The detection of trace volatiles from complex matrices using gas chromatography mass spectrometry (GC-MS) techniques and selected ion flow tube mass spectrometry (SIFT-MS) assessment of volatiles produced from nitric oxide producing smart dressings

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Copyright declaration

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Abstract

Volatile compounds (VCs) hold the potential to diagnose and monitor disease states in a cost effective, rapid, and most importantly non-invasive manner. Gas chromatography mass spectrometry (GC-MS) has been in use since the 1960s and remains the gold standard for qualitative VC analysis. Within this thesis three novel methods and/or utilisations of mass spectrometry are described.

Chapter 2 describes and benchmarks a metal oxide sensor (MOS) coupled to a standard GC-MS instrument. Testing this system to the headspace of 12 stool samples the sensor detected a mean 1.6 more peaks per sample then the MS. This superior sensitivity exhibited by the MOS sensor should allow for greater discriminatory abilities to differentiate samples into clinically relevant groups.

It has become increasingly important to qualitatively and quantitatively assess the VCs for use in monitoring health. Chapter 3 describes a novel method for the quantification of VCs from the headspace of stool samples analysed using GC-MS is presented. Using ^{13}C labelled carbon compounds as internal standards a method has been designed which quantifies the compounds within in the stool; 15 compounds were quantified.

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Aims and objectives

Mass spectrometry (MS) is a technique that dates back over 100 years and has been used over that time for numerous applications. MS is a technique that is continuously evolving and is more relevant today than it ever has been thanks to advances in the technology. In recent decades there has been growing interest in using MS for the biomedical sector, particularly to detect volatile compounds. Within this thesis 3 pieces of work have been carried out which use MS for biomedical applications.

Chapter 1 describes several real-time and non-real-time MS techniques, and reports on the current literature in which these techniques have been used for biomedical applications. This chapter also points out some of the benefits and challenges associated with each MS technique.

Chapter 2 introduces and benchmarks a novel gas chromatography mass spectrometry (GC-MS) instrument coupled to a metal oxide sensor which provide a binary output. This is the first time that these two types of detectors have been coupled in this way. This instrument is tested to a range of standards, health participant stool samples, and bacterial culture headspace, all pre-concentrated with solid phase micro extraction. This allows advantage to be taken of the superior sensitivity of the sensor while maintaining the qualitative GC-MS output.

Chapter 3 utilises GC-MS with a previously unreported technique to quantify compounds within stool using carbon 13 (^{13}C) labelled compounds as internal standards. This technique allows us to calculate the quantity of compound in the stool not just the headspace. Sodium hydroxide was also used to raise the pH of the stool prior to analysis. This allowed us to quantify trimethylamine and provided qualitative data on alkaline stool which has also not previously been reported.

Chapter 4 describes the development of a new method to quantify the products of a chemical reaction in real-time using SIFT-MS. Edixomed Ltd have developed a smart dressing that produces nitric oxide (NO) by the acidification of nitrite. NO is a known vasodilator and this dressing has been shown in clinical trials to promote better wound healing versus the standard care pathway. This chapter will not only seek to quantify products of the dressing reaction but also attempt to underpin some of the mechanisms of the reaction.

The work within this thesis will demonstrate that this 100-year-old technique has the potential to provide new insights into disease states and can be used to help develop new ways of treatment.

Chapter 1: A review of mass spectrometry (MS) for detection of volatile compounds (VCs) for biomedical applications.

1.1.0 Introduction

In 1989, a letter to the Lancet records the case of a dog becoming increasingly interested with a lesion on the owner's skin, after seeking clinical advice the lesion was confirmed to be a melanoma [1]. Since then numerous studies have been conducted attempting to demonstrate dogs ability for diagnosing disease from the smell of a sample, blood [2], urine [3–5], stool [6], breath [7,8], and tissue [9,10] samples have all been used for such work. Commonly dogs are assessed for their ability to diagnose cancer, and many of these studies have been included in critical reviews [11,12]. Dogs have also been assessed for their ability to detect *C. difficile* [13] and diabetic alert dogs are also used to alert their owners to hyper/hypoglycaemic episodes [14].

It is fair to state that many of these studies suffer from flaws in experimental design such as the use of inappropriate controls as in Pickel *et al.,* 2004 in which materials such as gauze and rubber gloves are used [9]; or McCulloch *et al.,* 2006 who collected breath on polypropylene tubes which were stored at room temperature for 60 days, with no available information on sample viability over this duration and no evidence that the tubes are capable of capturing breath volatiles [7]. Reproducibility is also an issue both within individual studies in which multiple dogs are used and from study to study, for example Amundson, 2014 attempted to detect lung cancer from breath and reported a sensitivity of 61.8%-67.6% and specificity of 8.3%-16.7% [15]. Ehmann *et al.,* 2012 also used breath to detect lung cancer and reported a sensitivity of 71% and specificity of 93% [8].

There is though on balance enough evidence to suggest that the dogs are responding to alterations in the volatile compounds (VCs) emitted from the samples; so much so that many traditional laboratory investigations have been conducted on the premise that VCs may be useful in the diagnoses of disease.

Understanding the pathways by which VCs are generated in the body is the key to understanding the mechanisms by which VCs can have clinical utility. Miekisch *et al.*, 2004 describe the mechanism of production for many families of compounds found on breath [16]. For instance acetone is a very commonly occurring compound on breath and is generally accepted to be derived from the decarboxylation of acetyl-CoA by the liver [16]. Elevated breath acetone is considered an indicator of beta-hydrobutyrate in the blood [17] and has been attributed to type 1 and type 2 diabetes in a number of studies [18–23]. However despite the biochemical mechanisms for breath acetone

being understood there are limits to its clinical utility, primarily due the fact that the acetone levels on the breath of healthy participants is so variable [23].

Shirasu and Touhara, 2011 briefly outline the basis for the use of VCs in breath, skin and sweat, urine, and faeces [24]. In short breath VCs are derived from ingestion of food, beverages, and cigarettes as well as gases in the atmosphere and blood borne compounds that are exhaled. Skin VCs are mostly derived from sweat, in this case most of the VCs from internal metabolism are altered from the bacteria on the skin. Urinary VCs are primarily derived from metabolic process throughout the body and contain a wide variety of functional groups such as ketones, alcohols, and furans; importantly it is possible for urinary VCs to be affected by ingested food and beverages, and are frequently diluted by water. Stool is primarily comprised of bacteria which generate a significant amount of VCs this gives stool excellent potential to diagnose gastro intestinal disorders [24]. Amann *et al.*, 2014 lay out all the compounds found in breath, skin emanations, urine, faeces, and saliva from heathy humans in a "megatable". This paper states that 874 compounds have been reported on breath, 279 compounds from urine, 504 form skin emanations, 353 from saliva, 130 compounds from blood, and 381 compounds from faeces [25]. Breath would seem, at first to have the most compounds, however, there are likely to be more studies conducted using breath as it is the most acceptable, instant, and cost-effective form of testing; thus more compounds have been identified versus other bodily excretions.

Within this project several mass spectrometry techniques and instruments will be assessed for their utility within the biomedical forum. This will include analysis of biological samples; and the evaluation of smart dressings which use generation of volatile oxides of nitrogen for improved wound healing.

1.1.1 Introduction to mass spectrometry (MS)

The primary function of mass spectrometry (MS) is to identify compounds within a substance and in some cases quantify compounds in a substance. The overall mechanisms along with applications and variations of MS is laid out in the book Mass Spectrometry: principles and applications [26], which describes multiple methods of mass spectrometry including different quadrupoles and detector types. The work presented in this thesis has been carried out on a gas chromatography mass spectrometry (GC-MS) instrument and selected ion flow tube mass spectrometer (SIFT-MS), both of which utilise a similar standard single quadrupole mass analyser.

Figure 1.1 shows a schematic of a standard quadrupole with detector; the analyte compound is ionised at the source, this is usually done by chemical (CI) or electron ionisation (EI) techniques, though there are multiple variants of this such as secondary electro-spray ionisation (SESI) as in Gaisl *et al.*, 2018 [27]. MS is a technique in which chemical species are ionized and sorted dependant on their mass to charge ratio (m/z). In a standard quadrupole detector (as in figure 1.1) the ion beams are focused by the first lens. The trajectory of the ion in the electric field allows separation of the ions according to the m/z [26]. A second lens focuses this beam to the detector. Again, there are a number of different detectors commercially available; however, in general the basic principles are the same in that the detection is dependent on the m/z and velocity of the ion and the signal is proportional to the abundance of ions.

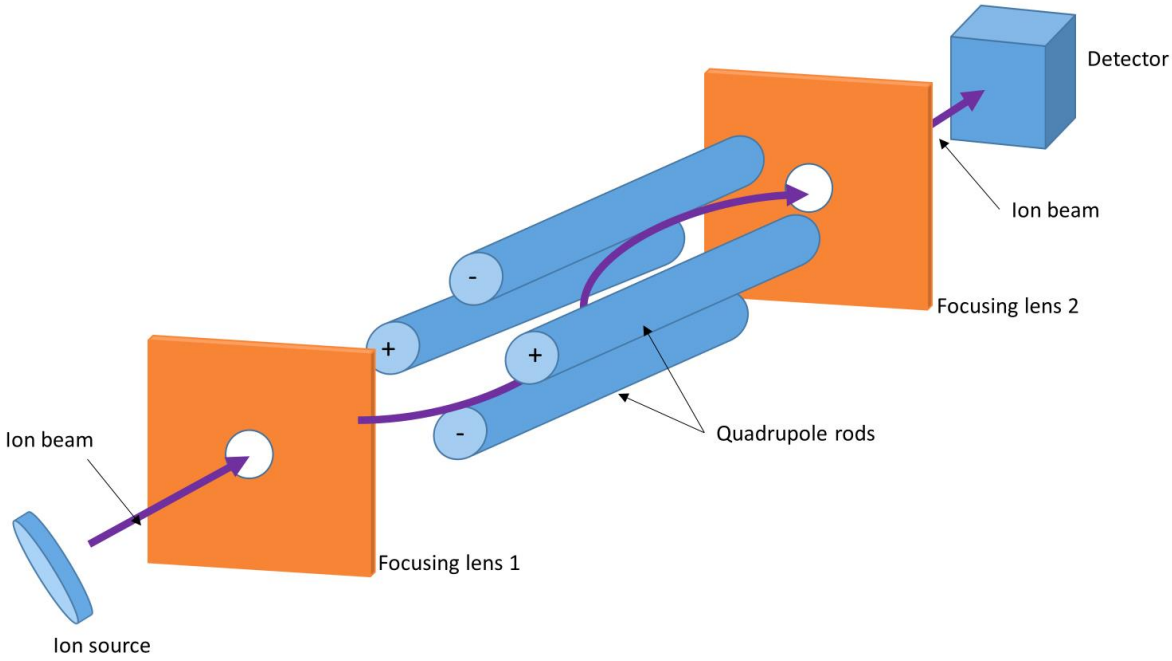


Figure 1.1 schematic of a standard quadrupole mass spectrometer as adapted from Kicman *et al* with © permission from Elsevier 2007 [28]

Mass spectrometry has been used for many applications over the years since its inception in the late 1800s [29] including environmental [30], biomedical [31–33], security[34–36], forensics[37–39], space exploration[40–42], agri-food [43,44] and numerous others. Even within the biomedical field there are numerous applications for MS including proteomics [45,46] and metabolomics [47–49].

Moreover, MS is used in many different instruments for example GC-MS, SIFT-MS, ion-mobility MS (I-MS), and liquid chromatography MS (LC-MS) Each have their own

advantages and limitations and are better suited for certain applications over others. For instance breath analysis in many cases can be improved by the use of real-time on-line techniques such as SIFT-MS and proton transfer reaction MS (PTR-MS) both of which negate the need for sample pre-concentration which can introduce contaminants or degrade the sample [50]. Ratiu *et al.,* 2017 present a review of mass spectrometry techniques for VC analysis of compounds generated by bacteria [51]; this review details many of the general advantages and disadvantages associated with the various techniques but also compares the costs of the different instruments.

This following review will outline the principles and mechanisms of the various mass spectrometry techniques and biomedical applications for them. Table 1.1 shows the acronyms for the instruments included in this chapter.

Acronym	Meaning
GC-MS	Gas chromatography mass spectrometry
GC-MS-MS	Gas chromatography tandem mass spectrometry
GC-TOF-MS	Gas chromatography time of flight mass spectrometry
GC-GC-TOF-MS	Two-dimensional gas chromatography time of flight mass spectrometry
PTR-MS	Proton transfer reaction mass spectrometry
PTR-TOF-MS	Proton transfer reaction time of flight mass spectrometry
SIFT-MS	Selected ion flow tube mass spectrometry
FAIMS	Field asymmetric ion mobility mass spectrometry

Table 1.1 the acronyms and meanings for the instruments and pre-concentrating methods that will be discussed throughout this review.

1.2.0 Non-real-time mass spectrometry techniques

1.2.1 Sample pre-concentrating methods

Sample pre-concentration techniques such as solid phase micro-extraction (SPME) and thermal desorption (TD) are required for GC-MS analysis to ensure sufficient analyte concentration is introduced to the column.

SPME offers time and cost efficiency, simplicity, reliability, and is easily portable [52]. Additionally, this technique does not exhaust the sample and allows direct input to the GC column. Garner *et al.*, 2007 used this technique to identify 297 compounds from

stool samples and demonstrate the potential for disease diagnosis using this technique [53]. Figure 1.2 shows a diagram of how a sample is pre-concentrated using a standard SPME fibre though it is important to note there are many variants on this technique which have undergone recent review [54]. Most commonly the sample is placed in a headspace vial allowing sufficient empty space to allow the fibre to enter the vial while not touching the sample. The needle of the SPME holder pierces the vial septa, the fibre can then be safely exposed to the gases in the vial, the VCs then bind to the fibre; the optimal duration of fibre exposure will be dependent on the sample type, analysis method, and analyte target. Before the fibre is removed it must first be retracted into the fibre holder, the fibres are delicate and easily broken if not properly handled. To introduce the sample to the GC-MS the needle of the SPME holder pierces the septa of the injector port the fibre is then exposed to the inlet liner which is heated, the heating of the fibre drives the VCs off the fibre and onto the column and the chromatography phase can begin.

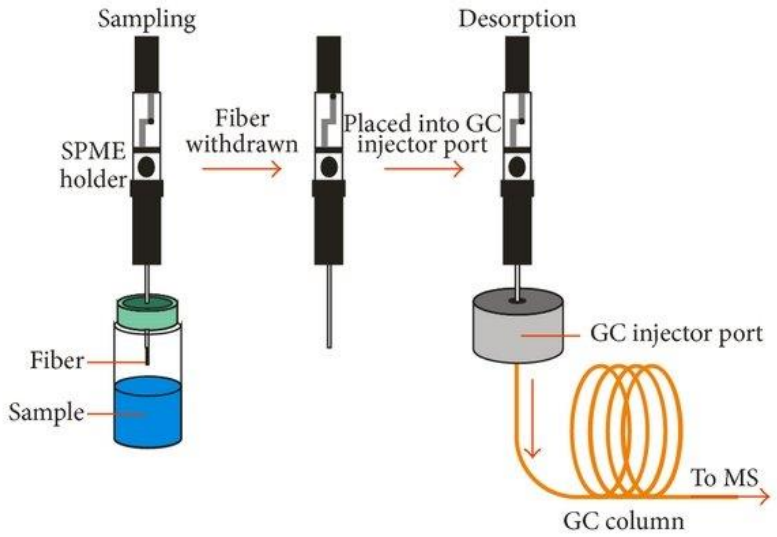


Figure 1.2 diagram of the operation of a solid phase micro-extraction technique. Diagram taken from Schmidt and Podmore (open access creative commons ©), 2015 [55]

There are many adsorbent coatings that can be used for SPME fibres many of which have described in a review by Silva *et al* [52]; the most appropriate adsorbent will be dependent on the target analyte(s). The most common adsorbent for complex matrices such as urine is PDMS, this strikes the balance between being able to adsorb a wide range of compounds while not falling having such a strong affinity to compounds that reuse becomes problematic due to carry over [52]. A 2012 study compared six

different SPME adsorbents PDMS, PA, DVB/CAR/PDMS, CAR/PDMS, CW/DVB, and PDMS/DVB. Each of these was used to analyse the urine of breast cancer patients. By comparing the number of identified compounds, the chromatographic peak areas, and relative standard deviations CAR/PDMS had the greatest extraction efficiency [56].

TD is a significantly more intricate technique which requires loading the TD tubes by flowing the gas to be analysed through the tube at a known flow rate to balance maximum sensitivity versus the possibility of overloading the tube. Figure 1.3 shows a diagram of a typical TD tube; the tube housing is traditionally glass or stainless steel. There are numerous sorbent materials that can be used to pack the TD tubes, these include: porous polymers, carbon blacks, molecular sieves, and activated charcoal. However, by far the most commonly used and most versatile is Tenax TA which a hydrophobic porous polymer. The mechanism of sorbent trapping has been described in a 2000 review [57]; there are three classified pore sizes macropore, mesopore, and micropore. Macropores have a multilayer adsorption which causes the vapour to enter a condensed phase and thus Henry's Law can apply. Micropores tend to hold the molecules more strongly as the molecule is held by more than one side of the pore. There is often a secondary sorbent bed to prevent breakthrough; this is where the analyte compounds are not retained in the tube. Breakthrough means that either the primary absorption bed is over capacity or compounds are being displaced [57].

One of the main advantages of TD is that once loaded the tubes can be stored for significant lengths of time provided correct tube fittings and storage is used. Kang and Thomas, 2016 report that stored at -80^{o}C breath samples retained 94% of the VCs after 1.5 months [58]. Harshman *et al*. performed a stability study using Tenax TA to trap compounds from breath samples; they found that after 14 days all the compounds were still present regardless of storage conditions and when stored below 4^{o}C the compounds kept for 31 days [59] In order to desorb the compounds on to the GC column a TD unit is required, an example of a direct injection and two stage desorb is shown in shown in Figure 1.4. The short path TD process is similar to a direct injection, the needle pierces the septa of the GC inlet once the pressures are stable the heating block heats the tube to desorb the VCs, which enter the GC oven via the needle there is a cryo-trap mounted in the GC oven which holds the VCs while the GC phase starts (Chromatography phase is discussed in section 1.2.2). The two stage desorb requires a separate unit, in this instance the tube is heated and the VCs are, as with the direct

desorb, held onto an internal trap. The VCs are then desorbed onto the heated transfer line which connects to the GC column.

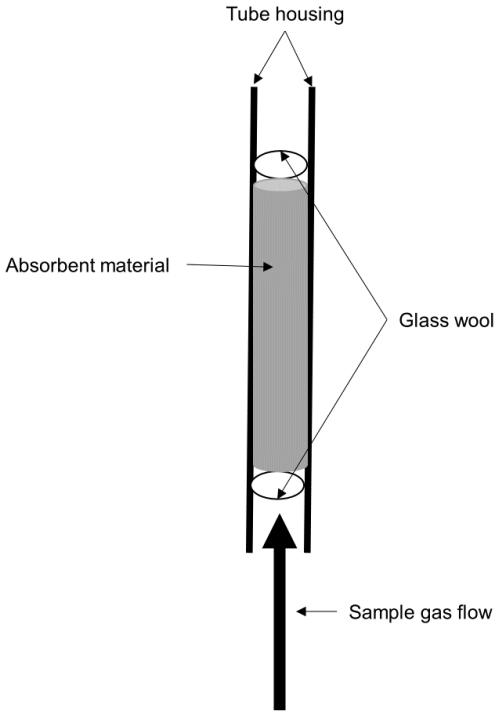


Figure 1.3 a diagram of an example thermal desorption tube glass wool keeps the adsorbent material in place while still allowing air flow through the tube.

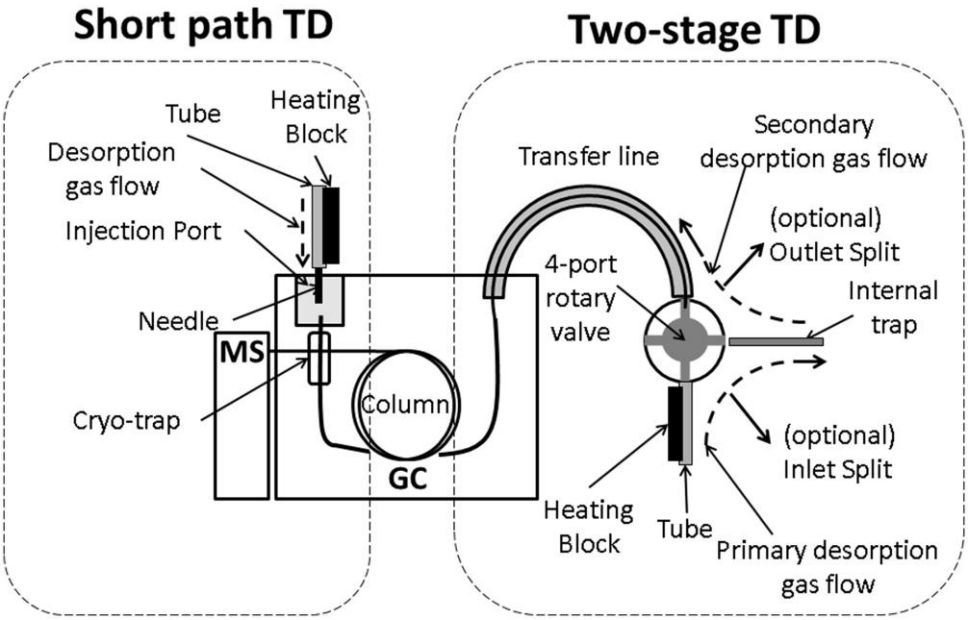


Figure 1.4 a simple schematic showing both short path and 2 stage thermal desorption processes, image used with © permission from Elsevier 2017 from Li and Zhu [60].

The drawback of TD as a pre-concentration method is that the initial cost of the thermal desorption unit is expensive; additionally, there is substantial method development required for optimal results. While the TD tubes and SPME fibres are a similar cost the time of analysis for TD per sample is generally double that of SPME. However, despite these drawbacks the enhanced sensitivity of this method is considered by many to be worth the additional costs and time [57].

1.2.2 Gas chromatography (GC)

Gas chromatography is a technique used to separate compounds from a matrix, using a column to do so. The mobile phase is an inert gas, most commonly helium or hydrogen which is used to carry the analyte compounds through the column. The stationary phase is usually a solid adsorbent which, can be polar or non-polar. The polarity of the column will have a significant impact on the elution order of compounds due to the affinity of the compound with the column. The order in which the compounds elute out are determined primarily by the chemical properties of the compound, as well as the affinity the compound has for the solid phase. The GC column is housed in an oven, as the oven temperature ramps up the compounds will begin flowing through the column the rate of travel is usually determined by the boiling point of the compound, in general lighter molecular weight compounds will flow elute out first due to having lower boiling, though this is not always the case.

There are many detectors that can be used in conjunction with GC, the most common is MS, and flame ionisation detectors (FID); other examples of detectors are metal oxide sensors [61], electron capture, flame photometric, nitrogen phosphorus, and thermal conductivity [62].

1.2.3 Gas chromatography mass spectrometry (GC-MS).

Gas chromatography mass spectrometry (GC-MS) is a very common analytical technique and is still considered a gold standard for qualitative gas analysis. There is such a vast quantity of biomedical based GC-MS papers that it is difficult to overestimate the role that GC-MS has played in the advancement of volatile analysis. GC-MS utilises two phases; the chromatography phase separates the compounds in the sample (see section 1.2.2).

The compounds are ionised as they enter the mass analyser which allows a spectrum to be mapped based on the mass to charge ratio (m/z) of the product ions which allows the detection phase to take place. This ionisation process makes this technique ideal

for identifying the compounds present in complex matrices such as stool and urine headspace. The national institute of standards and technology (NIST) library software provides identification of the compounds present by comparing the spectra produced from the ionised products of the analyte with those in the library. A full description of how this match is calculated has been published [63]; in short three values are used for the identification, the match, reverse-match (R-match) and probability value. The match directly compares the unknown spectrum to the library spectrum; the R-match also does this however ignores peaks in the unknown that are not in the library spectrum. The probability compares the hits adjacent to the unknown in the hit list to give a relative probability. Most modern GC-MS instruments have reported limits of detection (LOD) in the femtogram region however in reality when running a normal sample using a general method in total ion count most users report closer to the picogram LOD [64].

While GC-MS provides excellent sensitivity and is the most commonly used qualitative method of trace gas detection quantification requires considerable method development and analysis.

There have been numerous publications over many years attempting to utilise GC-MS for diagnostic purposes. For instance Tait *et al.*, 2013 used SPME GC-MS to identify *Clostridium difficile* from the headspace of stool samples with reported sensitivity and specificity of 83.1% and 100% respectively [65]. This study identified 2-fluoro-4-methylphenol, isobutyric acid, butyric acid, isocaproic acid, and p-cresol as the key compounds [65]. Another group also used SPME GC-MS to differentiate causes of diarrhoea and found that 5-methyl-2-furancarboxaldehyde was associated with *Clostridium difficile* and that this association improved when coupled with the absence of 3-methylindole (X^2=22.2, p=0.000002 and X^2=20.4, p=0.000002 respectively)[66]. Though it is worth noting that the total number of participants in this study was 35 with only six confirmed *Clostridium difficile* patients.

Colorectal cancer (CRC) is an excellent target for a diagnostic test based on VCs; currently the best method for diagnosing CRC is by colonoscopy, a procedure that is costly and very invasive. Moreover patients are less likely to come forward with symptoms due to embarrassment [67]. Therefore, if a VC test using urine or breath could be developed for the detection of CRC it may allow more accurate targeting of colonoscopy candidates and prevent many unnecessary procedures.

Altomare *et al,* 2013 used automated TD GC-MS to analyse the breath from 78 participants (37 cancer patients) in an attempt to differentiate colorectal cancer from healthy controls. The resultant VC patterns were fed into the probabilistic neural network which had an 85% overall accuracy; while this accuracy dropped during a validation phase to 76% this is still an encouraging result [68]. Amal *et al.,* 2016 also used breath, and demonstrated that acetone and ethyl acetate were increased in CRC patients versus controls and that ethanol and 4-methyl octane were decreased in CRC patients [69].

1.2.4 Gas chromatography tandem mass spectrometry (GC-MS-MS)

GC-MS-MS utilises two mass analysers in succession; the ions enter the first quadrupole and are filtered; from there the ions are focused into a collision cell which sits between the two quadrupoles. In the collision cell the ions are further fragmented before entering the second quadrupole, and finally the detector (as shown in Figure 1.5). McLafferty, 1980 sets out the mechanisms for MS-MS analysers [70]. Overall tandem mass spectrometry has greater selectivity and sensitivity over a single quadrupole MS. Moreover MS-MS lends itself to the analysis of heavier molecules due to the second fragmentation phase. Wang *et al.,* 2015 used GC-MS-MS to study the pharmacokinetics of Longu Rendan pills, a Chinese medicine used to prevent heat stroke and motion sickness. Using this technique, the team determined the concentrations of menthol, isoborneol, and borneol. They were able to determine the pharmacokinetics of the drug by feeding the drug to rats and analysing the plasma using the MS-MS technique [71].

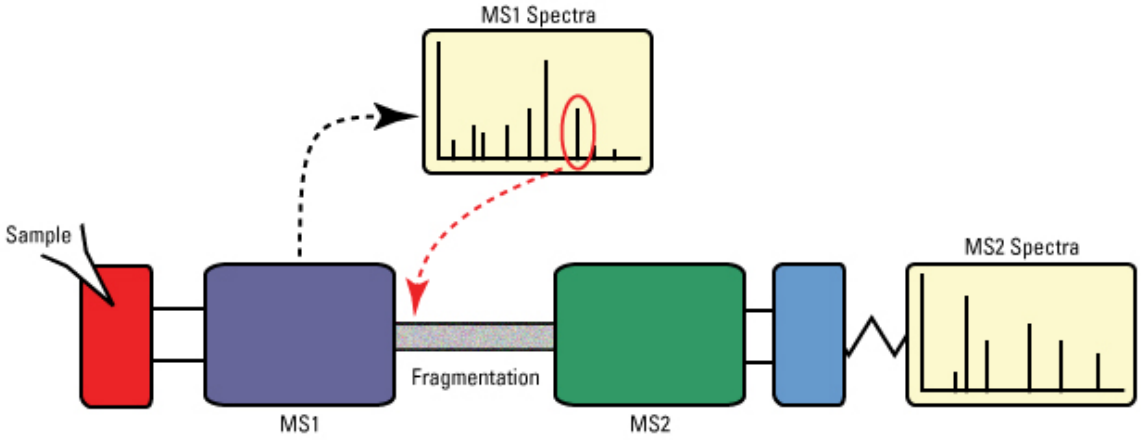


Figure 1.5 a simple schematic of a tandem mass spectrometer (image with © permission from Fisher Scientific [72])

Lomonaco, 2018 used MS-MS to detect a range of ketones and aldehydes at an LOD of less than 200 pptv. This technique was then used to determine carbonyl compounds in the breath of heart failure patients; they were able to derivatize on-sorbent [73]. Aldehyde and ketone concentrations are important as they are a feature of oxidative stress which is linked to a number of diseases such as diabetes, liver diseases, and heart failure.

Despite the benefits of GC-MS-MS, namely the increased sensitivity and specificity and superior ability to identify heavier mass molecules versus a single quadrupole, it is not a commonly used technique for biomedical investigations as the bulky equipment, extra expense, and often time consuming analysis is not optimally suited to the biomedical forum, where rapidity, low cost, and ease of use are often required attributes.

1.2.5 Gas chromatography time of flight mass spectrometry (GC-TOF-MS)

Time of flight (TOF) MS differs from standard MS as TOF does not use a quadrupole; in TOF-MS the ions are accelerated in a magnetic field so that all ions of the same charge have the same kinetic energy. These accelerated ions enter a field free drift region which is at a constant distance; the time it takes to cross this drift region will vary dependant on the m/z of the ion (see Figure 1.6). This gives TOF a higher acquisition rate versus quadrupole mass spectrometry, which in turn allows for excellent resolution particularly in semi-co-eluting compounds [74]. Moreover TOF-MS detectors have spectral continuity, this means that 5000 full mass range spectra can be analysed per second; spectral continuity means that concentration changes from peak elution does not affect the spectral continuity. This spectral continuity allows for greater performance of the deconvolution algorithms needed to identify a compound. As a result a TOF detector generally yields more accurate compound identification versus its quadrupole counterpart [74].

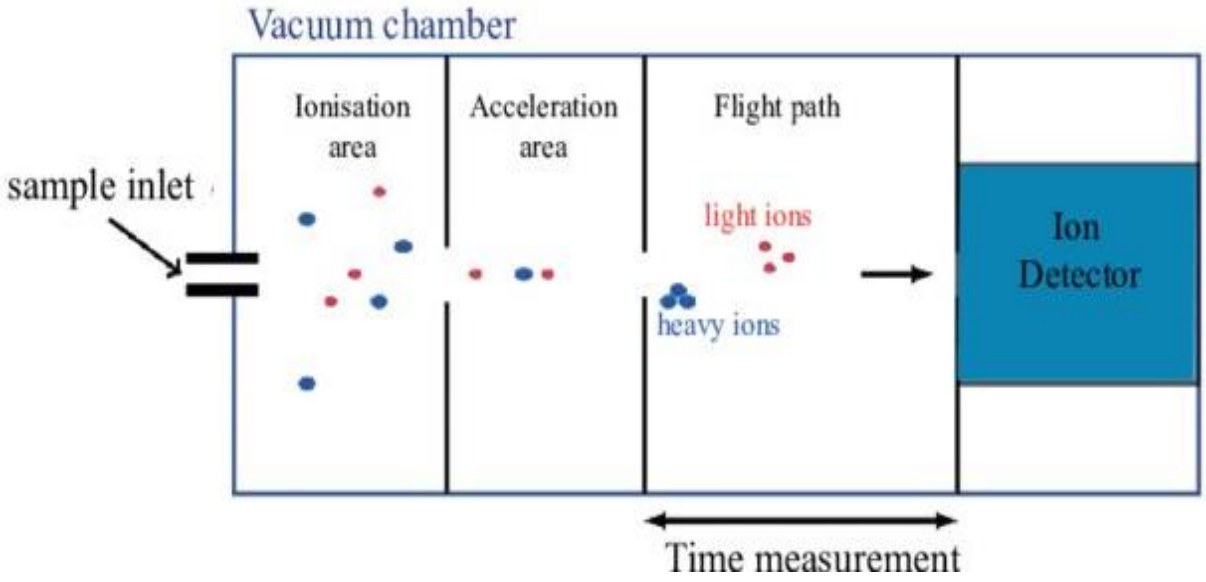


Figure 1.6 a diagram of a time of flight mass spectrometer (image from Plocoste *et al* 2016 open access cc ©)

Baranska *et al.,* 2016 analysed the breath samples from 1630 participants (170 irritable bowel syndrome (IBS) patients, 153 controls, and 1307 general population), 16 VOCs that could predict IBS in 89.4% of cases [75]. Similarly, Pijls *et al.*, 2016 identified a set of 11 volatiles to discriminate patients with chronic liver disease (CLD) from those with compensated cirrhosis (CIR). Over the course of this study the group claim a recorded data matrix of 3718 individual compounds from 152 chromatograms [76]. While these results are very encouraging both these examples lack details around the identification of the VCs. Neither paper offers a clear criterion by which a positive compound identification was achieved, for instance a set software matching threshold. Also, there is no verification with standards or retention indices.

VC analysis has enormous potential for clinical utility particularly when used in conjunction with other tests. Smolinska *et al.*, 2018 performed GC-TOF-MS analysis of breath samples from 184 patients and correlated these results with the results of microbiome tests from faecal samples in Crohn's disease patients [77]. This group demonstrated a link between the metabolites found on the breath and the microbiome found in the stool, either directly form the bodies metabolic processes or indirectly in the form of microbiota metabolites. This type of analysis should help provide a deeper mechanistic understanding of how the VCs can be clinically useful but also the pathogenesis of gastrointestinal diseases.

1.2.6 Two-dimensional gas chromatography time of flight mass spectrometry (GC-GC-TOF-MS)

The GC process is described in section 1.2.2; GC-GC couples 2 GC columns together one after another. Coupling together two columns allows better separation of compounds versus a standalone GC column. GC-GC is exceptionally good at separating out chemical families e.g. acids, ketones, and aldehydes etc.; additionally, GC-GC is also capable of separating the chemical isomers. Usually the two columns will use differing modes of separation such as polar and non-polar (see section 1.2.2). This system avoids co-elution problems whilst maintaining high sensitivity and specificity; moreover the ability to adjust and tune the parameters mean there is greater ability to optimise methods for the target range of analytes compared to a single GC column [78]. TOF-MS is a rapid detector which is much better suited to GC-GC chromatography phases [79].

Rees *et al.*, 2017 utilise this technique to determine the hypoxia VC signature of *Aspergillus fumigatus*, using SPME as a pre-concentration method. They were able to show the difference between early hypoxia and late hypoxia and early and late normoxia (normal oxygen level) [80]. By obtaining comprehensive VC data from this method this group was able to differentiate the oxygenation state of *aspergillus fumigatus* using 19 VCs however, more importantly this data obtained allowed the group to postulate possible pathways by which the fungus can continue metabolism under hypoxic conditions, involving 2,3-butandione and 3-hydroxy-2-butanone [80]. The idea of postulating metabolic pathways using VC profiles is important as this could provide crucial information in the pathogenesis of any number of disease states including infections, cancers, and metabolic disorders.

The same group also carried out similar work by characterising the VC metabolome of *Klebsiella pneumoniae* grown in human blood. In this instance the group used the GC-GC-TOF-MS and were able to identify compounds not previously reported as being produced by *Klebsiella pneumoniae.* This was done by comparing the sterile human blood sample compared to blood from the same participant inoculated with *K pneumoniae*; there was an increase in the production of VCs in the inoculated blood. By their own admission the authors acknowledge that their study is limited by only using the blood from one participant, nor were they able to compare the VCs produced by other strains of bacteria grown under the same conditions [81]. Despite the limited

sample size this study is still important as once again it demonstrates the utility of a powerful analytical technique such as GC-GC-TOF-MS.

1.2.7 Gas Chromatography-Sensor systems and sensor arrays for biomedical applications

A conventional GC-MS has been reported as being coupled to a metal oxide sensor (MOS); the full assessment of this novel analytical instrument is described in full detail in chapter 2 though the work has been published [61]. The MOS in the majority of cases display superior sensitivity to the mass spectral analyser, this has the potential to enhance the discriminatory capabilities whilst providing qualitative data simultaneously. A similar technique was used by Khalid *et. al.*, (2013) in this case a 30 m GC column was used for the chromatography phase in a conventional GC oven, however in this instance there was no MS, instead the column interfaced directly to a MOS sensor [82]. This method was successful in differentiating bladder cancer patients from patients with other urological conditions, the leave-one-out cross-validation predictive value for cancer patients was very high at 95.8%, this dropped only slightly for the control group to 94.6% [82]. While these numbers came from a small cohort (24 cancer, 74 controls) the numbers are very encouraging. The per sample run time for the method used in Khalid *et. al.* (2013) is 42 minutes, however the use of a high speed multi capillary column can be used to reduce this time as in McGuire *et. al.* (2014) who also used a GC column interfaced to a MOS sensor, on this occasion the use of a high speed multi capillary column was able to reduce the run time per sample down to 10 minutes [83]. McGuire *et. al.* (2014) analysed *C.difficile* positive stool samples and *C.difficile* negative stool samples and were able to differentiate the positive and negative samples with a sensitivity and specificity of 85% and 80% respectively [83].

Bartolazzi *et. al.* (2010), used a sensor array to analyse the VCs from five different cancer cell lines: three melanoma lines, synovial sarcoma, and thyroid cancer. An array of eight quartz microbalance sensors were used parallel to conventional GC-MS. The quartz microbalance sensors used metalloporphyrins to be activated copper, cobalt, zinc, iron, tin, and chromium were used as the metals [84]. This group found that the sensor array data provided good clustering and differentiation of the five cell lines; the parallel GC-MS analysis revealed 14 compounds that were most likely to be the cause of the clustering from the cell line [84].

Sensors and sensor array can have greater sensitivity versus MS techniques (as discussed in chapter 2). This means that sensitive sensor can enhance the differentiation of samples into clinically relevant groups, usually sensor outputs rely on pattern recognition techniques for data analysis. Moreover, sensors are rarely able to provide qualitative data to support the findings or infer mechanisms by which a change in the VCs present in a sample may occur. Despite this though the use of sensors could prove important in the development of high throughput, cost effective, and accurate clinical tests.

1.2.8 Summary and comparison of non-real time techniques

The chromatography phase of these techniques allows for the separation of compounds which is what makes the identification of compounds possible. However, this data is crucial to unlocking the mechanisms by which the VCs are created chromatographic methods require considerable development. Moreover, there are important decisions to be made about sample preparation and pre-concentration to ensure most efficient VC extraction without introducing confounding variables. In addition, GC-GC methods can have relatively long per sample analysis time.

	Advantages	Limitations
GC-MS	• Still considered the gold standard for VC qualitative analysis. • Capable of providing detailed data from complex matrices. • The most cost-effective method of non-real time MS analysis. • Can tailor pre-concentration methods to suit the sample being analysed. • Generally easy to operate once set up and data easy to analyse.	• Samples require pre-processing and pre-concentration which can be time consuming and expensive. • Method development requires experienced users. • Sample analysis can range from 30-60mins and require blank runs and QC qualification which limits throughput.
GC-MS-MS	• Much greater selectivity versus GC-MS. • Can measure a wide mass range including peptides • Enhanced signal-to noise ratio • Can provide more accurate identification of compounds versus GC-MS	• Can require derivitization of metabolites. • Both expensive and has a large lab. footprint. • Also requires experienced personnel for both method development and data analysis.
GC-TOF-MS	• Great mass resolution allows for more accurate identification of compounds. • Has a theoretically unlimited upper mass range. • Very high sensitivity.	• At high signal-to-noise levels reproducibility suffers. • Poor detector linearity compared to a standard quadrupole MS.

	Advantages	Limitations
	• More consistent performance for low concentration compounds. • Rapid acquisition rates.	
GC-GC-TOF-MS	• All advantages of GC-TOF-MS. • Compatible with single GC injection techniques. • Can provide sharper better resolved peaks. • The combination of GC-GC with a TOF-MS is considered the most powerful tool for quantitative VC analysis.	• Disadvantages of GC-TOF-MS. • Long sample run times. • Special software is required. • If the modulation between the two columns is not properly set then separation is compromised.
GC-Sensors	• Low cost. • Highly sensitive. • Rapid throughput. • Easy to operate. • Easy to customise for purpose.	• Lack of standardisation • No qualitative data • Analysis of data can require skilled personnel to set up • Low specificity (though high specificity sensor can be used).

Table 1.2 the advantages and limitations of the GC non-real time techniques discussed in chapter 1 section 2.

Table 1.2 summarises the advantages and limitations for the non-real time techniques discussed in this chapter. The distilled conclusion is that as the analytical power of the instrument increases so too does the cost, laboratory foot print, time, and effort required to analyse samples. Despite this GC-MS and related techniques are unparalleled for the qualitative analysis of VCs.

1.3.0 Real time mass spectrometry techniques

The use of real-time mass spectrometry is still a relatively new and emerging analytical method. These techniques can be highly sensitive and are better suited to providing quantitative data. Moreover, the sample analysis time can be reduced to seconds and in many cases, there is no need to pre-concentrate or prepare samples. However, the real time techniques often lack detailed qualitative data and thus elucidating metabolic data is difficult.

1.3.1 Selected ion flow tube mass spectrometry (SIFT-MS)

Selected ion flow tube mass spectrometry (SIFT-MS) provides real-time quantitative analysis of gases and is capable of detecting a wide variety of analytes in the low part per billion (ppb) range with the new generation of instruments reporting part per trillion levels of sensitivity for selected analytes [85]. In contrast to GC-MS SIFT-MS has no chromatographic phase. The SIFT-MS generates the reagent ions H_3O^+, NO^+, or O_2^+ by mixing air and water vapour in a microwave plasma source. These reagents are

selected by the front quadrupole and the ions used will be determined by their reactivity with the pre-selected analyte compounds. These reagents then enter the flow tube where they are reacted with the sample (see Figure 1.7). This soft chemical ionisation process forms ionised molecules which then are selected through a second quadrupole and on to the MS detector.

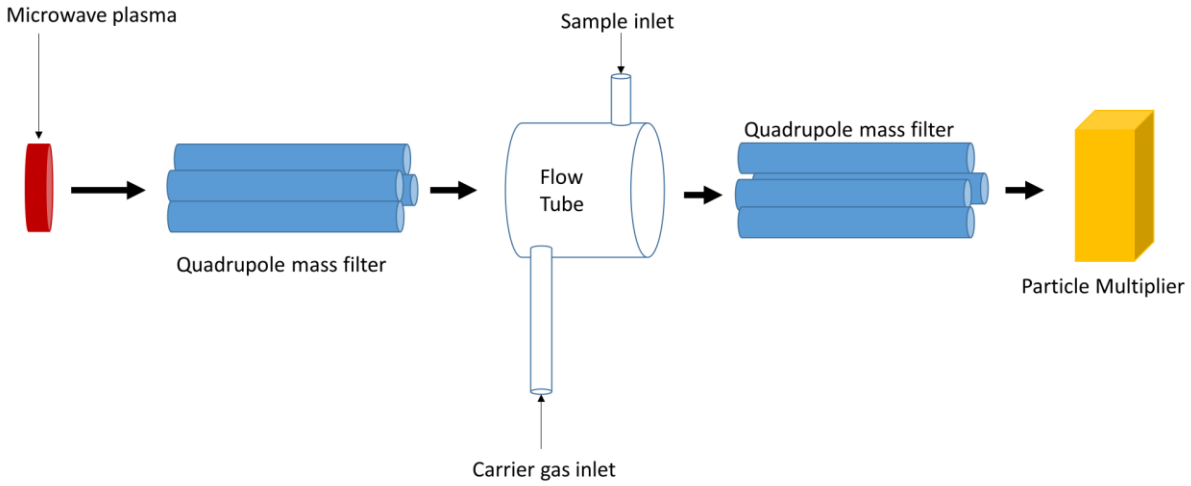


Figure 1.7 schematic of a SIFT-MS as adapted from siftms.com cc 3.0 copyright licence agreement [86] the reagent ions are generated by a mix of water and air in the microwave plasma. The ions are then filtered by the first quadrupole into the flow tube where they are mixed with the sample and carrier gas. The second quadrupole filters the desired molecules based on the selected m/z.

Due to the real-time quantitative output and no requirement for pre-concentration step SIFT-MS is ideally suited for assessment of breath VCs and bacterial headspace analysis, numerous papers exist on both applications; Spanel and Smith 2013, provide a review of SIFT-MS in breath analysis [87].

SIFT-MS offers real-time direct analysis and has excellent sensitivity, thus breath analysis has been used in attempts to diagnose many different disease states such as: breast cancer [88], liver disease [89], malignant biliary strictures [90], renal failure [91], chronic kidney disease [92], Crohn's disease and ulcerative colitis [93], inflammatory bowel disease [94,95], and gastro oesophageal cancer [96].

Breath was analysed on SIFT-MS in a study looking at the difference in VCs resulting from gastro-intestinal disorders including inflammatory bowel disease (IBD), Crohn's disease (CD), ulcerative colitis (UC), and ileal pouch anal anastomosis (IPAA) [97]. This group were able to determine that IBD could be differentiated from CD, UC, and

IPAA. Just as importantly they showed that CD and UC could not be differentiated from one another and that the VC profile was not altered by the inflammation in IPAA [97]. In 2017 Demirjian *et al.* used SIFT-MS breath analysis to attempt to identify a breath-print in patients with end stage renal disease (ESRD); this work showed that they were able to distinguish renal disease patients from healthy controls with a C statistic of 0.99 [91]. While this is a very encouraging result it should be noted that only 86 patients with renal disease were analysed and only 25 healthy volunteers served as controls. Moreover, patients either had ESRD or were completely healthy as such this does not give a realistic analogue for clinic patients. While it would be beneficial to be able to identify the different grades of renal disease from the early stages onward, that process would require numerous patients with a range of clinical stages of disease which, would be time consuming and very costly.

SIFT-MS has been used for the online breath monitoring of acetone, isoprene, and acetic acid while the patient was undergoing surgery ranging in time from 20 minutes to 60 minutes [98]. The results of this work showed that with the exception of isoprene the compounds remained relatively stable; isoprene however doubled in concentration in most cases over the course of the surgery [98]. This work shows that SIFT-MS is not only useful single sample analysis but also continuous monitoring over time. Sovova *et al.,* 2013 used continuous monitoring of bacteria from three species (*Serratia rubidea, Serritia marcescens,* and *E. coli*). Ammonia, ethanol, acetaldehyde, propanol, acetone, and acetic acid were quantitatively analysed for a continuous 24 hr period [99]. This group were able to determine that the bacterial species could be determined by the headspace composition; for instance, the presence of propanol was indicative of an *E. coli* population. This work also demonstrates that growth and death of the bacterial cultures could be monitored without interfering with the sample [99]. Similarly air changes in the atmospheric compounds can also be monitored over time, as in Prince, Milligan, and McEwan, 2010 who monitored toluene, 1,3-butadiene, benzene, ethanol, and ethene over a five day period [100]. The group monitored emissions from the Syft Technologies factory in order to remove organic compound, the inlet gas was heated to 300^{o}C. This group were able to record 1,3-butadiene at a level of 9 pptv with ±4 pptv precision and demonstrated the capabilities of SIFT-MS for the online real time monitoring of VCs [100].

The SIFT-MS allows not only the concentration of selected compounds to be calculated but also a full scan can show the m/z peaks present in the sample which

can be indicative of the compounds present. As shown by the examples discussed the rapid, real-time, non-invasive nature of breath testing makes it an ideal candidate for clinical utility.

1.3.2 Proton transfer reaction mass spectrometry (PTR-MS)

PTR-MS is similar technique to SIFT-MS in that there is a real-time soft chemical ionisation of compounds that results in less fragmentation of compounds than that of traditional quadrupole MS. As with SIFT-MS in most cases there is no need for sample preparation. Even the generation of H_3O^+ is similar to that of the SIFT-MS, however in a PTR-MS system there is no filtering quadrupole and no flow tube (see Figure 1.8). Instead the ions and analyte VCs are mixed in a drift cell, this drift cell has controlled pressure, temperature, and magnetic field thus there is no need for carrier gas which results in a claimed sensitivity several orders of magnitude greater than SIFT-MS [101]. The resulting ions are focused to a beam which are accelerated through different vacuum stages to account for the large pressure difference between the reaction chamber and the quadrupole mass spectrometer (see Figure 1.8).

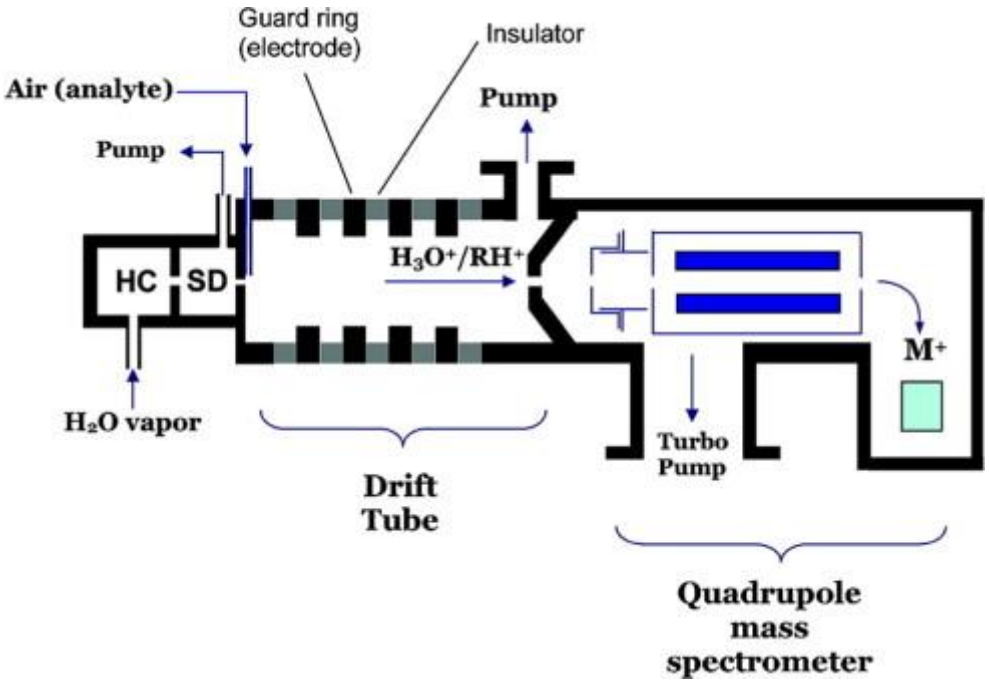


Figure 1.8 a simple schematic of a PTR-MS with permission © 2009 American Chemical Society from Blake, Monks, and Ellis 2009 [102].

Winkler, Herbig, and Ingrid (2013) demonstrated how this real-time technique with its excellent sensitivity (pptv) could be used to elucidate metabolism of VCs in the body [103]. In this paper isotope labelled ethanol was ingested and the breath was

monitored using a PTR-MS instrument, after 10 minutes metabolic products from the breakdown of the labelled ethanol could be seen (deuterated acetone and isoprene).
del Rio *et al.,* 2015 compared the breath of cirrhosis patients pre and post liver transplant using PTR-MS. Overall the group showed that limonene, methanol, 2-pentanone, 2-butanone, and carbon disulphide were all decreased following successful transplant. While limonene had the best individual diagnostic ability (ROC 0.91) this was enhanced when combined with methanol and 2-pentanone (ROC 0.95) [104]. This paper demonstrates nicely that while there may be a VC that serves well as a biomarker the likelihood is that a combination of markers will allow for greater discriminatory capabilities. More recently Zou *et al.,* 2018 used an ultrasonic nebulization technique to analyse urine using PTR-MS; this was used to analyse methanol, acetaldehyde, and acetone from healthy human urine which yielded a recovery of 88.89% and 94.54% this percentage was based on concentrations obtained from the groups previous work [105]. This method requires only 0.66mL of urine making it ideal for patients struggling to provide a full sample [105]. Moreover, this method yields LODs in the low μg/L (tested with standards of methanol (LOD 4.47 μg/L, acetaldehyde LOD 1.98 μg/L, and acetone LOD 3.47 μg/L), and can give a full scan result in 34 seconds.

1.3.3 Proton transfer reaction time of flight mass spectrometry (PTR-TOF-MS)

Another variant of the PTR-MS is with the use of a TOF-MS detector instead of a traditional single quadrupole MS. This retains all the benefits of the PTR system and combines it with the greater resolution and mass range of the TOF-MS detector.
In a study using this system to test coeliac disease patients from healthy controls Aprea *et al.,* 2014 were unable to find any significant difference between the 2 groups. However, their coeliac disease patients were all following a gluten free diet and were asymptomatic thus it is unsurprising that there would be little difference between the groups. Ideally the patients would have unmanaged symptoms or be suspected of having coeliac disease with an unconfirmed diagnosis but of course this would raise ethical issues because it would involve potentially delaying treatment, or encouraging known ceoliacs to eat gluten [106].
A more recent study also used PTR-TOF-MS to investigate dietary impact; Kistler *et al.,* 2016 found that in mice, diet induced obesity altered the VC profile [107]. This group found that when keto-bodies were present as were markers of lipid peroxidation.

(Methylthio) methanethiol was also reduced which is indicative of a link between metabolic changes and reproduction [107]. This paper suggests that breath can be used to not only diagnose potential metabolic changes early but also monitor the progression and regression of these changes in obesity cases. Again, this is an example of how powerful analytical techniques can serve to better our understanding of metabolic processes by determining how and why metabolites are generated in the body. This idea is further supported by work done in 2017 on patients with chronic kidney disease (CKD) in which ammonia was found to be elevated in stage 1 CKD patients in the later stages (2-4) isoprene, pentanal, and heptanal were also elevated, while methylamine was lower in patients versus controls (either patients post-transplant, or with acute infections) [108]. This suggests that it would be possible to detect the early metabolic changes associated with CKD and thus begin monitoring the condition early. The idea of being able to detect a disease such as CKD in the early stages often is crucial for improved recovery outcomes.

1.3.4 Field asymmetric Ion mobility mass spectrometry (FAIMS)

One drawback to both the quadrupole and time-of-flight mass detectors is the need for high vacuum conditions, this requirement adds bulk, expense, and additional equipment maintenance. FAIMS circumvents this obstacle by being able to perform analysis at atmospheric pressure and has been found to be functional at above 1500 torr [109]. Guevremont and Purves (1999) detail the mechanism of action for FAIMS [110] see Figure 1.9 taken from [111]. In short, the gas flow is ionized with a UV photo-ionization (photons are injected into the ion channel) or corona discharge source (which generates a reagent ion) before entering a magnetic field which causes the ions to drift, this allows separation of ions depending on their electrical properties. Once in the detector region the ions are deflected toward the detector, this ion beam

can be focused by using the electrical field to narrow the ion cloud [110,112].

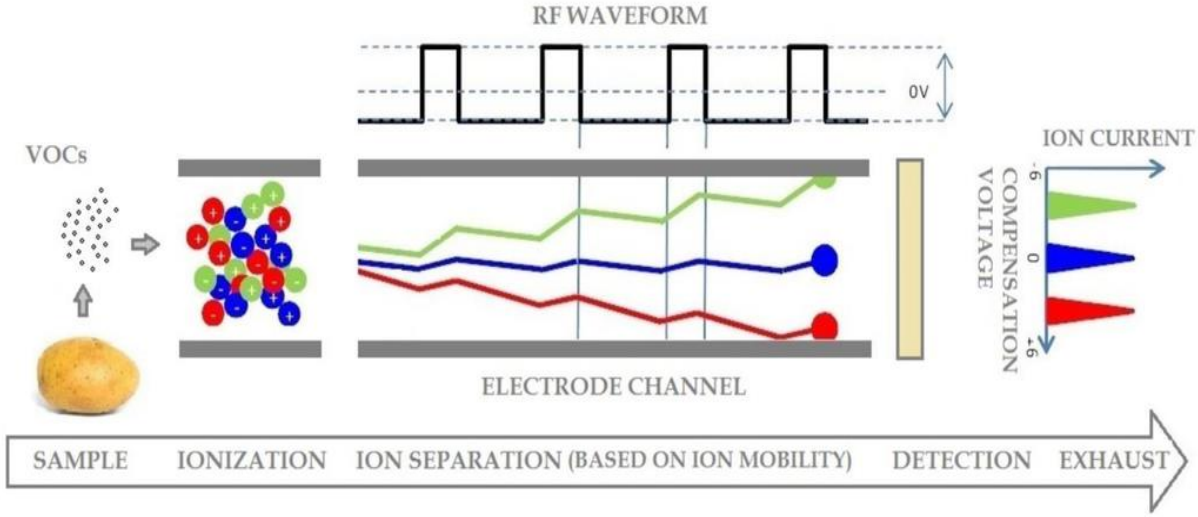


Figure 1.9 taken from Rutolo *et al,* 2014 [111] published in open access journal © cc shows a schematic for the mechanism by which a FAIMS system operates.

In section 1.3.3 it was noted Aprea *et al.,* 2014 used PTR-TOF-MS to investigate coeliac disease patients with little success. Arasaradnam *et al.* also in 2014 published work in which they attempt to differentiate coeliac disease from IBS. The FAIMS instrument in this instance was able to differentiate coeliac and IBS patients with a ROC of 0.91 [113]. While all the participants in this case were patients with diagnosed conditions and very few other variations were considered it nevertheless demonstrates the point that the ability to distinguish between diseases with overlapping symptoms has the most clinical utility. The paper also makes the point that an at home breath test for patients with chronic gastrointestinal conditions could be very useful for monitoring treatment compliance. The same group a year later used breath samples to determine if inflammatory bowel disease (IBD) could be differentiated from healthy controls. In this case the test had a sensitivity and specificity of 0.74 and 0.75 respectively [114], FAIMS is ideally suited to this type of testing as it can be done at the point-of-care.

FAIMS was also tested for the rapid detection of *C. difficile* infections and was found to have a ROC score of 0.86 on blinded samples [115]; this was done by training an algorithm with a batch of samples; then a second batch of samples was used to test this algorithm with double blind samples. Double blind validation of a new method is something that is rarely reported and yet is absolutely crucial to the development of a clinically useful test. By taking this extra measure the group show how the test could

be applied clinically; the benefit of FAIMS is that once everything is set up it can be operated by semi-skilled personnel.

1.3.5 Summary and comparisons of real-time techniques

Table 1.3 summarises the advantages and limitations of real time MS techniques, in general these techniques are able to provide accurate quantification (with the exception of FAIMS) at very low levels making them ideal for trace VC analysis. As such these instruments all have numerous applications such as: agri-food, environment, and, as discussed in this chapter, biomedical. Since these instruments offer rapid real time outputs, they are ideally suited for breath analysis and numerous studies have been performed to reflected this (see sections 1.3.1-1.3.4 for examples) However, the real time techniques usually require target analytes in order to achieve quantification. Moreover, the qualitative data obtained by these instruments is usually only indicative of the compounds present and offers no concrete identification. Perhaps though, the biggest challenge faced by real time MS is the overlap of compounds with the same m/z value [50].

	Advantages	Limitations
SIFT-MS	• Provides real time quantification of compounds. • Simple to operate. • Easy to interpret data. • Can discriminate isomers. • Highly sensitive (pptv levels) • Can detect compounds GC-MS can-not such as amines and thermally unstable compounds. • High throughput • No sample pre-processing. • Multiple reagent ions	• Quantification relies on having a target analyte with known kinetic parameters. • Very expensive *ca.* £250,000. • Difficult to get meaningful qualitative data. • Having two quadrupoles under vacuum can mean more maintenance.
PTR-MS	• No front quadrupole means cost is reduced versus SIFT-MS and PYR-TOF-MS • High throughput. • Can provide quantification within 20% accuracy without compound indentification. • Provides real time quantification of known compounds. • Can be used in conjunction to with GC methods. • More sensitive then SIFT-MS. • Easy to maintain.	• Only use the H_3O^+ reagent ion. • LOD determination requires instrument calibration with standards. • Possible interference from other compounds with same m/z ratio. • Low mass resolution means separation of isomers is not possible.

	Advantages	Limitations
PTR-TOF-MS	• High throughput. • Greater mass resolution means isomers can be resolved even in complex matrices. • No front quadrupole means the instrument in smaller than SIFT-MS. • Can rapidly detect the full mass spectrum in one TOF pulse. • Data can be mined to find unexpected compounds. • Easy to maintain. • Greater sensitivity versus SIFT-MS.	• Comparable cost to SIFT-MS. • Data processing can be complicated and time consuming. • Not as sensitive as PTR-MS. • Still vulnerable to m/z overlap in complex matrices.
FAIMS	• Compared to other real time techniques FAIMS is inexpensive. • High throughput. • No vacuum means very small lab. footprint. • Can be easily interfaced with other techniques such as GC, ESI, TD, and SPME. • Very easy to operate.	• Data processing can be difficult and is not standardised. • Reproducibility and calibration across instruments are difficult. • Ions can be separated differently across different instruments which makes method validation and centralised data processing challenging. • Prone to contamination from high concentration of compounds, • Humidity and contamination from the purge air are also common. • Difficult to interpret instrument performance from non-experienced users. • Does not provide quantitative outputs.

Table 1.3 advantages and limitations of the real time MS techniques discussed in chapter 1 section 3.

1.4.0 Overview

Sections 1.2 and 1.3 gives multiple examples of a number of applications for MS techniques in the biomedical sector.

While the chromatography based non real-time techniques require more sample preparation and in general by their nature take longer per sample analysis time; the metabolic insights they are capable of delivering with their qualitative data makes the cost to benefit worthwhile. Moreover, these techniques do require a reasonable skill level to operate and maintain. However, the qualitative data and the identification of VCs is crucial to understanding the metabolic changes that take place during disease states and thus understanding how VCs can be used in the clinical forum. The quality of data that can be collected from these instruments makes the shortcomings worthwhile.

Real-time techniques are in general simple to operate and can be done by semi-skilled operators. While non-real time techniques can also be easy to operate, they do require significantly more method development which does require expertise. The analysis time per sample of real-time techniques is very short (usually 2-3 minutes), which makes these techniques ideal for high throughput clinical laboratories. There is also the option to bring the patient to the instrument for breath analysis. However, the data collected from real-time techniques requires the use of algorithms or pattern recognition methods for differentiation. Moreover, in order to obtain quantitative data from the SIFT-MS target compounds must be identified. In the clinical setting this will require rigorous biomarker identification and validation.

Chapter 2: Assessment of a combined gas chromatography mass spectrometer sensor (GC-MSS) system for detecting biologically relevant volatile compounds (VCs).

The work in this chapter has been published in the Journal of Breath Research: -

Gould, O.; Wieczorek, T.; Costello, B. P. J. de L.; Persad, R.; Ratcliffe, N. Assessment of a combined gas chromatography mass spectrometer sensor (GC-MSS) system for detecting biologically relevant volatile compounds (VCs). *J. Breath Res.* **2017**, doi:10.1088/1752-7163/AA8EFE

2.1.0 Introduction

There is increasing interest in the design and fabrication of compact volatile compounds (VCs) sensor systems for disease diagnosis. Electronic nose (E-nose) systems based on: conducting polymers [116], cantilevers [117,118], ceramic sensors [119], colorimetric arrays [120], and GC-sensor systems [121–125] 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 [126–129]. 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 [130–133] to explosives detection [134] and waste management odour analysis [135]. Much of this research utilises ‘E-nose’ technology which uses an array of sensors combined with pattern recognition systems [130,131,133].

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 [123]. 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 [124]. 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 [125], ethylene and low molecular weight. hydrocarbons [136], aromatic volatiles [137], hydrogen fluoride vapours [138], benzene, toluene and xylene [139] and hydrogen, methane and carbon monoxide [140]. 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 [141,142]. 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 [143]; 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, [144] for five alcohols, acetaldehyde, acetone and ethyl acetate, which reports detection limits of several ppb and [145] 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 [83,126,129]. 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.

2.2.0 Methods

2.2.1 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 [146]. In short, a 3 mm alumina substrate was screen printed with gold interdigitated electrodes (four pairs of interpenetrating bars, electrode gap 100 µm) 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 <100 nm (Sigma Aldrich), and 50% tin oxide (IV) nanopowder <100 nm (Sigma Aldrich) (by weight) using the doctor blade technique detailed in [146–148].

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 2.4). 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 2.4). 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^{o}C; with a column flow of 3.1 mL/min at 40^{o}C dropping to 1.6 mL/min at 240^{o}C.

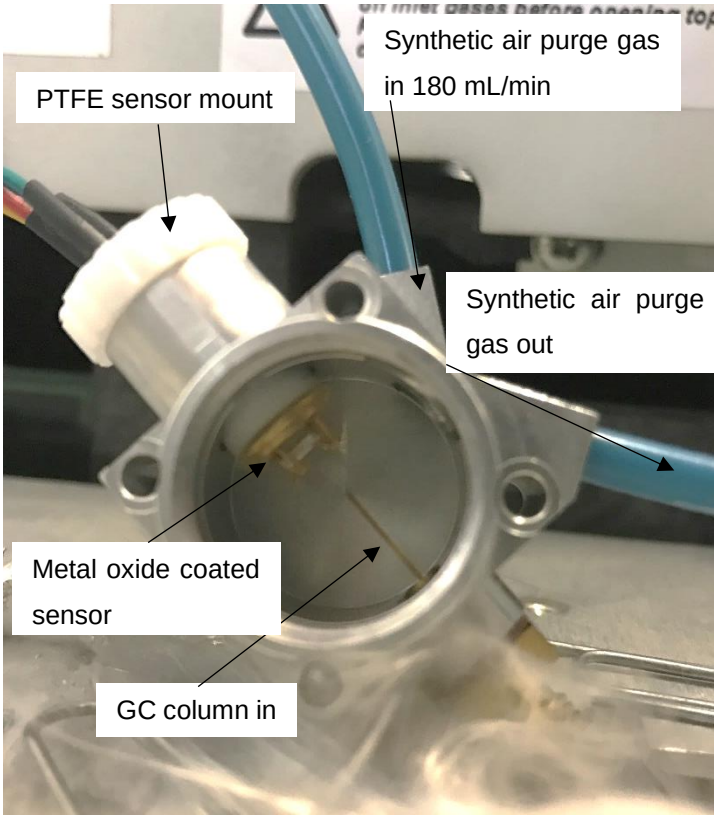


Figure 2.10 the aluminium sensor chamber with bespoke sensor mounted on PTFE interfaced with the GC column; purge gas (synthetic air) flows through the chamber at 180 mL/min.

2.2.2 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: 40ºC initial oven temperature, held for 2 minutes followed by a temperature ramp of 10ºC/minute up to 240ºC and hold at 240ºC 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 22.8 psi, 31.5 cm/s.

2.2.3 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 and each one analysed within 8 hours of preparation.

All chemicals used for this phase of testing are shown in Table 2.4 with grade and supplier. Compound groups were as follows:

Solution 1: Starting stock 10 g/L

Compound	Solubility in water
Methanol	Miscible
Ethanol	Miscible
Propanol	Miscible
Butanol	73 g/L (25^{o}C)
Acetone	Miscible
Phenol	83 g/L (25^{o}C)

Solution 2: Starting stock 1 g/L

Compound	Solubility in water
Acetaldehyde	Miscible
Propanal	540 g/L (20^{o}C)
Ethyl methanoate	88.2 g/L (25^{o}C)
Butanal	76 g/L (20^{o}C)
2-Butanone	290 g/L (20^{o}C)
Ethyl propanoate	25 g/L (15^{o}C)
Ethyl butanoate	4.9 g/L (20^{o}C)
Butyl propanoate	1.5 g/L (20^{o}C)

Solution 3: Starting stock 0.1 g/L

Compound	Solubility in water
Furan	10g/L (25^{o}C)
2,3-Butadione	200 g/L (20^{o}C)
Dimethyl disulphide	2.5 g/L (20^{o}C)
Pyrrole	45 g/L (25^{o}C)
Benzaldehyde	6.95 g/L (25^{o}C)
Benzyl alcohol	42.9 g/L (25^{o}C)
p-Cresol	21.5 g/L (25^{o}C)
2-Nitrotoluene	0.44 g/L (20^{o}C)
Methyl salicylate	0.7 g/L (30^{o}C)
Indole	3.56 g/L (25^{o}C)

Solution 4: Starting stock 10 g/L

Compound	Solubility in water
Ethanoic acid	Miscible
Propanoic acid	Miscible
Butanoic acid	Miscible
Pentanoic acid	24 g/L (25^{o}C)
Hexanoic acid	11 g/L (20^{o}C)

In order to make the dilutions 10 mL of the stock solution (for example 10 g/L) was added to a 100 mL volumetric flask and then topped to the fill line with deionized water, to give a 10 fold dilution in this example 1 g/L. For the next dilution 10 mL of the 1 g/L was added to a 100 mL volumetric flask and this was also topped up to the fill line with deionized water. This process was repeated as many times as required by the testing.
Solution 1 concentrations analysed were: 10 g/L, 1 g/L, 0.1 g/L, 0.01 g/L, 0.001 g/L, 0.0001 g/L, 0.00001 g/L, 0.000001 g/L.
Solution 2 concentrations analysed were: 1 g/L, 0.1 g/L, 0.01 g/L, 0.001 g/L, 0.0001 g/L, 0.00001 g/L, 0.000001 g/L, 0.0000001 g/L
Solution 3 concentrations analysed were: 0.1 g/L, 0.01 g/L, 0.001 g/L, 0.0001 g/L, 0.00001 g/L, 0.000001 g/L, 0.0000001 g/L
Solution 4 concentrations analysed were: 10 g/L, 1 g/L, 0.1 g/L, 0.01 g/L.
For sampling, 3 mL of a solution was removed to a headspace vial (PTFE/ silicone septa 10 mL 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 two minutes at ambient temp (21-25^{o}C) without stirring. The fibre was then inserted into the GC-MS port at 220^{o}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 carry over 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. This phase of experimentation took place over a four 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.

CAS-number	Compound	Grade	Supplier
75-07-0	Acetaldehyde	99.5%	Sigma-Aldrich
67-64-1	Acetone	99.9%	Fisher Scientific
100-52-7	Benzaldehyde	>99%	Sigma-Aldrich
100-51-6	Benzyl alcohol	>99%	Sigma-Aldrich
123-72-8	Butanal	99.8%	Sigma-Aldrich
431-03-8	2,3-Butadione	>99%	Sigma-Aldrich
71-36-3	Butanol	>99%	Sigma-Aldrich
78-93-3	2-Butanone	>99%	Acros organics
590-01-2	Butyl propanoate	>99%	Acros organics
107-92-6	Butanoic acid	>99%	Sigma-Aldrich
624-92-0	Dimethyl disulphide	>99%	Sigma-Aldrich
64-19-7	Ethanoic acid	99.5%	Sigma-Aldrich
64-17-5	Ethanol	100%	VWR chemicals
105-54-4	Ethyl butanoate	99%	Sigma-Aldrich
141-78-6	Ethyl methanoate	>97%	Sigma-Aldrich
105-37-3	Ethyl propanoate	>99%	Acros organics
110-00-9	Furan	>99%	Sigma-Aldrich
142-62-1	Hexanoic acid	99.5%	Sigma-Aldrich
120-72-9	Indole	>99%	Sigma-Aldrich
67-56-1	Methanol	>99.9%	Sigma-Aldrich
119-36-8	Methyl salicylate	>99%	Sigma-Aldrich
88-72-2	2-Nitrotoluene	>99%	Sigma-Aldrich
106-44-5	p-Cresol	>99%	Sigma-Aldrich
109-52-4	Pentanoic (valeric) acid	>99%	Acros organics
108-95-2	Phenol	>99%	Sigma-Aldrich
123-38-6	Propanal	>99%	Acros organics
79-09-4	Propanoic acid	>99.5%	Sigma-Aldrich
71-23-8	Propanol	>99.5%	BDH chemicals
109-97-7	Pyrrole	>99%	Acros organics

Table 2.4 the grade and suppliers of compounds used to make the 29 stock standard solutions.

2.2.4 Stool samples

Stool samples from healthy volunteers of diverse ethnic origins, gender and a mix of omnivore and vegetarian diets were collected (NHS Newcastle and North Tyneside research ethics committee reference 14/NE/0029). Three g were aliquoted into 20 mL headspace vials and stored in a freezer at -20^{o}C. For sampling the vials were defrosted in a water bath set to 60^{o}C for 30 minutes the SPME fibre was then added for 30 minutes while the vial was maintained at 60^{o}C..

2.2.5 Bacterial culture headspace analysis

Two colonies of *E. coli* were picked from an overnight culture on an agar plate (Oxoid CM0003) and used to inoculate 5 mL of nutrient broth (Oxoid CM0067) in a sterile glass universal bottle. The broths were incubated overnight at 37^{o}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 5 mL nutrient broth (Oxoid CM0067). One was frozen at -20^{o}C immediately with the others undergoing 2, 4- and 24-hours further incubation respectively before freezing. One mL of each sample was extracted prior to freezing for final absorbance measurements.
Prior to analysis vials were defrosted in a water bath set to 60^{o}C for 30 minutes before the SPME fibre (as used for both the stool and standards) was inserted for 30 minutes sample adsorption time, as for the stool samples the vial was maintained at 60^{o}C for the duration of the sampling.

2.2.6 Analysis

The same signal threshold of three times the noise was used for both the GC-MS chromatograms and the resistance trace from the sensor. The NIST library (version 2.2, 2014) was used for peak identification. 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 unidentified 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). The standard solutions were considered undetectable when sufficiently diluted such that the signal threshold was no longer met.

To analyse the sensor data the change in resistance from the base of the peak to the top of the peak was calculated for each response. Since the sensor output forms one continuous trace containing multiple GC-MS runs a marker was set as each GC-MS injection took place; this marker could then be used a T=0 minutes point to synchronise the chromatogram and sensor responses.

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 for data analysis; as this allows the data to be viewed as a function of the slope. ΔR/ΔT is a display function only and does not enhance the detection capabilities of the sensor. However, ΔR/ΔT 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.

2.3.0 Results and discussion

2.3.1 Results of the analysis of standard solutions

The 450^{o}C sensor operating temperature was derived experimentally. At 450^{o}C the water to ethanol response ratio was four times greater than when operated at 350^{o}C which gave a response ratio of close to one.

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 [149]. Figure 2.11 shows the full comparison of mode average LODs for all 29 VOCs, Figure 2.12 shows the data for each repeat. The MOS sensor showed greater LOD for 17 compounds 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 nine 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. Previous work shows there are advantages in using a mixed system for enhanced detection of a range of VOCs [147,148].

Table 2.5 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 as the solutions being used have factor of ten difference, the actual LOD would likely be at a value between the two solutions.

CAS-number	Compound	Vapour concentration (ppb)	Solution concentration (g/L)
75-07-0	Acetaldehyde	16215	$1.00x10^{-02}$
67-64-1	Acetone	57	$1.00x10^{-04}$
100-52-7	Benzaldehyde	242	$1.00x10^{-03}$
100-51-6	Benzyl alcohol	1027	$1.00x10^{00}$
123-72-8	Butanal	159	$1.00x10^{-04}$
431-03-8	2,3-Butadione	204	$1.00x10^{-03}$
71-36-3	Butanol	11	$1.00x10^{-04}$
78-93-3	2-Butanone	69	$1.00x10^{-04}$
590-01-2	Butyl propanoate	0.01	$1.00x10^{-05}$
107-92-6	Butanoic acid	5973	$1.00x10^{00}$
624-92-0	Dimethyl disulphide	115	$1.00x10^{-05}$
64-19-7	Ethanoic acid	3028	$1.00x10^{00}$
64-17-5	Ethanol	109	$1.00x10^{-03}$
105-54-4	ethyl butanoate	179	$1.00x10^{-04}$
141-78-6	Ethyl methanoate	329	$1.00x10^{-04}$
105-37-3	Ethyl propanoate	169	$1.00x10^{-04}$
110-00-9	Furan	8162	$1.00x10^{-04}$
142-62-1	Hexanoic acid	6149	$1.00x10^{00}$
120-72-9	Indole	1187	$1.00x10^{-01}$
67-56-1	Methanol	1419	$1.00x10^{-02}$

CAS-number	Compound	Vapour concentration (ppb)	Solution concentration (g/L)
119-36-8	Methyl salicylate	59	$1.00x10^{-02}$
88-72-2	2-Nitrotoluene	4289	$1.00x10^{-02}$
106-44-5	p-Cresol	711	$1.00x10^{-01}$
109-52-4	Pentanoic (valeric) acid	4451	$1.00x10^{00}$
108-95-2	Phenol	366	$1.00x10^{-01}$
123-38-6	Propanal	1324	$1.00x10^{-03}$
79-09-4	Propanoic acid	2177	$1.00x10^{00}$
71-23-8	Propanol	119	$1.00x10^{-03}$
109-97-7	Pyrrole	230	$1.00x10^{-03}$

Table 2.5 our concentrations in ppb for corresponding solution concentration to give indicative values for limits of detection; all concentrations calculated using Henry's law constants taken from Sander, Rolf. "Compilation of Henry's law constants (version 4.0) for water as solvent." *Atmospheric Chemistry & Physics* 15, no. 8 (2015).

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 these are 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 [150]. Siloxanes are relatively large, stable molecules yet they are volatile. While they are readily ionised in the mass spectral analyser the literature shows no instances where 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 six 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 (10 g/L) RT for the MS was 14.10 and the lowest

concentration (0.0001 g/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 slow that the next compound appears only as a change of gradient and not a peak response; in these cases, viewing the resistance as $\Delta R/\Delta T$ can help the resolution of responses from closely eluting compounds. In instances of the true co-elution of two 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.

The chamber was originally made from aluminium as it was easy to manufacture various iterations of the chamber design. We attempted to minimise all plastic 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 meant that we did not 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 four-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.

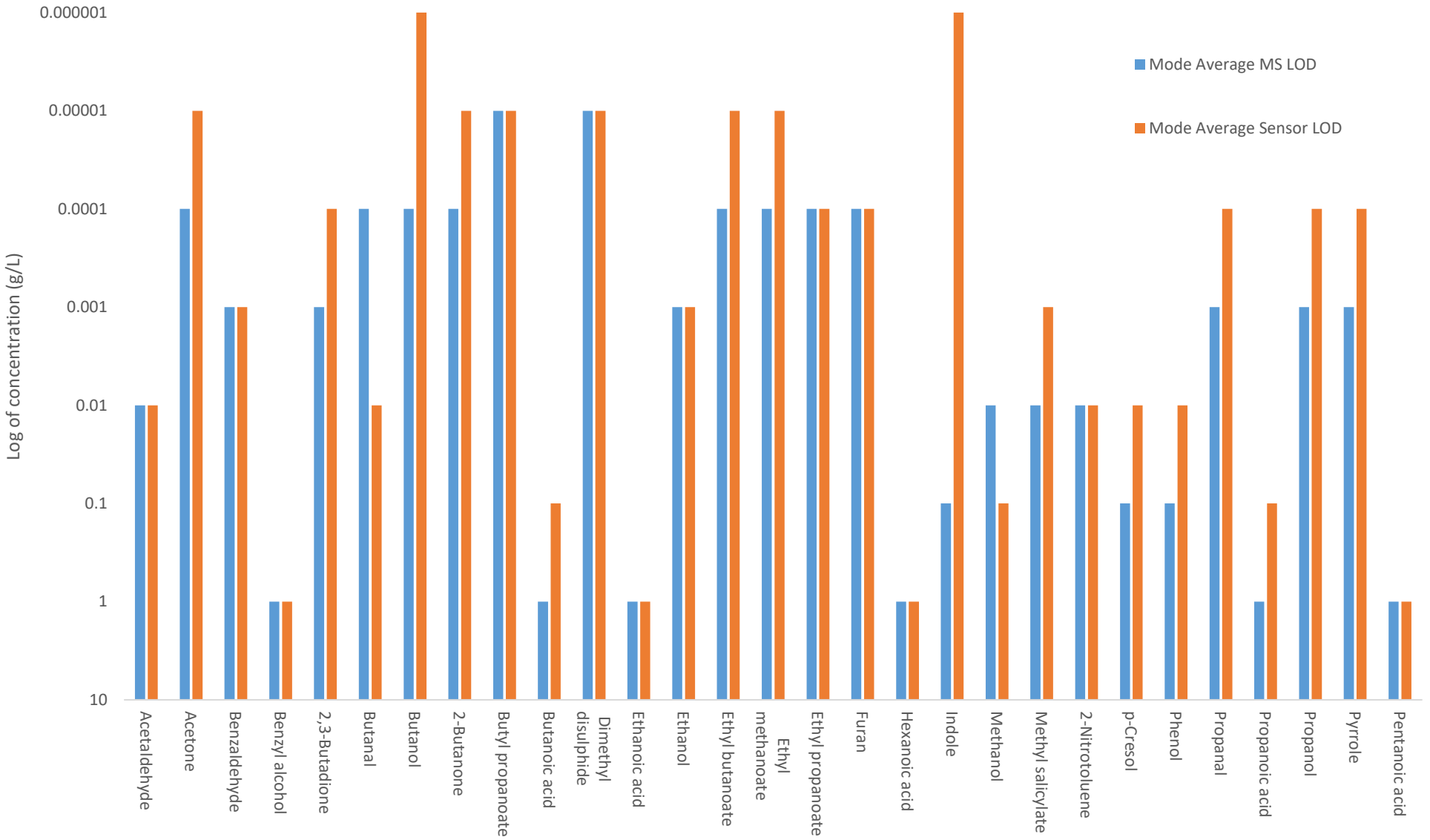


Figure 2.11 the limit of detection concentration of standard chemicals in deionised water, comparing a single quadrupole mass spectrometer with the mixed metal oxide (SnO_2 and ZnO) sensor, plotted on a log scale using the mode average.

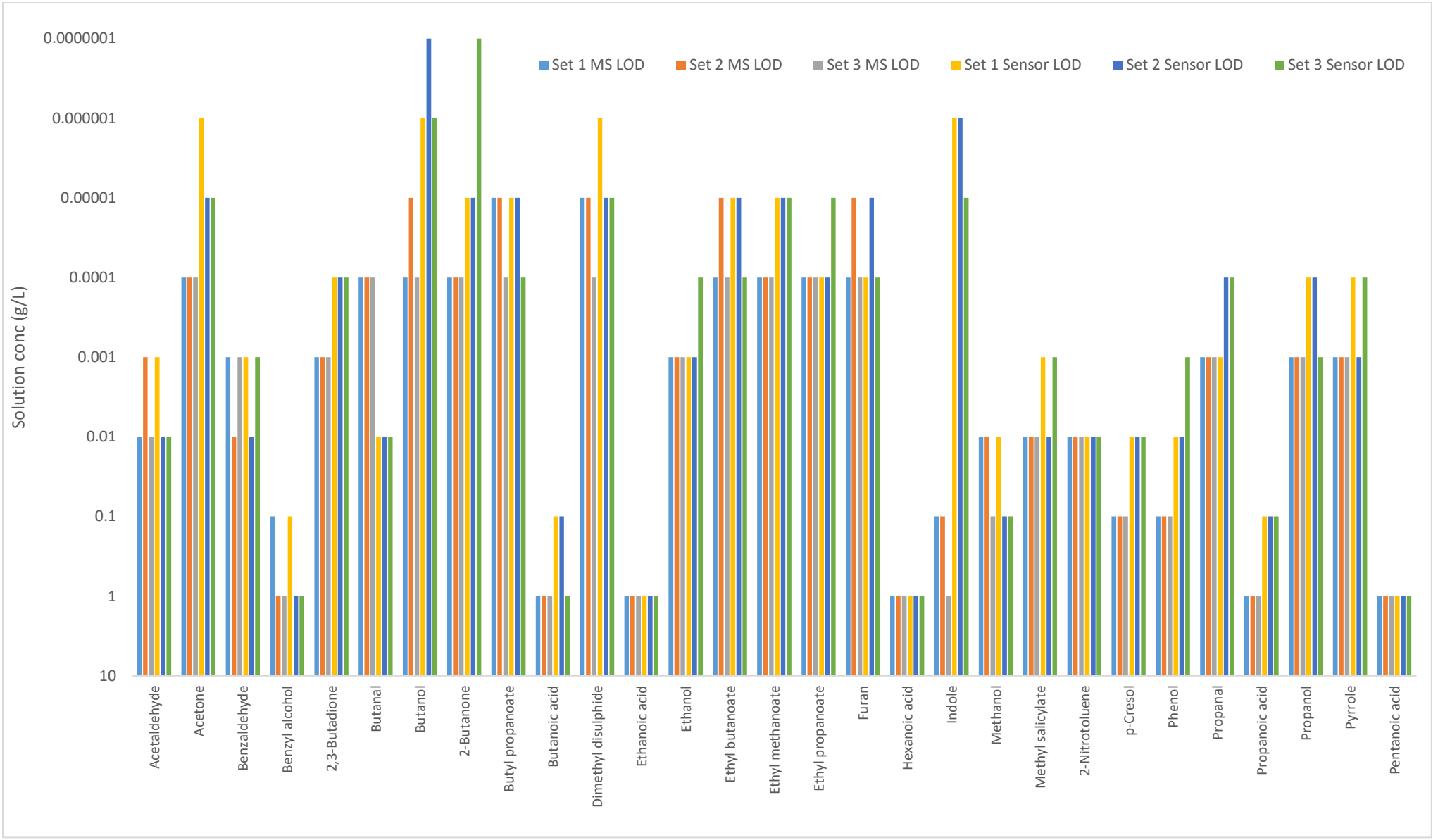


Figure 2.12 comparison of the detection limits for all the compounds used in standard solutions for the MS and sensor, plotted on a log scale for all repeats

2.3.2 Stool Samples

Across the 12 stool samples 84 compounds were detected, those which have a corresponding chemical standard from the standard solution experiments are shown in Table 2.6 along with the retention times. The remaining NIST matched compounds are shown in Table 2.7. On at least one occasion across the sample set each compound had a sensor response with a matching RT. Table 2.8 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 2.6 the list of the compounds detected from the headspace of stool from healthy participants that have been matched to the standards used to assess the LOD of the mass analyser verses the MOS.

CAS-number	Compound	MS RT (mins)	Sensor RT (mins)
74-93-1	Methanethiol	9.21	9.33
75-18-3	Dimethyl sulphide	10.86	10.9
79-20-9	Methyl ethanoate	11.07	11.14
78-84-2	2-Methylpropanal	11.79	11.85
141-78-6	Ethyl ethanoate	12.47	12.50
554-12-1	Methyl propanoate	12.95	12.92
78-83-1	2-Methyl-1-propanol	13.32	13.39
7803-49-8	Hydroxylamine	13.37	13.42
590-86-3	3-Methylbutanal	13.73	13.75
96-17-3	2-Methylbutanal	13.89	13.90
547-63-7	Methyl isobutyrate	13.95	13.94
107-87-9	2-Pentanone	14.42	14.47
563-80-4	3-Methyl 2-butanone	14.42	14.55
24653-75-6	Mercaptoacetone	14.51	14.53
109-60-4	Propyl ethanoate	14.52	14.55
110-62-3	Pentanal	14.55	14.51
623-42-7	Methyl butanoate	14.70	14.74
97-62-1	Ethyl-2-methylpropanoate	15.29	15.45
17220-38-1	Furazandiamine	15.40	15.39
137-32-6	2-Methyl-1-butanol	15.42	15.45
868-57-5	Methyl-2-methylbutanoate	15.71	15.99
71-41-0	1-Pentanol	15.95	16.00
106-36-5	Propyl propanoate	16.25	16.29
591-78-6	2-Hexanone	16.36	16.37
24552-04-3	3-Octanamine	16.36	N/D
123-86-4	Butyl ethanoate	16.37	16.40
66-25-1	Hexanal	16.49	16.52
624-24-8	Methyl pentanoate	16.58	16.60
540-42-1	2-Methylpropyl propanoate	16.78	16.98
108-64-5	Ethyl 3-Methylbutanoate	17.05	17.07
644-49-5	Propyl 2-methylpropanoate	17.08	17.05
111-84-2	Nonane	17.42	N/D
503-74-2	3-Methylbutanoic acid	17.66	17.70
111-27-3	1-Hexanol	17.77	17.80
116-53-0	2-Methylbutanoic acid	17.80	17.85
105-66-8	Propyl butanoate	17.86	17.87
110-43-0	2-Heptanone	18.16	18.18
57-06-7	Allyl isothiocyanate	18.35	18.40
106-70-7	Methyl hexanoate	18.35	18.39

CAS-number	Compound	MS RT (mins)	Sensor RT (mins)
79-31-2	2-methylpropanoic acid	18.37	18.46
	Terpene (1)	18.62	18.62
557-00-6	Propyl 3-methylbutanoate	18.73	18.83
539-90-2	2-Methylpropyl butanoate	18.83	18.83
23747-45-7	S-Methyl 3-methylbutanethioate	18.92	18.96
2179-60-4	Methyl propyl disulphide	19.00	19.03
928-68-7	6-methyl-2-heptanone	19.28	19.27
	Terpene (2)	19.36	19.39
109-21-7	Butyl butanoate	19.49	19.55
2137-56-0	Pentyl pentanoate	19.51	19.55
110-93-0	6-methyl 5-hepten-2-one	19.79	19.83
	Terpene (3)	19.93	19.89
3658-80-8	Dimethyl trisulphide	20.02	20.04
138-86-3	Limonene	20.21	20.24
7341-17-5	2-ethyl-1-hexanethiol	20.48	20.52
470-82-6	Eucalyptol	20.51	20.53
22104-69-4	Methyl 2-heptenoate	21.07	21.13
	Terpene (4)	21.40	21.42
3289-28-9	Ethyl cyclohexanecarboxylate	22.12	22.17
98-89-5	Cyclohexanecarboxylic acid	22.60	22.70
108-93-0	Cyclohexanol	23.18	23.24
	Terpene (5)	25.34	25.44
	Terpene (6)	26.33	26.37
	Terpene (7)	27.77	27.80
	Terpene (8)	28.60	28.63
83-34-1	3-Methylindole	28.87	28.97

Table 2.7 the list of NIST matched compounds detected in the headspace of 12 stool samples from healthy participants with MS and sensor RT.

CAS- number	Compound	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8		Sample 9		Sample 10		Sample 11		Sample 12	
		MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS
75-07-0	Acetaldehyde	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			*	*
67-64-1	Acetone	*		*	*	*	*	*	*	*	*	*		*		*		*	*	*	*	*	*	*	*
57-06-7	Allyl isothiocyanate											*	*							*					
123-72-8	Butanal	*	*	*	*	*	*			*	*					*	*	*	*						
431-03-8	2,3-Butadione							*	*			*	*												
107-92-6	Butanoic acid	*	*	*	*	*	*	*	*	*	*	*	*			*	*	*	*	*	*	*	*	*	*
71-36-3	Butanol	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*					*	*	*	*
78-93-3	2-Butanone			*	*	*	*	*	*	*	*	*	*	*	*										
109-21-7	Butyl butanoate	*	*	*	*	*	*	*	*									*	*			*	*		
123-86-4	Butyl ethanoate	*	*			*	*																		
590-01-2	Butyl propanoate	*		*	*	*		*												*		*	*	*	*
98-89-5	Cyclohexanecarboxylic acid																			*	*	*	*		
108-93-0	Cyclohexanol																			*	*			*	*
624-92-0	Dimethyl disulphide	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
75-18-3	Dimethyl sulphide	*	*	*	*	*	*			*		*	*	*	*	*	*	*	*						
3658-80-8	Dimethyl trisulphide	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
64-19-7	Ethanoic acid	*	*	*	*	*	*			*	*					*	*			*	*	*	*	*	*
64-17-5	Ethanol	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
105-54-4	Ethyl butanoate	*	*	*	*	*	*	*	*					*	*	*	*			*	*	*	*	*	*
3289-28-9	Ethyl cyclohexanecarboxylate	*	*																*	*	*				
141-78-6	Ethyl ethanoate	*	*																	*	*	*	*	*	*
7341-17-5	2-Ethyl-1-hexanethiol	*		*	*	*	*																		
108-64-5	Ethyl 3-methylbutanoate	*		*		*	*							*	*	*	*	*	*			*	*		
97-62-1	Ethyl-2-methylpropanoate	*	*			*																*		*	*
105-37-3	Ethyl propanoate													*						*	*	*		*	*

		Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8		Sample 9		Sample 10		Sample 11		Sample 12	
CAS- number	Compound	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS
470-82-6	Eucalyptol							*	*																
17220-38-1	Furazandiamine																			*	*				
110-43-0	2-Heptanone			*	*	*		*	*	*	*	*	*							*	*			*	
66-25-1	Hexanal			*	*	*	*			*	*	*	*	*	*	*	*	*	*	*	*				
111-27-3	1-Hexanol					*	*	*	*																
591-78-6	2-Hexanone			*	*			*	*	*	*	*	*	*	*	*	*	*	*			*	*		
7803-49-8	Hydroxylamine			*	*															*	*				
120-72-9	Indole	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
138-86-3	Limonene	*	*	*		*	*	*	*	*	*			*	*	*	*	*	*	*	*	*	*	*	*
24653-75-6	Mercaptoacetone	*	*	*	*	*	*	*	*							*	*								
74-93-1	Methanethiol	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*				
96-17-3	2-Methylbutanal	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*					*	*
590-86-3	3-Methylbutanal	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			*	*
623-42-7	Methyl butanoate	*		*	*	*		*						*		*				*	*	*	*	*	*
116-53-0	2-Methylbutanoic acid					*	*			*	*	*	*	*	*	*	*	*	*						
503-74-2	3-Methylbutanoic acid	*	*	*	*					*		*	*	*	*	*	*	*	*	*	*	*	*	*	*
137-32-6	2-methyl-1-butanol			*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*						
563-80-4	3-Methyl 2-butanone	*	*					*								*		*	*			*	*		
79-20-9	Methyl ethanoate	*																		*	*	*	*	*	
928-68-7	6-methyl-2-heptanone																			*	*				
22104-69-4	Methyl 2-heptenoate	*	*	*	*	*	*	*																	
110-93-0	6-methyl 5-hepten-2-one	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
106-70-7	Methyl hexanoate	*	*					*	*																
83-34-1	3-Methylindole			*	*	*	*	*	*			*	*	*	*			*	*						
547-63-7	Methyl isobutyrate																			*	*				
503-74-2	Methyl isopentanoic acid	*																				*			

		Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8		Sample 9		Sample 10		Sample 11		Sample 12	
CAS- number	Compound	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS
868-57-5	Methyl-2-methylbutanoate							*								*	*			*	*				
624-24-8	Methyl pentanoate	*				*		*	*											*	*	*	*		
554-12-1	Methyl propanoate	*		*	*	*	*	*	*							*	*					*	*	*	*
78-84-2	2-Methylpropanal	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*				
79-31-2	2-Methylpropanoic acid																								
78-83-1	2-Methyl-1-propanol							*	*	*	*	*	*	*	*			*	*						
539-90-2	2-Methylpropyl butanoate	*		*		*	*							*	*							*	*		
540-42-1	2-Methylpropyl propanoate			*	*																				
2179-60-4	Methyl propyl disulphide													*	*										
111-84-2	Nonane			*																					
24552-04-3	3-Octanamine																								
106-44-5	p-Cresol	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			*	*
110-62-3	Pentanal													*	*										
109-52-4	Pentanoic acid			*	*	*	*			*	*			*	*	*	*			*	*	*	*	*	*
71-41-0	1-Pentanol	*	*	*	*	*	*	*	*	*	*	*	*	*	*			*	*						
107-87-9	2-Pentanone			*	*	*				*	*	*													
2137-56-0	Pentyl pentanoate																			*	*			*	*
108-95-2	Phenol	*	*	*	*	*	*	*	*											*	*	*	*	*	*
79-09-4	Propanoic acid	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
71-23-8	Propanol	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*
105-66-8	Propyl butanoate	*	*	*		*		*	*					*	*	*	*	*	*	*	*	*	*	*	*
109-60-4	Propyl ethanoate											*	*							*	*	*	*	*	
644-49-5	Propyl 2-methylpropanoate			*	*	*	*													*	*				
557-00-6	Propyl 3-methylbutanoate	*		*		*														*	*	*	*		
106-36-5	Propyl propanoate	*		*	*	*				*	*			*		*	*			*	*	*	*	*	*

CAS- number	Compound	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8		Sample 9		Sample 10		Sample 11		Sample 12	
		MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS
	Siloxane (1)	*		*				*		*		*		*		*		*		*					
	Siloxane (2)	*		*		*		*		*		*		*				*		*		*		*	
	Siloxane (3)	*								*		*								*				*	
23747-45-7	Methyl 3-methylbutanethioate							*	*			*	*												
	Terpene (1)	*	*	*	*			*	*	*	*	*	*	*	*			*	*						
	Terpene (2)	*	*					*	*	*	*	*	*			*				*	*				
	Terpene (3)	*	*	*	*			*	*	*	*	*						*		*	*				
	Terpene (4)	*	*	*	*	*	*	*	*					*	*							*	*		
	Terpene (5)	*	*	*	*	*	*	*	*	*	*									*	*	*	*		
	Terpene (6)					*	*					*	*												
	Terpene (7)	*	*	*	*	*	*	*	*	*	*							*	*						
	Terpene (8)			*	*					*	*	*	*					*	*			*	*		
	Unknown MS/SS (1)	*	*	*	*	*	*			*	*	*	*	*	*	*	*	*	*	*	*			*	*
	Unknown MS/SS (2)	*	*	*	*	*	*	*	*	*	*					*	*	*	*	*	*			*	*
	Unknown MS/SS (3)	*	*	*	*						*						*	*	*	*	*				
	Unknown MS/SS (4)	*	*			*												*	*			*	*		
	Unknown MS/SS (5)	*	*	*	*	*	*										*						*		
	Unknown MS/SS (6)											*	*												
	Unknown MS/SS (7)										*	*	*					*	*		*				
	Unknown SS (1)								*		*						*						*		*
	Unknown SS (2)																						*		
	Unknown SS (3)														*										
	Unknown SS (4)																						*		
	Unknown SS (5)												*		*										
	Unknown SS (6)																						*		
	Unknown SS (7)																						*		

		Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8		Sample 9		Sample 10		Sample 11		Sample 12	
CAS- number	Compound	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS
	Unknown SS (8)																*								
	Unknown SS (9)																						*		
	Unknown SS (10)																								*
	Unknown SS (11)												*		*										
	Unknown SS (12)																						*		
	Unknown SS (13)														*				*			*	*		*
	Unknown SS (14)			*	*				*		*		*		*			*	*						
	Unknown SS (16)			*	*				*				*		*										
	Unknown SS (17)								*																
	Unknown SS (18)												*												

Table 2.8 data from individual stool samples including all responses (*) that met the signal to noise threshold criteria from both the mass spectral analyser (MS) and the MOS Sensor (SS).

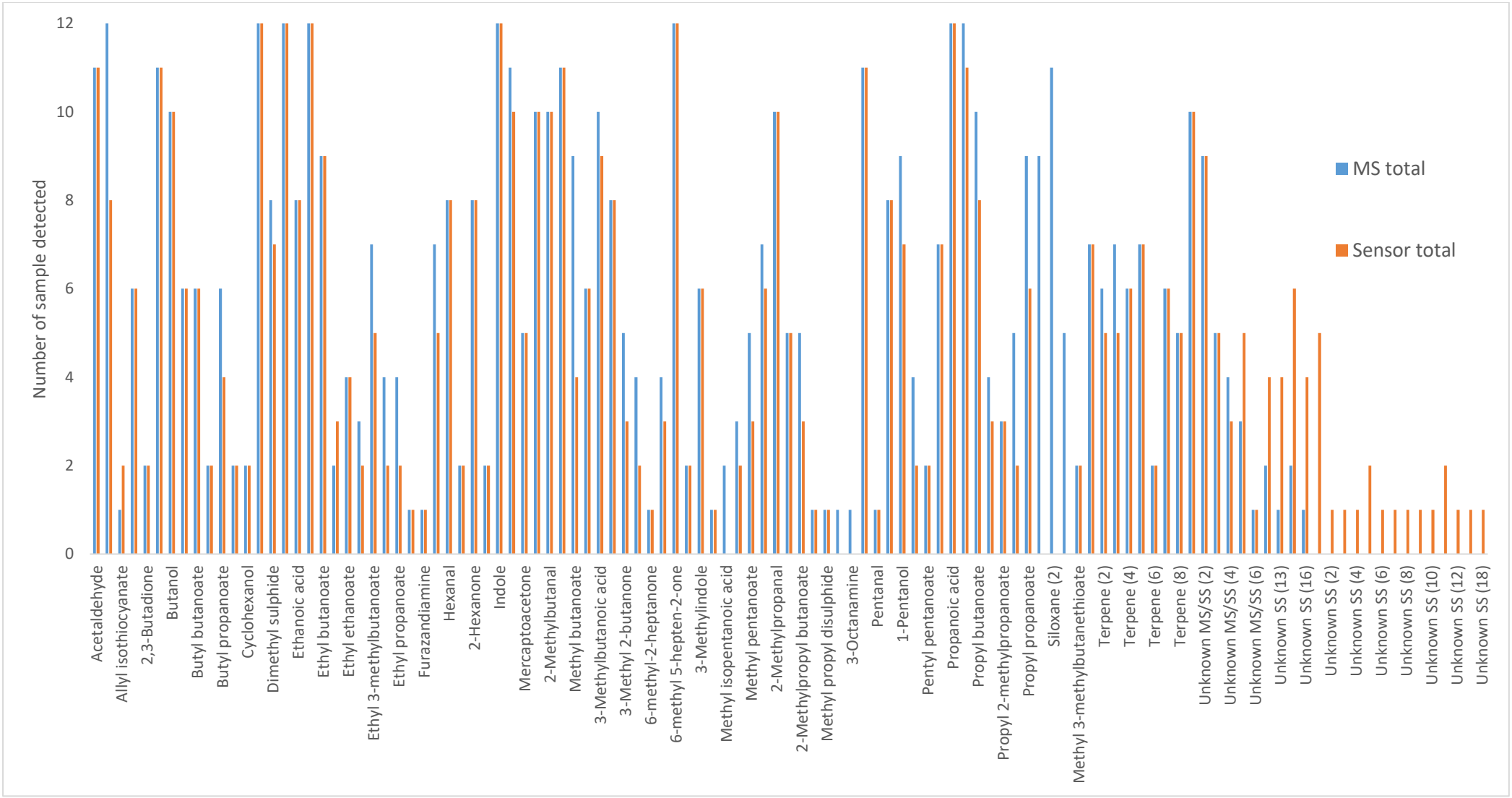


Figure 2.13 chart showing the number of times each compound was detected in the headspace of 12 stool samples collected from healthy participants on both the mass spectral analyser and the metal oxide sensor, sampled with SPME fibre.

Table 2.9 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) for the MOS sensor. Figure 2.14 shows an example of how the resistance trace compares to the chromatogram with several compounds marked. It is notable that the size of responses do not necessarily correlate across the two detectors. 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 2.9 shows a breakdown of the total number of responses detected on both the mass spectral analyser and MOS sensor from all 12 stool samples. Table 2.8 shows the raw data for each of the 12 stool samples, the total number of times each compound was detected are shown in Figure 2.13; 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 $\Delta R/\Delta T$ these responses are clearly visible on all but four 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 three 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 ten minutes of the sample run. Of the 85 detected compounds one (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 [143]. 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 [122]. 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 2.9 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 (±7.9)	42.5 (±8.3)
Unidentified responses	41	84	3.4 (±1.6)	7.0 (±2)
Siloxanes	27	0	2.3 (±1.0)	0.0
Total responses**	529	547	47.9 (±9.5)	49.5 (±10.3)

Table 2.9 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.

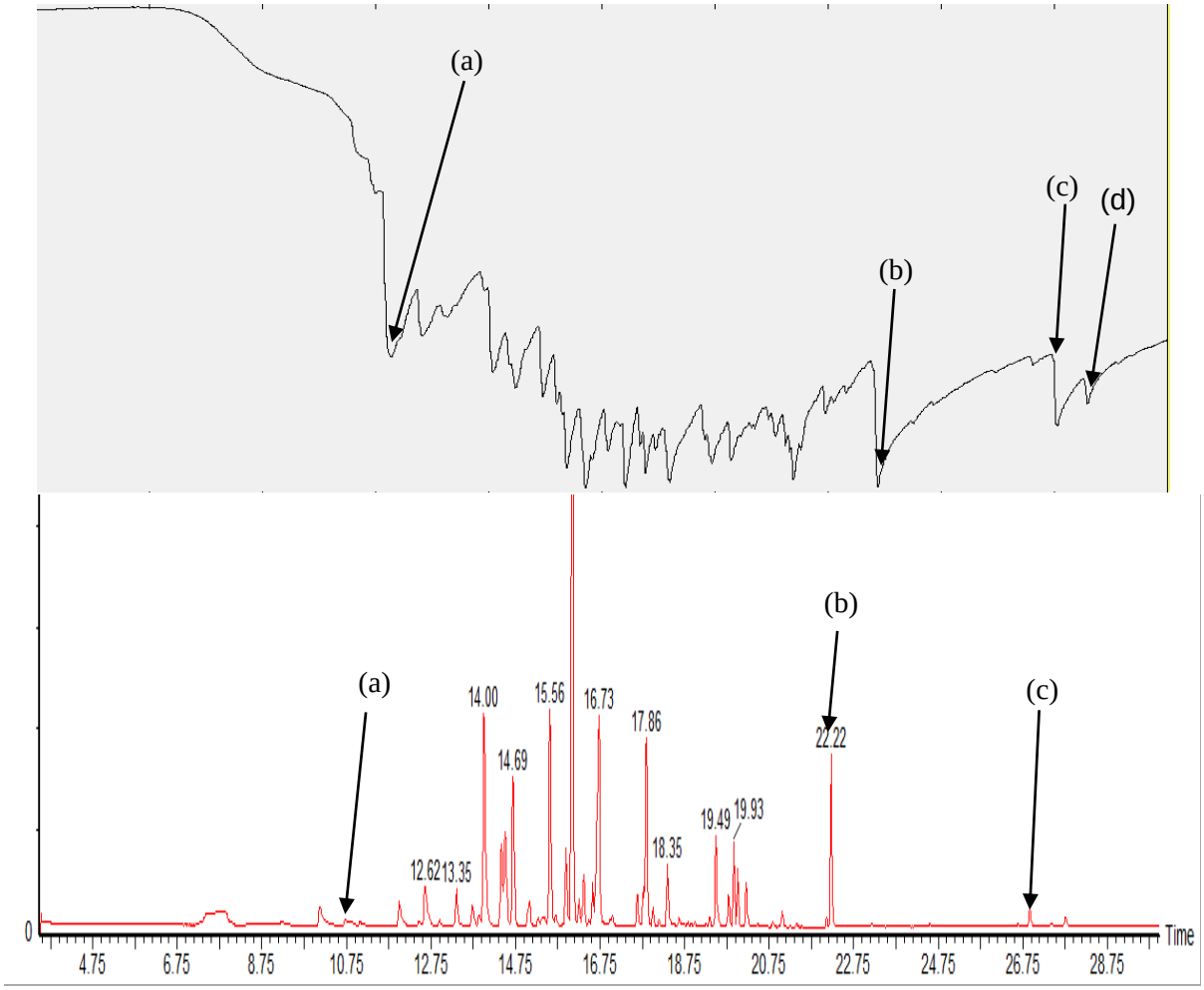


Figure 2.14 chromatogram of a typical stool sample MOS sensor trace (top) with corresponding chromatogram (bottom), time in minutes. (a) Dimethyl sulphide MOS Sensor RT 10.78 MS RT 10.86 minutes. (b) p-Cresol, MOS Sensor RT 22.23 MS RT 22.22 minutes. (c) Indole MOS Sensor RT 26.95 MS RT 26.92 minutes. (d) MOS Sensor peak RT 27.81 minutes unidentified by MS.

2.3.3 Bacterial headspace analysis

Twenty-nine 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 2.10 shows those that had been matched with a corresponding chemical standard in the standard solution experiments, the remaining compounds are shown in Table 2.11. All ten of the compounds shown in Table 2.10 have been mentioned previously in the literature; as shown in Table 2.12. There are 16 compounds detected in our experiments that 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 2.12. Despite this, Table 2.10 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 four 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 spectrometer detectors which often requires the use of solvent delays.

The difference in the MS and MOS sensor responses is shown clearly in Figure 2.15, which shows a GC-MS chromatogram and MOS sensor resistance trace (in $\Delta R/\Delta T$ negative only) overlaid on each other. Figure 2.15 illustrates just how well the responses correlate and in general 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 2.15).

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.

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

CAS-number	Compound	MS RT (mins)	MOS Sensor RT (mins)	Standard solution MS RT (mins)	Standard solution sensor RT (mins)
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

Table 2.10 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.

CAS-number	Compound	MS RT (mins)	MOS Sensor RT (mins)
75-09-2	Dichloromethane	11.37	N/D
110-54-3	Hexane	11.88	11.95
96-14-0	3-Methylpentane	11.90	12.05
141-78-6	Ethyl ethanoate	12.69	12.73
78-83-1	2-Methyl -1-propanol	13.41	13.47
74-98-6	Propane	13.80	13.85
590-86-3	3-Methyl -1-butanal	13.82	13.87
123-51-3	3-Methyl -1-butanol	15.44	15.53
109.97.7	Pyrrole	16.48	16.52
	Terpene 1	17.59	N/D
123-32-0	2,5-Dimethylpyrazine	18.58	18.63
	Terpene 2	18.98	19.05
109-21-7	Butyl butanoate	19.55	19.58
110-93-0	6-Methyl-5-hepten-2-one	19.85	19.87
	Terpene 3	20.11	20.14
	Terpene 4	20.26	20.33
	Terpene 5	23.23	23.25
107-91-5	2-Cyanoacetamide	23.62	23.68
	Terpene 6	26.54	26.62

Table 2.11 the list of NIST matched compounds detected in the headspace of *E. coli* culture along with MS and sensor RT

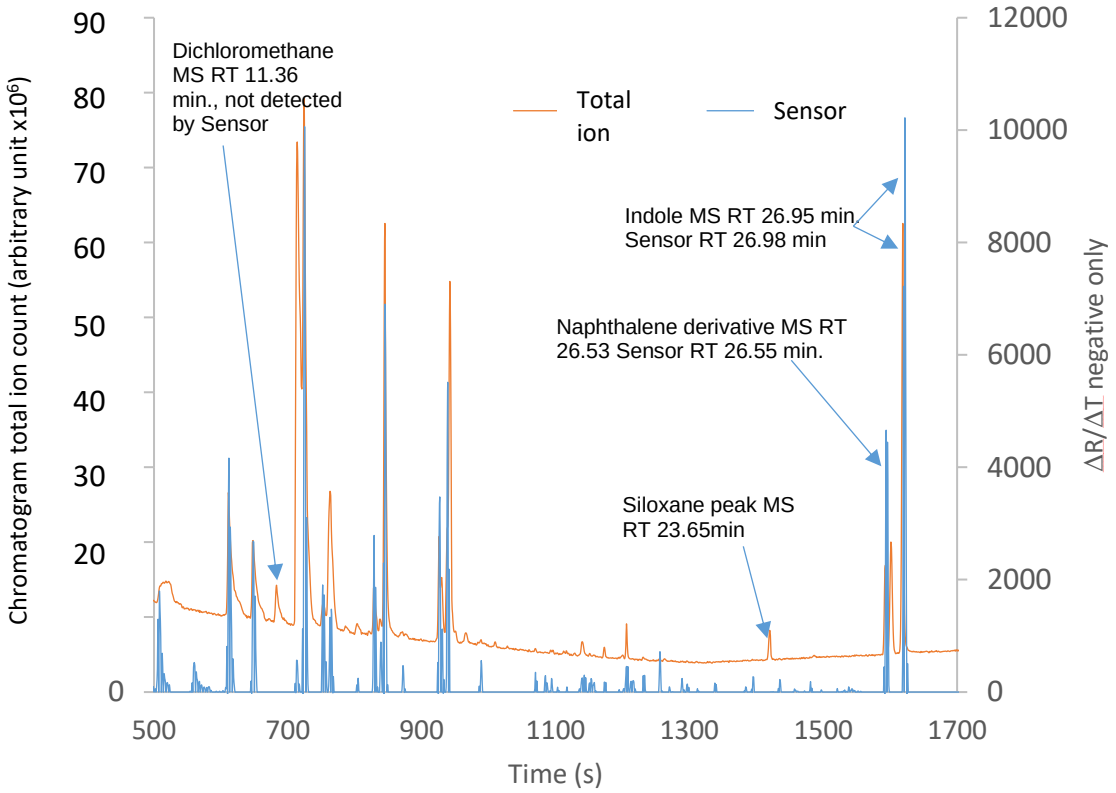


Figure 2.15 an example of an overlaid chromatogram from the GC-MS with a MOS sensor resistance trace in the ΔR/ΔT view (displaying only negative values).

Reference	Indole	Dimethyl disulphide	Ethanol	Propanol	2,3-Butadione	2-Butanone	Acetone	Methanol	Butanol	Benzaldehyde
Tait, E. *et al.* (2014) [151]	*									
Siripatrawan, U (2008) [152]	*	*	*							
Arnold, J.W (1998) [153]	*		*	*						
Hettinga, K.A (2008) [154]					*	*				
Hossain, K. Bojko, B. Pawliszyn, J (2013) [155]		*								
Storer, M. *et al.* (2011) [156]	*	*					*	*		
Allardyce, R. *et al.* (2006) [157]	*	*	*	*						
Thorn, R. Reynolds, D. Greenman, J (2011) [158]	*		*						*	
Allardyce, R. Hill, A. Murdoch, D (2006) [159]		*	*				*			
Concina, I. *et al.* (2009) [160]			*							
Bunge, M. *et al.* (2008) [161]	*		*			*	*	*	*	
Maddula, S. *et al.* (2009) [162]			*							
Zscheppank, C. *et al.* (2014) [163]	*					*			*	
Umber, B. *et al.* (2013) [164]		*	*	*						
Schulz, S. Dickschat, J (2007) [165]	*									
Chippendale, T. Spanel, P. Smith, D (2011) [166]		*	*	*			*	*		
Aathithan, S. *et al.* (2001) [167]			*							
Zhu, J. *et al.* (2010) [168]	*		*							
Boots, A. *et al.* (2014) [169]	*			*	*					*
Yu, K. *et al.* (2000) [170]	*	*								*

Table 2.12 compounds NIST matched in the headspace of E. coli from existing literature which were also NIST matched in our analysis (* = match).

Table 2.13 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. Although it should be noted that the bacterial broth headspace would be expected to have lower concentrations of compounds then the headspace of the stool

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

Table 2.14 shows the breakdown of all responses detected per sample on both the mass spectral analyser and the MOS sensor; Figure 2.16 shows how many times each compound was detected across the ten 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. There were six compounds in Table 2.10 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, 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.

CAS-number	Compound	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8		Sample 9		Sample 10	
		MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS
67-64-1	Acetone	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
100-52-7	Benzaldehyde					*	*	*	*	*	*					*	*	*	*	*	*
431-03-8	2,3-Butadione									*	*	*	*	*	*	*	*	*	*		
71-36-3	Butanol			*	*	*	*	*	*	*	*	*	*	*	*			*	*	*	*
78-93-3	2-Butanone	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		
109-21-7	Butyl butanoate									*	*	*	*	*	*	*	*	*	*	*	*
107-91-5	2-Cyanoacetamide									*	*										
75-09-2	Dichloromethane			*		*		*		*										*	
123-32-0	2,5-Dimethylpyrazine					*	*	*	*	*	*										
624-92-0	Dimethyl disulphide	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
64-17-5	Ethanol	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
141-78-6	Ethyl ethanoate																			*	*
110-54-3	Hexane																			*	*
120-72-9	Indole					*	*	*	*	*	*	*	*	*	*	*		*	*	*	*
590-86-3	3-Methyl -1-butanal					*	*			*	*	*	*	*	*	*	*	*	*	*	*
123-51-3	3-Methyl -1-butanol	*	*	*	*	*	*	*	*	*	*	*	*	*	*			*	*	*	*
110-93-0	6-Methyl-5-hepten-2-one									*	*			*	*	*	*	*	*		
96-14-0	3-Methylpentane	*	*	*	*	*	*	*	*												
78-83-1	2-Methyl -1-propanol			*	*	*	*					*	*	*	*	*	*	*	*		
106-44-5	p-Cresol									*	*	*	*	*	*	*	*	*	*		
74-98-6	Propane			*	*																
71-23-8	Propanol	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
109.97.7	Pyrrole											*	*	*	*	*	*	*	*		
	Siloxane (1)													*		*		*			
	Siloxane (2)													*				*			
	Siloxane (3)	*		*		*		*		*		*		*		*		*			
	Siloxane (4)	*		*				*		*		*		*				*			
	Siloxane (5)	*		*		*		*				*		*		*		*			

CAS-number	Compound	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8		Sample 9		Sample 10	
		MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS
	Siloxane (6)					*		*													
	Siloxane (7)							*				*									
	Siloxane (8)																	*			
	Siloxane (9)	*		*		*		*						*		*		*		*	
	Siloxane (10)	*		*		*		*		*		*		*		*		*		*	
	Terpene (1)											*	*					*	*		
	Terpene (2)									*	*	*	*	*	*	*	*	*	*		
	Terpene (3)													*	*						
	Terpene (4)													*	*						
	Terpene (5)													*							
	Terpene (6)													*	*	*	*	*	*	*	*
	Unknown MS/SS (1)										*	*	*	*	*			*	*	*	*
	Unknown MS (2)	*																			
	Unknown MS/SS (3)						*		*											*	*
	Unknown MS/SS (4)	*	*						*												*
	Unknown MS/SS (5)		*				*			*	*	*	*	*	*	*	*	*	*		
	Unknown SS (1)												*				*		*		
	Unknown SS (2)		*				*		*		*										*
	Unknown SS (3)		*		*		*				*		*		*		*				*
	Unknown SS (4)		*		*						*		*		*		*		*		*
	Unknown SS (5)		*												*		*				*
	Unknown SS (6)						*		*		*										
	Unknown SS (7)						*		*												
	Unknown SS (8)												*								
	Unknown SS (9)						*				*		*		*		*		*		
	Unknown SS (10)						*				*		*		*		*		*		*
	Unknown SS (11)						*						*		*		*				*
	Unknown SS (12)						*		*				*		*						*
	Unknown SS (13)																*		*		

		Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8		Sample 9		Sample 10	
CAS-number	Compound	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS
	Unknown SS (14)		*				*				*		*								
	Unknown SS (15)										*		*								
	Unknown SS (16)										*						*		*		
	Unknown SS (17)		*		*		*				*										
	Unknown SS (18)		*		*		*						*								

Table 2.14 data from individual bacterial headspace samples including all responses (*) 3 times the noise from both the mass spectral analyser (MS) and the MOS Sensor (SS)

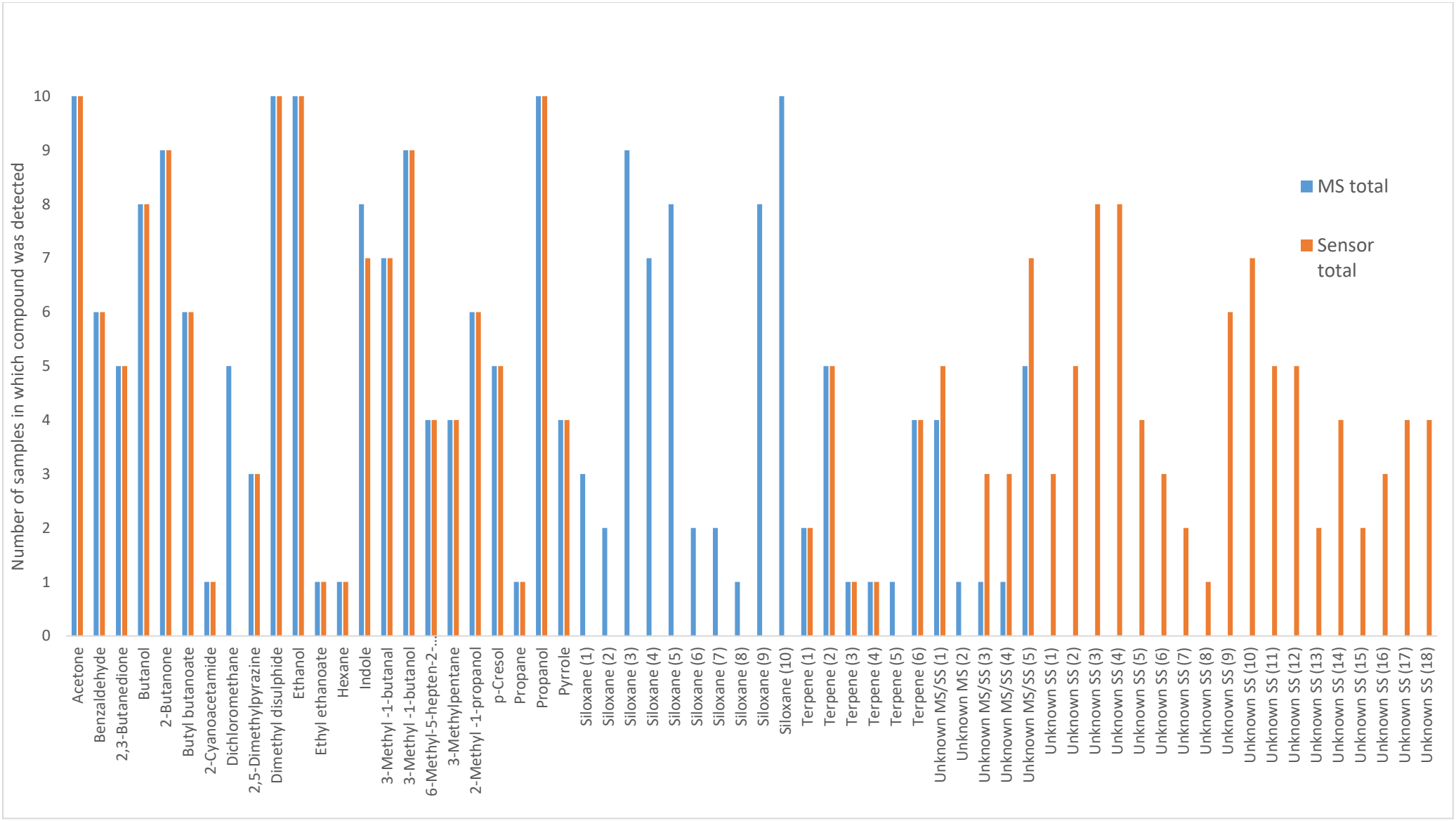


Figure 2.16 chart showing the number of times each compound was detected in the headspace of 10 *E.coli* broth culture samples on both the mass spectral analyser and the metal oxide sensor, sampled with SPME.

2.4.0 Conclusions

To summarise, we have developed a gas chromatography mass spectrometry sensor (GC-MSS) system which couples 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 verses the mass spectral analyser.

In testing to standard solutions, stool, and headspace samples we have demonstrated the potential for multiple applications of this combined GC-MS and sensor system. Although the response of MOS sensors is very fast, 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 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 [83].

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.

Chapter 3: Gas Chromatography Mass Spectrometry (GC-MS) Quantification of Metabolites in Stool Using ^{13}C Labelled Compounds

The work in this chapter has been published in the journal Metabolites: -

Gould O, de Lacy Costello B, Smart A, Jones P, Macmaster A, Ransley K, Ratcliffe N. Gas chromatography mass spectrometry (GC-MS) quantification of metabolites in stool using ^{13}C labelled compounds. Metabolites. 2018 Oct 31;8(4):75.

3.1.0 Introduction

Numerous studies have been conducted which present volatile compounds (VCs) as potential biomarkers for gastrointestinal (GI) disease states [53,171–173]. For instance, Garner *et al*. showed VCs were significantly different in infected patients with *C. difficile,* and *C. jejuni* compared to healthy participants [53]. Similarly, a pilot study suggested potential VC biomarkers of cholera [171]. Other work has suggested the VCs emitted from stool can have potential in the diagnosis of irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) [126,172,173]. Currently, GI diseases are commonly diagnosed invasively, via blood tests, endoscopy, and biopsy, while VC analysis of stools is non-invasive and is potentially more economical.

However, if VCs are to have real clinical utility, it is crucial that the healthy state volatilome is better understood. The gut is known to contain a vast and dynamic population of bacteria, with an estimated 100 trillion bacteria comprised of ca. 1000 species [174]. Currently, some 381 compounds have been identified from human stool, and this number may be unrepresentative considering over double this number of compounds have been identified in breath [25]. This discrepancy may simply be due to fewer studies attempting to identify compounds from stool rather than breath due to the increased complexity of sample gathering and difficulty in obtaining healthy stool samples. Determining the healthy gut volatilome is problematic as dietary alterations can both alter the composition of the microbiota and the metabolites produced [175]. For instance, Geypens *et al*. [176] investigated the effect of a high protein diet on VCs in stools, measuring the volatiles before and after a whey-protein supplemented diet in healthy volunteers. The study identified 120 VCs, ten of which appeared or increased after the protein-rich diet, particularly dimethyl trisulphide and short-chain fatty acids (SCFAs). Silvester *et al*. showed that high protein meals can also contribute to an increase in the production of N-nitroso compounds and ammonia by intestinal flora [177].

Currently, the quantification of volatiles from biological samples are mostly restricted to real-time methods, such as selected ion flow tube mass spectrometry (SIFT-MS) [178]. However, the use of GC-MS in the quantification of volatile compounds has previously been reported using solid phase micro extraction (SPME) to sample latrine models [179]; ten VOCs were quantified, with butanoic acid being found in the highest concentration with a range of 1.4-46.2 µg/g, whereas dimethyl sulphide was measured in the lowest concentration range of 0.02–2.1 µg/g [179].

Walton *et al*. quantitatively analysed VCs in the faecal headspace before and after treatment from patients with Crohn's disease (CD), ulcerative colitis (UC), and irritable bowel syndrome (IBS), and healthy controls. Following treatment, only propanoic acid ethyl ester remained significantly different across the groups [180]. Similarly, Baranska *et al.* used breath samples to differentiate IBS patients from healthy controls using GC time-of-flight mass spectrometry. This study suggests a set of 16 biomarkers that could be used to not only predict IBS, but also monitor its progression [75].

Wang *et al*. analysed stool samples of autistic spectrum disorder (ASD) children and healthy, age-matched controls for short chain fatty acids, phenols, and ammonia. Those with ASD had higher concentrations of acids than the controls and higher faecal ammonia [181].

De Preter *et al*. used purge and trap GC-MS analysis to quantify VCs in dried stool samples using calibration curves with diethyl sulphide, 2,6-dimethylphenol, and 2-ethylbutyric acid used as internal standards. A total of 135 different VCs were reported with 22 compounds common to all volunteers. Butanoic acid was found in the highest concentration with a range of 5–500 mg/L and dimethyl sulphide was found in the lowest concentration with a range of 5–1000 µg/L [182].

GC-MS analysis of VOCs from the human body has typically provided qualitative data and limited work exists on the quantification of metabolites in human stool. However, a method has been developed based on using internal ^{13}C labelled standards to calculate concentrations of key compounds in the solid stool sample and the headspace. This study used stool from participants of different nationalities and varying diets (vegetarian/omnivore) to assess the difference in VC composition. Knowing what constitutes a "healthy" profile across a range of geographic locations allows a better understanding of the deviations from this profile, which may be indicative of disease. No work appears to be published on VC analysis with pH alkaline alteration of stool. Altering the pH of stool to alkaline conditions was explored to allow quantification of some amine compounds.

This study qualitatively identified compounds associated with stool samples from healthy volunteers and thus adds knowledge to the established human volatilome.

3.2.0. Materials and methods

3.2.1 Instrumentation and separation methodology

A Clarus 600 gas chromatograph (GC) and Clarus 600T mass spectrometer (MS) (Perkin Elmer, Buckinghamshire, U.K.) was used for all the experiments described. The GC used a 30 m × 0.25 mm SOLGEL-WAX 0.25 µm column (Trajan scientific Europe Ltd). The GC method started at 40 °C with a four-minute hold, then ramped at a rate of 8 °C per minute to 240 °C, with a final four minute hold, and a total run time of 33 minutes. The MS was set to scan *m*/*z* 29–450 with electron ionisation selected. The automated thermal desorption (ATD) unit was a Turbo matrix 350 (Perkin Elmer, Buckinghamshire, U.K.). TD tubes were filled with Tenax TA 26 mg and Sulficarb 68 mg absorbents (Markes International Ltd, Llantrisant, U.K.). The valve was set to 215 °C with a tube temperature of 315 °C. The transfer temperature was set to 300 °C with a trap rate of 99 °C/second; the trap low and high was set to −20 °C and 320 °C, respectively. The dry purge time was 5 min and the desorb time was 10 min with a 1.2 mL/min column flow rate. The outlet split was 2 mL/min and the desorb was 180 mL/min; the inlet split was 25 mL/min with a 50 mL/min dry purge. The heated purge temperature was 50 °C.

3.2.2 Loading the thermal desorption (TD) tubes

Thermal desorption (TD) tubes were loaded using an adapted single shot heated auto sampler from an SRI GC instrument. This has a temperature-controlled heating block. In the modified version, two needles pierce the headspace vial, one that allows the nitrogen purge gas (BOC UN1066 99.998%) to enter the vial, and the other, which is securely attached to the TD tube with brass fittings (Swagelok). The vials were 10 mL glass headspace vials with a screw top phenolic cap and PTFE/silicone septa (Supelco, Bellefonte, U.S.A.). The SRI GC instrument uses an EPC valve to control the purge gas flow, which was set to 80 mL/min and flowed through the headspace vial containing the sample (3 g stool). The purge gas is flowed through the vial and out through the TD tube for two minutes; the flow rate through the tube was checked each time using a Perkin Elmer PE 1000 flow meter (Perkin Elmer, Buckinghamshire, U.K.). One µL standards dissolved in methanol were injected onto the TD tube using a 1 µL syringe (SGE analytical science, Ringwood, Australia) (see standard solutions section for details on solutions used) and loading rig (Markes International Ltd, Llantrisant, U.K.). Following the injection, nitrogen gas (BOC UN1066 99.998%) was flowed

through the tube at 80 mL/min for 2 min to remove excess solvent prior to analysis. As with the stool samples flow was measured each time using a Perkin Elmer PE 1000 flow meter (Perkin Elmer, Buckinghamshire, U.K.).

Once the tube was loaded, it was added immediately to the ATD unit carousel and analysed within 30 min using the ATD-GC-MS.

3.2.3 Standard Solutions

Two solutions were used for this work. Table 3.15 shows solution 1, and ^{13}C labelled compounds, which were used as internal standards in the samples and for the calibration curves (see Section 3.2.4 calibration curves). Table 3.16 shows solution 2, made of non-labelled compounds used for calibration curves. Both solutions were made up in methanol HPLC grade (Sigma Aldrich Company, Gillingham, U.K.) and stored at 4 °C.

CAS number	Compound ^{13}C (purity)	Supplier	Volume/ mass (in 50 mL methanol)
3881-06-9	Acetone 2-^{13}C (99%)	Sigma Aldrich Company	50µL/39.55 mg
1329835-35-9	Ethyl butanoate 1-^{13}C (>95%)	Precursor supplier, Cambridge Isotope Laboratories Ltd	50µL/43.25 mg
136321-14-7	Dimethyl disulphide $^{13}C2$ (99%)	Cambridge Isotope Laboratories Ltd	50µL/53.0 mg
1563-79-7	Ethanoic acid 1-^{13}C (99%)	Cambridge Isotope Laboratories Ltd	50µL/52.5 mg
38765-83-2	Butanoic acid 1-^{13}C (99%)	Cambridge Isotope Laboratories Ltd	50µL/47.5 mg
87994-84-1	3-Methylbutanoic acid 1-^{13}C (99%)	Cambridge Isotope Laboratories Ltd	50µL/46.25 mg
NA	Indole 2-^{13}C (98%)	Cambridge Isotope Laboratories Ltd	80 mg (solid)

Table 3.15 solution 1: 13C labelled compounds used as internal standards, purity, suppliers, and masses used.

CAS number	Compound	Supplier	Volume/ mass (in 100 mL methanol*)
75-18-3	Dimethyl sulphide	Sigma Aldrich Company	100µL/84.6 mg
67-64-1	Acetone	Sigma Aldrich Company	100µL/79.1 mg
7452-79-1	2-Methyl-butanoic acid, ethyl ester	Sigma Aldrich Company	100µL/86.5 mg
624-92-0	Dimethyl disulphide	Sigma Aldrich Company	100µL/106.0 mg
4312-99-6	1-Octen-3-one	Sigma Aldrich Company	100µL/84.3 mg
3658-80-8	Dimethyl trisulphide	Sigma Aldrich Company	10 µL/15.97 mg (in75 mL methanol)
3391-86-4	1-Octen-3-ol	Sigma Aldrich Company	100µL/84 mg
107-92-6	Butanoic acid	Sigma Aldrich Company	100µL/95.0 mg
503-74-2	3-Methylbutanoic acid	Sigma Aldrich Company	100µL/92.5 mg
109-52-4	Pentanoic acid	Sigma Aldrich Company	100µL/93.0 mg
120-72-9	Indole	Sigma Aldrich Company	80.5 mg (solid)
83-34-1	3-Methylindole	Sigma Aldrich Company	45 mg (solid) in 75 mL methanol

Table 3.16 solution 2: compounds used for standards, purity, supplier, and volume/masses used. *Unless stated otherwise.

3.2.4 Calibration Curves

Five mL of solution 1 was added to a 50 mL volumetric flask and topped to the fill line with methanol for a factor ten dilution. Five mL of this diluted solution was then placed into another 50 mL volumetric flask which was topped to the fill line with methanol which resulted in 100-fold dilution from the starting stock solution. Section 3.2.2 of loading TD tubes details how the solution was injected onto the tubes; 0.5 µL of the solutions 1 and 2, and the sequentially diluted solutions, were injected on to TD tubes. These were then analysed on the GC-MS, and the peak areas were recorded and noted for each compound. Calibration curves were created for each compound; none of the compounds in solution 1 or solution 2 yielded an r^2 of less than 0.99 (see Figures 3.17 and 3.18). Retention indices for all the standards were compared to that of the literature, and in all cases, the values obtained from our data matched the literature values.

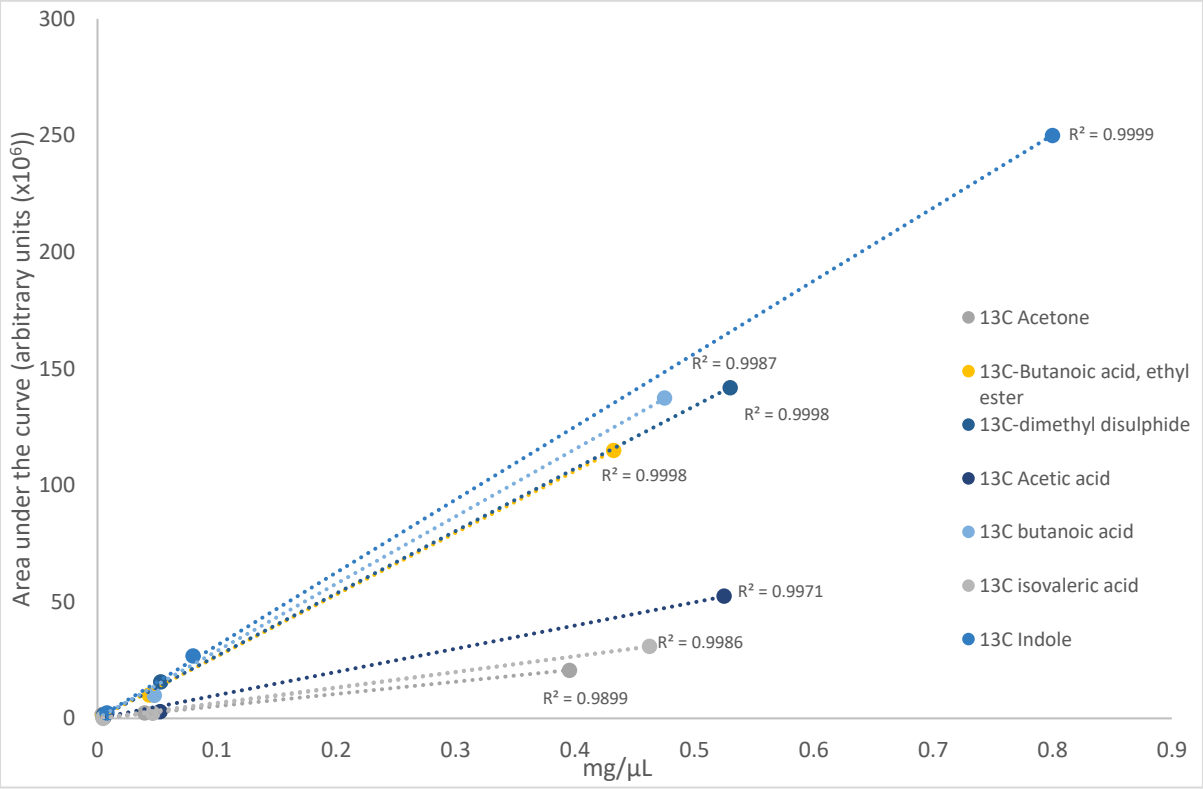


Figure 3.17 the three point calibration graphs generated from solution 1 which contains the ^{13}C labelled compounds.

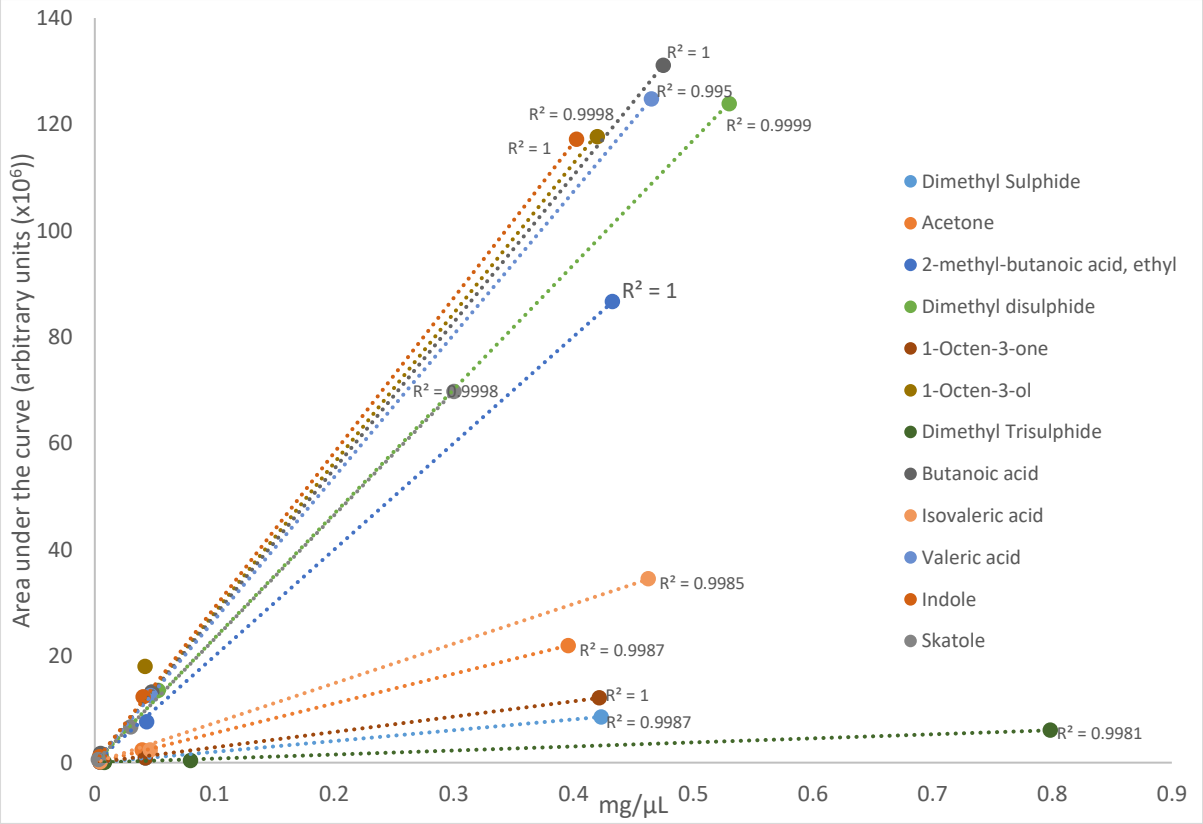


Figure 3.18 the three-point calibrations curves generated from solution 2 which contains the chemical standard of the compounds quantified in stool.

3.2.5 Sample Preparation

Stool samples were collected from 38 healthy volunteers, age range of 18–60 years, with full ethical consent (research ethics committee reference 14/NE/0029); these were immediately refrigerated (4 °C) on arrival and processed in a microbiological safety cabinet within 4 h. Samples were initially collected in aluminium containers, L 20 cm × W 11.5 cm × H 4 cm. Processing involved taking 6–8 aliquots weighing 3 g from each sample and placing them into 10mL headspace vials (Supelco), which were then frozen at −20 °C. The ethnic origin and the omnivore/ vegetarian status of the samples is shown in Table 3.17.

Number of Participants	Ethnic Origin	Omnivore	Vegetarian
16	UK	8	8
11	South America (Brazil, Mexico)	10	1
3	Mainland Europe (Czech Republic, Latvia, and Spain)	1	2
5	Asia (China, Vietnam, Iran)	4	1
3	Africa (Maldives, Nigeria)	1	2

Table 3.17 a breakdown of the participants by country of origin and omnivore/vegetarian status.

For analysis, the sample vial was removed from the freezer and immediately the screw cap was removed and a steel ball bearing (0.5 cm diameter, weighing approximately 0.5 g) was added and the vial recapped. Next, 1 µL of solution 1 (see 3.2.3 standard solutions section) was injected through the septa using a 1 µL syringe (SGE analytical science). The vial was then inserted into the TD loading rig heating block at 30 °C for 10 min, manually shaken for *ca.* 1 minute, and returned to the block. After a further 10 min, the vial was shaken for a second time; then, it was returned to the block for a further 10 min, giving a total 30 min of heating time (see Section 3.2.2 loading TD tubes section for details).

3.2.6 Trimethylamine Quantification in pH13 Stool Samples

Trimethylamine solution standards were made separately. 25 mg of ^{13}C trimethylamine (99% Cambridge Isotope Laboratories Ltd) was dissolved into 25 mL of methanol to give a final concentration of 1 mg/mL (most concentrated), and this 1 mg/mL solution was sequentially diluted to 0.1 mg/mL and 0.01 mg/mL. Trimethylamine, 400 mg (25 wt% in water, Sigma Aldrich Company, Gillingham, U.K.) was dissolved in 100 mL methanol to give the same 1 mg/mL concentration, which was again used as the most concentrated standard. This was diluted 10-fold to give 0.1 mg/mL and 0.01 mg/mL. These solutions were used to create calibration curves of the mass of compounds (*x*-axis) versus peak area recovered from the chromatogram (*y*-axis) (see calibration curves Section 3.2.4).

The same sample processing took place as described in the sample preparation section above; however, in the case when the ball bearing was added to the sample on removal from the freezer, 5 mL of aqueous 0.1 M sodium hydroxide (Fisher Scientific, Loughborough, U.K.) was also pipetted into the sample. The pH of the stool samples was checked post analysis with indicator paper. A total of 15 stool samples were run using this method.

3.2.7.0 Analysis

3.2.7.1 Qualitative Analysis

A signal threshold of three times the noise was set for all chromatograms. All peaks were searched manually using the NIST library (NIST 08). Compounds with a match and reverse match above 800 were identified. If the match and/or reverse match was under 800, then the peak was listed as unidentified. Terpene and siloxane compounds

were difficult to assign structures to and thus were listed under the chemical class name.

3.2.7.2 Mass Calculations Using ^{13}C Labelled Compounds

Using solution 1, the following compounds were quantified: Dimethyl sulphide, acetone, butanoic acid ethyl ester, 2-methylbutanoic acid ethyl ester, dimethyl disulphide, 1-octen-3-one, dimethyl trisulphide, 1-octen-3-ol, ethanoic acid, butanoic acid, 3-methylbutanoic acid, pentanoic acid, indole, and 3-methylindole.

To obtain the peak areas for each compound and the ^{13}C equivalent we obtained a separate trace for the single ion of interest and obtained the peak area from this trace. For example, the primary m/z product for 3-methylbutanoic acid is 87 and 88 for the ^{13}C isotope. Knowing the mass of ^{13}C compounds added to the sample we can calculate the proportion of ^{13}C compound recovered from the headspace using the calibration graphs constructed. We can then use the ratio of ^{13}C compound added to the sample versus the amount of ^{13}C compound recovered from the headspace to correct the peak area of the compound being quantified and therefore, quantify the amount in the stool samples. To demonstrate the calculations the example of 3-methylbutanoic acid will be used. Peak area units are marked as PAU.

- The calibration curves (Figures 3.17 and 3.18) gave equations y=66.62x and y=74.58x for ^{13}C 3-methylbutanoic acid and 3-methylbutanoic acid respectively.
- Since 0.5 µL of solution 1 was used for the calibration curve but 1 µL of solution 1 was added to the stool the first step was to calculate the theoretical peak area for 100% recovery of ^{13}C 3-methylbutanoic acid from the sample. In this example 66.62 (slope of ^{13}C 3-methylbutanoic acid) x 0.925 (mass (µg) in 1 µL solution 1) = 61.62 PAU.
- 61.62 PAU is then divided by the PAU of ^{13}C 3-methylbutanoic acid recovered from the sample; which in this case is 0.78 PAU so 61.62 PAU / 0.78 PAU = 79; this value is the correction factor that allows the calculation of the amount of compound within the stool.
- The correction factor is then multiplied by the peak area of the compound to be quantified from the sample, in this case the peak area of 3-methylbutanoic acid was 0.18 PAU, thus 79 x 0.18 PAU = 14.21 PAU.
- So the peak area for the amount of 3-methylbutanoic acid contained in the stool is 14.21 PAU, this can then be used with the calibration curve for 3-

methylbutanoic acid (y=74.58x) where y=peak area and x=mass of compound to solve for x so in our example x= 14.21 PAU/ 74.58= 0.19 µg

- Finally, 0.19 µg is divided by the mass of stool (3 g) = 0.063 µg/g which we then convert to ng/g = 63.3 ng/g.

3.2.7.3 Statistical analysis

All statistical analysis was carried out using IBM SPSS statistics version 24. Basic descriptive statistics (median, interquartile range, and range of masses)) were carried out on the quantitative data. Each chromatogram was analysed manually to identify the chromatographic peaks and the peak areas which provided the qualitative data.

The two largest groups of participants with different geographical origins were from the U.K. and South America, thus these groups were selected for comparison. Omnivore and vegetarian groups from the entire cohort of participants were also compared. To compare sample groups, the non-parametric test Mann Whitley U was carried out before a discriminant analysis using stepwise statistics (Wilks' Lambda). Leave-one-out cross validation was used to calculate a ROC curve.

The differences between samples (e.g., U.K. origin versus South American origin) have been discussed. Also discussed is the difference in chromatographic peaks both qualitatively and quantitatively for unmodified stool versus pH 13 stool.

3.3.0 Results

3.3.1 Qualitative data from unmodified stools

Tables 3.18-3.29 show the raw qualitative data from all 38 samples; in total, 174 distinct chromatographic peaks were recorded across these samples; the supplementary tables show these compounds separated into chemical class. The mean number of peaks above the noise threshold was 57 per sample with a range of 36–72. Of the total number of chromatographic peaks, 32 could not be identified by the NIST library and 30 were identified as unspecified terpenes. Table 3.30 shows retention indices (RI) values calculated from the experimental data and compared where possible to literature values. All RIs for the unidentified and terpene compounds have also been calculated. Table 3.30 also highlights which compounds have been validated using standards.

S1a)														
CAS	Aldehydes	Retention time (mins)	1	2	3	4	5	6	7	8	9	10	11	12
75-07-0	Acetaldehyde	2.16	169.44		230.78	72	53.08	166.03	97.55		91.36	81.08	260.13	82.12
123-38-6	Propionaldehyde	2.43												
123-72-8	Butyraldehyde	3.10		12.26	4.52	24.8	10.74	9.49		4.47			10.25	
66-25-1	Hexanaldehyde	6.76	9.23	10.2	8.89	19.19	18.18	37.13	14.9	12.28		9.52	15.09	17.01
100-52-7	Benzaldehyde	15.05	2.72	6.52	2.34	4.97	3.33	1.95	1.6		2.29	2.06	2.25	
55012-32-3	Isopropyl benzaldehyde	18.91										4.24		

S1b)														
CAS	Aldehydes	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
75-07-0	Acetaldehyde	2.16	173.9	65.69	143.82	31.3	156.33	170.17	60.17	30.89	39.56	42.08	65.72	30.64
123-38-6	Propionaldehyde	2.43							5.29					
123-72-8	Butyraldehyde	3.10			5.71			10.18	9.12	7.26			10.1	3.56
66-25-1	Hexanaldehyde	6.76	12.85	105.43	8.35	9.07	63.28	39.54	4.58	8.77	6.63	8.6	2.86	3.15
100-52-7	Benzaldehyde	15.05				1.22							2.06	1.29
55012-32-3	Isopropyl benzaldehyde	18.91			2.39									

S1c)																
CAS	Aldehydes	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
75-07-0	Acetaldehyde	2.16	22.78	122.52	77.79	54.3	35.32	42.75	81.05		33.97	24.51		43.3	12.15	13.02
123-38-6	Propionaldehyde	2.43				5.81			0.97	1.7		1.61	1.75	0.54		0.16
123-72-8	Butyraldehyde	3.10		3.88					8.65	3.36	3.39		4.7	3.64	2.79	3.58
66-25-1	Hexanaldehyde	6.76	15.61		6.98					5.35	5.24		16.45			

S1c)																
CAS	Aldehydes	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
100-52-7	Benzaldehyde	15.05				1.92										
55012-32-3	Isopropyl benzaldehyde	18.91														

Table 3.18 sections a, b, and c aldehydes found in unmodified stool samples for all participants with retention time and chromatographic peak area.

S2a)														
CAS	Alcohols	Retention time (mins)	1	2	3	4	5	6	7	8	9	10	11	12
64-17-5	Ethanol	3.91	54.64	163.04	244.98	113.15	95.58	129.81	88.27	74.59	61.08	150.72	291.37	925.93
78-92-2	2-Butanol	5.64	22.04		19.74	21.55	29.81	24.51	16.18		14.08	44.64	16.93	12.31
71-23-8	1-Propanol	5.92			87.79				44.53	132.26				
78-83-1	2-Methyl-1-propanol	7.27	11.68	13.1	25.51	24.08	41.83	21.15	14.48	22.89	7.71	15.85	37.54	
598-75-4	3-Methyl -2-butanol	7.80			4.64					4.87				
6032-29-7	2-Pentanol	7.83				7.2			3.1					3.97
71-36-3	1-Butanol	8.29	5.44		55.42	72.25	45.08	21.47	21.97	208.89	6.06		100.22	466.05
123-51-3	3-Methyl -1-butanol	9.51	18.33		13.04	32.73	43.3	24.12	11.86	23.5	20.74	21.16	43.48	17.77
108-11-2	4-Methyl -2-pentanol	9.78												
71-41-0	1-Pentanol	10.35	10		16.52	28.77	36.72	11.49	13.57	56.66	5.03	9.53	19.39	78.22
626-89-1	4-Methyl -1-pentanol	11.55	3.49	3.08	7.38	9.12			7.25	9.5			1.94	
543-49-7	2-Heptanol	11.59												
2313-61-3	4-Methyl -2-hexanol	11.66					3.38							
111-27-3	1-Hexanol	12.24				41.42	49.82			41.96		6.53	2.81	123.3
123-96-6	2-Octanol	13.40					4.94							
3391-86-4	1-Octen-3-ol	13.90	14.42	15.78	6.37	11.98	2.03	1.69						
111-70-6	1-Heptanol	13.99				2.78	15.37							
104-76-7	2-Ethyl-1-hexanol	14.49												
111-87-5	1-Octanol	15.64					4.92							
15356-70-4	Cyclohexanol, 5methyl-2-(1-methylethyl)-,(1a,2b,5a)	16.90						8.01						

S2b)														
CAS	Alcohols	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
64-17-5	Ethanol	3.91	491.15	113.67	156.52		12.27	253.7	339.14	254.14	139.93	137.65	58.64	151.62
78-92-2	2-Butanol	5.64	13.83	37.33	9.49	9.61	29.76	26.52	7.75	11.86	7.83	64.06	17.84	11.53
71-23-8	1-Propanol	5.92						22.39						7.88
78-83-1	1-Propanol,	7.27	11.54	9.14	13.78	5.55	11.31	54.5	86.4	20.77	10.26	27.88	11.98	44.08
598-75-4	3-Methyl -2-butanol	7.80	1.27				4.44	2.52	1.24					4.87
6032-29-7	2-Pentanol	7.83		11.2					2.35	4.69	1.98			
71-36-3	1-Butanol	8.29	61.48	12.91		55.51	12.25	1.68	132.28	147.5	46.15	28.17	8.71	72.26
123-51-3	3-Methyl -1-butanol	9.51			3.26	6.26	20.27	34.86	32.49	23.46	14.55	18.03	22.28	53.67
108-11-2	4-Methyl -2-pentanol	9.78		5.79										
71-41-0	1-Pentanol	10.35	10.61	6.63	6.33	10.66			29.39					
626-89-1	4-Methyl-1-pentanol	11.55	4.35			3.01		6.93				0.94		2
543-49-7	2-Heptanol	11.59												
2313-61-3	4-Methyl -2-hexanol	11.66												0.85
111-27-3	1-Hexanol	12.24	8.31	5.9	2.69	11.12	3.43	3.82	1.83	2.22	5.53	0.87	1.42	1.34
123-96-6	2-Octanol	13.40	0.6	1.26		0.35								
3391-86-4	1-Octen-3--ol	13.90							0.36					
111-70-6	1-Heptanol	13.99		1.57							7.49	20.46		
104-76-7	2-Ethyl -1-hexanol	14.49												
111-87-5	1-Octanol	15.64												
15356-70-4	Cyclohexanol, 5methyl-2-(1-methylethyl)-,(1a,2b,5a)	16.90												

S2c) CAS	Alcohols	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
64-17-5	Ethanol	3.91		200.44	96.72	422.76	292.36	159.97	100.69	1052.5	165.17	1479.78	307.18	286.75	54.48	51.63
78-92-2	2-Butanol	5.64	33.7	16.34			9.34		25.22	20.05	4.85		13.7	10.18		
71-23-8	1-Propanol	5.92				320.32										
78-83-1	2-Methyl-1-propanol,	7.27	27.48	37.51	9.11	4.46	41.82	5.77	73.17	19.88	5.75	17.65	8.98	11.93	3.77	9.47
598-75-4	3-Methyl -2-butanol	7.80	28.73						4.95		1.06					
6032-29-7	2-Pentanol	7.83		2.17		47.17										
71-36-3	1-Butanol	8.29	1106.38	40.2	9.3	5.03	20.55	6.73	116.09	307.81	20.58	481.3	49.07	84.58	11.69	13.62
123-51-3	3-Methyl -1-butanol	9.51	30.42	41.5	8.64	15.3	17.23	13.3	73.16	12.06	12.74	8.64	10.49	10.18	3.57	3.47
108-11-2	4-Methyl -2-pentanol	9.78	4.49			24.19								2.18		
71-41-0	1-Pentanol	10.35	23.17			6.24	9.65	4.58	32.7	30.36	0.8	49.63	10.92	9.57	4.3	5.22
626-89-1	4-Methyl -1-pentanol	11.55	15.49	0.73	3.68	0.87		1.49							0.59	
543-49-7	2-Heptanol	11.59					2.07									
2313-61-3	4-Methyl -2-hexanol	11.66			0.46											
111-27-3	1-Hexanol	12.24		9.8		1.88	6.26		26.29	2.55	0.9	1.77				4.38
123-96-6	2-Octanol	13.40				5.06					0.74					
3391-86-4	1-Octen-3--ol	13.90	50.03	3.05	0.73											
111-70-6	1-Heptanol	13.99			2.31											
104-76-7	2-Ethyl -1-hexanol	14.49						5.39								
111-87-5	1-Octanol	15.64		0.64												

S2c)																
CAS	Alcohols	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
15356-70-4	Cyclohexanol, 5methyl-2-(1-methylethyl)-,(1a,2b,5a)	16.90		6.06												

Table 3.19 sections a, b, and c alcohols found in unmodified stool samples for all participants with retention time and chromatographic peak area.

S3a)														
CAS	Esters and thioesters	Retention time (mins)	1	2	3	4	5	6	7	8	9	10	11	12
79-20-9	Ethanoic acid, methyl ester	2.69	107.77	101.23	117.83	136.73	104.72	206.31	107.89	177.06	88.00	73.82	104.92	
141-78-6	Ethanoic acid ethyl ester	3.21			13.13			20.11	24.31	17.81			11.33	483.39
105-37-3	Propanoic acid ethyl ester	4.28			18.47			31.27	38.51	26.72			7.5	368.55
97-62-1	2-Methylpropanoic acid ethyl ester	4.44			4.85							1.4		36.65
109-60-4	Ethanoic acid propyl ester	4.60												380.99
623-42-7	Butanoic acid methyl ester	4.79	11.88	9.6	133.9	48.71	10.79	287.95	252.51	268.02		7.24	34	776.39
868-57-5	2-Methylbutanoic acid methyl ester	5.28			12.78			14.28	16.27	15.87				
556-24-1	3-Methylbutanoic acid methyl ester	5.49								20.05				13.51
105-54-4	Butanoic acid ethyl ester	5.87												
106-36-5	Propanoic acid propyl ester	6.04							18.72	66.2				269.37
7452-79-1	2-Methylbutanoic acid ethyl ester	6.21			4.19	7.57		11.56	10.9					
123-86-4	Ethanoic acid butyl ester	6.61								36.17				214.65
624-24-8	Pentanoic acid methyl ester	6.87			20.52	11.51	4.83	52.98	41.21	62.74			13.26	106.62
105-66-8	Butanoic acid propyl ester	7.64			11.58	3.69	4.03	20.92	32.52	34.72			4.29	1205.79
539-82-2	Pentanoic acid ethyl ester	7.92			6.98			14.37	19.25	17.26				425.37
590-01-2	Propanoic acid butyl ester	8.06	77.7		13.23	20.74	16.6	10.96	31.24	50.47	25.32	56.09	1.97	205.3
97-87-0	Propanoic acid 2-methylbutyl ester	8.18												10
539-90-2	Butanoic acid 2-methylpropyl ester	8.45						10.19	9.16	5.29		30.82		20.77
628-63-7	Ethanoic acid pentyl ester	8.68												
123-92-2	Ethanoic acid 3-methylbutanyl ester	8.75								6.02		4.00		27.47
106-70-7	Hexanoic acid methyl ester	9.01				15.44	6.28		21.85	25.74		0.88		293.84

S3a)														
CAS	Esters and thioesters	Retention time (mins)	1	2	3	4	5	6	7	8	9	10	11	12
109-21-7	Butanoic acid butyl ester	9.68			5.45	3.25		8.18	7.27	13.29			2.07	877.59
141-06-0	Pentanoic acid propyl ester	9.73							7.66	10.11				
123-66-0	Hexanoic acid ethyl ester	9.99							4.93	10.3				1089.71
624-54-4	Propanoic acid pentyl ester	10.12								4.53				
109-19-3	3 -Methylbutanoic acid butyl ester	10.29								7.41				
106-27-4	Butanoic acid 3-methylbutyl ester	10.60												14.59
142-92-7	Ethanoic acid hexyl ester	10.73												38.28
106-73-0	Heptanoic acid methyl ester	11.02				7.86			4.03	5.8				20.31
591-68-4	Pentanoic acid butyl ester	11.50												
5870-93-9	Butanoic acid heptyl ester	11.51												
626-77-7	Hexanoic acid propyl ester	11.63												438.07
106-30-9	Heptanoic acid ethyl ester	11.88												
4630-82-4	Cyclohexane carboxylic acid methyl ester	12.71								21.93				12.22
111-11-5	Octanoic acid methyl ester	12.82												
626-82-4	Hexanoic acid butyl ester	13.30												149.2

S3b)														
CAS	Esters and thioesters	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
79-20-9	Ethanoic acid methyl ester	2.69	118.44	130	72.01	118.43	124.64	86.24	109.89	155.81	64.69	103.66	54.19	53.44
141-78-6	Ethanoic acid ethyl ester	3.21	124.04	2.6		138.53		23.95		15.17				
105-37-3	Propanoic acid ethyl ester	4.28	105.99		7.63	152.64	7.6	50.66		13	4.86			9.48
97-62-1	2-Methylpropanoic acid ethyl ester	4.44	22.33			66.71		30.57		4.51	38.36	16.98		2.35
109-60-4	Ethanoic acid propyl ester	4.60	66.09			56.77	223.64			70.46			44.68	5.2
623-42-7	Butanoic acid methyl ester	4.79	104.48	25.53	11.77	248.03	40.94	17.27	16.92		21.62	45.44	7.9	8.67
868-57-5	2-Methylbutanoic acid methyl ester	5.28	3.77			23.62		23.76		2.73				1.7
556-24-1	3-Methylbutanoic acid methyl ester	5.49	11.54		16.09	68.49	37.21	27.56	8.34	8.26	11.34		64.57	26.41
105-54-4	Butanoic acid ethyl ester	5.87	519.8		171.54	630.87		426.27	463.79	141.16		120.9		

S3b)														
CAS	Esters and thioesters	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
106-36-5	Propanoic acid propyl ester	6.04	22.66			15.18								
7452-79-1	2-Methylbutanoic acid ethyl ester	6.21	9.82	4.8		47.99		22.48	6.87	3.74	1.58			2.78
123-86-4	Ethanoic acid butyl ester	6.61	14.5		0.93	22.03				6.2				
624-24-8	Pentanoic acid methyl ester	6.87	18.01	5.43	4.84	41.96	21.58	2						
105-66-8	Butanoic acid propyl ester	7.64	49.85		2.4	20.98		50.3		8.19	7.79		2.32	
539-82-2	Pentanoic acid ethyl ester	7.92	58.21		2.49	93.66		51.21	1.55	2.87	4.63		89.42	3.57
590-01-2	Propanoic acid butyl ester	8.06	9.91		16.35	19.29		2.53			5.07	7.68	16.4	
97-87-0	Propanoic acid 2-methyl butyl ester	8.18			11.58		11.47	8.79						
539-90-2	Butanoic acid 2-methylpropyl ester	8.45		4.81	1.1	9.24		57.99						
628-63-7	Aethanoic acid pentyl ester	8.68												
123-92-2	Ethanoic acid 3-methyl butanyl ester	8.75	1.44			3.03	18.62	1.93		0.71	4.23	1.1	4.04	1.47
106-70-7	Hexanoic acid methyl ester	9.01	14.52	5.54	1.43	2.49			4.32	7.74		1.36		22.93
109-21-7	Butanoic acid butyl ester	9.68	18.3			9.62	2.32	17.78	2.56	4.17		1.45		2.92
141-06-0	Pentanoic acid propyl ester	9.73	8				1.45	16.69						1.67
123-66-0	Hexanoic acid ethyl ester	9.99	42.59			70.59	0.68	1.74			1.77		0.79	
624-54-4	Propanoic acid pentyl ester	10.12	1.22	38.46	1.46			4.07					7.56	
109-19-3	3- Methylbutanoic acid butyl ester	10.29					10.09	18.27		21.9	10.56	10.36	4.38	26.15
106-27-4	Butanoic acid 3-methylbutyl ester	10.60	1.39				3.01						6.72	
142-92-7	Ethanoic acid hexyl ester	10.73	0.92			1.55	0.99	0.98			1.11	0.91	2.4	
106-73-0	Heptanoic acid methyl ester	11.02	3.2		2.4	4.47								
591-68-4	Pentanoic acid butyl ester	11.50												
5870-93-9	Butanoic acid heptyl ester	11.51												
626-77-7	Hexanoic acid propyl ester	11.63	5.72	4.29		2.59	0.73							
106-30-9	Heptanoic acid ethyl ester	11.88	3.78			2.66	30.09	17.84	30.75	20.37	6.8	9.82	50.34	71.99
4630-82-4	Cyclohexane carboxylic acid methyl ester	12.71					1.02						0.42	
111-11-5	Octanoic acid methyl ester	12.82												
626-82-4	Hexanoic acid, butyl ester	13.30	1.35		2.15	1.28	0.4							0.39

S3c)																
CAS	Esters and thioesters	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
79-20-9	Ethanoic acid, methyl ester	2.69	506.42	216.74	75.02	120.83	79.75	129.05	129.68	242.88	203.39	556.94	4.12	69.11	11.85	18.14
141-78-6	Ethanoic acid ethyl ester	3.21		13.79		93.82	2.96		11.35	229.27	26.4		2.42	2.02	0.7	0.84
105-37-3	Propanoic acid ethyl ester	4.28				449.67			11.76	81.22	48.23	833.25	46.61	3.07	2.89	1.7
97-62-1	2-Methyl propanoic acid ethyl ester	4.44	49.1			15.19			78.57	5.26						
109-60-4	Ethanoic acid propyl ester	4.60				17.25				80.21	54.21	482.53			5.19	
623-42-7	Butanoic acid methyl ester	4.79		8.32	5.46	84.59	38.56	11.42	204	597.33	335.208	2314.9	17.12	58.6	9.25	7.53
868-57-5	2-Methyl butanoic acid methyl ester	5.28				31.96			12.26		4.83			1.25	51.36	
556-24-1	3-Methyl butanoic acid methyl ester	5.49	13.25	19.77								16.16				
105-54-4	Butanoic acid ethyl ester	5.87		164.15						1075.92	287.35			198.35		
106-36-5	Propanoic acid propyl ester	6.04		2.8							68.87					
7452-79-1	2-Methyl butanoic acid ethyl ester	6.21	472.99		2.5	19.33			2.12		4.92					
123-86-4	Ethanoic acid butyl ester	6.61	435.4			4.98				30.89		277.58				
624-24-8	Pentanoic acid methyl ester	6.87	67.82	3.09	1.7	5.6	8.43	5.75	29.36	61.61	24.68	332.4	49.45	11.04		
105-66-8	Butanoic acid propyl ester	7.64	1043.23		1.46	25.92	1.12		21.4	94.32	39.03	136.07	1.68	4.41	2.38	

S3c)																
CAS	Esters and thioesters	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
539-82-2	Pentanoic acid ethyl ester	7.92		6.21	1.55	3.35			2.91	59.3	2.94	788.31		1.89	1.04	
590-01-2	Propanoic acid butyl ester	8.06	361.95	15.37		12.49			7.61			208.25		2.07	0.95	0.74
97-87-0	Propanoic acid 2-methylbutyl ester	8.18				4.88										
539-90-2	Butanoic acid 2-methylpropyl ester	8.45			0.85				7.83			67.33				
628-63-7	Aethanoic acid pentyl ester	8.68							3.24			19.12				
123-92-2	Ethanoic acid 3-methyl butanyl ester	8.75	8.39		3.17	2.91										
106-70-7	Hexanoic acid methyl ester	9.01				2.98	7.81	1.33	29.21	1.24		5.36				3.1
109-21-7	Butanoic acid butyl ester	9.68		2.55		17.29			18.27	55.87	4.81	715.41		2.03	1.25	0.78
141-06-0	Pentanoic acid propyl ester	9.73			0.43											
123-66-0	Hexanoic acid ethyl ester	9.99	8.2		0.47				2.31			19.14			0.46	0.44
624-54-4	Propanoic acid pentyl ester	10.12	2.53			0.47			1.18		3.59	9.77				
109-19-3	3- Methyl butanoic acid butyl ester	10.29	14.78	18.36	4.82	2.53					4.57				1.25	
106-27-4	Butanoic acid 3-methylbutyl ester	10.60							4.9			7.82				
142-92-7	Ethanoic acid hexyl ester	10.73	3.57			14.43			1.55			2.76				
106-73-0	Heptanoic acid methyl ester	11.02							8.64							0.73
591-68-4	Pentanoic acid butyl ester	11.50										78.93				
5870-93-9	Butanoic acid heptyl ester	11.51							10.84	7.5						
626-77-7	Hexanoic acid propyl ester	11.63	3.03	2.14												
106-30-9	Heptanoic acid ethyl ester	11.88	57.61	27.71	10.9	1.23							1.04			
4630-82-4	Cyclohexane carboxylic acid methyl ester	12.71	3.29			1.29					12.16					
111-11-5	Octanoic acid methyl ester	12.82						2.21								

S3c)															
CAS	Esters and thioesters	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
626-82-4	Hexanoic acid, butyl ester	13.30			0.2				6.12			2.65				

Table 3.20 section a, b, and c esters and thioesters found in unmodified stool samples for all participants with retention time and chromatographic peak area.

S4a)														
CAS	Ketones	Retention time (mins)	1	2	3	4	5	6	7	8	9	10	11	12
67-64-1	Acetone	2.61	148.03	187.16	184.56	179.32	193.52	404.75	363.6	146.87	162.02	170.04	202.18	210.3
78-93-3	2-Butanone	3.37		546.72										
563-80-4	3-Methyl 2-butanone	4.56												
107-87-9	2-Pentanone	4.61		37.44		198.34	58.79	141.58	108.36	117.22		29.05	42.67	
431-03-8	2,3-Butanedione	4.61			47.06									
108-10-1	Methyl isobutyl ketone	5.19				27.36								
591-78-6	2-Hexanone	5.21						12.93						
565-61-7	3-Methyl 2-pentanone	5.37				12.83					5.82			
600-14-6	2,3- Pentanedione	6.33											19.55	
105-42-0	4-Methyl 2-hexanone	8.84												
110-43-0	2-Heptanone	8.91			3.94	17.39	26.59	4.68	8.23	1.96	4.03	3.86	2.35	
110-93-0	6-Methyl -5-hepten-2-one	11.94	68.91	98.65	43.13	37.69	15.41	101.06	9.14	4.33	52.48	12.54	43.31	100.71

S4b)														
CAS	Ketones	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
67-64-1	Acetone	2.61	206.65	340.64	25.04	55.52	533.55	186.84	109.9	95.74	154.69	148.61	466.96	151.9
78-93-3	2-Butanone	3.37												
563-80-4	3-Methyl 2-butanone	4.56												
107-87-9	2-Pentanone	4.61		557.96				27.73	26.39					

S4b)														
CAS	Ketones	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
431-03-8	2,3-Butanedione	4.61												
108-10-1	Methyl isobutyl ketone	5.19	7.02	20.15					6.1					
591-78-6	2-Hexanone	5.21					3.29			2.65	3.4	5.38		
565-61-7	3-Methyl -2-pentanone	5.37		15.59					9.35		5.04	6.85		
600-14-6	2,3- Pentanedione	6.33	7.47		10.75			6.9		11.51	2.21		5.59	
105-42-0	4-Methyl- 2-hexanone	8.84												
110-43-0	2-Heptanone	8.91	5.63	23.09		2.53	11.61		0.98	1.59	2.83		3.91	
110-93-0	6-Methyl -5-hepten-2-one	11.94	0.78	13	7.47	3.2		1.57	0.86					

S4c)																
CAS	Ketones	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
67-64-1	Acetone	2.61	211.47	240.61	78.31	43.06	73.78	356.22	286.22	103.38	56.55	110.4	340.88	185.94	37.79	20.18
78-93-3	2-Butanone	3.37														
563-80-4	3-Methyl -2-butanone	4.56														2.89
107-87-9	2-Pentanone	4.61		48.74	85.84											
431-03-8	2,3-Butanedione	4.61					6.95	17.86								
108-10-1	Methyl isobutyl ketone	5.19			2.54	4.53					5.31					
591-78-6	2-Hexanone	5.21	14.19													
565-61-7	3-Methyl -2-pentanone	5.37	24.08											1.46		
600-14-6	2,3,- Pentanedione	6.33	65.04	9.48					2.04		2.22					
105-42-0	4-Methyl -2-hexanone	8.84					1.58	0.99	3.91	1.47	2.69		1			
110-43-0	2-Heptanone	8.91	15.36	2.32	0.99											
110-93-0	6-Methyl -5-hepten-2-one	11.94	3.27				1.54			1.81		2.17				

Table 3.21 section a, b, and c ketones found in unmodified stool samples for all participants with retention time and chromatographic peak area.

S5a)														
CAS	Acids	Retention time (mins)	1	2	3	4	5	6	7	8	9	10	11	12
64-19-7	Ethanoic acid	14.11	36.5	49.85	39.26	84.42	29.61	27.56	29.65	184.72	83.28	60.75	56.5	61.11
79-09-4	Propanoic acid	15.42								116.3	44.51	20.4	13.88	27.17
79-31-2	2-Methylpropanoic acid	15.93	1.99	1.48	2.26	2.96	1.84	2.46	9.25	63.54	21.57	10.37	6.5	12.15
107-92-6	Butanoic acid	16.82	17.25	13.78	18.45	46.21	18.42	12.8	25.93	322.75	133.6	60.27	44.23	78.16
503-74-2	3-Methylbutanoic acid	17.33	8.63	10.39	11.6	10.3	10.31	10.46	19.8	110.89	39.97	18.27	17.16	32.86
109-52-4	Pentanoic acid	18.40	2.19		4.54			4.04	14.06	89.51		12.82	13.84	20.04
142-62-1	Hexanoic acid	19.77												
111-14-8	Heptanoic acid	21.23												3.71
98-89-5	Cyclohexanecarboxylic acid	22.89								9.94				
65-85-0	Benzoic acid	27.00				25.97								

S5b)														
CAS	Acids	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
64-19-7	Ethanoic acid	14.11	56.46	120.09	5.05	68.57		52.97	20.96	15.61			31.51	20.94
79-09-4	Propanoic acid	15.42	20.73	34.6	8.38		8.3	15.66	4.93	2.89	1.61	3.98	3.22	3.44
79-31-2	2-Methylpropanoic acid	15.93	9.67	11.61	3.24	11.81	6.15	12.07	3.41	2.14	0.75	2.29	1.26	2.11
107-92-6	Butanoic acid	16.82	71.31	99.34	29.87	85.42	40.99	57.1	16.49	9.48	4.36	12.36	10.47	13.74
503-74-2	3-Methylbutanoic acid	17.33	19.9	23.13	7.08	20.46	12.43	22.91	6.66	4.53		4.43	3	4
109-52-4	Pentanoic acid	18.40	15.51	12.86	2.96	11.14	6.92	11.14	4.74	1.93		2.47	1.86	

S5b)														
CAS	Acids	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
142-62-1	Hexanoic acid	19.77												
111-14-8	Heptanoic acid	21.23					0.91							
98-89-5	Cyclohexanecarboxylic acid	22.89												
65-85-0	Benzoic acid	27.00												

S5c)																
CAS	Acids	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
64-19-7	Ethanoic acid	14.11		53.75		10.72	20.82	26.06	230.18	101.34	8.05	179.82	87.46	24.03	12.41	14.98
79-09-4	Propanoic acid	15.42		12.27	14.99	3.32		5.56	94.61	30.23	30.4	74.44	32.75	5.37	2.67	3.09
79-31-2	2-Methylpropanoic acid	15.93	92.22	3.49				2.81	71.8	21.83	16.47	30.53	15.91	2.84	2.24	1.59
107-92-6	Butanoic acid	16.82	637.09	48.91	18.02	11.88	13.49	23	364.97	116.71	102.47	274.48	135.55	25.23	12.58	16.38
503-74-2	3-Methylbutanoic acid	17.33	162.97	9.08				5.74	133.1	45.8	30.86	41.62	22.97	5.53	2.56	5.54
109-52-4	Pentanoic acid	18.40		5.77			5.12	3.35	64.32	28.85	16.77	37.22	22.59	7.38	2.75	2.3
142-62-1	Hexanoic acid	19.77							2.16	12.38	6.69	17.93	7.8		1.72	
111-14-8	Heptanoic acid	21.23			2.66				4.32							
98-89-5	Cyclohexanecarboxylic acid	22.89			1.43											
65-85-0	Benzoic acid	27.00														

Table 3.22 sections a, b, and c acid found in unmodified stool samples for all participants with retention time and chromatographic peak area.

S6a)														
CAS	Nitrogen containing compounds	Retention time (mins)	1	2	3	4	5	6	7	8	9	10	11	12
75-05-8	Acetonitrile	5.10	5.42	13.59	9.33						10.6	9.69	17.7	
7149-26-0	1,6-Octadien-3-ol, 3,7-dimethyl-2-aminobenzoate	15.63		12.59		1.18								

S6a)															
CAS	Nitrogen containing compounds	Retention time (mins)	1	2	3	4	5	6	7	8	9	10	11	12
120-72-9	Indole	26.78	22.53	26.36	14.06	12.37	14.87	13.82	39.7	3.13				
83-34-1	3-Methylindole	27.27	16.84	27.74	19.94	12.67	20.08	7.09		0.5				

S6b)														
CAS	Nitrogen containing compounds	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
75-05-8	Acetonitrile	5.10	5.01		12.58		3.55		3.85	3.7	5.9	4.44	9.5	2.89
7149-26-0	1,6-Octadien-3-ol, 3,7-dimethyl-2-aminobenzoate	15.63		1.82		1.98								
120-72-9	Indole	26.78					17.61	40.36	18.46	6.19	5.89	6.72	5.88	8.99
83-34-1	3-Methylindole	27.27					11.87	13.09	9.63	3.35	15.19	3.98	7.81	7.62

S6c)																
CAS	Nitrogen containing compounds	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
75-05-8	Acetonitrile	5.10		12.72	4.43	3.94	4.44	5.72	6.37	3.06			7.52	2		
7149-26-0	1,6-Octadien-3-ol, 3,7-dimethyl-2-aminobenzoate	15.63			1.68											
120-72-9	Indole	26.78	20.56	31.73	7.11	12.9	71	14.5	21.78	19.25		13.5	9.8	18.1		
83-34-1	3-Methylindole	27.27	10.89	12.14	3.77	3.14	3.16	6.62	5.7	12.42						

Table 3.23 sections a, b, and c nitrogen containing compounds found in unmodified stool samples for all participants with retention time and chromatographic peak area.

S7a)														
CAS	Sulphur compounds	Retention time (mins)	1	2	3	4	5	6	7	8	9	10	11	12
75-18-3	Dimethyl sulphide	2.30	203.39	56.77	16.53		23.15	45.02	223.11	203.39	374.49	42.98	281.73	106.79
624-92-0	Dimethyl disuphide	6.52	231.45	325.68	86.61	197.58	195.1	259.84	213.6	178.59	200.21	168.97	834.04	188.52
5925-75-7	S-Methyl propanethioate	7.45	42.57	20.19	6.1	1.22	9.1	13.4		2	0.79	36.37		
23747-45-7	S-Methyl 3-methylbutanethioate	9.69												
2179-60-4	Methyl propyl disulphide	9.87	3.4		1.94		3.87			0.93			2.59	
1618-26-4	2,4-Dithiapentane	10.60											7.07	
57-06-7	Allyl isothiocyanate	12.32				13.45					6.97		5.42	
3658-80-8	Dimethyl trisulphide	12.65	9.41	17.77	4.59	9.3	9.92	9.62	14.5		3.81	2.17	52.99	

S7b)														
CAS	Sulphur compounds	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
75-18-3	Dimethyl sulphide	2.30	16.7	17.12		55.47	24.21	238.77	37.98	44.42	19.91	165.05		32.7
624-92-0	Dimethyl disulphide	6.52	296.83	516.85	224.59	362.51	166.39	566.67	484.93	200.35	212.21	237.7	200.09	604.71
5925-75-7	S-Methyl propanethioate	7.45			5.73	10.85							13.79	
23747-45-7	S-Methyl 3-methylbutanethioate	9.69												
2179-60-4	Methyl propyl disulphide	9.87	1.58	2.99										
1618-26-4	2,4-Dithiapentane	10.60							0.54					
57-06-7	Allyl isothiocyanate	12.32	0.55	2.01		0.7		2.3	1.8					0.89
3658-80-8	Dimethyl trisulfide	12.65	20.62	20.93	3	15.81	5.99	39.63	24.67	1.82	4.67	10.05	2.82	2.69

S7c)																
CAS	Sulphur compounds	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
75-18-3	Dimethyl sulphide	2.30	8.79	282.86	20.75	28.7	57.14	81.34	34.74	137.39		46.26	65.97	129.46	350.25	18.64
624-92-0	Dimethyl disuphide	6.52	491.65	412.1	23.49	197.74	488.56	183.41	762.2	267.79	117.79	136.73	464.7	496.07	196.26	71.58
5925-75-7	S-Methyl propanethioate	7.45					1.45									

S7c)																	
CAS	Sulphur compounds	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
23747-45-7	S-Methyl 3-methylbutanethioate	9.69					3.57		18.2	2.08			1.8	1.6		
2179-60-4	Methyl propyl disulphide	9.87		3.02			4.78	1.34	1.77		0.84				0.51	
1618-26-4	2,4-Dithiapentane	10.60		1.13												
57-06-7	Allyl isothiocyanate	12.32	13.61	1.24		1.23										
3658-80-8	Dimethyl trisulfide	12.65	63.43	19.61	13.68	13.54	43.15	4.32	62.39	27.27		2.94	36.14	39.72	11.54	4.25

Table 3.24 sections a, b, and c sulphur containing compounds found in unmodified stool samples for all participants with retention time and chromatographic peak area.

S8a)															
CAS	Miscellaneous	Retention time (mins)	1	2	3	4	5	6	7	8	9	10	11	12
75-09-2	Dichloromethane	3.78	82.96	144.14	46.62						92.1			
1073-91-2	1,2,4,5- Tetroxane, 3,3,6,6-tetramethyl	4.63	18.86								13.29			

S8b)														
CAS	Miscellaneous	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
75-09-2	Dichloromethane	3.78	73.89		77.16		227.47	92.56	68.45	151.42		117.81	148.9	95.33
1073-91-2	1,2,4,5- Tetroxane, 3,3,6,6-tetramethyl	4.63			33.38									

S8c)																
CAS	Miscellaneous	Retention time (mins)	25	26	27	28	3029	30	31	32	33	34	35	36	37	38
75-09-2	Dichloromethane	3.78	97.01	92.4	107.9	55.36	44.6	130.81	83.48		44.97		95.41	84.58	31.43	20.89
1073-91-2	1,2,4,5- Tetroxane, 3,3,6,6-tetramethyl	4.63												22.79		

Table 3.25 sections a, b, and c uncategorised compound found in unmodified stool samples for all participants with retention time and chromatographic peak area.

S9a)														
CAS	Aromatic compounds	Retention time (mins)	1	2	3	4	5	6	7	8	9	10	11	12
7149-26-0	1,6-Octadien-3-ol, 3,7-dimethyl-2-aminobenzoate	15.63		12.59		1.18								
140-67-0	Estragole	17.32												
108-95-2	Phenol	21.84	10.65	12.26	20.53	21.72	9.4	10.36	13.1	16.14	11.66	10.85	14.11	12.48
106-44-5	4-Methylphenol	22.76	49.39	76.34	67.8	40.56	81.97	97.82	79.73	52.38	63.66	37.6	61.66	72.45
120-72-9	Indole	26.78	22.53	26.36	14.06	12.37	14.87	13.82	39.7	3.13				
83-34-1	3-Methyl indole	27.27	16.84	27.74	19.94	12.67	20.08	7.09		0.5				

S9b)														
CAS	Aromatic compounds	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
7149-26-0	1,6-Octadien-3-ol, 3,7-dimethyl-,2-aminobenzoate	15.63		1.82		1.98								
140-67-0	Estragole	17.32				4.39					2.38			
108-95-2	Phenol	21.84	5.33	5.27	4.94	5.18	4.2	5.79	6.03	3.94	3.39		15.67	10.76
106-44-5	4-Methylphenol	22.76	84.54	97.97	112.53	55.98	47.26	144.16	102.33	72.75	65.4	97.56	91.74	114.85
120-72-9	Indole	26.78					17.61	40.36	18.46	6.19	5.89	6.72	5.88	8.99
83-34-1	3-Methylindole	27.27					11.87	13.09	9.63	3.35	15.19	3.98	7.81	7.62

S9c)																
CAS	Aromatic compounds	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
7149-26-0	1,6-Octadien-3-ol, 3,7-dimethyl-,2-aminobenzoate	15.63			1.68											
140-67-0	Estragole	17.32														
108-95-2	Phenol	21.84	22.65	4.8	8.49	7.39	12.26	8.46	5.98	5.92	14.57	11.32	4.98	4.94	2.12	1.72
106-44-5	4-Methylphenol	22.76	179.67	250.49	60.76	42.3	39.49		155.92	54.74	15.67	13.52	32.45	22.78	5.48	3.89
120-72-9	Indole	26.78	20.56	31.73	7.11	12.9	71	14.5	21.78	19.25		13.5	9.8	18.1		
83-34-1	3-Methylindole	27.27	10.89	12.14	3.77	3.14	3.16	6.62	5.7	12.42						

Table 3.26 sections a, b, and c aromatic compounds found in unmodified stool samples for all participants with retention time and chromatographic peak area.

S10a)													
Siloxanes	**Retention time (mins)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Siloxane (1)	4.31				19.99								
Siloxane (2)	13.03		10.44	4.62		5.28	3.64	4.06	8.42	3.48	2.89	3.97	
Siloxane (3)	15.32	4.81	5.16	4.57	5.4	6.49	3.88	5.51		2.03	1.94	2.34	

S10b)													
Siloxanes	**Retention time (mins)**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Siloxane (1)	4.31												
Siloxane (2)	13.03	1.48	2.94		4.09	1.62	2.61	2.43	0.92		2.89	1.97	1.31
Siloxane (3)	15.32				1.08								

S10c)															
Siloxanes	**Retention time (mins)**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
Siloxane (1)	4.31														
Siloxane (2)	13.03	20.89	2.66	29.28			1.38	7.13	11.49	7.12	2.57		2.38	1.46	
Siloxane (3)	15.32	18.68			2.87										

Table 3.27 sections a, b, and c siloxanes found in unmodified stool samples for all participants with retention time and chromatographic peak area.

S11a)													
Terpenes	**Retention time (mins)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Terpene (1)	3.12	18.15								11.49	10.61		
Terpene (2)	4.13												
Terpene (3)	5.30												
Terpene (4)	5.52	60.25	187.79	27.85	21.87	44.69	27.29	28.09		18.77	113.72		
Terpene (5)	5.78												
Terpene (6)	6.39					8.39				12.39	4.15		
Terpene (7)	7.13	69.38	35.86	10.72	14.75	134.4	17.7	11.42	7.53	14.13	262.89		16.46
Terpene (8)	7.37												
Terpene (9)	8.16					27.09	4.43	8.42	6.65		4.37	6.97	
Terpene (10)	8.42	13.47	531.5			10.12						1.8	
Terpene (11)	8.47									7			
Terpene (12)	9.16	81.61	15555.7	33.19	13.06	21.47	243.65	26.07	60.62	21.39	79.09	9.22	6.75
Terpene (13)	9.34	6.68				4.89	5.8			4.38	9.65		
Terpene (14)	9.92												
Terpene (15)	10.16	27.03	11.71	22.36	6.54	79.37	5.37	2.21		4.1	114.69	1.24	
Terpene (16)	10.38		14.73										
Terpene (17)	10.63	13.3	42.15	14.48	3.27	32.28	10.11	3.14	1.9	3.27	132.89		
Terpene (18)	10.95		14.7			16.42							
Terpene (19)	13.69		63.44								0.8		
Terpene (20)	13.73			7.17									
Terpene (21)	14.56					2.07							
Terpene (22)	14.73		40.71	0.66		3.45	1.33	0.71			2.58		
Terpene (23)	15.47		4.6	4.52	7.8	3.62	3.41						
Terpene (24)	16.16	3.9											
Terpene (25)	16.35	15.42	27.56	3.8	5.09	27.95	7.87	6.64	1.87	4.79	29.53	3.31	
Terpene (26)	17.99					1.97							
Terpene (27)	18.12									6.66			

S11a)													
Terpenes	**Retention time (mins)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Terpene (28)	18.22	1.53											
Terpene (29)	20.71		33.18	7.86						10.91	6.86		
Terpene (29)	23.86		2.27	1.21	3.21				1.1			0.91	
Terpene (30)	24.64				14.18	6.41							

S11b)													
Terpenes	**Retention time (mins)**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Terpene (1)	3.12			34.48									
Terpene (2)	4.13												
Terpene (3)	5.30												
Terpene (4)	5.52		17.42										
Terpene (5)	5.78					106.26			126.39	67.9		97.28	150.86
Terpene (6)	6.39												
Terpene (7)	7.13		60.76	11.9	171.24	30.42						38.37	
Terpene (8)	7.37												
Terpene (9)	8.16	1.37		4.62	9.99	66.92							
Terpene (10)	8.42	3.26		1.99	3.49	4.07							
Terpene (11)	8.47					0.79	13.37		0.49				
Terpene (12)	9.16	6.94		6.44	42.26	36.39	3.51		2.7	28.21		57.74	
Terpene (13)	9.34			2.05	3.63	1.1	0.88		3.74			3.24	2.59
Terpene (14)	9.92												
Terpene (15)	10.16				84.68								
Terpene (16)	10.38					2.15							2.23
Terpene (17)	10.63		21.08	1.22	28.48								
Terpene (18)	10.95		4.67			4.22	2.32	0.95					1.48
Terpene (19)	13.69					39.61						0.39	
Terpene (20)	13.73												

S11b)													
Terpenes	**Retention time (mins)**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Terpene (21)	14.56									1.1			
Terpene (22)	14.73			1.3	1.07	2.85						1.25	
Terpene (23)	15.47				25.27								
Terpene (24)	16.16												
Terpene (25)	16.35	1.84	3.8	10.77	4.62	3.19	2.81	1.44		0.99			1.28
Terpene (26)	17.99												
Terpene (27)	18.12		3.22	4.81					0.45				
Terpene (28)	18.22		28.78	2.73									2.63
Terpene (29)	20.71												
Terpene (29)	23.86												
Terpene (30)	24.64												

S11c)															
Terpenes	**Retention time (mins)**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
Terpene (1)	3.12														
Terpene (2)	4.13					4.25									
Terpene (3)	5.30						1.57		3.19		14.17		1.02		
Terpene (4)	5.52			7.23		12.62	27.49		21.48	14.43					
Terpene (5)	5.78			47.1			103.61								
Terpene (6)	6.39					5.39	7.25				18.53				
Terpene (7)	7.13	16.09	3.39	2.91	1.52	3.59	4.83		17.83	5.59	2.12		3.23	1.38	
Terpene (8)	7.37						2.65		4.2	3.91	2.65				
Terpene (9)	8.16	16.58				4.72	2.54		45.33	14.67				3.74	
Terpene (10)	8.42					2.84	0.75			2.91				0.76	
Terpene (11)	8.47		8.03						2.01						
Terpene (12)	9.16	53.23	4.56	6.7		10.97	26.35	2.76	23.68	7.94	287.27	1.34	4.17	5.28	0.74
Terpene (13)	9.34	1.2	2			22.72	2.67		2.05	1.38	13.76				

S11c)															
Terpenes	**Retention time (mins)**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
Terpene (14)	9.92					3.84			1.18						
Terpene (15)	10.16					1.67	5.04							0.48	
Terpene (16)	10.38	43.96													
Terpene (17)	10.63					6.12	1.89			2.49					
Terpene (18)	10.95	2.49	2.5		3.15		2.97					2.55			
Terpene (19)	13.69				3.97						0.72				
Terpene (20)	13.73														
Terpene (21)	14.56		2.5	0.77											
Terpene (22)	14.73		1.17												
Terpene (23)	15.47			4.76		7.02									
Terpene (24)	16.16														
Terpene (25)	16.35	3.85	4.7		5.37	4.53	7.08		2.37	1.75			2		
Terpene (26)	17.99														
Terpene (27)	18.12	100.88					1.56								
Terpene (28)	18.22			1.77											
Terpene (29)	20.71		26.1		5.52	13.81	17.34		0.89	1.07					
Terpene (29)	23.86										1.15				
Terpene (30)	24.64														

Table 3.28 sections a, b, and c terpenes found in unmodified stool samples for all participants with retention time and chromatographic peak area.

S12a)													
Unidentified	**Retention time (mins)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Unidentified (1)	3.46		954.93										1149.81
Unidentified (2)	3.81				103.68			111.2	122.12		47.91	73.41	
Unidentified (3)	4.38												
Unidentified (4)	5.19			10.58		13.99							
Unidentified (5)	5.37			10.07		5.87					9.93		
Unidentified (6)	5.84	10.02	116.36	88.28	160.57							114.57	

S12a)													
Unidentified	**Retention time (mins)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Unidentified (7)	5.87					97.72	186.13	175.49	144.23		66.06		
Unidentified (8)	6.04												
Unidentified (9)	6.23								12.49				55.16
Unidentified (10)	6.36	3.38	6.95		6.61				0.8				
Unidentified (11)	6.67												
Unidentified (12)	6.92		13.3										
Unidentified (13)	7.60	2.27	6.74										
Unidentified (14)	7.91	5.42									1.72	6.18	
Unidentified (15)	9.58							3.37					
Unidentified (16)	10.43											5.88	
Unidentified (17)	10.53												
Unidentified (18)	11.26	2.03						5.05	2.97			1.67	
Unidentified (19)	12.04											3.82	
Unidentified (20)	12.29												
Unidentified (21)	13.31								2.82				
Unidentified (22)	13.35							1.31					
Unidentified (21)	15.02								5.04				17.57
Unidentified (22)	17.58					1.27							
Unidentified (23)	17.75					5.47					1.82	1.18	
Unidentified (24)	19.86												
Unidentified (25)	20.31	4.41	7.07	3.5		62.59							
Unidentified (26)	20.82								2.73				
Unidentified (27)	21.22						2.19	3.4	6.69	4.54	2.32		
Unidentified (28)	21.56						2.62	2.2	3.18	2.22			
Unidentified (29)	21.99	13.55	7.47										
Unidentified (30)	25.61						8.1						
Unidentified (31)	25.88	12.58	10.95	10.14		5.53							

S12a)													
Unidentified	**Retention time (mins)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Unidentified (32)	26.97					18.77	7.7						

S12b)													
Unidentified	**Retention time (mins)**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Unidentified (1)	3.46												
Unidentified (2)	3.81		85.19			97.28				151.12			
Unidentified (3)	4.38												
Unidentified (4)	5.19					7.92			4.68		11.4	14.33	
Unidentified (5)	5.37	11.82											
Unidentified (6)	5.84		74.7										
Unidentified (7)	5.87												
Unidentified (8)	6.04												
Unidentified (9)	6.23												
Unidentified (10)	6.36		12.36		2.82								
Unidentified (11)	6.67						9.8	4.9	9.34	4.53		24.08	14.01
Unidentified (12)	6.92												
Unidentified (13)	7.60							5.83					5.34
Unidentified (14)	7.91												
Unidentified (15)	9.58												1.07
Unidentified (16)	10.43	2.11	2.64	6.62	1.89		2.14	3.02	1.47	1.28	1.79	1.83	
Unidentified (17)	10.53						6.09		0.79				1.19
Unidentified (18)	11.26	0.48	1.19		0.97		1.14						
Unidentified (19)	12.04	1.36	0.77		1.58								
Unidentified (20)	12.29												
Unidentified (21)	13.31												
Unidentified (22)	13.35	1.17											
Unidentified (21)	15.02		1.35	3.12			2.41	1.2	0.64		0.8		

S12b)														
Unidentified	Retention time (mins)	13	14	15	16	17	18	19	20	21	22	23	24
Unidentified (22)	17.58												
Unidentified (23)	17.75			2.49									
Unidentified (24)	19.86												
Unidentified (25)	20.31						1.2						
Unidentified (26)	20.82				1.88					23.07	23.97		
Unidentified (27)	21.22	1.47	1.34										
Unidentified (28)	21.56												
Unidentified (29)	21.99												
Unidentified (30)	25.61												
Unidentified (31)	25.88												
Unidentified (32)	26.97											6.64	

S12c)															
Unidentified	Retention time (mins)	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Unidentified (1)	3.46														
Unidentified (2)	3.81														
Unidentified (3)	4.38							2.33							
Unidentified (4)	5.19														
Unidentified (5)	5.37			2.76						2.76					
Unidentified (6)	5.84					138.32		206.48				237.85			21.89
Unidentified (7)	5.87														
Unidentified (8)	6.04										7023.54				
Unidentified (9)	6.23														
Unidentified (10)	6.36														
Unidentified (11)	6.67		10.06				2.14	11.77			7.02		2.7		
Unidentified (12)	6.92														

S12c)															
Unidentified	**Retention time (mins)**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
Unidentified (13)	7.60		2.27				4.42								
Unidentified (14)	7.91	365.96				0.86							0.78		
Unidentified (15)	9.58	419.43													
Unidentified (16)	10.43	3.52		5.45								2			
Unidentified (17)	10.53	12.84			1.44				6.49						
Unidentified (18)	11.26	1.41			0.26			3.89							0.5
Unidentified (19)	12.04													0.5	
Unidentified (20)	12.29					1.86									
Unidentified (21)	13.31					0.53									
Unidentified (22)	13.35	13.55				0.62			5.1						
Unidentified (21)	15.02	16.88						3.1							
Unidentified (22)	17.58			9.13		2.08									
Unidentified (23)	17.75			0.51											
Unidentified (24)	19.86														1.22
Unidentified (25)	20.31						3.88								
Unidentified (26)	20.82			8.81											
Unidentified (27)	21.22														
Unidentified (28)	21.56			3.96											
Unidentified (29)	21.99														
Unidentified (30)	25.61			0.31									1.72		
Unidentified (31)	25.88			2.02											
Unidentified (32)	26.97				0.51										

Table 3.29 sections a, b, and c unidentified compounds found in unmodified stool samples for all participants, with retention time and chromatographic peak area.

CAS	Compound name	RT (mins)	RI exp (KI)	RI lit [A] (F. Bianchi *et al.*. 2007)	RI lit [B] (V.I. Babushok *et al.*. 2011)	RI lit other source [Ref]	Confirmed by Standard
75-07-0	Acetaldehyde	2.16	788				
123-38-6	Propionaldehyde	2.43	804	801			
123-72-8	Butyraldehyde	3.1	882	878			
66-25-1	Hexanaldehyde	6.76	1087	1080			
100-52-7	Benzaldehyde	15.05	1513	1528			
55012-32-3	Isopropyl benzaldehyde (cuminaldehyde)	18.91	1782		1784.1		
64-17-5	Ethanol	3.91	938	932			
78-92-2	2-Butanol	5.64	1027	1035			
71-23-8	1-Propanol	5.92	1043	1052			
78-83-1	2-Methyl-1-propanol	7.27	1112		1089.3*		
6032-29-7	2-Pentanol	7.83	1137	1142			
71-36-3	1-Butanol	8.29	1157	1152			
123-51-3	3-Methyl -1-butanol	9.51	1203	1215			
108-11-2	4-Methyl -2-pentanol	9.78	1218				
71-41-0	1-Pentanol	10.35	1248	1256			
626-89-1	4-Methyl -1-pentanol	11.55	1306				
543-49-7	2-Heptanol	11.59	1308		1315.3		
2313-61-3	4-Methyl -2-hexanol	11.66	1313				
111-27-3	1-Hexanol	12.24	1346	1354			
123-96-6	2-Octanol	13.4	1409				
3391-86-4	1-Octen-3-ol	13.9	1442	1456			yes
111-70-6	1-Heptanol	13.99	1448	1460			

CAS	Compound name	RT (mins)	RI exp (KI)	RI lit [A] (F. Bianchi *et al.*. 2007)	RI lit [B] (V.I. Babushok *et al.*. 2011)	RI lit other source [Ref]	Confirmed by Standard
104-76-7	2-Ethyl-1-hexanol	14.49	1479	1492			
111-87-5	1-Octanol	15.64	1552	1561			
15356-70-4	Cyclohexanol, 5methyl-2-(1-methylethyl)-,(1a,2b,5a)	16.9	1631		1630.4		
79-20-9	Ethanoic acid, methyl ester	2.69	846	828			
141-78-6	Ethanoic acid ethyl ester	3.21	890	893			
105-37-3	Propanoic acid ethyl ester	4.28	958				
97-62-1	2-Methylpropanoic acid ethyl ester	4.44	966	960			
109-60-4	Ethanoic acid propyl ester	4.6	974	976			
623-42-7	Butanoic acid methyl ester	4.79	982	982			
868-57-5	2-Methylbutanoic acid methyl ester	5.28	1003				
556-24-1	3-Methylbutanoic acid methyl ester	5.49	1017	1024			
105-54-4	Butanoic acid ethyl ester	5.87	1040	1040			
106-36-5	Propanoic acid propyl ester	6.04	1050	1056			
7452-79-1	2-Methylbutanoic acid ethyl ester	6.21	1060				
123-86-4	Ethanoic acid butyl ester	6.61	1080	1077			
624-24-8	Pentanoic acid methyl ester	6.87	1093	1087			
105-66-8	Butanoic acid propyl ester	7.64	1129	1123			
539-82-2	Pentanoic acid ethyl ester	7.92	1141	1138			
590-01-2	Propanoic acid butyl ester	8.06	1147				
97-87-0	Propanoic acid 2-methylbutyl ester	8.18	1152	1154			
539-90-2	Butanoic acid 2-methylpropyl ester	8.45	1163	1152			

CAS	Compound name	RT (mins)	RI exp (KI)	RI lit [A] (F. Bianchi *et al.*. 2007)	RI lit [B] (V.I. Babushok *et al.*. 2011)	RI lit other source [Ref]	Confirmed by Standard
628-63-7	Aethanoic acid pentyl ester	8.68	1172	1180			
123-92-2	Ethanoic acid 3-methylbutanyl ester	8.75	1175	1125			
106-70-7	Hexanoic acid methyl ester	9.01	1184	1190			
109-21-7	Butanoic acid butyl ester	9.68	1212	1223			
141-06-0	Pentanoic acid propyl ester	9.73	1215			1233 Peng *et al*..	
123-66-0	Hexanoic acid ethyl ester	9.99	1229	1238			
624-54-4	Propanoic acid pentyl ester	10.12	1236	1239			
109-19-3	3 -Methylbutanoic acid butyl ester	10.29	1244				
106-27-4	Butanoic acid 3-methylbutyl ester	10.6	1260	1267			
142-92-7	Ethanoic acid hexyl ester	10.73	1266	1269			
106-73-0	Heptanoic acid methyl ester	11.02	1280	1288			
591-68-4	Pentanoic acid butyl ester	11.5	1303				
5870-93-9	Butanoic acid heptyl ester	11.51	1304				
626-77-7	Hexanoic acid propyl ester	11.63	1311	1324			
106-30-9	Heptanoic acid ethyl ester	11.88	1325	1331			
4630-82-4	Cyclohexane carboxylic acid methyl ester	12.71	1371				
111-11-5	Octanoic acid methyl ester	12.82	1377	1387			
626-82-4	Hexanoic acid butyl ester	13.3	1403	1420			
108-64-5	3-Methylbutanoic acid ethyl ester	6.48	1074				
5870-93-9	Butanoic acid heptyl ester	11.51	1304				
3289-28-9	Cyclohexanecarboxylic acid ethyl ester	13.42	1411				

CAS	Compound name	RT (mins)	RI exp (KI)	RI lit [A] (F. Bianchi *et al.*. 2007)	RI lit [B] (V.I. Babushok *et al.*. 2011)	RI lit other source [Ref]	Confirmed by Standard
67-64-1	Acetone	2.61	814	814			yes
78-93-3	2-Butanone	3.37	901	901			
563-80-4	3-Methyl 2-butanone	4.56	972				
107-87-9	2-Pentanone	4.61	974	980			
431-03-8	2,3-Butanedione	4.61	974	986			
108-10-1	Methyl isobutyl ketone	5.19	998	1008			
591-78-6	2-Hexanone	5.21	999				
565-61-7	3-Methyl 2-pentanone	5.37	1009				
600-14-6	2,3- Pentanedione	6.33	1066	1071			
105-42-0	4-Methyl 2-hexanone	8.84	1178				
110-43-0	2-Heptanone	8.91	1180	1185			
110-93-0	6-Methyl -5-hepten-2-one	11.94	1329	1340			
107-92-6	Butanoic acid	16.82	1626	1630			yes
109-52-4	Pentanoic acid	18.4	1737				yes
142-62-1	Hexanoic acid	19.77	1854				
503-74-2	3-Methylbutanoic acid	17.33	1659				yes
64-19-7	Ethanoic acid	14.11	1455	1480			
65-85-0	Benzoic acid	27	2266				
79-09-4	Propanoic acid	15.42	1538	1554			
79-31-2	2-Methylpropanoic acid	15.93	1570				
111-14-8	Heptanoic acid	21.23	1972				

CAS	Compound name	RT (mins)	RI exp (KI)	RI lit [A] (F. Bianchi *et al.*. 2007)	RI lit [B] (V.I. Babushok *et al.*. 2011)	RI lit other source [Ref]	Confirmed by Standard
98-89-5	Cyclohexanecarboxylic acid	22.89	2081				
75-50-3	Trimethylamine	1.95	775				yes
124-40-3	Dimethylamine	3.19	889				
75-05-8	Acetonitrile	5.1	995				
926-64-7	Acetonitrile (dimethylamino)	10.02	1230			1243 (Pub Chem)	
7149-26-0	1,6-Octadien-3-ol, 3,7-dimethyl-2-aminobenzoate	15.63	1551				
120-72-9	Indole	26.78	2253				yes
83-34-1	3-Methylindole	27.27	2282				yes
75-18-3	Dimethyl sulphide	2.3	795				yes
624-92-0	Dimethyl disuphide	6.52	1076	1075			yes
5925-75-7	S-Methyl propanethioate	7.45	1121				
23747-45-7	S-Methyl 3-methylbutanethioate	9.69	1213				
2179-60-4	Methyl propyl disulphide	9.87	1223				
1618-26-4	2,4-Dithiapentane	10.6	1260			1260 (Shluter *et al.*)	
57-06-7	Allyl isothiocyanate	12.32	1350	1372			
3658-80-8	Dimethyl trisulphide	12.65	1368	1383	1376.2		yes
75-09-2	Dichloromethane	3.78	930				
1073-91-2	1,2,4,5- Tetroxane, 3,3,6,6-tetramethyl	4.63	975				

CAS	Compound name	RT (mins)	RI exp (KI)	RI lit [A] (F. Bianchi *et al.*. 2007)	RI lit [B] (V.I. Babushok *et al.*. 2011)	RI lit other source [Ref]	Confirmed by Standard
7149-26-0	1,6-Octadien-3-ol, 3,7-dimethyl-2-aminobenzoate	15.63	1551				
140-67-0	Estragole	17.32	1658				
108-95-2	Phenol	21.84	1959				
106-44-5	4-Methylphenol	22.76	2014				
120-72-9	Indole	26.78	2253				yes
83-34-1	3-Methyl indole	27.27	2282				yes
	Terpene (1)	3.12	884				
	Terpene (2)	4.13	950				
	Terpene (3)	5.3	1005				
	Terpene (4)	5.52	1019				
	Terpene (5)	5.78	1035				
	Terpene (6)	6.39	1069				
	Terpene (7)	7.13	1105				
	Terpene (8)	7.37	1117				
	Terpene (9)	8.16	1151				
	Terpene (10)	8.42	1162				
	Terpene (11)	8.47	1164				
	Terpene (12)	9.16	1190				
	Terpene (13)	9.34	1196				
	Terpene (14)	9.92	1225				
	Terpene (15)	10.16	1238				

CAS	Compound name	RT (mins)	RI exp (KI)	RI lit [A] (F. Bianchi *et al.*. 2007)	RI lit [B] (V.I. Babushok *et al.*. 2011)	RI lit other source [Ref]	Confirmed by Standard
	Terpene (16)	10.38	1249				
	Terpene (17)	10.63	1261				
	Terpene (18)	10.95	1277				
	Terpene (19)	13.69	1428				
	Terpene (20)	13.73	1431				
	Terpene (21)	14.56	1483				
	Terpene (22)	14.73	1493				
	Terpene (23)	15.47	1541				
	Terpene (24)	16.16	1584				
	Terpene (25)	16.35	1595				
	Terpene (26)	17.99	1700				
	Terpene (27)	18.12	1712				
	Terpene (28)	18.22	1721				
	Terpene (29)	20.71	1929				
	Terpene (29)	23.86	2079				
	Terpene (30)	24.64	2125				
	Unidentified (1)	3.46	908				
	Unidentified (2)	3.81	931				
	Unidentified (3)	4.38	963				
	Unidentified (4)	5.19	998				
	Unidentified (5)	5.37	1009				

CAS	Compound name	RT (mins)	RI exp (KI)	RI lit [A] (F. Bianchi *et al.*. 2007)	RI lit [B] (V.I. Babushok *et al.*. 2011)	RI lit other source [Ref]	Confirmed by Standard
	Unidentified (6)	5.84	1039				
	Unidentified (7)	5.87	1040				
	Unidentified (8)	6.04	1050				
	Unidentified (9)	6.23	1061				
	Unidentified (10)	6.36	1068				
	Unidentified (11)	6.67	1083				
	Unidentified (12)	6.92	1095				
	Unidentified (13)	7.6	1127				
	Unidentified (14)	7.91	1141				
	Unidentified (15)	9.58	1207				
	Unidentified (16)	10.43	1252				
	Unidentified (17)	10.53	1257				
	Unidentified (18)	11.26	1291				
	Unidentified (19)	12.04	1334				
	Unidentified (20)	12.29	1349				
	Unidentified (21)	13.31	1403				
	Unidentified (22)	13.35	1406				
	Unidentified (21)	15.02	1512				
	Unidentified (22)	17.58	1675				
	Unidentified (23)	17.75	1685				
	Unidentified (24)	19.86	1861				
	Unidentified (25)	20.31	1896				

CAS	Compound name	RT (mins)	RI exp (KI)	RI lit [A] (F. Bianchi *et al.*. 2007)	RI lit [B] (V.I. Babushok *et al.*. 2011)	RI lit other source [Ref]	Confirmed by Standard
	Unidentified (26)	20.82	1938				
	Unidentified (27)	21.22	1971				
	Unidentified (28)	21.56	1998				
	Unidentified (29)	21.99	2010				
	Unidentified (30)	25.61	2183				
	Unidentified (31)	25.88	2199				
	Unidentified (32)	26.97	2264				

Table 3.30 shows the retention indices calculated from the experimental data and where possible compared to available literature retention indices for PEG based polar columns. [A] F. Bianchi *et al*. J. Sep. Sci. 2007, 30, 563-572 [B] V.I. Babushok *et al*. J. Phys. Chem. Ref. Data, Vol. 40, No. 4, 2011, 043101-1, C.T.Peng *et al*.., J. Chromatogr., 1991, 586, 1, 85-112, S. Schlüter *et al*.., J. Agric. Food Chem., 1999, 47, 12, 5146-5150

3.3.2 Quantitative data from unaltered stools

Table 3.31 shows the mean and standard deviation for each compound in nanograms per gram of stool for all samples. The raw data for each individual sample can be found in Table 3.32 Table 3.31 also shows the mean concentration (ng/g) and standard deviation for each compound separated by samples of UK and South American origin, as these were the two largest groups.

	Origin	Number of Samples	Number of Times Detected	Median (ng/g)	Inter. Range (ng/g)	Range (ng/g)
Dimethyl sulphide CAS 75-18-3	Total	38	37	1682	4999	25–25626
	UK	16	16	1058	4124	26–8637
	South America	11	10	1644	3719	621– 8079
Acetone CAS 67-64-1	Total	38	38	1874	925	442– 3005
	UK	16	16	1227	743	442– 2521
	South America	11	11	1768	641	819– 2377
Ethyl butanoate CAS 105-54-4	Total	38	21	290	1097	39–2468
	UK	16	14	152	287	39–2468
	South America	11	3	393	355	118–828
Ethyl 2-methylbutanoate CAS 7452-79-1	Total	38	12	13	10	0.34–180
	UK	16	10	11	11	0.3–180
	South America	11	2	26	17	9–42
Dimethyl disulphide CAS 624-92-0	Total	38	38	513	890	35–1302
	UK	16	16	228	282	48–864
	South America	11	11	374	755	36–1313
1-Octen-3-one CAS 4312-99-6	Total	38	1	12	0	12
	UK	16	1	12	0	12
	South America	11	0	0	0	Not detected
Dimethyl trisulphide CAS 3658-80-8	Total	38	38	105	289	10–409
	UK	16	16	61	95	10–253
	South America	11	11	143	168	13–364
1-Octen-3-ol CAS 3391-86-4	Total	38	13	3	33	0.4–58
	UK	16	10	8	30	1–58
	South America	11	3	1	1	0.4–2
Ethanoic acid CAS 64-19-7	Total	38	38	9455	2519	672–12963
	UK	16	16	7849	2889	672–11343
	South America	11	11	9250	1253	7968–12963
Butanoic acid CAS 107-92-6	Total	38	38	8200	4661	2493–11553

	UK	16	16	6673	3592	2493–8376
	South America	11	11	8633	855	7043–9953
	Total	38	36	3878	3807	63–8262
3-Methylbutanoic acid	UK	16	16	1033	1824	64–3602
CAS 503-74-2	South America	11	10	4785	4097	220–6885
	Total	38	37	4516	5149	88–21886
Pentanoic acid	UK	16	16	1437	2345	88–5970
CAS 109-52-4	South America	11	11	6712	5896	946–21886
	Total	38	38	4495	2076	290–5477
Indole	UK	16	16	3079	924	1508–4309
CAS 120-72-9	South America	11	11	4232	847	3286–5477
	Total	38	38	1062	654	37–3483
3-Methyl-1*H*-indole	UK	16	16	1146	1092	425–3054
CAS 83-34-1	South America	11	11	1485	558	636–3483
Trimethylamine **CAS 75-50-3**	Total	15	15	161	108	40–920.5

Table 3.31 the mean concentration (ng/g) and standard deviation for compounds quantified from the stool of healthy participants using GC-MS. The mean concentration (ng/g) and S.D. of each compound from the UK and South American participants has also been compared.

Sample	Diet	Origin	Dimethyl Sulphide	Acetone	Butanoic acid, ethyl ester	2-Methyl-butanoic acid, ethyl	Dimethyl disulphide	1-Octen-3-one	Dimethyl Trisulphide	1-Octen-3-ol	Ethanoic acid	Butanoic acid	3-Methylbutanoic acid	Pentanoic acid	Indole	3-methyl indole	Trimethyl amine
1	Veg	UK	5238.15	598.82	0	0	148.61	11.88	43.95	35.07	4297.67	2492.6	63.56	88.3	2213.48	626.3	180.94
2	Meat	UK	916.89	1478.2	40.47	0	285.53	0	89.11	58.2	4989.53	2681.27	642.62	816.26	2342.28	830.68	132.49
3	Veg	UK	1328.85	1844.24	175.28	13.47	222.07	0	76.82	26.05	6193.06	2785.31	826.22	1113.08	2862.26	726.45	304.47
4	Veg	UK	153.99	1098.05	39.28	0.34	118.12	0	52.36	38.05	9030	4103.38	1074.09	1760.06	1508.71	670.22	
5	Veg	UK	502.9	1539.91	39.33	0.84	233.34	0	68.58	12.27	6953.33	4177.96	422.29	734.63	1862.04	1040.24	163.22
6	Meat	UK	669.77	2222.98	311.08	16.6	245.71	0	53.77	3.03	672	3328.19	991.47	1010.12	2500.71	585.03	
7	Meat	UK	8088.71	2198.58	339.64	18.1	295.84	0	119.51	2.37	6087.5	5548.11	2295.07	2380.76	2910.77	425.42	920.5
8	Meat	UK	5408.83	869.02	264.7	8.14	75.94	0	30.22	2.7	9146.98	7427.16	3602.64	5970.98	3318.1	677.29	
9	Veg	UK	8636.56	1356.9	0	0	91.31	0	19.23	0	11343.44	8228.25	406.55	558.75	4309.01	1251.73	
10	Veg	UK	1199.38	1494.35	43.41	4.66	48.47	0	10.83	0	8830	7700.68	372.04	565.21	3278.59	1629.85	
11	Meat	UK	4368.62	697.32	129.1	8.29	863.74	0	253.34	1.08	7236.49	6463.85	1927.86	2984.45	3247.19	3054	
12	Veg	UK	91.42	442.04	1659.32	0	52.95	0	22.66	0	8685.19	7331.51	2224.45	3531.9	2698.7	2185.55	
13	Veg	UK	565.56	874.6	1166.05	0	590.75	0	183.08	1.83	7007.78	7496.09	2506.21	4943.48	3577.83	1529.18	
14	Meat	UK	341.06	2520.59	69.78	15.41	725.96	0	135.15	0	10337.8	8376.46	366.05	730.37	4127.2	1457.4	
15	Meat	UK	25.8	1049.61	53.49	0	62.03	0	10.15	0	9133.87	6881.94	1707.53	2708.97	3961.73	2583.96	
16	Meat	UK	2055.58	1086.78	2468.23	179.98	755.98	0	141.86	0	8462.22	7518.38	2386.87	3360.7	3320.22	2180.09	
17	Meat	South America	746.73	2376.78	0	0	315.09	0	62.65	0.42	9434.78	9009.96	3084.57	4667.99	4313.84	1485.49	175.92
18	Meat	South America	6961.49	819.28	828.15	42.43	1012.95	0	315.66	0	9154.69	9363.66	0	6012.64	4756.23	1472.41	185.18
19	Meat	South America	1333.53	1011.08	0	0	957.42	0	207.74	1.23	7967.65	8624.19	5645.87	21886	4443.32	2561.2	
20	Meat	South America	1910.67	2048.95	0	0	373.75	0	199.6	0	8137.5	8632.79	575.85	946.12	3836.98	1085.39	
21	Meat	South America	621.54	1369.21	0	0	145.66	0	30.67	0	10750	8153.84	220.33	3015.06	3286.3	1646.75	
22	Meat	South America	5311.77	1440.22	0	0	355.64	0	91.73	0	9250	8547.05	5508.16	9259.46	3465.66	1162.92	5311.77
23	Meat	South America	0	1993.51	0	0	36.04	0	12.65	0	10519.44	8157.61	716.06	1117.26	4232.06	3483.23	351.83
24	Meat	South America	1376.92	1768.06	0	0	1302.92	0	241.31	0	9611.54	9050.63	6885.2	11172.57	3701.86	2103.59	104.16
25	Meat	South America	1294.29	1347.73	392.53	8.98	121.09	0	50.73	0	12963.32	7042.55	4838.42	6711.54	4677.54	1622.99	158.33
26	Veg	South America	8078.67	2000.63	0	0	712.09	0	142.55	2.22	8686.36	9953.48	5095.05	10216.02	5477.47	1479.56	39.88

Sample	Diet	Origin	Dimethyl Sulphide	Acetone	Butanoic acid, ethyl ester	2-Methyl-butanoic acid, ethyl	Dimethyl disulphide	1-Octen-3-one	Dimethyl Trisulphide	1-Octen-3-ol	Ethanoic acid	Butanoic acid	3-Methylbutanoic acid	Pentanoic acid	Indole	3-methyl indole	Trimethyl amine
27	Veg	Africa	611.89	1370.4	0	0	619.84	0	162.57	0	9590	9784.61	1836.05	2015.14	4776.4	1389.45	
28	Meat	Asia	613.44	453.09	1770.12	0	98.52	0	36.1	0	9975	8838.37	0	0	5050.3	956.18	
29	Veg	Africa	2704.33	2287.17	0	0	227.25	0	33.37	0	9203.51	9344.76	5220.03	6311.4	4543.73	2267.31	
30	Meat	Europe	1532.28	785.92	0	0	963.02	0	319.58	0	7670.83	4368.24	8262.24	13774.4	5070.29	790.31	
31	Meat	Africa	579.1	2156.48	0	0	1137.74	0	409.57	0	9670.6	7391.69	4914.36	4364.38	4714.12	1085.59	
32	Meat	Africa	5747.89	1747.46	2333.28	0	828.49	0	286.25	0	9475.73	9324.14	3606.73	3163.39	4117.27	774.76	
33	Veg	Asia	124.57	957.07	290.43	0	37.16	0	15.66	0	9763.67	8923.94	3558.52	3050.35	4586.6	686.74	
34	Meat	Asia	1546.13	1845.72	859.88	0	35.01	0	14.89	0	9982.29	8872.95	4098.55	5810.87	4605	403.99	545.95
35	Meat	Asia	2070.65	3005.62	0	0	1189.27	0	362.32	0	9984.12	9632.85	4149.28	5829.42	4782.7	993.61	
36	Meat	South America	4157.13	1998.93	118.41	0	1084.08	0	364.48	0	8937.71	9604.83	4732.02	7429.37	3725.53	636.13	
37	Veg	Europe	25626.1	1101.97	0	0	1058.28	0	199.02	0	12233.44	10448.77	3041.42	3295.83	289.94	37.25	55.35
38	Veg	Europe	3689.88	1028.97	0	0	809.81	0	162.32	0	11374.26	11552.97	5821.2	5532.56	4770.44	717.13	119.94

Table 3.32 raw data of concentrations of a range of stool compounds, in ng/g, calculated by adding ^{13}C labelled internal standards to stool samples and analysing the headspace using automated thermal desorption gas chromatography mass spectrometry.

3.3.3 UK samples versus South American samples

Mann Whitney U tests revealed six compounds to be significantly different between UK and South American samples, and these are shown in Table 3.33.

Compound	Sig.
Ethyl butanoate	0.023
1-Octen-3-ol	0.034
Ethanoic acid	0.011
Butanoic acid	0.000
Pentanoic acid	0.001
Indole	0.000

Table 3.33 statistical analyses showing significant differences (95% significance level) for six compounds using Mann Whitney U tests to determine differences in the quantities of compounds measured in stool from UK and South American participants.

Discriminant analysis using stepwise statistics (Wilks' Lambda) calculated butanoic acid and ethyl butanoate as being the two significant grouping variables.

Figure 3.19 shows a plot of the discriminant scores versus country of origin. There is a clear difference between the groups, with the South American sample set having a smaller range of scores versus the UK samples. This demonstrates that, with the exception of the outliers, the two means are very well separated. Figure 3.20 shows the resultant receiver operator curve (ROC) from the calculated discriminant scores; the area under the curve was 0.937 with a standard error of 0.044. This shows close to perfect separation of the U.K. and South American samples based on differences in the concentration of butanoic acid and ethyl butanoate. These two compounds alone can predict group membership. Figures 3.21 and 3.22 show the box plots for the masses of ethyl butanoate and butanoic acid respectively. Butanoic acid provides the better separation of the of UK and South American samples as a single variable (see Figure 3.22) however, the addition of ethyl butanoate into the model adds an extra level of differentiation (as in Figure 3.19). Figure 3.21 shows that the majority of samples fall into a relatively narrow band with only 5 outliers from the whole sample set. Figure 3.22 shows that in the South American samples the level of butanoic acid has a small range at a higher average versus the UK samples which have a wider range with a significantly lower average mass.

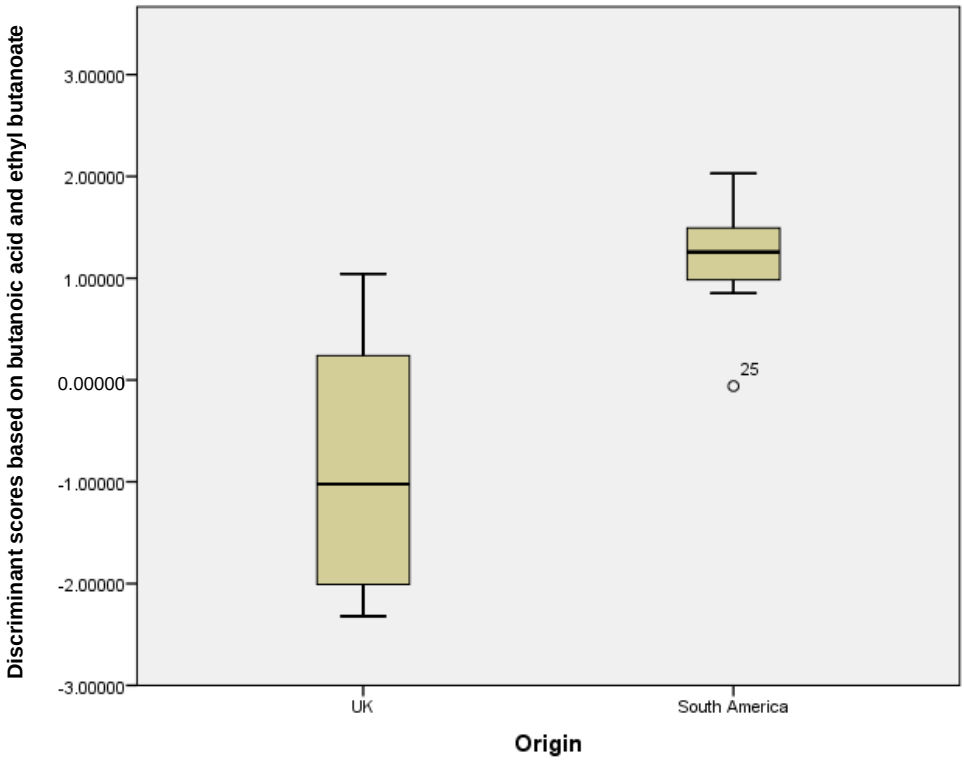


Figure 3.19 discriminant scores' box plots showing the differentiation between UK and South American stool samples (Wilks' Lambda analysis showed butanoic acid and ethyl butanoate to be the significant grouping variables).

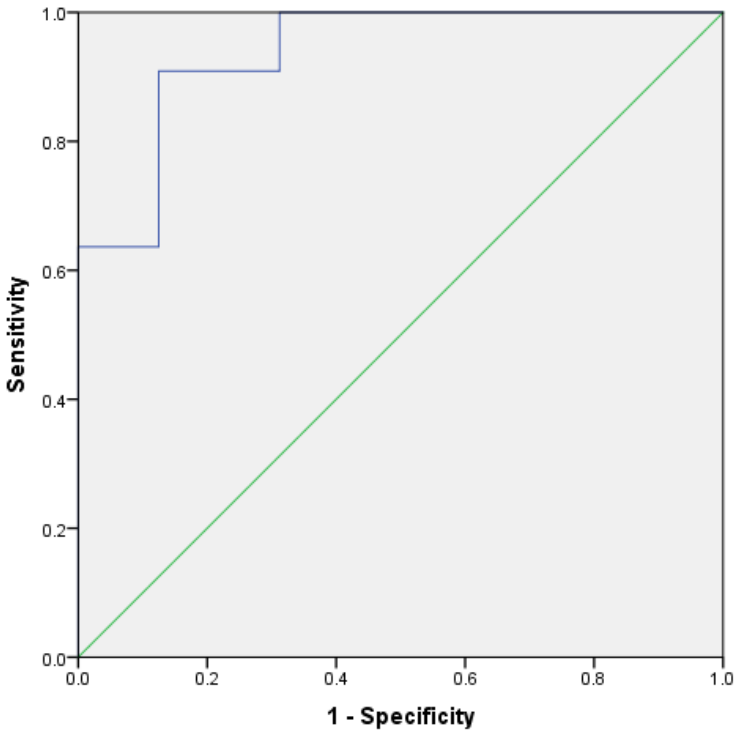


Figure 3.20 ROC curve based on leave-one-out cross validation discriminant scores, using butanoic acid and ethyl butanoate for different UK and South American stool samples.

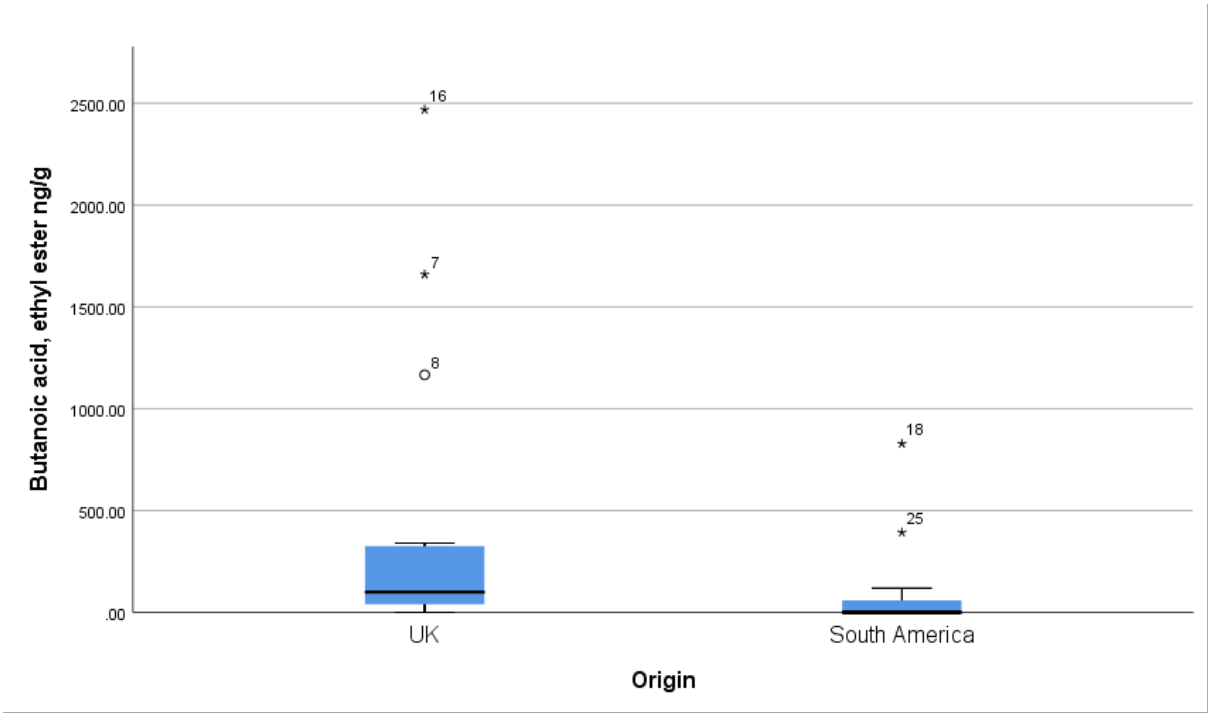


Figure 3.21 box plot of ethyl butanoate ng/g for UK versus South American participants

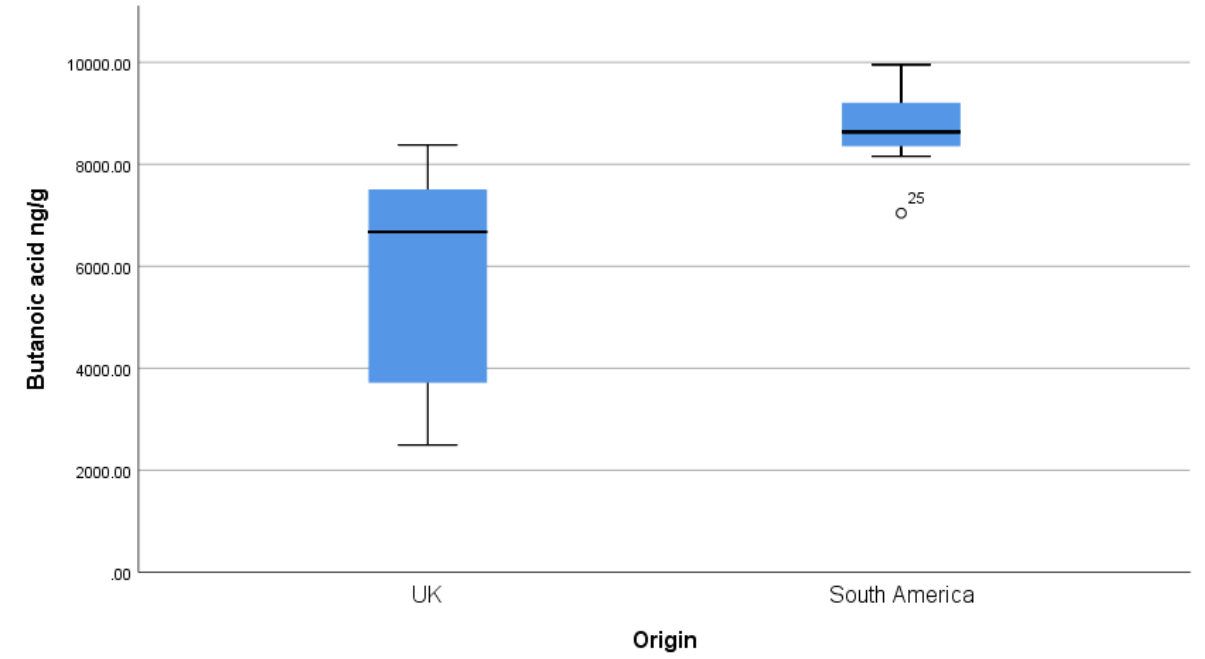


Figure 3.22 box plot of butanoic acid ng/g for UK versus South American participants

3.3.4 Omnivore versus vegetarian

The same series of statistical tests were carried out comparing vegetarians and omnivores; Table 3.34 shows the mean concentration (ng/g) and standard deviation for each compound. However, in this instance, there was no statistically significant differences in those compounds analysed. Discriminant analysis using stepwise statistics (Wilks' Lambda) calculated that the most significantly different compound

was dimethyl trisulphide with a $p = 0.055$. The discriminant scores for this compound measured in the omnivore and vegetarian groups are plotted in Figure 3.23. While the range of discriminant scores is wider for omnivore participants, the two groups completely overlap, illustrating the lack of any significant difference between the two groups.

	Diet	Number of Samples	Number of Times Detected	Median (ng/g)	Inter. Range (ng/g)	Range (ng/g)
Dimethyl sulphide	Vegetarian	14	14	1264	4333	91–25626
75-18-3	Omnivore	24	23	1532	3555	26–8089
Acetone	Vegetarian	14	14	1229	553	442–2287
CAS 67-64-1	Omnivore	24	24	1613	1036	453–3006
Ethyl butanoate	Vegetarian	14	7	175	687	39–1659
CAS 105-54-4	Omnivore	24	14	325	731	40–2468
Ethyl 2-methylbutanoate	Vegetarian	14	4	3	6	0.3–13
CAS 7452-79-1	Omnivore	24	8	16	15	8–180
Dimethyl disulphide	Vegetarian	14	14	225	225	37–1058
CAS 624-92-0	Omnivore	24	24	365	365	35–1303
1-Octen-3-one	Vegetarian	14	1	12	12	12
CAS 4312-99-6	Omnivore	24	0	0	0	Not detected
Dimethyl trisulphide	Vegetarian	14	14	60	60	11–199
CAS 3658-80-8	Omnivore	24	24	127	127	10–410
1-Octen-3-ol	Vegetarian	14	6	19	19	2–38
CAS 3391-86-4	Omnivore	24	7	2	2	0.4–58
Ethanoic acid	Vegetarian	14	14	8930	2293	4298–12233
CAS 64-19-7	Omnivore	24	24	9202	1882	672–12963
Butanoic acid	Vegetarian	14	14	7964	4708	2493–11553
CAS 107-92-6	Omnivore	24	24	8267	1905	2681–9633
3-Methylbutanoic acid	Vegetarian	14	14	2030	2906	64–5821
CAS 503-74-2	Omnivore	24	22	3344	3641	220–8262
Pentanoic acid	Vegetarian	14	14	2533	3761	88–10216
CAS 109-52-4	Omnivore	24	23	4364	3817	730–21886
Indole	Vegetarian	14	14	3428	2241	290–5477
CAS 120-72-9	Omnivore	24	24	4040	1303	2342–5070
3-Methyl-1*H*-indole	Vegetarian	14	14	1146	822	37–2267
CAS 83-34-1	Omnivore	24	24	1124	975	404–3483

Table 3.34 the mean concentration (ng/g) and measured concentration range (ng/g) for each compound in the omnivore and vegetarian diet groups.

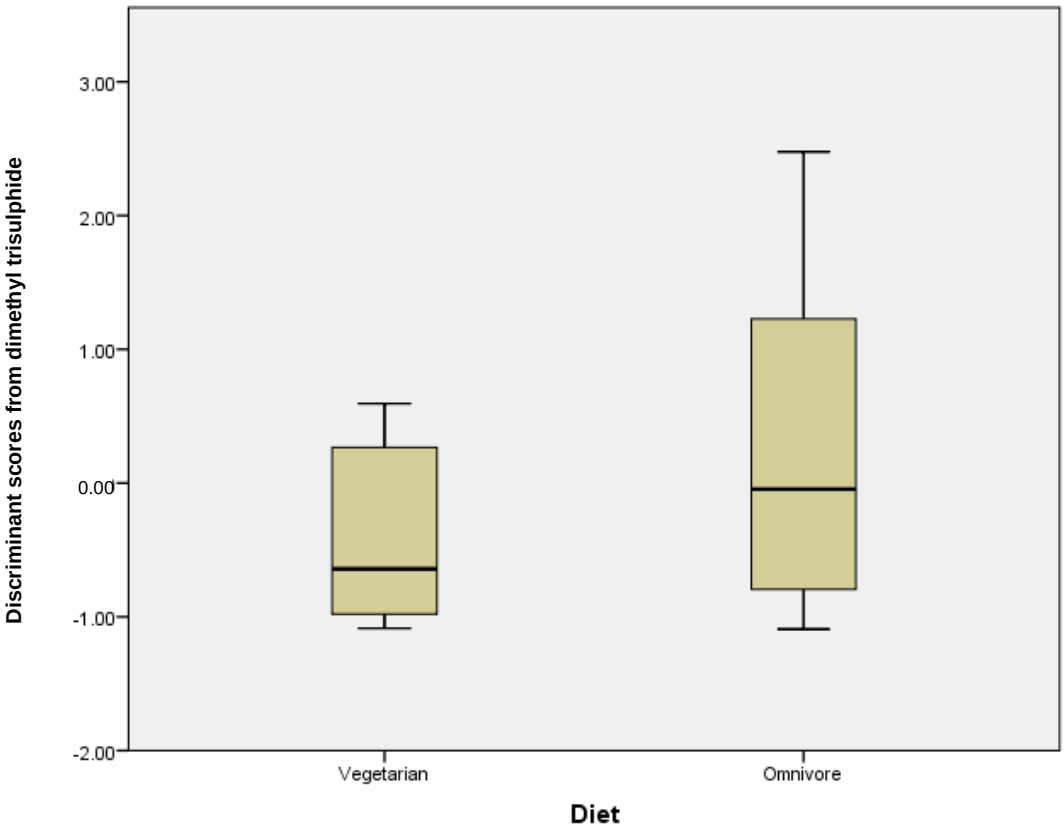


Figure 3.23 discriminant scores' box plots showing a lack of differentiation of stool samples from vegetarian and omnivore volunteers.

3.3.5 Qualitative data from alkaline (pH13) stools

A total of 15 stool samples were analysed after the addition of sodium hydroxide to alter the pH to 13. A total of 133 chromatographic peaks were recorded across the 15 samples with a mean of 43 peaks per chromatogram: with the lowest number of peaks being 29 and the highest 53. Of the total number of peaks, 16 were unidentified and 43 were categorised as terpenes. Table 3.35– 3.45 shows the qualitative data separated by chemical class.

CAS	Nitrogen containing compounds	RT (mins)	1	2	5	7	17	18	22	23	24	25	26	32	34	37	38
75-50-3	Trimethylamine	1.95	8.26	7.76	31.95	11.95	7.42	8.39	4.19	18.21	40.65	40.01	97.26	5.65			1.59
124-40-3	Dimethylamine	3.19						9.51	215.77			314.32			86.69		
75-05-8	Acetonitrile	5.1	16.55	10.99		15.94	17.05	13.13		21.01	13.99	8.53	12.68	8.51	5.47	16.82	21.23
926-64-7	Acetonitrile (dimethylamino)	10.02	6.41	51.86			7.94	22.61	32.54	4.41	23.03			13.57	8.64	21.88	13.52
120-72-9	Indole	26.78	58.63		31.28		62.31	39.9	23.14	11.88	39.84	30.13	46.36	15.01	56.04		
83-34-1	3-Methylindole	27.27						26.31		30.13	3.66			23.48			

Table 3.35 nitrogen containing compounds found in stool samples adjusted to pH 13 with sodium hydroxide for 15 participants with retention time and chromatographic peak area.

CAS	Aldehydes	RT (mins)	1	2	5	7	17	18	22	23	24	25	26	32	34	37	38
75-07-0	Acetaldehyde	2.16	12.37	2.08	4.31	31.59	2.23	10.56	1.84	2.45		19.32	9.04	5.36	36.89	5.16	5.1
123-72-8	Butyraldehyde	3.1	25.28				17	1.85					8.34		3.65	1.89	
66-25-1	Hexanaldehyde	6.76	5.29														

Table 3.36 aldehyde compounds found in stool samples adjusted to pH 13 with sodium hydroxide for 15 participants with retention time and chromatographic peak area.

CAS	Sulphides	RT (mins)	1	2	5	7	17	18	22	23	24	25	26	32	34	37	38
75-18-3	Dimethyl sulphide	2.3	110.78	32.67	17.83	104.6	4.69	243.23	102.33		16.1	73.12	115.65	3.39	34.36	332.31	12.93
624-92-0	Dimethyl disuphide	6.52	41.24				50.8	148.71	21.35	4.85	51.92	83.43	46.39	14.18			46.16
23747-45-7	S-methyl 3-methylbutanethioate	9.69					3.1										

Table 3.37 sulphide compounds found in stool samples adjusted to pH 13 with sodium hydroxide for 15 participants with retention time and chromatographic peak area.

CAS	Ketones	RT (mins)	1	2	5	7	17	18	22	23	24	25	26	32	34	37	38
67-64-1	Acetone	2.61	80.3	200.02	179.45	174.92	434.16	69.08	46.68	314.15	117.53	234.79	82.31	59.39	105.27	196.82	55.35
107-87-9	2-pentanone	4.61		27.89	48.5		133.37			32.29			13.75			19.26	
591-78-6	2-hexanone	6.75					28.63			14.89							
105-42-0	4-Methyl 2-hexanone	8.84				3.73	14.3										
110-43-0	2-Heptanone	8.91								3.19							
111-13-7	2-Octanone	10.91			14.42												

110-93-0	6-Methyl 5-hepten-2-one	11.94	58.17	64.95	9.81	2.93	9.01	7.72	3.28	17.36	67.41	32.06	9.16	10.43	3.52	3.78	52.32

Table 3.38 ketone compounds found in stool samples adjusted to pH 13 with sodium hydroxide for 15 participants with retention time and chromatographic peak area.

CAS	Esters	RT (mins)	1	2	5	7	17	18	22	23	24	25	26	32	34	37	38
79-20-9	Ethanoic acid, methyl ester	2.69	67.5	117.34	74.36	72.72	84.96	56.36	70.26	52.96	58.36		78.99	85.11	394.92	48.61	61.7
141-78-6	Ethanoic acid ethyl ester	3.21				40.51	99.29							12.32			
105-37-3	Propanoic acid ethyl ester	4.28						39.5				448.29		26.95	886.65		
97-62-1	Propanoic acid, 2-methyl- ethyl ester	4.44						27.03				53.51			39.29		
109-60-4	Ethanoic acid propyl ester	4.6				62.1						200.57			319.61		
623-42-7	Butanoic acid methyl ester	4.79				223.7		87.46	17.03	5.14	5.03	778.65	2.21	115.95	1265.08		
868-57-5	2-Methylbutanoic acid methyl ester	5.28				20.92		24.95				19.76		16.28	4.18		
556-24-1	3-Methylbutanoic acid methyl ester	5.49				27.8											
105-54-4	Butanoic acid methyl ester	5.87				42.67		208.41	51.57		55.49	2199.01		78.62			
106-36-5	Propanoic acid propyl ester	6.04										23.62		8.07	268.18		
7452-79-1	2-Methylbutanoic acid ethyl ester	6.21				26.14		25.14	6.42			62.44		7.04	25.28		
108-64-5	3-Methylbutanoic acid ethyl ester	6.48		13.13	30.12	110									51.64	50.48	
123-86-4	Ethanoic acid butyl ester	6.61										127.97			182.48		
624-24-8	Pentanoic acid methyl ester	6.87				32.36		25.41						14.08	135.44		
105-66-8	Butanoic acid propyl ester	7.64				89.12		45.96	7.95		5.63	560.89		9.22	578.32		
539-82-2	Pentanoic acid ethyl ester	7.92										52.24		5.65	593.2		
590-01-2	Propanoic acid butyl ester	8.06													134.41		
97-87-0	Propanoic acid 2-methyl butyl ester	8.18													2.28		
539-90-2	Butanoic acid 2-methylpropyl ester	8.45						11.43	1.78								
628-63-7	Ethanoic acid pentyl ester	8.68													13.76		
123-92-2	Ethanoic acid 3-methyl butanyl ester	8.75															
106-70-7	Hexanoic acid methyl ester	9.01				6.03											
109-21-7	Butanoic acid butyl ester	9.68				26.75		26.14	5.78			306.99		3.26	178.47		
141-06-0	Pentanoic acid propyl ester	9.73									5.67						1.28
123-66-0	Hexanoic acid ethyl ester	9.99				11.1							49.67				

109-19-3	3- Methylbutanoic acid butyl ester	10.29		10.69						33.21					4.74		
591-68-4	Pentanoic acid butyl ester	11.5				2.51				11.79							
5870-93-9	Butanoic acid heptyl ester	11.51												15.71			
4630-82-4	Cyclohexanecarboxylic acid methyl ester	12.65								33.8							
3289-28-9	Cyclohexanecarboxylic acid ethyl ester	13.42								44.71							

Table 3.39 ester compounds found in stool samples adjusted to pH 13 with sodium hydroxide for 15 participants with retention time and chromatographic peak area.

CAS	Alcohols	RT (mins)	1	2	5	7	17	18	22	23	24	25	26	32	34	37	38
64-17-5	Ethanol	3.91				128.5		161.7		122.42		74.94	222.59	217.58	1184.95		
78-92-2	2-Butanol	5.64					13.61										
73-23-8	1-Propanol	5.92													3777.17		206.89
78-83-1	2-Methyl -1-propanol	7.27						19.44	14.18		20.11			7.69	15.82		
1572-93-6	3-Methyl-2-butanol	7.78									2.06			1.81			
6032-29-7	2-Pentanol	7.83				3.93											
71-36-3	1-Butanol	8.29						29.81	24.73		32.63	320.62	6.55	19.73	438.54	5.74	10.95
137-32-6	2-Methyl-1-butanol	9.51	5.18			14.19	13.4	11.57	9.12	7.94	22.76	12.4	9.53	5.48		10.37	9.62
71-41-0	1-Pentanol	10.35				13.3	9.36	10.46	8.03		14.41			6.98			
626-89-1	4-Methyl-1-pentanol	11.51									1.49						

Table 3.40 alcohols found in stool samples adjusted to pH 13 with sodium hydroxide for 15 participants with retention time and chromatographic peak area.

CAS	Acids	RT (mins)	1	2	5	7	17	18	22	23	24	25	26	32	34	37	38
79-31-2	2-Methylpropanoic acid	6.7						4.68									
64-19-7	Ethanoic acid	14.11	26.14		19.23	31.94	15.4	19	18.72		12.84	16.95		20.32	36.52	20.27	23.15
107-92-6	Butanoic acid	16.82	18.14	13.15		24.67									34.47	10.52	

Table 3.41 acids found in stool samples adjusted to pH 13 with sodium hydroxide for 15 participants with retention time and chromatographic peak area.

CAS	Aromatic compounds	RT (mins)	1	2	5	7	17	18	22	23	24	25	26	32	34	37	38
7149-26-0	1,6-Octadien-3-ol, 3,7-dimethyl-,2-aminobenzoate	15.63													52.57		

108-95-2	Phenol	21.84	6.32	7.81	6.18	5.94	8.59	7.38	19.75	5.15	9.6	9.98	10.15	5.66	6.85	5.38	4.77
106-44-5	4-Methylphenol	22.76	59.39	70.1	100.66	60.66	120.73	69.62	76.09	83.48	113.1	35.04	34.77	49.98	30.72	40.6	44.75
120-72-9	Indole	26.78	58.63		31.28		62.31	39.9	23.14	11.88	39.84	30.13	46.36	15.01	56.04		
83-34-1	3-Methylindole	27.27						26.31		30.13	3.66			23.48			

Table 3.42 aromatic compounds found in stool samples adjusted to pH 13 with sodium hydroxide for 15 participants with retention time and chromatographic peak area.

Siloxanes	RT (mins)	1	2	5	7	17	18	22	23	24	25	26	32	34	37	38
Siloxane (1)	4.31	23.43	33.97	17.49	110.4	24.75			15.45	31.05		11.31			12.6	
Siloxane (2)	6.83					5.38					16.75					
Siloxane (3)	7.58			3.72		1.97									2.78	
Siloxane (4)	10.53								5.21					13.13		
Siloxane (5)	13.03											5.63				
Siloxane (6)	13.13	9.95	7.58	3.97	3	5.61	2.94	2.32	2.98	10.19	2.29		5.07		3.18	2.34
Siloxane (7)	15.32	8.53												9.16		

Table 3.43 siloxane compounds found in stool samples adjusted to pH 13 with sodium hydroxide for 15 participants with retention time and chromatographic peak area.

Terpenes	RT (mins)	1	2	5	7	17	18	22	23	24	25	26	32	34	37	38
Terpene (1)	3.02									29.63			6.39			
Terpene (2)	5.3			5.08			46.18			1.12						
Terpene (3)	5.52	78.67	332.62	68.07		46.87			67.66	39.98	68.77	28.22	31.64	49.67	13.5	
Terpene (4)	5.59	22.2						21.66			16.54		11.15			
Terpene (5)	5.78	10.31	64.32	20.43		46.26			16.12			32.03			26.52	
Terpene (6)	6.39		10.4	19.85					8.51			10.2		29.68		
Terpene (7)	6.87	6.57														
Terpene (8)	7.13	61.47	61.46	145.04	10.19	56.33	7.64		22.71		112.28	17.89	24.61	12.1	10.33	
Terpene (9)	7.37	45.91	32.57	11.38	8.62	1.23	6.1		8.66		32.81			15.76		18.15
Terpene (10)	7.4								11.42				7.58			
Terpene (11)	7.86					155.85	55.22	3.02	1.26	3.18		1.78				

Terpene (12)	7.99	72.26	72.86	24.83				2.18	34.55	5.4	179.23	4.52	15.04		2.89	
Terpene (13)	8.16		68.87	67.35		7.81	1.56	14.58	15.06	1.21	17.75	14.04			30.04	12.98
Terpene (14)	8.37								3.6							
Terpene (15)	8.42	2.1	698.76			39.18							5.84	510.92	3.02	
Terpene (16)	8.47											1.27				
Terpene (17)	8.69	3.27	6.79						4.23		83.44					
Terpene (18)	8.88			21.35							3.81	3.07	1.29		3.78	
Terpene (19)	9.16	74.57		64.69	21.49	64.24	10.9	3.57	28.66	31.28	470.09	5.61	21.22	1328.47	39.74	57.75
Terpene (20)	9.34	7.38	1486.21	7.54		2.88	1.12		5.5	2.77	5.39	3.76	1.95	50.39		
Terpene (21)	9.92													47.96		
Terpene (22)	10.16	11	18.56	99.67				0.26	7.45		105.84		28.74	136.34		5.77
Terpene (23)	10.38	5.71							3.8			4.3				
Terpene (24)	10.51	14.65		71	11.78	14.21	12.55	10.09		10.03	45.09	8.68			4.64	7.23
Terpene (25)	10.6								11.55				28.98			
Terpene (26)	10.63		70.69													
Terpene (27)	10.83		25.54													
Terpene (28)	10.95		17.05			4.81										
Terpene (29)	12.21			9.89	4.84	3.82	3.69				3.08	3.42			1.4	4.8
Terpene (30)	12.53		4.4		4.88									24.63		
Terpene (31)	12.92		10.4													
Terpene (32)	13.69		24.13	1.13												
Terpene (33)	14.73			2.13											5.32	
Terpene (34)	15.47				7.93	14.08	5.27	6.54				12.65			6.84	5.59
Terpene (35)	16.35	14.94	23.15	18.19		5.74	4.67		8.71		10.84	4.41	4.21		18.77	
Terpene (36)	17.46		4.93	3.72		3.89	3.85				3.67	3.56	4.61		4.19	5.81
Terpene (37)	17.99			1.67												
Terpene (38)	18.12			2.42												
Terpene (39)	18.22			3.12												
Terpene (40)	19.58		12.23													

Terpene (41)	20.71	40.4	39.44	22.93	39.12	45.63	29.39	26.86	23.71	22.01	25.07	5.19	17.4	39.5	33.56	26.66
Terpene (42)	23.86		3.14	1.88	1.05		1.46	1.5		3.03	1.61				2.8	
Terpene (43)	24.64		0.75	0.64	0.58		0.69	0.55	0.72	0.92	0.65		0.74		1.13	

Table 3.44 terpene compounds found in stool samples adjusted to pH 13 with sodium hydroxide for 15 participants with retention time and chromatographic peak area.

Unidentified	RT (mins)	1	2	5	7	17	18	22	23	24	25	26	32	34	37	38
Unidentified (1)	4.56	8.29					10.18	9.11		24.2			19.73			
Unidentified (2)	6.08								0.82							
Unidentified (3)	6.23															2.29
Unidentified (4)	6.67			13.68						28.6						
Unidentified (5)	7.55	2.5														
Unidentified (6)	7.6											2.46				
Unidentified (7)	8.39									2.3						
Unidentified (8)	9.58			14.62										13.56		
Unidentified (9)	11.53				2.02											
Unidentified (10)	12.29														3.63	
Unidentified (11)	14.25											13.69				
Unidentified (12)	15.49												4.91			
Unidentified (13)	16.84			9.66		11.58	12.11	13.6	8.33	12.44	13.74	8.37	12.35			13.09
Unidentified (14)	17.38				7.93				2.84					9.72		
Unidentified (15)	18.38		3.88		9.29	2.78	8.64	8.27			7.29	2.1			2.14	6.53
Unidentified (16)	19.86							0.8								

Table 3.45 identified compounds found in stool samples adjusted to pH 13 with sodium hydroxide for 15 participants with retention time and chromatographic peak area.

3.3.6 Quantitative data from alkaline (pH13) stools

Figure 3.24 shows the comparison between chromatograms of unaltered stool (Figure 3.24a) and the same sample with 5 mL of 0.1M sodium hydroxide added (Figure 3.24b). As expected, the region containing the majority of the short chain fatty acids (retention time 7–18 min) has both less chromatographic peaks and smaller peak areas when the sodium hydroxide is added. However, Figures 3.24 and 3.25 show that when sodium hydroxide is added to make the stool alkali the trimethylamine can be detected. In the unmodified stool (Figure 3.24a) there is no 58 *m*/*z* peak at the 1.94-minute retention time, which is indicative of trimethylamine. The peak with a retention of 1.91 in Figure 3.24 does not have any clear matches in the NIST library. This peak is no longer visible when only the 58 *m*/*z* for trimethylamine is displayed as shown in the section of the chromatogram displayed in Figure 3.25a. Whereas the identical section of the chromatogram for the pH altered stool gives a peak at the earlier retention time with a library match for trimethylamine (Figure 3.25b).

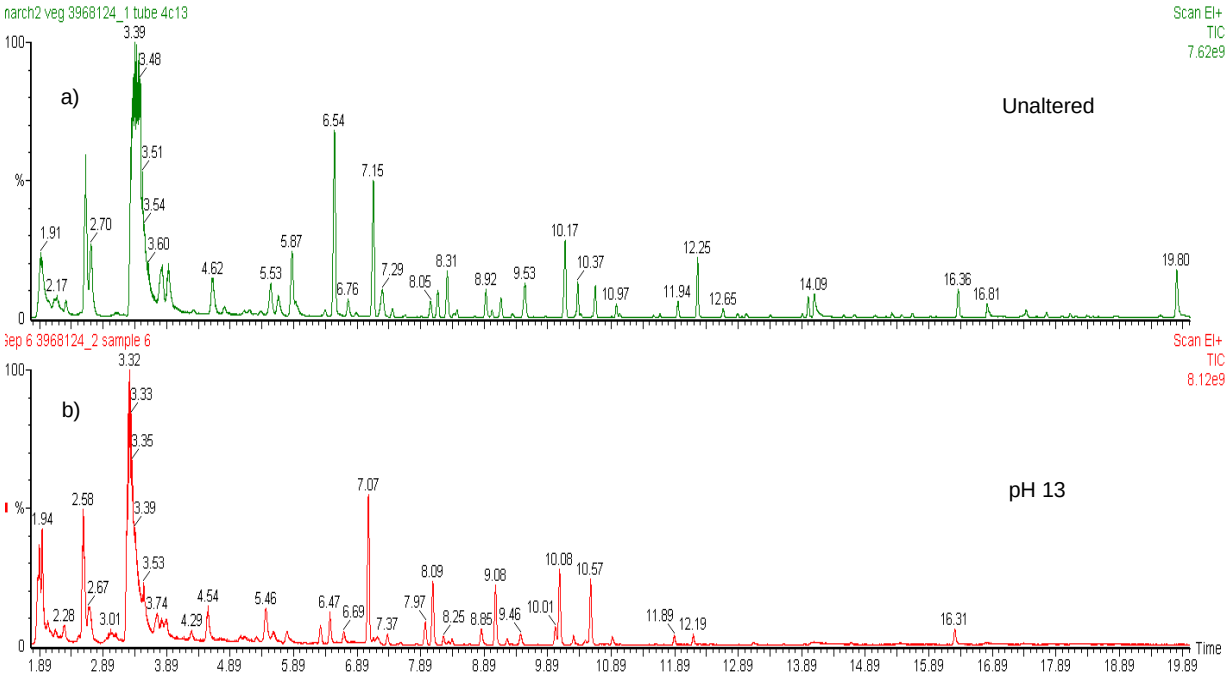


Figure 3.24 a) Chromatogram produced from unmodified stool sample spiked with ^{13}C labelled internal standards (solution 1) b) chromatogram produced from another aliquot of the sample with 5mL of 0.1M aqueous sodium hydroxide added in addition to ^{13}C labelled trimethylamine.

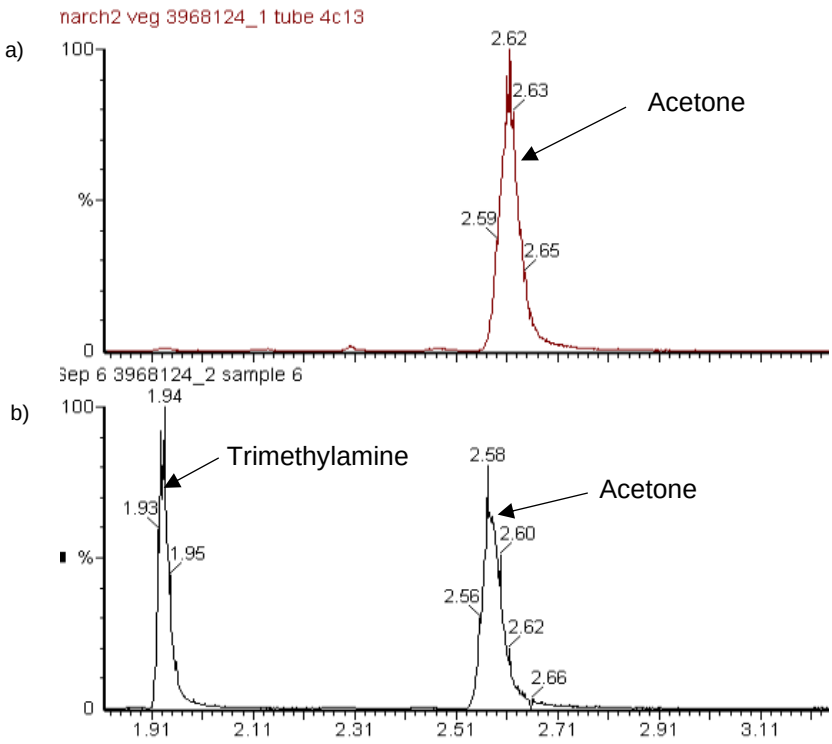


Figure 3.25 a) the same sample as Figure 3.22a with only m/z 58 ions displayed vs. time. The peak at retention time 2.62 min was identified as acetone b) the same sample as Figure 3.22b (the pH altered stool) with only m/z 58 ions displayed vs. time. The peak at retention time 1.94 min was identified by the NIST library as trimethylamine, and the peak at retention time 2.58 min as acetone.

3.4.0 Discussion

It is considered that some diseases could be linked to the microbiome [183]. There is a lack of knowledge of gut chemistry, due in part to the complexity of the stool mixture. Recently, hundreds of new compounds have been identified in the gut mainly due to the VC analyses in headspace studies, and much of this work is of a qualitative nature [66,173]. We have developed a method for quantifying key compounds from stool samples, which minimizes chemical alterations to the stool using ^{13}C isotope labelled internal standards. It quantifies headspace concentrations and accounts for dissolved stool concentrations by comparing the distribution and relative recovery of isotope labelled compounds from the headspace above stool.

We have also devised a method to quantify trimethylamine in stool by using isotope ^{13}C labelled trimethylamine in conjunction with elevating the pH to 13. Although addition of base to stool samples is not common, Wang *et al*. also added sodium hydroxide to stool prior to the sample being centrifuged and filtered to measure lactic acid and short chain fatty acids (SCFAs) with high-performance liquid chromatography [184]. However, in the Wang *et al*. study, no healthy participants were used, and all the participants were premature infants. This study also differs from our study as only

1 mL of 10 mmol/L sodium hydroxide was added along with 5 mmol/L of crotonic acid, with no mention as to the effect these additions had on the pH.

Walton *et al*. used TD-GC-MS, a similar method to that reported here, to analyse the headspace of stool samples. They found levels of acetone that were 142 ng/L whereas butanoic acid was 33 ng/L, 3-methylbutanoic acid was 7 ng/L, and Indole was 9 ng/L. Measuring the headspace concentration underestimates the level of free acid and other compounds, such as indole, in the stool, due to the relatively low concentrations portioning into the headspace. To determine the differences in quantification of the headspace concentrations versus the in-stool concentrations, headspace quantification was done on a subset of samples (16 UK samples). Although the levels of acetone, dimethyl sulphide, and other lower molecular weight, less water soluble, and lower boiling point compounds were equivalent to the values measured during the quantitation studies for the same samples (Table 3.16 and Table 3.35, UK participants), much lower amounts of acids and indole were determined if just pure headspace concentrations were measured (Table 3.46). Thus, the method we devised considered the headspace concentration vs. dissolved concentration, by dissolving a known ^{13}C isotope with similar chemical properties into the stool and measuring headspace concentrations, but correcting for lower than expected recovery and applying this same correction factor to the levels detected (peak areas) of the non-isotope labelled naturally occurring compounds.

As mentioned, fatty acids and indole concentrations in stool are disproportionately underestimated if just the headspace concentration is considered. The devised method does rely on distributing the ^{13}C labelled compound throughout the stool sample.

Compound	2	6	7	8	11	14	15	16	Mean omnivore	1	3	4	5	9	10	12	13	Mean vegetarian
Trimethylamine	ND	ND	ND	ND	ND	2.6	ND	ND	**2.6**	ND	ND	ND	ND	ND	ND	ND	ND	**ND**
Dimethyl Sulphide	213.6	400.0	1786.2	850.4	3922.0	50.5	34.9	827.1	**1010.6**	2159.0	229.1	28.0	178.6	2423.1	260.2	314.5	621.3	**776.7**
Acetone	290.4	2384.9	3771.6	1666.2	602.6	2149.0	72.6	145.2	**1385.3**	842.2	2029.2	410.2	1847.7	838.5	254.1	653.4	700.6	**947.0**
Propanethiol	9.5	ND	ND	23.8	ND	33.3	ND	ND	**22.2**	ND	ND	ND	ND	7.6	ND	ND	ND	**7.6**
Pantanal	ND	ND	ND	ND	ND	ND	ND	ND	**ND**	ND	ND	ND	ND	ND	ND	ND	ND	**ND**
Ethyl-2-methyl butanoate	ND	ND	ND	ND	ND	ND	ND	ND	**ND**	ND	ND	ND	ND	ND	ND	ND	ND	**ND**
Dimethyl disulphide	365.0	206.0	223.7	164.8	1106.7	129.5	64.8	688.8	**368.7**	288.5	482.7	14.1	223.7	88.3	306.1	100.1	370.9	**234.3**
1-Octen-3-one	ND	ND	ND	ND	ND	ND	ND	ND	**ND**	ND	2.4	1.3	ND	ND	ND	ND	2.8	**2.1**
Dimethyl trisulphide	94.7	78.9	98.6	68.7	560.3	51.3	5.5	284.1	**155.3**	75.0	161.8	5.5	56.0	20.5	75.0	39.5	94.7	**66.0**
1-Octen-3-ol	ND	ND	ND	ND	0.2	ND	ND	ND	**0.2**	0.2	ND	ND	ND	ND	ND	ND	0.2	**0.2**
Butanoic acid	88.1	363.5	82.6	170.7	269.8	19.8	66.1	253.3	**164.2**	27.5	82.6	44.1	38.5	25.9	38.5	1921.9	126.7	**288.2**
3-Methylbutanoic acid	23.0	140.4	25.5	70.2	44.7	3.8	22.3	51.1	**47.6**	5.7	23.6	10.9	6.4	7.0	6.4	197.9	25.5	**35.4**
Pentanoic acid	12.8	98.9	19.1	59.4	36.4	ND	9.6	44.7	**40.1**	3.2	14.7	6.4	6.4	3.8	4.5	217.0	17.9	**34.2**
Indole	6.6	22.9	14.8	46.5	9.6	13.3	7.4	11.1	**16.5**	31.0	14.0	1.5	22.1	11.1	5.2	25.8	11.8	**15.3**
3-Methyl indole	9.8	11.5	5.7	29.5	57.4	4.9	32.0	13.1	**20.5**	11.5	12.3	16.4	24.6	19.7	18.0	8.2	11.5	**15.3**

Table 3.46 quantities of compounds determined from the headspace of healthy stool samples from the U.K. omnivore and vegetarian samples. Quantities were calculated by collecting a known volume of headspace on TD tubes and using calibration curves of standards spiked onto the same TD tubes at different concentrations to calculate concentration in ng/g of stool. N.D. =not detected

Extensive method development was undertaken using standard mixtures containing 18 compounds previously identified in stool to optimise the chromatography method and automated thermal desorption (ATD) tube loading. Prior to this, a number of different solvents had been used for the standard mixture and methanol was selected due to its compatibility with a wide range of compounds, ease of removal from ATD tubes when loading calibration standards, and minimal solvent peak, which did not interfere with the compounds of interest in unmodified stool.

The method relies on correcting recovered values based on the ^{13}C standard recovery. This gives a more realistic quantitation for the amount of certain compounds in stool. However, if the naturally occurring compounds of interest are below the limit of detection for the headspace analysis method, then there is no way to apply the correction to ascertain the actual concentration of the compound in stool. Therefore, a solvent extraction method, which does not rely on working close to the limit of detection, may have enabled quantitation of the full range of samples. Prior to developing the headspace-based method reported here, a solvent extraction method had been developed for quantifying selected compounds in stool. However, it proved difficult to obtain a reliable solvent extraction method that enabled simultaneous quantitation of the compounds of interest, which span a broad range of chemical classes.

Wang *et al*. utilized a vacuum distillation process to isolate the short chain fatty acids (SCFAs) in stool [181]. These studies identified higher levels of SCFA than the method reported here, with levels in the high µg/g range compared to this study where values were in the low µg/g range. Wang *et al*. obtained median values of 3705 µg/g for ethanoic acid, 1756 µg/g for butanoic acid, and 285 µg/g for 3-methylbutanoic acid. These values are higher than those reported here due to the methodology used, whereby both the free acid and anionic forms would be extracted and analysed.

De Preter *et al*. freeze dried their stool samples before they were salted with sodium sulphate and acidified with sulphuric acid [182]. Acidification of stool samples is a reasonably common technique as detailed in a comprehensive review of SCFA analysis via GC and other methods [185]. Their reported values for the SCFAS measured (ethanoic acid, butanoic acid, and 3-methylbutanoic acid) were in the high µg/g range in agreement with the studies of Wang *et al*. detailed above [181]. Interestingly, de Preter also quantified dimethyl sulphide and found levels of 0–402 ng/g whereas this study identified DMS at a mean concentration of 3058 ng/g with a

range of 26–25626 ng/g. So, there is fairly good agreement between the two different analysis methods for quantifying volatile sulphur compounds, and this is because the alteration of the chemistry does not affect the quantification of sulphides in the same way as SCFAs. De Preter also quantified indole and found levels ranging from 24 µg/g to 44 µg/g whereas this study found mean values of 3 µg/g. Therefore, there is fairly good agreement that can be explained by the different effects altering the stool chemistry has on quantifying indole vs. quantifying SCFAs.

As mentioned, by the action of acidifying stool, both the free acid and the previously anionic form is measured. However, in the gut, the acid exists as free acid, but also more so in the anionic form [186] (with the counter cation being H^+, metal cations, and, to a lesser extent, ammonium ions). Our contention is that quantification of the free SCFA concentration in addition to the total SCFA concentration is important to know in a study of the gut chemistry. The amount of free acid will dictate the amount of acid detectable in the headspace and thus the amount available for potential diagnostic purposes [187].

Preliminary work on the basification of stool was reported. The unmodified stools yielded a mean of 174 peaks across 38 samples with a mean of 57 per sample (range 36–73). The addition of the sodium hydroxide reduced both values significantly to 133 and 43 (range 29–53), respectively. Moreover, as demonstrated in Figure 3.24a and 3.24b, after the first 10–12 min, the frequency and size of the chromatographic peaks notably reduces. By making the stool alkali, we have shown that it is possible to not only detect, but also quantify, trimethylamine, which is likely to be a result of the conversion of protonated trimethylamine to trimethylamine in the high pH conditions. Figure 3.25b shows a clear peak with an RT of 1.94 after the sodium hydroxide is added, which is identified by the NIST library with a match and reverse-match at 999 and 992, respectively. Figure 3.25a shows the same sample with unmodified stool in which the 1.94-minute peak is barely visible. Lin *et al*. found that in their latrine models, the amine smell became more prominent as the pH increased to 9 [179]. They also found that trimethylamine could not be detected from their field samples, and this could be due to the presence of acids in the sample protonating the amine compounds [177], resulting in a lack of free trimethylamine within the samples. Simenhoff *et al*. suggested that secondary and tertiary amines were in high levels on the breath of patients with end stage renal disease [188]; a finding that was also supported by Davis, Spanel, and Smith [189]. Moreover, ammonia has been associated with hepatic

encephalopathy [190]. Thus, improving amine detection techniques could have clinical utility in the future. While we were unable to quantify any other amine compounds in this instance, we are confident that with further method development we should be able to quantify more amine compounds. We were able to identify methylamine in one of the stools modified to pH 13, and this peak eluted as a shoulder to the methanol solvent peak; this is suggestive that methylamine may be in other samples but co-elutes with the solvent peak. Thus, developing the method further to reduce or eliminate this solvent peak, for instance, by increasing the tube collection purge time to drive off more methanol, may reveal more amine compounds. Table 3.47 shows the differences in the number of compounds detected for each chemical class between the unaltered and alkaline stool samples. There were fewer compounds detected across all the chemical classes, with the exception of nitrogen containing compounds, in the alkaline stool; not surprisingly, the largest percentage decrease in the number of compounds came from the acids with a 70% decrease. The least change came from the esters, which only decreased by 17% following pH alteration. As expected, the only class of compounds that showed an increase in numbers were the nitrogen containing compounds. 1,6-Octadien-3-ol-3,7-dimethyl-2-aminobenzoate was detected in the unmodified stool but was not detected in the alkaline stool. Trimethylamine, dimethylamine, and acetonitrile (dimethylamino) were detected in the alkaline stool, but not in the unmodified stool. Acetonitrile, indole, and 3-methyl indole were seen in both unmodified and pH modified stool.

Chemical Family	Unmodified	pH Modified	Percentage change (Unmodified to pH13 Modified)
Aldehydes	6	3	-50%
Esters and thioesters	36	30	-17%
Ketones	12	7	-42%
Alcohols	20	11	-45%
Acids	10	3	-70%
Nitrogen containing	4	6	+50%
Sulphides	8	3	-63%
Aromatic compounds	6	5	-29%
Miscellaneous	2	0	-100%
Terpenes	30	22	-40%
Unidentified	32	16	-50%

Table 3.47 comparison of compounds found across different classes of chemicals in unmodified stool and the stool modified to pH 13.

The origins of a majority of the esters could arise from reactions between the alcohols and acids reported here. A homologous series of alcohols from ethanol to octanol was found, and from ethanoic acid to heptanoic acid, in agreement with previous work [191], and their reaction would produce many straight chain esters. Branched chain esters can similarly be explained by a reaction of, for example, 3-methylbutanoic acid with alcohols. Interestingly, a large number of methyl esters (nine in total) were found although no free methanol was observed. It may be that that the body, or bacterial enzymes, particularly “trap” methanol as esters, reduce the methanol’s toxic effects on cells.

The statistical tests on the gathered data showed that five compounds were in significantly different quantities in stool gathered from UK and South American participants; significantly, these participants were temporarily living in the UK with little exposure to a UK diet due to the short duration of their time in the UK. Moreover, using one cross validation, we were able build an example model using butanoic acid and ethyl butanoate to differentiate UK and South American samples; albeit, this was based on a small dataset. However, there was very little overlap between the two groups. In many clinical studies, there is little attention paid to the ‘healthy’ participants; however, developing a comprehensive understanding of how healthy samples can differ from population to population could be significant in the development of VC based diagnostics. For example, a stool volatile based diagnostic test that has high accuracy in the UK may not exhibit the same accuracy in a South American population. The results presented here do seem to suggest that further work is required to assess the difference in volatile profiles of healthy participants of different geographical origins.

A 2010 study compared the microbiota of children from Burkina Faso with children from the European Union (EU) [192]. This team suggested that there was a significant difference in the composition of gut microbiota between the two groups; they proposed the increased sugar, animal fats, and general calorie dense foods as the reason for this difference [192]. A difference in microbiota composition will inevitably lead to differences in the faecal volatilome. Similarly, a research team from China assessed the gut microbiota of 314 healthy participants of different geographical origins within China. This group was also able to determine differences in the composition of gut microbiota from these geographical origin groups; interestingly, this study was unable to determine any difference as a result of lifestyle. However, importantly, the team

state they did not process the necessary dietary information to make any inferences on the role diet plays in the formation of microbiota [193]. A 2006 study examined the gut microbiota of four different European countries; in this instance, very few differences were noted as a result of country of origin, however, this study suggested that other factors, such as age and gender, conferred significant differences [194]. Taken together, there is sufficient evidence to say that there are a number of variables that can influence the gut microbiota and, in turn, the associated volatilome.

Comparing the quantities of the compounds in omnivore samples versus vegetarian samples revealed no significant differences between the two groups, as evidenced by the discriminant scores plot shown in Figure 3.19. There have been numerous publications that suggest why this might be the case, for instance, Kabeerdoss *et al*. compared the microbiota of female southern Indian omnivores and vegetarians. This groups found that, with the exception of *Clostridium* cluster XIVa and some butanoate producing bacteria, which were more prevalent in omnivores, the groups were very similar [195]. While our results showed no significant difference due to the large standard deviations, the butanoic acid, ethyl butanoate, and ethyl 2-methylbutanoate were all higher in the omnivore group versus the vegetarian group. Van Faassen *et al*. demonstrated that while stool mass and frequency was higher in vegetarians versus omnivores, the pH of the stool was not significantly different; this was attributed to both vegetarians and omnivores having similar calcium intake [196]. In a recent 2015 study, Ferrocino *et al*. assessed the gut microbiota of 153 healthy participants from five different regions of Italy. This group also found that significant differences in gut microbiota could be attributed to region of origin rather than dietary habits (vegetarian/omnivore) [197].

A 2014 review of the healthy human volatilome found 381 distinct compounds from human stool [191]. Overall, we were able to identify 106 distinct compounds; moreover, we have tentatively identified additional compounds that have not previously been reported in the literature from stool samples of healthy participants (see Table 3.48). However, 3-Methyl-2-butanone has previously been reported in the urine samples of healthy individuals [197]. The compound, 2,4-dithiapentane, has been associated with white truffles [198] and truffle oil [199], and is likely to be directly derived from diet. Cyclohexanol, 5-methyl-2-(1-methylethyl)-,(1a,2b,5a) is a none verified isomer of menthol [200], and 4-isopropyl benzaldehyde is better known as a cuminaldehyde and both of these compounds are found in food [201]. Table 3.49

shows a further three compounds that were identified in the alkali treated stool samples that have not previously been reported in the current literature concerning volatile compounds emanating from stool of healthy individuals, but acetonitrile (dimethylamino) was identified in the headspace above stool of patients with C. *difficile* [53]. It is important to understand more about the volatilome if volatile compounds are going to prove useful in monitoring health. It may be particularly important to understand the gut derived volatilome both directly and indirectly due to the important role the microbiome plays in human health. Tables 3.48 and 3.49 show the retention indices for each compound calculated from the sample versus the literature values, in all cases where literature values exist or samples matched closely. Table 3.48 shows the RI values for all chromatographic peaks detected across all samples and compares those to the literature. In the vast majority of cases, our experimental value is in-line with those in the literature. Figures 3.26– 3.37 shows the mass spectra for each of the compounds in Tables 3.48 and 3.49. Trimethylamine was checked against the standards used for quantification. Future work will include verifying all of these compounds with chemical standards.

CAS	Compound	Retention Time (min)	Retention Indices Sample	Retention Indices Literature
563-80-4	3-Methyl-2-butanone	4.56	972	970 [202]
556-24-1	3-Methylbutanoic acid methyl ester	5.49	1017	1024 [203]
97-87-0	Propanoic acid 2-methylbutyl ester	8.18	1152	1154 [203]
539-90-2	Butanoic acid 2-methylpropyl ester	8.45	1158	1,152 [203]
141-06-0	Pentanoic acid propyl ester	9.73	1215	1,200–1233 [204]
1618-26-4	2,4-Dithiapentane	10.6	1260	1260 [205]
2313-61-3	2-heptanol	11.66	1308	1315.3 [206]
15356-70-4	Cyclohexanol, 5methyl-2-(1-methylethyl)-,(1a,2b,5a) (Menthol)	16.9	1631	1630.4 [206]
55012-32-3	4-Isopropyl benzaldehyde (cuminaldehyde)	18.91	1783	1781.4 [206]

Table 3.48 details of 12 compounds identified from the headspace above stool samples from healthy individuals that have not been previously reported in the literature. Babushok, Linstrom, and Zenkevich reported the retention indices for 505 compounds frequently occurring in plant essential oils [206]. Bianchi *et al*.. evaluated the retention indices for 250 compounds based on a polar column similar to that used to carry out our work [203].

CAS	Compound	Retention Time (mins)	Retention Indices Sample	Retention Indices Literature
75-50-3	Trimethylamine	1.95	-	-
108-64-5	3-Methyl butanoic acid ethyl ester	6.48	1074	1068 [204]
926-64-7	Acetonitrile (dimethylamino)	10.02	1230	1243 [207]

Table 3.49 three compounds, previously unreported in the literature, identified from the headspace of alkaline treated stool samples.

Compound	2	6	7	8	11	14	15	16	Mean omnivore	1	3	4	5	9	10	12	13	Mean vegetarian
Trimethylamine	ND	ND	ND	ND	ND	2.6	ND	ND	**2.6**	ND	ND	ND	ND	ND	ND	ND	ND	**ND**
Dimethyl Sulphide	213.6	400.0	1786.2	850.4	3922.0	50.5	34.9	827.1	**1010.6**	2159.0	229.1	28.0	178.6	2423.1	260.2	314.5	621.3	**776.7**
Acetone	290.4	2384.9	3771.6	1666.2	602.6	2149.0	72.6	145.2	**1385.3**	842.2	2029.2	410.2	1847.7	838.5	254.1	653.4	700.6	**947.0**
Propanethiol	9.5	ND	ND	23.8	ND	33.3	ND	ND	**22.2**	ND	ND	ND	ND	7.6	ND	ND	ND	**7.6**
Pantanal	ND	ND	ND	ND	ND	ND	ND	ND	**ND**	ND	ND	ND	ND	ND	ND	ND	ND	**ND**
Ethyl-2-methyl butanoate	ND	ND	ND	ND	ND	ND	ND	ND	**ND**	ND	ND	ND	ND	ND	ND	ND	ND	**ND**
Dimethyl disulphide	365.0	206.0	223.7	164.8	1106.7	129.5	64.8	688.8	**368.7**	288.5	482.7	14.1	223.7	88.3	306.1	100.1	370.9	**234.3**
1-Octen-3-one	ND	ND	ND	ND	ND	ND	ND	ND	**ND**	ND	2.4	1.3	ND	ND	ND	ND	2.8	**2.1**
Dimethyl trisulphide	94.7	78.9	98.6	68.7	560.3	51.3	5.5	284.1	**155.3**	75.0	161.8	5.5	56.0	20.5	75.0	39.5	94.7	**66.0**
1-Octen-3-ol	ND	ND	ND	ND	0.2	ND	ND	ND	**0.2**	0.2	ND	ND	ND	ND	ND	ND	0.2	**0.2**
Butanoic acid	88.1	363.5	82.6	170.7	269.8	19.8	66.1	253.3	**164.2**	27.5	82.6	44.1	38.5	25.9	38.5	1921.9	126.7	**288.2**
3-Methylbutanoic acid	23.0	140.4	25.5	70.2	44.7	3.8	22.3	51.1	**47.6**	5.7	23.6	10.9	6.4	7.0	6.4	197.9	25.5	**35.4**
Pentanoic acid	12.8	98.9	19.1	59.4	36.4	ND	9.6	44.7	**40.1**	3.2	14.7	6.4	6.4	3.8	4.5	217.0	17.9	**34.2**
Indole	6.6	22.9	14.8	46.5	9.6	13.3	7.4	11.1	**16.5**	31.0	14.0	1.5	22.1	11.1	5.2	25.8	11.8	**15.3**
3-Methyl indole	9.8	11.5	5.7	29.5	57.4	4.9	32.0	13.1	**20.5**	11.5	12.3	16.4	24.6	19.7	18.0	8.2	11.5	**15.3**

Table 3.50 quantities of compounds determined from the headspace of healthy stool samples from the U.K. omnivore and vegetarian samples. Quantities were calculated by collecting a known volume of headspace on TD tubes and using calibration curves of standards spiked onto the same TD tubes at different concentrations to calculate concentration in ng/g of stool. N.D. =not detected

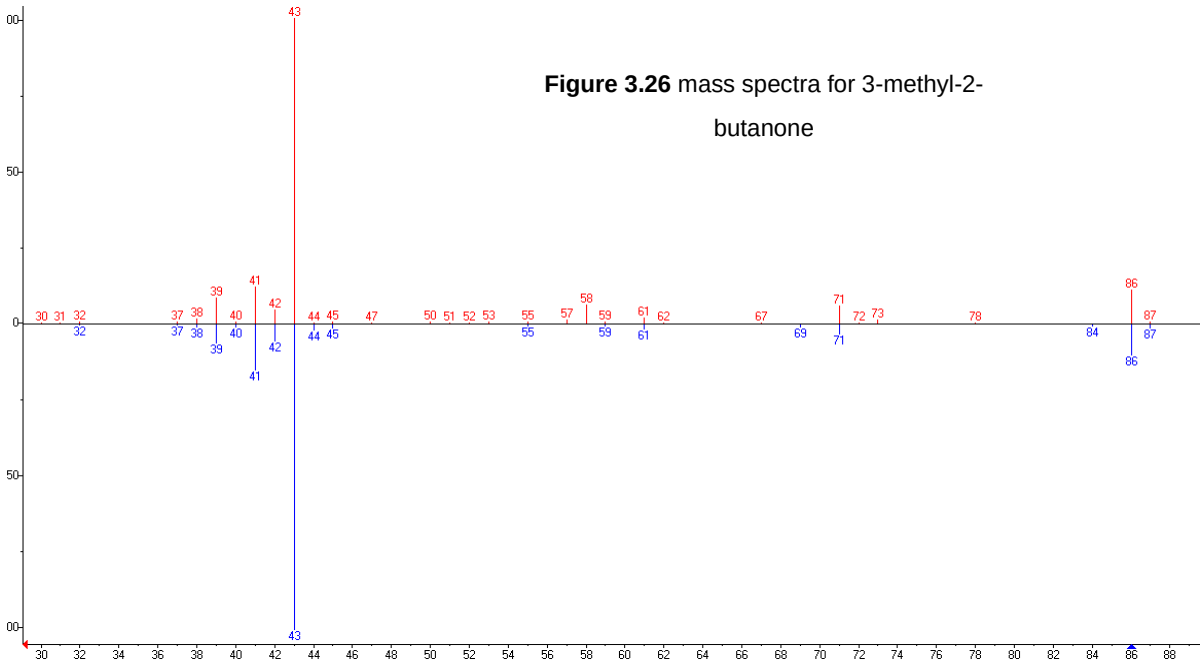


Figure 3.26 mass spectra for 3-methyl-2-butanone

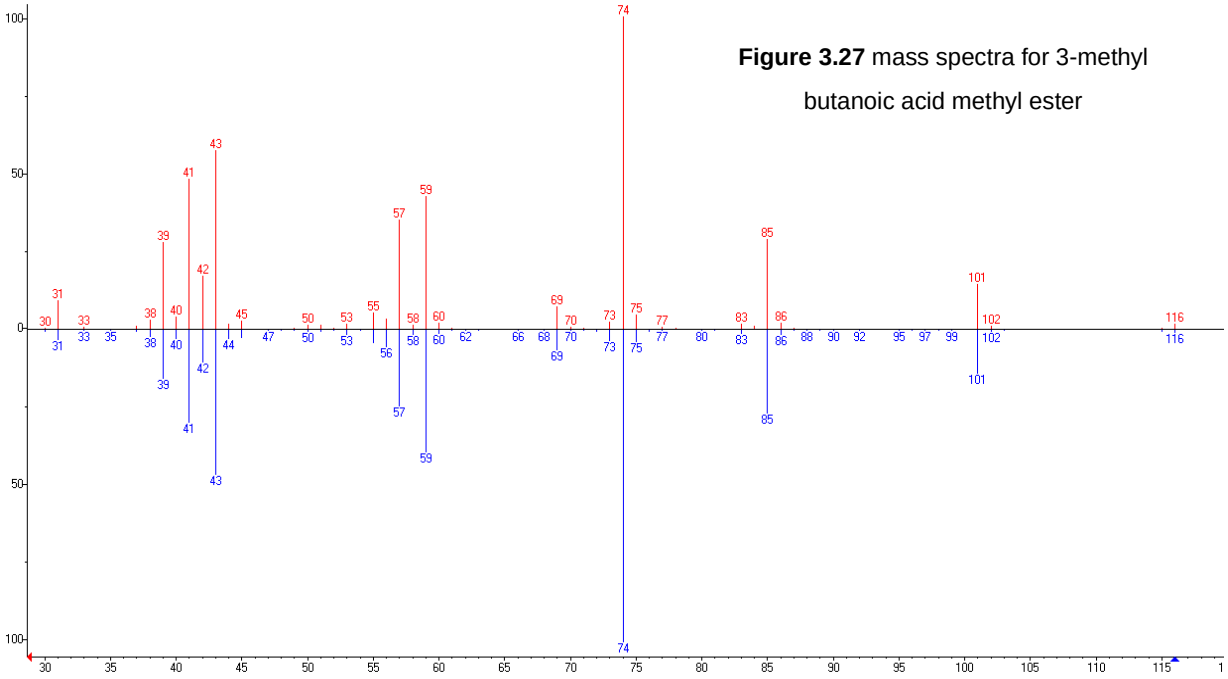


Figure 3.27 mass spectra for 3-methyl butanoic acid methyl ester

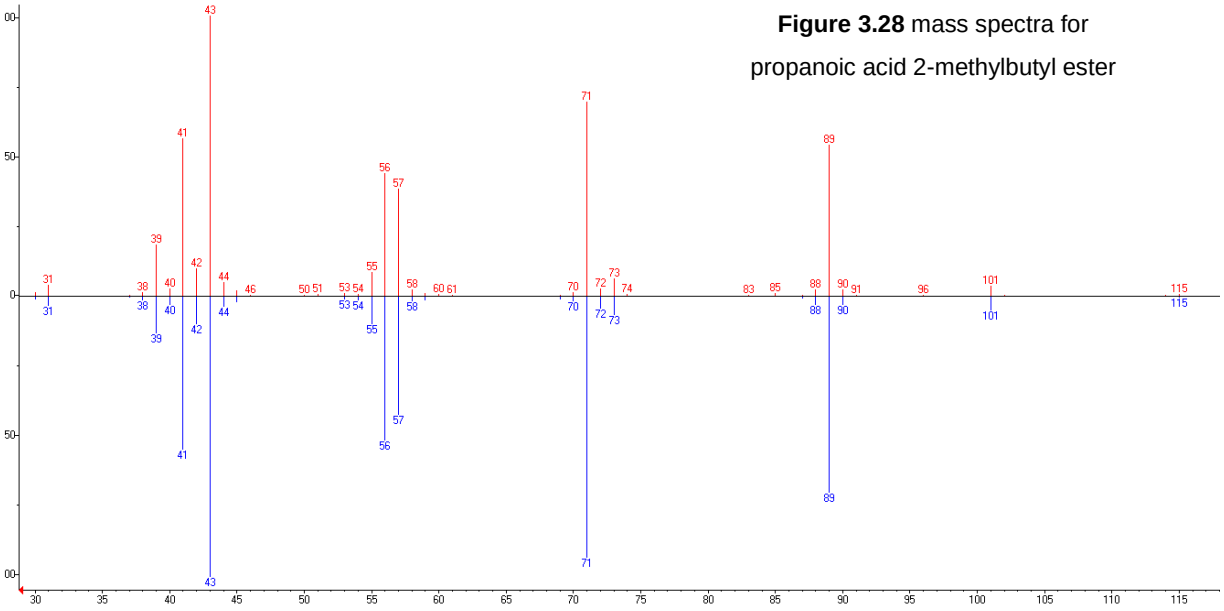


Figure 3.28 mass spectra for propanoic acid 2-methylbutyl ester

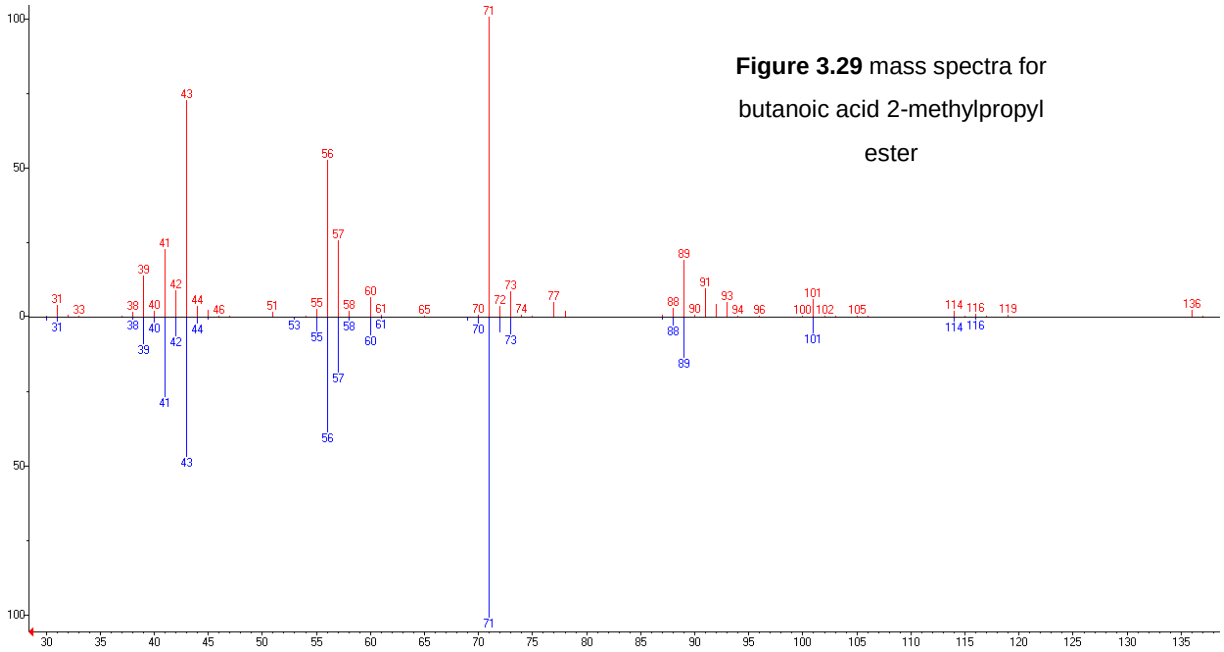


Figure 3.29 mass spectra for butanoic acid 2-methylpropyl ester

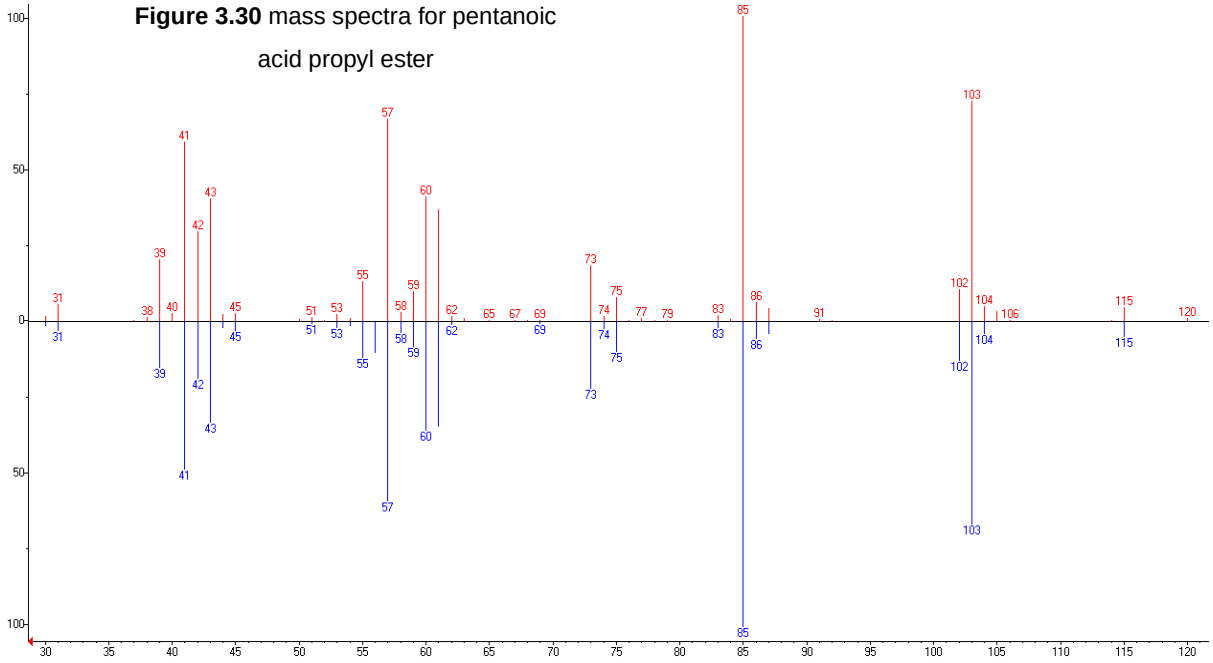


Figure 3.30 mass spectra for pentanoic acid propyl ester

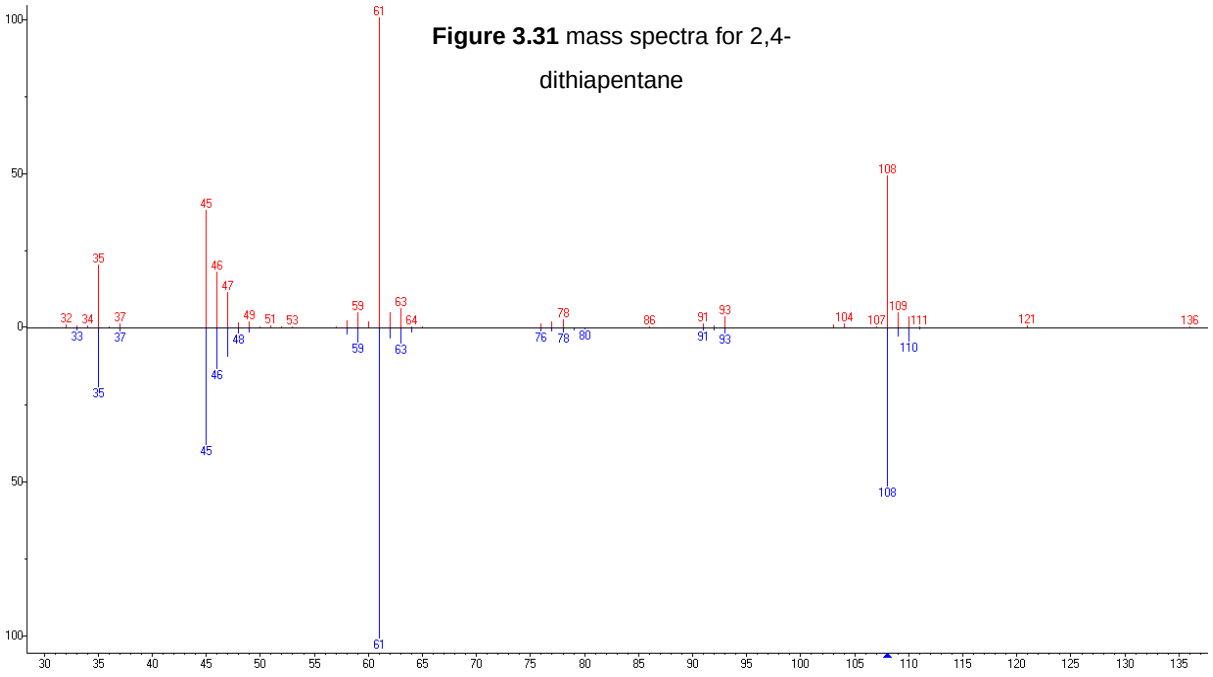


Figure 3.31 mass spectra for 2,4-dithiapentane

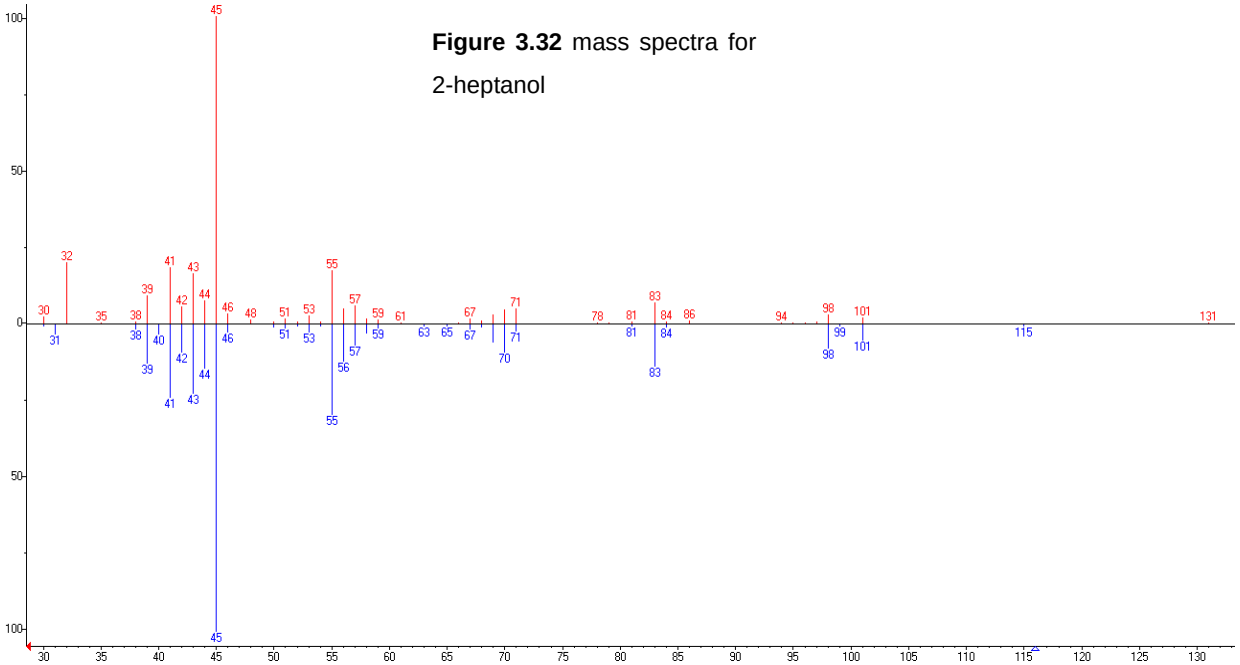


Figure 3.32 mass spectra for 2-heptanol

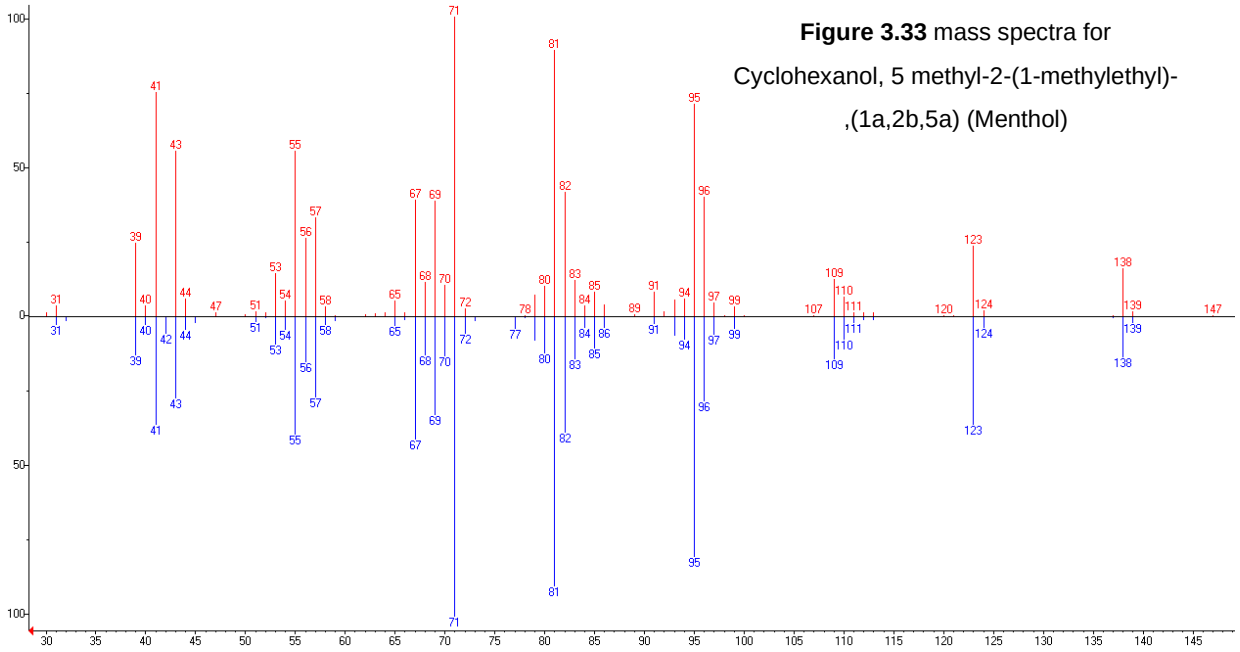


Figure 3.33 mass spectra for Cyclohexanol, 5 methyl-2-(1-methylethyl)-,(1a,2b,5a) (Menthol)

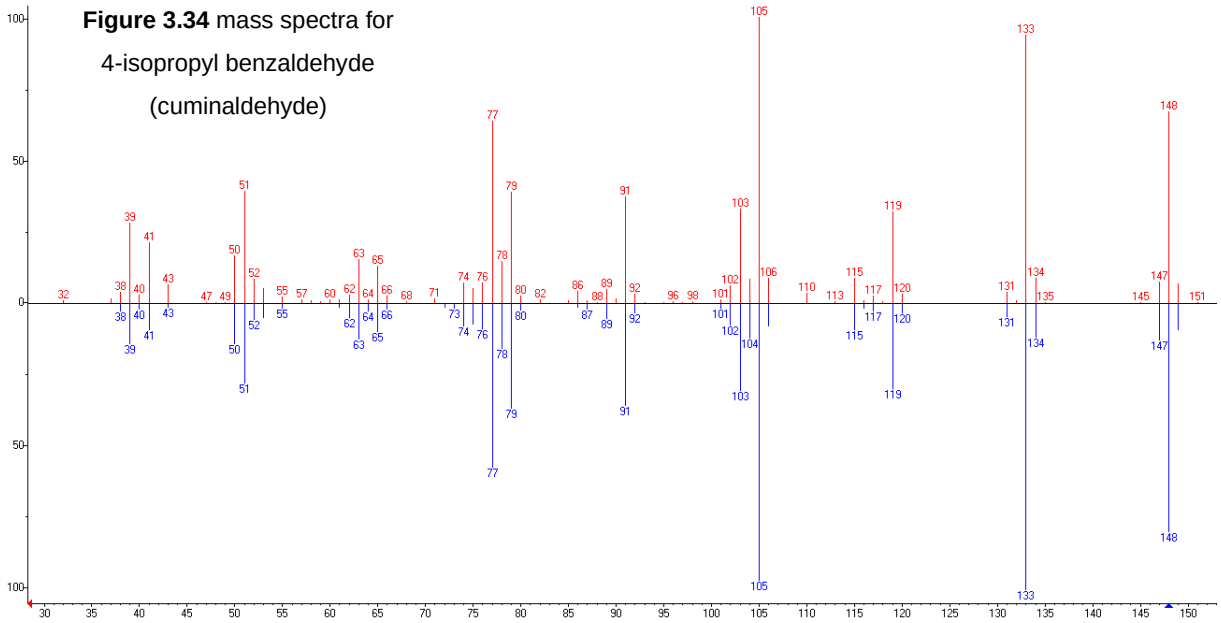


Figure 3.34 mass spectra for 4-isopropyl benzaldehyde (cuminaldehyde)

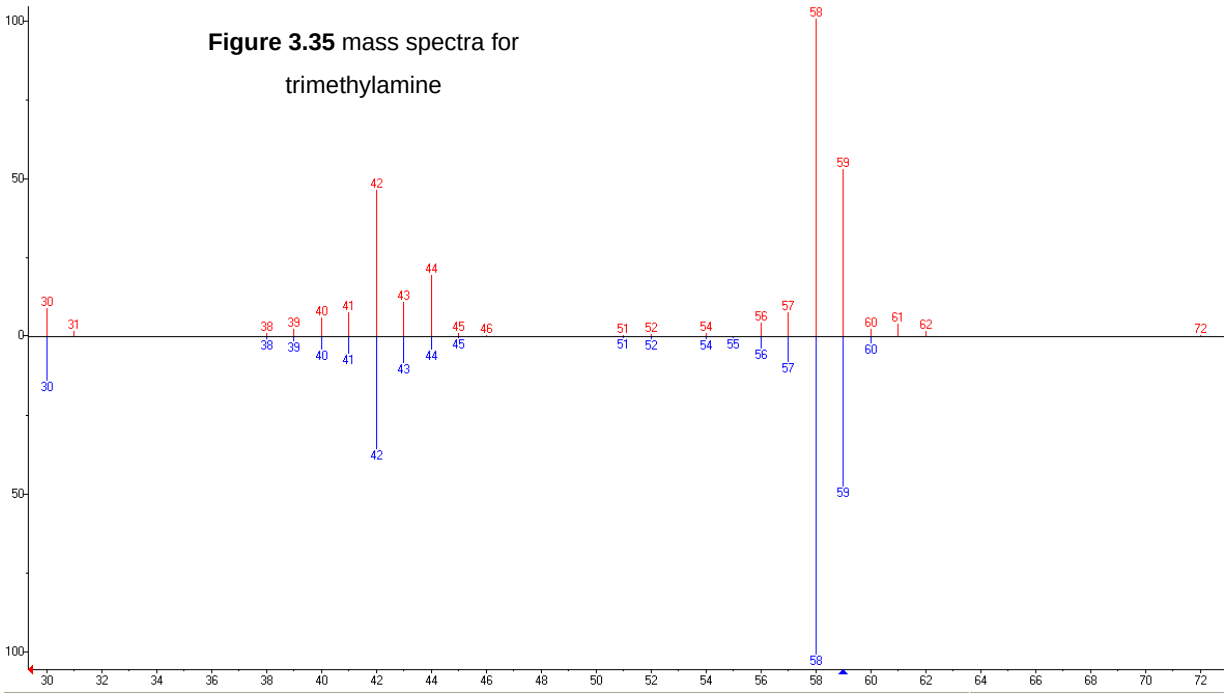


Figure 3.35 mass spectra for trimethylamine

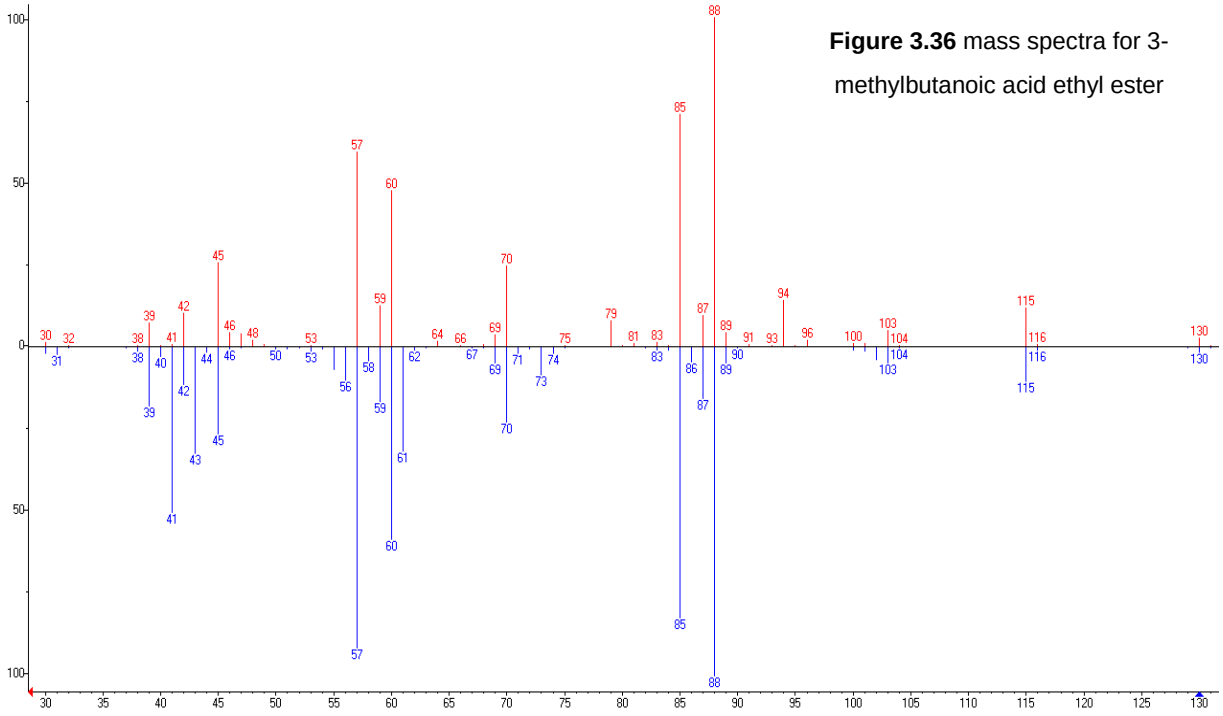


Figure 3.36 mass spectra for 3-methylbutanoic acid ethyl ester

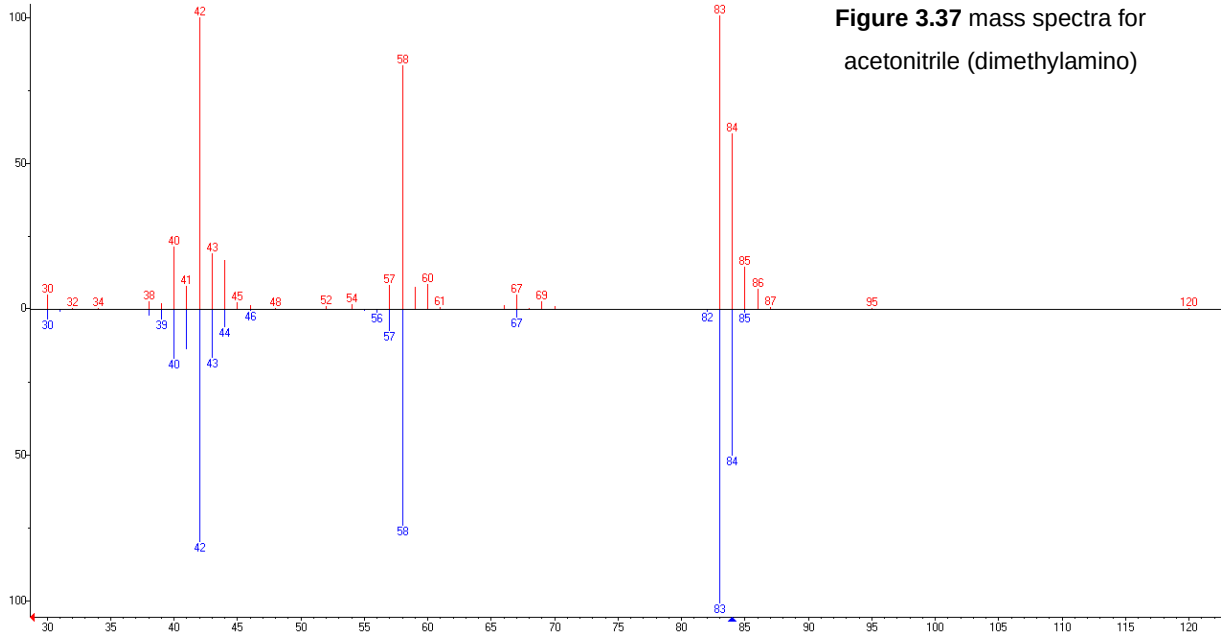


Figure 3.37 mass spectra for acetonitrile (dimethylamino)

3.5.0 Conclusions

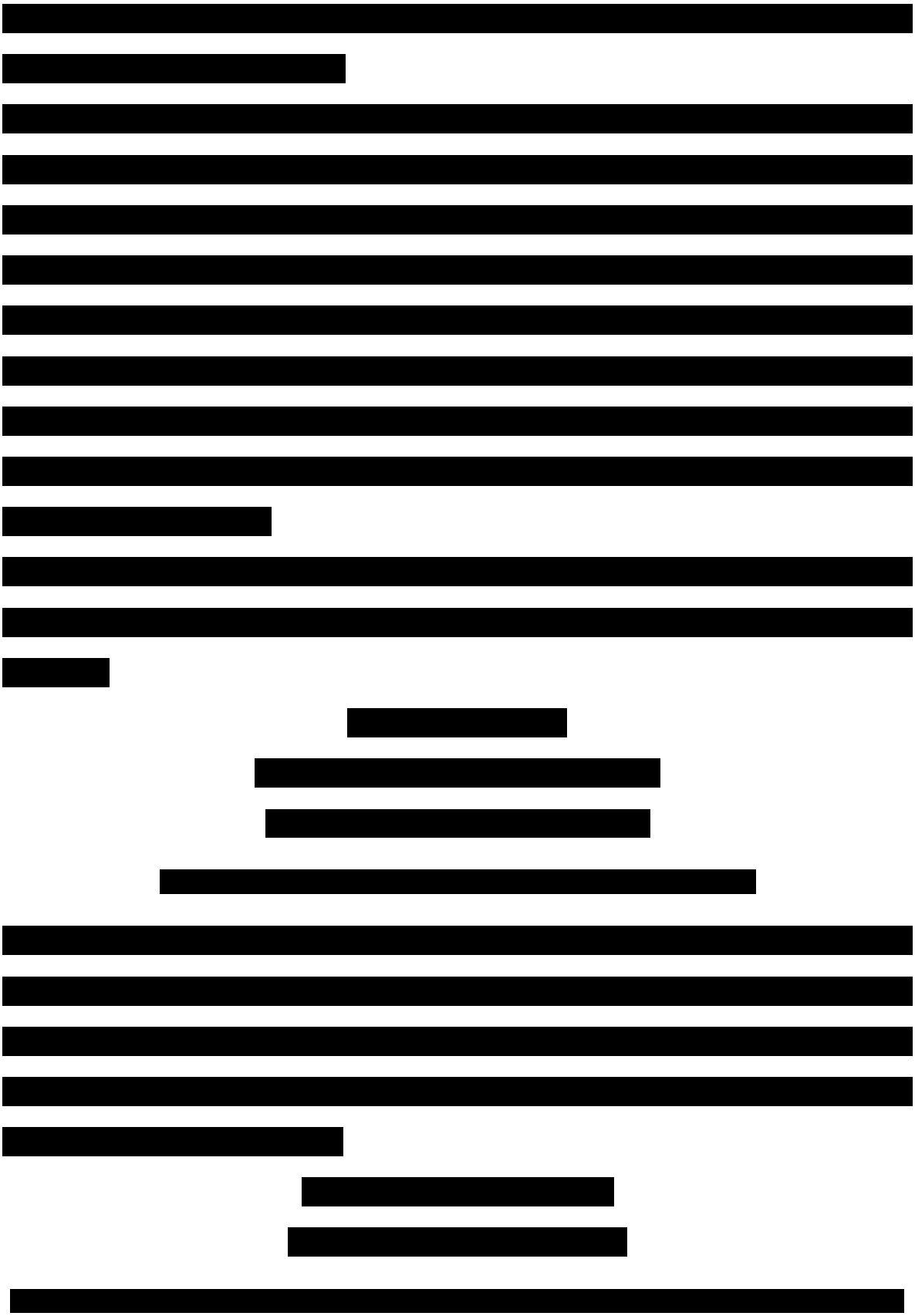
We have presented a method for the analysis of stool samples whereby the addition of ^{13}C compounds has allowed us to quantify a range of VOCs in stool: Dimethyl sulphide (26–2,5626 ng/g), acetone (442–3006 ng/g), ethyl butanoate (39–2468 ng/g), ethyl 2-methylbutanoate (0.3–180 ng/g), dimethyl disulphide (35–1303 ng/g), 1-octen-3-one (12 ng/g), dimethyl trisulphide (10–410 ng/g), 1-octen-3-ol (0.4–58 ng/g), ethanoic acid (672–1,2963 ng/g), butanoic acid (2493–1,1553 ng/g), 3-methylbutanoic acid (64–8262 ng/g), pentanoic acid (88–2,1886 ng/g), indole (290–5477 ng/g), and 3-methyl indole (37–3483 ng/g). Moreover, by altering the pH of the stool to pH 13, in conjunction with the addition of ^{13}C trimethylamine, we have also been able to detect and quantify trimethylamine for the first time (range 40–5312 ng/g). We were able to gather stool samples from participants of different countries of origin, which allowed us to compare the quantities of compounds from samples of UK origin with those of South American origin. Using a Mann Whitney U test, five compounds, ethyl butanoate, 1-octen-3-ol, ethanoic acid, butanoic acid, pentanoic acid, and indole, were calculated to be significantly different between South American and UK samples. Wilks' Lambda analysis showed that butanoic acid and ethyl butanoate could be used to differentiate the two groups. This has important implications for future studies looking to develop diagnostic tests based on VCs, especially where these diagnostics are not based on distinct markers of disease, but on changes in a number of VCs that are commonly observed in healthy individuals. This is because in order for diagnostic tests to be effective there must be a baseline range for which to compare the disease state to.

Logic might dictate that the difference in diet would lead to changes in produced metabolites. However, our work shows no significant differences in metabolite quantities between omnivores and vegetarians were observed in this study, in agreement with previous studies. Additionally, we have been able to tentatively

identify 15 compounds that have not previously been reported from stool samples. This data adds to the understanding of the human volatilome.

Chapter 4: Selected ion flow tube mass spectrometry (SIFT-MS) assessment of the oxides of nitrogen produced by EDX 110 dressings.

[redacted]



[redacted]

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Figure 4.38 the 10 cm x 10 cm hydrogel pad (left) shown adhesive side up, and the 5 cm x 5 cm sodium nitrite soaked mesh (right) which holds approximately 140 mg of 1M sodium nitrite.

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Figure 4.39 the EDX110 with the sodium nitrite approximately (140 mg 1 M) soaked layer applied to the centre of the adhesive hydrogel which contains a carboxylic acid at an approximate pH 4.

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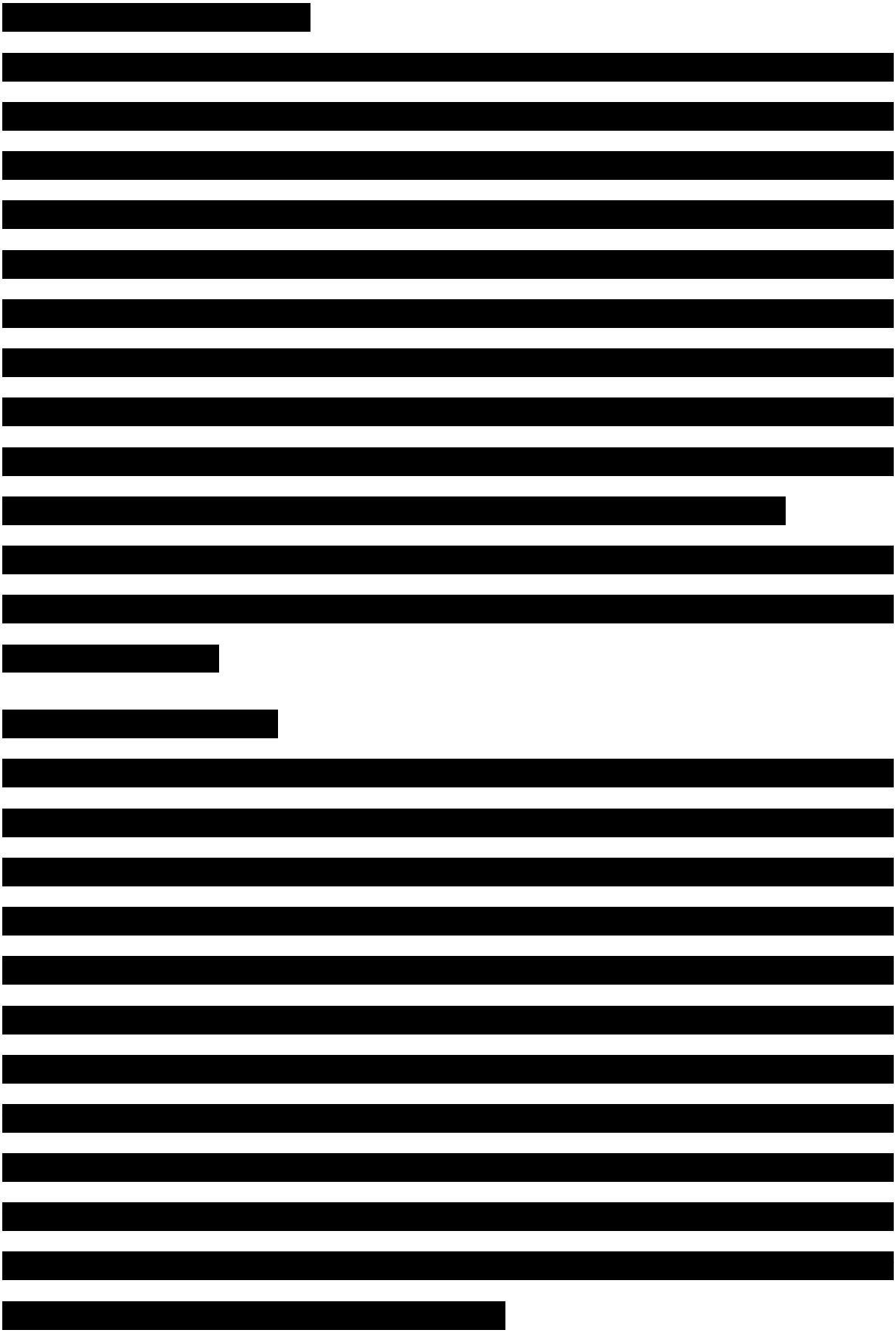
Figure 4.40 the polypropylene plastic chamber for chemical reactions to take place in. Brass Swagelok union adapters are used to secure the tubing in place and provide a gas tight seal. The pump draws the gas through the chamber and pushes the gas over the SIFT-MS capillary inlet.

Figure 4.41 the sampling system for real-time SIFT-MS monitoring of the products from chemical reactions. 1. The air inlet 2. Drechsel bottles to humidify the air in the reaction chamber 3. Chamber inlet 4. NMP015B pump (KNF Neuburger UK Ltd) 5. Analyte air is pushed over the SIFT-MS inlet capillary at a rate of 670mL/min.



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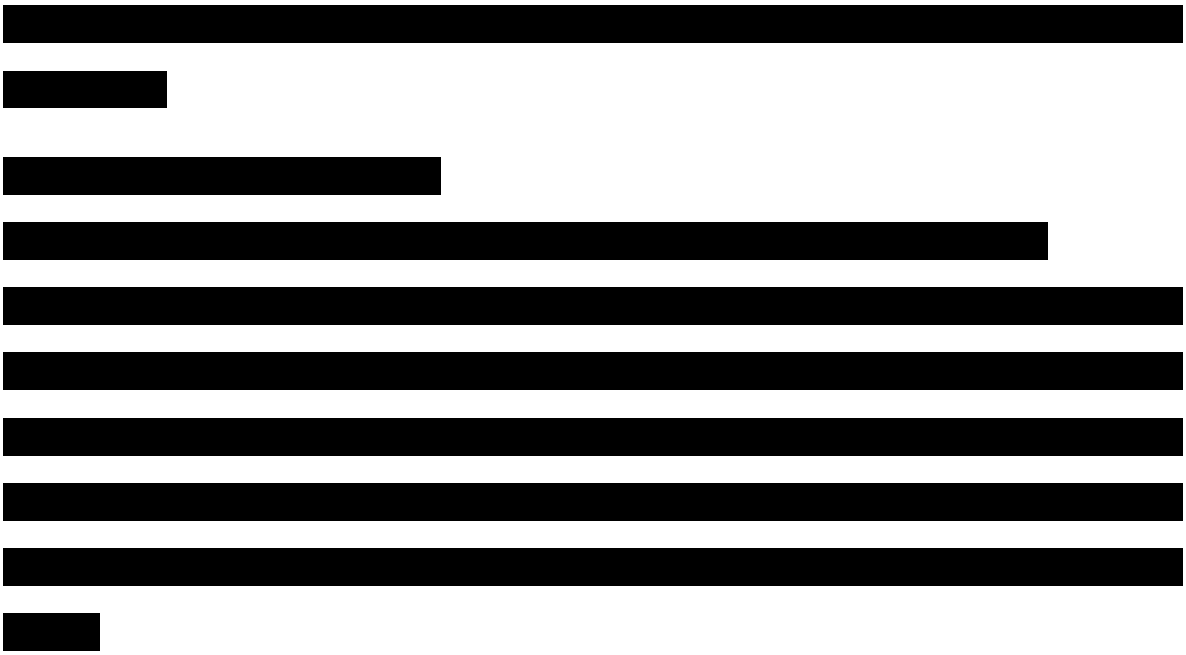

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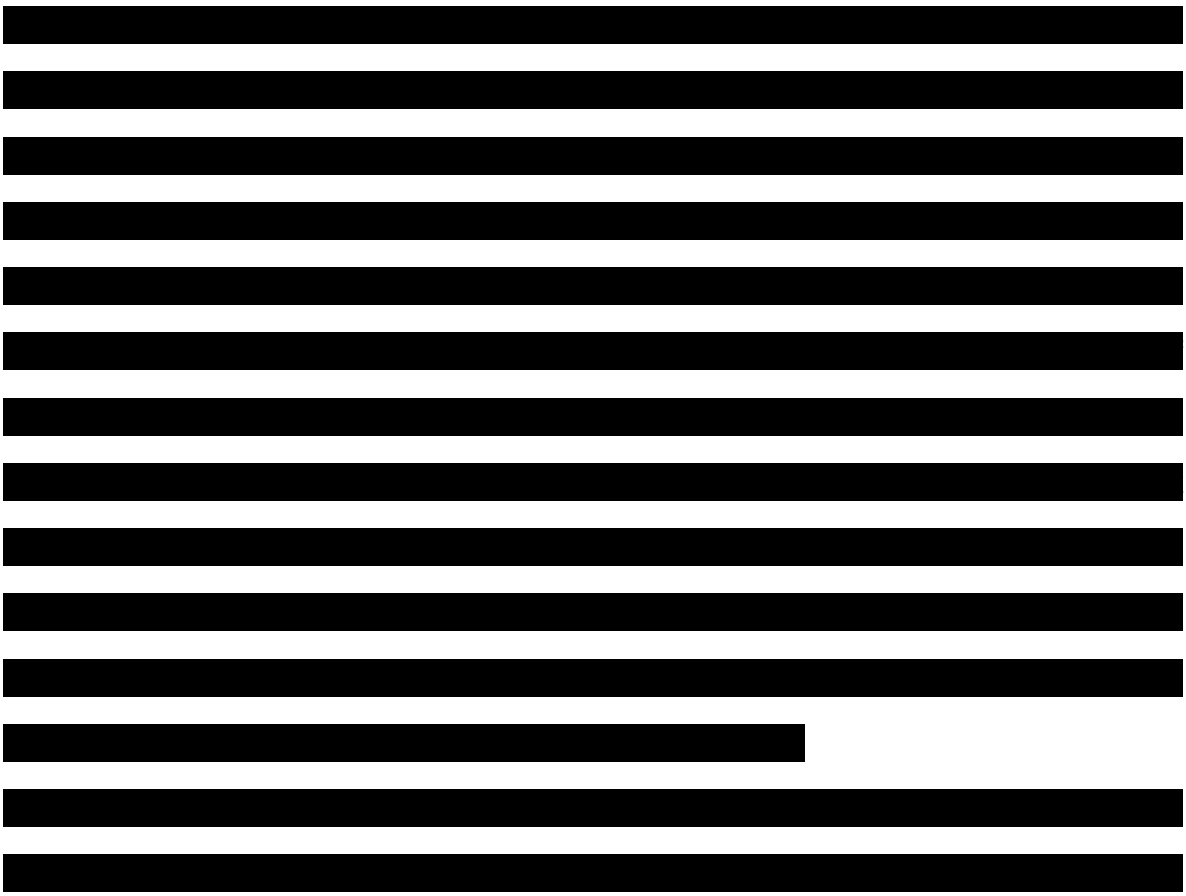
Figure 4.42 shows the evolution of NO at 6 different pH values by reacting buffered citric acid (0.1M) with 1M sodium nitrite.



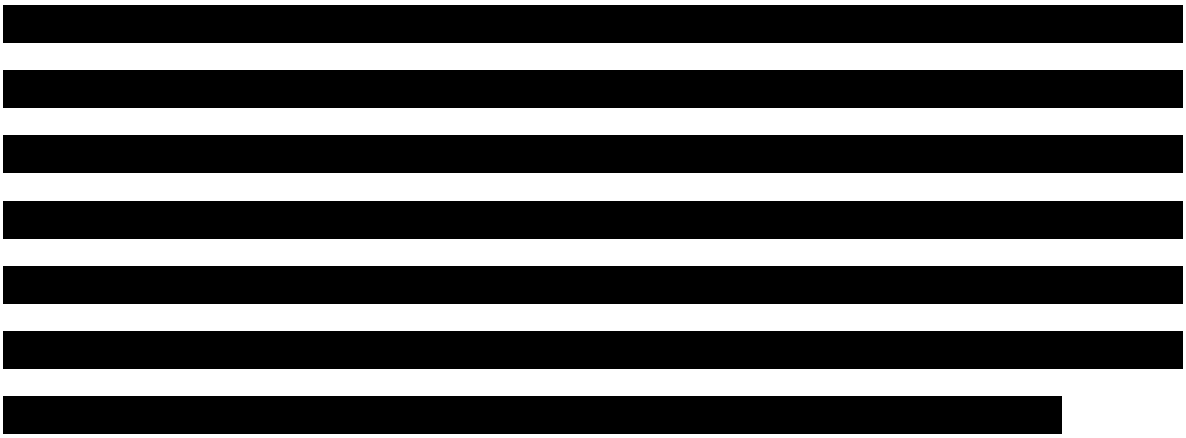


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Figure 4.43 cumulative plot in nmol per mg nitrite of nitric oxide evolved by the reaction of 0.1 M citric acid buffered with 0.1 M sodium citrate with 1 M sodium nitrite at different pH values.

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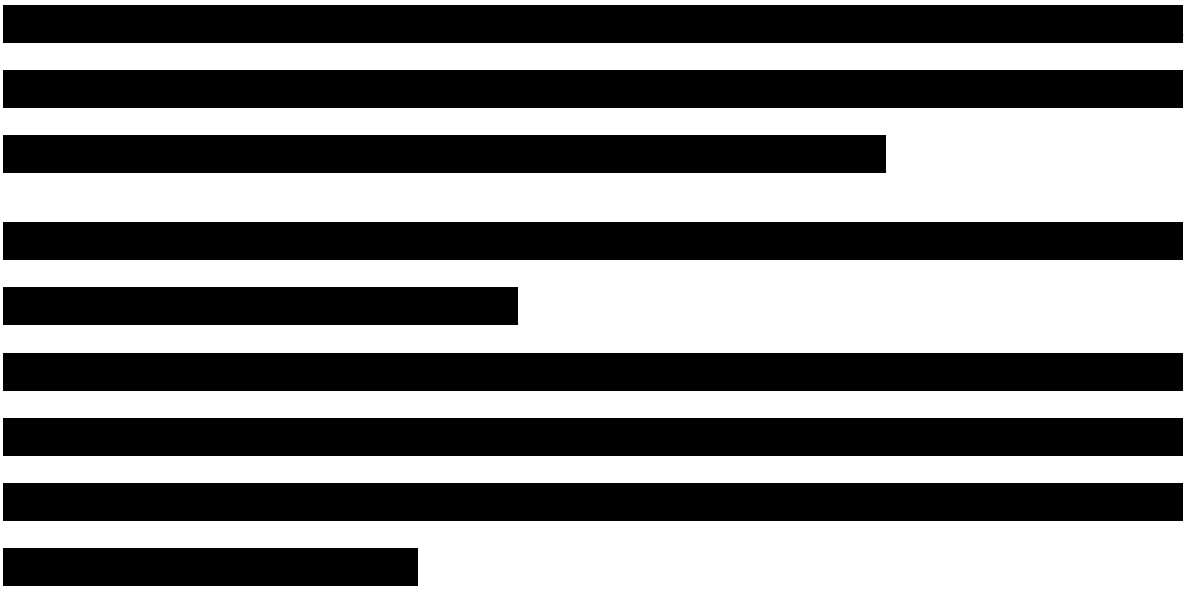
Figure 4.44 cumulative plot in nmol per mg nitrite of nitrogen dioxide evolved by the reaction of 0.1 M citric acid buffered with 0.1 M sodium citrate with 1M sodium nitrite at different pH values.



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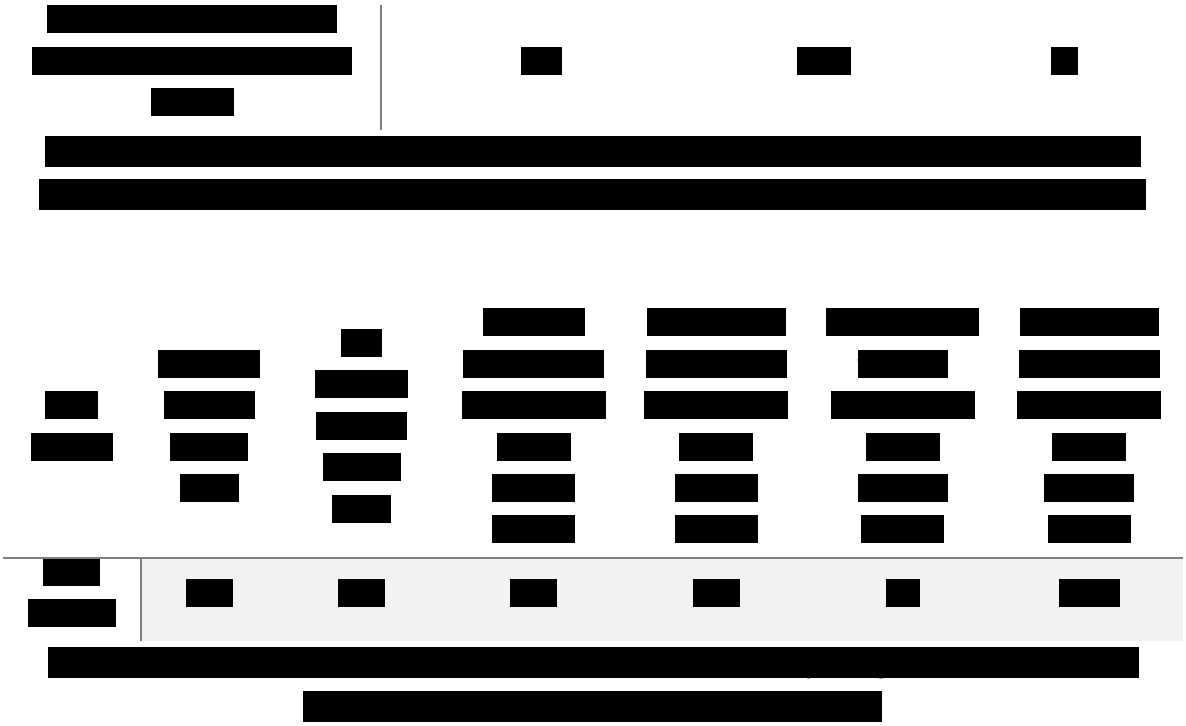

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Figure 4.45 the rate of evolution of nitric oxide from reacting citric or ascorbic acid with sodium nitrite with and without the presence of polyols.

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Figure 4.46 the cumulative plots of nitric oxide in nmol per mg sodium nitrite comparing citric and ascorbic acid with and without the presence of polyols.

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Figure 4.47 show the cumulative nitrogen dioxide in nmol per mg sodium nitrite comparing citric and ascorbic acid with and without the presence of polyols.

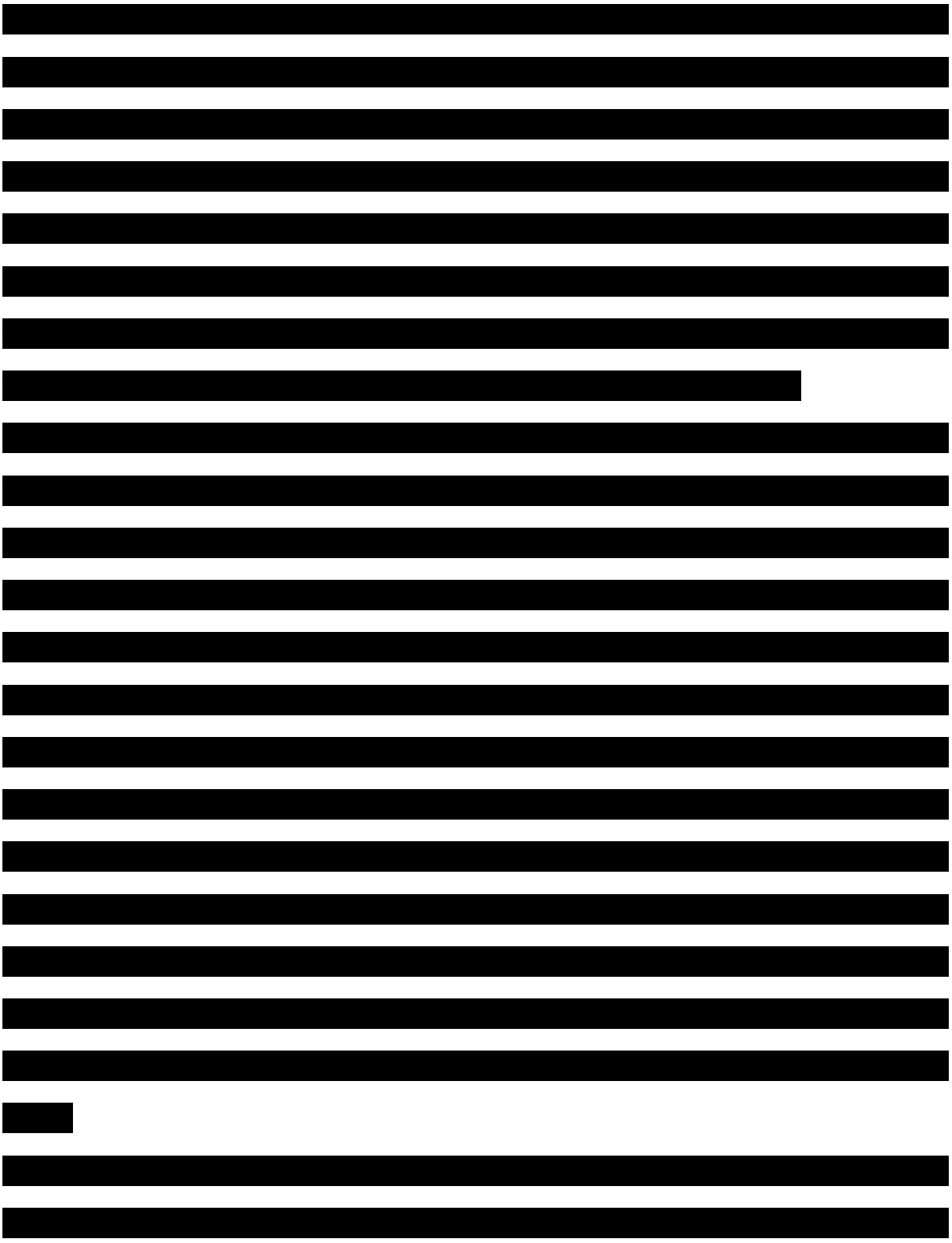

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Figure 4.48 a) shows the evolution of nitric oxide from EDX 110 dressings analysed at UWE and **b)** shows the evolution of nitric oxide from the EDX110 dressing by IPS. Both analysed on the Voice 200 SIFT-MS.

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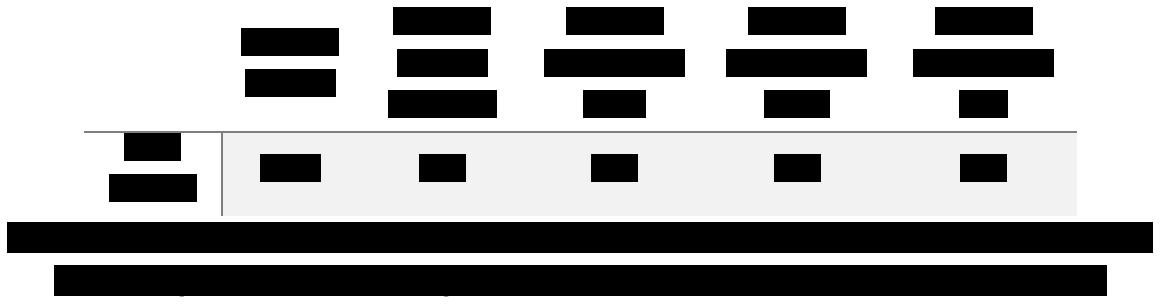

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Figure 4.49 the rate of evolution of nitric oxide produced from 1 quarter EDX 110 dressing with added filter paper saturated with one of either water, bovine serum albumin solution (BSA), hanks balanced salt solution, and saline.

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Figure 4.50 the cumulative plot of nitric oxide produced from 1 quarter EDX 110 dressing over 3 hours with added filter paper saturated with one of either water, bovine serum albumin solution (BSA), hanks balanced salt solution, and saline.

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Figure 4.51 the cumulative plot of nitrogen dioxide produced from 1 quarter EDX 110 dressing over 3 hours with added filter paper saturated with one of either water, bovine serum albumin solution (BSA), hanks balanced salt solution, and saline.

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Chapter 5: Summary, future work, and closing remarks

5.0.0 Summary

Over the last 4 chapters 3 novel mass spectrometry techniques have been described for biomedical applications.

5.1.1 Chapter 1 conclusions

Section 1.2 discusses and describes non-real time MS techniques and cites a number of studies in which these techniques have been used for biomedical applications. Chapter 1 describes how mass spectrometry has been developed over the years and is constantly being updated with ever increasing sensitivity and accuracy. Techniques such as two-dimensional gas chromatography time of flight mass spectrometry (GC-GC-TOF-MS) is providing such detailed information that we are able to gain new insights into the mechanisms underpinning the pathogenesis of disease states. In general, most chromatography-based techniques suffer from similar limitations; the time per analysis tends to range from 30 minutes to over one hour. Since there is so much data to process from a single sample data analysis can often be time consuming and require skilled personnel (as exemplified by chapter 3 which took several months of methods development). Similarly, the method development of these instruments also requires experienced users and can take significant time. Often samples require pre-processing and pre-concentration such as SPME or TD; this allows detectable concentrations of VCs to be injected into the instrument and while TD offers significantly improved sensitivity over SPME it adds time and expense to the process. However, the metabolic insights they can deliver with their qualitative data makes the cost to benefit worthwhile. This is because the qualitative data and the identification of VCs is crucial to understanding the metabolic changes that take place during disease states and thus understanding how VCs can be used in the clinical forum. The quality of data that can be collected from these instruments makes the shortcomings worthwhile.

Section 1.3 describes and discusses real-time techniques which, in general are simple to operate and can be done by semi-skilled operators. While non-real time

techniques can also be easy to operate, they do require significantly more method development which does require expertise. All the real time instruments discussed in chapter 1 are high throughput with very short time per sample analysis ranging from 1-10 minutes. This short analysis time means that it is possible to have these instruments at the point-of-care and perform the tests rapidly. Moreover, these instruments usually provide very high sensitivity and can often detect compounds that can-not be detected by non-real time techniques for instance thermally unstable compounds. SIFT-MS, PTR-MS, and PTR-TOF-MS are all quantitative with relatively good accuracy (usually within 20%). However, in order to achieve quantification a target analyte must first be identified; the biggest challenge facing real time quantitative techniques is the inability to differentiate between compounds with the same m/z value. This greatly limits the qualitative ability of these instruments.

5.2.0 Chapter 2 conclusions

In chapter 2 we present a novel instrument which couples a metal oxide sensor to a standard gas chromatography mass spectrometry instrument such that the GC column is split with half of the analyte gas entering the mass analyser and half entering the sensor chamber. Across the stool samples and bacterial headspace samples the sensor detected over 100 peaks that were not seen by the mass spectral analyser or were sub-threshold. We tested this system to 29 standards across a range of masses and functional groups. In all but two instances the MOS sensor showed superior or the same performance then the mass spectral analyser. By testing three complex matrices we have shown that this GC-MS-S system can be used for multiple applications. Although the response of MOS sensors is very fast, 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 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 [83]. However, it is important to note that the sensor only provides a resistance output so the extra comes at the expense of any qualitative data. By coupling the two detectors on a binary output we are able to take advantage of the qualitative MS data and the extra sensitivity of the sensor by using this data for differentiation of samples into groups e.g. disease versus non-disease.

5.2.1 Chapter 2 future work

Currently our system is set up for SPME pre-concentration however we plan to adapt this system to use automated thermal desorption (ATD). As discussed in section 1.2.1 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 the potential for 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.

Work is under way to compare our GC-MS-S system with both a SIFT-MS instrument and a FAIMS instrument with a view to diagnosing colorectal cancer in the fast track diagnosis pathway.

5.3.0 Chapter 3 conclusions

In chapter 3 a standard GC-MS system was coupled to an ATD unit for the analysis of the compounds from stool samples, again from healthy participants. In this case the goal was to quantify some common stool metabolites and test how this data could be applied for use (potentially clinically). This required the development of a novel sampling method which involved spiking the stool samples with a solution comprised of ^{13}C labelled compounds. Dimethyl sulphide (26–2,5626 ng/g), acetone

(442–3006 ng/g), ethyl butanoate (39–2468 ng/g), ethyl 2-methylbutanoate (0.3–180 ng/g), dimethyl disulphide (35–1303 ng/g), 1-octen-3-one (12 ng/g), dimethyl trisulphide (10–410 ng/g), 1-octen-3-ol (0.4–58 ng/g), ethanoic acid (672–1,2963 ng/g), butanoic acid (2493–1,1553 ng/g), 3-methylbutanoic acid (64–8262 ng/g), pentanoic acid (88–2,1886 ng/g), indole (290–5,477 ng/g), and 3-methyl indole (37–3483 ng/g). We have also made a subset of samples alkaline by adding sodium hydroxide and with this we have been able to detect and quantify trimethylamine (range 40–5312 ng/g). Additionally, we record 12 compounds that have not been previously recorded as being found in stool. We compared this data to the results from the UK participants to those from South America and found that there were five compounds that were significantly different between the two groups. This demonstrates that our novel method has the capability of differentiating samples into groups, which could have utility in future projects. Moreover, it highlights the idea that understanding what constitutes a healthy sample is important as ethnic origin can affect intestinal flora composition.

5.3.1 Chapter 3 future work

The technique described in chapter 3 could be used to quantify some of the compounds in clinical stool and/or urine. The data gathered from these could then be used to differentiate into disease positive and disease negative groups using the statistics described in section 3.3.3. It could even be possible to identify biomarkers that increase in proportion to disease progression and regression.

There needs to be more work done identifying the human volatilome; a review has been carried out in which all of the healthy human volatilome compounds identified in the literature have been tabulated [25]. However, one could question if this goes far enough in trying to elucidate what a healthy volatilome is. Most studies focus on a cohort of patients and/or participants with a sample taken at one point in time. To develop a truly comprehensive overview of the healthy volatilome there would need to be multiple longitudinal studies across multiple populations over many years.

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5.5.0 Closing remarks

Chapter 1 highlights some of advances in mass spectrometry and some of the new types of data that we can gather faster and more accurately than ever before. Moreover, chapter 1 shows there is an ever-expanding body of evidence that mass

spectrometry and related analytical techniques could pave the way to providing low cost, rapid, and non-invasive testing for numerous disease states.

In chapter 2 a standard GC-MS instrument is coupled to a MOS sensor such that from a single sample run a binary output achieved; an instrument of this type has not previously been reported in the literature. Chapter 2 demonstrates that the MOS sensor is capable of superior sensitivity in several cases and that this instrument can be used for a range of applications. By combining the enhanced sensitivity of the MOS sensor with the qualitative output of the GC-MS this instrument shows great potential for the clinical setting.

Chapter 3 presents a novel method for the quantification of VCs in stool samples by using ^{13}C labelled compounds as internal standards. This method was used successfully and quantifies the mass of compounds retained in the stool and not just what is evolved into the headspace. This chapter also presents analysis of stool samples after being made alkaline (pH 13), this had a profound effect on the compounds evolved and allowed for the identification and quantification of amine compounds. The literature search did not show any other instances in which stools samples had been made alkaline prior to GC-MS analysis. Several compounds identified from the NIST library had not been previously reported in stool.

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This thesis presents three novel methods of the use of MS for biomedical applications. Thus, showing that despite recent advances in technology and large body of pre-existing literature there are still many methods and techniques yet to be

developed. As such this thesis shows that while mass spectrometry might be over 120 years old it is more relevant today than it ever has been.

6.0 References

[1] Williams H and Pembroke A 1989 Sniffer dogs in the melanoma clinic? *Lancet* **333** 734

[2] Horvath G, Andersson H and Paulsson G 2010 Characteristic odour in the blood reveals ovarian carcinoma *BMC Cancer* **10** 643

[3] Willis C M, Church S M, Guest C M, Cook W A, McCarthy N, Bransbury A J, Church M R T and Church J C T 2004 Olfactory detection of human bladder cancer by dogs: proof of principle study. *BMJ* **329** 712

[4] Taverna G, Tidu L, Grizzi F, Torri V, Mandressi A, Sardella P, La Torre G, Cocciolone G, Seveso M, Giusti G, Hurle R, Santoro A and Graziotti P 2015 Olfactory system of highly trained dogs detects prostate cancer in urine samples. *J. Urol.* **193** 1382–7

[5] Willis C M, Britton L E, Harris R, Wallace J and Guest C M 2011 Volatile organic compounds as biomarkers of bladder cancer: Sensitivity and specificity using trained sniffer dogs *Cancer Biomarkers* **8** 145–53

[6] Sonoda H, Kohnoe S, Yamazato T, Satoh Y, Morizono G, Shikata K, Morita M, Watanabe A, Morita M, Kakeji Y, Inoue F and Maehara Y 2011 Colorectal cancer screening with odour material by canine scent detection. *Gut* **60** 814–9

[7] McCulloch M, Jezierski T, Broffman M, Hubbard A, Turner K and Janecki T 2006 Diagnostic Accuracy of Canine Scent Detection in Early- and Late-Stage Lung and Breast Cancers *Integr. Cancer Ther.* **5** 30–9

[8] Ehmann R, Boedeker E, Friedrich U, Sagert J, Dippon J, Friedel G and Walles T 2012 Canine scent detection in the diagnosis of lung cancer: revisiting a puzzling phenomenon. *Eur. Respir. J.* **39** 669–76

[9] Pickel D, Manucy G P, Walker D B, Hall S B and Walker J C 2004 Evidence for canine olfactory detection of melanoma *Appl. Anim. Behav. Sci.* **89** 107–16

[10] Horvath G, Järverud G af K, Järverud S and Horváth I 2008 Human Ovarian Carcinomas Detected by Specific Odor *Integr. Cancer Ther.* **7** 76–80

[11] Moser E and McCulloch M 2010 Canine scent detection of human cancers: A review of methods and accuracy *J. Vet. Behav. Clin. Appl. Res.* **5** 145–52

[12] Gould O, Smart A, Ratcliffe N, arXiv B C preprint and 2015 undefined Canine Olfactory Differentiation of Cancer: A Review of the Literature *arxiv.org*

[13] Bomers M K, van Agtmael M A, Luik H, van Veen M C, Vandenbroucke-Grauls C M J E and Smulders Y M 2012 Using a dog's superior olfactory sensitivity to identify Clostridium difficile in stools and patients: proof of principle study. *BMJ* **345** e7396

[14] Rooney N J, Morant S and Guest C 2013 Investigation into the Value of Trained Glycaemia Alert Dogs to Clients with Type I Diabetes ed W Hughes *PLoS One* **8** e69921

[15] Amundsen T, Sundstrøm S, Buvik T, Gederaas O A and Haaverstad R 2014 Can dogs smell lung cancer? First study using exhaled breath and urine screening in unselected patients with suspected lung cancer *Acta Oncol. (Madr).* **53** 307–15

[16] Miekisch W, Schubert J K and Noeldge-Schomburg G F . 2004 Diagnostic potential of breath analysis—focus on volatile organic compounds *Clin. Chim. Acta* **347** 25–39

[17] Sun M, Chen Z, Gong Z, Zhao X, Jiang C, Yuan Y, Wang Z, Li Y and Wang C 2015 Determination of breath acetone in 149 Type 2 diabetic patients using a ringdown breath-acetone analyzer *Anal. Bioanal. Chem.* **407** 1641–50

[18] Nelson N, Lagesson V, Nosratabadi A R, Ludvigsson J and Tagesson C 1998 Exhaled Isoprene and Acetone in Newborn Infants and in Children with Diabetes Mellitus *Pediatr. Res.* **44** 363–7

[19] Tassopoulos C N, Barnett D and Russell Fraser T 1969 Breath-acetone and blood-sugar measurements in diabetes *Lancet* **293** 1282–6

[20] American Diabetes Association. M J, Karger B A and Wrenshall G 1952 *Diabetes.* vol 1 ([American Diabetes Association])

[21] Rooth G and Östenson S 1966 Acetone in alveolar air, and the control of diabetes *Lancet* **288** 1102–5

[22] Turner C, Walton C, Hoashi S and Evans M 2009 Breath acetone concentration decreases with blood glucose concentration in type I diabetes mellitus patients during hypoglycaemic clamps *J. Breath Res.* **3** 046004

[23] Storer M, Dummer J, Lunt H, Scotter J, McCartin F, Cook J, Swanney M, Kendall D, Logan F and Epton M 2011 Measurement of breath acetone concentrations by selected ion flow tube mass spectrometry in type 2 Diabetes *J. Breath Res.* **5** 046011

[24] Shirasu M and Touhara K 2011 The scent of disease: volatile organic compounds of the human body related to disease and disorder *J. Biochem.* **150** 257–66

[25] Amann A, Costello B de L, Miekisch W, Schubert J, Buszewski B, Pleil J, Ratcliffe N and Risby T 2014 The human volatilome: volatile organic compounds (VOCs) in exhaled breath, skin emanations, urine, feces and saliva *J. Breath Res.* **8** 034001

[26] de Hoffmann E and Stroobant V *Mass Spectrometry Principles and Applications* Date accessed: 19.11.2019

[27] Gaisl T, Bregy L, Stebler N, Gaugg M T, Bruderer T, García-Gómez D, Moeller A, Singer F, Schwarz E I, Benden C, M-L Sinues P, Zenobi R and Kohler M 2018 Real-time exhaled breath analysis in patients with cystic fibrosis and controls *J. Breath Res.* **12** 036013

[28] Witasse, O., Huber, L., Zender, J., Lebreton, J.P., Beebe, R., Heather, D., Matson, D.L., Zarnecki, J., Wheadon, J., Trautner, R. and Tomasko, M., 2008. The Huygens scientific data archive: Technical overview. *Planetary and Space Science*. **56** 770-777.

[29] Kevin Downard 2004 Mass Spectrometry's Beginnings 1.1 A brief history 1.1.1 Early Pioneers and Cathode Rays Date accessed: 12.09.2018

[30] Richardson S D 2001 Mass Spectrometry in Environmental Sciences *chem. Rev.* **101** 211-254

[31] van Belkum A, Welker M, Erhard M and Chatellier S 2012 Biomedical mass spectrometry in today's and tomorrow's clinical microbiology laboratories. *J. Clin. Microbiol.* **50** 1513–7

[32] Déglon J, Thomas A, Mangin P and Staub C 2012 Direct analysis of dried blood spots coupled with mass spectrometry: concepts and biomedical applications *Anal. Bioanal. Chem.* **402** 2485–98

[33] Chughtai K and Heeren R M A 2010 Mass Spectrometric Imaging for Biomedical Tissue Analysis *Chem. Rev.* **110** 3237–77

[34] Wes E. Steiner, Brian H. Clowers, Laura M. Matz, William F. Siems and and Herbert H. Hill J . 2002 Rapid Screening of Aqueous Chemical Warfare Agent Degradation Products: Ambient Pressure Ion Mobility Mass Spectrometry *Anal. Chem.* **74** 4343-52.

[35] Steiner W E, Clowers B H, Fuhrer K, Gonin M, Matz L M, Siems W F, Schultz A J and Hill H H 2001 Electrospray ionization with ambient pressure ion mobility separation and mass analysis by orthogonal time-of-flight mass spectrometry *Rapid Commun. Mass Spectrom.* **15** 2221–6

[36] Wes E. Steiner, Steve J. Klopsch, William A. English, Brian H. Clowers and and Hill* H H 2005 Detection of a Chemical Warfare Agent Simulant in Various Aerosol Matrixes by Ion Mobility Time-of-Flight Mass Spectrometry *Anal. Chem.* **77** 4792-4799

[37] Guinan T, Kirkbride P, Pigou P E, Ronci M, Kobus H and Voelcker N H 2015 Surface-assisted laser desorption ionization mass spectrometry techniques for application in forensics *Mass Spectrom. Rev.* **34** 627–40

[38] Lawton Z E, Traub A, Fatigante W L, Mancias J, O'Leary A E, Hall S E, Wieland J R, Oberacher H, Gizzi M C and Mulligan C C 2017 Analytical Validation of a Portable Mass Spectrometer Featuring Interchangeable, Ambient Ionization Sources for High Throughput Forensic Evidence Screening *J. Am. Soc. Mass Spectrom.* **28** 1048–59

[39] Pavlovich M J, Musselman B and Hall A B 2018 Direct analysis in real time-Mass spectrometry (DART-MS) in forensic and security applications *Mass Spectrom. Rev.* **37** 171–87

[40] Goesmann F, Rosenbauer H, Bredehöft J H, Cabane M, Ehrenfreund P, Gautier T, Giri C, Krüger H, Le Roy L, MacDermott A J, McKenna-Lawlor S, Meierhenrich U J, Muñoz Caro G M, Raulin F, Roll R, Steele A, Steininger H, Sternberg R, Szopa C, Thiemann W and Ulamec S 2015 COMETARY SCIENCE. Organic compounds on comet 67P/Churyumov-Gerasimenko revealed by COSAC mass spectrometry. *Science* **349** aab0689

[41] Stern J C, Sutter B, Freissinet C, Navarro-González R, McKay C P, Archer P D, Buch A, Brunner A E, Coll P, Eigenbrode J L, Fairen A G, Franz H B, Glavin D P, Kashyap S, McAdam A C, Ming D W, Steele A, Szopa C, Wray J J, Martín-Torres F J, Zorzano M-P, Conrad P G, Mahaffy P R and MSL Science Team the M S 2015 Evidence for indigenous nitrogen in sedimentary and aeolian deposits from the Curiosity rover investigations at Gale crater, Mars. *Proc. Natl. Acad. Sci. U. S. A.* **112** 4245–50

[42] Cmarik G, Knox J and Huff T 2018 Analysis of Performance Degradation of Silica Gels after Extended use Onboard the ISS *48th International Conference on Environmental Systems* Date Accessed: 01.10.2018

[43] Ruan E D, Aalhus J L, Juárez M and Sabik H 2015 Analysis of Volatile and Flavor Compounds in Grilled Lean Beef by Stir Bar Sorptive Extraction and Thermal Desorption—Gas Chromatography Mass Spectrometry *Food Anal. Methods* **8** 363–70

[44] Black C, Chevallier O P and Elliott C T 2016 The current and potential applications of Ambient Mass Spectrometry in detecting food fraud *TrAC Trends Anal. Chem.* **82** 268–78

[45] Bantscheff M, Schirle M, Sweetman G, Rick J and Kuster B 2007 Quantitative mass spectrometry in proteomics: a critical review *Anal. Bioanal. Chem.* **389** 1017–31

[46] Aebersold R and Mann M 2003 Mass spectrometry-based proteomics *Nature* **422** 198–207

[47] Dettmer K, Aronov P A and Hammock B D 2007 Mass spectrometry-based metabolomics *Mass Spectrom. Rev.* **26** 51–78

[48] Villas-Bôas S G, Mas S, Åkesson M, Smedsgaard J and Nielsen J 2005 Mass spectrometry in metabolome analysis *Mass Spectrom. Rev.* **24** 613–46

[49] Garcia A and Barbas C 2011 Gas Chromatography-Mass Spectrometry (GC-MS)-Based Metabolomics (Humana Press) pp 191–204

[50] Smith D, Španěl P, Herbig J and Beauchamp J 2014 Mass spectrometry for real-time quantitative breath analysis *J. Breath Res.* **8** 027101

[51] Ratiu I A, Ligor T, Bocos-Bintintan V and Buszewski B 2017 Mass spectrometric techniques for the analysis of volatile organic compounds emitted from bacteria *Bioanalysis* **9** 1069–92

[52] Souza-Silva É A, Jiang R, Rodríguez-Lafuente A, Gionfriddo E and Pawliszyn J 2015 A critical review of the state of the art of solid-phase microextraction of complex matrices I. Environmental analysis *TrAC Trends Anal. Chem.* **71** 224–35

[53] Garner C E, Smith S, de Lacy Costello B, White P, Spencer R, Probert C S J and Ratcliffe N M 2007 Volatile organic compounds from feces and their potential for diagnosis of gastrointestinal disease. *FASEB J.* **21** 1675–88

[54] Ocaña-González J A, Fernández-Torres R, Bello-López M Á and Ramos-Payán M 2016 New developments in microextraction techniques in bioanalysis. A review *Anal. Chim. Acta* **905** 8–23

[55] Schmidt K and Podmore I 2015 Current Challenges in Volatile Organic Compounds Analysis as Potential Biomarkers of Cancer *J. Biomarkers* **2015** 1–16

[56] Silva C L, Passos M and Câmara J S 2012 Solid phase microextraction, mass spectrometry and metabolomic approaches for detection of potential urinary cancer biomarkers—A powerful strategy for breast cancer diagnosis *Talanta* **89** 360–8

[57] Harper M 2000 Sorbent trapping of volatile organic compounds from air *J. Chromatogr. A* **885** 129–51

[58] Kang S and Paul Thomas C L 2016 How long may a breath sample be stored for at −80 °C? A study of the stability of volatile organic compounds trapped onto a mixed Tenax:Carbograph trap adsorbent bed from exhaled breath *J. Breath Res.* **10** 026011

[59] Harshman S W, Mani N, Geier B A, Kwak J, Shepard P, Fan M, Sudberry G L, Mayes R S, Ott D K, Martin J A and Grigsby C C 2016 Storage stability of exhaled breath on Tenax TA *J. Breath Res.* **10** 046008

[60] Li Y and Zhu J 2017 Identification of sink spots in two thermal desorption GC/MS systems for the analysis of polycyclic aromatic hydrocarbons *Anal. Chim. Acta* **961** 67–73

[61] Gould O, Wieczorek T, Costello B P J de L, Persad R and Ratcliffe N 2017 Assessment of a combined gas chromatography mass spectrometer sensor (GC-MSS) system for detecting biologically relevant volatile compounds (VCs) *J. Breath Res.***12** 016009

[62] Dimbat M, Porter P E and Stross F H 1956 Apparatus Requirements for Quantitative Applications *Anal. Chem.* **28** 290–7

[63] Stein S E, Linstrom P, Mirokhin Y, Tchekhovskoi D, Yang X, Gary W, Sparkman M O D and Sparkman J A 1987 *NIST Standard Reference Database 1A NIST/EPA/NIH Mass Spectral Database (NIST 11) and NIST Mass Spectral Search Program (Version 2.0g) For Use with Microsoft ® Windows User's Guide The NIST Mass Spectrometry Data Center*

[64] Fialkov A B, Steiner U, Lehotay S J and Amirav A 2007 Sensitivity and noise in GC-MS: Achieving low limits of detection for difficult analytes *Int. J. Mass Spectrom.* **260** 31–48

[65] Tait E, Hill K A, Perry J D, Stanforth S P and Dean J R 2014 Development of a novel method for detection of Clostridium difficile using HS-SPME-GC-MS *J. Appl. Microbiol.* **116** 1010–9

[66] Probert C S J, Jones P R H and Probert S J 2004 A novel method for rapidly diagnosing the causes of diarrhoea *Gut* **53** 68-61

[67] Vega P, Valentín F and Cubiella J 2015 Colorectal cancer diagnosis: Pitfalls and opportunities. *World J. Gastrointest. Oncol.* **7** 422–33

[68] Altomare D F, Di Lena M, Porcelli F, Trizio L, Travaglio E, Tutino M, Dragonieri S, Memeo V and De Gennaro G 2013 Exhaled volatile organic compounds identify patients with colorectal cancer *Br. J. Surg.* **100** 144–50

[69] Amal H, Leja M, Funka K, Lasina I, Skapars R, Sivins A, Ancans G, Kikuste I, Vanags A, Tolmanis I, Kirsners A, Kupcinskas L and Haick H 2016 Breath testing as potential colorectal cancer screening tool *Int. J. Cancer* **138** 229–36

[70] McLafferty F W, Levsen of Bonn K, Maquestiau A, Flammang of Mons R, Burlingame of Berkeley L and Anbar M 1978 *A*ccounts of chemical research volume 13 number Tandem Mass Spectrometry (MS/MS): A Promising New Analytical Technique for Specific Component Determination in Complex Mixtures *Elsevier* vol 50 (UTC)

[71] Wang T M, Ding L Q, Jin H J, Shi R, Wu J S, Zhu L, Jia Y Q and Ma Y M 2015 Simultaneous quantification of multiple volatile active components in rat plasma using a headspace-solid phase dynamic extraction method coupled to gas chromatography-tandem mass spectroscopy: Application in a pharmacokinetic study of Longhu Rendan pills *RSC Adv.* **5** 29631–8

[72] Overview of Mass Spectrometry for protein analysis *ThermoFisher Scientific* https://www.thermofisher.com/uk/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-mass-spectrometry.html.html Accessed: 18.01.2019

[73] Biagini D, Lomonaco T, Ghimenti S, Bellagambi F G, Onor M, Scali M C, Barletta V, Marzilli M, Salvo P, Trivella M G, Fuoco R and Di Francesco F 2017 Determination of volatile organic compounds in exhaled breath of heart failure patients by needle trap micro-extraction coupled with gas chromatography-tandem mass spectrometry *J. Breath Res.* **11**

[74] Brinkley J, Libarondi M 2010 Comparing the Capabilities of Time-of-Flight and Quadrupole Mass Spectrometers *Current Trends in Mass Spectrometry* 1-5

[75] Baranska A, Mujagic Z, Smolinska A, Dallinga J W, Jonkers D M A E, Tigchelaar E F, Dekens J, Zhernakova A, Ludwig T, Masclee A A M, Wijmenga C and van Schooten F J 2016 Volatile organic compounds in breath as markers for irritable bowel syndrome: a metabolomic approach *Aliment. Pharmacol. Ther.* **44** 45–56

[76] Pijls K E, Smolinska A, Jonkers D M A E, Dallinga J W, Masclee A A M, Koek G H and Van Schooten F J 2016 A profile of volatile organic compounds in exhaled air as a potential non-invasive biomarker for liver cirrhosis *Sci. Rep.* **6** 1–8

[77] Smolinska A, Tedjo D I, Blanchet L, Bodelier A, Pierik M J, Masclee A A M, Dallinga J, Savelkoul P H M, Jonkers D M A E, Penders J and van Schooten F J 2018 Volatile metabolites in breath strongly correlate with gut microbiome in CD patients *Anal. Chim. Acta* **1025** 1–11

[78] Kalinová B, Jiroš P, Žďárek J, Wen X and Hoskovec M 2006 GC × GC/TOF MS technique—A new tool in identification of insect pheromones: Analysis of the persimmon bark borer sex pheromone gland *Talanta* **69** 542–7

[79] S Haglund P, Löfstrand K, Siek K and Asplund L 2013 Powerful GC-TOF-MS Techniques for Screening, Identification and Quantification of Halogenated Natural Products. *Mass Spectrom. (Tokyo, Japan)* **2** S0018

[80] Mellors T R, Rees C A, Wieland-alter W F, Bean H D, Rees C A, Hill J E, Phan J, Meinardi S and Barletta B 2017 Sniffing out the hypoxia volatile metabolic signature of Aspergillus fumigatus *J. Breath Res.* **11** 036003

[81] Rees C A, Smolinska A and Hill J E 2016 The volatile metabolome of Klebsiella pneumoniae in human blood *J. Breath Res.* **10** 027101

[82] Khalid T, White P, De Lacy Costello B, Persad R, Ewen R, Johnson E, Probert C S and Ratcliffe N 2013 A Pilot Study Combining a GC-Sensor Device with a Statistical Model for the Identification of Bladder Cancer from Urine Headspace ed W-C Chin *PLoS One* **8** e69602

[83] McGuire N D, Ewen R J, De Lacy Costello B, Garner C E, Probert C S J, Vaughan K and Ratcliffe N M 2014 Towards point of care testing for C. difficile infection by volatile profiling, using the combination of a short multi-capillary gas chromatography column with metal oxide sensor detection *Meas. Sci. Technol.* **25** 065108

[84] Bartolazzi A, Santonico M, Pennazza G, Martinelli E, Paolesse R, D'Amico A and Di Natale C 2010 A sensor array and GC study about VOCs and cancer cells *Sensors and Actuators B: Chemical* **146** 483–8

[85] Perkins M, Langford V and McEwan M 2017 High-Throughput VOC and Inorganic Gas Analysis: Automated SIFT-MS *Am. Lab.* **49** 17–9

[86] Anon SIFT-MS selected ion flow tube mass spectrometry

[87] Spanel P, Smith D. 2013 Advances in on-line absolute trace gas analysis by SIFT-MS. *Current Analytical Chemistry.* **9** 525-39.

[88] Phillips M, Beatty J D, Cataneo R N, Huston J, Kaplan P D, Lalisang R I, Lambin P, Lobbes M B I, Mundada M, Pappas N and Patel U 2014 Rapid Point-Of-Care Breath Test for Biomarkers of Breast Cancer and Abnormal Mammograms ed S A Aziz *PLoS One* **9** e90226

[89] Hanouneh I A, Zein N N, Cikach F, Dababneh L, Grove D, Alkhouri N, Lopez R and Dweik R A 2014 The breathprints in patients with liver disease identify novel breath biomarkers in alcoholic hepatitis *Clin. Gastroenterol. Hepatol.* **12** 516–23

[90] Navaneethan U, Parsi M A, Gutierrez N G, Bhatt A, Venkatesh P G K, Lourdusamy D, Grove D, Hammel J P, Jang S, Sanaka M R, Stevens T, Vargo J J and Dweik R A 2014 Volatile organic compounds in bile can diagnose malignant biliary strictures in the setting of pancreatic cancer: A preliminary observation *Gastrointest. Endosc.* **80** 1038–45

[91] Demirjian S, Paschke K M, Wang X, Grove D, Heyka R J and Dweik R A 2017 Molecular breath analysis identifies the breathprint of renal failure *J. Breath Res.* **11** 062009

[92] Wang M H, Yuk-Fai Lau S, Chong K C, Kwok C, Lai M, Chung A H Y, Ho C S, Szeto C C and Chung-Ying Zee B 2017 Estimation of clinical parameters of chronic kidney disease by exhaled breath full-scan mass spectrometry data and iterative PCA with intensity screening algorithm *J. Breath Res.* **11** 036007

[93] Dryahina K, Smith D, Bortlík M, Machková N, Lukáš M and Španěl P 2018 Pentane and other volatile organic compounds, including carboxylic acids, in the exhaled breath of patients with Crohn's disease and ulcerative colitis *J. Breath Res.* **12** 016002

[94] Dryahina K, Španěl P, Pospíšilová V, Sovová K, Hrdlička L, MacHková N, Lukáš M and Smith D 2013 Quantification of pentane in exhaled breath, a potential biomarker of bowel disease, using selected ion flow tube mass spectrometry *Rapid Commun. Mass Spectrom.* **27** 1983–92

[95] Patel N, Alkhouri N, Eng K, Cikach F, Mahajan L, Yan C, Grove D, Rome E S, Lopez R and Dweik R A 2014 Metabolomic analysis of breath volatile organic compounds reveals unique breathprints in children with inflammatory bowel disease: A pilot study *Aliment. Pharmacol. Ther.* **40** 498–507

[96] Huang J, Kumar S, Abbassi-Ghadi N, Španěl P, Smith D and Hanna G B 2013 Selected ion flow tube mass spectrometry analysis of volatile metabolites in urine headspace for the profiling of gastro-esophageal cancer *Anal. Chem.* **85** 3409–16

[97] Rieder F, Kurada S, Grove D, Cikach F, Lopez R, Patel N, Singh A, Alkhouri N, Shen B, Brzezinski A, Baker M, Fiocchi C and Dweik R A 2016 A Distinct Colon-Derived Breath Metabolome is Associated with Inflammatory Bowel Disease, but not its Complications *Clin. Transl. Gastroenterol.* **7** e201

[98] Boshier P R, Cushnir J R, Mistry V, Knaggs A, Španěl P, Smith D and Hanna G B 2011 On-line, real time monitoring of exhaled trace gases by SIFT-MS in the perioperative setting: a feasibility study *Analyst* **136** 3233-3237

[99] Sovová K, Čepl J, Markoš A and Španěl P 2013 Real time monitoring of population dynamics in concurrent bacterial growth using SIFT-MS quantification of volatile metabolites *Analyst* **138** 4795

[100] Prince B J, Milligan D B and McEwan M J 2010 Application of selected ion flow tube mass spectrometry to real-time atmospheric monitoring *Rapid Commun. Mass Spectrom.* **24** 1763–9

[101] Anon What is Proton Transfer Reaction Mass Spectrometry (PTR-MS)? - TOFWERK 2018 https://www.tofwerk.com/proton-transfer-reaction-mass-spectrometry/ Accessed: 16.02.2018

[102] Blake R S, Monks P S and Ellis A M 2009 Proton-transfer reaction mass spectrometry *Chem. Rev.* **109** 861–96

[103] Winkler K, Herbig J and Kohl I 2013 Real-time metabolic monitoring with proton transfer reaction mass spectrometry *Journal Bres. Res.* **7** 036006

[104] Fernández R, Hara M E O, Holt A, Pemberton P, Shah T, Whitehouse T and Mayhew C A 2015 EBioMedicine Volatile Biomarkers in Breath Associated With Liver Cirrhosis — Comparisons of Pre- and Post-liver Transplant Breath Samples *EBIOM* **2** 1243–50

[105] Zou X, Lu Y, Xia L, Zhang Y, Li A, Wang H, Huang C, Shen C and Chu Y 2018 Detection of Volatile Organic Compounds in a Drop of Urine by Ultrasonic Nebulization Extraction Proton Transfer Reaction Mass Spectrometry *Anal. Chem.* **90** 2210-2215

[106] Aprea E, Cappellin L, Gasperi F, Morisco F, Lembo V, Rispo A, Tortora R, Vitaglione P, Caporaso N and Biasioli F 2014 Application of PTR-TOF-MS to investigate metabolites in exhaled breath of patients affected by coeliac disease under gluten free diet *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **966** 208–213

[107] Kistler M, Muntean A, Szymczak W, Rink N, Fuchs H, Gailus-Durner V, Wurst W, Hoeschen C, Klingenspor M, Hrabě De Angelis M and Rozman J 2016 Diet-induced and mono-genetic obesity alter volatile organic compound signature in mice *J. Breath Res.* **10** 016009

[108] Obermeier J, Trefz P, Happ J, Schubert J K, Staude H, Fischer D C and Miekisch W 2017 Exhaled volatile substances mirror clinical conditions in pediatric chronic kidney disease *PLoS One* **12** 1–18

[109] What is High-Field Asymmetric Waveform Ion Mobility Spectrometry? http://www.faims.com/what.htm Accessed: 28.01.2019

[110] Guevremont R and Purves R W 1999 Atmospheric pressure ion focusing in a high-field asymmetric waveform ion mobility spectrometer *Rev. Sci. Instrum.* **70** 1370–83

[111] Rutolo M, Covington J, Clarkson J and Iliescu D 2014 Detection of Potato Storage Disease via Gas Analysis: A Pilot Study Using Field Asymmetric Ion Mobility Spectrometry *Sensors* **14** 15939–52

[112] Field Asymmetric Ion Mobility Spectrometry | LMIS1 https://lmis1.epfl.ch/page-134527.htm Accessed: 28.01.2019

[113] Arasaradnam R P, Westenbrink E, McFarlane M J, Harbord R, Chambers S, O'Connell N, Bailey C, Nwokolo C U, Bardhan K D, Savage R and Covington J A 2014 Differentiating Coeliac Disease from Irritable Bowel Syndrome by Urinary Volatile Organic Compound Analysis – A Pilot Study ed J Green *PLoS One* **9** e107312

[114] Arasaradnam R P, McFarlane M, Daulton E, Skinner J, O'Connell N, Wurie S, Chambers S, Nwokolo C, Bardhan K, Savage R and Covington J 2016 Non-invasive exhaled volatile organic biomarker analysis to detect inflammatory bowel disease (IBD) *Dig. Liver Dis.* **48** 148–53

[115] Bomers M K, Menke F P, Savage R S, Vandenbroucke- Grauls C M J E, van Agtmael M A, Covington J A and Smulders Y M 2015 Rapid, Accurate and On-Site Detection of C. difficile in Stool Samples *Am. J. Gastroenterol.* **110** 588–94

[116] Pavlou A K, Magan N, Jones J M, Brown J, Klatser P and Turner A P F 2004 Detection of Mycobacterium tuberculosis (TB) in vitro and in situ using an

electronic nose in combination with a neural network system *Biosens. Bioelectron.* **20** 538–44

[117] Lang H P, Ramseyer J P, Grange W, Braun T, Schmid D, Hunziker P, Jung C, Hegner M and Gerber C 2007 An Artificial Nose Based on Microcantilever Array Sensors *J. Phys. Conf. Ser.* **61** 663–7

[118] Shekhawat G and Dravid V P 2007 Microelectronics meets biomedicine: Integrated MOSFET-embedded cantilever sensor and diagnostics system *2007 International Workshop on Physics of Semiconductor Devices* (IEEE) pp 693–4

[119] Rao B B 2000 Zinc oxide ceramic semi-conductor gas sensor for ethanol vapour *Mater. Chem. Phys.* **64** 62–5

[120] Shepherd R L, Yerazunis W S, Lau K T and Diamond D 2006 Low-Cost Surface-Mount LED Gas Sensor *IEEE Sens. J.* **6** 861–6

[121] Hou, N., Jin, Z., Sun, B., Sun, Y., Shen, W., Guo, Z., Kong, L., Li, M. and Meng, F. 2015 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*, ***15*** 3668-3674.

[122] García-González D L and Aparicio R 2010 Coupling MOS sensors and gas chromatography to interpret the sensor responses to complex food aroma: Application to virgin olive oil *Food Chem.* **120** 572–9

[123] Dimbat M, Porter PE, Stross FH. 1956 Apparatus Requirements for Quantitative Applications. *Anal. Chem*. **28** 290-7.

[124] Povarov V G and Lopatnikov A I 2016 A simple multisensor detector based on tin dioxide in capillary gas chromatography *J. Anal. Chem.* **71** 901–6

[125] Tzeng, T.H., Kuo, C.Y., Wang, S.Y., Huang, P.K., Huang, Y.M., Hsieh, W.C., Huang, Y.J., Kuo, P.H., Yu, S.A., Lee, S.C. and Tseng, Y.J. 2015 A portable micro gas chromatography system for lung cancer associated volatile organic compound detection. *IEEE Journal of Solid-State Circuits*, **51** 259-272.

[126] Shepherd S F, McGuire N D, de Lacy Costello B P J, Ewen R J, Jayasena D H, Vaughan K, Ahmed I, Probert C S and Ratcliffe N M 2014 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 *J. Breath Res.* **8** 026001

[127] Ahmed I, Greenwood R, Costello B, Ratcliffe N and Probert C S 2016 Investigation of faecal volatile organic metabolites as novel diagnostic biomarkers in inflammatory bowel disease *Aliment. Pharmacol. Ther.* **43** 596–611

[128] Khalid T, Aggio R, White P, De Lacy Costello B, Persad R, Al-Kateb H, Jones P, Probert C S and Ratcliffe N 2015 Urinary Volatile Organic Compounds for the Detection of Prostate Cancer ed F L Chan *PLoS One* **10** e0143283

[129] Aggio R B M, de Lacy Costello B, White P, Khalid T, Ratcliffe N M, Persad R and Probert C S J 2016 The use of a gas chromatography-sensor system combined with advanced statistical methods, towards the diagnosis of urological malignancies *J. Breath Res.* **10** 017106

[130] Feng T, Zhuang H, Ye R, Jin Z, Xu X and Xie Z 2011 Analysis of volatile compounds of Mesona Blumes gum/rice extrudates via GC–MS and electronic nose *Sensors and Actuators B: Chemical* **160** 964–73

[131] Park M H, Jeong M K, Yeo J, Son H-J, Lim C-L, Hong E J, Noh B-S and Lee J 2011 Application of Solid Phase-Microextraction (SPME) and Electronic Nose Techniques to Differentiate Volatiles of Sesame Oils Prepared with Diverse Roasting Conditions *J. Food Sci.* **76** C80–8

[132] Messina V, Sancho A and Walsöe de Reca N 2015 Monitoring odour of heated extra-virgin olive oils from Arbequina and Manzanilla cultivars using an electronic nose *Eur. J. Lipid Sci. Technol.* **117** 1295–300

[133] Tahri K, Tiebe C, Bougrini M, Saidi T, El Alami El Hassani N, El Bari N, Hübert T and Bouchikhi B 2015 Characterization and discrimination of saffron by

multisensory systems, SPME-GC-MS and UV-Vis spectrophotometry *Anal. Methods* **7** 10328–38

[134] Brudzewski K, Osowski S and Pawlowski W 2012 Metal oxide sensor arrays for detection of explosives at sub-parts-per million concentration levels by the differential electronic nose *Sensors and Actuators B: Chemical* **161** 528–33

[135] Giungato P, de Gennaro G, Barbieri P, Briguglio S, Amodio M, de Gennaro L and Lasigna F 2016 Improving recognition of odors in a waste management plant by using electronic noses with different technologies, gas chromatography–mass spectrometry/olfactometry and dynamic olfactometry *J. Clean. Prod.* **133** 1395–402

[136] Sklorz A, Janßen S and Lang W 2013 Application of a miniaturised packed gas chromatography column and a SnO2 gas detector for analysis of low molecular weight hydrocarbons with focus on ethylene detection *Sensors and Actuators B: Chemical* **180** 43–9

[137] Zampolli S, Elmi I, Mancarella F, Betti P, Dalcanale E, Cardinali G C and Severi M 2009 Real-time monitoring of sub-ppb concentrations of aromatic volatiles with a MEMS-enabled miniaturized gas-chromatograph *Sensors and Actuators B: Chemical* **141** 322–8

[138] Sanchez J-B, Berger F, Daniau W, Blind P, Fromm M and Nadal M-H 2006 Development of a gas detection micro-device for hydrogen fluoride vapours *Sensors and Actuators B: Chemical* **113** 1017–24

[139] Sanchez J-B, Berger F, Fromm M and Nadal M-H 2006 A selective gas detection micro-device for monitoring the volatile organic compounds pollution *Sensors and Actuators B: Chemical.* **119** 227–33

[140] Keiichi Ohta *,†, Hisayoshi Terai †, Ikuhiko Kimura ‡ and and Tanaka§ K 1999 Simultaneous Determination of Hydrogen, Methane, and Carbon Monoxide in Water by Gas Chromatography with a Semiconductor Detector *Analytical Chemistry* **71** 2697-2699

[141] Kohl D, Heinert L, Bock J, Hofmann T and Schieberle P 2000 Systematic studies on responses of metal-oxide sensor surfaces to straight chain alkanes, alcohols, aldehydes, ketones, acids and esters using the SOMMSA approach *Sensors and Actuators B: Chemical* **70** 43–50

[142] Nishizaka Y, Yokoyama C, Inumaru K, Okuhara T and Misono M 1993 Effect of additives and particle size on the sensitivity of SnO2-based sensors for offensive-odor components *Sensors and Actuators B Chemical* **13** 355–7

[143] Mildner-Szkudlarz S and Jeleń H H 2008 The potential of different techniques for volatile compounds analysis coupled with PCA for the detection of the adulteration of olive oil with hazelnut oil *Food Chem.* **110** 751–61

[144] Meng H, Yang W, Yan X, Zhang Y, Feng L and Guan Y 2015 A highly sensitive and fast responsive semiconductor metal oxide detector based on In2O3 nanoparticle film for portable gas chromatograph *Sensors and Actuators B: Chemical* **216** 511–7

[145] Sanchez J-B, Berger F, Fromm M and Nadal M-H 2005 Use of a chromatographic column to improve the selectivity of the SnO2 gas sensors: first approach towards a miniaturised device and selective with hydrogen fluoride vapours *Sensors and Actuators B Chem.* **106** 823–31

[146] Vaughan K 2013 An investigation into the fabrication of a new mini gas sensor for medical diagnostic applications Diss. University of the West of England https://uwe-repository.worktribe.com/output/929556

[147] de Lacy Costello B P ., Ewen R ., Jones P R ., Ratcliffe N . and Wat R K . 1999 A study of the catalytic and vapour-sensing properties of zinc oxide and tin dioxide in relation to 1-butanol and dimethyldisulphide *Sensors and Actuators B Chemical.* **61** 199–207

[148] de Lacy Costello B P J, Ewen R J, Ratcliffe N M and Sivanand P S 2003 Thick film organic vapour sensors based on binary mixtures of metal oxides *Sensors and Actuators B: Chemical* **92** 159–66

[149] De B, Costello L, Amann A, Al-Kateb H, Flynn C, Filipiak W, Khalid T, Osborne D and Ratcliffe N M 2014 A review of the volatiles from the healthy human body *J. Breath Res. J. Breath Res* **8** 14001–29

[150] Sun P, Ayre C and Wallace M 2003 Characterization of Organic Contaminants Outgassed from Materials Used in Semiconductor Fabs/Processing *AIP Conference Proceedings* vol 683 (AIP) pp 245–53

[151] Tait E, Perry J D, Stanforth S P and Dean J R 2014 Identification of Volatile Organic Compounds Produced by Bacteria Using HS-SPME-GC–MS *J. Chromatogr. Sci.* **52** 363–73

[152] Siripatrawan U 2008 Rapid differentiation between E. coli and Salmonella Typhimurium using metal oxide sensors integrated with pattern recognition *Sensors and Actuators B: Chemical.* **133** 414–9

[153] Arnold J W and Senter S D 1998 Use of digital aroma technology and SPME GC-MS to compare volatile compounds produced by bacteria isolated from processed poultry *J. Sci. Food Agric.* **78** 343–8

[154] Hettinga K A, van Valenberg H J F, Lam T J G M and van Hooijdonk A C M 2008 Detection of Mastitis Pathogens by Analysis of Volatile Bacterial Metabolites *J. Dairy Sci.* **91** 3834–9

[155] Hossain S M Z, Bojko B and Pawliszyn J 2013 Automated SPME–GC–MS monitoring of headspace metabolomic responses of E. coli to biologically active components extracted by the coating *Anal. Chim. Acta* **776** 41–9

[156] Storer M K, Hibbard-Melles K, Davis B and Scotter J 2011 Detection of volatile compounds produced by microbial growth in urine by selected ion flow tube mass spectrometry (SIFT-MS) *J. Microbiol. Methods* **87** 111–3

[157] Allardyce R A, Langford V S, Hill A L and Murdoch D R 2006 Detection of

volatile metabolites produced by bacterial growth in blood culture media by selected ion flow tube mass spectrometry (SIFT-MS) *J. Microbiol. Methods* **65** 361–5

[158] Thorn R M S, Reynolds D M and Greenman J 2011 Multivariate analysis of bacterial volatile compound profiles for discrimination between selected species and strains in vitro *J. Microbiol. Methods* **84** 258–64

[159] Allardyce R A, Hill A L and Murdoch D R 2006 The rapid evaluation of bacterial growth and antibiotic susceptibility in blood cultures by selected ion flow tube mass spectrometry *Diagn. Microbiol. Infect. Dis.* **55** 255–61

[160] Concina I, Falasconi M, Gobbi E, Bianchi F, Musci M, Mattarozzi M, Pardo M, Mangia A, Careri M and Sberveglieri G 2009 Early detection of microbial contamination in processed tomatoes by electronic nose *Food Control* **20** 873–80

[161] Bunge M, Araghipour N, Mikoviny T, Dunkl J, Schnitzhofer R, Hansel A, Schinner F, Wisthaler A, Margesin R and Märk T D 2008 On-line monitoring of microbial volatile metabolites by proton transfer reaction-mass spectrometry. *Appl. Environ. Microbiol.* **74** 2179–86

[162] Maddula S, Blank L M, Schmid A and Baumbach J I 2009 Detection of volatile metabolites of Escherichia coli by multi capillary column coupled ion mobility spectrometry *Anal. Bioanal. Chem.* **394** 791–800

[163] Zscheppank C, Wiegand H L, Lenzen C, Wingender J and Telgheder U 2014 Investigation of volatile metabolites during growth of Escherichia coli and Pseudomonas aeruginosa by needle trap-GC-MS *Anal. Bioanal. Chem.* **406** 6617–28

[164] Umber B J, Shin H-W, Meinardi S, Leu S-Y, Zaldivar F, Cooper D M and Blake D R 2013 Gas signatures from Escherichia coli and Escherichia coli-inoculated human whole blood *Clin. Transl. Med.* **2** 13

[165] Schulz S and Dickschat J S 2007 Bacterial volatiles: the smell of small organisms *Nat. Prod. Rep.* **24** 814

[166] Chippendale T W E, Španěl P and Smith D 2011 Time-resolved selected ion flow tube mass spectrometric quantification of the volatile compounds generated by E. coli JM109 cultured in two different media *Rapid Commun. Mass Spectrom.* **25** 2163–72

[167] Aathithan S, Plant J C, Chaudry A N and French G L 2001 Diagnosis of bacteriuria by detection of volatile organic compounds in urine using an automated headspace analyzer with multiple conducting polymer sensors. *J. Clin. Microbiol.* **39** 2590–3

[168] Zhu J, Bean H D, Kuo Y-M and Hill J E 2011 Fast Detection of Volatile Organic Compounds from Bacterial Cultures by Secondary Electrospray Ionization-Mass Spectrometry *J. Clin. Microbiol.* **49** 769–769

[169] Boots A W, Smolinska A, van Berkel J J B N, Fijten R R R, Stobberingh E E, Boumans M L L, Moonen E J, Wouters E F M, Dallinga J W and Van Schooten F J 2014 Identification of microorganisms based on headspace analysis of volatile organic compounds by gas chromatography–mass spectrometry *J. Breath Res.* **8** 027106

[170] Keshun Yu †, Thomas R. Hamilton-Kemp *,†, Douglas D. Archbold †, Randall W. Collins † and and Newman‡ M C 2000 Volatile Compounds from Escherichia coli O157:H7 and Their Absorption by Strawberry Fruit *Journal of agricultrural and food chemistry* **48** 413-417

[171] Garner C E, Smith S, Bardhan P K, Ratcliffe N M and Probert C S J 2009 A pilot study of faecal volatile organic compounds in faeces from cholera patients in Bangladesh to determine their utility in disease diagnosis *Trans. R. Soc. Trop. Med. Hyg.* **103** 1171–3

[172] Probert C S, Reade S and Ahmed I 2014 Fecal volatile organic compounds: a novel, cheaper method of diagnosing inflammatory bowel disease? *Expert Rev. Clin. Immunol.* **10** 1129–31

[173] Ahmed I, Greenwood R, Costello B de L, Ratcliffe N M and Probert C S 2013 An Investigation of Fecal Volatile Organic Metabolites in Irritable Bowel Syndrome ed D L Boone *PLoS One* **8** e58204

[174] Zhang Y-J, Li S, Gan R-Y, Zhou T, Xu D-P and Li H-B 2015 Impacts of Gut Bacteria on Human Health and Diseases *Int. J. Mol. Sci.* **16** 7493–519

[175] Flint H J, Duncan S H, Scott K P and Louis P 2015 Links between diet, gut microbiota composition and gut metabolism *Proc. Nutr. Soc.* **74** 13–22

[176] Geypens B, Claus D, Evenepoel P, Hiele M, Maes B, Peeters M, Rutgeerts P and Ghoos Y 1997 Influence of dietary protein supplements on the formation of bacterial metabolites in the colon. *Gut* **41** 70–6

[177] Silvester K R, Bingham S A, Pollock J R A, Cummings J H and Neill I K O ' 2009 Effect of meat and resistant starch on fecal excretion of apparent N-nitroso compounds and ammonia from the human large bowel Effect of Meat and Resistant Starch on Fecal Excretion of Apparent N-Nitroso Compounds and Ammonia From the Human Large Bowel *Nutr. Cancer* **29** 13–23

[178] Sethi S, Nanda R and Chakraborty T 2013 Clinical application of volatile organic compound analysis for detecting infectious diseases. *Clin. Microbiol. Rev.* **26** 462–75

[179] Lin J, Aoll J, Niclass Y, Velazco M I, Wünsche L, Pika J and Starkenmann C 2013 Qualitative and Quantitative Analysis of Volatile Constituents from Latrines *Environ. Sci. Technol.* **47** 7876–82

[180] Walton C, Fowler D P, Turner C, Jia W, Whitehead R N, Griffiths L, Dawson C, Waring R H, Ramsden D B, Cole J A, Cauchi M, Bessant C and Hunter J O 2013 Analysis of volatile organic compounds of bacterial origin in chronic gastrointestinal diseases *Inflamm. Bowel Dis.* **19** 2069–78

[181] Wang L, Christophersen C T, Sorich M J, Gerber J P, Angley M T and Conlon M A 2012 Elevated Fecal Short Chain Fatty Acid and Ammonia Concentrations in Children with Autism Spectrum Disorder *Dig. Dis. Sci.* **57** 2096–102

[182] De Preter V, Staeyen V, Esser D, Rutgeerts P and Verbeke K 2009 Development of a screening method to determine the pattern of fermentation metabolites in faecal samples using on-line purge-and-trap gas chromatographic–mass spectrometric analysis *J. Chromatogr. A* 1216–476

[183] Shreiner A B, Kao J Y and Young V B 2015 The gut microbiome in health and in disease. *Curr. Opin. Gastroenterol.* **31** 69–75

[184] Wang C, Shoji H, Sato H, Nagata S, Ohtsuka Y, Shimizu T and Yamashiro Y 2007 Effects of Oral Administration of Bifidobacterium breve on Fecal Lactic Acid and Short-chain Fatty Acids in Low Birth Weight Infants *J. Pediatr. Gastroenterol. Nutr.* **44** 252–7

[185] Primec M, Mičetić-Turk D and Langerholc T 2017 Analysis of short-chain fatty acids in human feces: A scoping review *Anal. Biochem.* **526** 9–21

[186] Fordtran J 1971 Organic anions in fecal contents *N. Engl. J. Med.* **284** 329-330

[187] Rodríguez-Carrio J, Salazar N, Margolles A, González S, Gueimonde M, de los Reyes-Gavilán C G and Suárez A 2017 Free Fatty Acids Profiles Are Related to Gut Microbiota Signatures and Short-Chain Fatty Acids *Front. Immunol.* **8** 823

[188] Simenhoff M L, Burke J F, Saukkonen J J, Ordinario A T, Doty R and Dunn S 1977 Biochemical Profile of Uremic Breath *N. Engl. J. Med.* **297** 132–5

[189] Davies S, Spanel P and Smith D 1997 Quantitative analysis of ammonia on the breath of patients in end-stage renal failure *Kidney Int.* **52** 223–8

[190] Ong J P, Aggarwal A, Krieger D, Easley K A, Karafa M T, Lente F Van, Arroliga A C and Mullen K D 2003 Correlation between Ammonia Levels and the Severity of Hepatic Encephalopathy *American Journal of Medicine* **114** 188-

193

[191] de Lacy Costello B, Amann A, Al-Kateb H, Flynn C, Filipiak W, Khalid T, Osborne D and Ratcliffe N M 2014 A review of the volatiles from the healthy human body *J. Breath Res.* **8** 014001

[192] De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet J B, Massart S, Collini S, Pieraccini G and Lionetti P 2010 Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl. Acad. Sci. U. S. A.* **107** 14691–6

[193] Kwok L, Zhang J, Guo Z, Gesudu Q, Zheng Y, Qiao J, Huo D and Zhang H 2014 Characterization of Fecal Microbiota across Seven Chinese Ethnic Groups by Quantitative Polymerase Chain Reaction ed S Bereswill *PLoS One* **9** e93631

[194] Mueller S, Saunier K, Hanisch C, Norin E, Alm L, Midtvedt T, Cresci A, Silvi S, Orpianesi C, Verdenelli M C, Clavel T, Koebnick C, Zunft H-J F, Doré J and Blaut M 2006 Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. *Appl. Environ. Microbiol.* **72** 1027–33

[195] Kabeerdoss J, Shobana Devi R, Regina Mary R and Ramakrishna B S 2012 Faecal microbiota composition in vegetarians: comparison with omnivores in a cohort of young women in southern India *Br. J. Nutr.* **108** 953–7

[196] van Faassen A, Hazen M J, van den Brandt P A, van den Bogaard A E, Hermus R J and Janknegt R A 1993 Bile acids and pH values in total feces and in fecal water from habitually omnivorous and vegetarian subjects *Am. J. Clin. Nutr.* **58** 917–22

[197] Ferrocino I, Di Cagno R, De Angelis M, Turroni S, Vannini L, Bancalari E, Rantsiou K, Cardinali G, Neviani E and Cocolin L 2015 Fecal Microbiota in Healthy Subjects Following Omnivore, Vegetarian and Vegan Diets: Culturable Populations and rRNA DGGE Profiling ed M M Heimesaat *PLoS One* **10** e0128669

[198] Falasconi M, Pardo M, Sberveglieri G, Battistutta F, Piloni M and Zironi R 2005 Study of white truffle aging with SPME-GC-MS and the Pico2-electronic nose *Sensors and Actuators B: Chemical* **106** 88–94

[199] Wernig F, Buegger F, Pritsch K and Splivallo R 2018 Composition and authentication of commercial and home-made white truffle-flavored oils *Food Control* **87** 9–16

[200] National Institute of Standards and Technology Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1α,2β,5α)- https://webbook.nist.gov/cgi/cbook.cgi?ID=C15356704 Accessed:17.10.2018

[201] Masood N, Chaudhry A and Tariq P 2008 In vitro antibacterial activities of kalonji, cumin and poppy seed *Pakistan Journal of Botany* **40** 461

[202] Methyl isopropyl ketone | C5H10O - PubChem https://pubchem.ncbi.nlm.nih.gov/compound/11251 Accessed: 24.10.2018

[203] Bianchi F, Careri M, Mangia A and Musci M 2007 Retention indices in the analysis of food aroma volatile compounds in temperature-programmed gas chromatography: Database creation and evaluation of precision and robustness *J. Sep. Sci.* **30** 563–72

[204] Peng C T, Yang Z C and Ding S F 1991 Prediction of retention indexes: II. Structure-retention index relationship on polar columns *J. Chromatogr. A* **586** 85–112

[205] Schlüter, S., Steinhart, H., Schwarz, F.J. and Kirchgessner, M., 1999. Changes in the odorants of boiled carp fillet (Cyprinus carpio L.) as affected by increasing methionine levels in feed. *Journal of agricultural and food*

chemistry, **47** 5146-5150.

[206] Babushok V I, Linstrom P J and Zenkevich I G 2011 Retention Indices for Frequently Reported Compounds of Plant Essential Oils *J. Phys. Chem. Ref. Data* **40** 043101

[207] Liu J-L, Yan C-W, Li Y-T, Wu Z-Y and Zhang W-J 2008 Diacetonitrile-1κ *N* ,3κ *N* -bis{μ- *trans* - *N* -[3-(dimethylamino)propyl]- *N* ′-(2-hydroxyethyl)oxamidato(2–)}-1:2κ ^{5} *N* , *N* ′, *O* : *O* ′, *N* ″;2:3κ ^{5} O′, *N* ″: *N* , *N* ′, *O* -dithiocyanato-1κ *N* ,3κ *N* -tricopper(II) *Acta Crystallogr. Sect. C Cryst. Struct. Commun.* **64** m149–52

[208] Diabetes Prevalence 2017 (November 2017) | Diabetes UK https://www.diabetes.org.uk/professionals/position-statements-reports/statistics/diabetes-prevalence-2017 Accessed: 08.02.2019

[209] Facts and Figures | Diabetes UK https://www.diabetes.org.uk/professionals/position-statements-reports/statistics Accessed: 08.02.2019

[210] Sen C K, Gordillo G M, Roy S, Kirsner R, Lambert L, Hunt T K, Gottrup F, Gurtner G C and Longaker M T 2009 Human skin wounds: a major and snowballing threat to public health and the economy. *Wound Repair Regen.* **17** 763–71

[211] Falanga V 2005 Wound healing and its impairment in the diabetic foot *Lancet* **366** 1736–43

[212] Hardwick J B, Tucker A T, Wilks M, Johnston A and Benjamin N 2001 A novel method for the delivery of nitric oxide therapy to the skin of human subjects using a semi-permeable membrane. *Clin. Sci. (Lond).* **100** 395–400

[213] Kruse I and Edelman S 2006 Evaluation and Treatment of Diabetic Foot Ulcers *Clin. Diabetes* **24**

[214] Edmonds M E, Bodansky H J, Boulton A J M, Chadwick P J, Dang C N, D'Costa R, Johnston A, Kennon B, Leese G, Rajbhandari S M, Serena T E, Young M J, Stewart J E, Tucker A T and Carter M J 2018 Multicenter, randomized controlled, observer-blinded study of a nitric oxide generating treatment in foot ulcers of patients with diabetes-ProNOx1 study *Wound Repair Regen.* **26** 228–37

[215] Opländer C, Römer A, Paunel-Görgülü A, Fritsch T, Van Faassen E E, Mürtz M, Bozkurt A, Grieb G, Fuchs P, Pallua N and Suschek C V. 2012 Dermal application of nitric oxide in vivo: Kinetics, biological responses, and therapeutic potential in humans *Clin. Pharmacol. Ther.* **91** 1074–82

[216] Kamoun E A, Chen X, Mohy Eldin M S, Kenawy E-R S and Journal A 2015 Crosslinked poly(vinyl alcohol) hydrogels for wound dressing applications: A review of remarkably blended polymers Production and hosting by Elsevier *Arab. J. Chem.* **8** 1–14

[217] Yang X, Yang K, Wu S, Chen X, Yu F, Li J, Ma M and Zhu Z 2010 Cytotoxicity and wound healing properties of PVA/ws-chitosan/glycerol hydrogels made by irradiation followed by freeze–thawing *Radiat. Phys. Chem.* **79** 606–11

[218] Park J-Y and Lee Y-N 1988 Solubility and Decomposition Kinetics of Nitrous Acid in Aqueous Solution *Journal of Physical Chemistry* **92** 6294-6302

[219] Dunlea E J, Herndon S C, Nelson D D, Volkamer R M, San Martini F, Sheehy P M, Zahniser M S, Shorter J H, Wormhoudt J C, Lamb B K, Allwine E J, Gaffney J S, Marley N A, Grutter M, Marquez C, Blanco S, Cardenas B, Retama A, Ramos Villegas C R, Kolb C E, Molina L T and Molina M J 2007 Evaluation of nitrogen dioxide chemiluminescence monitors in a polluted urban environment *Atmos. Chem. Phys.* **7** 2691–704

[220] Spanel P and Smith D 2000 An investigation of the reactions of H_3O^+ and O^{2+} with NO, NO_2, N_2O and HNO_2 in support of selected ion flow tube mass spectrometry *Rapid Commun. Mass Spectrom.* **14** 646–51

[221] Eklund B 1992 Practical guidance for flux chamber measurements of fugitive volatile organic emission rates *J. Air Waste Manag. Assoc.* **42** 1583–91

[222] Marty B. Rhoades M B, David B. Parker D B, Brent Auvermann B, N. Andy Cole N A, Zena Perscbacher-Buser Z, Robert E. DeOtte R E and Jr. 2005 Factors Affecting Emission Measurements with Surface Isolation Flux Chambers *2005 Tampa, FL July 17-20, 2005* (St. Joseph, MI: American Society of Agricultural and Biological Engineers) p 1

[223] Buffer-Reference-Center @ Www.Sigmaaldrich.Com http://www.sigmaaldrich.com/life-science/core-bioreagents/biological-buffers/learning-center/buffer-reference-center.html/citric

[224] Weller R, Price R J, Ormerod A D, Benjamin N and Leifert C 2001 Antimicrobial effect of acidified nitrite on dermatophyte fungi, Candida and bacterial skin pathogens *J. Appl. Microbiol.* **90** 648–52

[225] Zacharia I G and Deen W M 2005 Diffusivity and solubility of nitric oxide in water and saline. *Ann. Biomed. Eng.* **33** 214–22

[226] Fite A, Dykhuizen R, Litterick A, Golden M and Leifert C 2004 Effects of Ascorbic Acid, Glutathione, Thiocyanate, and Iodide on Antimicrobial Activity of Acidified Nitrite *Antimicrob. Agents Chemother.* **48** 655–8

[227] Hobizal K B and Wukich D K 2012 Diabetic foot infections: current concept review. *Diabet. Foot Ankle* **3** 18409

[228] Anon Viscosity of Water – viscosity table and viscosity chart :: Anton Paar Wiki https://wiki.anton-paar.com/en/water/ Accessed 01.03.2019

[229] Calculate density and viscosity of glycerol/water mixtures http://www.met.reading.ac.uk/~sws04cdw/viscosity_calc.html Accessed: 01.03.2019

[230] Ashland Speciality Ingredients 2011 *Product Data Natrosol 250* https://www.brenntag.com/media/documents/bsi/product_data_sheets/material_science/ashland_cellulose_rheology_modifiers/natrosol_250_pds.pdf

Accessed:01.03.2019

[231] Ninni L, Camargo M S and Meirelles A J A 2000 Water activity in polyol systems *J. Chem. Eng. Data* **45** 654–60

[232] Hughes E D, Ingold C K and Kidd J H 1958. Nitrosation, Diazotisation, and Deamination. Part VI Com-parative Discussion of Mechanisms of N-and 0-Nitrosation with 8pecial Reference to Diazotisation *Journal of the Chemical Society* **0**

[233] Alowitz M J and Scherer M M 2002 Kinetics of nitrate, nitrite, and Cr(vi) reduction by iron metal *Environ. Sci. Technol.* **36** 299–306

[234] Summers D P and Chang S 1993 Prebiotic ammonia from reduction of nitrite by iron (II) on the early Earth *Nature* **365** 630–3

[235] Kampschreur M J, Kleerebezem R, de Vet W W J M and Van Loosdrecht M C M 2011 Reduced iron induced nitric oxide and nitrous oxide emission *Water Res.* **45** 5945–52

[236] Han D, Wan J, Ma Y, Wang Y, Li Y, Li D and Guan Z 2015 New insights into the role of organic chelating agents in Fe(II) activated persulfate processes *Chem. Eng. J.* **269** 425–33

[237] Blunt J 2001 Wound cleansing: Ritualistic or research-based practice? *Nursing Standard* **16** 33-36

[238] Derby A 1978 Wound healing in tadpole tailfin pieces in vitro *J. Exp. Zool.* **205** 277–83

[239] Jones V, Grey J E and Harding K G 2006 Wound dressings. *BMJ* **332** 777–80

[240] Rizk M, Witte M B and Barbul A 2004 Nitric Oxide and Wound Healing *World J. Surg.* **28** 301–6

[241] Woolford G, Cassens R G, Greaser M L and Sebranek J G 1976 The fate of nitrite: reaction with protein *J. Food Sci.* **41** 585–8

[242] Kharitonov V G, Sundquist A R and Sharma V S 1995 Kinetics of Nitrosation

of Thiols by Nitric Oxide in the Presence of Oxygen *Journal of Biological Chemistry* **270** 28158-28164

[243] Ewing J F, Young D V, Janero D R, Garvey D S and Grinnell T A 1997 Nitrosylated Bovine Serum Albumin Derivatives as Pharmacologically Active Nitric Oxide Congeners *J. Pharmacol. Exp. Ther.* **283** 947–54

[244] Pietraforte D, Castelli M, Metere A, Scorza G, Samoggia P, Menditto A and Minetti M 2006 Salivary uric acid at the acidic pH of the stomach is the principal defense against nitrite-derived reactive species: Sparing effects of chlorogenic acid and serum albumin *Free Radic. Biol. Med.* **41** 1753–63

[245] Vallance P and Charles I 1998 Nitric oxide as an antimicrobial agent: Does NO always mean NO? *Gut* **42** 313–4

[246] Klebanoff S J 1993 Reactive nitrogen intermediates and antimicrobial activity: Role of nitrite *Free Radic. Biol. Med.* **14** 351–60

[247] Pielesz A and Bąk M K K 2008 Raman spectroscopy and WAXS method as a tool for analysing ion-exchange properties of alginate hydrogels *Int. J. Biol. Macromol.* **43** 438–43

[248] Waal D de, Staden J F van and Makhafola M A 1996 Kinetic Study of the Decomposition of Nitrite to Nitrate in Acid Samples Using Raman Spectroscopy *Appl. Spectrosc. Vol. 50, Issue 8, pp. 991-994* **50** 991–4

[249] Cheng C F and Tsang C W 1998 Simultaneous determination of nitrite, nitrate and ascorbic acid in canned vegetable juices by reverse-phase ion-interaction HPLC *Food Addit. Contam.* **15** 753–8

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ProductInformation

Basal Salt Mixtures

The use of a balanced salt solution (BSS) in tissue culture is generally attributed to early workers in the field. In 1885, Sydney Ringer developed a solution of inorganic salts designed to maintain contractility of mammalian heart tissue. A less specific salt solution was designed by Tyrode for use in work with mammalian cells in general. Tyrode's salt solution became the accepted fluid for diluting protein components of media of natural origin. Since that time, many other balanced salt solutions have been developed for use in tissue culture. The current role of a balanced salt solution in cell culture is multifaceted and can be divided into four principal functions:

- serves as an irrigating, transporting and diluting fluid while maintaining intra- and extracellular osmotic balance;
- provides cells with water and certain bulk inorganic ions essential for normal cell metabolism;
- combined with a carbohydrate, such as glucose, provides the principal energy source for cell metabolism;
- provides a buffering system to maintain the medium within the physiological pH range (7.2-7.6).

HANKS' BALANCED SALTS

COMPONENT	H 6136 H 6393 g/L	H 9269 H 2513 [1X] g/L	H 1387 g/L	H 8264 [1X] g/L	H 2387 g/L
INORGANIC SALTS					
$CaCl_2 \cdot 2H_2O$	0.185	0.185	0.185	0.185	—
$MgSO_4$ (anhyd)	0.09767	0.09767	0.09767	0.09767	—
KCl	0.4	0.4	0.4	0.4	0.4
KH_2PO_4 (anhyd)	0.06	0.06	0.06	0.06	0.06
$NaHCO_3$	—	0.35	—	0.35	—
NaCl	8.0	8.0	8.0	8.0	8.0
Na_2HPO_4 (anhydrous)	0.04788	0.04788	0.04788	0.04788	0.04788
OTHER					
D-Glucose	1.0	1.0	1.0	1.0	1.0
Phenol Red•Na	0.011	0.011	—	—	0.011
ADD					
$NaHCO_3$	0.35	—	0.35	—	0.35
Grams of powder required to prepare 1 L	9.8	N/A	9.8	N/A	9.5

COMPONENT	H 9394 H 8389 [1X] g/L	H 4891 g/L	H 1641 [10X] g/L	H 4641 [10X] g/L	H 6648 [1X] g/L	H 5899 [1X] g/L
INORGANIC SALTS						
$CaCl_2 \cdot 2H_2O$	—	—	1.85	—	—	0.1855
$MgSO_4$ (anhyd)	—	—	0.9767	—	—	0.09767
KCl	0.4	0.4	4.0	4.0	0.4	0.4
KH_2PO_4 (anhyd)	0.06	0.06	0.6	0.6	0.06	0.06
$NaHCO_3$	0.35	—	—	—	0.35	—
NaCl	8.0	8.0	80.0	80.0	8.0	8.0
Na_2HPO_4 (anhydrous)	0.04788	0.04788	0.4788	0.4788	0.04788	0.0475
OTHER						
D-Glucose	1.0	1.0	10.0	10.0	1.0	1.0
Phenol Red•Na	0.011	—	0.11	0.11	—	—
ADD						
$NaHCO_3$	—	0.35	0.35 at 1X	0.35 at 1X	—	0.35 at
Grams of powder required to prepare 1 L	N/A	9.5	N/A	N/A	N/A	N/A

REFERENCES

1. Hanks, J. (1976) Hanks' Balanced Salt Solution and pH Control. Tissue Culture Association Manual. 3, 3.