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Identification of volatile organic compounds secreted from cancer tissues and bacterial cultures

Bogusław Buszewski[a,*], Agnieszka Ulanowska[a], Tomasz Ligor[a], Marek Jackowski[b], Ewa Kłodzińska[a], Jacek Szeliga[b]

[a] *Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Gagarin Street 7, 87-100 Torun, Poland*
[b] *Department of Surgery, Collegium Medicum, Nicolaus Copernicus University, St. Joseph Street 53-59, 85-067 Bydgoszcz, Poland*

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ABSTRACT

The early cancer diagnosis increases the possibility of total recovery. The infection of *Helicobacter pylori* is associated with gastric cancer, the second most common cancer in the world. The determination of volatile organic compounds (VOCs) excreted by stomach tissue and bacteria culture has been investigated. Solid-phase microextraction (SPME) was used for preconcentration and the determination was accomplished by gas chromatography coupled with mass spectrometry (GC/MS). The samples of tissue were taken from five patients (ten samples) with stomach cancer and normal (non-cancerous) segments from other parts of the stomach were used as a control. Eighteen compounds were identified in stomach tissue and seven of them were present both in healthy and cancer tissue. These compounds assumed to be endogenous and acetone ratio (AR) was calculated for ethanol, butane, carbon disulfide, 1-propanol, 2-butanone and 2-pentanone. The data shows that amount of 1-propanol and carbon disulfide in the gaseous composition is higher in cancer tissue than in normal tissue. Eight compounds were identified both in bacteria and tissue. These data suggest that bacteria present in the stomach might cause the increase in the concentration of 1-propanol and carbon disulfide in emission from cancer tissue.

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1. Introduction

The early diagnosis of cancer tissues in human body allows recovery in most cases. Many methods which are known enable identifying tumor, e.g. computer tomography (CT), magnetic resonance imaging (MRI), endoscopy and ultrasonography [1–4]. These methods are time-consuming, unpleasant for patients, require skilled medical staff and need expensive device. Most recently the breath analysis has been proposed as a convenient and safe complementary method to blood and urine sampling due to a number of advantages in comparison with the traditional diagnostic techniques [5,6].

Despite the obvious advantages, it has not been introduced as a standard method in clinical diagnosis yet. Only a few breath tests are used in the routine applications: the nitric oxide test to recognize asthma, the evaluation of ethanol concentrations in expiratory gas after alcohol consumption, and the ^{13}C-urea breath tests for the diagnosis of *Helicobacter pylori* (*H. pylori*) infection [7–9]. At present the ^{13}C breath test is considered to be one of the most sensitive and specific techniques for the early diagnosis of *H. pylori* infection. However, the requirement for an isotope ratio mass spectrometer, laser or infrared spectroscopy together with the production of ^{13}C labelled urea, results in a significant cost per sample [10]. Additionally, if the analytical apparatus is not readily available, results may be delayed by 48 h or more due to the need for transportation [11].

The different species of bacteria existing in human body may cause many serious diseases. *H. pylori* culture is widely recognized as a gastrointestinal pathogen, which causes chronic superficial gastritis and is a major factor contributing to the pathogenesis of gastric and duodenal ulcer disease [11–15].

The International Agency for Research on Cancer has classified *H. pylori* as a group I carcinogen and a definite cause of gastric cancer in humans. In most cases, it is diagnosed at advanced stage; therefore, most patients who develop this cancer will not survive [16]. Because of the high morbidity and mortality associated with duodenal and gastric inflammation caused by *H. pylori*, the rapid and accurate diagnostic method is desirable.

Many volatile organic compounds (VOCs) are generated during metabolic processes which occur in the presence of different bacte-

Abbreviations: AR, acetone ratio; CT, computer tomography; DMS, dimethyl sulfide; DMDS, dimethyl disulfide; GC, gas chromatography; HS, head space; LOD, limit of detection; LOQ, limit of quantitation; MRI, magnetic resonance imaging; MS, mass spectrometry; PHA, phytohemagglutinin; SPME, solid-phase microextraction; R^2, correlation coefficient; VOCs, volatile organic compounds.

* Corresponding author. Tel.: +48 56 6114308; fax: +48 56 6114837.
E-mail address: bbusz@chem.uni.torun.pl (B. Buszewski).

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ria cultures [17]. So far nine volatile metabolites (i.e. acetaldehyde, acetic acid, ethanol, ammonia, hydrogen sulfide, methane thiol, dimethylsulfide, dimethyl disulfide) were identified and determined at ppb level in emission from *Escherichia coli* [17]. These VOCs were produced by five bacteria strains cultured in the blood culture media [18,19]. Barret et al. present that the microorganisms which are responsible for the urinary tract infection produce acetic acid [20]. This compound can be detected by gas chromatography.

The activity of bacteria may cause carcinogenic changes in the cells [21]; therefore, a lot of studies have been carried out for characterising substances in breath from patients with lung [22–26], breast [27] and gastric cancer [28–30]. It is known that the composition and quantity of VOCs in the exhaled breath from patient with lung cancer varies from healthy volunteers. Amann et al., on the basis of many research years of VOCs detected in exhaled breath, tried to connect biomarkers with diseases [31]. The advantageous method for sampling, preconcentration and detection of VOCs in exhaled breath was recently discussed in review [32]. This method based on breath sampling is non-invasive, painless, and sensitive and allows to determine gaseous substances at ppb or ppt level.

In order to diagnose stomach cancer in early stage, the development of a method based on the emission of volatiles from tissue has been performed. Due to headspace solid-phase microextraction technique and gas chromatography–mass spectrometry, it is possible to identify substances secreted from cancer cells.

In this paper, headspace solid-phase microextraction technique coupled with gas chromatography–mass spectrometry (HS-SPME GC/MS) method was applied for the analyses of VOCs emitted from stomach cancer and non-cancer tissue as well as from *H. pylori* culture. The cancer and healthy tissue were collected from patients during surgery. In addition, gaseous substances excreted by *H. pylori* was investigated and have been compared to compounds released by stomach tissues.

2. Materials and methods

2.1. Apparatus

The GC/MS analysis was performed on Agilent 5975 Inert XL MSD coupled with 6890N gas chromatograph (Agilent Technologies, Waldbronn, Germany) with the split–splitless injector. The temperature of split–splitless injector was 200 °C. Desorptions in hot injector were performed in spiltless mode within 1 min splitless time at split ratio 1:35. Helium was used as a carrier gas with linear velocity 40 cm s^{-1}. The MS analyses were carried out in a full-scan mode, with scan range 15–220 amu. A scan rate was 3.46 scan/s. The electron impact ionisation was used at energy 70 eV. The temperature of ion source and quadrupole was 190 °C and 150 °C, respectively. The acquisition of chromatographic data was performed by means of Chemstation Software (Agilent). The 25 m × 0.25 mm × 3 μm capillary column CPQ (Varian Inc., Middelburg, The Netherlands) was used. The oven temperature programme was as follows: initial 40 °C held for 2 min, then ramped 10 °C min^{-1} to 140 °C, next ramped 5 °C min^{-1} to 270 °C and held for 3 min.

Samples were prepared with SPME method. A manual SPME holder and Carboxen-PDMS coated fibre (Supelco, Bellefonte, USA) were used.

2.2. Reagents

Alcohols, aldehydes, alkanes and ketones were purchased from Sigma–Aldrich (Steinheim, Germany); Scotty gas mixture containing C1–C6 aliphatic hydrocarbons was purchased from Supelco (Supelco, Bellefonte, PA, USA). Helium and nitrogen-purity 99.999% were purchased from B.O.C. (Bydgoszcz, Poland).

2.3. Preparation of gaseous standards

Calibration gases were prepared by the evaporation of liquid substances (aldehydes, ketones, alcohols and branched hydrocarbons) in a glass gas bulb. 1 ml of each compounds injected into evacuated bulb. This stock mixture was sequentially diluted with nitrogen in other Tedlar bags.

2.4. SPME sorption

During exposition, the SPME fibre was introduced into the glass vial containing tissue sample or gaseous standards, through a silicone septum and was exposed for 15 min. All vials were thermostated at 40 °C for 10 min before SPME sorption. For the bacteria culture the SPME extraction was done within 1 h and the vials were thermostated at 36 °C. After that, the fibre was desorbed in hot GC injector port during 1 min at 200 °C.

2.5. Stomach tissues

The tissues from five patients (two men and three women) with stomach cancer were investigated. All of them were above 50 years old. The study was approved by the Nicolaus Copernicus University Ethic Commission.

Stomach cancer tissue specimen and normal stomach tissues (approx. 2 g) from patients with diagnosed stomach cancer were provided by University Surgical Clinic in Torun. Cancer tissues were cut out from different parts of tumor and normal stomach tissues were used as a control sample. The presence of cancerous cells in tissues was confirmed or excluded by histopathological examination. The stomach tissues were collected in 20 ml vials after surgical resection then transported to laboratory and analysed. Vials were tightly crimped. Before analysis the vials were weighed and then the SPME extraction and GC/MS analysis were performed. The chromatographic analysis was done no longer than 25 min after surgical resection.

The identification of VOCs was done using the mass spectra and retention times of the chromatographic standards. First of all, peaks were distinguished with the help of the NIST mass spectrum library with the similarity index higher than 75%. Afterwards main peaks were confirmed with chromatographic standards.

2.6. Multiplying H. pylori strain CCUG 17874

H. pylori strain CCUG 17874 was multiplying on the selective medium BD BBL™ Stacker™ Plates (Becton Dickinson GmbH) and the medium prepared according to Chmiela (1999) (Bulion Broucella (Difco) 28 g/l, Bacto Agar (Difco) 15 g/l, and sterile added: sheep blood (EMAPOL sp. z o. o.) 50 ml/l, veal serum 25 ml/l, vankomycin 10 mg/l). Bacterial strain was incubated in 37 °C during 3–6 days in microaerofilic conditions (BD Gas Pack™ EZ Campy Gas Generating Pouch System, bio Mérieux® SA). Applied generators keep concentration of O_2 at the level of 5% and CO_2 at the level of 10%.

2.7. Preparation of H. pylori strain CCUG 17874 for GC/MS analysis

After 3–6 days of incubation the bacterial cells were removed from the surface of medium and diluted in 5 ml of sterile water. Suspension was centrifuged at 4 °C (Janetzki 23 kD) in sterile tubes (Falcon®, 15 ml) at 8000 rpm for 6 min. Supernatant was

removed, pellet diluted again in 5 ml of sterile water and centrifuged (conditions as above). The above procedure of washing bacterial cells was repeated four times. After the last wash bacterial cells were diluted in 5 ml of sterile water and placed in sterile falcons with membrane. Additionally, approximate number of bacterial cells was assessed (estimated number of equivalents of *H. pylori* cells in 1 ml) with the use of McFarland curve model. Estimated number of cells (in equivalent of *H. pylori*) amounted 9.22×10^8 ml^{-1}.

The vials with bacteria strains received from laboratory were placed in the thermostat at 36 °C. In order to perform SPME extraction, Carboxen PDMS fibre was introduced into the vial for 1 h and then GC/MS analysis was performed. Analysis of gaseous substances released by the bacteria strains from the same vials was done after 24 h.

3. Results and discussion

It is known that live organisms produce many volatile organic compounds during metabolic processes. These substances can be detected using proposed method. Some attempts were done to identify compounds secreted from cancer tissue by Ligor et al. [7]. Fig. 1 shows the GC/MS chromatogram of emission from mononuclear cells stimulated by phytohemagglutinin (PHA). On this chromatogram four VOCs such as 2-propanol, 4-methylheptane, 4-methyloctane and 2-ethyl-1-hexanol were distinguished (Table 1). These compounds were recognized by other authors as biomarkers of cancer disease [22–30]. However, this chromatogram is very complicated because the cells release a lot of VOCs and some of them are difficult to identify due to frail peaks separation or low detection limit (Fig. 1). This chromatogram gives insufficient information about the state of patient health and it is not possible to analyse all of identified substances. In this case it is necessary to find some biomarkers linked to cancer or other diseases or pathogenic states.

3.1. Method calibration

The qualitative and quantitative analyses were done for compounds more often present in emission from bacteria and tissues.

The calibration curves for chosen VOCs were calculated from the results obtained for the analysis of gaseous mixture of compounds diluted with nitrogen. The precision of the method was determined by performing six consecutive analyses by SPME–GC/MS. The linearity between the analytical signal and the amount of the analyte is another important characteristic for quantitative analysis. A linear regression analysis of peak area versus the analyte concentration was performed, using standard mixture with the concentration ranges depending on compound, for aliphatic hydrocarbons concentrations were in the range: 4.5–136.0 ppb, and for oxygen containing molecules: 3.0–97.3 ppb (Table 2). The reproducibility was estimated by the relative standard deviation (% RSD, $n = 6$) of the peak areas of analytes. The values of the RSD ranged from 6% to 10% for analyzed compounds, which are sufficient for SPME method. The linearity is satisfactory with the correlation coefficients (R^2) ranging from 0.996 to 0.999 (Table 2).

3.2. Limit of detection (LOD) and quantitation (LOQ)

All analyses were done using a sensitive SPME–GC/MS technique. Signal to noise ratio was defined as three times of detection limit (LOD) and signal to noise ratios equalled to 9 as the quantitation limit (LOQ). The detection limits and quantitation limits of the chosen VOCs are shown in Table 2. The lowest detection limit was achieved generally for oxygenated species. The LODs were on the level of 0.6–2.8 ppb (Table 2).

3.3. Samples of tissues

Fig. 2a and b shows chromatograms of stomach cancer tissues. These tissues were originated from two patients and they were taken out from two different parts of the stomach. This fact has considerable influence of emission from human cells. Eighteen VOCs were identified in gases produced by stomach tissues. The total number of VOCs found in normal (non-cancer) tissues was 17 (Table 1). In normal tissue 2-methyl-1-propene was not found but it was also identified in the cancer tissue. On the other hand, in normal tissue 1,2-diethoxyethane was identified but it was not detected in cancer tissue. This compound is supposed to be exogenous. Ethanol, butane, acetone, carbon disulfide, 1-propanol, 2-butanone and 2-

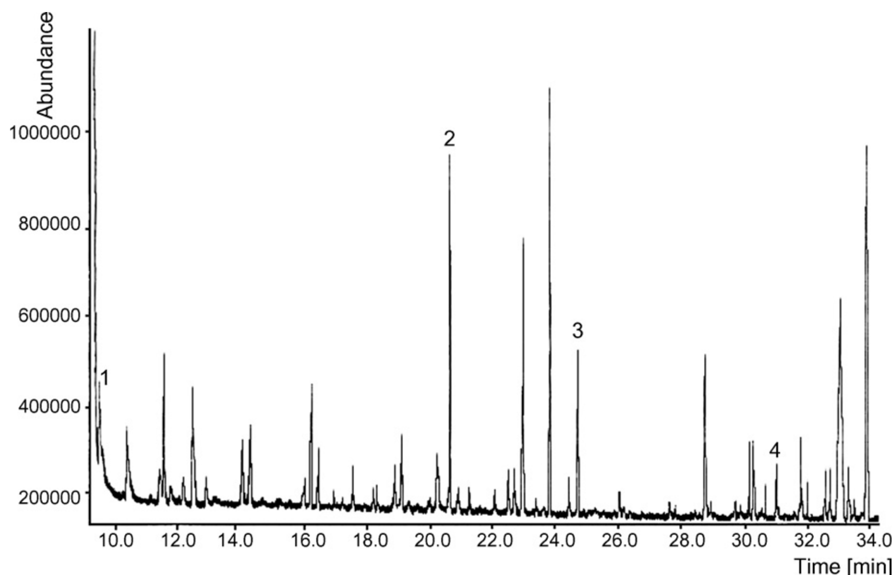

Fig. 1. The exemplary GC/MS chromatogram of emission from mononuclear cells stimulated by phytohemagglutinin (PHA): (1) 2-propanol; (2) 4-methylheptane; (3) 4-methyloctane; (4) 2-ethyl-1-hexanol.

Table 1
Compounds identified in exhaled breath, healthy tissue, cancer tissue and *H. pylori* culture

Compound	Identification ions (*m*/*z*)	Origin	*H. pylori*	Normal tissue	Cancer tissue
Ethanol	31, 45	en		+	+
Isobutane	43, 41	?	+		
2-Methyl-1-propene	41, 39	?	+		+
Acetonitrile	41, 40	ex		+	+
1-Butene	41, 56	?	+		
Butane	43, 29	en	+	+	+
Acetone	43, 58	en	+	+	+
Carbon disulfide	76, 44	en	+	+	+
1-Propanol	31, 29	en		+	+
2-Propanol	45, 27	ex/en			
1,2-Diethoxyethane	59, 31	ex		+	
Ethylcyclopropane	42, 55	?	+		
Sevoflurane	131, 181	ex		+	+
2-Butanone	43, 72	en		+	+
Trichloromethane	83, 85	?	+		
2-Methyl-1-pentene	56, 41	?	+		
3-Methylpentane	57, 56	?	+		
Benzene	78, 77	ex	+	+	+
Hexane	57, 43	?	+		
Methylcyclopentane	56, 41	?	+		
Cyclohexane	64, 46	?	+		
2-Ethoxy-2-methylpropane	59, 87	?	+		
2-Pentanone	43, 86	en		+	+
Toluene	91, 92	ex	+	+	+
2,4,4-Trimethyl-1-pentene	57, 41	?	+		
4-Methylheptane	43, 70	?	+		
5-Azauracil	113, 70	?	+		
Ethylbenzene	91, 106	ex	+	+	+
p-Xylene	91, 106	ex	+	+	+
Styrene	104, 103	ex	+		
o-Xylene	91, 106	ex	+	+	+
4-Methylheptane	43, 70	?			
2,4-Dimethyl-1-heptene	43, 70	?	+		
4-Methyl-2-heptanone	43, 58	?	+		
4-Methyloctane	43, 85	?			
1,4-Dichlorobenzene	146, 148	ex		+	+
α-Pinene	93, 92	ex		+	+
2-Ethyl-1-hexanol	57, 41	?			

Abbreviations: +, compound identified in gaseous mixture; en, endogenous; ex, exogenous. Bolded compounds were recognized as cancer biomarkers.

pentanone are endogenous compounds and they were identified in normal and cancer tissues. In order to characterize the physiological meaning and the diagnostic potential of these substances, the biochemical pathways and the way of generation have to be known. For these substances acetone ratio (AR) was calculated (Table 3) [7].

The AR for ethanol in normal tissue (78.02–220.24) is higher than in cancer tissue (12.29–54.02). It means that the amount of ethanol in the gaseous mixture of cancer tissue is higher than in healthy tissue. This fact can be explained because the potential source of endogenous ethanol is the bacterial flora [33]. However, AR for butane was found to be higher for cancer tissue (174.24–423.52) than for emission from normal tissue (86.47–192.08) (Table 3).

Butane is a stable end product of lipid peroxidation. This compound is related to oxidative stress and/or inflammatory state [33]. Therefore, increased emission of butane can be linked to inflammation caused by tumor.

In case of ketones (e.g. acetone, 2-butanone) which mainly arise from carbohydrate metabolism and also from the lipid oxidation processes [34]. In pathological states, carbohydrates are changed and the amounts of ketones in the body increase [35]. Acetone has been regarded as an important disease marker of diabetes and ketoacidosis [36]. 2-Butanone was identified in normal tissue only once; however, it was always present in cancer tissue but amount of this compound in emission from tissue is

Table 2
The linearity, limit of detection, limit of quantization and repeatability obtained for volatile organic compounds

Compound	Calibration range (ppb)	RSD (%)	R^2	LOD (ppb)	LOQ (ppb)
Acetone	10.1–83.7	7.08	0.997	2.8	8.4
2-Butanone	2.2–97.3	7.02	0.999	0.6	1.8
2-Propanol	3.0–81.0	6.31	0.997	0.7	2.0
2-Pentanone	2.8–81.6	8.14	0.997	0.6	1.8
Ethanol	3.2–8.6	6.07	0.997	0.7	2.1
Butane	45.2–136.0	9.01	0.996	14.0	42.0
Hexane	4.5–136.0	8.34	0.997	1.1	3.2
2-Methylpentane	15.2–99.8	7.82	0.995	1.4	4.2
3-Methylpentane	16.4–101.5	6.42	0.996	1.4	4.2
2-Methylhexane	22.8–89.3	8.14	0.995	2.1	6.3
3-Methylhexane	22.9–90.3	9.08	0.997	2.1	4.3

Table 3
The individual acetone ratio

Compound	AR for *H. pylori*	AR for healthy tissue	AR for cancer tissue
Ethanol		78.02–220.24	12.29–54.02
Butane	0.18–0.06	86.47–192.08	174.24–423.52
Acetone	–	–	–
Carbon disulfide	0.33–3.65	1.08–12.10	0.27–1.95
1-Propanol		73.62–277.88	13.39–52.48
2-Butanone		495.42	572.67–895.17
2-Pentanone		285.23–350.25	257.41–440.47

It was calculated as a ratio between acetone peak area and the peak area of other compounds for each sample of tissue.

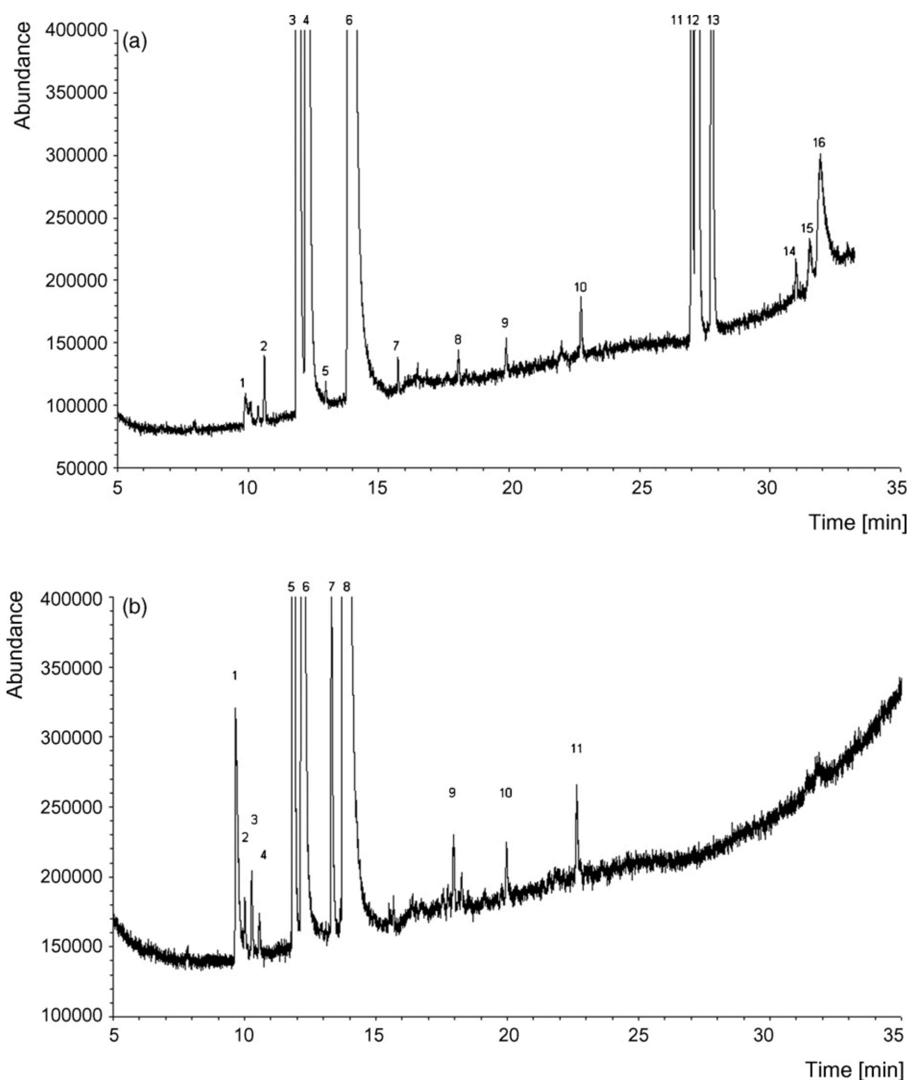

Fig. 2. (a) The GC/MS chromatogram of cancer tissue from front part of stomach: (1) ethanol; (2) butane; (3) acetone; (4) carbon disulfide; (5) 1-propanol; (6) sevoflurane; (7) 2-butanone; (8) benzene; (9) 2-pentanone; (10) toluene; (11) ethylbenzene; (12) *p*-xylene; (13) *o*-xylene; (14) 1,4-dichlorobenzene; (15) α-pinene; (16) hexamethylcyclotrisiloxane. (b) The GC/MS chromatogram of cancer tissue from other part of stomach: (1) ethanol; (2) 2-methyl-1-propene; (3) acetonitrile; (4) butane; (5) acetone; (6) carbon disulfide; (7) 1-propanol; (8) sevoflurane; (9) benzene; (10) 2-pentanone; (11) toluene.

very low (AR, 572.67–895.17). The amount of 2-pentanone emitted from cancer and normal tissue was on similar level. The AR value being in the range of 257.41–440.47 and 285.23–350.25. The data show that the concentration of 1-propanol and carbon disulfide excreted by cancer tissue is higher than in normal tissue (Table 3). The AR for 1-propanol in normal tissue was in the range of 73.62–277.88 and for cancer tissue was 13.39–52.48 and for carbon disulfide AR was 1.08–12.10 and 0.27–1.95 for normal and cancer tissue. The high level of these compounds in cancer patients might be caused by an inflammation which accompanies tumor.

Sulfur-containing compounds like carbon disulfide are generated as a by-product of methionine metabolism and may act as a clear marker for organ rejection after the lung transplantation. It is known that bacteria can generate this compound too. The high carbon disulfide concentrations have also been found in patients with schizophrenia [33].

In both cancer and normal tissue from one patient ethylbenzene, xylenes are present. The primary sources of these compounds are tobacco smoke and exhaust from internal combustion engines [37]. In work by Poli at al. xylene isomers, ethylbenzene, benzene, toluene and styrene were recognized as markers of lung cancer [38].

Sevoflurane is not taken into account because it was used as a volatile anaesthetic during surgery. It has also been identified on the breath of medical staff [39]. Hexamethylcyclotrisiloxane is very often identified on the chromatograms (peak 16, Fig. 2a) because it is a residue from column, a part of the stationary phase which is eluted from the column [40].

3.4. Samples of bacteria culture

H. pylori infection is known as the most common gastrointestinal bacterial disease [11]. An important environmental risk factor for stomach cancer is the infection with the gastric spiral bacterium *H. pylori* [30]. The research also showed that the relationship between *H. pylori* infection and stomach cancer varies strongly by age, showing a much stronger relationship among younger than among older subjects. It is also suggested that stomach cancer is more strongly linked with *H. pylori* strains that have the cytotoxin-associated gene (cagA) [29]. Given the high rate of patient morbidity and mortality associated with gastric cancer, any method by which one can

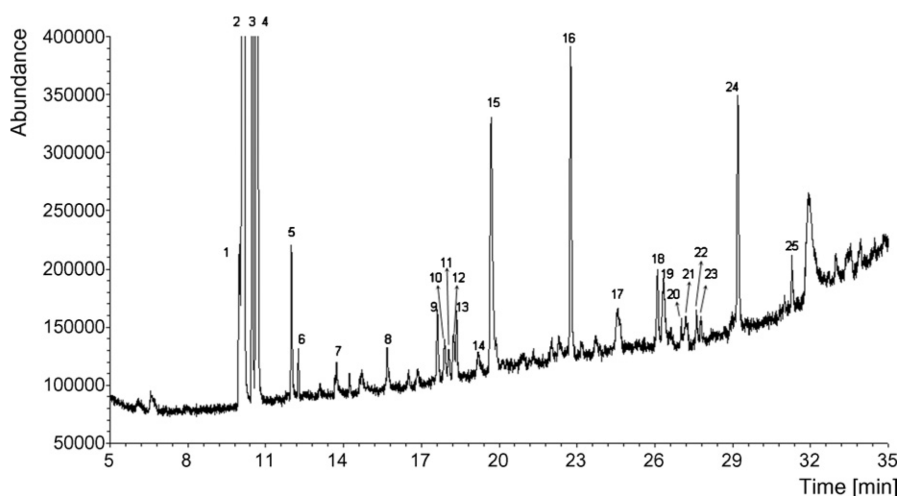

Fig. 3. The GC/MS chromatogram of gaseous substances released by *H. pylori* culture: (1) isobutene; (2) 2-methyl-1-propene; (3) 1-butene; (4) butane; (5) acetone; (6) carbon disulfide; (7) ethylcyclopropane; (8) trichloromethane; (9) 2-methyl-1-pentene; (10) 3-methylpentane; (11) benzene; (12) methylcyclopentane; (13) hexane; (14) cyclohexane; (15) 2-ethoxy-2-methylpropane; (16) toluene; (17) 2,4,4-trimethyl-1-pentene; (18) 4-methylheptane; (19) 5-azauracil; (20) ethylbenzene; (21) *p*-xylene; (22) styrene; (23) *o*-xylene; (24) 2,4-dimethylheptene; (25) 4-methyl-2-heptanone.

reduce the occurrence of disease or increase its early detection is desirable [16].

The presence of bacteria in stomach tissue was confirmed by the urease test. The rapid urease test detects *H. pylori* cells by detecting urease present in the bacterial cell [41]. The procedure is very simple and a tissue sample is placed in a medium containing urea and phenol red. Urease is an enzyme that breaks the carbon–nitrogen bond of amides to form carbon dioxide, ammonia, and water. Urease can be detected by plating bacteria onto an amide-containing medium, specifically urea. When urea is broken down, ammonia is released and the pH of the medium increases (becomes more basic). This pH change is detected by a pH indicator that turns pink in a basic environment. A pink medium indicates a positive test for urease. The rapid urease test has high sensitivity (90–95%) and specificity, and it is simple and cheap. It is rapid and convenient to perform but provides only general information about the presence of bacteria. More detailed information gives an analysis of volatile compounds excreted by bacteria.

In Fig. 3 the emission of VOCs released from *H. pylori* is presented. The total number of identified substances was 25 (Table 1). Eight of identified VOCs are identical with compounds recognized in stomach tissue. This fact might result in that *H. pylori* culture were bred on stomach segments delivered from one of the surgery patients. Butane, acetone and carbon disulfide are endogenous substances present in both normal and cancer tissue and bacteria culture. AR was calculated for butane (0.08–0.18) and carbon disulfide (0.33–3.65) identified in each sample of bacteria culture. These compounds are present in emission from tissues. It might be possible that bigger amount of butane and carbon disulfide in cancer tissue is due to the presence of bacteria.

Fifteen volatile compounds are characteristic for the bacteria because they are not present in normal and cancer tissues (Table 1). These substances might originate from the solution used for bacteria strains purification and separation.

4. Conclusions

In this paper SPME GC/MS technique was used as a very applicatory tool for preconcentration and analysis of VOCs released by stomach tissue and *H. pylori* culture. Due to this technique we are able to separate and identify most of the gaseous compounds. Some of them might indicate the presence of pathological state in human body. Eighteen compounds were found in stomach tissues and seven of them are endogenous. Acetone ratio was calculated for ethanol, butane, carbon disulfide, 1-propanol, 2-butanone and 2-pentanone. The AR of 1-propanol and carbon disulfide is higher for normal tissue than for cancer tissue. The data shows that stomach tissues release many different compounds and the composition of VOCs depends on which part of the stomach they were taken out. The concentration of ketones, butane, ethanol, 1-propanol and carbon disulfide differs significantly in gaseous emission from healthy and cancer tissue. In comparison with the results obtained for *H. pylori* some of VOCs were identified both in bacteria and tissue (8 compounds). The AR was calculated for butane and for carbon disulfide. It is possible that bacteria present in the stomach might cause the increase of these two compounds in emission from cancer tissue.

The proposed method might be applied as a rapid screening method for detecting the early carcinogenic processes in the stomach. Due to this method it will be possible to select a group of patients who needed a further diagnosis and treatment.

The amount of VOCs excreted by tissue and bacteria is very huge and some of them are at the low level. In this case the identification and quantitation of all compounds are very difficult. Therefore, this research work should be continued using a large number of tissues and bacterial culture samples.

In order to diagnose stomach cancer in early stage, the development of a method based on the emission of volatiles from tissue is very promising.

Acknowledgments

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