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Determination of volatile organic compounds as biomarkers of lung cancer by SPME–GC–TOF/MS and chemometrics

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ABSTRACT

A method for qualitative and quantitative the determination of concentrations volatile organic compounds (VOCs) in human breath samples using solid phase microextraction (SPME) and gas chromatography–time of flight–mass spectrometry (GC–TOF/MS) has been carried out. They are employed for the preconcentration, separation and analysis of biological samples. The technique to rapid determination compounds present in human air, at the level of parts per billion (ppb) is applied. This method was optimized and evaluated. It showed linear correlations ranging from 0.83 to 234.05 ppb, limit of detection in the range of 0.31 to 0.75 ppb and precision, expressed as the RSD, was less then 10.00%. The unique combination of statistical methods allowed reduce the number of compounds to significant ones only and indicate the potential way to find the biomarkers of the lung cancer. Presented an analytical and statistical methods for detection composition of exhaled air could be applied as a potential non-intrusive tool for screening of lung cancer.

1. Introduction

Lung cancer belongs to most often of malicious tumours being a main cause death both men and women in industrialized countries. Predominant factor of lung cancer is active and passive smoking because cigarette smoke contain about 200 substances with influence carcinogenic and mutagenic. Other common initiator cause of lung cancer belong to exposure to radon, cadmium, arsenic, beryllium, asbestos [1–3]. Lung cancer is classified into two broad groups: small cell lung carcinoma (SCLC) (20–25% frequency of occurrence) and non-small cell lung carcinoma (NSCLC) (70–75%). The letter category includes adenocarcinomas (25–30%), squamous cell (30–35%) and large cell carcinomas (10–15%). This classification takes into account histological type of lung cancer as well as facilitate method of treatment and prognosis of disease. The key difference between lung carcinoma is that, the time of diagnosis SCLC has metastases and cannot be cure by surgery. Therefore, they are cured by apply chemotherapy or radiotherapy. In contrast, NSCLC is usually treated by surgery because it is relatively insensitive to chemotherapy [4,5].

In the recent years scientific interest to search of non-invasive technique, painless and agreeable for patients which would facilitate diagnosis of early-stage of lung cancer without necessary using invasive medical routine has been increased. Exemplary denouement could be analysis of breath which has numerous advantages in comparison with traditional diagnostic methods [6–9]. In the 1970th Linus Pauling selected about 200 volatile organic compounds (VOCs) in exhaled breath, at the level of parts per million by volume–parts per trillion by volume (ppmv–pptv) [10,11]. Volatile organic compounds (VOCs) are generated in human body as products of metabolic process. However, biochemical ways of create most of compounds which were detected in breath have not precisely and scientifically explained. Except biochemical process, the other sources of organic compounds are external factures e.g. environmental pollutions, food additions, because they are introduced alimentary or respiratory way [10].

Volatile organic compounds (VOCs) in exhaled breath provide valuable information about state of human health. The composition of the breath is variable and depends on the types of diseases for example sweet smell indicates diabetes, while the odor of rotten eggs, which are caused by sulfur-containing compounds suggests liver problems [12,13]. Currently, intensive search are carried out for compounds that could be the potential markers of cancer and in the future to facilitate diagnosis [14]. The function of this substance may perform low weight and macromolecules, such as: volatile organic compounds, presumably are hydrocarbons, alcohols, aldehydes, ketones, hydrocarbons containing nitrogen and sulfur, as well as protein, or carbohydrate component of the lipid, glycolipids, nucleic acids [12,15,16].

Presence of VOC in breath air at the trace levels makes breath analysis difficult, therefore, as the sampling technique, solid

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phase microextraction (SPME) [11,13] and thermal desorption (TD) [17,18] are mainly applied. After sampling of VOCs, identification of the components is performed most often by using: gas chromatography–mass spectrometry (GC/MS) [7,13,19], selected ion flow tube-mass spectrometry (SIFT-MS) [20,21], proton transfer reaction-mass spectrometry (PTR-MS) [10,22]. Nowadays, the promising method for detecting of VOC is the electronic nose [23]. However, chromatographic technique is still preferred for the analysis of breath, because is providing more information about the molecular composition of exhaled breath.

In this paper, solid phase microextraction technique and gas chromatography coupled with time of flight and mass spectrometry (GC–TOF/MS) were used for the analyses of VOCs of exhaled air from patients with lung cancer and healthy persons. Additionally, to the selection of compounds as potential markers of lung cancer statistic methods were applied, namely Mann–Whitney's test *U* followed by discriminant function analysis (DFA) and factor analysis (FA). It allowed to determine the individual chemical pattern for groups of patients with cancer diagnosis and healthy ones.

2. Experimental

2.1. Instrumentation

The GC–TOF/MS analysis was performed on gas chromatograph 7890A (Agilent, Waldbronn, Germany) coupled with spectrometer TruTOF (Leco, St. Joseph, MI, USA) equipped with CP-Porabond-Q (Varian Inc., Middelburg, The Netherlands) 25 m × 0.25 m × 3 μm column. Oven temperature program was as follows: initial 40 °C held for 2 min, then ramped at 10 °C/min to 140 °C and the ramped at 5 °C/min to 270 °C and held for 5 min. The temperature of the split–splitless injector was 200 °C. Acquisition was performed at mass range m/z 30–300, acquisition rate 30 spectra/s. Spectra were collected at electron ionization (EI) 70 eV, both ion source and line transfer temperatures were set to 200 °C. The acquisition of chromatographic data was performed by means of Chroma TOF software (Leco).

A manual SPME holder and carboxen/polydimethylsiloxane (CAR/PDMS) (75 μm) coated fiber (Supelco, Bellefonte, USA) were used for the SPME method.

2.2. Chemicals

Scotty gas mixture containing C1–C6 aliphatic hydrocarbons was purchased from Supelco (Supelco), 1-propanol, isopropyl alcohol, propanal, butanal, 2-methylpropanal, acetone, 2-butanone, 2-methylpentane, 3-methylpentane, benzene, toluene, ethylbenzene, o-xylene, furan, acetonitrile were purchased from Sigma–Aldrich (Steinheim, Germany). Helium and argon, purity 99.999%, were purchased from B.O.C. (Bydgoszcz, Poland).

2.3. Calibration

Prior to the use, the bulb was cleaned with methanol and dried in oven at 60 °C for at least 12 h. Afterwards, it was purged with pure argon for 15 min. Then, the bulb was evacuated by using vacuum pump within 30 min. Gaseous standard were prepared by injection of 1 μL of each compound into 1 L glass bulb and its evaporation. Afterwards, the mixture was moved using gas syringe to 1 L Tedlar bag filled of 0.5 L of pure argon. During the sampling SPME fiber was introduced into vial through the septum to obtain concentrations in the range 0.5–200 ppb and exposed to gas mixture. Each measurement was repeated three times.

Table 1
Number and type of carcinoma for cancer patients.

	Male	Female
Amount research persons	17	6
Age	51–78	52–72
Period of smoking	25–50 years	25–50 years
Number of cigarettes	20–60/24 h (16 persons)	10–20/24 h (5 persons)
Histologic type		
Squamous cell carcinoma	10	5
Adenocarcinoma	2	1
Small celled carcinoma	2	–
Lung tumour	3	–

Table 2
Characteristic of healthy persons group.

	Male	Female
Number of persons	10	20
Mean age (range)	43 (22–58)	32 (20–45)
Number of smokers	4	2

2.4. Solid phase microextraction

Before the first use, the fiber was conditioned in an injector at 200 °C for 5 h. During exposition, the SPME fiber was introduced into the glass vial containing sample of breath, through a silicone septum and was exposed for 15 min, at 25 °C. After extraction, the fiber was withdrawn to the needle, pulled out from the vial and injected into the GC. The compounds were desorbed in the hot GC injector port for 2 min at 200 °C.

2.5. Breath collection

Breath samples were collected in a 1 L Tedlar bags using breath sampler. Before collection of breath, all bags were cleaned by flushing with argon gas and then filled with argon and heated at 60 °C for 12 h to remove any contaminations. Afterwards, a 10 mL sample was transferred into glass vial. Before transfer, glass vial was crimped and evacuated by using a glass syringe. Ambient air samples were taken for measurement blank.

2.6. Human subjects

Sample of breath was collected from 30 healthy adult volunteers, ten men and twenty women. Every person was asked to fill in a questionnaire inscribing their smoking status, coexistent diseases, received drugs, consume meal, etc. Relevant information about the carcinoma patients and healthy persons are shown in Tables 1 and 2, respectively. Breath samples from patients with lung cancer were collected in hospital in Torun. The study was approved by the Nicolaus Copernicus University Ethic Commission.

2.7. Statistics

The chemometric calculations were performed in Statistica 7.1 Data Miner (Statsoft, Krakow, Poland) software running on Windows XP platform. The peak area of the identified analyte was used for calculations. Due to significant skewness of variables nonparametric Mann–Whitney's test *U* was performed. For data classification and dimensionality reduction discriminant function analysis (DFA) have been applied. DFA is a supervised method of classification that maximizes the ratio between-class variance to the within-class variance in any particular data set thereby guaranteeing maximal separability. DFA seeks the minimum number of parameters to classify the data on strictly defined groups with defined tolerance. Furthermore, factor analysis (FA) was applied,

Table 3
Compounds detected in human breath of smoking and non-smoking volunteers and patients with lung cancer. The total number research persons: healthy non-smoker was 24, healthy smoker 6, patients non-smoker 2 and patients smoker 21.

No	Compound	t_R [s]	The total number person in which identified compound				CAS-number
			Healthy person		Person with lung cancer		
			Non-smoker	Smoker	Non-smoker	Smoker	
1	Methyl alcohol	356.053	24	6	2	21	67-56-1
2	Propyne	361.918	0	2	0	0	74-99-7
3	Propane*	364.317	7	3	2	21	74-98-6
4	Acetaldehyde	442.298	24	6	2	21	75-07-0
5	Ethyl alcohol	556.069	24	6	2	21	64-17-5
6	1-Buten-3-yn	562.068	0	2	0	0	689-97-4
7	Isobutane	569.599	11	3	2	21	75-28-5
8	1-Butene	576.331	24	6	2	21	106-98-9
9	1,3-Butadiene	576.864	0	3	0	3	106-99-0
10	Acetonitrile*	591.594	24	6	2	21	75-05-8
11	Butane*	609.189	24	6	2	21	106-97-8
12	2-Propenal	655.511	24	6	2	21	107-02-8
13	Furan*	677.306	24	6	2	21	110-00-9
14	Propanal	682.838	24	6	2	21	123-38-6
15	Acetone*	688.503	24	6	2	21	67-64-1
16	Carbon disulfide	705.032	24	6	2	21	75-15-0
17	Isopropyl alcohol*	712.763	24	6	2	21	67-63-0
18	Dimethyl sulfide	718.429	24	6	2	21	75-18-3
19	1-Hydroxy-2-propanone	748.954	24	6	2	21	116-09-6
20	1-Propanol*	771.415	0	0	2	21	71-23-8
21	2-Pentene	791.744	6	5	0	12	109-68-2
22	2-Methyl-1,3-butadiene	796.676	24	6	2	21	78-79-5
23	Pentane*	820.203	24	6	2	21	109-66-0
24	Cyclopentane	828.468	10	5	1	4	287-92-3
25	1,3-Pentadiene	835.333	0	5	1	4	504-60-9
26	Methacrolein	842.264	24	6	2	21	78-85-3
27	2-Methylpropanal*	859.193	24	6	2	21	78-84-2
28	Methyl vinyl ketone	875.056	24	6	2	21	78-94-4
29	2,3-Butanedione	900.317	24	6	2	21	431-03-8
30	2-Methylfuran	901.383	24	6	2	21	534-22-5
31	Butanol	902.183	24	6	2	21	71-36-3
32	2-Butanone*	908.048	24	6	2	21	78-93-3
33	3-Methylfuran	917.512	6	6	0	3	930-27-8
34	Ethyl acetate	951.504	24	6	2	21	141-78-6
35	2-Methylpentane*	1014.95	24	6	2	21	107-83-5
36	3-Methylpentane*	1031.82	24	6	2	21	96-14-0
37	Benzene*	1040.41	24	6	2	21	71-43-2
38	Methylcyclopentane	1050.81	24	6	2	21	96-37-7
39	Hexane*	1059.61	24	6	2	21	110-54-3
40	Cyclohexane	1107.20	9	3	1	12	110-82-7
41	2-Pentanone	1148.72	24	6	2	21	107-87-9
42	2,5-Dimethylfuran	1151.79	1	3	0	0	625-86-5
43	Pentanal*	1164.12	24	6	2	21	110-62-3
44	Toluene*	1317.55	24	6	2	21	108-88-3
45	2,4,4-Trimethyl-1-pentene	1424.59	24	6	2	21	107-39-1
46	Hexanal	1432.65	9	3	1	12	66-25-1
47	Butyrolacetone	1445.58	24	6	2	21	96-48-0
48	Ethylbenzene*	1571.08	24	6	2	21	100-41-4
49	o-Xylene*	1616.80	24	6	2	21	95-47-6
50	Benzaldehyde	1683.25	12	2	1	4	100-52-7
51	Nonane	1821.15	24	6	2	21	111-84-2
52	6-Metyl-5-hepten-2-on	1870.27	17	2	2	10	110-93-0
53	Acetophenone	1932.86	12	1	1	10	98-86-2
54	Decane	2044.76	24	6	2	21	124-18-5
55	Styrene	2364.55	8	3	2	21	100-42-5

* Confirmed with standards.

aiming at finding and interpreting complex relationships between variables in the data set. FA was based on the calculation of principal components analysis with Varimax rotation of its loads.

3. Results and discussion

3.1. Breath analysis

All compounds detected in human breath were compared to the ambient air and only compounds with amount more than the ambient level were reported. Exemplary GC–TOF/MS chromatogram of breath for human with lung cancer is shown in Fig. 1. Analytes such as methanol, acetaldehyde, ethanol, 1-butene, acetonitrile, butane, 2-propenal, furan, propanal, acetone, carbon disulfide, 2-propanol, dimethyl sulfide, 1-hydroxy-2-propanone, 2-methyl-1,3-butadiene, pentane, methacrolein, 2-methylpropanal, methyl vinyl ketone, 2,3-butanedione, 2-methylfuran, butanal, 2-butanone, ethyl acetate, 2-mkethylpentane, 3-methylpentane, benzene, methylcyclopentane, hexane, 2-pentanone, pentanal, toluene, 2,4,4-trimethyl-1-pentene, butyrolacetone, ethylbenzene, o-xylene, nonane, and decane were found in all breath samples. In exhaled air of healthy and cancer smoker persons 1,3-butadiene and 1,3-pentadiene were detected. The same compounds were found in both groups except 2,5-dimethylfuran and 1-propanol.

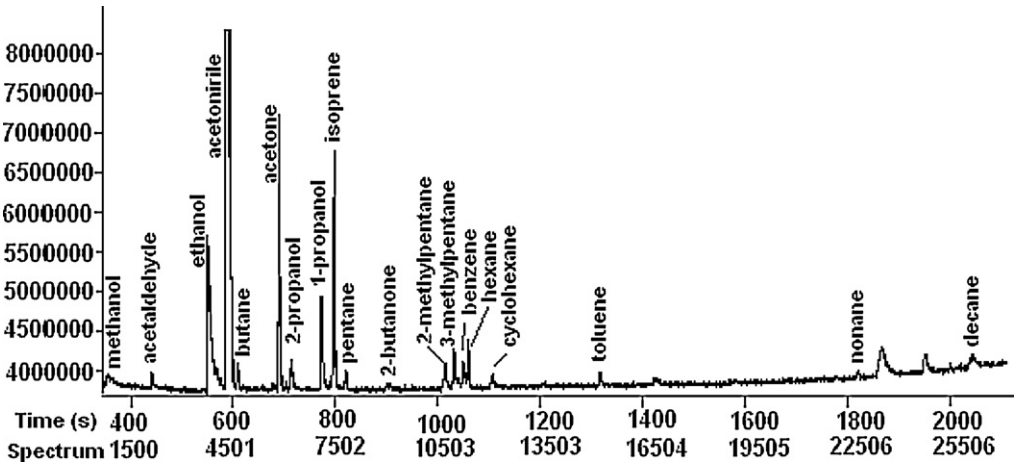

Fig. 1. The GC–TOF/MS chromatogram of exhaled air of human with lung cancer.

The 1-propanol was determined only in breath of hospitalised persons, because it was applied in disinfectants. However, 2,5-dimethylfuran was detected in breath of healthy smoker peoples. There are listed in Table 3. Compounds such as alkanes, ketones, aldehydes are produced in human body as product of metabolic processes. Acetone is formed in decarboxylation of acetoacetate and acetyl-CoA, pentane is produced during lipid peroxidation, however other part have origin exogenous [11]. The level propane, 2-methylpropanal, isopropanol is higher in patients with lung cancer than in case of healthy volunteers (Table 4).

3.2. Validation of the method

The linearity, precision and detection limits for selected VOCs determination in human breath are shown in Table 5. The precision of the method was determined by performing three analyses. The value of the relative standard deviation (RSD) was in the range from 3.36% to 9.54% for hydrocarbons, alcohols, aldehydes, ketones and aromatic compounds. RSD values less than 10% show that the present method has good repeatability. A calibration curve was linear for aliphatic hydrocarbons in the range 0.93–150.00 ppb, for alcohols 1.63–163.51 ppb, for aldehydes 1.34–170.46 ppb, for ketones 1.37–166.46 ppb and aromatic

Table 4
Concentration range of compounds detected in human breath of healthy persons and cancer patients.

Compounds	Concentration range [ppb] for healthy person (number of person)	Concentration range [ppb] for lung cancer patients (number of person)
Propane	3.45–5.96 (4)	3.19–9.74 (9)
Butane	0.46–16.63 (22)	0.58–2.71 (10)
Pentane	6.84–94.36 (21)	0.73–17.50 (13)
Hexane	1.75–6.31 (19)	0.82–1.88 (11)
2-Methylpentane	2.37–107.80 (23)	0.31–3.77 (18)
3-Methylpentane	1.05–8.76 (19)	0.61–8.87 (17)
1-Propanol	Not detected	4.37–93.15 (15)
Isopropanol	3.21–14.17 (22)	3.32–19.19 (28)
Butanal	0.52–1.87 (18)	0.78–2.55 (10)
Propanal	0.56–3.44 (25)	0.66–3.74 (11)
2-Methylpropanal	5.10–9.57 (21)	6.84–94.36 (13)
Acetone	14.44–531.45 (30)	34.57–390.60 (23)
2-Butanone	0.49–3.18 (23)	0.49–2.86 (13)
Benzene	1.15–14.97 (22)	0.88–3.82 (19)
Toluene	1.45–37.21 (9)	1.51–17.10 (9)
Ethylbenzene	2.22–18.38 (9)	1.45–3.16 (7)
o-Xylene	2.06–74.44 (10)	1.99–7.64 (4)
Furan	0.53–3.25 (21)	1.17–2.81 (12)
Acetonirile	5.99–782.98 (30)	10.96–423.60 (23)

Table 5
Validation parameters for volatile organic compounds.

Compounds	Linearity [ppb]	R^2	RSD %	LOD [ppb]	LOQ [ppb]
Propane	1.50–150.00	0.9938	4.05	0.47	1.41
Butane	1.50–150.00	0.9988	4.74	0.46	1.38
Pentane	1.50–150.00	0.9983	5.27	0.49	1.47
Hexane	1.50–150.00	0.9949	3.44	0.48	1.44
2-Methylpentane	0.93–92.61	0.9950	9.54	0.31	0.93
3-Methylpentane	0.94–94.17	0.9880	9.37	0.32	0.94
1-Propanol	1.64–163.51	0.9952	5.15	0.53	1.58
Isopropanol	1.60–159.65	0.9984	9.42	0.52	1.57
Butanal	1.36–135.60	0.9979	8.48	0.44	1.32
Propanal	1.70–170.46	0.9986	7.23	0.52	1.56
2-Methylpropanal	1.34–133.91	0.9963	6.26	0.44	1.32
Acetone	1.66–166.46	0.9914	8.97	0.54	1.62
2-Butanone	1.37–136.51	0.9973	3.36	0.45	1.35
Benzene	1.38–136.77	0.9951	4.66	0.43	1.29
Toluene	1.15–114.75	0.9918	5.95	0.37	1.11
Ethylbenzene	1.00–99.81	0.9921	4.54	0.32	0.96
o-Xylene	1.00–100.16	0.9943	4.85	0.33	0.99
Furan	1.66–165.61	0.9994	4.91	0.51	1.53
Acetonirile	2.34–234.03	0.9963	3.40	0.75	2.25

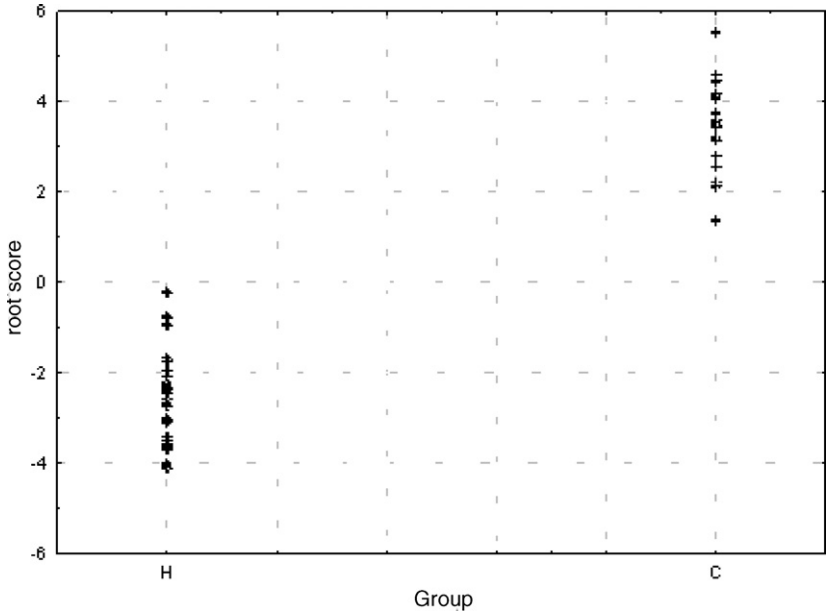

Fig. 2. Scores of canonical root for all breath samples (H - healthy and C - with cancer diagnosis).

compounds 1.00–165.61 ppb. The linear correlation coefficients were higher than 0.991.

The sensitivity of the method restricts detecting the limits of the SPME/GC–TOF/MS technique. The detection limit (LOD) was defined as signal-to-noise ratios equaled to three and signal-to-noise ratios equaled ten as the quantification limits (LOQ). The lowest values of LOD were obtained for hydrocarbons and aromatic compounds in the range of 0.31–0.49 ppb and 0.32–0.43 ppb, respectively.

3.3. Statistical analysis of VOC detected in sample of human exhaled air

55 compounds investigated (Table 3) has been detected only in cancer group. The absence of 1-propanol in the group of healthy persons was related with existence of this compound in hospital air. Statistics were therefore performed on 54 variables excluding 1-propanol.

Due to high right skewness of data, all variables were normalized by transformation (1):

$$z_i = \log(x_i + 10^6) \quad (1)$$

where z_i stands for transformed value and x_i is the peak area.

Most of the transformed variables fail to Shapiro–Wilks test of normal distribution therefore nonparametric Mann–Whitney's test *U* has been performed to select the variables for further classification. 20 of 54 transformed parameters have significant ($p < 0.05$) *U*-value indicating important difference between two groups of patients: H-healthy and C-with cancer diagnosis. The results of Mann–Whitney's test *U* for only those compounds are listed in Table 6.

Forward stepwise method of discriminant function analysis (DFA) has been performed on those twenty parameters. The DFA analysis was performed with desired tolerance at 0.1 and values *F*-enter and *F*-remove at 1 and 0, respectively. During the classification, further reduction of variables to fourteen was achieved. The summary of DFA analysis is presented in Table 7. Marked compounds have *F*-remove values significant ($p < 0.05$) and much higher than threshold value (1.38). The biggest discriminant ability is expressed by partial Wilks' Lambda. The impact of isopropyl

Table 6
Summary of Mann–Whitney's test *U* performed on 54 parameters.

	Rank sum H	Rank sum C	*U*	*p*-Level
Propane	575	856	110	0.000
Ethyl alcohol	583	848	118	0.000
Isobutane	638	793	173	0.002
2-Propenal	653	778	188	0.005
Furan	687	744	222	0.027
Propanal	657	774	192	0.006
Carbon disulfide	607	824	142	0.000
Isopropyl alcohol	493	938	28	0.000
Dimethyl sulfide	938	493	217	0.022
Pentane	960	471	195	0.007
Cyclopentane	906	525	249	0.048
Propanal, 2-methyl-	690	741	225	0.031
2,3-Butanedione	684	747	219	0.024
Furan, 2-methyl-	974	457	181	0.003
Butanal	928	503	227	0.034
Furan, 3-methyl-	924	507	231	0.012
Pentanal	674	757	209	0.015
Ethylbenzene	964	467	191	0.006
Nonane	930	501	225	0.031
Styrene	636	795	171	0.001

Table 7
Summary of discriminant function analysis.

	Wilks' Lambda	Partial Wilks' Lambda	*F*-remove (1.38)	*p*-Level
Isopropyl Alcohol	0.206	0.500	38.01	0.00
Styrene	0.147	0.703	16.08	0.00
Pentanal	0.106	0.971	1.14	0.29
Carbon disulfide	0.131	0.786	10.33	0.00
Furan, 2-methyl-	0.108	0.956	1.75	0.19
Ethylbenzene	0.132	0.783	10.53	0.00
Isobutane	0.112	0.919	3.35	0.08
2-Propenal	0.116	0.884	4.98	0.03
Propane	0.118	0.870	5.68	0.02
Furan, 3-methyl-	0.109	0.949	2.06	0.16
Propanal	0.108	0.956	1.74	0.19
Cyclopentane	0.111	0.924	3.11	0.09
Butanal	0.106	0.968	1.27	0.27
Pentane	0.106	0.969	1.22	0.28

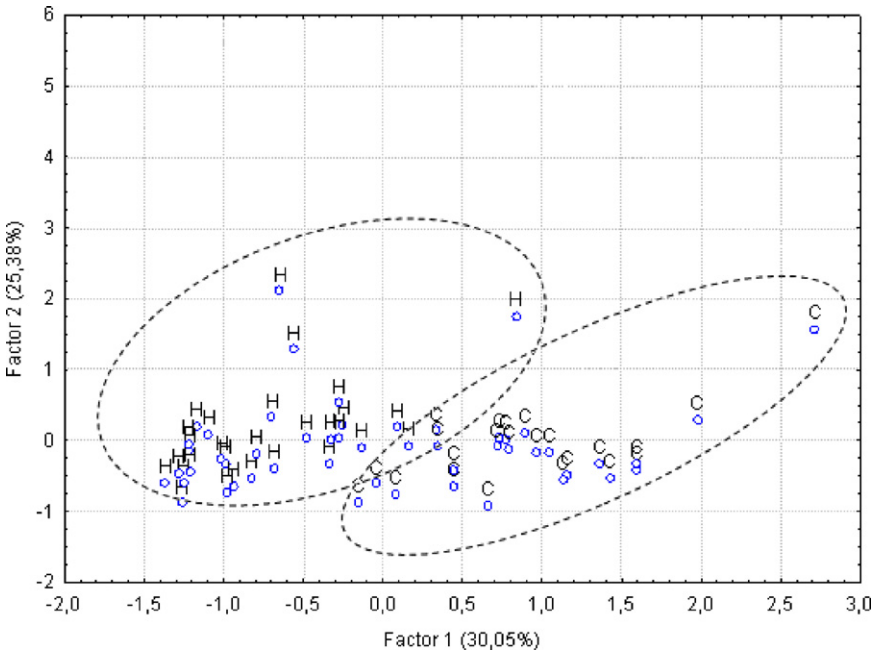

Fig. 3. Factor scores of breath samples. The explained variance of particular factors are written in brackets.

alcohol on discrimination is highest followed by styrene, carbon disulfide, ethylbenzene and 2-propenal, propane.

On the base on 14 variables canonical analysis were performed. The scores of only one canonical root allow to discriminate two groups for all the samples as is presented at Fig. 2.

The relation between calculated canonical root and the original fourteen variables has been investigated by means of correlation coefficients (R). Some of R values were significant but does not indicate simple interpretation. The variables with significant F value taken from Table 7 were used to perform factor analysis (FA). This method has well known ability to replace original variables by so called factors related to particular variables and gives the opportunity to describe the impact of particular compound on the classification of breath samples to predefined groups. The calculation of factors was based on principal components analysis. Two factors were obtained by Varimax rotation with Eigenvalue bigger than 1. They explained more than 65% of variance. The first one was loaded mainly by propane and carbon disulfide where second one by 2-propenal and ethylbenzene (Table 8). In the first group were selected compounds which have origin endogenous opposite the second group.

The presentation of scores of breath samples in the factors space give good classification to the predefined groups. Samples taken from the patients with diagnosed cancer have significantly higher scores of factor 1. The samples are not fully discriminated into two groups (Fig. 3) but it has been achieved from six selected compounds only.

Table 8
Factor loadings.

	Factor 1	Factor 2
Propane	**0.75**	0.08
2-Propenal	0.13	**0.82**
Carbon disulfide	**0.75**	−0.02
Isopropyl alcohol	0.68	−0.24
Ethylbenzene	−0.10	**0.87**
Styrene	0.44	0.19
Explained variance [%]	30.05	25.38

Bold values indicates the influence these compounds on factors 1 and 2 are significant.

4. Conclusions

A combination the solid phase microextraction and gas chromatography–time of flight–mass spectrometry (SPME/GC–TOF/MS) application to detection compounds in human breath samples were discussed. The technique was applied to determination composition breath the 23 patients with lung cancer and 31 volunteers. The total number of compounds identified in sample of breath equal 55. On the basis of the analysis using GC–TOF/MS compound which enables an indication group of persons with lung cancer was isopropyl alcohol.

The statistical analysis allowed to extract the compounds with concentrations level significantly different between groups of healthy persons and patients with cancer diagnosis. Combination of non-parametric test with supervised and unsupervised classification methods enabled to predict five compounds propane, carbon disulfide, 2-propenal, ethylbenzene and isopropyl alcohol which separated two research groups of patients and healthy controls.Volatile organic compounds present in human breath which were selected by statistical analysis are origin endogenous and formed by biochemical process. A 2-propenal and propane are products lipid peroxidation of polyunsaturated fatty acids, whereas isopropyl alcohol is obtained as a result of metabolism acetone. A source of carbon disulfide could be process occur in the body by activity of bacteria. A ethylbenzene is compound origin exogenous which introduced by inhalation to the human body [11,24,25].

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