Clinical Application of SPME: Analysis of VOCs in Exhaled Breath as Cancer Biomarkers

Contributed Article

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Introduction

Characteristic odors in breath have long been used as a tool for medical diagnosis. Familiar examples are diabetes with the odor of overripe apples, renal diseases with the fishy smell of amines and ammonia, and dental or liver diseases with the cabbage-like odor of organic sulfides[1]. Linus Pauling applied formal science to the analysis by using gas chromatography (GC) to detect volatile organic compounds (VOC) in breath.[2] More recently, Michael Philips and his Menssana coworkers focused on determination of breath compounds that are attracting attention in clinical and toxicological analysis[3]. Although breath analysis is of great importance in disease detection, toxicology, and the study of metabolic processes, its use by doctors and clinicians as a diagnostic tool is a lost art.

The classes of VOCs that can be present in exhaled breath include hydrocarbons, alcohols, ketones, aldehydes, esters, and organic sulfides[4]. The determination of VOCs in exhaled air requires the detection of very low concentrations. Hence, the analytical methods employed must include a preconcentration step. The common preconcentration methodologies currently utilized for VOCs are sorption onto an adsorbent and cold trapping.

However, solid phase microextraction (SPME) is a viable alternative to these methods, as will be shown here. SPME has been widely used for the determination of volatile organic compounds in various matrices, including exhaled breath[5]. Compared to other preconcentration techniques, SPME is simple, inexpensive, and solvent-free. It is fully automatable, and no thermal desorption unit or modifications to the GC instrument are necessary. Compatible with all GC systems, SPME can be used by practically every laboratory. The objective of this study was to use SPME with GC-MS analysis to identify volatile biomarkers of lung cancer.

Experimental

Breath samples were collected from ten healthy volunteers and twelve patients with lung cancer. Each participant provided via questionnaire their age, sex, other diseases, medications, smoking habits, and composition of recent meals. Breath samples were collected in 1 L Tedlar® bags which were kept at a constant 25 °C. A gas standard containing the compounds of interest was made by vaporizing a liquid mixture of the compounds in a glass bulb. A defined volume of the mixture was transferred into the Tedlar bag prior to sampling. During extraction, the CAR/PDMS SPME fiber was introduced into the bag containing breath sample or gas standards through a septum and exposed for 15 minutes. Ambient air samples were collected for background. External calibration was employed. The fiber desorption and sample analysis (GC-MS) conditions are shown in **Figure 1**.

Figure 1. GC-MS Analysis of VOC in Breath from Lung Cancer Patient after SPME Using CAR/PDMS Fibers

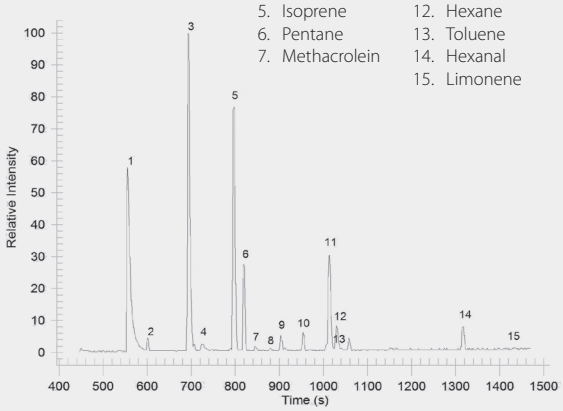
SPME Conditions

sample:	exhaled breath, 1 L in Tedlar bag
fiber:	Carboxen®/Polydimethylsiloxane (CAR/PDMS), 75 µm film (57318)
holder:	manual SPME holder (57330-U)
extraction:	15 min at 25 °C
desorption:	1 min at 220 °C

GC-MS Conditions

column:	GC PLOT column (Q-type), 25 m x 0.25 mm I.D., 3 µm
inj. temp.:	200 °C
oven:	40 °C (2 min), 10 °C/min to 140 °C, 5 °C/min to 270 °C (3 min)
carrier gas:	helium, 40 cm/sec, constant
injection:	splitless 1 min, then split at 35:1
detector:	MS, full scan, m/z 15-220, rate 3.46 scans/sec, EI ion source and transfer line temp: 220 °C

1. Ethanol
2. Acetonitrile
3. Acetone
4. 2-Propanol
5. Isoprene
6. Pentane
7. Methacrolein
8. Methyl vinyl ketone
9. Ethyl acetate
10. 2-Methylpentane
11. 3-Methylpentane
12. Hexane
13. Toluene
14. Hexanal
15. Limonene



Results

The linearity, precision, and detection limits for VOCs determination in human breath are presented in **Table 1**. The relative standard deviation (RSD) was in the range of 3.4% to 9.4%. Linear regression coefficient values (r^2) were close to 1. The lowest LOD values obtained for hydrocarbons varied from 0.3 to 0.49 ppb.

Table 1. Validation Parameters for Volatile Organic Compounds (VOCs)*

Compounds	Linear Range (ppb)	r^2	%RSD	LOD (ppb)	LOQ (ppb)
Acetone	1.6 - 920.7	0.991	8.9	0.54	1.62
Acetonitrile	2.3 - 234.0	0.996	3.4	0.75	2.25
Ethyl acetate	1.3 - 136.7	0.995	4.6	0.43	1.29
Methyl vinyl ketone	9.9 - 135.6	0.997	8.4	3.30	9.90
3-Methylpentane	1.3- 136.5	0.997	3.3	0.45	1.35
Ethanol	1.0 - 99.8	0.992	4.5	0.33	1.00
2-Methylfuran	1.6 - 165.6	0.999	4.9	0.54	1.63
2-Methylpentane	0.9 - 87.8	0.992	4.5	0.32	0.96
Hexanal	9.9 - 133.9	0.996	6.2	3.30	9.90
Hexane	1.5 - 150.0	0.994	3.4	0.49	1.47
Isoprene	2.6 - 380.2	0.998	3.7	0.87	2.62
Pentane	1.5 - 150.0	0.998	5.2	0.49	1.47
Methacrolein	11.7 - 170.4	0.998	7.2	3.91	11.73
1-Propanol	1.6 - 163.5	0.995	5.1	0.53	1.59
2-Propanol	1.6 - 159.6	0.998	9.4	0.52	1.57
Toluene	1.1 - 114.7	0.991	5.9	0.41	1.11
o-Xylene	1.0 - 100.1	0.994	4.8	0.33	0.99

* Conditions same as Figure 1 except sample is a mixture of VOC standards. n=3. Bolded compounds had statistical significance.

Figure 1 shows a typical GC-MS chromatogram of breath from a lung cancer patient. Analysis of exhaled air from healthy volunteers and cancer patients identified seventeen volatile compounds, mainly hydrocarbons, ketones, aldehydes, and alcohols. Similar compounds were found in both healthy and cancer patients, except furan derivatives which are considered to be markers for tobacco smoking. Statistical tests were applied to distinguish cancer patients from the healthy control group. The VOC concentration data were log-transformed and tested for normality using the Shapiro-Wilks W test, ($p<0.05$). Full details of the statistical analysis is beyond the scope of this brief report; however, they are available upon request. Summarizing: Although there was variation between the patients and not all patients exhibited the same biomarker pattern, four compounds stood out statistically from the others: methyl vinyl ketone, 1-propanol, 2-propanol, and o-xylene. These compounds showed statistically higher levels in cancer patients compared to the healthy control group.

Conclusion

This brief report is intended to demonstrate the potential of solid phase microextraction (SPME) as a clinical research tool, in this case toward the extraction from human breath of VOCs associated with lung cancer. By using SPME with GC-MS analysis and applying rigorous statistical methods, we found the VOC profiles between a small set of healthy individuals and those diagnosed with lung cancer were significantly different. These promising findings would necessarily be followed up with studies on larger populations for definitive associations. The SPME-GC-MS method presented here had the requisite linearity and sensitivity, and could be easily adopted by laboratories as an investigational tool into biomarker discovery, among many other applications relevant to the clinical and biochemical fields.

Acknowledgments

This research work was supported by Grant N N 204 026238 (2010-2013).

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Description	Cat. No.
SPME fiber assembly Carboxen®/Polydimethylsiloxane (CAR/PDMS), needle size 24 ga, coating 75 µm, for use with manual holder	57318
SPME fiber holder for use with manual sampling	57330-U
Capillary GC Column	
Supel-Q™ PLOT, 30 m x 0.32 mm I.D.	24242
Standards	
For a complete listing of our volatile organic standards, visit sigma-aldrich.com/standards	

96-well plates are also available, but were not used in this study.

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