

Halitosis associated volatiles in breath of healthy subjects

Sandra van den Velde^a, Marc Quiryren^{a,*}, Paul van Hee^b, Daniel van Steenberghe^a

^a Department of Periodontology, Catholic University of Leuven, Kapucijnenvoer 33, 3000 Leuven, Belgium

^b Laboratory for Biochemistry and Toxicology, Ziekenhuis Netwerk Antwerpen Stuivenberg, Lange Beeldekensstraat 267, 2060 Antwerpen, Belgium

Received 15 October 2006; accepted 23 February 2007

Available online 14 March 2007

Abstract

Background: Halitosis can have an intra- or extra-oral origin. In all cases, bad breath is caused by the presence of volatile organic compounds originating from the mouth or the expired air. They can be specific for certain diseases or infections.

Study objective: This study explored the presence and concentration of these volatile compounds normally associated with halitosis in the breath of healthy symptomless volunteers.

Methods: Alveolar and mouth air of 40 healthy volunteers as well as environmental air were analyzed by gas chromatography–mass spectrometry (GC–MS) and by a commercially available GC device (OralChroma[®]).

Results: 14 compounds, associated with halitosis could be detected. All of them except carbon disulfide, appeared to be (partly) produced endogenously and/or in the mouth. Acetone, 2-butanone, 2-pentanone and 1-propanol were common to all volunteers in both alveolar and mouth air and indole and dimethyl selenide in alveolar air.

Conclusions: GC–MS seems a promising tool for differential diagnosis of halitosis, with the possibility to detect extra-oral causes, which often remain undetected unless characterized by a specific smell.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Halitosis; VOC's; GC/MS; Alveolar air; Breath air

1. Introduction

Halitosis (bad breath) has a significant socio-economic impact and may reveal disease. It was neglected until recently by scientists and clinicians and is hardly covered in the medical curricula [1]. Halitosis is caused by a number of volatiles, which originate from the oro-pharynx or from alveolar air. For oral malodor, the sulfur containing gases (hydrogen sulfide, methyl mercaptan and dimethyl sulfide), derived from bacterial degradation of sulfur containing amino acids in the oropharynx, play a significant role. Other gases such as indole, skatole, putrescine, cadaverine and acetone are also relevant and sometimes even the dominant cause of halitosis [2].

Mostly (85%) the pathology causing halitosis lies within the oropharynx (tongue coating, gingivitis, periodontitis, tonsillitis)

[3]. In 10–15% of the patients, however, breath malodor has an extra-oral cause [4,5]. Bad smelling metabolites can be formed/absorbed at any place in the body and be transported by the bloodstream to the lungs. Exhalation of these volatiles causes then halitosis. According to literature, these extra-oral causes are sometimes associated with a typical odor as a result of specific volatile organic compounds (VOC's) in breath [5–7] (Table 1). In clinical practice, diabetes mellitus is characterized by the sweet smell of acetone, liver diseases by the smell of sulfur and kidney failure results in a fishy odor. These findings suggested that VOC's in exhaled breath could provide, in a non-invasive way, valuable information about the subjects' physiological and pathophysiological condition [6–9]. Most patients who complain about breath malodor consult a periodontologist or dentist. There is a risk that too much focus is on the oropharynx, thus neglecting eventual extra-oral causes, which may also play a role.

Until now, breath was mostly analyzed subjectively (organoleptic score by smelling). As a result of inter-examiner variation, the objectivity and reproducibility of this method are rather low [10]. Many clinicians suffer from (partial) anosmia,

* Corresponding author at: Department of Periodontology, Catholic University of Leuven, Kapucijnenvoer 33, BE-3000 Leuven, Belgium. Tel.: +32 16 332485; fax: +32 16 332484.

E-mail address: Marc.Quiryren@med.kuleuven.be (M. Quiryren).

Table 1
Intra- and extra-oral causes of halitosis and their related compounds

Cause	Specific compounds
Oral malodor	Hydrogen sulfide, methyl mercaptane, dimethyl sulfide and dimethyl disulfide
Diabetes mellitus – weight reduction	Acetone, other ketones
Uremia – kidney failure	Dimethylamine, trimethylamine, ammonia
Liver diseases	Dimethyl sulfide, ethanethiol, C ₂ –C ₅ aliphatic acids (acetic acid, propionic acid), butyric acid, isobutyric acid, isovaleric acid
Lung carcinoma	Acetone, 2-butanone, <i>n</i> -propanol, aniline, <i>o</i> -toluidine
Upper respiratory/oropharyngeal carcinoma	C ₂ –C ₈ normal and branched organic acids
Trimethylaminuria	Trimethylamine
Food: garlic/onions	Allyl methyl sulfide
Other potential compounds	Indole, skatole, cadaverine, putrescine, carbon disulfide, dimethyl selenide

References [5,6,9].

which makes organoleptic assessment even impossible. Some VOC's have a high detection level threshold or a low offensive effect and might be more difficult to detect [11]. The portable sulphide monitors, which are used in many clinical centres, are intended to improve the objectivity but are limited to the measurement of the global concentration of sulphur containing gases. In this way, extra-oral causes cannot be detected. Gas chromatography–mass spectrometry (GC–MS) makes it possible to identify, next to the sulphur containing gases, also the other VOC's [12–14]. However, this approach implies a substantial investment and expertise.

Before one can draw any conclusion from such GC–MS analysis, it is essential to know the compounds normally present in breath of healthy volunteers, to act as a reference. This study examines for the first time by GC–MS the presence and concentration of the volatile compounds that are known to be associated with halitosis (i.e. all compounds given in Table 1) in the breath of 40 healthy non halitosis subjects. A distinction is made between alveolar air (originating from the lungs) and the air present in the oropharynx.

2. Subjects, materials and methods

2.1. Subject selection

Forty healthy volunteers (25 females) were enrolled (mean age 41). They were thoroughly questioned on their medical antecedents and on their absence of breath malodor perceived by themselves or by their environment except for morning bad breath. All confirmed they were not suffering from any known disease and were not receiving any medical treatment. Some took low-dose estrogen–progestagen associations. Volunteers were asked to refrain from eating garlic and onions or any spicy food, 24 h before measurement. All subjects signed informed consent and the protocol was approved by the Clinical Trials Committee of the University Hospital of the Catholic University Leuven.

2.2. Sample collection

Per subject three samples were taken: alveolar, mouth and environmental air; the latter to correct for differences in inhaled air. An adult expires approximately 500 mL air with each breath

of which the first 150 mL is dead-space air from the upper airways and nasopharynx, and the subsequent 350 mL is alveolar breath from within the lungs. Alveolar air was collected using a commercial device (Bio-VOC[®] sampler, Markes International Limited, Rhondda Cynon Taff, UK). After 60 minutes' rest, the subjects performed a single slow vital capacity breath, into an inert, non-emitting Teflon[®]-bulb, which has an open end so that the first part of the breath passes through the sampler and only the last portion of exhaled air (150 mL) is trapped. Alveolar breath was then transferred immediately from the sampler to a sorbent tube to capture all VOC's present in a sample. This procedure was repeated three times.

In order to collect air from the mouth, a new Bio-VOC[®] sampler was used. A specially designed coupling device, which fits a disposable mouthpiece, was placed at the open end of the sampler. Subjects were instructed not to breathe for 30 s. Then, air was drawn from the mouth to the Teflon[®]-bulb using a manual piston and transferred to a sorbent tube (three times repeated). The Bio-VOC[®] sampler was also used to take a sample of room air (same procedure). Two layer sorbent tubes containing 200 mg TenaxTA and 200 mg Unicarb (carbonized molecular sieve) (Markes International Limited) were used. The sorbent tubes were preconditioned with constant flow (90 mL/min) of nitrogen (purity 6.0, a nitrogen purifier (Alltech Associates, Lokeren, Belgium) was used to further increase the purity) using the following temperature program: 1 h at 100 °C, 1 h at 200 °C, 1 h at 300 °C and 30 min at 335 °C. They were then sealed by both Swagelok fitting and PTFE ferrules and stored at 4 °C.

2.3. VOC extraction and analysis

Analysis of samples was performed by GC–MS combined with thermal desorption. VOC's were desorbed and concentrated in a thermal desorber (Unity[®], Markes International Limited) at 250 °C onto a –10 °C cold trap for 6 min (helium flow 50 mL/min). The cold trap, packed with the same sorbents as the sorbent tubes, was then heated rapidly to 250 °C and VOC's were transferred to a gas chromatograph (HP6890N, Agilent Technologies, Diegem, Belgium). Column (capillary column, HP5MS, 30 m × 0.25 mm × 0.25 μm film thicknesses, Agilent Technologies) temperatures were ramped as follows: –40 °C for 1 min, 4 °C/min to 180 °C, 0.10 min hold and 30 °C/min to

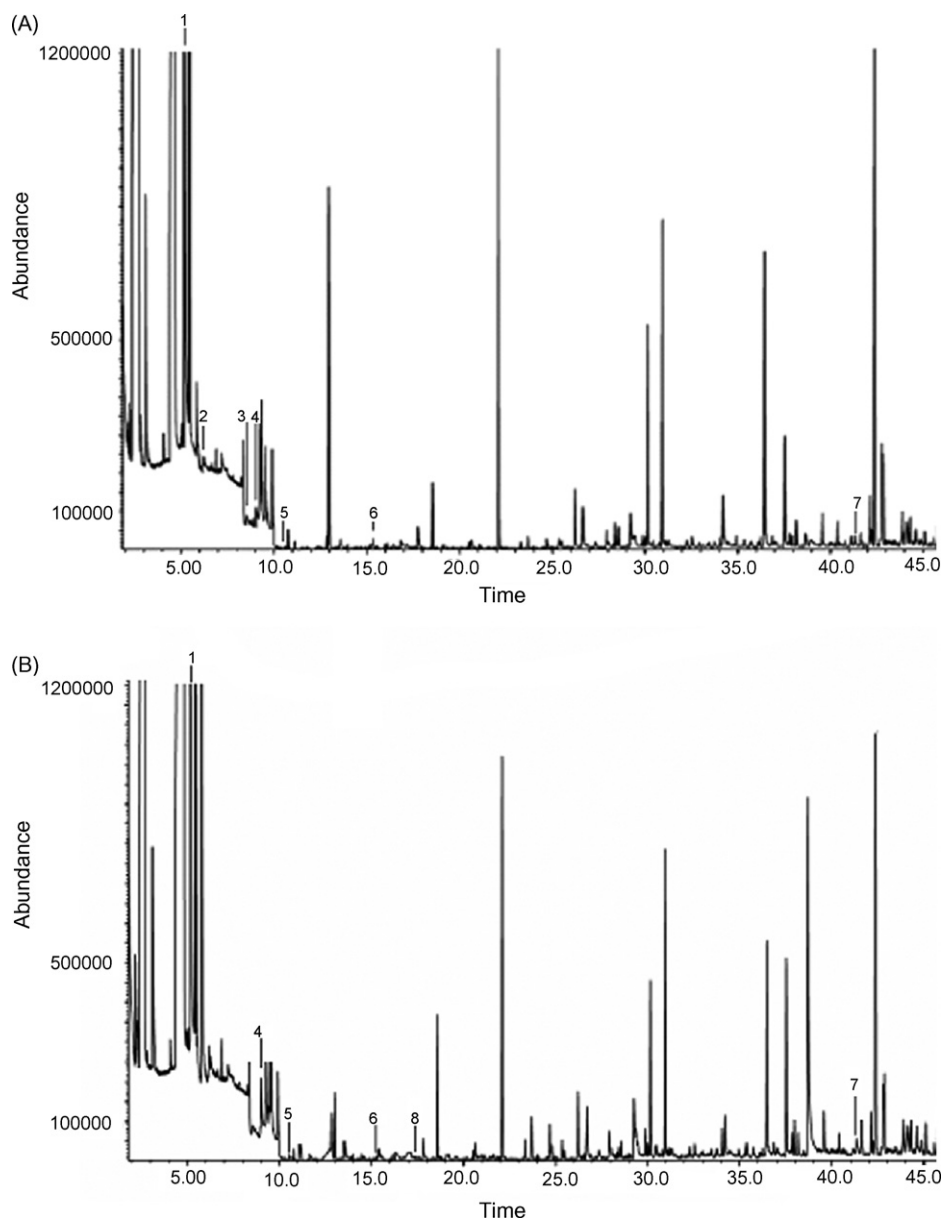


Fig. 1. Typical chromatogram of alveolar (A) and mouth (B) air of a healthy volunteer. The drop at 10 min is the result of the chosen mass range (initially from 10 to 350, after 10 min from 35 to 350) (1) acetone; (2) dimethyl sulfide; (3) dimethyl selenide; (4) 1-propanol; (5) 2-butanone; (6) 2-pentanone; (7) indole; (8) dimethyl disulfide.

300 °C, 0.25 min hold. Liquid nitrogen was used as cryogen. Column head pressure of helium carrier gas was set to 10 psi. Purity of helium was at least 6.0 and a helium purifier (Alltech Associates) was used to further increase the purity. Identification of VOC's occurred in a mass spectrometer (HP5973, Agilent Technologies). Mass range was initially applied from 10 to 350 amu and after 10 min from 35 to 350 (Fig. 1).

2.4. Data management

The presence of all compounds given in Table 1 was examined in all breath and environmental samples. Therefore, for each compound an extracted ion chromatogram of the ions, specific for that compound, was made using the Chemstation software (Agilent Technologies). For double-checking, the observed

SCAN spectrum was compared with the spectrum in the NIST98 library.

2.5. Quantification

For each compound, detected in at least one breath sample, a calibration curve was made. Liquid standards (analytical grade) of the compounds were obtained from Sigma–Aldrich (Bornem, Belgium). Briefly, setting up the calibration curve occurred as follows: a methanolic solution of the standards was injected onto a sorbent tube using a calibration solution loading rig (CSLR, Markes International). This loading rig allows introducing calibration solutions in the vapour phase. The CSLR consist of an unheated injector port with a controlled carrier gas supply (helium, purity 6.0 with helium purifier, Alltech

Associates) and a sorbent tube connection point. The sampling end of a sorbent tube is connected to the CSLR via Swagelok fitting and combined PTFE ferrule. The carrier gas is set at 80 mL/min. This sweeps the injector port and passes down through the sorbent tube. The calibration solution (2 μ L) is introduced through the injector septum using a standard GC syringe (Agilent Technologies). The solution vaporizes in the flow of gas and reaches the sorbent bed in the vapour phase. Then, the same procedure was followed as described for the samples (TD-GC-MS). For each compound 6 different concentrations (5 repeated measurements) were used: 8, 4, 1, 0.5, 0.25 and 0.05 mg/L for indole, dimethyl trisulfide, 2-butanone, 2-pentanone, 1-propanol, dimethyl sulfide, dimethyl disulfide, skatole, allyl methyl sulfide, dimethyl selenide and carbon disulfide and 480, 240, 120, 90, 60 and 45 mg/L for acetone. Each calibration curve was set up using the calibration tool of the Chemstation software (Agilent Technologies) (X-axis: concentration of the compound, Y-axis: response, i.e. peak area of a chosen target ion in the mass spectrum of each compound). For each compound one target ion and at least two qualifier ions were used for identification and quantification. The following target and qualifier ions were used: for indole (117, 90, 89), dimethyl trisulfide (126, 111, 79), 2-butanone (43, 72, 57), 2-pentanone (43, 86, 71, 58) 1-propanol (31, 59, 42), dimethyl sulfide (62, 47, 45), dimethyl disulfide (94, 79, 45), skatole (130, 131, 77), allyl methyl sulfide (88, 73, 41), dimethyl selenide (110, 95, 92), carbon disulfide (76, 78, 44) and acetone (43, 58, 42). The percentage uncertainty, which defines the acceptable range for the relative response of the qualifier ions, was set at 20% relative to the relative response of the calibrator.

Based on the calibration curve, the concentration of each compound was automatically calculated in both breath and environmental samples and a conversion to parts per billion per volume (ppbv or nmol/mol) in air was made. In each sample and for each compound, quality of quantification was checked using the 'QEdit quantitation results tool' of the Chemstation software. If the compound was also present in the environment, the environmental concentration was subtracted from the concentration in the breath samples [13,14].

2.6. Statistical analysis

For each compound, a paired Wilcoxon test was performed in order to detect significant differences between alveolar air and mouth air. To correct for multiple testing a Bonferoni correction was included. The spearman correlation coefficient was used to detect correlations both between alveolar air and mouth air for a certain compound as well as between the different compounds.

2.7. Measurement of sulfur containing compounds

Next to the described method, a commercially available system (OralChroma[®], Abilit Corporation, Osaka City, Japan) was used. It only measures the concentration of hydrogen sulfide, methyl mercaptan and dimethyl sulfide, three major contributors to halitosis of oro-pharyngeal origin. This portable gas chromatograph is equipped with an indium oxide semiconductor

gas sensor. Sample collection occurred by use of a disposable syringe (1 mL), which was inserted into the oral cavity of the volunteers. Subjects had to close their mouth for 30 s before sample collection. 0.5 mL of mouth air was then injected into the measuring device. After 8 min the measurement process is completed and the concentration values of the three gases are displayed in either ng/10 mL or ppbv (nmol/mol).

3. Results

3.1. Gas chromatography-mass spectrometry

Twelve of the compounds given in Table 1 were detected by GC-MS in the expired breath of the 40 volunteers. For each compound, r^2 of the calibration curve was at least 0.99 indicating a good fit of the data points. Limits of detection (LOD) were determined empirically. By this method, the LOD is defined as the concentration at which all routine GC/MS acceptance criteria (retention time within 2% of the calibrator, ion ratios within 20% of calibrator) are met. Dilutions were made until these criteria were not met anymore [15]. LOD in ppbv (nmol/mol) were: for acetone (0.081), 2-butanone (0.045), 2-pentanone (0.038), indole (0.021), skatole (0.019), 1-propanol (0.095), dimethyl selenide (0.067), dimethyl sulfide (0.9), dimethyl disulfide (0.017), dimethyl trisulfide (0.032), allyl methyl sulfide (0.029) and carbon disulfide (0.043). Relative standard deviations (RSD) were determined by performing 5 replicate measurements of the lowest level of the calibration curve and using the following formula: $[(\sigma/\bar{x}) \cdot 100]$. σ is the standard deviation of the response of the compound for the 5 measurements; \bar{x} is the mean slope of the calibration curve. RSD were below 5% for each compound except for dimethyl disulfide (10.55%) and acetone (6.78%). Method accuracy was checked by performing triplicate measurements of a known concentration lying in the middle of the calibration curve (100 mg/L for acetone and 2 mg/L for the other compounds). For each compound and each measurement, the observed concentrations did not deviate more than 10% of the injected concentrations. Data of the compounds in ppbv (nmol/mol) are shown in Table 2. Median values are given because of the presence of some outliers and lots of zeros for some compounds. Acetone, 2-butanone, 2-pentanone, 1-propanol, dimethyl disulfide, dimethyl trisulfide and carbon disulfide were found in at least one of the environmental samples (10 different environmental samples were taken). Only carbon disulfide had a negative median concentration. For dimethyl disulfide, dimethyl trisulfide, carbon disulfide and 2-butanone negative concentration values were obtained for some volunteers after subtraction of the environmental sample. Acetone showed the highest concentration. Six compounds were common to all volunteers in alveolar air and four compounds in mouth air. A significant difference between alveolar air and mouth air was found for acetone ($p < 0.001$), 2-pentanone ($p < 0.001$), dimethyl selenide ($p < 0.001$), 1-propanol ($p < 0.001$), dimethyl sulphide ($p < 0.001$), dimethyl disulphide ($p < 0.001$), dimethyl trisulphide ($p = 0.003$) and allyl methyl sulfide ($p < 0.001$). For every compound there was a positive correlation between alveolar air and mouth air. For acetone, 2-pentanone, indole, dimethyl disul-

Table 2
Descriptive statistics of the compounds detected by GC/MS in ppbv (nmol/mol)

	Median	Min	Max	LQ	UQ	N
Alveolar						
Acetone	199.19	74.14	7909.82	144.69	325.37	40
2-Butanone	0.25	−0.82	71.39	0.017	0.73	40
2-Pentanone	0.38	0.028	37.50	0.23	0.55	40
Indole	0.20	0.021	1.62	0.056	0.34	40
Skatole	0	0	0.037	0	0	8
Dimethyl selenide	0.56	0.15	1.24	0.41	0.80	40
1-Propanol	7.30	0.31	29.05	2.65	10.76	40
Dimethyl sulfide	14.48	0	157.27	7.74	23.70	37
Dimethyl disulfide	0	−0.095	0.33	0	0.043	11
Dimethyl trisulfide	0	−0.058	0.26	0	0	6
Allyl methyl sulfide	0.10	0	16.04	0.037	0.20	35
Carbon disulfide	−0.021	−0.16	698.01	−0.054	0.027	39
Mouth						
Acetone	101.67	9.07	1821.36	59.37	157.79	40
2-Butanone	0.32	−0.034	21.06	0.20	0.73	40
2-Pentanone	0.11	0	10.94	0.066	0.17	40
Indole	0.15	0	1.91	0.063	0.20	39
Skatole	0	0	0.037	0	0	3
Dimethyl selenide	0.13	0	0.55	0.075	0.23	33
1-Propanol	25.7	1.55	54.52	8.98	35.92	40
Dimethyl sulfide	4.29	0	116.62	0	9.18	25
Dimethyl disulfide	0.061	−0.095	0.75	0.017	0.19	31
Dimethyl trisulfide	0	0	0.60	0	0.087	16
Allyl methyl sulfide	0	0	4.55	0	0.074	14
Carbon disulfide	−0.021	−0.16	273.10	−0.054	0.021	37

LQ: Lower quartile; UQ: upper quartile; N: number of persons in whom the compound has been detected.

phide, carbon disulphide and allyl methyl sulfide this correlation was strong ($R \geq 0.66$). For 1-propanol, the correlation was weak ($R \leq 0.33$). Between the compounds, the strongest correlation was found between dimethyl disulfide and dimethyl trisulfide in both alveolar air and mouth air (R is respectively 0.73 and 0.75, $p < 0.001$).

3.2. Measurement of sulfur containing compounds

Data in ppbv (nmol/mol) for hydrogen sulfide, methyl mercaptan and dimethyl sulfide are given in Table 3. In the breath of seven persons none of the compounds was present. In eleven volunteers all three compounds could be detected. For every person, the concentration values for hydrogen sulfide were below the cognitive threshold (the level at which the human nose detects malodor) proposed by the manufacturer of the device. For methyl mercaptane, four people showed a higher level than the threshold and one person had a concentration of more than 100 ppbv

Table 3
Data of the Oralchroma® device in ppbv (nmol/mol)

	Conc (ppb)	Number	Range	SD	TH
Hydrogen sulfide	11.78	25	0–68	19.87	112
Methyl mercaptane	9.7	16	0–164	27.17	26
Dimethyl sulfide	20.3	24	0–233	42.57	8

Conc: mean concentration; Number: number of subjects in whom the compound has been detected; SD: standard deviation; TH: cognitive threshold provided by the manufacturer.

(nmol/mol). For dimethyl sulfide, the concentration was higher than the threshold in eighteen volunteers and for two of them a concentration of more than 100 ppbv (nmol/mol) was registered.

A weak positive but not significant correlation could be found for the dimethyl sulfide concentration obtained by the GC/MS system and the Oralchroma device ($R = 0.32$; $p = 0.20$).

4. Discussion

In the breath of the 40 volunteers, fourteen compounds normally associated with bad breath could be detected (Table 4). Seven of these compounds were also detected in at least one of the environmental samples. In order to make a distinction between endogenous and exogenous compounds, it is thus necessary to make corrections for background concentrations of volatile compounds in the inspired air. In our study, background corrections were made by subtracting inspired from expired air [13,14]. Only carbon disulfide had a negative median concentration and this compound is believed to be the result of background contamination. For some other compounds (dimethyl disulfide, dimethyl trisulfide and 2-butanone) negative values were obtained for some volunteers, which indicates that the compound was more likely to be derived from environmental origin. In addition, compounds may be stored in different compartments of the body and exhaled after days or weeks or may be excreted by another pathway than exhalation. All other compounds had a positive or zero median and minimum concentration, indicating they were produced endogenously and/or by bacterial

Table 4
Detected compounds with their potential origin and some characteristics

Compound	Origin	Odor qualification	O.I. ^a	RT ^b
Acetone	Decarboxylation of acetoacetate	Sweet	720	300
2-Butanone	Degradation of fatty acids	Acetone-like	3800	30
2-Pentanone	Degradation of fatty acids	Acetone-like	2000	8
Indole	Bacterial breakdown of tryptophan	Recal, nauseating	–	–
Skatole	Bacterial breakdown of tryptophan	Fecal, nauseating	–	–
Dimethyl selenide	Metabolism of selenium	Garlic-like	–	–
1-Propanol	Bacterial fermentation of threonine	Alcoholic, slightly stupefying	480	45
Dimethyl sulfide	Bacterial breakdown of methionine	Unpleasantly sweet	2760000	0.1
Dimethyl disulfide	Bacterial breakdown of amino acids	Pungent	–	0.007
Dimethyl trisulfide	Bacterial breakdown of amino acids	Pungent	–	–
Allyl methyl sulfide	Garlic intake	Garlic-like	–	–
Carbon disulfide	Metabolism of methionine	Slightly pungent	1600000	0.9
Hydrogen sulfide	Bacterial breakdown of cysteine/methionine	Rotten eggs	17000000	1
Methyl mercaptane	Bacterial breakdown of cysteine/methionine	Pungent, rotten cabbage	53300000	0.035

References [17,30].

^a O.I.: Odor index = ratio between the vapor pressure and the 100% recognition threshold.

^b RT: 100% recognition threshold in ppmv ($\mu\text{mol/mol}$) = concentration at which 100% of the odor panel defined the odor as being representative of the odorant being studied.

processes in the mouth. For most compounds except for dimethyl disulfide, dimethyl trisulfide, carbon disulfide, 1-propanol and 2-butanone, endogenous production is more important (higher concentration) than production in the mouth.

In the study, a distinction was made between alveolar and mouth air. This distinction is very important because if mouth air, dead space air and alveolar air are mixed, a dilution takes place. This dilution factor cannot be ignored because the dilution factor unavoidably varies, e.g. when the test subject is breathing deeply or shallowly. In a previous study, performed with the same GC–MS system and sampling method, we made clear that there is an important difference between the composition of alveolar air and mouth air (Van den Velde et al., submitted for publication).

Acetone, the most abundant metabolite in alveolar air, is derived from decarboxylation of acetyl-CoA when lipolysis or lipid peroxidation occurs. Acetone levels are elevated in diabetes mellitus, which causes the sweet smell of the breath of these patients [16]. Other ketones that were detected are 2-pentanone and 2-butanone, which have an acetone-like odor [17]. High concentrations of 2-butanone are known to be a marker for lung cancer [5].

Dimethyl sulfide is together with other sulfides responsible for the characteristic odor in the breath of cirrhotic patients. Sulfur compounds are generated by incomplete metabolism of methionine. Under normal conditions, concentrations of these compounds are very low [7]. Dimethyl sulfide can also be produced in the mouth by anaerobic bacterial breakdown of sulfur containing amino acids such as from the tongue coating or from impacted food remnants, and can thus cause oral malodor [7]. This is also the case for the other sulfides, dimethyl disulfide and dimethyl trisulfide which are strongly correlated with each other. Allyl methyl sulfide has been associated with garlic intake through expression by the cervical fluid which reflects the circulating molecules in the bloodstream [18]. However, the latter compound was found in the alveolar breath of 35 of the 40 volunteers, indicating that there has to be another origin too. Carbon

disulfide seems to be generated as a by-product of methionine metabolism [7]. Levels of this compound were negative for most volunteers, indicating environmental origin. However, carbon disulfide was highly elevated in breath of one volunteer. Further questioning of the involved person made clear that this was the result of the intake of disulfiram, a medicine used to control alcohol abuse, something he did not reveal at enrolment in the study [19]. Due to the intake, acetone, 2-pentanone, 2-butanone and dimethyl sulfide levels were also highly increased.

Indole and skatole are by-products of the metabolic breakdown of tryptophan in the digestive tract but can also be produced by bacteria in the mouth [20].

1-Propanol is a normal constituent of the human metabolism. High levels have been associated with lung carcinoma [5]. It has an alcoholic and slightly stupefying odor [17]. The higher gradient in mouth air is believed to be the result of bacterial fermentation of threonine in the mouth.

Dimethyl selenide is a product of the metabolism of selenium, which is an essential micronutrient. Excessive intake of selenium leads to a garlic-like breath [21].

Some other compounds that are believed to be associated with halitosis were not detected (Table 1). First of all, some compounds are not likely to be present in the breath of healthy volunteers but can be expected in patients with certain diseases. This could be the case for metabolites like the organic acids butyric acid, isobutyric acid and isovaleric acid which appear to be only present in patients with liver diseases [6]. Secondly, it is possible that some compounds cannot be detected with the GS/MS system used in this study. Ammonia for example is known to be present in everyone's breath but because of its low boiling point detection becomes impossible [7]. For some compounds, concentrations in healthy persons can be below the detection limit of the device.

Hydrogen sulfide and methyl mercaptane were not detectable by the GC/MS system mainly because of their enormous reactivity and volatility, which can lead to their removal during thermal desorption. Moreover, the HP5MS column gives little or no

retention of these sulfur compounds as separation is based on the boiling point of the compounds. We did detect however these sulfur compounds together with dimethyl sulfide in mouth air using a commercially available device (OralChroma[®]). Most volunteers had at least one of the three gases in their breath. Only a weak positive correlation was found between the levels of dimethyl sulfide measured with the GC/MS system and the OralChroma[®]. A previous study has shown that the indium oxide gas sensor can measure the concentration of the compounds quantitatively over a range from 50 to 1000 ppbv (nmol/mol) [22]. In our study, for most volunteers concentrations were below 50 ppbv which could explain the poor correlation. Moreover, two previous independent studies already made clear that the commercial device gives good correlations for hydrogen sulfide and methyl mercaptan but not for dimethyl sulfide [23,24]. The detector of the OralChroma[®] shows less sensitivity for dimethyl sulfide than for hydrogen sulfide and methyl mercaptan. Low concentrations are thus more difficult to detect.

VSC monitors with a semiconductor gas sensor detect not only VSC's but also other volatile compounds like alcohols and ketones, which might interfere with the measurements, although this is considered to have only a limited influence [22,25]. The differences in sample collection and manipulation might also have had an impact.

With the OralChroma[®] device hydrogen sulfide and methyl mercaptan were only measured in mouth air and not in alveolar air. However, *in vitro* experiments have shown that the free –SH group of methyl mercaptan immediately reacts with blood within seconds, which results in an irreversible binding and oxidation. In this way, transport of methyl mercaptan from the blood into the alveolar air is not easy. The same holds for H₂S. This is not the case for dimethyl sulfide which is a neutral molecule that is stable in blood and can be transported from blood into the alveolar air and be expired [26].

Food intake could have had some impact on the detected concentrations of the compounds. However, because this method will be used in clinical practice for diagnosis, we chose for a general view of the breath composition of healthy, non-fasting people and did not ask the volunteers to fast during a period of 24 h. Fasting is known to cause elevated concentrations of the ketones acetone, 2-pentanone and 2-butanone. Acetone concentrations after fasting (4.1 ppmv (μmol/mol)) were even very close to the range of diabetic breath (1.7–3.7 ppmv) in a study conducted with seven fasting monks [27]. We did however asked the volunteers to refrain from eating garlic, onions or any spicy food 24 h before measurements. Breath samples were also taken at least 30 min after consumption of any food or beverages and before lunch.

Due to some technical difficulties of the methodology used (e.g. high water content), there might be a slight underestimation of the detected concentrations. However, acetone and propanol concentrations for example were very close to the detected normal range of previous studies [28,29].

It is important to take into account that the threshold to smell differs according to the type of gas. Some gases can cause a striking odor at very low concentrations while others need to be present in much higher quantities to give a noticeable odor. This

is important for drawing conclusions regarding the origin of halitosis whether with an intra or extra-oral cause when using an organoleptic approach. In literature some lists are known with the odor index and odor recognition threshold for a number of organic gases (Table 4) [30]. Compounds with a high odor index and a low odor recognition threshold are odorous. Of all chemical classes, mercaptans, disulfides and sulfides are the most odorous and these metabolites (methyl mercaptan, hydrogen sulfide, dimethyl sulfide, dimethyl disulfide) are the most important contributors to bad breath.

Breath analysis by GC/MS can thus become a powerful non-invasive tool for the differential diagnosis of halitosis. Further research will be necessary to find out which compounds play a crucial role in the different pathologies that can cause bad breath. Because of the possible influence of environmental compounds and because halitosis associated compounds are also present in the breath of normal volunteers, care should be taken when using breath analysis for diagnosis. It will be necessary to search for gas profiles rather than focusing on just one compound.

Acknowledgements

This study was supported (financially) in part by the Fund for Scientific Research – Flanders (FWO Vlaanderen) and GABA International, Münchenstein, Switzerland.

Prof. D. van Steenberghe was principal investigator and had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

- [1] D. van Steenberghe, *Breath Malodor: A Step-by-Step Approach*, Quintessence, Copenhagen, 2004.
- [2] J. Tonzetich, *J. Periodont.* 48 (1977) 13.
- [3] D. van Steenberghe, M. Quirynen, in: J. Lindhe, T. Karring, N. Lang (Eds.), *Clinical Periodontology and Implant Dentistry*, Blackwell Munksgaard, Oxford, 2003, p. 512.
- [4] G. Delanghe, J. Ghyselen, L. Feenstra, D. van Steenberghe, *Lancet* 350 (1997) 187.
- [5] G. Preti, H. Lawley, C. Hormann, in: M. Rosenberg (Ed.), *Bad Breath: Research Perspectives*, Ramot Publishing, Tel-Aviv University, 1995, p. 149.
- [6] A. Manolis, *Clin. Chem.* 29 (1983) 5.
- [7] W. Miekisch, J.K. Schubert, G. Noeldge-Schomburg, *Clin. Chim. Acta* 347 (2004) 25.
- [8] N. Marczin, S.A. Kharitonov, M.H. Yacoub, P. Barnes, *Disease markers in exhaled breath*, Marcel Dekker, New York, 2002.
- [9] A. Tangerman, *Int. Dent. J.* 52 (2002) 201.
- [10] M. Rosenberg, C.A. McCulloch, *J. Periodont.* 63 (1992) 776.
- [11] J. Greenman, J. Duffield, P. Spencer, M. Rosenberg, D. Corry, S. Saad, P. Lenton, G. Majerus, S. Nachnani, M. El-Maaytah, *J. Dent. Res.* 83 (2004) 81.
- [12] D. Claus, B. Geypens, P. Rutgeers, J. Ghyselen, K. Hoshi, D. van Steenberghe, Y. Ghos, in: D. van Steenberghe, M. Rosenberg (Eds.), *Bad Breath: A Multidisciplinary Approach*, University Press, Leuven, 1996, p. 15.
- [13] M. Phillips, *Anal. Biochem.* 247 (1997) 272.
- [14] M. Phillips, J. Herrera, S. Krishnan, M. Zain, J. Greenberg, R.N. Cataneo, *J. Chromatogr. B* 729 (1999) 75.
- [15] D.A. Armbruster, M.D. Tillman, L.M. Hubbs, *Clin. Chem.* 40 (1994) 1233.
- [16] R.D. Stewart, E.A. Boettner, *New Eng. J. Med.* 270 (1964) 1035.
- [17] A. Smith, P.A. Heckelman, M.J. O'Neil, S. Budavari, Merck, *The Merck Index*, Merck Publishing, N.J., 2001.

- [18] F. Suarez, J. Springfield, J. Furne, M. Levitt, *Am. J. Physiol. Gastrointest. Liver Physiol.* 276 (1999) 425.
- [19] R.N. Bloor, P. Spanel, D. Smith, *Addict. Biol.* 11 (2006) 163.
- [20] M.T. Jensen, B.B. Jensen, *J. Chromatogr. B Biomed. Appl.* 655 (1994) 275.
- [21] W.J. Hunter, L.D. Kuykendall, *J. Chromatogr. A* 1038 (2004) 295.
- [22] M. Hanada, H. Koda, K. Onaga, K. Tanaka, T. Okabayashi, T. Itoh, H. Miyazaki, *Anal. Chim. Acta* 475 (2003) 27.
- [23] P. Lenton, G. Majerus, M. Levitt, J.S. Hodges, *J. Dent. Res.* 83 (2004), No 3330 (abstract).
- [24] J. Duan, D. Choi, K. Yaegaki, IADR Conference, Göteborg (2003) No 2019 (abstract).
- [25] K. Yaegaki, in: M. Rosenberg (Ed.), *Bad Breath: Research Perspectives*, second ed., Ramot Publishing, Tel-Aviv University, 1997, p. 225.
- [26] H.J. Blom, A. Tangerman, *J. Lab. Clin. Med.* 111 (1988) 606.
- [27] M. Statheropoulos, A. Agapiou, A. Georgiadou, *J. Chromatogr. B* 832 (2006) 274.
- [28] T. Qin, X. Xiaobai, T. Polak, V. Pacakova, K. Stulik, L. Jech, *Talanta* 44 (1997) 1683.
- [29] C. Turner, P. Spanel, D. Smith, *Physiol. Meas.* 27 (2006) 321.
- [30] K. Verschuere, *Handbook of environmental data on organic chemicals*, Van Nostrand Reinhold Company, New York, 1983.