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The portable gas chromatograph OralChroma™: a method of choice to detect oral and extra-oral halitosis

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Abstract
It is now generally accepted that the volatile sulfur compounds (VSCs) hydrogen sulfide, methyl mercaptan and dimethyl sulfide are the main contributors to halitosis when of oropharyngeal origin. Gas chromatography using a specific sulfur detector is the most appropriate method to detect halitosis of different origin (intra-oral and extra-oral halitosis) and should be considered as the gold standard. However, a gas chromatograph is an expensive apparatus and needs trained personnel. The less specific Halimeter is the most used apparatus in halitosis research. In this study a newly developed portable gas chromatograph, the OralChroma™ (Abilit Corporation, Japan), was evaluated for use in the field of halitosis. The results show that the OralChroma is a very sensitive apparatus for measuring VSCs. Just like standard gas chromatography, it can perfectly differentiate between intra-oral and extra-oral blood-borne halitosis, while the Halimeter can only detect intra-oral halitosis. The hardware of the OralChroma meets all the needs for becoming the apparatus of choice in the field of halitosis. However, the software needs a major revision. Sometimes, the concentrations given for the different VSCs are completely incorrect due to a wrong assignment of the place of the VSCs in the chromatogram.

1. Introduction
Reports now agree that about 90% of halitosis originates within the oral cavity. Bacterial formation of the odorous volatile sulfur compounds (VSCs) hydrogen sulfide (H₂S) and methyl mercaptan (MM, CH₃SH) within the oral cavity, especially in the tongue coating, is the main cause of oral malodour [1–4]. An estimated 10% has non-oral causes, mostly due to the presence of dimethyl sulfide (DMS, CH₃SCH₃) [1, 5]. In a group of patients visiting our clinic for reasons of bad breath, 6 out of 58 patients had blood-borne extra-oral halitosis, all due to the presence of DMS [5]. In a group of patients visiting our clinic for reasons of bad breath, 6 out of 58 patients had blood-borne extra-oral halitosis, all due to the presence of DMS [5]. Examples of extra-oral halitosis not due to DMS, like the fish odour syndrome due to elevated trimethylamine, are very rare, except diabetes which is characterized by a sweet fruity smell of acetone and might therefore not be classified as ‘bad breath’ [1]. Diabetes can be easily detected organoleptically. Blood-borne extra-oral halitosis might be a manifestation of a serious disease. Therefore, it is of utmost importance to differentiate between intra-oral and extra-oral halitosis. This can be easily done by comparing mouth breath with nose breath. Among the host of patients with extra-oral halitosis, only a few have been investigated using analytical techniques to identify the volatile odorous compounds associated with the odour [1]. This is highly important, in order to diagnose the cause and to find a possible treatment.

Besides organoleptic scoring (OLS), gas chromatography is the most appropriate method to detect halitosis of different origin and should be considered as the ‘gold standard’ [1, 5, 6]. However, a gas chromatograph with a specific sulfur detector (FPD) to detect the VSCs is an expensive apparatus and needs trained personnel. Most breath clinics make use of the Halimeter [7] to measure the VSCs and to detect oral malodour. However, the Halimeter cannot differentiate
between the three VSCs. Moreover, it is more sensitive to H$_2$S than to MM and it is almost insensitive to DMS [7, 8]. The Halimeter is therefore not suited to detect extra-oral halitosis. Very recently, a portable gas chromatograph OralChroma$^\text{TM}$ (Abilit Corporation, Japan) has been marketed to detect the VSCs [9]. This apparatus is relatively cheap ($6000) and is very easy to use. It only needs electricity and the apparatus can be used everywhere. It does not need a carrier gas such as helium or nitrogen, like in standard gas chromatography. It uses the room air as a carrier gas for the chromatographic column. Recent publications show that this apparatus is also used in clinical practice [10, 11]. The aim of this study was to evaluate the use of the OralChroma in the field of halitosis.

2. Experimental details

Gaseous standards of the VSCs H$_2$S, MM and DMS were prepared in essentially the same way as described previously [12, 13, 15]. To evaluate the potential of the portable gas chromatograph OralChroma in the field of halitosis, standard gas chromatography, using a sulfur-specific flame photometric detector, was used for comparison [5, 12, 13]. For measuring the VSCs in breath, the latter needs a preconcentration step onto Tenax trap tubes. Patients were asked to breathe out 500 ml of mouth breath into a foil balloon. These foil balloons, provided with a three-way valve, were coated inside with polyethylene and metallized on the outside [15]. When using nose breath, a cap, connected to the sampling balloon, was placed over the nose. Patients were then instructed to breathe out 500 ml of nose breath via the cap into the balloon, while closing the mouth. 100 ml was then collected out of the balloon into a large sampling syringe and cryogenically preconcentrated onto a Tenax trap tube ($-196 \, ^{\circ}C$, liquid nitrogen). The trap tube was then inserted in the injection port of the gas chromatograph where the adsorbed sulfur compounds were thermally liberated (200 $^{\circ}C$) directly into the carrier gas stream and transferred to the gas chromatographic column (glass column 2 m $\times$ 4 mm i.d., packed with 20% SE-30 on Chromosorb P, 60–80 mesh, Varian Chrompack, Middelburg, The Netherlands, column temperature: 80 $^{\circ}C$). A Packard gas chromatograph, type 429, equipped with a sulfur-specific detector (flame photometric detector; Packard, Model 906) was used (Varian Chrompack, Middelburg, The Netherlands).

The instructions for the use of the Halimeter [7] and the OralChroma [9] have been described. For measuring the oral malodour with the OralChroma, the air inside the oral cavity was sampled directly with a 1 ml syringe after closing the mouth for 60 s. The amount of sample injected into the OralChroma was set at 0.5 ml. To make the method more sensitive, we use an amount of 1 ml, which can be done because a linear relationship was found between the peak height and the amount injected (0.5–2.0 ml, see figure 7). For sampling nose breath, one nostril was closed and the 1 ml syringe, without a plunger, was placed in the other nostril, with the tip of the syringe in the nostril cavity. The patient was then asked to breathe out through the nostril containing the syringe. After breathing for 5–10 s, the plunger was replaced and the collected 1 ml of nose breath was immediately injected into the OralChroma. The OralChroma column for the gas chromatographic analysis was made of a Teflon tube (5 mm i.d., 300 mm length) packed with 25% ODPN supported on Celite (GL Science). The column temperature was 35 $^{\circ}C$ and ambient air was used for the carrier gas. The three VSCs are detected by a highly sensitive semiconductor (In$_2$O$_3$) gas sensor. The OralChroma digitally displays the concentrations of H$_2$S, MM and DMS in ng/ml and ppb. Preferably, it is connected to a computer to also show the results in a chromatographic form. The OralChroma does not have a specific sulfur detector and might also detect other non-sulfur compounds. Possible interfering non-sulfur gases are isoprene, acetaldehyde, acetone and ethanol, which can be detected at high concentrations by the OralChroma. Although there is some overlapping of the peak of isoprene with that of H$_2$S and of acetaldehyde with that of MM, no influence of these compounds was seen in the VSCs analysis, because the sensitivities to these gases were much lower than those to VSCs [9].

3. Results and discussion

3.1. Detection limits and reproducibility

The detection limit, corresponding to a peak height equal to two times the background noise, was experimentally determined by us. When injecting 1 ml of a standard gas mixture into our standard gas chromatograph, a detection limit of 240 ppb for each VSC was obtained. This is not sensitive enough to measure halitosis, where much lower detection limits are required. A preconcentration step of 100 ml of breath onto Tenax trap tubes lowered the detection limit about 100 times, enough to measure the concentrations of the VSCs in halitosis. The OralChroma is about 60 times more sensitive. Injection of 1 ml of a standard gas mixture resulted in detection limits of 4 ppb for each VSC, by far sensitive enough to measure halitosis. Both standard gas chromatography and the OralChroma gave very accurate results, the latter only when using peak height measurements (see section 3.4). The reproducibility of the standard gas chromatography was studied by passing ten samples of 100 ml of air, each containing 50 ppb of MM and DMS, through ten different Tenax trap tubes. The gas chromatographic analysis of these trap tubes gave a recovery of 97 $\pm$ 5% (mean $\pm$ SD) for both volatiles [12]. Similar good recoveries above 90% were obtained for H$_2$S [13]. The recoveries for the OralChroma, when injecting ten samples of 1 ml of an artificial breath sample containing 250 ppb of each of the three VSCs, amounted to 96 $\pm$ 3% for H$_2$S, 97 $\pm$ 2% for MM and 98 $\pm$ 2% (mean $\pm$ SD) for DMS. The Halimeter measures the sum of the three VSCs H$_2$S, MM and DMS and cannot distinguish between the three VSCs. A Halimeter result above 160 ppb is considered as halitosis (www.halimeter.com/halcal.htm).
3.2. Detection of and differentiation between intra- and extra-oral halitosis

As recently shown by us [5], standard gas chromatography can perfectly detect intra-oral (oral malodour) and extra-oral blood-borne halitosis and can easily differentiate between these two forms of halitosis. Figure 1 shows some characteristic gas chromatographic spectra of mouth and nose breath of patients with intra-oral and extra-oral halitosis. All patients had complaints of halitosis and were referred to our clinic for diagnosis and treatment of halitosis. Patients with oral halitosis have only elevated concentrations of H₂S and MM in mouth breath. Patients with pure extra-oral blood-borne halitosis have elevated concentrations of DMS in mouth and nose breath. The OralChroma is also perfectly capable of detecting intra- as well as extra-oral halitosis and to differentiate between these two forms. Figure 2 shows OralChroma chromatograms of a patient with oral malodour before therapy (left) and after therapy (right). Before therapy elevated levels of the bad-smelling H₂S and MM, the cause of the malodour, are seen. DMS is almost absent. Again, H₂S and MM were absent in nose breath (not shown). After therapy (tongue scraping and gargling with Halita mouthwash [5, 14]), H₂S and MM have disappeared. Patients can see these chromatograms on the spot. It is highly stimulating for the patients to see the results of the therapy. Figure 3 shows the OralChroma chromatogram of the mouth breath of a patient with extra-oral blood-borne halitosis.

Figure 1. Gas chromatograms of the mouth breath (a) and nose breath (b) of a patient with oral halitosis, and of the mouth breath (c) and nose breath (d) of a patient with extra-oral halitosis. Peak assignment: 1 = H₂S, 2 = MM, 3 = DMS (adopted from [3]).

Figure 2. OralChroma chromatograms of the mouth breath of a patient with oral malodour before therapy (left) and after therapy (right).
with pure extra-oral blood-borne halitosis. This was the same patient as shown in figures 1(c) and (d), but measured on a different occasion. H$_2$S and MM are absent whereas DMS, the cause of the extra-oral halitosis, is elevated. The nose breath of this patient (not shown) gave a similar result as the mouth breath. Note that the background peaks ‘a’, which are always present in breath and which belong to non-sulfur volatiles [9], are higher than those in figure 2, due to the use of a more sensitive output scale. The concentration of DMS in this patient amounted to 72 ppb. The threshold of objectionability of DMS amounts to 24 ppb as experimentally determined by us [5]. These concentrations of DMS show why the Halimeter is unable to detect extra-oral halitosis. The Halimeter measures the sum of the three VSCs and cannot differentiate between these VSCs. Moreover, the Halimeter underestimates DMS by some 70% [8], resulting in ppb levels of DMS far below the detection limits (estimated: 50–80 ppb) of the Halimeter. These Halimeter detection limits were estimated as follows. Nose samples containing no VSCs, having an organoleptic score of 0 and OralChroma values of 0, always showed Halimeter readings from the nose between 50 and 80 ppb. These nose samples closely mimic oral samples, containing no VSCs. Moreover, control patients after therapy, with an organoleptic score of 0 and OralChroma values of 0, showed Halimeter readings from the mouth between 50 and 80 ppb (own observations). Human standard readings (readings of individuals with a clean mouth and not having any halitosis problem) should be between 80 and 110 ppb (www.halimeter.com/halcal.htm), also pointing to a detection limit around 80 ppb for the Halimeter.

3.3. Major advantages and disadvantages of the use of the OralChroma

The major advantages of the OralChroma are as follows. (1) It has a very low detection limit of 4 ppb, allowing detection of halitosis by injection of 1 ml of breath, without the need for a preconcentration step. (2) It can easily detect oral and extra-oral halitosis and can differentiate between these two forms of halitosis. (3) The manufacturer advises injection of 0.5 ml of mouth air. It is possible to enhance sensitivity by injection of larger amounts, up to 2 ml (see section 3.4). (4) It is extremely easy to use. The operator is only required to draw a mouth sample and inject it into the inlet of the OralChroma. (5) After using the OralChroma for a couple of years, we observed only a limited decrease in sensitivity per year (10–20%). We therefore advise to calibrate the OralChroma once a year with standards of known concentrations of the VSCs. (6) Its relatively low cost (about $6000) as compared with a standard gas chromatograph.

The major disadvantages concern the software used in the OralChroma. The hardware of the OralChroma meets all the needs for becoming the apparatus of choice in the field of halitosis. However, the software needs a major revision. The major points are outlined below. (1) Sometimes, the concentrations given by the OralChroma for the different VSCs are completely incorrect due to a wrong assignment of the place of the VSCs in the chromatogram. This is clearly shown in figure 4. On two occasions injection of the same standard mixture of H$_2$S, MM and DMS gave totally different results. On the left panel of figure 4, the assignment of H$_2$S, MM and DMS (dotted lines) is correct, resulting in reliable concentrations. However, on the right panel the assignment is
incorrect, especially for MM and DMS, resulting in a lower concentration of H$_2$S and in zero concentrations of MM and DMS. Probably, the little negative peak at 0.5 min, just before H$_2$S, deregulates the software and might be responsible for the incorrect assignment. This incorrect assignment would not have been noticed when the OralChroma was used as a stand-alone apparatus without connection to a computer to show the chromatographic results. It is therefore important to use the OralChroma in connection with a computer. (2) The outcome in ppb as given by the OralChroma is generally somewhat too low, also dependent on the age of the OralChroma. This can be easily overcome by calibrating the OralChroma with gaseous standards of H$_2$S, MM and DMS. (3) Sometimes, large broad peaks appear in the OralChroma chromatograms (see figure 5). These peaks originate from the rubber barrel seal of the 1 ml plastic syringe used and come from a previous injection. Probably, these peaks do not belong to a sulfur compound because these peaks were not seen in the chromatograms of the standard gas chromatograph with a specific sulfur detector, when using the same syringe. The use of all-plastic syringes is highly advocated to avoid these broad peaks. Again, the software does not know how to handle these broad peaks. On the left panel, an OralChroma chromatogram of the breath of a normal person is shown. This breath did not contain measurable concentrations of H$_2$S, MM or DMS. Nevertheless, the OralChroma falsely assigns the broad peak to DMS. On the right panel, an OralChroma chromatogram of the breath of a patient with extra-oral halitosis is shown. Here, the elevated DMS is seen as a shoulder on the large broad peak. The OralChroma again falsely assigns the broad peak to DMS, resulting in much too high values for DMS. In both spectra, large background peaks are seen due to the use of the most sensitive output scale. Again, the incorrect assignment of DMS would not have been noticed when the OralChroma was used as a stand-alone apparatus without the chromatographic results.

### 3.4. Peak height measurements

Because the software of the OralChroma is sometimes inadequate to calculate the VSC concentrations by peak area measurements, we now use peak heights (expressed in mV) measured by hand to calculate the concentrations. Perfect linear correlations were found (figure 6) between the concentrations of H$_2$S, MM or DMS injected and the measured peak heights in the concentration range studied (0–1000 ppb).

The manufacturer advises injection of 0.5 ml of mouth air. It is possible to enhance sensitivity by injection of larger amounts, up to 2 ml. Figure 7 shows that there is a linear correlation between the peak heights and the concentrations of the injected VSCs.
Figure 7. Plot of the volume (ml) injected against the peak height (mV).

relationship between the volume of gas injected (0.5–2.0 ml) and the measured peak height.

4. Conclusions

The OralChroma™ can, within the limitations described above for this apparatus, measure intra-oral as well as extra-oral halitosis and can discriminate between these two forms of halitosis. After some major adjustments in the software, the OralChroma™ might become the apparatus of choice in the field of halitosis.

References