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Assessment of a combined gas chromatography mass spectrometer sensor (GC-MSS) system for detecting biologically relevant volatile compounds (VCs).

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Abstract

There have been a number of studies in which metal oxide sensors (MOS) have replaced conventional analytical detectors in gas chromatography systems. However, despite the use of these instruments in a range of applications including breath research the sensor responses (i.e. resistance changes w.r.t. concentration of VCs) remain largely unreported. This paper addresses that issue by comparing the response of a metal oxide sensor directly with a mass spectrometer (MS), whereby both detectors are interfaced to the same GC column using an s-swafer.

It was demonstrated that the sensitivity of an in-house fabricated ZnO/ SnO₂ thick film MOS was superior to a modern MS for the detection of a wide range of volatile compounds (VCs) of different functionalities and masses. Better techniques for detection and quantification of these VCs is valuable, as many of these compounds are commonly reported throughout the scientific literature. This is also the first published report of a combined GC-MS sensor system. These 2 different detector technologies when combined, should enhance discriminatory abilities to aid disease diagnoses using volatiles from e.g. breath, and bodily fluids.

29 chemical standards have been tested using solid phase micro-extraction; 25 of these compounds are found on human breath. In all but 2 instances the sensor exhibited the same or superior limit of detection compared to the MS.

12 stool samples from healthy participants were analysed, the sensor detected, on average 1.6 peaks more per sample than the MS. Similarly analysing the headspace of *E. coli* broth cultures the sensor detected 6.9 more peaks per sample versus the MS. This greater sensitivity is primarily a function of the superior limits of detection of the metal oxide sensor. This shows that systems based on the combination of chromatography systems with solid state sensors shows promise for a range of applications.

Introduction

There is increasing interest in the design and fabrication of compact volatile organic compound (VOC) sensor systems for disease diagnosis. Electronic nose (E-nose) systems based on: conducting polymers (1), cantilevers (2,3), ceramic sensors (4), colorimetric arrays (5), and GC-sensor systems (6-10) for volatile detection with potential applications in disease diagnoses have, for instance, been reported. GC-sensor systems, using metal oxide semiconductor (MOS) sensors, have shown promise for detecting gastrointestinal and urinary tract diseases (11-14). MOS sensors are low cost and easy to manufacture while maintaining high sensitivity and stability.

MOS gas sensor technology has been extensively investigated in the past few decades in research ranging from food odour sensing (15-18) to explosives detection (19) and waste management odour analysis (20). Much of this research utilises 'E-nose' technology which uses an array of sensors combined with pattern recognition systems (15,16,18).

The idea of combining MOS sensors with a gas chromatography (GC) column to separate mixtures of compounds and record the analyte responses was first investigated in 1962 (8). This makes compound identification easier once retention times (RT) (for a particular system) are known, and allows for analysis of more complex mixtures of gases, when compared to a sensor array alone. The development of MOS sensors has seen intensive research over several decades, whilst the combined MOS sensor-GC systems has received relatively little attention. Povarov and Lopatnikov (2016) estimate only 20 papers on the subject were published between 1960 and 2010 (9). Current research has been focused on the development of miniaturized portable devices, frequently using micro-machined GC columns for separation prior to MOS sensor detection. Systems of this type have been used for a range of applications including detection of lung cancer associated volatiles (10), ethylene and low mwt. hydrocarbons (21), aromatic volatiles (22), hydrogen fluoride vapours (23), benzene, toluene and xylene (24) and hydrogen, methane and carbon monoxide (25). Such devices could provide quick, easy, on-site analysis potentially in the hands of unskilled operators. Systematic studies have been carried out on quantifying the relationship between sensor response and factors such as type of volatile compound, metal oxide additives and surface structure (26,27). Numerous

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3 studies comparing detector types have been reported, for example Mildner-Szkudlarz
4 and Jelen (2008) who compared a solid phase micro extraction (SPME)-fast GC-FID
5 with an MOS e-nose array, and SPME direct to MS (28); despite this there are no
6 cases we could find in which the MOS sensor is integrated into the same system as a
7 common detector type (e.g. MS or FID). A small number of publications have also
8 reported the limit of detection (LOD) of MOS sensor-GC systems, (29) for five alcohols,
9 acetaldehyde, acetone and ethyl acetate, which reports detection limits of several ppb
10 and (30) for hydrogen fluoride, with detection limits of 800 ppb.
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14 This investigation will assess the sensing abilities of a MOS sensor comprising a
15 binary mixture of ZnO and SnO₂ when used as a secondary detector for a Clarus 500
16 GC-MS (the combination of this MOS sensor with GC has previously been
17 demonstrated as highly sensitive and effective (11,14,31)). This combined GC-MS
18 MOS sensor system is referred to as gas chromatography mass spectrometer sensor
19 (GC-MSS) system. A broad and diverse range of volatile organic compounds (VOCs)
20 were measured, and their limits of detection determined using standards; volatiles
21 from the headspace of stool and bacteria in broth have also been analysed.
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24 **Experimental**

25 **Sensor preparation and sensor chamber**

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27 The details of the sensor substrate design and manufacture and the coating procedure
28 are described in detail by Vaughan *et al.*, 2013 (32). In short, a 3mm alumina substrate
29 was screen printed with gold interdigitated electrodes (4 pairs of interpenetrating bars,
30 electrode gap 100µm) on one side and with a platinum heater track on the reverse.
31 This was wire bonded to a TO39 transistor can. The sensor substrate was coated with
32 a metal oxide paste comprising 50% zinc oxide nanopowder <100nm (Sigma Aldrich),
33 and 50% tin oxide (IV) nanopowder <100nm (Sigma Aldrich) (by weight) using the
34 doctor blade technique detailed in (32-34).
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38 An aluminium chamber (volume 5 cm³) was used to house the sensor with a PTFE
39 mount to hold the sensor in place (see figure 1). The GC column (see GCMS and
40 sensor set up section for details) enters the sensor chamber directly opposite the
41 sensor and is positioned circa 5mm from the sensor surface (Figure 1). A purge gas
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(100% synthetic air 287478-L-C from BOC Ltd) with a constant flow rate of 180 mL/min was used for all the experiments described herein. The sensor operating temperature was 450°C; with a column flow of 3.1 mL/min at 40°C dropping to 1.6 mL/min at 240°C.

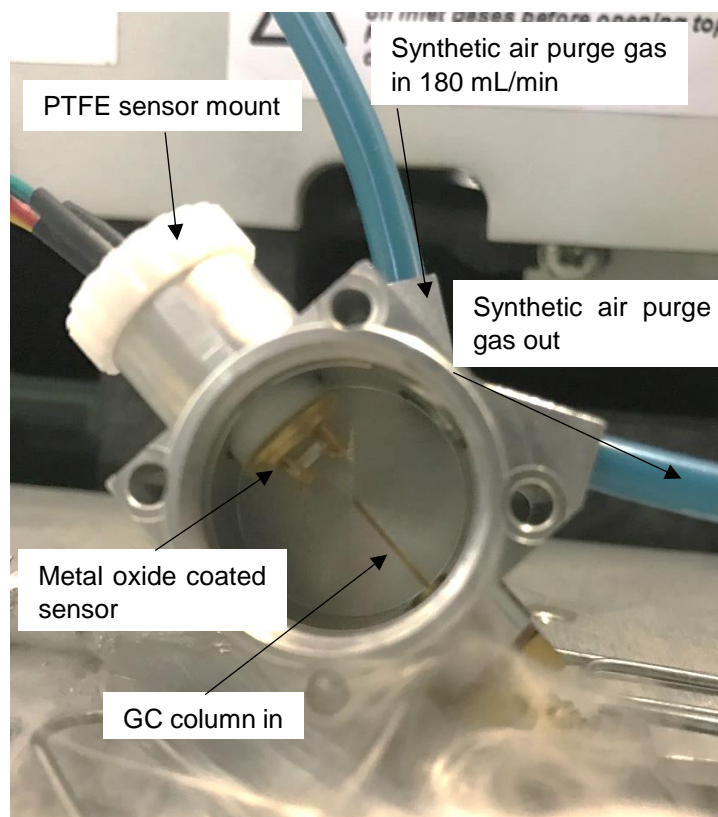


Figure 1 the aluminium sensor chamber with bespoke sensor mounted on PTFE interfaced with the GC column; purge gas (synthetic air) flows through the chamber at 180 mL/min.

GC-MS and sensor setup

A Clarus 500 GC-MS (Perkin Elmer) with single quadrupole detector was used for all samples with the GC output split by S-Swafer technology (Perkin-Elmer) 50% going to the MS and 50% to the MOS sensor. The GC method was as follows: 40°C initial oven temperature, held for 2 minutes followed by a temperature ramp of 10 °C/minute for 20 minutes up to 240°C and hold at 240°C for 8 minutes. A Zebron ZB-624 column, 60 m length, I.D: 0.52 mm, film thickness: 1.40 µm, helium carrier gas at 22.8psi, 31.5cm/s, with a column flow of 3.1 mL/min at 40°C dropping to 1.6 mL/min at 240°C was used throughout this work.

Standard solutions

The 29 organic compound standards used in this investigation were divided into four solutions (detailed in the supplementary materials along with solubility for each compound) to avoid co-elution and significant in-solution reactions. Stock solutions in deionised water were made for each of these groups at a concentration of 10 g/L, 1 g/L, and 0.1 g/L according to the solubility of the compounds in the solution. From these original stock solutions serial 10 fold dilutions were made (10mL into 100mL volumetric flask) and each one analysed within 8 hours of preparation. All chemicals used for this phase of testing are shown in supplementary table S1 with grade and supplier.

For sampling, 3 ml of a solution was removed to a headspace vial (PTFE/ silicone septa 10mL Supelco, Sigma Aldrich), and the headspace was sampled with a polydimethylsiloxane/carboxen PDMS-CAR solid phase micro-extraction (SPME) portable field sampling fibre (Supelco, Sigma Aldrich Company Ltd.) for 2 minutes at ambient temp (21-25°C) without stirring. The fibre was then inserted into the GC-MS port at 220°C constant temperature and left for the first three minutes of each run to fully desorb the analyte.

Each mixture of organic compounds was run from most dilute to most concentrated to avoid carryover from the SPME fibre and/or column between samples. Where necessary blank runs were also used to further mitigate this issue. The above method was repeated three times for each of the four groups of organic compounds. Each set of data was gathered within 48 hours of the solution being made to limit the interactions between the chemicals while in solution. This phase of experimentation took place over a 4-month period with the response of both the mass spectral analyser and sensor being tested with a control standard solution (1% w/v solution of ethanol, methanol, propanol, butanol, and acetone) to ensure consistent performance of both detectors. In order to mimic the process of analysing an unknown gas mixture LOD optimisation techniques such as searching the chromatogram for individual product masses and/or running a single ion monitoring (SIM) MS method was not undertaken. Similarly, analysis of the MOS sensor trace as a function of Δ resistance/ Δ time ($\Delta R/\Delta T$) was not used; as this allows the data to be viewed as a function of the slope. This is a display function only and does not enhance the detection capabilities of the

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3 sensor. However, $\Delta R/\Delta T$ allows changes in the gradient of a response recovery to
4 become apparent; these changes in gradient can be small responses that would
5 otherwise go unnoticed.
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8 9 **Stool samples**

10 Stool samples from healthy volunteers of mixed ethnic origin and gender were
11 collected this also included vegetarian and meat eaters (research ethics committee
12 reference 14/NE/0029). 3g were aliquoted into 20mL headspace vials and stored in a
13 freezer at -20°C. For sampling the vials were defrosted in a water bath set to 60°C for
14 30 minutes the SPME fibre was then added and for a further 30 minutes while the vial
15 was maintained at 60°C in the water bath.
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22 **Bacterial culture headspace analysis**

23 2 Colonies of *E. coli* were picked from an overnight culture on an agar plate (Oxoid
24 CM0003) and used to inoculate 5mL of nutrient broth (Oxoid CM0067) in a sterile glass
25 universal. The broths were incubated overnight at 37°C. Absorbance was measured
26 and colony forming units / mL (CFU/mL) was estimated using the Agilent online
27 calculator (Agilent Genomics). Overnight cultures were diluted as appropriate to give
28 between 10⁵ CFU/mL and 10⁶ CFU/mL in four glass headspace vials with 5mL nutrient
29 broth (Oxoid CM0067). One was frozen at -20°C immediately with the others
30 undergoing 2, 4 and 24 hours further incubation respectively before freezing. 1mL of
31 each sample was removed with needle and syringe prior to freezing for final
32 absorbance measurements.
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43 Prior to analysis vials were defrosted in a water bath set to 60°C for 30 minutes before
44 the SPME fibre (as used for both the stool and standards) was added for a further 30
45 minutes, as for the stool samples the vial was maintained at 60°C for the duration of
46 the sampling. 10 of the bacteria samples were selected at random for analysis on the
47 GC-MSS.
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53 **Analysis**

54 The same signal threshold of 3 times the noise was used for both the GCMS
55 chromatograms and the resistance trace from the sensor. The NIST library (version
56 2.2, 2014) was used on the chromatograms to identify the peak, due to the unreliable
57 nature of siloxane and terpene identification these were recorded by family (siloxane
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3 or terpene respectively). All responses were searched manually and in order to be
4 classed as NIST matched response, a minimum threshold of 800 match and reverse
5 match was used, in cases where the threshold was not achieved the peak was
6 characterised as unknown; similarly cases in which a peak appeared on the sensor
7 trace that was not visible on the total ion chromatogram the peak noted as unidentified
8 mass spectrometer (unidentified MS), similarly responses on the sensor system that
9 could not be NIST matched by the mass spectral analyser were noted as unidentified
10 sensor system (unidentified SS). For the standard solutions, the compound was
11 determined as undetectable when the 3 times the noise peak threshold was no longer
12 met.
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21 **Results and discussion**

22 **Standard solutions**

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27 The 450°C sensor operating temperature was derived experimentally. At 450°C the
28 water to ethanol response ratio was 4 times greater than when operated at 350°C
29 which gave a response ratio of close to 1.
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33 Of the 29 chemical standards investigated, 25 have been found on the breath of
34 healthy humans, and three others have been found as volatiles emitted from the
35 human body from other sources such as faeces, urine, and saliva (35). Figure 2 shows
36 the full comparison of mode average LODs for all 29 VOCs, Supplementary figure SF1
37 shows the data for each repeat. 17 compounds showed better LOD with the MOS
38 sensor including significantly improved sensitivity for butanol, 2-butanone and indole.
39 The mass spectrometer only showed increased sensitivity for two compounds,
40 methanol and butanal. The mass spectrometer and the MOS sensor exhibited the
41 same sensitivity for the remaining 9 compounds. The MOS sensor comprised two
42 metal oxides, tin oxide and zinc oxide both of which on their own are well known to be
43 capable of sensing a wide range of VOCs. We have shown there are advantages in
44 using a mixed system for enhanced detection of a range of VOCs (33,34).
45 Supplementary table 2 (S2) shows indicative headspace concentration values based
46 on Henry's law constants; to give some indication as to the range of concentrations
47 being detected above the solutions. Both the mass spectral analyser and MOS sensor
48 show a wide dynamic range from low part per billion to part per million. Calculating an
49 accurate headspace concentration is problematic as the headspace is pre-
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3 concentrated onto an SPME fibre thus the quantity absorbed will be subject to the
4 selectivity of the fibre. Moreover, in a complex solution such as those analysed it is
5 possible that Henry's law will not be fully applicable due to inter compound
6 interactions, although the solutions are extremely dilute. It should also be noted that
7 since we are using solutions with a factor of 10 difference, the actual LOD would likely
8 be at a value between the 2 solutions.
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14 While the sensor showed high sensitivity to a wide range of analytes it unexpectedly
15 elicited zero responses to siloxanes. Siloxanes were observed in many of the GC-MS
16 chromatograms in this work (a common GC contaminant often coming from the
17 injection septum, sample vial septum, SPME fibre, column bleed or indeed the sample
18 itself, as they are widely used in biomedical and cosmetic applications (36)). Siloxanes
19 are relatively large, stable molecules yet they are volatile. While they are readily
20 ionised in the mass spectral analyser we have been unable to find any instances in
21 the scientific literature in which they are oxidised/ catalytically broken down, by metal
22 oxides. Any reaction that may occur at the sensor surface is too slight to produce a
23 sensor response. For analysis of biologically derived VOCs this is an advantage, as
24 unwanted contaminant responses would reduce the ability of the sensor to recover
25 and may obscure analyte responses. Additionally, if applying these resistance traces
26 to algorithms in order to differentiate them into clinically relevant groups, siloxanes will
27 interject false information into the equation. The lack of sensitivity of the MOS sensor
28 to column bleed compounds such as the siloxanes means that at higher retention
29 times the sensor has a more stable baseline and thus the potential to detect lower
30 levels of "target" compounds in this region e.g. 3-methyl indole.
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45 A mean RT delay of 6 seconds across the chromatogram was observed between the
46 mass spectrometer and MOS sensor with a standard deviation ± 2 seconds; these RT
47 differences are constant across the duration of the sample time. Using butanol as an
48 example the highest concentration (10g/L) RT for the MS was 14.10 and the lowest
49 concentration (0.0001g/L) was 14.06. The sensor recorded RTs of 14.14 and 14.15
50 respectively. These RT drifts are typical across the whole range of tested standards
51 for both the MS and sensor.
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58 After responding to a VOC, the sensor tends to return to its previous oxygen surface
59 state equilibrium and the resistance can take time to recover to the previous baseline
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3 value. During this recovery, which is enhanced by the purge gas, another compound
4 can cause a sensor response which means that the size of the response is often very
5 different between the MS and MOS. The helium carrier gas of the GC-MS can have a
6 detrimental effect on sensor response over time as the oxygen species are depleted;
7 this effect is countered by using air as a purge gas which allows the oxygen to be
8 continuously replenished. In cases of very large responses the recovery can be
9 sufficiently rapid that the next compound appears only as a change of gradient and
10 not a peak response; in these cases, viewing the resistance as $\Delta R/\Delta T$ can help the
11 resolution of responses from closely eluting compounds. In instances of the true co-
12 elution of 2 or more compounds this function would have limited utility as there is no
13 change in the slope visible. A mass spectral analyser might be able to separate
14 compounds based on product ions within the total ion chromatogram although this is
15 not always the case. In contrast to the sensor, the mass spectral analyser will produce
16 a response as long as the product ions are being detected and will cease when the
17 ions are no longer detected thus the recovery time is typically shorter meaning the MS
18 has superior peak resolution.
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32 An important point to reiterate is that the mass spectrometer was not run optimally in
33 terms of maximum sensitivity (e.g. not SIM mode) but it was run using the same
34 parameters as would be applied for the analysis of an unknown sample for example.
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38 The metal oxide sensor could also be run using parameters which increased
39 sensitivity (column positioned closer to sensor) but which resulted in a loss of peak
40 resolution (recovery time increased). Therefore, the sensor was optimised by altering
41 the column position and carrier gas, purge gas flow rates to produce maximum
42 sensitivity but appropriate separation/resolution for the compounds of interest. The
43 directionality of the carrier gas stream exiting the column and its close proximity to the
44 sensor ensures efficient transit of the analytes to the surface and minimal dilution by
45 the purge gas. Thus the purge gas flow rate was selected based upon providing a
46 balance between maximal recovery between responses without sacrificing sensitivity.
47 Low purge gas flow increased the level of noise which was disadvantageous when
48 analysing complex samples. The chamber flushing rate was also selected so that a
49 high concentration of analyte presented at the exhaust and around the seals of the
50 sensor chamber gave no sensor response. This was an important consideration to
51 ensure the sensor baseline was not altered by environmental volatiles.
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3 We produced the chamber originally from aluminium as it was easy to manufacture
4 various iterations of the chamber design. We attempted to minimise all
5 plastics/materials within the chamber which might outgas additional compounds or
6 adsorb compounds. On testing the current chamber with the standards, we did not get
7 baseline issues with the sensor indicating that outgassing compounds were
8 minimised. The directional transport of analytes to the sensor surface and efficient
9 removal via the purge gas mean that we didn't have issues with carryover or
10 contamination. If issues had been identified then we may have considered other
11 sensor chamber materials, or inert surface treatment of the current chamber. In terms
12 of chamber size, it is possible that different designs or a smaller size may further
13 optimise performance. We intend to continue to develop the current system which has
14 gone through a number of iterations to reach its current state of development.
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25 Over the 4-month testing period a slight change of $\leq 5\%$ in the sensitivity per month
26 was observed. During the test period, slight increases in sensitivity were observed for
27 some compounds (butanol, propanol, acetone) and others slightly decreased (ethanol,
28 methanol). Overall the sensor system gave good stability in terms of response to
29 standard analytes at a known and relevant concentration. Baseline stability was also
30 high with no significant drop during the testing period.
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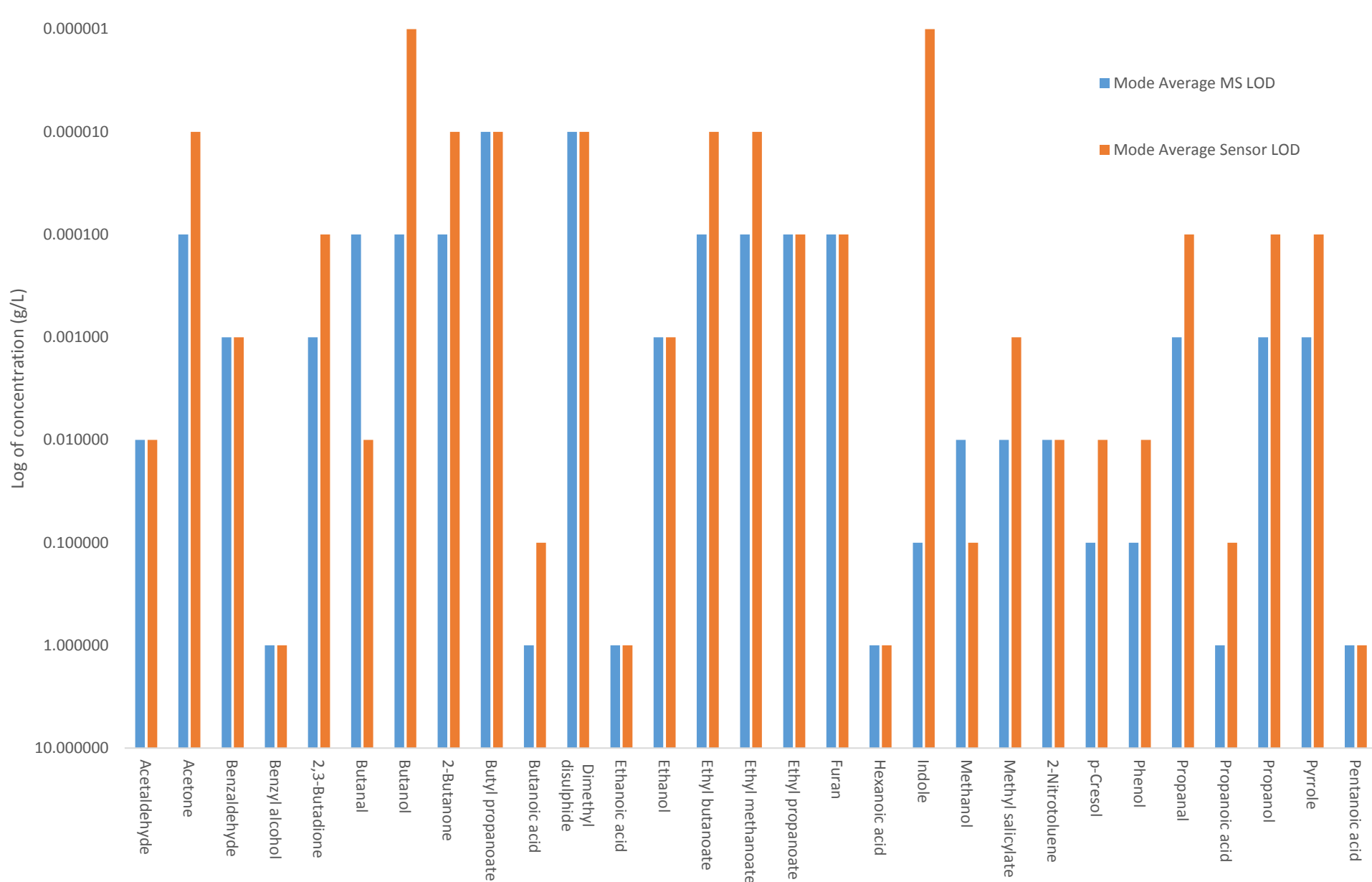


Figure 2 the limit of detection concentration of standard chemicals in deionised water, comparing a single quadrupole mass spectrometer with the mixed metal oxide (SnO₂ and ZnO) sensor, plotted on a log scale using the mode average.

Stool Samples

84 compounds were detected across the 12 stool samples those which have a corresponding chemical standard from the standard solution experiments are shown in table 1 along with the retention times. The remaining NIST matched compounds are shown in supplementary table S3. On at least one occasion across the sample set each compound had a sensor response with a matching RT. Supplementary table S4 shows which compounds were detected in which sample and if they were detected by the mass spectral analyser, the MOS sensor or both. Very few of the terpenes seen could accurately be NIST matched thus they were denoted simply as terpenes however they were still included as NIST matched responses provided they met the thresholds described in the method. Siloxanes are known common contaminants and so were not included as NIST matched compounds.

CAS-number	Compound	MS RT (mins)	Sensor RT (mins)	Chemical standard RT MS (mins)	Chemical standard RT Sensor (mins)
75-07-0	Acetaldehyde	8.93	8.98	8.94	9.08
64-17-5	Ethanol	10.13	10.20	10.19	10.33
67-64-1	Acetone	10.71	10.82	10.74	10.88
71-23-8	Propanol	12.00	12.07	12.03	12.16
123-72-8	Butanal	12.48	12.53	12.51	12.61
431-03-8	2,3-Butadione	12.65	12.71	12.44	12.68
78-93-3	2-Butanone	12.66	12.70	12.68	12.77
64-19-7	Ethanoic acid	13.35	13.33	13.37	13.42
71-36-3	Butanol	14.00	14.05	14.07	14.15
105-37-3	Ethyl propanoate	14.42	14.45	14.43	14.54
79-09-4	Propanoic acid	15.09	15.12	15.12	15.20
624-92-0	Dimethyl disulphide	15.56	15.59	15.56	15.73
105-54-4	Ethyl butanoate	16.09	16.14	16.10	16.19
107-92-6	Butanoic acid	16.73	16.80	16.72	16.82
590-01-2	Butyl propanoate	18.01	18.20	18.05	18.12
109-52-4	Pentanoic acid	18.35	18.40	18.40	18.47
108-95-2	Phenol	20.84	20.88	20.98	20.99
106-44-5	p-Cresol	22.22	22.28	22.21	22.39
120-72-9	Indole	26.92	27.04	26.90	27.17

Table 1 the list of the compounds detected from the headspace of stool from healthy participants that have been certified with chemical standards from standard solution experiments.

Table 2 shows the total number of responses detected by both detectors across all the samples, as previously mentioned the MOS sensor provides a resistance trace and so

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3 the identification of compounds causing resistance responses is based on the RTs
4 from the mass spectral analyser. There was a mean difference between the standards
5 and the sample of 2.5 (± 3 seconds) for the mass spectral analyser and 5 (± 2 seconds).
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7 Table 1 shows a mean difference between the mass spectral analyser and the sensor
8 of 3.6 seconds (± 2.4 seconds). Over the course of our experiments the sensor has
9 shown a consistently longer RT versus the mass spectral analyser. This could be in
10 part because the sensor takes more time to reach the peak value from which we derive
11 the RT. Moreover, between repeats of the same sample including standards there will
12 be slight variation in the RT. Although we have made efforts to minimise differences
13 there may also be slight variation in the column lengths between the swafer and MS,
14 sensor detectors. There may also be differences in temperature profiles across these
15 transfer lines as one exits the GC oven. These combined factors could explain both
16 the difference in RT and the consistency of the difference between sensor and MS.
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20 Table 2 shows a breakdown of the total number of responses detected on both the
21 mass spectral analyser and MOS sensor from all 12 stool samples; Supplementary
22 table S4 shows the raw data for each of the 12 stool samples the total number of times
23 each compound was detected can be shown in supplementary figure SF2; in total 25
24 more NIST matched responses were detected on the mass spectral analyser. In most
25 cases this is due to the resistance responses being lost in the recovery of the previous
26 peak. When the resistance trace is viewed as a function of $\Delta R/\Delta T$ (an example of this
27 is shown in figure 3) these responses are clearly visible on all but 4 occasions. Across
28 the 12 samples the mass spectral analyser was unable to identify via the NIST library
29 41 responses at the defined threshold; these unidentified MS peaks were detected on
30 the MOS sensor. However, the MOS sensor detected 43 additional responses across
31 the 12 samples that did not appear 3 times above the noise on the chromatogram or
32 had no peak at the matching RT. The mass spectral analyser detected 27 siloxanes
33 across the 12 samples no responses were detected on the MOS sensor at the
34 matching RT. The majority of the unidentified responses from the MOS sensor traces
35 appear during the last 10 minutes of the sample run. Of the 85 detected compounds
36 1 (nonane) did not have a corresponding resistance response. Due to the high number
37 of carbons, stability, and lack of oxygen species compounds like nonane (and many
38 other alkanes) are difficult to catalytically break down. The superior LOD of the sensor
39 will account for many of the additional peaks; low concentrations in the samples will
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mean that the mass spectral analyser response will drop to a level indistinguishable from the noise, or disappear altogether.

Several papers have utilized both GC-FID and GC-MOS systems, Mildner-Szkudlarz and Jelen (2008) reported similar capabilities for the detection of olive oil impurities (28). García-González and Aparicio (2010) conclude that their MOS sensor array is very useful in the analysis of food aroma but had not yet reached the same performance as that of an FID (7). FID is also far less expensive than mass spectrometry with a cost more in line with that of an E-nose or single MOS sensor system. That said the MOS sensor employed in this work would be considerably less expensive than an FID. However, an FID system is incapable of providing the qualitative data provided by the mass spectral analyser. It is this qualitative data that can provide detail about the biochemistry and possible biomarkers when utilised for clinical applications. Therefore, we wanted to benchmark our sensor system against a gold standard MS technique to investigate the range of compounds detected particularly when dealing with unknown samples.

Table 2 also shows the mean number of responses per sample for both the mass spectral analyser and the MOS sensor. In general, the performance of both detectors is very similar though the MOS sensor exhibits twice as many unidentified responses than the MS; this is a benefit of the enhanced sensitivity of the MOS sensor and this coupled with the lack of response to siloxanes may have benefits in the correct classification of samples into groups (e.g. disease from non-disease).

	MS complete set	MOS Sensor complete set *	MS mean per chromatogram	MOS Sensor mean per chromatogram *
NIST matched responses	488	463	44.5 (\pm 7.9)	42.5 (\pm 8.3)
Unidentified responses	41	84	3.4 (\pm 1.6)	7.0 (\pm 2)
Siloxanes	27	0	2.3 (\pm 1.0)	0.0
Total responses**	529	547	47.9 (\pm 9.5)	49.5 (\pm 10.3)

Table 2 the total number of responses from the mass spectral analyser and MOS sensor detected from 12 stool samples from healthy participants; and the mean responses per sample for both the mass spectral analyser and MOS sensor. *Sensor response with RT corresponding with a NIST matched compound. **Total responses not including siloxanes.

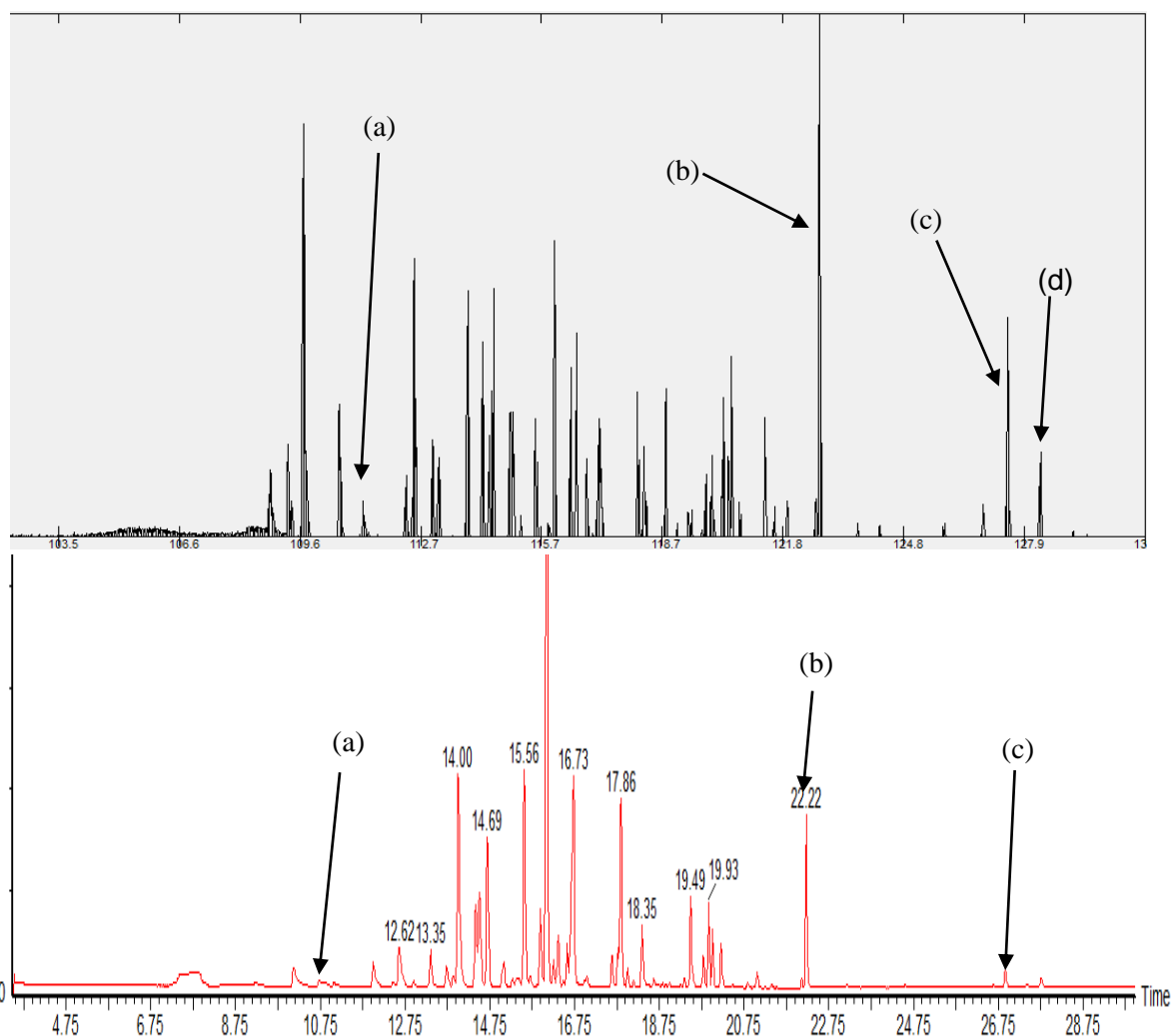


Figure 3 Chromatogram of a typical stool sample $\Delta R/\Delta T$ negative only MOS sensor trace (top) with time in minutes and corresponding chromatogram (bottom), time in minutes. (a) Dimethyl sulphide MOS Sensor RT 10.78 MS RT 10.86 minutes. (b) p-Cresol, MOS Sensor RT 22.23 MS RT 22.22 minutes. (c) Indole MOS Sensor RT 26.95 MS RT 26.92 minutes. (d) MOS Sensor peak RT 27.81 minutes unidentified by MS.

Bacterial headspace analysis

29 compounds were NIST matched using the defined analytical parameters as set out in the experimental analysis section, this is relatively few compared to the 84 NIST matched compounds of the stool samples. Table 3 shows those that had been certified with a corresponding chemical standard in the standard solution experiments, the remaining compounds are shown in supplementary table S5. All 10 of the compounds shown in table 3 have been mentioned previously in the literature; as shown in table 4. 16 compounds detected in our experiments were not previously reported. However, our method was not developed with a specific focus but more as a general method to test the abilities of the system. There was no attempt in this study to replicate the

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3 methods of the cited work in table 4. Despite this, table 3 shows that we are able
4 to detect a number of key compounds, this leads us to believe that our system is not only
5 able to detect a wide range of compounds, in terms of mass and functional groups,
6 but also that there are multiple applications including bacterial VC analysis.
7 Dichloromethane was present in 4 of the samples analysed, this is likely to be a
8 contaminant however is included as a compound as it was clearly identifiable and
9 interestingly produced no response from the MOS sensor. This is fortuitous as in many
10 instances dichloromethane is used as a solvent and can produce responses capable
11 of overloading mass spectrum detectors which often requires the use of solvent
12 delays.
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22 The difference in the MS and MOS sensor responses is shown clearly in figure 4,
23 which shows a GC-MS chromatogram and MOS sensor resistance trace (in $\Delta R/\Delta T$
24 negative only) overlaid on each other. Figure 4 illustrates just how well the responses
25 match up and generally larger responses on the chromatogram will result in larger
26 responses from the MOS sensor; though this is not always the case, for example
27 Indole (figure 4). In this instance, the mass spectral analyser standards had a mean
28 difference of 3 seconds ± 2 versus the bacterial samples. The sensor also had a mean
29 of 3 seconds ± 2 seconds difference between the standards and bacterial headspace.
30 The mean difference between the mass spectral analyser and sensor for the bacterial
31 headspace samples was 4.5 seconds ± 2 ; these values are consistent with those
32 calculated from the stool samples.
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CAS-number	Compound	MS RT (mins)	MOS Sensor RT (mins)	Standard solution MS RT (mins)	Standard solution sensor RT (mins)
64-17-5	Ethanol	10.20	10.30	10.19	10.33
67-64-1	Acetone	10.80	10.93	10.74	10.88
71-23-8	Propanol	12.09	12.17	12.03	12.16
431-03-8	2,3-Butadione	12.54	12.60	12.44	12.68
78-93-3	2-Butanone	12.66	12.71	12.68	12.77
71-36-3	Butanol	14.09	14.17	14.07	14.15
624-92-0	Dimethyl disulphide	15.64	15.70	15.56	15.73
100-52-7	Benzaldehyde	20.08	20.10	20.03	20.18
106-44-5	p-Cresol	22.27	22.35	22.21	22.39
120-72-9	Indole	26.98	27.07	26.90	27.17

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Table 3 the list of compounds detected on the GC-MSS certified with chemical standards from standard solution experiments detected from the headspace of 10 E.coli broth samples.

Plot comparing 1st differential of sensor trace against corresponding chromatogram for strain CC354 T=24.

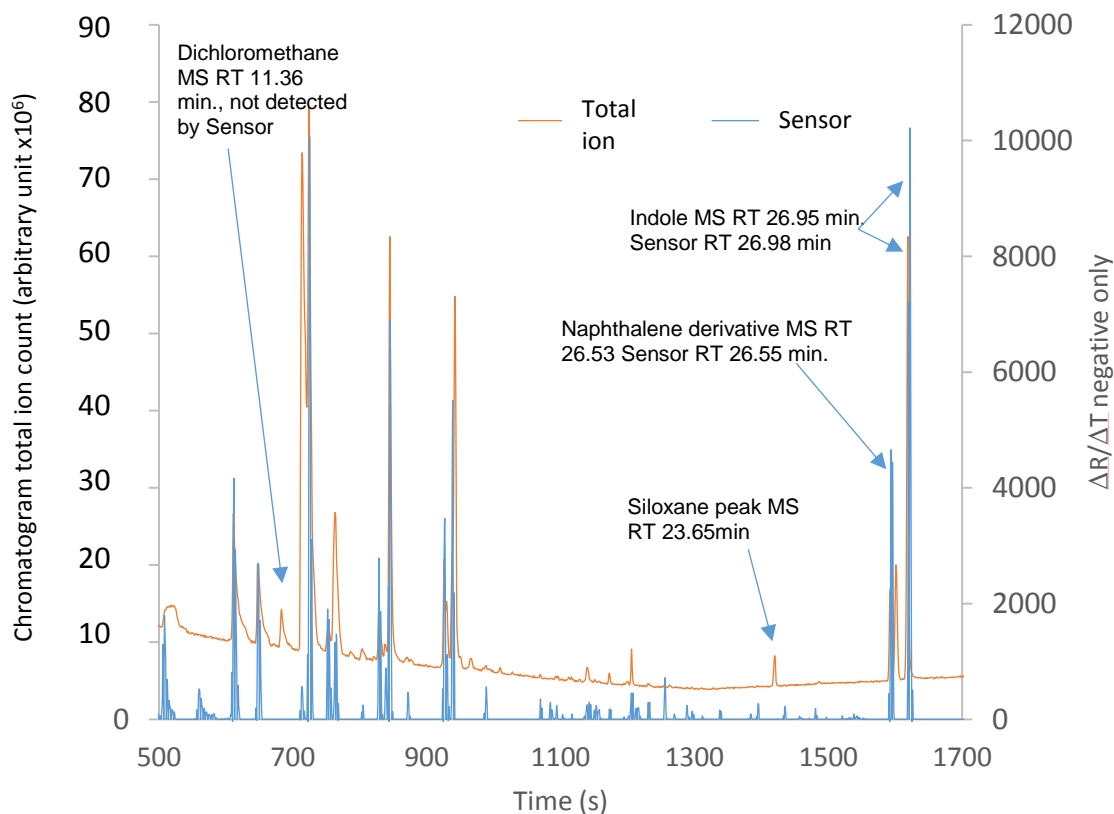


Figure 4 An example of an overlaid chromatogram from the GC-MS with a MOS sensor resistance trace in the $\Delta R/\Delta T$ negative only view.

	MS complete set	MOS Sensor complete set *	MS mean per chromatogram	MOS Sensor mean per chromatogram *
NIST matched responses	156	143	15.6 (± 3.0)	14.3 (± 3.3)
Unidentified	11	93	1.1 (± 0.9)	9.3 (± 2.4)
Siloxanes	57	0	5.7 (± 1.7)	0.0
Total responses**	167	236	16.7 (± 3.9)	23.6 (± 5.7)

Table 5 comparison of the total responses found from 10 E. coli samples grown in nutrient broth and the mean response per sample. *Sensor response with RT corresponding with a NIST matched compound. **Total responses not including siloxanes

Table 5 shows that as with the stool samples the MOS sensor is able to detect more responses versus the mass spectral analyser. In this case the sensor detected 69 additional peaks when compared to the MS. This constitutes a much larger % increase (41%) when comparing the two detectors than observed for the stool samples. This highlights the enhanced LOD of the sensor for a range of compounds. Supplementary table S6 shows the breakdown of all responses detected per sample on both the mass

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3 spectral analyser and the MOS sensor; supplementary figure SF3 shows how many
4 times each compound was detected across the 10 samples on both detectors. In many
5 cases the unknown responses from the MOS sensor trace do have a very small
6 corresponding MS peak that was not above the noise threshold. Moreover, the
7 bacterial samples produced lower relative abundances on the chromatogram across
8 the whole chromatogram when compared to those from the stool samples. For
9 instance, the mean peak area from the MS for dimethyl disulphide from the stool
10 samples was 85×10^6 compared to just $1. \times 10^6$ for the bacterial headspace studies. This
11 is suggestive that the concentrations in the bacterial headspace are significantly lower
12 than those in the stool. 6 of the compounds in table 3 that were present in both stool
13 and the bacterial headspace had a mean 80% smaller chromatographic peak area in
14 bacteria versus stool.
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25 As with the stool sample analysis, we see that the sensor detects additional responses
26 consistently across each sample with a mean of 23.6 (± 5.7) from the MOS sensor
27 versus 16.7 (± 3.9) from the mass spectral analyser. The lower relative abundances
28 from the bacterial samples are indicative of lower concentrations of compounds;
29 despite this the MOS sensor shows clear superiority over the mass spectral analyser
30 in terms of responses detected. This enhanced sensitivity should allow for greater
31 discriminatory abilities by detecting more points of differences between samples.
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Reference	Indole	Dimethyl disulphide	Ethanol	Propanol	2,3-Butadione	2-Butanone	Acetone	Methanol	Butanol	Benzaldehyde
Tait, E. <i>et al</i> (2014) (37)	*									
Siripatrawan, U (2008) (38)	*	*	*							
Arnold, J.W (1998) (39)	*		*	*						
Hettinga, K.A (2008) (40)					*	*				
Hossain, K. Bojko, B. Pawliszyn, J (2013) (41)		*								
Storer, M. <i>et al</i> (2011) (42)	*	*					*	*		
Allardyce, R. <i>et al</i> (2006) (43)	*	*	*	*						
Thorn, R. Reynolds, D. Greenman, J (2011) (44)	*		*						*	
Allardyce, R. Hill, A. Murdoch, D (2006) (45)		*	*				*			
Concina, I. <i>et al</i> (2009) (46)			*							
Bunge, M. <i>et al</i> (2008) (47)	*		*			*	*	*	*	
Maddula, S. <i>et al</i> (2009) (48)			*							
Zscheppank, C. <i>et al</i> (2014) (49)	*					*			*	
Umber, B. <i>et al</i> (2013) (50)		*	*	*						
Schulz, S. Dickschat, J (2007) (51)	*									
Chippendale, T. Spanel, P. Smith, D (2011) (52)		*	*	*			*	*		
Aathithan, S. <i>et al</i> (2001) (53)			*							
Zhu, J. <i>et al</i> (2010) (54)	*		*							
Boots, A. <i>et al</i> (2014) (55)	*			*	*					*
Yu, K. <i>et al</i> (2000) (56)	*	*								*

Table 4 Compounds NIST matched in the headspace of E. coli from existing literature which were also NIST matched in our analysis.

Conclusions

To summarise, we have developed a gas chromatography mass spectrometry sensor (GC-MSS) system which combines a metal oxide sensor with a standard quadrupole detector. This system has shown the ability to detect a broad range of compounds at trace concentrations with a variety of different functional groups and masses. Moreover, in many cases the MOS sensor has shown superior sensitivity over the mass spectral analyser, particularly when applied to challenging matrices such as the headspace of bacterial culture. Overall the sensor detected over 100 peaks that were not seen by the mass spectral analyser or were sub-threshold. This is borne out by the standard solutions work whereby the sensor system was found to give superior LOD to a range of standard compounds vs. the mass spectral analyser.

In testing to standard solutions, stool, and headspace samples we have demonstrated the potential for multiple applications of this combined GCMS and sensor system. Although the response of MOS sensors is very fast with volatiles, recovery, especially with high concentrations of VOCs can be slow, which may obscure very small peaks which subsequently elute from the column. Although this may also happen with the MS, it does possess faster response and recovery than the sensor generally and thus better peak resolution. In previous work the same sensor has been operated continuously for 6 months while assessing hundreds of stool samples for *C. difficile* infection and has retained its sensitivity when tested to certified gas standards (31).

Currently our system is set up for SPME pre-concentration however we plan to adapt this system to use automated thermal desorption (ATD). Despite the additional costs and time associated with ATD the increased sensitivity and efficient sample storage (particularly of breath) make it a very desirable technique. We believe that systems of this type which incorporate chromatographic separation with MOS or other sensitive sensor technology have great potential utility in analysing a range of samples including those that are medically derived. For stool and urine headspace this may be possible via direct headspace analysis, but for breath and other matrices then appropriate sample collection and pre-concentration may be required either in system or offline. The purpose of this work was to assess the range and relative detection limits of the sensor as a detector when compared to a standard mass analyser. The sensor showed equivalent or better performance to a broad range of chemical compounds

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3 whilst exhibiting selectivity against siloxanes and other common chromatographic
4 contaminants such as chlorinated solvents. Therefore, the development of sensor
5 systems combined with chromatographic separation can be seen to have potential
6 utility in developing instruments with applications in the medical field. However, in
7 order for this to occur work has to continue in developing sensors with high sensitivity,
8 selectivity (if disease markers are known) and stability (baseline and response) as well
9 as appropriate algorithms for deconvolution of “chromatographic” data and
10 subsequent pattern classification.
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References

- (1) Pavlou AK, Magan N, Jones JM, Brown J, Klatser P, Turner AP. Detection of Mycobacterium tuberculosis (TB) in vitro and in situ using an electronic nose in combination with a neural network system. *Biosensors and Bioelectronics* 2004;20(3):538-544.
- (2) An artificial nose based on microcantilever array sensors. *Journal of Physics: Conference Series: IOP Publishing*; 2007.
- (3) Microelectronics meets biomedicine: integrated MOSFET-embedded cantilever sensor and diagnostics system. *Physics of Semiconductor Devices, 2007. IWPSD 2007. International Workshop on: IEEE*; 2007.
- (4) Rao BB. Zinc oxide ceramic semi-conductor gas sensor for ethanol vapour. *Mater Chem Phys* 2000;64(1):62-65.
- (5) Shepherd RL, Yerazunis WS, Lau KT, Diamond D. Low-cost surface-mount LED gas sensor. *IEEE Sensors Journal* 2006;6(4):861-866.
- (6) Hou N, Jin Z, Sun B, Sun Y, Shen W, Guo Z, et al. New Strategy for Rapid Detection of the Simulants of Persistent Organic Pollutants Using Gas Sensor Based on 3-D Porous Single-Crystalline ZnO Nanosheets. *IEEE Sensors Journal* 2015;15(7):3668-3674.
- (7) García-González DL, Aparicio R. Coupling MOS sensors and gas chromatography to interpret the sensor responses to complex food aroma: Application to virgin olive oil. *Food Chem* 2010;120(2):572-579.
- (8) Siyama T, Kato A. A new detector for gaseous components using semiconductor thin film. *Anal Chem* 1962;34(11):1502-1503.
- (9) Povarov V, Lopatnikov A. A simple multisensor detector based on tin dioxide in capillary gas chromatography. *Journal of Analytical Chemistry* 2016;71(9):901-906.
- (10) Tzeng T, Kuo C, Wang S, Huang P, Huang Y, Hsieh W, et al. A Portable Micro Gas Chromatography System for Lung Cancer Associated Volatile Organic Compound Detection. *IEEE J Solid State Circuits* 2016;51(1):259-272.
- (11) Shepherd S, McGuire ND, de Lacy Costello B, Ewen R, Jayasena D, Vaughan K, et al. The use of a gas chromatograph coupled to a metal oxide sensor for rapid assessment of stool samples from irritable bowel syndrome and inflammatory bowel disease patients. *Journal of breath research* 2014;8(2):026001.
- (12) Ahmed I, Greenwood R, Costello B, Ratcliffe N, Probert C. Investigation of faecal volatile organic metabolites as novel diagnostic biomarkers in inflammatory bowel disease. *Aliment Pharmacol Ther* 2016;43(5):596-611.

1
2
3 (13) Khalid T, Aggio R, White P, Costello BDL, Persad R, Al-Kateb H, et al. Urinary
4 volatile organic compounds for the detection of prostate cancer. PloS one
5 2015;10(11):e0143283.
6

7
8 (14) Aggio RB, de Lacy Costello B, White P, Khalid T, Ratcliffe NM, Persad R, et al.
9 The use of a gas chromatography-sensor system combined with advanced statistical
10 methods, towards the diagnosis of urological malignancies. Journal of breath research
11 2016;10(1):017106.
12

13
14 (15) Feng T, Zhuang H, Ye R, Jin Z, Xu X, Xie Z. Analysis of volatile compounds of
15 Mesona Blumes gum/rice extrudates via GC-MS and electronic nose. Sensors
16 Actuators B: Chem 2011;160(1):964-973.
17

18
19 (16) Park MH, Jeong MK, Yeo J, Son H, Lim C, Hong EJ, et al. Application of solid
20 phase-microextraction (SPME) and electronic nose techniques to differentiate
21 volatiles of sesame oils prepared with diverse roasting conditions. J Food Sci
22 2011;76(1):C80-C88.
23

24
25 (17) Messina V, Sancho A, Walsöe de Reca N. Monitoring odour of heated extra-virgin
26 olive oils from Arbequina and Manzanilla cultivars using an electronic nose. European
27 Journal of Lipid Science and Technology 2015;117(8):1295-1300.
28

29
30 (18) Tahri K, Tiebe C, Bougrini M, Saidi T, El Hassani, N El Alami, El Bari N, et al.
31 Characterization and discrimination of saffron by multisensory systems, SPME-GC-
32 MS and UV-Vis spectrophotometry. Analytical Methods 2015;7(24):10328-10338.
33

34
35 (19) Brudzewski K, Osowski S, Pawlowski W. Metal oxide sensor arrays for detection
36 of explosives at sub-parts-per million concentration levels by the differential electronic
37 nose. Sensors Actuators B: Chem 2012;161(1):528-533.
38

39
40 (20) Giungato P, de Gennaro G, Barbieri P, Briguglio SC, Amodio M, de Gennaro L,
41 et al. Improving recognition of odors in a waste management plant by using electronic
42 noses with different technologies, gas chromatography-mass
43 spectrometry/olfactometry and dynamic olfactometry. J Clean Prod 2016.
44

45
46 (21) Sklorz A, Janßen S, Lang W. Application of a miniaturised packed gas
47 chromatography column and a SnO₂ gas detector for analysis of low molecular weight
48 hydrocarbons with focus on ethylene detection. Sensors Actuators B: Chem
49 2013;180:43-49.
50

51
52 (22) Zampolli S, Elmi I, Mancarella F, Betti P, Dalcanale E, Cardinali G, et al. Real-
53 time monitoring of sub-ppb concentrations of aromatic volatiles with a MEMS-enabled
54 miniaturized gas-chromatograph. Sensors Actuators B: Chem 2009;141(1):322-328.
55

56
57 (23) Sanchez J, Berger F, Daniau W, Blind P, Fromm M, Nadal M. Development of a
58 gas detection micro-device for hydrogen fluoride vapours. Sensors Actuators B: Chem
59 2006;113(2):1017-1024.
60

1
2
3 (24) Sanchez J, Berger F, Fromm M, Nadal M. A selective gas detection micro-device
4 for monitoring the volatile organic compounds pollution. *Sensors Actuators B: Chem*
5 2006;119(1):227-233.
6

7
8 (25) Ohta K, Terai H, Kimura I, Tanaka K. Simultaneous determination of hydrogen,
9 methane, and carbon monoxide in water by gas chromatography with a semiconductor
10 detector. *Anal Chem* 1999;71(14):2697-2699.
11

12
13 (26) Kohl D, Heinert L, Bock J, Hofmann T, Schieberle P. Systematic studies on
14 responses of metal-oxide sensor surfaces to straight chain alkanes, alcohols,
15 aldehydes, ketones, acids and esters using the SOMMSA approach. *Sensors*
16 *Actuators B: Chem* 2000;70(1):43-50.
17

18
19 (27) Nishizaka Y, Yokoyama C, Inumaru K, Okuhara T, Misono M. Effect of additives
20 and particle size on the sensitivity of SnO₂-based sensors for offensive-odor
21 components. *Sensors Actuators B: Chem* 1993;13(1):355-357.
22

23
24 (28) Mildner-Szkudlarz S, Jeleń HH. The potential of different techniques for volatile
25 compounds analysis coupled with PCA for the detection of the adulteration of olive oil
26 with hazelnut oil. *Food Chem* 2008;110(3):751-761.
27

28
29 (29) Meng H, Yang W, Yan X, Zhang Y, Feng L, Guan Y. A highly sensitive and fast
30 responsive semiconductor metal oxide detector based on In₂O₃ nanoparticle film
31 for portable gas chromatograph. *Sensors Actuators B: Chem* 2015;216:511-517.
32

33
34 (30) Sanchez J, Berger F, Fromm M, Nadal M. Use of a chromatographic column to
35 improve the selectivity of the SnO₂ gas sensors: first approach towards a miniaturised
36 device and selective with hydrogen fluoride vapours. *Sensors Actuators B: Chem*
37 2005;106(2):823-831.
38

39
40 (31) McGuire ND, Ewen R, de Lacy Costello B, Garner CE, Probert C, Vaughan K, et
41 al. Towards point of care testing for *C. difficile* infection by volatile profiling, using the
42 combination of a short multi-capillary gas chromatography column with metal oxide
43 sensor detection. *Measurement Science and Technology* 2014;25(6):065108.
44

45
46 (32) Vaughan K. An investigation into the fabrication of a new mini gas sensor for
47 medical diagnostic applications 2013.
48

49
50 (33) de Lacy Costello B, Ewen R, Jones P, Ratcliffe N, Wat R. A study of the catalytic
51 and vapour-sensing properties of zinc oxide and tin dioxide in relation to 1-butanol and
52 dimethyl sulphide. *Sensors Actuators B: Chem* 1999;61(1):199-207.
53

54
55 (34) de Lacy Costello B, Ewen RJ, Ratcliffe NM, Sivanand P. Thick film organic vapour
56 sensors based on binary mixtures of metal oxides. *Sensors Actuators B: Chem*
57 2003;92(1):159-166.
58

59
60 (35) de Lacy Costello B, Amann A, Al-Kateb H, Flynn C, Filipiak W, Khalid T, et al. A
review of the volatiles from the healthy human body. *Journal of breath research*
2014;8(1):014001.

1
2
3 (36) Sun P, Ayre C. Characterization of organic contaminants outgasses from
4 materials used in semiconductor fabs/ processing. 2003.
5

6
7 (37) Tait E, Perry JD, Stanforth SP, Dean JR. Identification of volatile organic
8 compounds produced by bacteria using HS-SPME-GC-MS. *J Chromatogr Sci* 2014
9 Apr;52(4):363-373.
10

11 (38) Siripatrawan U. Rapid differentiation between *E. coli* and *Salmonella typhimurium*
12 using metal oxide sensors integrated with pattern recognition. *Sensors Actuators B:*
13 *Chem* 2008;133(2):414-419.
14

15 (39) Arnold JW, Senter SD. Use of digital aroma technology and SPME GC-MS to
16 compare volatile compounds produced by bacteria isolated from processed poultry. *J*
17 *Sci Food Agric* 1998;78(3):343-348.
18

19 (40) Hettinga K, Van Valenberg H, Lam T, Van Hooijdonk A. Detection of mastitis
20 pathogens by analysis of volatile bacterial metabolites. *J Dairy Sci* 2008;91(10):3834-
21 3839.
22

23 (41) Hossain SZ, Bojko B, Pawliszyn J. Automated SPME–GC–MS monitoring of
24 headspace metabolomic responses of *E. coli* to biologically active components
25 extracted by the coating. *Anal Chim Acta* 2013;776:41-49.
26

27 (42) Storer MK, Hibbard-Melles K, Davis B, Scotter J. Detection of volatile compounds
28 produced by microbial growth in urine by selected ion flow tube mass spectrometry
29 (SIFT-MS). *J Microbiol Methods* 2011;87(1):111-113.
30

31 (43) Allardyce RA, Langford VS, Hill AL, Murdoch DR. Detection of volatile metabolites
32 produced by bacterial growth in blood culture media by selected ion flow tube mass
33 spectrometry (SIFT-MS). *J Microbiol Methods* 2006;65(2):361-365.
34

35 (44) Thorn RMS, Reynolds DM, Greenman J. Multivariate analysis of bacterial volatile
36 compound profiles for discrimination between selected species and strains in vitro. *J*
37 *Microbiol Methods* 2011;84(2):258-264.
38

39 (45) Allardyce RA, Hill AL, Murdoch DR. The rapid evaluation of bacterial growth and
40 antibiotic susceptibility in blood cultures by selected ion flow tube mass spectrometry.
41 *Diagn Microbiol Infect Dis* 2006;55(4):255-261.
42

43 (46) Concina I, Falasconi M, Gobbi E, Bianchi F, Musci M, Mattarozzi M, et al. Early
44 detection of microbial contamination in processed tomatoes by electronic nose. *Food*
45 *Control* 2009;20(10):873-880.
46

47 (47) Bunge M, Araghipour N, Mikoviny T, Dunkl J, Schnitzhofer R, Hansel A, et al. On-
48 line monitoring of microbial volatile metabolites by proton transfer reaction-mass
49 spectrometry. *Appl Environ Microbiol* 2008 Apr;74(7):2179-2186.
50

51 (48) Maddula S, Blank LM, Schmid A, Baumbach JI. Detection of volatile metabolites
52 of *Escherichia coli* by multi capillary column coupled ion mobility spectrometry.
53 *Analytical and bioanalytical chemistry* 2009;394(3):791-800.
54
55
56
57
58
59
60

1
2
3 (49) Zscheppank C, Wiegand H, Lenzen C, Wingender J, Telgheder U. Investigation
4 of volatile metabolites during growth of *Escherichia coli* and *Pseudomonas aeruginosa*
5 by needle trap-GC-MS. *Analytical and bioanalytical chemistry* 2014;406(26):6617-
6 6628.
7

8
9 (50) Umber BJ, Shin H, Meinardi S, Leu S, Zaldivar F, Cooper DM, et al. Gas
10 signatures from *Escherichia coli* and *Escherichia coli*-inoculated human whole blood.
11 *Clinical and translational medicine* 2013;2(1):13.
12

13
14 (51) Schulz S, Dickschat JS. Bacterial volatiles: the smell of small organisms. *Nat Prod*
15 *Rep* 2007;24(4):814-842.
16

17
18 (52) Chippendale TW, Španěl P, Smith D. Time-resolved selected ion flow tube mass
19 spectrometric quantification of the volatile compounds generated by *E. coli* JM109
20 cultured in two different media. *Rapid Communications in Mass Spectrometry*
21 2011;25(15):2163-2172.
22

23
24 (53) Aathithan S, Plant JC, Chaudry AN, French GL. Diagnosis of bacteriuria by
25 detection of volatile organic compounds in urine using an automated headspace
26 analyzer with multiple conducting polymer sensors. *J Clin Microbiol* 2001
27 Jul;39(7):2590-2593.
28

29
30 (54) Zhu J, Bean HD, Kuo YM, Hill JE. Fast detection of volatile organic compounds
31 from bacterial cultures by secondary electrospray ionization-mass spectrometry. *J Clin*
32 *Microbiol* 2010 Dec;48(12):4426-4431.
33

34
35 (55) Boots A, Smolinska A, van Berkel J, Fijten R, Stobberingh E, Boumans M, et al.
36 Identification of microorganisms based on headspace analysis of volatile organic
37 compounds by gas chromatography–mass spectrometry. *Journal of breath research*
38 2014;8(2):027106.
39

40
41 (56) Yu K, Hamilton-Kemp TR, Archbold DD, Collins RW, Newman MC. Volatile
42 compounds from *Escherichia coli* O157: H7 and their absorption by strawberry fruit. *J*
43 *Agric Food Chem* 2000;48(2):413-417.
44
45
46
47
48
49
50
51
52
53
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57
58
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