Relation between ambient air and breath volatile organic compounds

H. Dhondt[1,2], E. Goelen[1], G. Koppen[1] & L. Verschaeve[1,2]
[1]*Flemisch Institute of Technological Research, VITO, Belgium*
[2]*University of Antwerp, UA, Belgium*

Abstract

Breath analysis is a non-invasive tool that can be used to measure body impact following exposure to air pollutants. A method for collecting and analyzing exhaled breath was developed and used to compare individual exhaled breath volatile organic compounds (VOCs) with ambient air VOCs. Exhaled breath was collected in Teflon bags and analyzed using thermal desorption gas chromatography – mass spectrometry. The repeatability of this method was examined following analysis of 10 breath samples of each of three subjects. An environmental health study including 56 3-year-old children in Flanders examined abundances of VOCs in exhaled breath in relation to the presence of these VOCs in ambient air. It was demonstrated that a number of VOCs were either significantly retained or cleared by the body.
Keywords: exhaled breath, volatile organic compounds, ambient air.

1 Introduction

Monitoring the influence of environmental pollution on human health has increased remarkably during the past decades. So far, methods to evaluate exposure to or impact of these environmental pollutants mainly involved the collection and analysis of biological media such as blood or urine. Measuring biomarkers in breath, however, is a very attractive approach to monitoring environmental impact because it is non-invasive and makes repeated sampling possible [1]. Breath contains valuable information because the pulmonary alveolar membrane consists of a thin barrier separating the air in the alveoli of the lung from the blood in the capillaries.

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The structure of this membrane allows diffusion of many volatiles. Consequently, inhalation of VOCs – present in the ambient air – will result in absorption of these VOCs by the pulmonary blood supply followed by subsequent distribution of these VOCs throughout the body. Following exhalation, the alveolar air will subsequently be enriched by the VOCs contained within the pulmonary blood to an extent determined by the concentration of the VOCs in the blood and blood-gas partition coefficients [2]. This blood – gas exchange model has resulted in extensive research with regard to the breath content and the potential to use breath analysis as a diagnostic tool able to link breath components with pathologies and their early onset. Almost 30 years ago Pauling and co-workers [3] reported that normal human breath contains a mixture of several hundred volatile organic compounds (VOCs). Since then new techniques have been developed and explored that allow detection of > 1000 volatiles in human exhaled breath [4]. Most studies, however, focus rather on singular compounds in exhaled breath as a potential biomarker for specific biological effects. Recently, profiling of VOCs in exhaled breath is gaining interest in the field of biomarker research [5, 6]. Metabolic profiling considers a set of compounds, their combinations and specific relations as a potential biomarker rather than increase or decrease of singular compounds. The aim of this study is to develop a method for collecting and analyzing exhaled breath of a variety of human subjects (children, patients, healthy controls) using gas chromatography – mass spectrometry. The method will be used firstly to compare individual exhaled breath VOCs with ambient air VOCs and secondly to find exhaled breath VOC patterns specific for oxidative stress.

2 Methodology

In order to find suitable combinations of compounds or specific ratios of volatile organic compounds that relate to specific health effects (metabolic VOC profiles), analytical methods are required that are able to detect, identify and possibly quantify as many VOCs as possible in exhaled breath. Such screening methods, however, often require large sample volumes because of the low concentrations of most VOCs present in the exhaled breath. For this reason a thermal desorption gas chromatography – mass spectrometry (GC-MS) method was further developed using sorbent tubes to preconcentrate large amounts of exhaled breath.

2.1 Sampling and sample preparation

Exhaled breath of study subjects was collected in Teflon bags. A 3-way valve was used to facilitate the collection of breath for children and for patients with severe airway obstruction. A Gillian® personal sampler was used to draw the breath content of this sampling bag over a sorbent tube containing 3 cm Carbograph 1TD/ 3 cm Carbopack X. For each breath test an equivalent amount of ambient air – present in the room which the subjects occupied during the breath test – was sampled on a sorbent tube. Although breath consists of a

relatively 'clean' sample matrix compared to urine or blood, the high CO_2 content and humidity can turn out to be a serious challenge to GC-MS analysis. Because the moisture trapped onto the sorbent tubes was found to interfere with GC-MS output, sorbent tubes were purged with 500 mL Helium (50 mL/min) prior to analysis to expel the moisture.

2.2 Thermal desorption gas chromatography: mass spectrometry

Sampled VOCs were recovered from the adsorbent traps by thermal desorption (Markes International Ltd.). Analysis was performed by GC (HP 6890 series) – MS (HP 5973 Mass Selective Detector). The column was an RTX 502.2 column with a Crossbond phenyl methyl polysiloxane phase (105 m long, 0.32 mm ID and 1.8 µm film thickness). Thermal desorption, gas chromatography and mass spectrometry parameters are summarized in Table 1.

Table 1: Analytical settings of thermal desorption unit, gas chromatograph and mass spectrometer.

Parameter Desorption Unit	Setting
Primary desorption flow	20 mL/min
Primary split	Splitless
Primary desorption temperature	250 °C
Primary desorption time	15 min
Cold trap volume	0.02 mL
Cold trap temperature	-10 °C
Cold trap packing	Carbograph 1TD/Carbograph 2TD
Trap heating rate	MAX
Secondary desorption temperature	325 °C
Secondary desorption time	3 min
Prepurge time	1 min
Prepurge flow	20 mL/min

Parameter Gas Chromatograph	Setting
Column pressure	140 kPa
Initial temperature held 1 min	35 °C
Final temperature	270 °C
Temperature ramp	5 °C/min → 35-200 °C; 70 °C/min → 200-270 °C

Parameter Mass Spectrometer	Setting
Scan mode	EI
Temperature	230 °C
Transfer line temperature	175 °C
Scan range	25-200 amu
Scan frequency	2.14 scans/s

3 Results and discussion

3.1 Repeatability experiment

Repeatability of this method was evaluated by determining coefficients of variance for 56 VOCs present in exhaled breath of 3 subjects. Three subjects were asked to fill a 56 L Teflon bag with exhaled breath. 10 x 5 L of exhaled breath of each of the 3 subjects was captured on sorbent tubes and samples were subsequently analyzed. Coefficients of variance for these 56 VOCs are summarized in table 3. Coefficients of variance for these VOCs were well within acceptable range with 89% of the coefficients being ≤ 30%. Multiple ANOVA indicated that coefficients of variance were both subject ($p < 0.00$) and component ($p < 0.00$) dependent (Table 2).

Table 2: Multiple ANOVA results for coefficients of variance.

Source	Sum of Squares	Df	Mean Square	F-ratio	P-value
Subject	4086	2	2034	16.3	**0.00**
VOC	18334	71	258	2.1	**0.00**
Residual	17690	142	125		
Total (corrected)	40092	215			

Table 3 also shows abundances for VOCs in exhaled breath and ambient air for 2 non-smoking subjects and 1 smoker. VOCs for which areas under the curve (AUC) in exhaled breath were at least twice these in ambient air are marked. These compounds represent VOCs which are most likely to be metabolites produced by the body. Some of these well known metabolites include 2-methyl-1,3-butadiene, acetone, dimethyl sulfide, dimethyl disulfide, ethanol and 1-propanol. AUCs for VOCs in ambient air that exceed twice the amount of VOC in exhaled breath are also marked and represent those substances that are most likely to be retained by the body.

Data for subject 3 are somewhat different to these of subjects 1 and 2 as it seems that most VOCs are rather produced by the body than retained. A possible explanation could be that smokers are more exposed to these substances and that build-up of these VOCs leads to a release of these compounds rather than a steady state situation. Of course a larger sample is needed to test this hypothesis more fully and to gain more certainty concerning this indication.

3.2 Environmental health study

An environmental health monitoring study performed in Flanders allowed for collection and analysis of 7 L exhaled breath of 56 three-year-old children living in urban and rural areas in Flanders.

Table 3: Coefficients of variance and mean AUCs for 56 VOCs present in ambient air and exhaled breath of 3 subjects.

VOC	subject 1 (non-smoker)			subject 2 (non-smoker)			subject 3 (smoker)		
	CV (%)	Mean AUC[1] (counts x 10^4)	Mean AUC[2] (counts x 10^4)	CV (%)	Mean AUC[1] (counts x 10^4)	Mean AUC[2] (counts x 10^4)	CV (%)	Mean AUC[1] (counts x 10^4)	Mean AUC[2] (counts x 10^4)
1-pentene	9.2	11.1	12.1	10.8	7.3	5.8	9.3	84.2	27.8
pentane	6.9	36.0	39.9	13.1	12.0	10.9	9.3	35.8	23.6
2-methyl-1,3-butadiene	2.0	11889.4	203.7	5.1	6205.2	33.1	1.7	16190.6	217.5
1-hexene	3.6	6.3	6.8	16.5	3.9	3.6	5.2	30.7	4.2
hexane	3.6	13.2	14.1	14.9	9.9	9.0	20.5	26.3	18.1
ethyl acetate	19.0	249.4	1348.0	45.3	104.6	318.6	4.0	14806.3	137.8
2-methyl hexane	12.0	33.7	41.5	10.0	32.0	24.8	19.8	171.1	78.3
3-methyl hexane	5.1	25.3	25.0	9.5	32.0	28.6	18.0	118.7	82.7
1,1,1-trichloroethane	6.0	5.1	6.5	5.5	10.0	10.0	27.5	4.9	19.6
cyclohexane	3.2	46.6	52.8	10.5	49.9	43.9	16.6	79.4	65.5
2,2,4-trimethyl pentane	2.3	51.7	51.9	12.2	75.8	67.0	17.2	106.6	0.0
heptane	1.8	12.3	14.9	8.1	7.8	6.5	13.0	23.2	14.2
benzene	3.4	273.8	483.9	15.5	327.5	449.1	5.1	5020.9	463.2
trichloroethylene	2.9	3.0	4.7	8.9	5.9	8.6	8.5	25.9	8.9
methylcyclohexane	2.5	31.6	39.2	9.3	20.1	17.2	16.6	36.1	25.8
octane	1.7	31.0	41.7	16.4	9.3	6.1	11.5	34.2	9.8
toluene	3.0	815.1	1292.1	13.9	504.7	685.4	10.2	7752.0	2049.8
tetrachloroethylene	1.4	22.0	13.6	9.5	15.5	14.6	21.7	43.6	15.3
nonane	3.2	53.6	64.0	21.0	7.4	5.2	12.8	16.6	7.4
ethylbenzene	6.2	107.9	223.1	18.2	62.3	127.0	10.4	547.2	196.5
m/p-xylene	6.3	220.0	453.7	19.2	115.8	255.5	12.4	940.3	423.7
o-xylene	6.3	94.8	196.4	20.3	41.9	89.4	17.3	202.1	143.4
styrene	9.3	42.3	196.4	21.3	13.8	29.0	4.3	202.7	56.1
a-pinene	4.5	210.9	325.4	12.3	63.8	20.3	23	77.3	74.2
decane	2.0	237.2	316.8	16.4	32.9	25.4	13.7	78.8	50.1
propylbenzene	4.2	78.4	170.9	35.4	23.0	33.7	15.3	80.0	50.5
1-ethyl-2-methyl benzene	3.9	93.5	218.9	21.7	22.3	36.2	20.0	55.1	53.6
1,2,4-trimethyl benzene	5.1	326.9	804.7	25.5	65.6	108.5	27.7	130.3	168.9
1,2,3-trimethyl benzene	5.0	92.2	210.7	24.4	17.0	28.3	18.6	45.3	43.1
ethanol	10.4	1495.4	2443.2	16.8	1085.1	182.3	10.2	28124.7	126.7
acetone	3.0	43032.3	2673.7	6.3	148773.2	420.2	2.9	52176.3	285.4

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Table 3: Continued.

VOC	subject 1 (non-smoker) CV (%)	subject 1 (non-smoker) Mean AUC[1] (counts x 10^4)	subject 1 (non-smoker) Mean AUC[2] (counts x 10^4)	subject 2 (non-smoker) CV (%)	subject 2 (non-smoker) Mean AUC[1] (counts x 10^4)	subject 2 (non-smoker) Mean AUC[2] (counts x 10^4)	subject 3 (smoker) CV (%)	subject 3 (smoker) Mean AUC[1] (counts x 10^4)	subject 3 (smoker) Mean AUC[2] (counts x 10^4)
dimethyl sulfide	9.8	67.8	8.6	8.9	29.3	0.0	8.3	160.7	0.0
1-propanol	8.7	449.0	28.7	17.3	166.6	10.0	1.4	1406.4	2.4
2-butenal	13.4	510.3	18.9	23.9	36.4	9.8	6.9	219.4	12.5
2-butanone	7.6	214.4	243.8	9.3	94.6	74.8	1.9	11111.8	11.1
2-methyl furan	11.6	96.1	6.1	16.2	42.5	2.1	10.5	149.9	4.8
thiophene	64.3	5.0	1.6	8.9	5.3	3.5	6.5	36.2	2.5
2-pentanone	3.1	278.6	28.9	8.4	87.7	9.3	3	1753.9	13.2
1-methylthiopropane	8.2	57.4	0.6	10.8	3.7	0.0	6	207.9	0.0
3-penten-2-one	46.2	167.6	4.8	40.7	4.9	1.1	28.4	87.1	2.0
dimethyl disulfide	31.1	105.3	14.3	43	8.9	1.7	30.4	83.8	1.8
3-methylthiophene	60.5	271.0	3.1	27.9	72.7	3.3	48.1	64.6	3.3
hexanal	27.9	27.9	462.8	66.1	9.6	2.6	42	17.3	17.1
4-methyl octane	4.9	34.1	41.5	15.1	9.3	8.7	18.2	7.6	5.5
3-furaldehyde	30	15.4	1.4	31.3	3.4	0.4	23.2	11.1	0.3
2,6-dimethyl octane	3.8	28.7	28.4	11.2	5.9	5.0	23.1	11.0	6.8
2-heptanone	10.9	18.0	32.4	51.2	6.9	1.1	9.9	25.2	1.2
2-methyl nonaan	6.4	20.9	22.4	14.9	5.0	4.5	17.3	9.9	8.2
camphene	8.5	28.9	59.2	23.4	14.7	9.8	4.8	72.1	18.2
1-ethyl-3-methyl benzene	5.4	292.9	677.2	22.6	14.7	20.3	22	179.9	17.3
b-pinene	10.4	47.0	168.5	34	17.1	0.9	17.2	17.8	5.2
dimethyl sulfone	21.8	318.2	7.8	53.5	59.2	0.0	29.9	271.7	1.8
phenol	18.5	572.7	113.4	37.6	58.2	3.8	18.6	161.8	0.0
D-limonene	4.9	434.6	840.5	9.9	155.4	11.1	4.7	691.9	70.6
eucalyptol	11	10.3	16.5	31.3	6.8	0.0	13.5	3.7	0.0
methyl-ethenyl benzene	13.1	78.0	157.1	29.4	24.7	5.7	17.5	47.7	15.4

mean AUC breath ≥ 2 x mean AUC ambient air

mean AUC ambient air ≥ 2 x mean AUC breath

CV (coefficient of variation) > 30 %

[1] breath

[2] ambient air

A standard gas mixture containing 52 VOCs of the 189 hazardous air pollutants mentioned in 1990 Clean Air Act Amendment [7] was used as a means to monitor these compounds in human breath. Of these 52 VOCs, 43 could be identified in the breath of these 3-year old subjects. Abundance of these 43 VOCs was compared to corresponding abundances of these VOCs in the ambient air the children inhaled previous to the breath test. A logarithmic (with base 2) transformation was applied to normalize both ambient air and breath abundance data. A paired student t-test at significance level of 0.01 was performed taking differences between abundances of VOCs in ambient air and exhaled breath. Table 4 shows the results of this t-test. A negative t-value indicates production or release of this VOC by the body. A positive t-value indicates retention of the VOC by the body.

Table 4: Retention / release of VOCs by the body.

VOC	t	p-value	VOC	t	p-value
isobutane	3.76	0.000	chlorobenzene	-2.80	0.007
2-methyl butane	4.20	0.000	butane	2.76	0.008
2-methyl-1,3-butadiene	-24.46	0.000	2,2,4-trimethyl pentane	-1.82	0.074
dichloromethane	14.93	0.000	heptane	-1.76	0.084
chloroform	5.32	0.000	propylbenzene	-1.71	0.093
3-methyl hexane	-4.64	0.000	3-methyl heptane	-1.63	0.108
1,1,1-trichloroethane	6.51	0.000	hexane	-1.54	0.130
cyclohexane	-3.88	0.000	methylcyclohexane	-1.45	0.153
octane	-4.19	0.000	2-methyl heptane	-1.43	0.157
tetrachloroethylene	-5.39	0.000	2-methyl pentane	-1.20	0.235
nonane	-4.19	0.000	trichloroethylene	-1.18	0.245
styrene	-14.57	0.000	ethylbenzene	1.15	0.256
decane	-6.13	0.000	1,3-Butadiene	0.88	0.381
1-hexene	-3.47	0.001	3-methyl pentane	-0.62	0.536
2-methyl hexane	-3.21	0.002	benzene	-0.50	0.622
1,2-dichloroethane	3.28	0.002	methylcyclopentane	-0.48	0.631
α-pinene	3.07	0.003	pentane	0.46	0.648
carbon Tetrachloride	3.01	0.004	toluene	-0.32	0.754
m/p-xylene	3.02	0.004	1,2,3-trimethyl benzene	-0.20	0.842
1-pentene	2.92	0.005	m-methyl toluene	0.15	0.882
o-xylene	2.86	0.006	1,2,4-trimethyl benzene	-0.13	0.900
ethylacetate	2.78	0.007			

4 Conclusion

A sampling and thermal desorption gas chromatography – mass spectrometry method was developed that allows monitoring of C_5-C_{12} VOCs in exhaled breath of subjects as young as 3 years old. A repeatability experiment demonstrated that the method can be considered reliable for at least 56 VOCs present in exhaled breath with 89% of the coefficients of variance being less than 30% (and 85% ≤

20%). Bearing in mind that coefficients of variance of 20% are normal for standard chemical analysis methods and that we are evaluating a screening method rather than a method optimized to monitor a small selection of compounds, we can conclude that coefficients of variance up to 30% are acceptable and even better than a lot of other whole organism bioassays [8]. The abundances of 43 VOCs in inhaled and exhaled air were compared for 56 3-year-old children and revealed that for this study group certain compounds were significantly retained in the body while the clearance rate for other compounds was systematically larger than the absorption.

The non-invasive nature of breath sampling makes this test more convenient than monitoring blood; however several factors, including the high concentration of water vapor and the short biological half-lives of many absorbed vapors make screening methods difficult to validate. In these experiments issues such as breakthrough, background, stability of compounds on sorbent material and stability of mass detector signal over time were avoided by comparing samples of ambient air and exhaled breath samples, prepared and analyzed at the same time, following the same procedure and correcting for background. However, before a fully validated method can be presented, there are still important issues about stability, variability and sensitivity that need to be addressed.

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