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

Oliver Gould¹, Tom Wieczorek¹, Ben de Lacy Costello¹, Raj Persad² and Norman Ratcliffe¹.

¹ Institute of Biosensor Technology, University of the West of England, Coldharbour Lane, Frenchay, Bristol, BS16 1QY

² Bristol Urological Institute, North Bristol NHS Trust, Southmead Hospital, Southmead Road, Westbury-on-Trym, Bristol, BS10 5NB
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-swarer.

It was demonstrated that the sensitivity of an in-house fabricated ZnO/ SnO$_2$ 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
studies comparing detector types have been reported, for example Mildner-Szkudlarz and Jelen (2008) who compared a solid phase micro extraction (SPME)-fast GC-FID with an MOS e-nose array, and SPME direct to MS (28); despite this there are no cases we could find in which the MOS sensor is integrated into the same system as a common detector type (e.g. MS or FID). A small number of publications have also reported the limit of detection (LOD) of MOS sensor-GC systems, (29) for five alcohols, acetaldehyde, acetone and ethyl acetate, which reports detection limits of several ppb and (30) for hydrogen fluoride, with detection limits of 800 ppb.

This investigation will assess the sensing abilities of a MOS sensor comprising a binary mixture of ZnO and SnO\(_2\) when used as a secondary detector for a Clarus 500 GC-MS (the combination of this MOS sensor with GC has previously been demonstrated as highly sensitive and effective (11,14,31)). This combined GC-MS MOS sensor system is referred to as gas chromatography mass spectrometer sensor (GC-MSS) system. A broad and diverse range of volatile organic compounds (VOCs) were measured, and their limits of detection determined using standards; volatiles from the headspace of stool and bacteria in broth have also been analysed.

**Experimental**

**Sensor preparation and sensor chamber**

The details of the sensor substrate design and manufacture and the coating procedure are described in detail by Vaughan et al., 2013 (32). In short, a 3mm alumina substrate was screen printed with gold interdigitated electrodes (4 pairs of interpenetrating bars, electrode gap 100\(\mu\)m) on one side and with a platinum heater track on the reverse. This was wire bonded to a TO39 transistor can. The sensor substrate was coated with a metal oxide paste comprising 50% zinc oxide nanopowder <100nm (Sigma Aldrich), and 50% tin oxide (IV) nanopowder <100nm (Sigma Aldrich) (by weight) using the doctor blade technique detailed in (32-34).

An aluminium chamber (volume 5 cm\(^3\)) was used to house the sensor with a PTFE mount to hold the sensor in place (see figure 1). The GC column (see GCMS and sensor set up section for details) enters the sensor chamber directly opposite the sensor and is positioned circa 5mm from the sensor surface (Figure 1). A purge gas
(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 (ΔR/ΔT) 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
sensor. However, $\Delta R/\Delta T$ allows changes in the gradient of a response recovery to become apparent; these changes in gradient can be small responses that would otherwise go unnoticed.

**Stool samples**

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

**Bacterial culture headspace analysis**

2 Colonies of *E. coli* were picked from an overnight culture on an agar plate (Oxoid CM0003) and used to inoculate 5mL of nutrient broth (Oxoid CM0067) in a sterile glass universal. The broths were incubated overnight at 37°C. Absorbance was measured and colony forming units / mL (CFU/mL) was estimated using the Agilent online calculator (Agilent Genomics). Overnight cultures were diluted as appropriate to give between $10^5$ CFU/mL and $10^6$ CFU/mL in four glass headspace vials with 5mL nutrient broth (Oxoid CM0067). One was frozen at -20°C immediately with the others undergoing 2, 4 and 24 hours further incubation respectively before freezing. 1mL of each sample was removed with needle and syringe prior to freezing for final absorbance measurements.

Prior to analysis vials were defrosted in a water bath set to 60°C for 30 minutes before the SPME fibre (as used for both the stool and standards) was added for a further 30 minutes, as for the stool samples the vial was maintained at 60°C for the duration of the sampling. 10 of the bacteria samples were selected at random for analysis on the GC-MSS.

**Analysis**

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

**Results and discussion**

**Standard solutions**

The 450°C sensor operating temperature was derived experimentally. At 450°C the
water to ethanol response ratio was 4 times greater than when operated at 350°C
which gave a response ratio of close to 1.

Of the 29 chemical standards investigated, 25 have been found on the breath of
healthy humans, and three others have been found as volatiles emitted from the
human body from other sources such as faeces, urine, and saliva (35). Figure 2 shows
the full comparison of mode average LODs for all 29 VOCs, Supplementary figure SF1
shows the data for each repeat. 17 compounds showed better LOD with the MOS
sensor including significantly improved sensitivity for butanol, 2-butanone and indole.
The mass spectrometer only showed increased sensitivity for two compounds,
methanol and butanal. The mass spectrometer and the MOS sensor exhibited the
same sensitivity for the remaining 9 compounds. The MOS sensor comprised two
metal oxides, tin oxide and zinc oxide both of which on their own are well known to be
capable of sensing a wide range of VOCs. We have shown there are advantages in
using a mixed system for enhanced detection of a range of VOCs (33,34).

Supplementary table 2 (S2) shows indicative headspace concentration values based
on Henry’s law constants; to give some indication as to the range of concentrations
being detected above the solutions. Both the mass spectral analyser and MOS sensor
show a wide dynamic range from low part per billion to part per million. Calculating an
accurate headspace concentration is problematic as the headspace is pre-
concentrated onto an SPME fibre thus the quantity absorbed will be subject to the selectivity of the fibre. Moreover, in a complex solution such as those analysed it is possible that Henry's law will not be fully applicable due to inter compound interactions, although the solutions are extremely dilute. It should also be noted that since we are using solutions with a factor of 10 difference, the actual LOD would likely be at a value between the 2 solutions.

While the sensor showed high sensitivity to a wide range of analytes it unexpectedly elicited zero responses to siloxanes. Siloxanes were observed in many of the GC-MS chromatograms in this work (a common GC contaminant often coming from the injection septum, sample vial septum, SPME fibre, column bleed or indeed the sample itself, as they are widely used in biomedical and cosmetic applications (36)). Siloxanes are relatively large, stable molecules yet they are volatile. While they are readily ionised in the mass spectral analyser we have been unable to find any instances in the scientific literature in which they are oxidised/ catalytically broken down, by metal oxides. Any reaction that may occur at the sensor surface is too slight to produce a sensor response. For analysis of biologically derived VOCs this is an advantage, as unwanted contaminant responses would reduce the ability of the sensor to recover and may obscure analyte responses. Additionally, if applying these resistance traces to algorithms in order to differentiate them into clinically relevant groups, siloxanes will interject false information into the equation. The lack of sensitivity of the MOS sensor to column bleed compounds such as the siloxanes means that at higher retention times the sensor has a more stable baseline and thus the potential to detect lower levels of "target" compounds in this region e.g. 3-methyl indole.

A mean RT delay of 6 seconds across the chromatogram was observed between the mass spectrometer and MOS sensor with a standard deviation ±2 seconds; these RT differences are constant across the duration of the sample time. Using butanol as an example the highest concentration (10g/L) RT for the MS was 14.10 and the lowest concentration (0.0001g/L) was 14.06. The sensor recorded RTs of 14.14 and 14.15 respectively. These RT drifts are typical across the whole range of tested standards for both the MS and sensor.

After responding to a VOC, the sensor tends to return to its previous oxygen surface state equilibrium and the resistance can take time to recover to the previous baseline
value. During this recovery, which is enhanced by the purge gas, another compound can cause a sensor response which means that the size of the response is often very different between the MS and MOS. The helium carrier gas of the GC-MS can have a detrimental effect on sensor response over time as the oxygen species are depleted; this effect is countered by using air as a purge gas which allows the oxygen to be continuously replenished. In cases of very large responses the recovery can be sufficiently rapid that the next compound appears only as a change of gradient and not a peak response; in these cases, viewing the resistance as ΔR/ΔT can help the resolution of responses from closely eluting compounds. In instances of the true co-elution of 2 or more compounds this function would have limited utility as there is no change in the slope visible. A mass spectral analyser might be able to separate compounds based on product ions within the total ion chromatogram although this is not always the case. In contrast to the sensor, the mass spectral analyser will produce a response as long as the product ions are being detected and will cease when the ions are no longer detected thus the recovery time is typically shorter meaning the MS has superior peak resolution.

An important point to reiterate is that the mass spectrometer was not run optimally in terms of maximum sensitivity (e.g. not SIM mode) but it was run using the same parameters as would be applied for the analysis of an unknown sample for example.

The metal oxide sensor could also be run using parameters which increased sensitivity (column positioned closer to sensor) but which resulted in a loss of peak resolution (recovery time increased). Therefore, the sensor was optimised by altering the column position and carrier gas, purge gas flow rates to produce maximum sensitivity but appropriate separation/resolution for the compounds of interest. The directionality of the carrier gas stream exiting the column and its close proximity to the sensor ensures efficient transit of the analytes to the surface and minimal dilution by the purge gas. Thus the purge gas flow rate was selected based upon providing a balance between maximal recovery between responses without sacrificing sensitivity. Low purge gas flow increased the level of noise which was disadvantageous when analysing complex samples. The chamber flushing rate was also selected so that a high concentration of analyte presented at the exhaust and around the seals of the sensor chamber gave no sensor response. This was an important consideration to ensure the sensor baseline was not altered by environmental volatiles.
We produced the chamber originally from aluminium as it was easy to manufacture various iterations of the chamber design. We attempted to minimise all plastics/materials within the chamber which might outgas additional compounds or adsorb compounds. On testing the current chamber with the standards, we did not get baseline issues with the sensor indicating that outgassing compounds were minimised. The directional transport of analytes to the sensor surface and efficient removal via the purge gas mean that we didn’t have issues with carryover or contamination. If issues had been identified then we may have considered other sensor chamber materials, or inert surface treatment of the current chamber. In terms of chamber size, it is possible that different designs or a smaller size may further optimise performance. We intend to continue to develop the current system which has gone through a number of iterations to reach its current state of development.

Over the 4-month testing period a slight change of ≤ 5% in the sensitivity per month was observed. During the test period, slight increases in sensitivity were observed for some compounds (butanol, propanol, acetone) and others slightly decreased (ethanol, methanol). Overall the sensor system gave good stability in terms of response to standard analytes at a known and relevant concentration. Baseline stability was also high with no significant drop during the testing period.
Figure 2: The limit of detection concentration of standard chemicals in deionised water, comparing a single quadrupole mass spectrometer with the mixed metal oxide (SnO$_2$ 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.

<table>
<thead>
<tr>
<th>CAS-number</th>
<th>Compound</th>
<th>MS RT (mins)</th>
<th>Sensor RT (mins)</th>
<th>Chemical standard RT MS (mins)</th>
<th>Chemical standard RT Sensor (mins)</th>
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</thead>
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<tr>
<td>75-07-0</td>
<td>Acetaldehyde</td>
<td>8.93</td>
<td>8.98</td>
<td>8.94</td>
<td>9.08</td>
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<td>64-17-5</td>
<td>Ethanol</td>
<td>10.13</td>
<td>10.20</td>
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<td>67-64-1</td>
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<td>10.74</td>
<td>10.88</td>
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<td>Propanol</td>
<td>12.00</td>
<td>12.07</td>
<td>12.03</td>
<td>12.16</td>
</tr>
<tr>
<td>123-72-8</td>
<td>Butanal</td>
<td>12.48</td>
<td>12.53</td>
<td>12.51</td>
<td>12.61</td>
</tr>
<tr>
<td>431-03-8</td>
<td>2,3-Butadione</td>
<td>12.65</td>
<td>12.71</td>
<td>12.44</td>
<td>12.68</td>
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<td>13.37</td>
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<td>71-36-3</td>
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<td>14.05</td>
<td>14.07</td>
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<td>79-09-4</td>
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<td>624-92-0</td>
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<td>15.59</td>
<td>15.56</td>
<td>15.73</td>
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<tr>
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<td>16.14</td>
<td>16.10</td>
<td>16.19</td>
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<tr>
<td>107-92-6</td>
<td>Butanoic acid</td>
<td>16.73</td>
<td>16.80</td>
<td>16.72</td>
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<td>590-01-2</td>
<td>Butyl propanoate</td>
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<td>18.20</td>
<td>18.05</td>
<td>18.12</td>
</tr>
<tr>
<td>109-52-4</td>
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<td>18.40</td>
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<td>18.47</td>
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<tr>
<td>108-95-2</td>
<td>Phenol</td>
<td>20.84</td>
<td>20.88</td>
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<tr>
<td>106-44-5</td>
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<td>22.28</td>
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<tr>
<td>120-72-9</td>
<td>Indole</td>
<td>26.92</td>
<td>27.04</td>
<td>26.90</td>
<td>27.17</td>
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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
the identification of compounds causing resistance responses is based on the RTs from the mass spectral analyser. There was a mean difference between the standards and the sample of 2.5 (±3 seconds) for the mass spectral analyser and 5 (±2 seconds). Table 1 shows a mean difference between the mass spectral analyser and the sensor of 3.6 seconds (±2.4 seconds). Over the course of our experiments the sensor has shown a consistently longer RT versus the mass spectral analyser. This could be in part because the sensor takes more time to reach the peak value from which we derive the RT. Moreover, between repeats of the same sample including standards there will be slight variation in the RT. Although we have made efforts to minimise differences there may also be slight variation in the column lengths between the swafer and MS, sensor detectors. There may also be differences in temperature profiles across these transfer lines as one exits the GC oven. These combined factors could explain both the difference in RT and the consistency of the difference between sensor and MS.

Table 2 shows a breakdown of the total number of responses detected on both the mass spectral analyser and MOS sensor from all 12 stool samples; Supplementary table S4 shows the raw data for each of the 12 stool samples the total number of times each compound was detected can be shown in supplementary figure SF2; in total 25 more NIST matched responses were detected on the mass spectral analyser. In most cases this is due to the resistance responses being lost in the recovery of the previous peak. When the resistance trace is viewed as a function of \( \Delta R/\Delta T \) (an example of this is shown in figure 3) these responses are clearly visible on all but 4 occasions. Across the 12 samples the mass spectral analyser was unable to identify via the NIST library 41 responses at the defined threshold; these unidentified MS peaks were detected on the MOS sensor. However, the MOS sensor detected 43 additional responses across the 12 samples that did not appear 3 times above the noise on the chromatogram or had no peak at the matching RT. The mass spectral analyser detected 27 siloxanes across the 12 samples no responses were detected on the MOS sensor at the matching RT. The majority of the unidentified responses from the MOS sensor traces appear during the last 10 minutes of the sample run. Of the 85 detected compounds 1 (nonane) did not have a corresponding resistance response. Due to the high number of carbons, stability, and lack of oxygen species compounds like nonane (and many other alkanes) are difficult to catalytically break down. The superior LOD of the sensor will account for many of the additional peaks; low concentrations in the samples will
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 *
<table>
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<td>NIST matched responses</td>
<td>488</td>
<td>463</td>
<td>44.5 (±7.9)</td>
<td>42.5 (±8.3)</td>
</tr>
<tr>
<td>Unidentified responses</td>
<td>41</td>
<td>84</td>
<td>3.4 (±1.6)</td>
<td>7.0 (±2)</td>
</tr>
<tr>
<td>Siloxanes</td>
<td>27</td>
<td>0</td>
<td>2.3 (±1.0)</td>
<td>0.0</td>
</tr>
<tr>
<td>Total responses**</td>
<td>529</td>
<td>547</td>
<td>47.9 (±9.5)</td>
<td>49.5 (±10.3)</td>
</tr>
</tbody>
</table>

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
methods of the cited work in table 4. Despite this, table 3 shows that we are able to
detect a number of key compounds, this leads us to believe that our system is not only
able to detect a wide range of compounds, in terms of mass and functional groups,
but also that there are multiple applications including bacterial VC analysis.
Dichloromethane was present in 4 of the samples analysed, this is likely to be a
contaminant however is included as a compound as it was clearly identifiable and
interestingly produced no response from the MOS sensor. This is fortuitous as in many
instances dichloromethane is used as a solvent and can produce responses capable
of overloading mass spectrum detectors which often requires the use of solvent
delays.

The difference in the MS and MOS sensor responses is shown clearly in figure 4,
which shows a GC-MS chromatogram and MOS sensor resistance trace (in $\Delta R/\Delta T$
negative only) overlaid on each other. Figure 4 illustrates just how well the responses
match up and generally larger responses on the chromatogram will result in larger
responses from the MOS sensor; though this is not always the case, for example
Indole (figure 4). In this instance, the mass spectral analyser standards had a mean
difference of 3 seconds ±2 versus the bacterial samples. The sensor also had a mean
of 3 seconds ±2 seconds difference between the standards and bacterial headspace.
The mean difference between the mass spectral analyser and sensor for the bacterial
headspace samples was 4.5 seconds ±2; these values are consistent with those
calculated from the stool samples.

<table>
<thead>
<tr>
<th>CAS-number</th>
<th>Compound</th>
<th>MS RT (mins)</th>
<th>MOS Sensor RT (mins)</th>
<th>Standard solution MS RT (mins)</th>
<th>Standard solution sensor RT (mins)</th>
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<td>64-17-5</td>
<td>Ethanol</td>
<td>10.20</td>
<td>10.30</td>
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<td>71-23-8</td>
<td>Propanol</td>
<td>12.09</td>
<td>12.17</td>
<td>12.03</td>
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<td>12.54</td>
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<td>12.71</td>
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<td>20.03</td>
<td>20.18</td>
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<td>26.98</td>
<td>27.07</td>
<td>26.90</td>
<td>27.17</td>
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</table>

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.
Figure 4 An example of an overlaid chromatogram from the GC-MS with a MOS sensor resistance trace in the ΔR/ΔT negative only view.

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
spectral analyser and the MOS sensor; supplementary figure SF3 shows how many times each compound was detected across the 10 samples on both detectors. In many cases the unknown responses from the MOS sensor trace do have a very small corresponding MS peak that was not above the noise threshold. Moreover, the bacterial samples produced lower relative abundances on the chromatogram across the whole chromatogram when compared to those from the stool samples. For instance, the mean peak area from the MS for dimethyl disulphide from the stool samples was $85 \times 10^6$ compared to just $1.1 \times 10^6$ for the bacterial headspace studies. This is suggestive that the concentrations in the bacterial headspace are significantly lower than those in the stool. 6 of the compounds in table 3 that were present in both stool and the bacterial headspace had a mean 80% smaller chromatographic peak area in bacteria versus stool.

As with the stool sample analysis, we see that the sensor detects additional responses consistently across each sample with a mean of 23.6 (±5.7) from the MOS sensor versus 16.7 (±3.9) from the mass spectral analyser. The lower relative abundances from the bacterial samples are indicative of lower concentrations of compounds; despite this the MOS sensor shows clear superiority over the mass spectral analyser in terms of responses detected. This enhanced sensitivity should allow for greater discriminatory abilities by detecting more points of differences between samples.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Indole</th>
<th>Dimethyl disulphide</th>
<th>Ethanol</th>
<th>Propanol</th>
<th>2,3-Butadione</th>
<th>2-Butanone</th>
<th>Acetone</th>
<th>Methanol</th>
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<th>Benzaldehyde</th>
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</table>

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

Acknowledgements

Dr Cheryl Flynn, for her assistance in calculating vapour concentrations. Dr Barbara Rees for culturing and aliquoting the bacterial samples. Dr Rick Ewen for development and installation of the sensor electronics and software. Katie Vaughan for work developing the sensor design. Funding support from the Above and Beyond charities, Bristol.
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