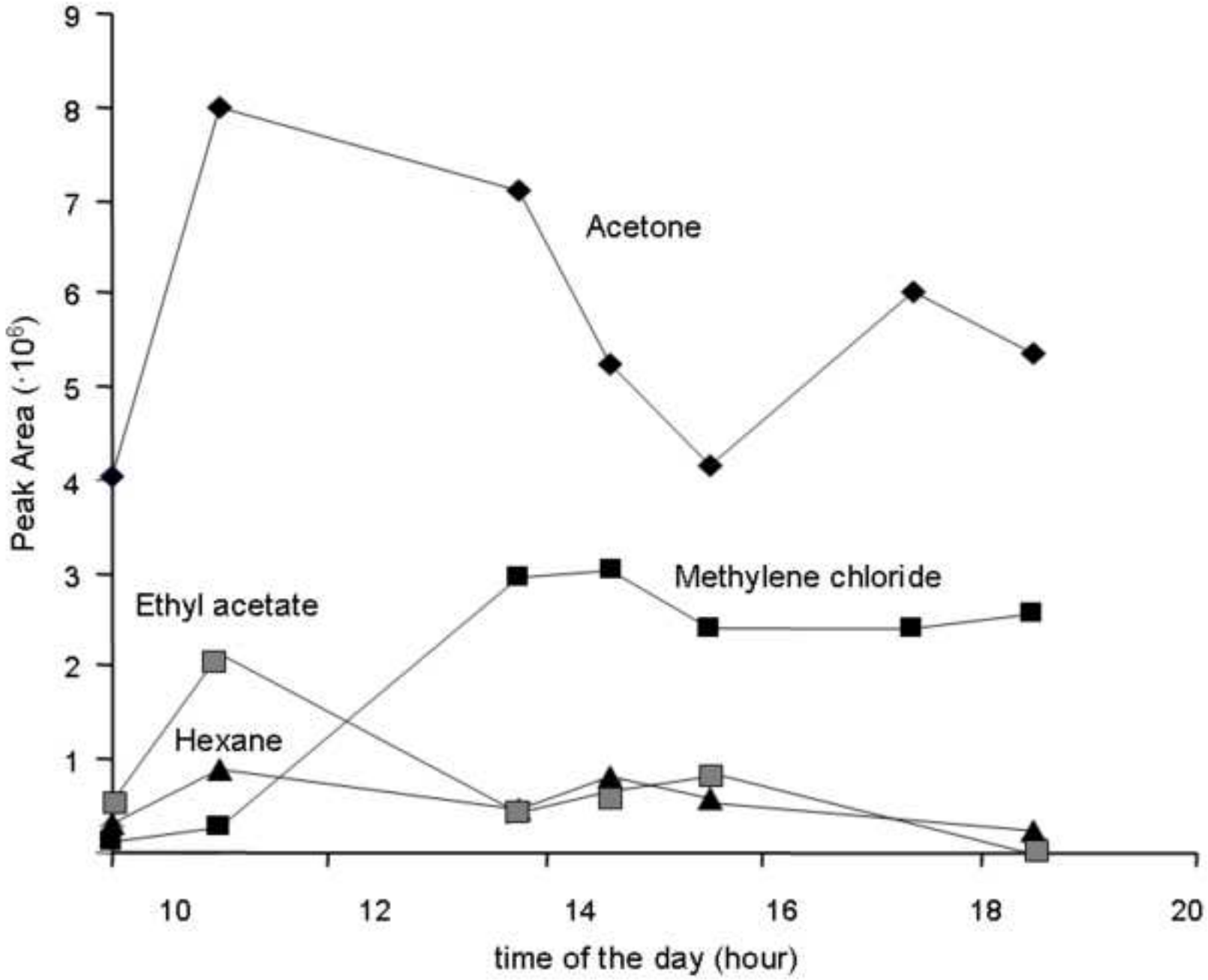
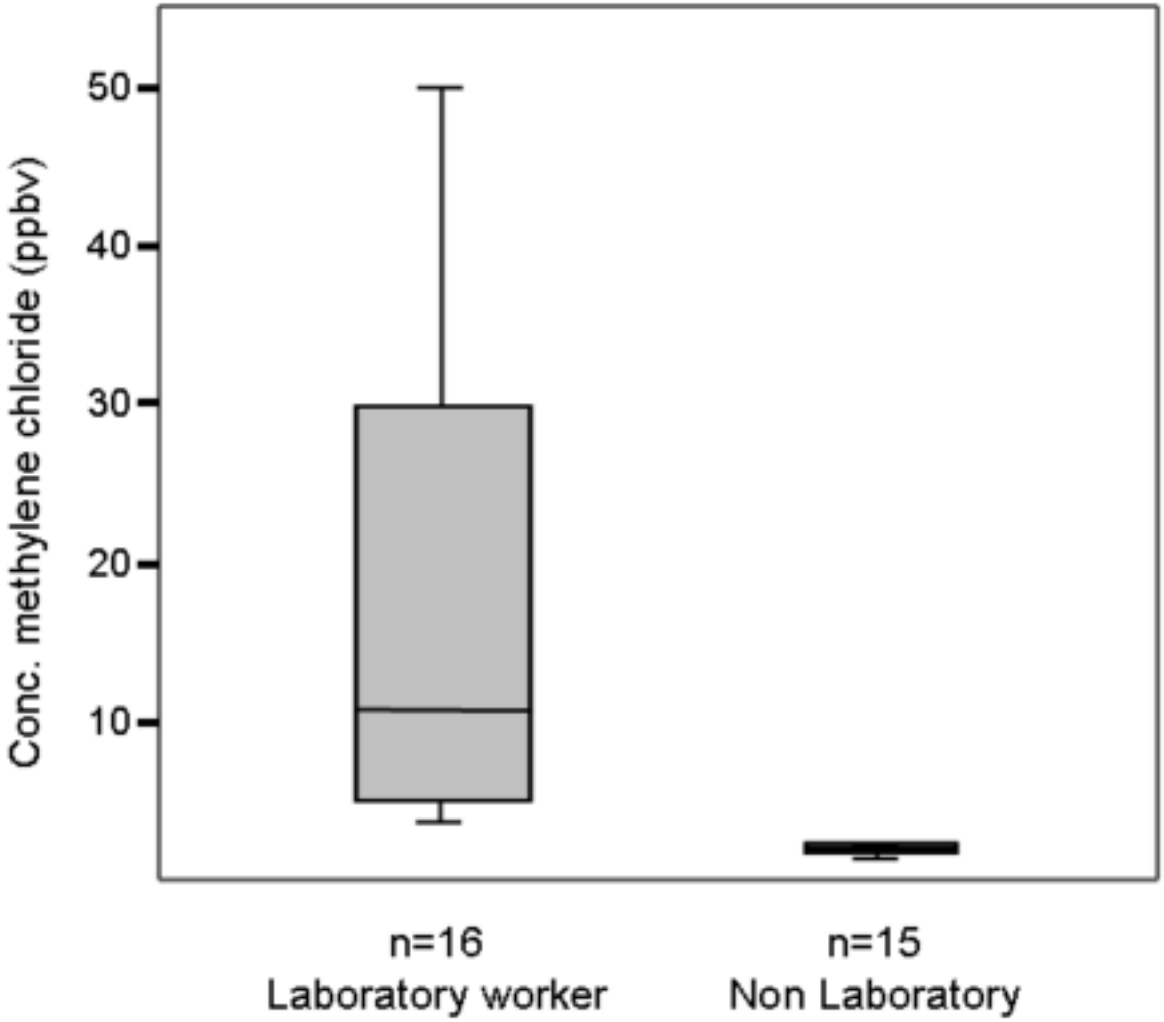

Peak Area (·10^6)
9
8
7
6
5
4
3
2
1
Acetone
Methylene chloride
Ethyl acetate
Hexane
10
12
14
16
18
20
time of the day (hour)

Figure 7


Conc. methylene chloride (ppbv)
50
40
30
20
10
n=16
Laboratory worker
n=15
Non Laboratory