Volatile sulphur compound measurement with OralChroma™: a methodological improvement

Anna Szabó[1,2,5], Zsófia Tarnai[3], Csaba Berkovits[4], Péter Novák[4], Árpád Mohácsi[2], Gábor Braunitzer[4], Zoltán Rakonczay[3], Kinga Turzó[3], Katalin Nagy[4] and Gábor Szabó[1,2]

[1]Department of Optics and Quantum Electronics, Faculty of Science and Informatics, University of Szeged, Dóm tér 9, Szeged 6720, Hungary

[2]MTA-SZTE Research Group on Photoacoustic Spectroscopy, Dóm tér 9, 6720 Szeged, Hungary

[3]Department of Oral Biology and Experimental Dental Research, Faculty of Dentistry, University of Szeged, Tisza L. krt. 83, Szeged, 6720, Hungary

[4]Department of Oral Surgery, Faculty of Dentistry, University of Szeged, Tisza L. krt. 64, Szeged 6720, Hungary

E-mail: aszabo@titan.physx.u-szeged.hu

[5]Author to whom any correspondence should be addressed.

Abstract

Instrumental measurement of volatile sulphur compounds is a common practice to assess halitosis. One of the most widespread devices for that purpose is OralChroma™, a combination of a semiconductor gas sensor and a compact gas chromatograph (GC) system. Several lines of evidence indicate that although the hardware of OralChroma™ is fit for the precise measurement of volatile sulphur compounds (VSCs), its software needs revision to allow that precision. In this study we sought to develop a software to solve this problem, and to test the utility of the new software in a population of patients and controls. The results were also compared with VSC measurements done with Halimeter®, another widespread device, so as to assess correlation. A set of measurements involving volunteers (21 controls and 14 oral cancer patients) were conducted. The analysis of the chromatograms recorded by OralChroma™ indicated that the majority of the studied breath samples contained significant amounts of isoprene (peak around 100 s) and acetaldehyde (peak around 350 s), therefore OralChroma™ was also calibrated for both isoprene and acetaldehyde. Linear relationship was found between the concentration (in the range of 80-1400 ppbv for acetaldehyde and 40-560 ppbv for isoprene) and the area under the corresponding peak. In numerous cases the concentrations of VSCs calculated by the software of OralChroma™ required revision. In the new software, the concentrations of the VSCs, isoprene and acetaldehyde were determined by fitting the chromatograms with the sum of six Gaussian functions. Based on the findings of the present study we conclude that our new software allows improved and instantaneous evaluation of OralChroma™ chromatograms with the additional possibility of determining isoprene and acetaldehyde concentrations from breath samples.

Keywords: halitosis, volatile sulphur compound, gas chromatograph, semiconductor sensor

1. Introduction

Halitosis is oral malodour that can lead to both intrapsychic and social problems in the affected. The prevalence of this condition is still not well established due to the lack of consensus regarding diagnostic criteria and the limited accuracy and sensitivity of detection methods. However, there is evidence to suggest that its prevalence is somewhere between twenty and fifty percent [1-4].

In about 80-90% of the cases, halitosis is of intraoral origin, which also makes it the most studied type. Several studies showed that oral malodour is caused mainly by the bacterial biofilm coating the tongue [5, 6]. Oral factors, like periodontal disease, peri-implantitis, deep carious lesions, exposed necrotic tooth pulp, or mucosal ulcerations can also lead to halitosis [5-7]. Furthermore, Scully and Felix, in a review, proposed that patients with oral cancer can develop oral malodour [8], which seems to be supported by the observation that the breath of head and neck cancer patients contains volatile organic compounds (VOCs) in elevated concentrations [9-11]. In most of the cases, malodour comes about as a result of the microbial degradation of organic substrates present in the saliva, the crevicular fluid exudate, oral soft tissues and retained debris. During the process, volatile sulphur compounds (VSCs), diamines (e.g. cadaverine, putrescine) and phenyl compounds (e.g. indole, skatole) are formed [5, 12, 13]. VSCs include hydrogen sulphide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulphide ($(CH_3)_2S$). The measurement of the concentration of these compounds in breath offers an objective assessment of halitosis, as opposed to organoleptic assessment, which has a strong subjective element, even if two or more different examiners (trained and calibrated judges) analyze the exhaled air [14].

Of the several objective methods, such as the benzoyl-DL-arginine-α-naphthylamide (BANA) test, ammonia monitoring, salivary incubation test, beta-galactosidase activity, and PCR [5, 7] of sampled microorganisms, gas chromatography and halimetry (e.g. the sulphide-monitoring Halimeter®) are the most widespread [2].

In halitosis research, Halimeter® is traditionally the instrument of choice, while its specificity is quite limited. It cannot properly differentiate between the three VSCs, as it is the most sensitive to hydrogen sulphide, less sensitive to methyl mercaptan and it is almost insensitive to dimethyl sulphide [15, 16].

Several studies indicated that gas chromatography is the appropriate method for the precise quantification of oral malodour, which also allows differentiation between halitosis subtypes based on their origin [17, 18]. Only this method can differentiate between the individual VSCs, which is crucial for the determination of origin [1, 17, 19].

Hanada *et al.* developed a portable oral malodour analyzer for the quantitative detection of VSCs in mouth air using a combination of a semiconductor gas sensor and a compact gas chromatograph (GC) system [20, 21]. This became known as OralChroma™, a commercially available GC device [22]. This instrument serves the purpose of quick VSC assessment, optimized for the measurement of those gas components that are considered to be of key importance in the development of oral malodour.

Van den Velde *et al.* analyzed alveolar and mouth air by gas chromatography–mass spectrometry (GC–MS) and by OralChroma™ [22]. They proposed that GC–MS is the most promising tool for the differential diagnosis of halitosis. However, the method is expensive and sample preparation and data analysis require special knowledge, whereby this method is not used in the everyday practice [20].

In their recent study, Tangerman and Winkel pointed out that the hardware of OralChroma™ meets the requirements for an accurate gas chromatograph [18] distinguishing quantitatively all three major VSCs. However, the software needs major revision, given the often erroneous assignment of VSC peaks, and the resulting false results [18]. Moreover, although the OralChroma™ semiconductor sensor is particularly sensitive to VSCs, it is not specific for these compounds at the desirable level.

The last few years have seen an increasing demand for commercially available GC systems to detect halitosis, which brought on the realization that the software of these systems (including OralChroma™) often fails to meet the expectations. The aim of this study was to develop a new, accurate and reliable real-time evaluation software for OralChroma™. An additional aim was to enable the system to identify and measure isoprene and acetaldehyde too, so that the reliability of OralChroma™ - based diagnostics could be enhanced. Finally, as the poor correlation between instrumental measurements in this field is a well-known problem, OralChroma™ measurements were compared to Halimeter® measurements in order to find out if the new software can also address this problem.

2. Subjects, materials and methods

35 volunteers participated in the study. Of the participants, 21 were healthy controls of excellent oral hygiene (n_{female}=11, n_{male}=10, average age: 35.6 years), and 14 were oral cancer patients (n_{female}=2, n_{male}=12, average age: 59.8 years). The oral cancer group consisted entirely of patients diagnosed with squamous cell carcinoma. Oral cancer patients were chosen because, based on the literature, we assumed that the composition of their breath would be significantly different from that of healthy controls [8-11]. The measurements bore out this assumption (see later). As the sole purpose of the study was to test the new software with breath samples of significantly different compositions, and given that the measurements indicated that the samples of the two groups indeed differed to a considerable extent in their composition, we did not consider it necessary to set up a diagnosis of halitosis with the help of a calibrated judge.

All measurements were performed at least three hours after the last meal, drink or oral hygienic measure (e.g. toothbrushing, flossing, etc.). All measurements were carried out in triplicate in each case between 8:30 and 12:30.

Exclusion criteria included antibiotic treatment in four weeks prior to the measurements, and the consumption of onions, garlic or alcohol over two days prior to the measurements.

The study protocol conformed to the tenets of the Declaration of Helsinki in all respects. All subjects gave their informed consent and the protocol was approved by the Ethics Committee of the University of Szeged.

The two most common devices used in small breath clinics – OralChroma™ (Abimedical Corporation, Japan) and Halimeter® (Interscan Corporation, CA, USA) – were utilized. OralChroma™ is a portable GC, which uses ambient air as carrier gas and a semiconductor (In_2O_3) gas sensor to detect the VSCs [20, 21]. Halimeter® is a portable sulphur monitor that uses an electrochemical sensor that generates a signal when exposed to sulphur-containing gases [15, 16]. Halimeter® has a good time resolution, therefore it shows changes and short time variation (e.g. over 5 or 10 minutes) which other instruments (including GC or GC-MS) would miss.

First of all, the influence of sampling time, sampled volume and syringe material on the reproducibility of the OralChroma™ chromatograms was examined. Syringes with rubber barrel seal (provided by the manufacturer) and all-plastic syringes (2 ml B. Braun Inject® Luer

Solo, B. Braun Medical Inc., Germany) were tested. In accordance with a previous study [18] it was found that all-plastic syringes are preferable over rubber-containing syringes. Furthermore, our experience suggests that a sampling time longer than what is recommended by the manufacturer (30 s) allows somewhat better reproducibility. Consequently, syringes were held in the oral cavity for 2 minutes. Halimeter® was operated according to the manufacturer's instructions.

The calibration of OralChroma™ and Halimeter® was performed with humidified (~2% water vapour) hydrogen sulphide mixed in synthetic air. Various H_2S concentrations were prepared from certified cylinders (200 ppmv H_2S in N_2 and synthetic air, Messer Hungarogas, Hungary) using mass flow controllers. The cross-sensitivity of OralChroma™ for volatile organic compounds (VOCs) was investigated quantitatively. Liquid standards (analytical grade) of isoprene and acetaldehyde (Sigma Aldrich, Schnelldorf, Germany) were used to prepare gases for calibration of OralChroma™. Gas samples with a known amount of isoprene were prepared by adding isoprene with Hamilton syringes (Hamilton Messtechnik GmbH, Germany) through a septum into a closed glass flask filled with air. Gas samples containing 46, 93, 185, 276, and 555 ppbv isoprene were prepared. The same procedure was used for acetaldehyde and samples containing 85, 250, 460, 505, 755, 925, 965, and 1385 ppbv acetaldehyde were prepared.

A new software (written in LabVIEW™; National Instruments, TX, USA) was developed to simplify and accelerate the re-evaluation of the OralChroma™ chromatograms. The program reads the files that are automatically generated by the OralChroma™ when a measurement is saved, and detects local maxima in ± 10 second intervals of the expected peak locations (at 30, 60, 100, 150, 250, 350 s). Then it fits the sum of six Gaussian (18-parameter) functions using the Levenberg-Marquardt method. As far as the initial parameters of the fitting procedure are concerned, local maxima are used as initial peak heights, while initial full widths at half maxima and the peak centres are constant. The concentrations of hydrogen sulphide, isoprene, methyl mercaptan, dimethyl sulphide and acetaldehyde are calculated from the areas under the peaks at 60, 100, 150, 250 and 350 s, respectively. The sensitivity of OralChroma™ to methyl mercaptan and dimethyl sulphide was determined from chromatograms with regular peaks (without overlaps or retention time shift) at 150 and 250 s.

Statistical analysis was carried out using Statistica for Windows 11.0 (StatSoft, Inc., OK, USA).

3. Results

3.1 Calibrations

3.1.1 Isoprene calibration

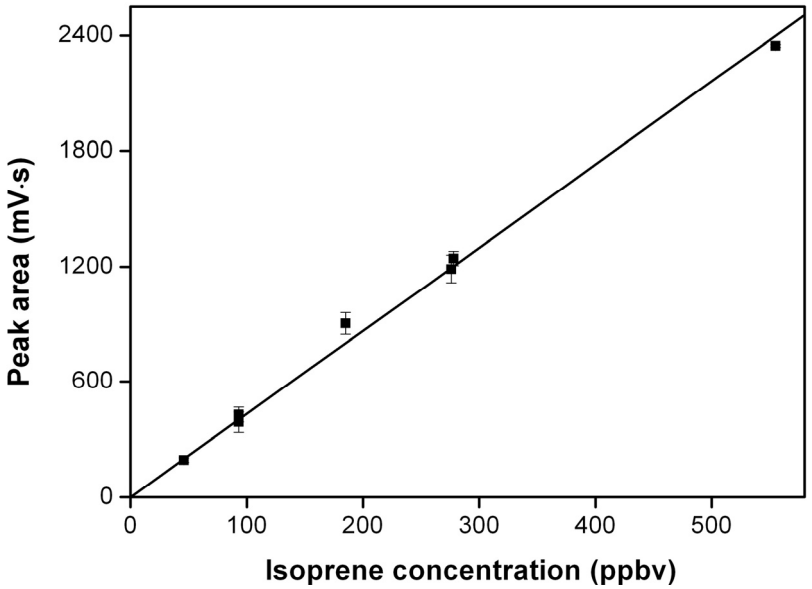

Figure 1. Area under the peak at 100 s as a function of isoprene concentration measured by OralChroma^{TM}. The solid line shows the linear regression of the data. Error bars indicate the standard deviation of three independent measurements.

A distinct peak at 100 s was noticed on the chromatograms of 34 volunteers (97%) indicating the isoprene content of the sample. Linear relationship (R = 0.9980) was found between the peak area and the concentration of isoprene (Figure 1). Sensitivity to isoprene was found to be 4.32 ± 0.07 (mV·s)/ppbv.

3.1.2 Acetaldehyde calibration

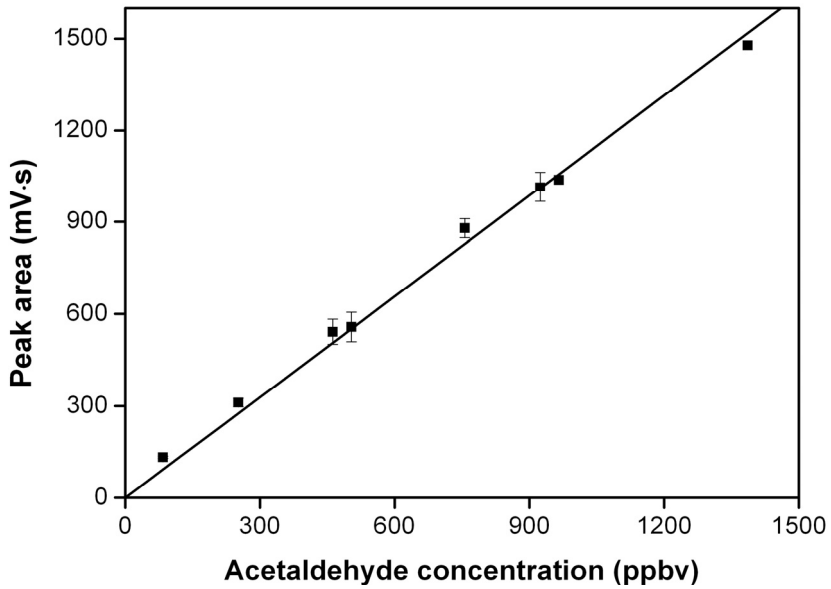

Figure 2. Area under the peak at 350 s as a function of acetaldehyde concentration. The solid line reflects the linear regression of the data. Error bars indicate the standard deviation of three independent measurements.

In the majority of the cases (77%), the chromatograms contained a broad peak around 350 s indicating the acetaldehyde content of the sample. Linear relationship (with R = 0.9990) was found between the peak area and the concentration of acetaldehyde (Figure 2). Sensitivity to acetaldehyde was found to be 1.10 ± 0.02 (mV·s)/ ppbv.

Table 1 summarizes the sensitivity of the OralChroma™ for the measurable components.

Table 1. Sensitivity of OralChroma™ for VSCs, isoprene and acetaldehyde.

	Retention time (s)	Sensitivity of OralChroma™ (mV·s/ppbv)	Sensitivity of OralChroma™ given by Hanada et al. [20] (mV·s/ppbv)
hydrogen sulphide	60	2.57	3.26
isoprene	100	4.32	–
methyl mercaptan	150	3.70	4.44
dimethyl sulphide	250	2.65	3.2
acetaldehyde	350	1.10	–

3.2 Results yielded by the new software

An example of the incorrect results yielded by the default software of OralChroma™ is shown in Figure 3a. The default software returned zero ppbv for CH_3SH; at the same time, there is a distinct, unambiguous peak around 150 s. The new software returned 28 ppb for this CH_3SH peak. In addition, significant peaks are noticeable at 100 s and 400 s, indicating an isoprene and acetaldehyde content of the breath sample as shown in the re-evaluated chromatogram (Figure 3b). Additionally, there is a low peak of $(CH_3)_2S$ around 270 s (with a concentration of 2 ppb), which may strongly overlap with the broad acetaldehyde peak.

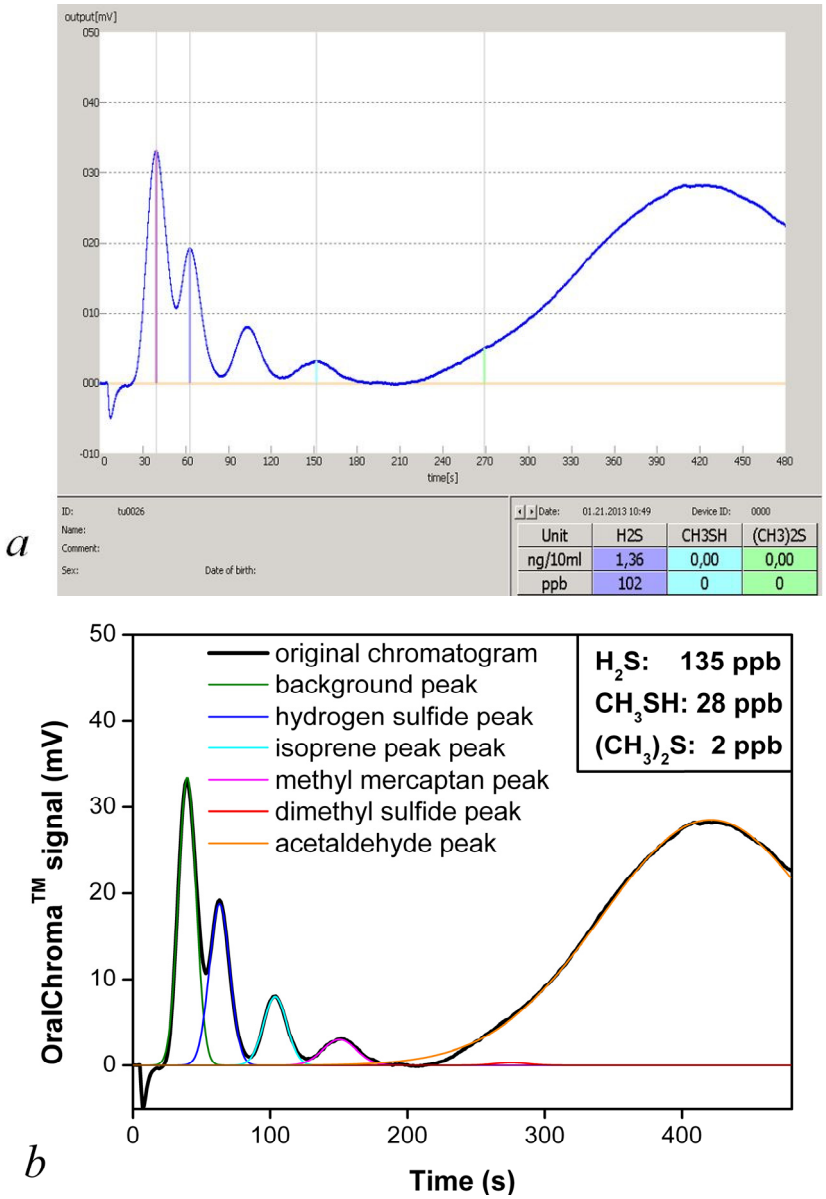

Figure 3 a-b. Example of incorrect evaluation of an OralChroma™ chromatogram (a) software display (b) after re-evaluation. A peak around 150 s clearly indicates the CH_3SH

content of the sample; however the OralChroma™ software assigned 0 ppb to that peak. Nevertheless, the new software returned 28 ppb for the CH_3SH concentration.

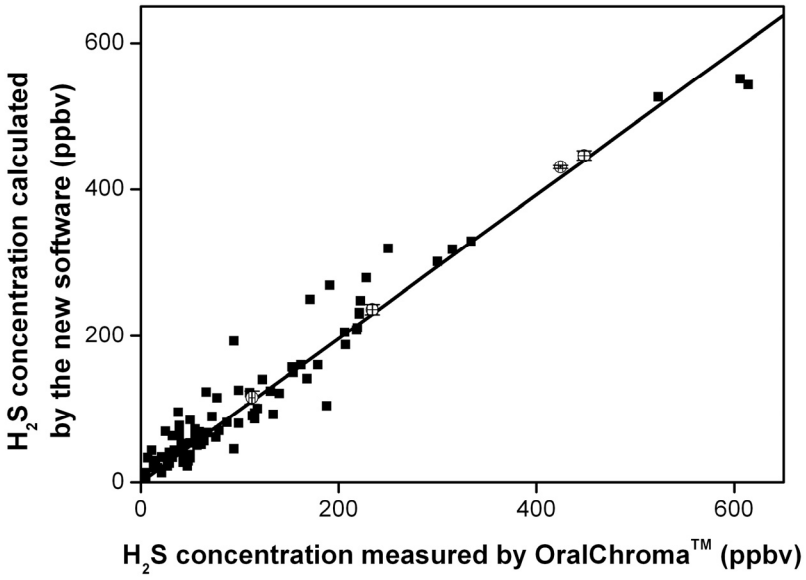

Figure 4. H_2S concentration measured by OralChroma™, calculated from the area under the curve of the fitted Gaussian function. Closed squares show data from breath samples and open circles denote data of measurements with H_2S in synthetic air. Error bars of open circles indicate standard deviation of three independent measurements.

H_2S concentration measured by the OralChroma™ and calculated from the area under the curve of the fitted Gaussian function agreed within 2% (R = 0.9691) for the corrected chromatograms, indicating the reliability of the evaluation software (Figure 4). In Figure 4 closed squares denote H_2S in breath samples and open circles indicate measurement of H_2S (112, 234, 424, 448 ppbv) in synthetic air; error bars show the standard deviation of three independent measurements.

3.3 Correlation coefficients

Of the 35 volunteers, 14 had oral cancer (patients). Table 2 shows the descriptive statistics of the measurements in patients and controls, as measured by Halimeter® and OralChroma™. All data showed normal distribution.

Table 2. Halimeter® data, concentration of H_2S and sum of the VSCs (sumVSC) measured by the OralChroma™ in breath of the subjects.

		original OralChroma™ evaluation (ppbv)		re-evaluated OralChroma™ chromatograms (ppbv)		Halimeter® (ppb)	
		mean ± SEM	range	mean ± SEM	range	mean ± SEM	range
healthy volunteers (n=21)	H_2S	63 ± 16	0–264	66 ± 16	2–285		
	CH_3SH	28 ± 8	0–373	16 ± 5	0–67		
	$(CH_3)_2S$	29 ± 14	0–285	16 ± 8	0–165		
	sumVSC	119 ± 43	0–906	98 ± 27	4–477	77 ± 9	24–199
patients (with oral cancer) (n=14)	H_2S	274 ± 92	1–996	285 ± 91	2–1025		
	CH_3SH	122 ± 42	0–430	138 ± 38	0–401		
	$(CH_3)_2S$	32 ± 9	0–108	28 ± 9	0–113		
	sumVSC	428 ± 132	4–1351	451 ± 131	3–1367	323 ± 81	52–949

SEM: standard error of mean, sumVSC = $[H_2S] + [CH_3SH] + [(CH_3)_2S]$

As expected, the mean values of VSC concentrations for the patient group were higher (2 to 9 times) than those of controls. SEM values decreased with the re-evaluation in the case of CH_3SH and $(CH_3)_2S$ and also sumVSC (sum of H_2S, CH_3SH and $(CH_3)_2S$).

Table 3. Pearson's correlation coefficient between Halimeter® and OralChroma™ results with original and re-evaluated data.

	Pearson's correlation coefficient between Halimeter® and OralChroma™			
	original OralChroma™ evaluation		re-evaluated OralChroma™ data	
	H_2S	sumVSC	H_2S	sumVSC
healthy volunteers (n=21)	0.788*	0.581*	0.819*	0.723*
patients (with oral cancer) (n=14)	0.570*	0.571*	0.634*	0.689*

(*) $p < 0.01$, sumVSC = $[H_2S] + [CH_3SH] + [(CH_3)_2S]$

Significant correlations were found between the Halimeter® values and the OralChroma™ levels for H_2S and sumVSC in case of both OralChroma™ chromatogram re-

evaluations (Table 3). Additionally, as shown in table 3, stronger correlations were found in case of re-evaluated data for both groups.

Table 4. Relative standard errors from three consecutive measurements.

	Halimeter®	original OralChroma™ evaluation		re-evaluated OralChroma™ data	
		H_2S	sumVSC	H_2S	sumVSC
healthy volunteers (n=21)	0.131	0.455	0.463	0.289	0.285
patients (with oral cancer) (n=14)	0.121	0.365	0.343	0.281	0.264

The relative standard errors of the subjects' three consecutive measurements (Table 4) clearly show that the Halimeter® provides better reproducibility than OralChroma™. However, data indicate that the reproducibility of OralChroma™ can be improved by the new software.

Table 5. Concentration of isoprene and acetaldehyde estimated from the OralChroma™ chromatograms.

	isoprene (ppbv)		acetaldehyde (ppbv)	
	mean ± SEM	range	mean ± SEM	range
healthy volunteers (n=21)	70 ± 10	7–164	810 ± 220	0–4120
patients (with oral cancer) (n=14)	36 ± 10	0–143	729 ± 490	0–7050

SEM: standard error of mean

Table 5 shows isoprene and acetaldehyde concentration of the volunteers' samples assessed from the re-evaluation of the OralChroma™ chromatograms. Measurable isoprene was found in the breath samples of all healthy volunteers, and in those of 13 patients. As for acetaldehyde, this compound could be measured in the samples of only 16 controls (of 21) and 11 patients (of 14). No significant difference between the patient and control group was found for isoprene and acetaldehyde concentrations. At the same time, isoprene concentrations were significantly higher than acetaldehyde concentrations in both groups.

4. Discussion

OralChroma™ is a commonly used device that allows differentiation between the three major VSCs in breath air. While its hardware meets the requirements of the field of halitosis research, its software has several limitations that make it less suitable for routine use [18]. It was Tangerman and Winkel who suggested that OralChroma™ chromatograms should always be inspected visually to correct the erroneous VSC peak assignment of the default software [18]. In the majority of the investigated breath samples, incorrect assignments occurred because of baseline disturbances, retention time shifts, peak tailing and cross sensitivity effects. Other studies [19, 23-25] also recommend calculating the concentrations of the VSCs by determining the peak heights of the chromatograms manually. Nevertheless, this process is time-consuming, cannot eliminate errors resulting from overlaps, and the uncertainty of analysis can be significant.

The results of the present study indicate that our newly developed software allows real-time and more precise re-evaluation of the chromatograms than the default software of OralChroma™. It fits the sum of six Gaussian functions to the chromatogram (Figures 3b) and calculates the concentrations of five components (hydrogen sulphide, isoprene, methyl mercaptan, dimethyl sulphide, and acetaldehyde) from the areas under the peaks.

In the case of optimal chromatograms (i.e. without retention time shift, no baseline disturbance, no peak tailing or overlapping), excellent correlation ($R = 0.9619$) was found between H_2S levels calculated by the original software and those calculated by the new software (Figure 4). This correlation is particularly high for samples of H_2S in synthetic air, indicating the correct assignment of the re-evaluation software.

Based on the literature we assumed that patients with oral cancer would have an increased VOC concentration in their exhaled air [9-11], and that they might also have a higher VSC concentration in their breath [8]. Data from Table 2 proved that concentrations of VSCs (for each compound, with both instruments) in the oral cavity were indeed higher in the samples of patients with oral cancer than in those of healthy controls. This difference enabled us to test our new software at both low and high concentrations.

Significant correlations (Table 3) were found between OralChroma™ and Halimeter® measurements for H_2S and sumVSC as it was previously reported [19]. However, the correlation showed further improvement after the re-evaluation of OralChroma™ data, indicating a better agreement between the devices. It was found that correlation coefficients

are higher in the case of healthy volunteers, which may stem from the wider range of VSC concentrations of patients (Table 2) including several extremely high VSC concentrations. The observed increase in correlation (Table 3), decrease in SEM (Table 2) and relative standard error of a subject's three consecutive measurements (Table 4) also support the new software's superiority over the default one.

Peak heights and peak areas were in very strong correlation ($R > 0.98$) for each studied component. It can be inferred, therefore, that peak height can be applied to recalculate concentrations, as it was also suggested by previous works [18, 19, 23-25]. However, it must be taken into consideration that errors may arise from the lack of proper de-convolution of the chromatogram due to the overlap of the peaks and background changes.

At the same time, it can be seen that the reproducibility of the chromatograms was still inadequate. It might be due to erroneous sampling or analysis, but other factors cannot be excluded either [26]. For instance, Springfield *et al.* suggested that minute-to-minute variability in oral VSC concentrations can be a true biological phenomenon [26]. If so, this effect must be taken into consideration as a potential confounder. The low relative standard error values of the Halimeter® (Table 4) possibly reflect the effect of continuous sampling and the shorter time intervals between two consecutive measurements.

The peak of isoprene overlaps with the peak of H_2S and the peak of acetaldehyde with the peak of $(CH_3)_2S$. Previous studies claimed that isoprene and acetaldehyde do not influence VSC analysis significantly due to their relatively small concentration in the oral cavity [17, 20]. Our results appear to indicate the contrary (see Table 5). Due to the significant cross correlation effects of isoprene and acetaldehyde, not only 4 peaks but 6 peaks must be taken into account for the correct evaluation of the chromatograms. Linear relationship was found for both isoprene and acetaldehyde between the concentration (in the range of 85-1385 ppbv for acetaldehyde and 46-555 ppbv for isoprene) and the area under the corresponding peak (Figures 1 and 2). Table 1 shows sensitivity of the semiconductor gas sensor of OralChroma™. It can be established that sensitivities for the five compounds are in the same order of magnitude, but the sensor has the highest sensitivity for isoprene. Sensitivity values obtained in the present study and that of Hanada *et al.* [20] are in good agreement.

Khalid *et al.* determined the VOC profiles of bacterial species associated with halitosis and suggested that the contribution of VOCs from oral anaerobes cannot be ignored and more research is required to identify the major source of breath compounds [27]. Our data indicate that the isoprene and acetaldehyde contents of breath significantly influenced the chromatograms; therefore they should not be disregarded. Although levels of isoprene and

acetaldehyde may or may not contribute to the overall malodour, they can interfere with the VSC profile analysis, and some VOC with long retention times (e.g. acetaldehyde) may delay the period at which the instrument can be re-used for the next sample.

In addition, the precise separation of the hydrogen sulphide, isoprene and methyl mercaptan peaks can be of particular importance in the case of discriminating patients with periodontal disease, as their methyl mercaptan/hydrogen sulphide ratio is elevated [28].

5. Conclusions

Re-evaluation of the chromatograms by de-convolution and calculation of the VSC concentrations considering the areas under the peaks significantly improve the accuracy of breath analysis by OralChroma^{TM}. Furthermore, the new software allows the determination of the concentrations of two VOCs (isoprene and acetaldehyde) in the oral cavity. It must be kept in mind, however, that the individual variability is high, which necessitates several consecutive measurements to reduce errors arising from fluctuations of VSC concentrations.

Acknowledgements

We are grateful for the financial support of the TÁMOP-4.2.2.A-11/1/KONV-2012-0035 project. The authors would like to express thanks to Professor John Greenman for his suggestions on the manuscript.

The research of Anna Szabó was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/2-11/1-2012-0001 ‘National Excellence Program’.

Disclosure

The authors report no conflicts of interest.

References

[1] Harvey-Woodworth C N 2013 Dimethylsulphidemia: the significance of dimethyl sulphide in extra-oral, blood borne halitosis *Brit. Dent. J.* **214** E20

[2] Scully C and Greenman J 2012 Halitology (breath odour: aetiopathogenesis and management) *Oral Dis.* **18** 333–45

[3] Sanz M, Roldán S and Herrera D 2001 Fundamentals of breath malodour *J. Contemp. Dent. Practice* **2** 1–17

[4] Liu X N, Shinada K, Chen X C, Zhang B X, Yaegaki K and Kawaguchi Y 2006 Oral malodor-related parameters in the Chinese general population *J. Clin. Periodontol.* **33** 31–6

[5] van den Broek A M W T, Feenstra L and de Baat C 2008 A review of the current literature on management of halitosis *Oral Dis.* **14** 30–9

[6] Porter S R and Scully C 2006 Oral malodour (halitosis) *Brit. Med. J.* **333** 632–5

[7] van den Broek A M W T, Feenstra L and de Baat C 2007 A review of the current literature on aetiology and measurement methods of halitosis *J. Dent.* **35** 627–35

[8] Scully C and Felix D H 2005 Oral Medicine – Update for the dental practitioner – Oral malodour *Brit. Dent. J.* **199** 498–500

[9] Schmutzhard J, Rieder J, Deibl M, Schwentner I M, Schmid S, Lirk P, Abraham I, Gunkel A R 2008 Pilot study: volatile organic compounds as a diagnostic marker for head and neck tumors *Head Neck* **30** 743–9

[10] Hakim M, Billan S, Tisch U, Peng G, Dvrokind I, Marom O, Abdah-Bortnyak R, Kuten A and Haick H 2011 Diagnosis of head-and-neck cancer from exhaled breath *Brit. J. Cancer* **104** 1649–55

[11] Gruber M, Tisch U, Jeries R, Amal H, Hakim M, Ronen O, Marshak T, Zimmerman D, Israel O, Amiga E, Doweck I and Haick H 2014 Analysis of exhaled breath for diagnosing head and neck squamous cell carcinoma: a feasibility study *Brit. J. Cancer* **111** 790–8

[12] Hughes F J and McNab R 2008 Oral malodour – a review *Arch. Oral Biol.* **53** Suppl. S1–S7

[13] Thorn R M S and Greenman J 2012 Microbial volatile compounds in health and disease conditions *J. Breath Res.* **6** 024001

[14] Wozniak W T 2005 The ADA guidelines on oral malodor products *Oral Dis.* **11** 7–9

[15] Rosenberg M, Kulkarni G V, Bosy A and McCulloch C A G 1991 Reproducibility and sensitivity of oral malodor measurements with a portable sulfide monitor *J. Dent. Res.* **70** 1436–40

[16] Furne J, Majerus G, Lenton P, Springfield J, Levitt D G and Levitt M D 2002 Comparison of volatile sulfur compound concentrations measured with a sulfide detector vs. gas chromatography *J. Dent. Res.* **81** 140–3

[17] Tangerman A and Winkel E G 2007 Intra- and extra-oral halitosis: finding of a new form of extra-oral blood-borne halitosis caused by dimethyl sulphide *J. Clin. Periodontol.* **34** 748–55

[18] Tangerman A and Winkel E G 2008 The portable gas chromatograph OralChroma^{TM}: a method of choice to detect oral and extra-oral halitosis *J. Breath Res.* **2** 017010

[19] Vandekerckhove B, van den Velde S, de Smit M, Dadamio J, Teughels W, van Tornout M and Quirynen M 2009 Clinical reliability of non-organoleptic oral malodour measurements *J. Clin. Periodont.* **36** 964–9

[20] Hanada M, Koda H, Onaga K, Tanaka K, Okabayashi T, Itoh T and Miyazaki H 2003 Portable oral malodor analyzer using highly sensitive In_2O_3 gas sensor combined with a simple gas chromatography system *Anal. Chim. Acta* **475** 27–35

[21] Murata T, Rahardjo A, Fujiyama Y, Yamaga T, Hanada M, Yaegaki K and Miyazaki H 2006 Development of a compact and simple gas chromatography for oral malodor measurement *J. Periodontol.* **77** 1142–7

[22] van den Velde S, Quirynen M, van Heeb P and van Steenberghe D 2007 Halitosis associated volatiles in breath of healthy subjects *J. Chromatogr. B* **853** 54–61

[23] Yaegaki K, Brunette D M, Tangerman A, Choe Y S, Winkel E G, Ito S, Kitano T, Ii H, Calenic B, Ishkitiev N and Imai T 2012 Standardization of clinical protocols in oral malodor research *J. Breath Res.* **6** 017101

[24] Laleman I, Dadamio J, Geest S D, Dekeyser C and Quirynen M 2014 Instrumental assessment of halitosis for the general dental practitioner *J. Breath Res.* **8** 017103

[25] Snel J, Burgering M, Smit B, Noordman W, Tangerman A, Winkel E G and Kleerebezem M 2011 Volatile sulphur compounds in morning breath of human volunteers *Arch. Oral Biol.* **56** 29–34

[26] Springfield J, Suarez F, Majerus G, Lenton P, Furne J and Levitt M 2001 Spontaneous fluctuations in the concentrations of oral sulfur containing gases *J. Dent. Res.* **80** 1441–4

[27] Khalid T Y, Saad S, Greenman J, de Lacy Costello B, Probert C S J and Ratcliffe N M 2013 Volatiles from oral anaerobes confounding breath biomarker discovery *J. Breath Res.* **7** 017114

[28] Yaegaki K and Sanada K 1992 Volatile sulfur compounds in mouth air from clinically healthy subjects and patients with periodontal disease *J. Periodontal. Res.* **27** 233–8