Norwegian University
of Life Sciences

Master's Thesis 2023 60 ECTS
Faculty of Chemistry, Biotechnology and Food Sciences

Optimising the Extraction and Quantification of Geosmin and 2-Methylisoborneol by Use of LLE, SPE and GC Orbitrap

Even Holm Hansen
Master's Degree in Chemistry

Acknowledgements

This thesis constitutes 60 ECTS as a partial fulfilment for a 120 ECTS Master's degree in Chemistry at the Faculty of Chemistry, Biotechnology and Food Science (KBM) at the Norwegian University of Life Sciences (NMBU).

I will forever treasure the incredible knowledge my supervisor Prof. Dag Ekeberg and co-supervisor Senior Engineer Hanne Marie Devle have shared with me, as well as their invaluable guidance throughout the work on my thesis. Additionally, I am grateful for the helpful feedback I have received from PhD candidate Rannei Skaali and research scientist Carl Fredrik Næss-Andresen.

To the wonderful people I got to know in my research group, Einar, Live and Mehdi, as well as the other Master's students on the first floor KBM labs: thank you all for a great time. I will blissfully remember our hilarious lunch breaks and cake Tuesdays. Wish you all the very best in the future. It will be as bright as you all!

To my dear friends, my joyful student choir Noe Ganske Annet (distracting me at necessary times) and of course everyone else dear to me in Norway, the UK and Australia: thank you so much for your endless support. You know who you are, albeit being in fishy soups or having endless minch.

I am forever grateful to my parents, for always reminding me that my best is enough. Thank you to my sister, my grandparents and my dearest niece and nephew for bringing me the sweet pearls of joy a 26-year old needs while balancing a fair bit of work and a full-time master's degree.

Even Holm Hansen

Ås, August 2023

Norwegian University of Life Sciences (NMBU)

Abstract

Water is an incredibly valuable resource to humans. Concerns for distasteful contaminants in water sources emerged in the 70's and has been observed to cause significant losses of water resources in recent years. Geosmin and 2-methylisoborneol are hydroxylated irregular sesquiterpenes and monoterpenes respectively and are widely recognised as two of the main compounds responsible for off-flavour and odour contamination in water. Humans possess incredibly low sensory thresholds for these compounds, ranging from a couple to around 50 ng/L. Because of these low sensory thresholds, these contaminants have gained worldwide attention, especially with regards to appropriate analytical methods to detect and quantify these contaminants. In this thesis, experiments were conducted in order to optimise aspects of extraction and quantification by liquid-liquid extraction (LLE) and solid-phase extraction (SPE), as well as GC Orbitrap. With respect to LLE, experiments involved an internal standard, solvent evaporation and microextractions. Factors including conditioning, optimisation of extraction, as well as quantification by use of internal standards and standard addition were investigated with regards to SPE. Temperature programming and split/splitless conditions were investigated with respect to GC Orbitrap. It was observed that these extraction methods in general did not perform well when compared to literature values, often including the use of other extraction techniques such as closed-loop stripping analysis and purge & trap, in addition to selected ion monitoring in mass spectrometry. Additionally, standard addition did not yield a reproducible method. However, novel improvements were made with the conditioning of SPE columns in order to extract and elute the analytes, increasing their quantification limits. A new internal standard candidate, 1-methylcyclohexanol, yet to be utilised in the literature, did exhibit promising response factors with geosmin in SPE analysis. Further research should include the application of more successful extraction methods from the literature with GC Orbitrap, as well as further investigations of 1-methylcyclohexanol as an internal standard candidate.

Sammendrag

Vann er en svært viktig ressurs for mennesker. Bekymringer for forurensninger i vannkilder som gir avsmak og luktproblemer på vannet oppstod spesielt på 70-tallet og har skap betydelige tap av vannressurser i nåværende tid. Geosmin og 2-metylisoborneol er henholdsvis hydroksylerte irregulære sesquiterpener og monoterpener og er godt kjent for å være hovedgrunnene til smak- og luktproblemer i vann. Mennesker har svært lave sansetersklер for disse stoffene, i litteraturen beskrevet som noen få til rundt 50 ng/L. Grunnet disse sansetersklene, har forbindelsene fått global oppmerksomhet, spesielt med hensyn til analytiske metoder til å detektere og kvantifisere disse stoffene. I denne oppgaven ble forsøk utført for å optimalisere ekstraksjon og kvantifisering via væske-væskeekstraksjon (LLE) og fast-faseekstraksjon (SPE) med GC Orbitrap. Med hensyn til LLE, lå fokuset rundt internstandard, avdamping av løsemiddel og mikroekstraksjoner. Aspekter som kondisjonering, optimalisering av ekstraksjon og kvantifisering med internstandard og standardaddisjon ble undersøkt med tanke på SPE. Temperaturprogrammering og split/splitless-forhold ble undersøkt med hensyn til GC Orbitrap. Det ble observert at ekstraksjonsmetodene generelt sett ikke sammenliknet tilfredsstillende med litteraturverdier, spesielt verdier oppnådd ved hjelp av andre ekstraksjonsmetoder som closed-loop stripping analysis og purge & trap, i tillegg til selected ion monitoring i massespektrometri. Standardaddisjon ga heller ikke reproduserbare resultater og kunne ikke brukes som en ny kvantifiseringsmetode. På den andre siden ble det utviklet nye forbedringer i kondisjoneringen av SPE-kolonner, hvor kvantifiseringsgrenser ble økt. I tillegg ble det observert at 1-metylsykloheksanol, som ikke er benyttet i litteraturen så langt, kan fungere som en ny internstandardkandidat som følge av lovende responsfaktorer i SPE (også observert med kamfer). Framtidig forskning bør inkludere mer vellykkede ekstraksjonsmetoder fra litteraturen med GC Orbitrap, i tillegg til videre undersøkelser med 1-metylsykloheksanol som internstandard.

Table of contents

LIST OF ABBREVIATIONS

BPC	Base peak chromatogram
CLSA	Closed-loop stripping analysis
DCM	Dichloromethane
EI	Electron ionisation
EIC	Extracted ion chromatogram
FID	Flame-ionisation detection
FPP	Farnesyl pyrophosphate
GC	Gas chromatography
GLC	Gas-liquid chromatography
GPP	Geranyl pyrophosphate
GSC	Gas-solid chromatography
GSM	Geosmin
HETP	Height equivalent of theoretical plate
LC	Liquid chromatography
LLE	Liquid-liquid extraction
LLME	Liquid-liquid microextraction
LOD	Limit of detection
LOQ	Limit of quantification
MEOH	Methanol
MIB	2-Methylisoborneol
MS	Mass spectrometry
PLOT	Porous-layer open-tubular
P&T	Purge and trap
RSTD	Relative standard deviation
SAM	*S*-adenosylmethionine
SCOT	Support-coated open-tubular
SIM	Selected ion monitoring
S/N	Signal to noise ratio
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
STD	Standard deviation
TIC	Total ion chromatogram
WCOT	Wall-coated open-tubular

1.0 Introduction

Water is arguably the most valuable resource known to humankind. Access to clean water is in general heavily appreciated, where absence of odour and/or taste often is associated with pristineness. In addition to drinking and cooking, water is an essential resource utilised industrially, especially in agriculture. The quality of the water applied in these instances is therefore of utmost importance and thus methods of analysation to verify said quality.

A number of chemical compounds influence the taste and/or odour of water. Two considerable compounds which possess such properties are geosmin (GSM) and 2-methylisoborneol (MIB). These chemicals are terpenes, more specifically hydroxylated irregular sesqui- and monoterpenoids, respectively (Lin et al., 2019). GSM and MIB are both known to emanate a strong mouldy and earthy taste and odour, readily detected by humans due to significantly low sensory thresholds (Jüttner & Watson, 2007; Lindholm-Lehto & Vielma, 2018). As both compounds demonstrate lipophilic characteristics, they have been observed to accumulate in fatty tissues of animals present in water bodies, i.e. fish in freshwater farming facilities, thus altering the taste and odour of said animals (Petersen et al., 2014).

Cyanobacteria and algae in aquatic systems are the main sources of GSM and MIB (Jüttner & Watson, 2007). Interestingly, it has been suggested in the literature that MIB concentrations peak in summer months with warmer temperatures and access to light, while geosmin concentrations tend to remain stable throughout the different seasons (Ma et al., 2007). Accordingly, the accretion of these taste and odour compounds tends to be greater in eutrophic and stagnant water bodies where algae and cyanobacteria are allowed to accumulate. Figure 1.0 visualises the motion of GSM and MIB from water bodies to aquatic livestock and drinking water

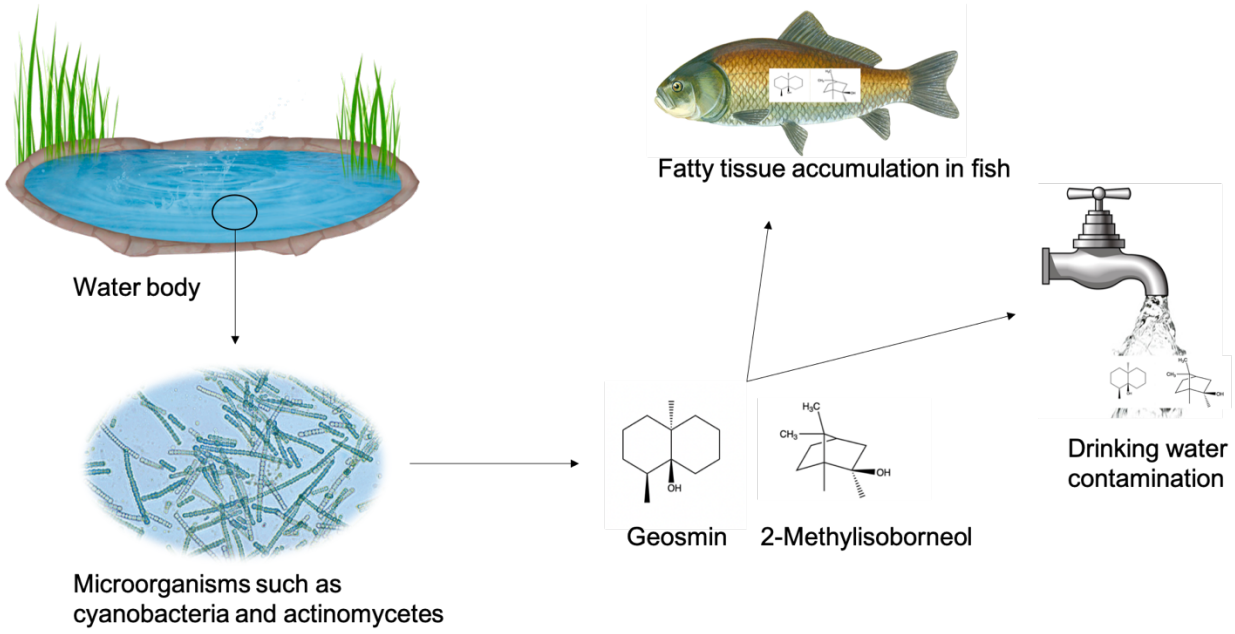


Figure 1.0: *A simplified illustration of the pathway in which geosmin and 2-methylisoborneol end up in drinking water and fatty tissues in aquatic fauna.*

Presence of GSM and MIB in water evidently impose significant industrial, economic and environmental consequences. Water polluted by these odour and flavour compounds is frequently discarded and has consequently been related to drastic decreases in water consumption and thus extensive waste of water (Cees et al., 1974; Zoeteman & Piet, 1973). Additionally, accumulation of GSM and MIB in fatty tissues in fish often result in fish products not being distributed and polluted specimens are often subjected to depuration procedures requiring large quantities of pristine water in order to remove the off flavours (Dionigi et al., 1998; Howgate, 2004; Tucker & Martin, 1991). Similar issues have been observed in other seafood products as well, such as shrimps and clams (Hsieh et al., 1988; Lovell & Broce, 1985).

Proper detection and quantification of GSM and MIB is of utmost importance. With severely low sensory thresholds in humans, methods allowing for quantification at minimal concentrations are crucial. Several methods are reported in the literature, but prevalent challenges are appreciable recoveries of analytes, impracticalities and the requirement of large water samples (Ma et al., 2007). Current methods tend to combine either solid-phase extraction (SPE), solid-phase microextraction (SPME), liquid-liquid extraction (LLE), purge and trap (P&T) or closed-loop stripping analysis (CLSA) with

gas-chromatography – mass spectrometric analysis (GC-MS) (Krasner et al., 1983; Ma et al., 2007). Flame-ionisation detection (FID) has in some instances been used instead of MS (Romero et al., 2007). Optimisation of current methodologies should aim to achieve simplistic, economical and pertinent analytical procedures.

1.1 Aims and objectives of the project

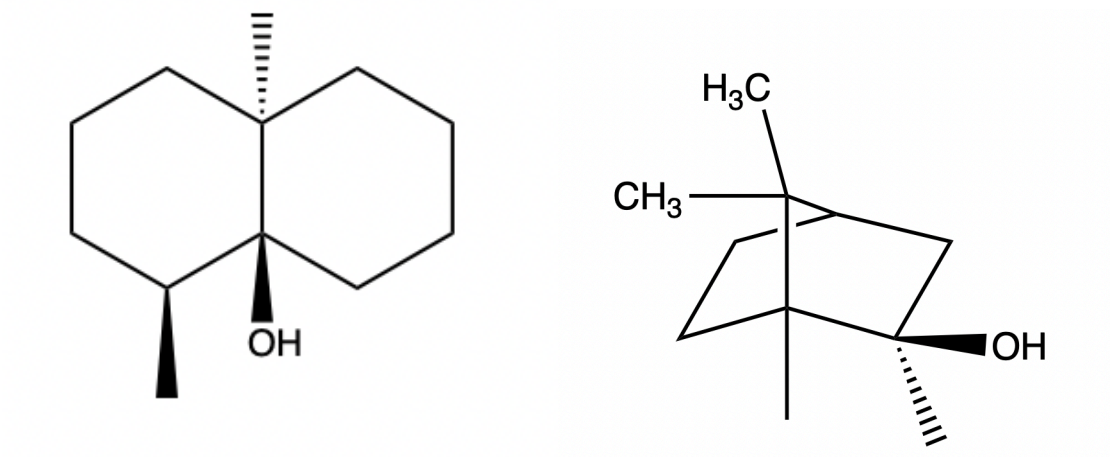
This thesis has involved investigations and optimisation efforts with respect to GSM and MIB analysis via GC Orbitrap. No known experiments involving this instrument has been reported in the literature to date and analyses executed in this thesis aimed to observe the possible benefits of utilising this relatively novel and sensitive technique for detection and quantification. Therefore, proper optimisation of conditions involving the instrumentation was emphasised.

Emphasis has as well been laid on extraction of the compounds from water samples. A major objective was to achieve sufficient extraction of analytes via simple and well-established extraction methods such as LLE and SPE by investigating factors such as partition, flow rates, conditioning and solvent optimisation.

2.0 Background

2.1 Odour compounds of interest

Geosmin and 2-methylisoborneol, displayed in Figure 2.0, are arguably the two taste and odour compounds in water which have gathered the most interest and attention. Both compounds are produced by various microorganisms and are described individually in the following chapters. Sensory thresholds for GSM and MIB have been observed to be extremely low, where GSM in the literature has demonstrated odour thresholds at 1.3 - 3.8 ng/L, while MIB has demonstrated odour thresholds at approximately 6.3 - 20 ng/L (Young et al., 1996; Zoeteman & Piet, 1973). With regards to taste thresholds, GSM and MIB have demonstrated levels at 16 and 15 ng/L respectively (Young et al., 1996).



Geosmin 2-Methylisoborneol

Figure 2.0: *Geosmin (left) and 2-methylisoborneol (right). Both compounds are tertiary alcohols of respectively 12 and 11 carbons and do therefore possess low water solubility.*

2.1.1 Geosmin

Geosmin, or (4*S*,4*aS*,8*aR*)-4,8*a*-dimethyloctahydronaphthalen-4*a*(*2H*)-ol (IUPAC name), is a hydroxylated bicyclic irregular sesquiterpene. All though GSM is a 12-carbon terpenoid, its irregular sesquiterpenoid characteristics is likely a result of isopropyl elimination (Jiang et al., 2007; Tucker, 2000). With regards to physicochemical properties, GSM has an exact molar mass of 182.1671 g/mol, a boiling point of 270 °C, a vapour pressure of 0.0030 mmHg at 25 °C, an estimated solubility in water of 156.7 mg/L, a log K_{ow} of 3.570 and exists as two enantiomers; (+)- and (-) - GSM (NIH, 2023c; TGSC, N.d.-b). The biosynthesis of GSM in cyanobacteria and algae has been proposed in several studies and has also been found to occur in several microorganisms, namely many of the actinomycetes (such as the Streptomyces), in addition to myxobacteria, fungi and cyanobacteria. (Dickschat et al., 2004; Gerber & Lechevalier, 1965; Izaguirre & Taylor, 2004; La Guerche et al., 2005). A major biogenic pathway of GSM production in Streptomyces has been proposed via cyclisation of the precursor farnesyl pyrophosphate (FPP) into germacradienol, following isopropyl elimination into an octalin and finally addition of water into geosmin (Cane et al., 2006; Jiang et al., 2006; Jiang et al., 2007). Interestingly, one single enzyme, geosmin synthase, has been revealed to be responsible for the synthesis from FPP in the presence of Mg^{2+} (Cane et al., 2006; Jiang et al., 2007). A simplified representation of the formation of GSM is presented in Figure 2.1 (summarisation of biosynthesis described in Jiang et al., 2007).

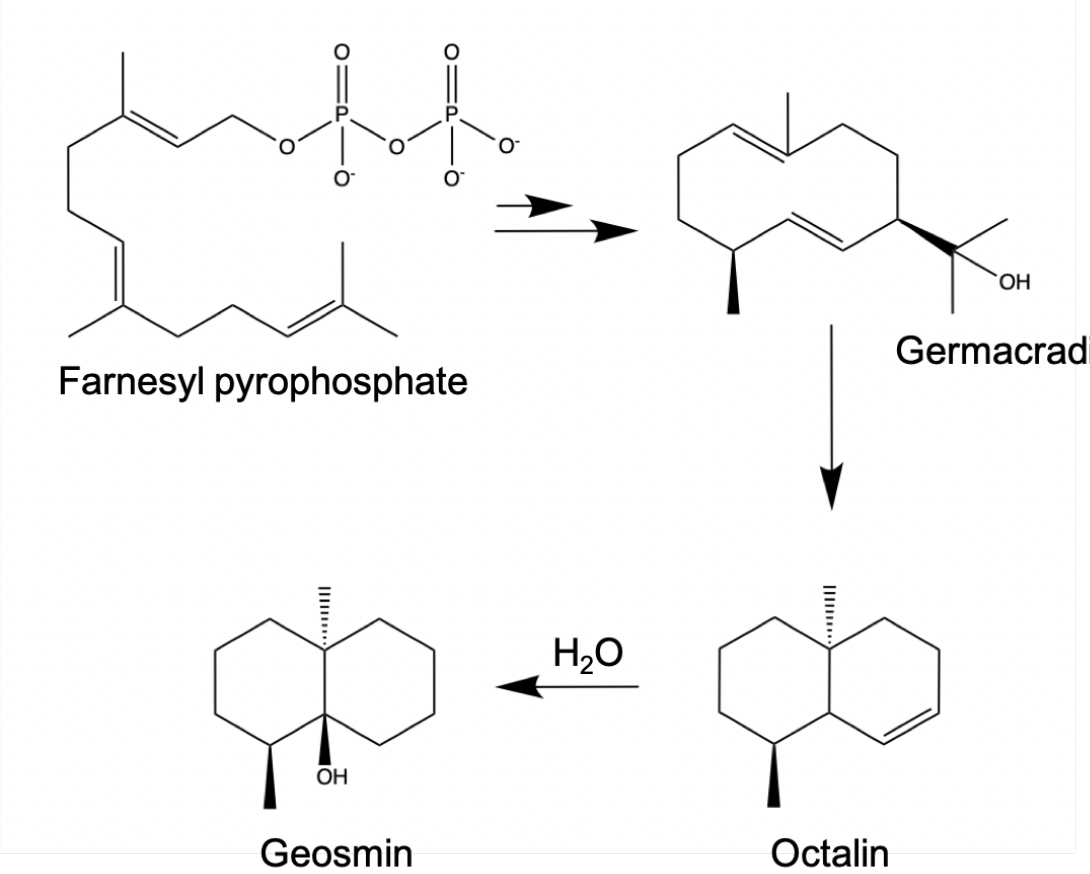


Figure 2.1: *Proposed simplified biosynthesis pattern of geosmin. In most microbial GSM producers, the compound is synthesised from farnesyl pyrophosphate by cyclisation reactions, in addition to water addition, resulting in the characteristic hydroxylation of GSM. Adapted from Jiang et al. (2007).*

Yielding an earthy odour and taste, GSM is the main flavour compound in beets (Tyler et al., 1978). On the contrary, the intense odour and taste of geosmin poses significant challenges when present in water and seafood. As formerly mentioned, odour and taste thresholds of GSM are extremely low in humans. The two known enantiomers, (+)- and (-)-geosmin, interestingly induce somewhat different sensory thresholds in humans (Polak & Provasi, 1992).

In mass spectrometry, electron ionisation causes a proposed fragmentation pattern as illustrated in Figure 2.2 (Jeleń et al., 2003). It should be revealed that one modification has been made in the base peak fragment at *m/z* 112, adding a double bond hypothesised to yield the actual mass of the fragment (the figure in the article incorrectly yields a base peak at 114).

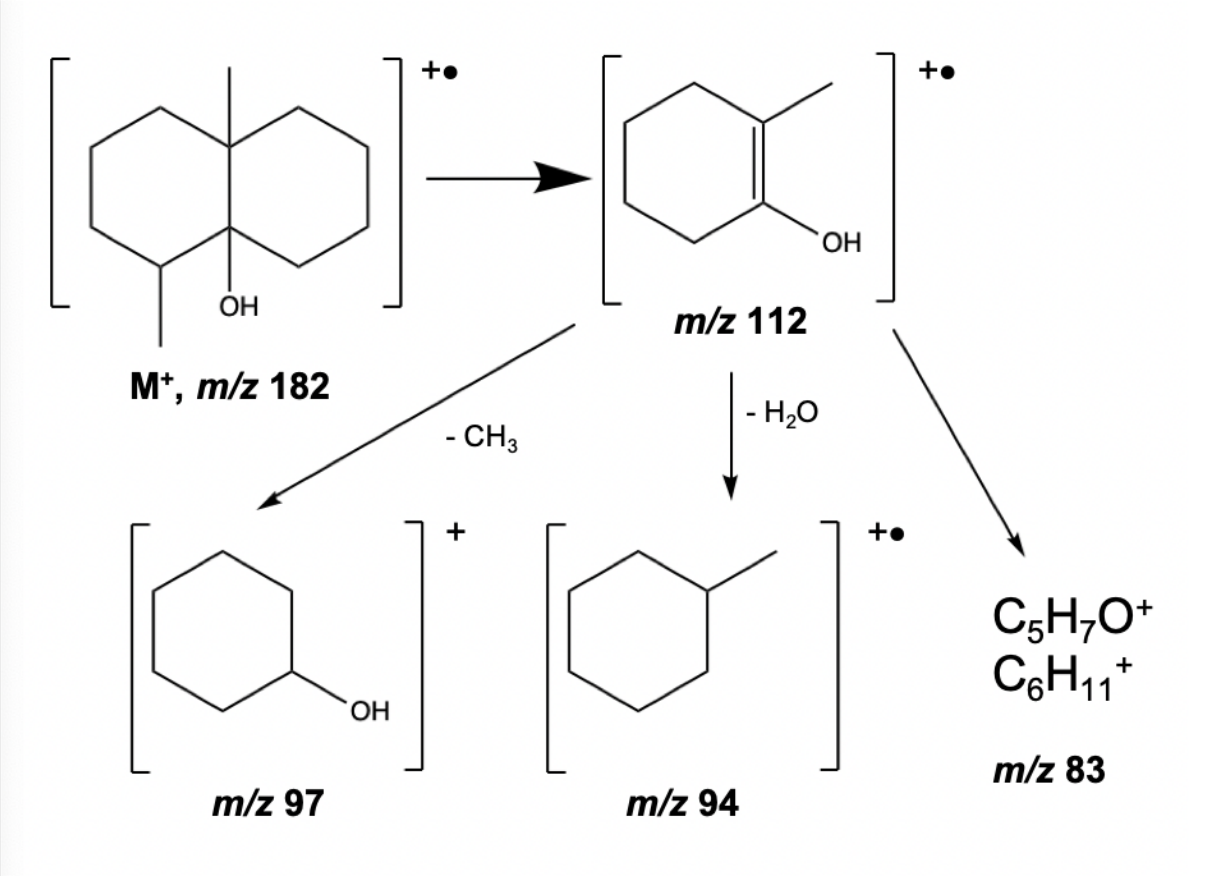


Figure 2.2: *Proposed fragmentation pattern of geosmin following electron ionisation. The base peak is yielded initially as the m/z 112 fragment. Adapted from Jeleń et al. (2003), with a modification in the structure of the base peak fragment.*

2.1.2 2-Methylisoborneol

2-Methylisoborneol, or 1,2,7,7-tetramethylbicyclo[2.2.1]heptan-2-ol, is a hydroxylated irregular monoterpene. MIB is interestingly an 11-carbon terpenoid, but has been revealed to be a methylated monoterpenoid (Dairi, 2010). The compound has certain physicochemical properties; a molar mass of 168.28 g/mol, a boiling point of 208 °C, vapour pressure of 0.0490 mmHg at 25 °C, water solubility of 305 mg/L and an estimated log K_{ow} of 2.931 (NIH, 2023b; TGSC, N.d.-a). Comparably to GSM, microorganisms such as cyanobacteria and actinomycetes (again mostly Streptomyces) produce MIB naturally (Dairi, 2010; Jüttner & Watson, 2007). Its monoterpenoid structure is in actinomycetes and cyanobacteria synthesised via a methylation assisted by *S*-adenosylmethionine (SAM) of the precursor geranyl pyrophosphate (GPP), following cyclisation and subsequent addition of water (Dairi, 2010; Giglio et al., 2011). The enzyme MIB synthase is responsible for the cyclisation and finalisation of the bicyclic product (Giglio et al., 2011). A simplified representation

of the formation of MIB is presented in Figure 2.3 (summarisation of biosynthesis described in Dairi, 2010).

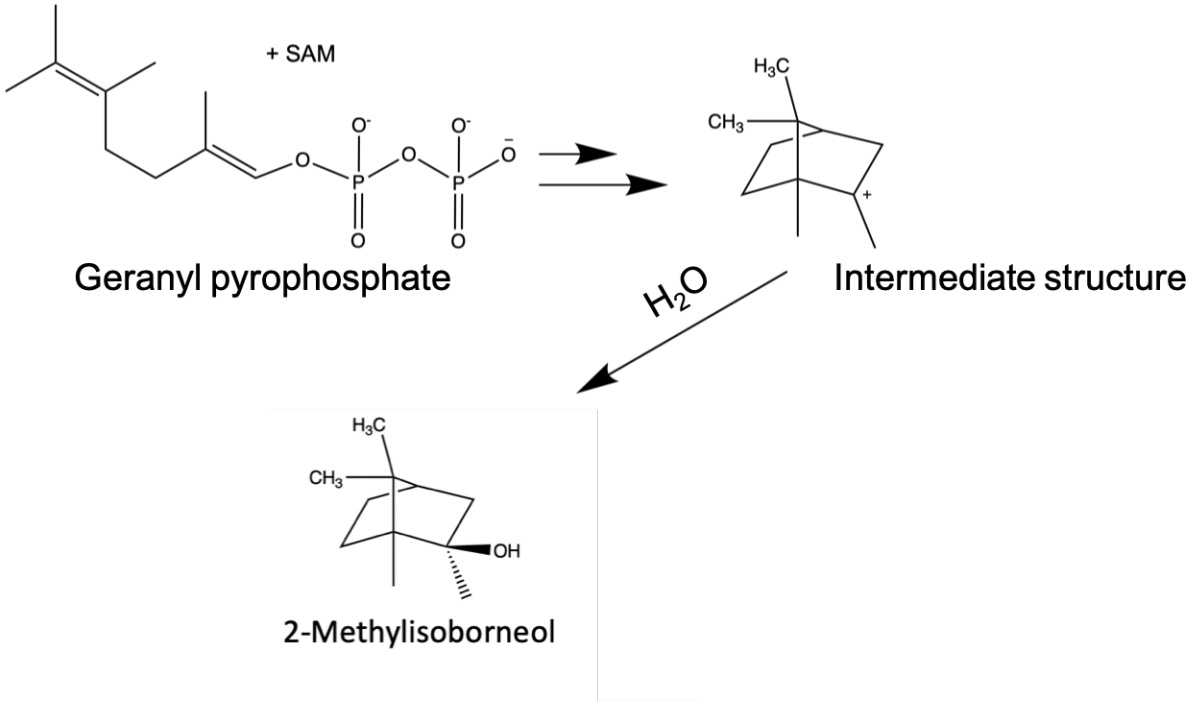


Figure 2.3: *Proposed biosynthesis of 2-methylisoborneol. Microorganisms synthesise the compound from the precursor GPP and analogously to GSM hydroxylate a cyclic intermediate species.*

Figure 2.4 elucidates a proposed fragmentation pattern of MIB in mass spectrometry following electron ionisation (Xu et al., 2010).

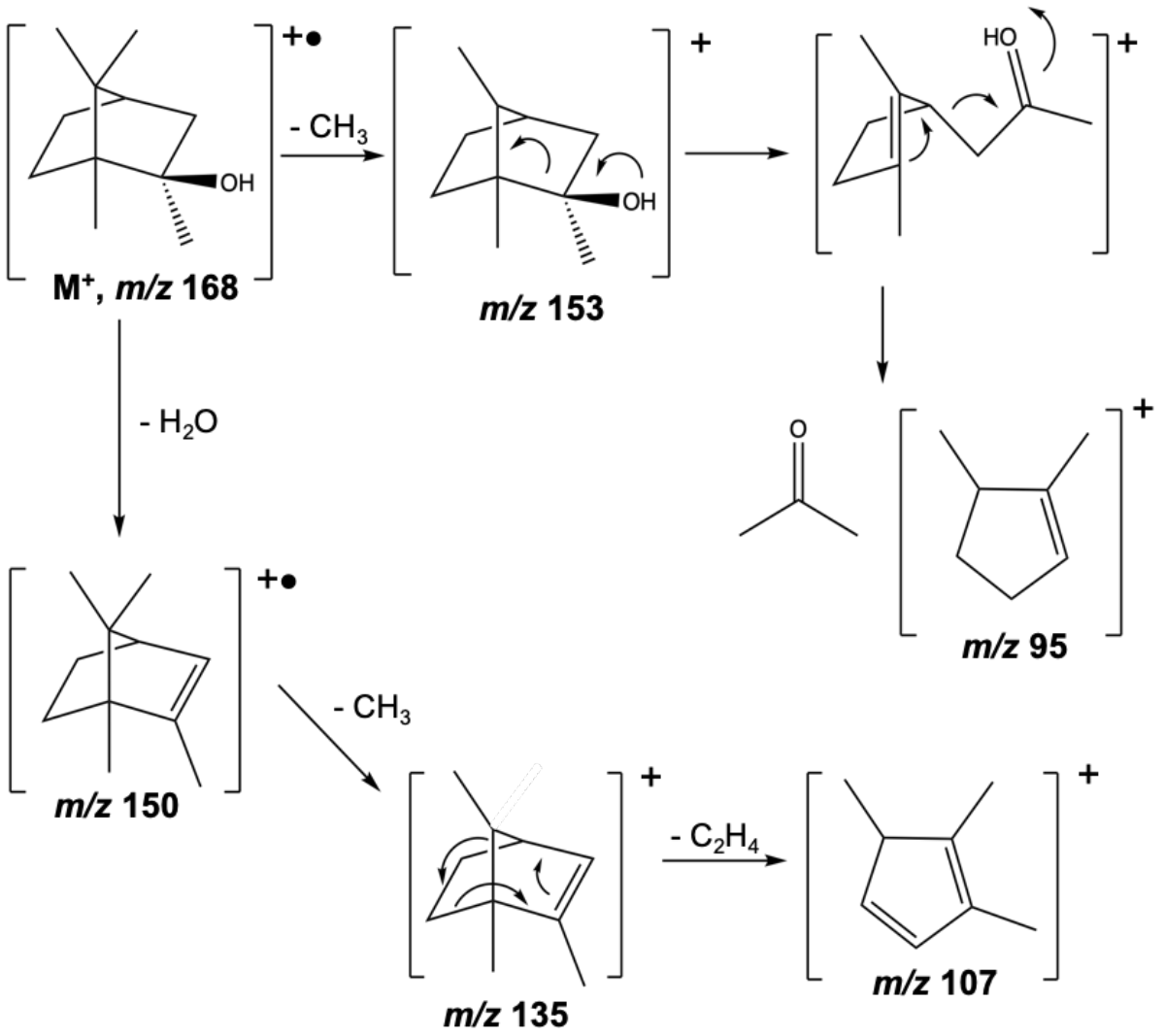


***Figure 2.4**. Proposed fragmentation pattern of MIB. The fragment of m/z 95 constitutes the base peak.*

2.2 Analysis of odour compounds in water

Arguably, the aspect of highest concern with respect to GSM and MIB analysis are quantification and detection limits. The limit of quantification (LOQ) refers to the lowest concentration of a compound a method yields a reliable, accurate, repeatable and determinable result, where the limit of detection (LOD) on the other hand describes the lowest concentration of which a response can be distinguished from zero (Konieczka, 2012; Miller, 2009). In chromatographical terms, the LOQ requires a signal to noise ratio (S/N) of 10 or more in the chromatographical peak, while the LOD requires an S/N of 3 or more (Miller, 2009). GSM and MIB usually occur with levels in the ng/L level and induce serious taste and odour problems at these levels, which is why the development of reliable methods able to quantify the compounds at these

concentrations is crucial. With regards to quantification, two approaches have been investigated in this thesis; internal standards and standard addition. Quantification by use of internal standards involves the addition of a compound structurally similar and/or with similar properties to the analyte. The chromatographical response of the internal standard will reflect the response of the analyte through a response factor, which often is the ratio of peak area and concentration ratios of the analyte and the internal standard. Standard addition involves adding increasing increments of known concentrations of the analyte, where the negative x-intercept of the resulting linear regression reflects the concentration of the sample. Methods described in the literature are further described in Chapter 2.2.1.

2.2.1 Methods described in the literature

GSM and MIB have formerly been analysed with several different methods. The prevalent instrumental parts of current methodologies are GC coupled with MS and occasionally FID (Bristow et al., 2019). GC and MS are individually described in the following chapters of this thesis. The extraction methods investigated in this thesis include LLE and SPE, all though there is a considerable amount of various other methods applicable for GSM and MIB analysis described in the literature.

Liquid-liquid extraction applies solute partitioning between two immiscible solvents (Berk, 2018). The technique has been utilised over several decades commercially with respect to analysis, extraction and/or isolation of various compounds (Ferguson et al., 2022). A common variant of LLE is the extraction of lipophilic solutes from an aqueous solution into an organic solvent, for example dichloromethane (DCM), ethyl acetate (EA) or heptane, by adding the aqueous solution and the organic solvent into a separatory funnel, mixing and shaking the heterogenous mixture and finally extracting the organic solvent which will contain the solutes of intertest (Cook-Botelho et al., 2017; Wilson et al., 2000). With respect to GSM and MIB, organic solvents utilised in LLE have been methylene chloride, DCM, pentane and hexane (Brownlee et al., 2004; Johnsen & Kuan, 1987; Lu et al., 2016; Shin & Ahn, 2004). It should be elucidated that the most successful LLE experiments have been liquid-liquid microextractions (LLME), which involves the use of a small volume of extraction solvents dispersed in the sample, some yielding quantification limits below 1 ng/L of GSM and MIB (Assadi et

al., 2012; Bristow et al., 2019). Figure 2.5 illustrates the main principles of LLE (Targuma et al., 2021).

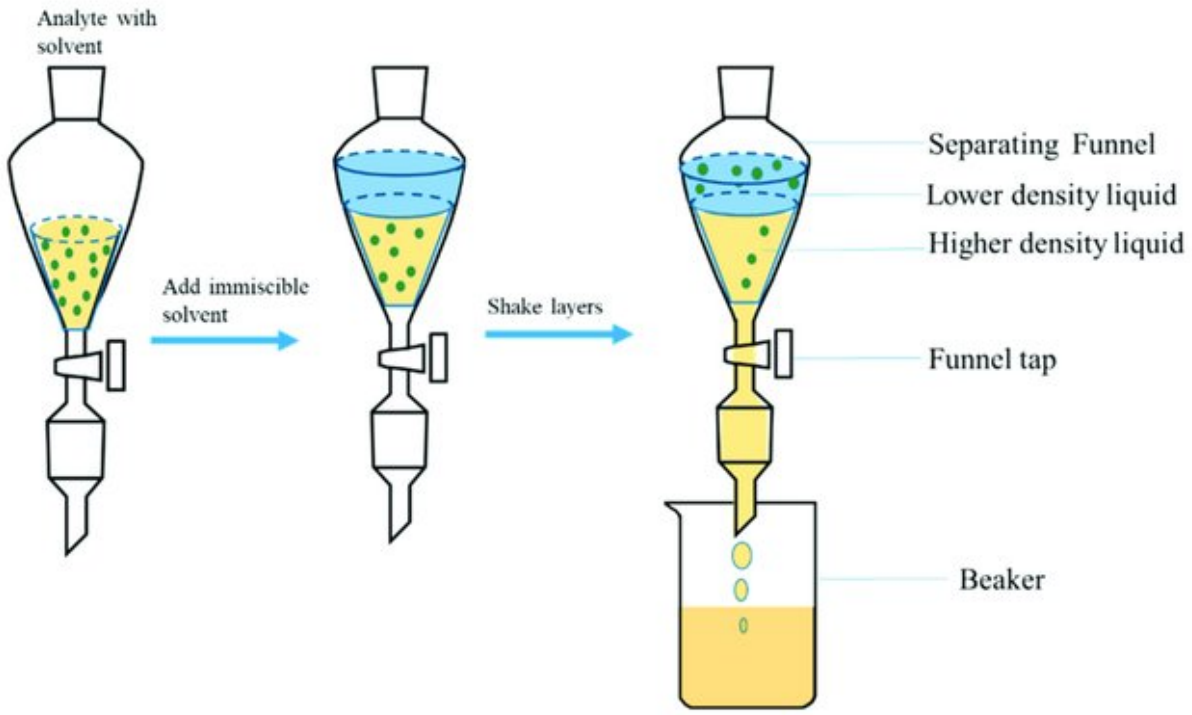


Figure 2.5: *Illustration of a liquid-liquid extraction. The figure elucidates the principle of partitioning allowing for the separation of analytes via this method. Unmodified and redistributed under license CC BY 4.0. (Targuma et al., 2021)*

Solid-phase extraction involves the use of a solid sorbent which adsorbs solutes from a liquid sample (Lehotay & Schenck, 2000). Analogously to liquid chromatography (LC), the solid phase may possess polar or non-polar properties, often composed of similar materials as used in LC (Lehotay & Schenck, 2000). SPE is composed of the following steps: conditioning, equilibration, sample loading, washing and elution (Chen et al., 2016; Raynie & Watson, 2014). Conditioning serves the purpose of removing impurities and soaking the sorbent, equilibration creates a chemically similar environment to that of the sample, loading is the action of passing the sample through the column, washing removes contaminants originating from the sample matrix and elution is when a solvent is applied to remove the analyte from the sorbent (Maranata et al., 2021; Raynie & Watson, 2014). SPE has its significant benefits in concentrating the analyte sample into the extract, as the eluent volume does not need to be large (Raynie & Watson, 2014). GSM and MIB have successfully been quantified with the use of SPE, all though larger sample volumes (> 1000 mL) are usually required

(Bristow et al., 2019). The literature suggests organic solvents such as EA, ethanol and hexane for elution, where quantification limits below 1 ng/L has been achieved (Bristow et al., 2019; Wright et al., 2014). Figure 2.6 visualises SPE (Alkarawi, 2016).

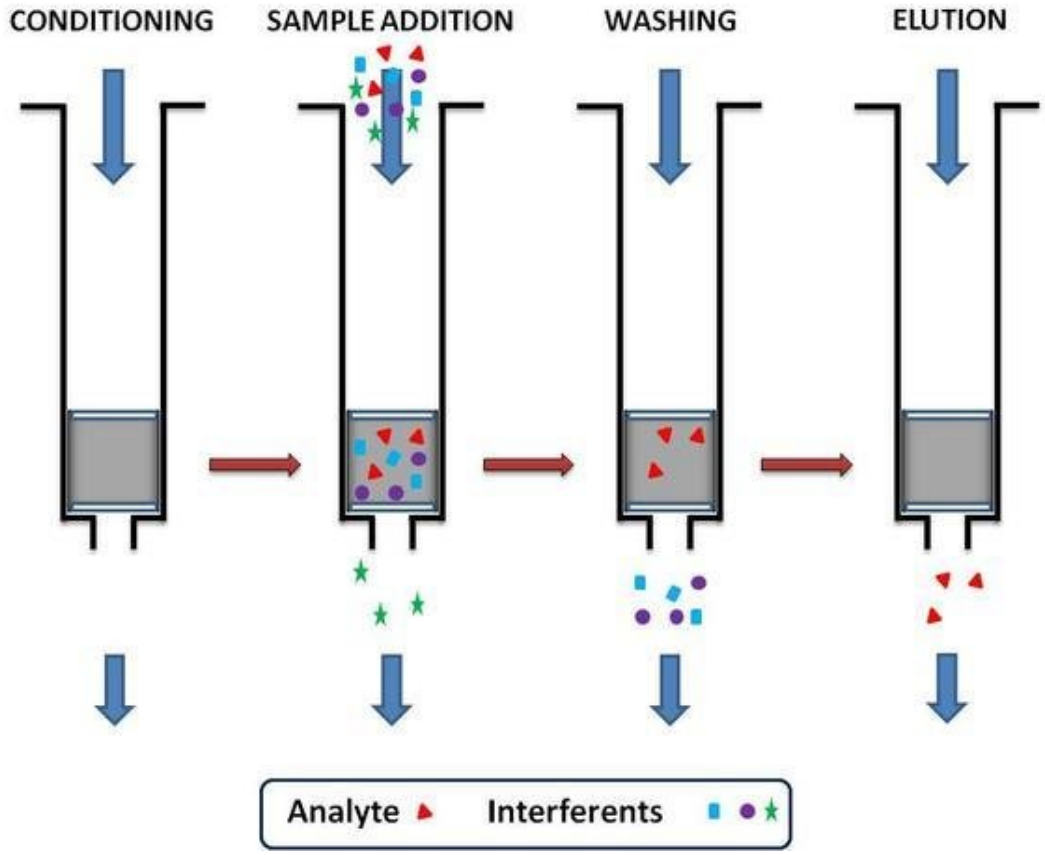


Figure 2.6: *Illustration of the principle behind solid-phase extraction. The figure displays how analytes are adsorbed to the sorbent and ultimately eluted by flushing with an appropriate elution solvent. Redistributed from Alkarawii (2016).*

A grand variety of other methods have been developed and successful in the identification of these odorous compounds. One prominent example is the closed-loop stripping analysis, which involves the concept of acquiring semi-volatile compounds, such as GSM and MIB, from a recirculated air flow on a material such as an activated carbon trap (McGuire et al., 1981). The method has yielded decent achievements with respect to quantification and detection limits, some in the pg/L level and most require sample volumes in the range of 250 mL to 1000 mL (Bruchet, 2006; Hwang et al., 1984; Malleret et al., 2001). Another successful extraction method is the utilisation of headspace, which essentially is the collection of vapour from a volatile compound

existing above a sample in the condensed phase (Bruno & Harries, 2019; Ikai et al., 2003). The P&T method is prevalent in the literature and involves the expulsion of volatile compounds by running an inert gas through the sample, followed by collection and analysis via GC-MS (Bruno & Harries, 2019; Stahl & Parkin, 1994). All though the abovementioned methods arguably are the most prominent, there are additional methods that will not be discussed in this thesis.

2.2.2 Gas chromatography

Gas chromatography is undoubtedly the most popular mode of separation utilised for volatile compounds. GC applies differences primarily in polarity and vapour pressure to achieve separation of compounds and has indeed through its development in the last century proven to be a method capable of separating volatile chemicals in complex mixtures (Stauffer et al., 2008). GC may be divided into two main categories; gas-solid chromatography (GSC) and gas-liquid chromatography (GLC) (Miller, 2009). GSC involves utilising an inorganic support material (such as alumina and silica) as the stationary phase, achieving retention via adsorption and is mostly favourable when analysing analytes of low boiling points (Engewald et al., 2014; Miller, 2009). In the present, GLC is more commonly applied and involves an immobilised liquid stationary phase on a solid inert packing or directly on the capillary tubing walls (Shellie, 2013). The common constituents of a GC are the injector, an oven-heated column and a detector, as seen in Figure 2.7. In addition, the GC will be connected to a supply of carrier gas (Miller, 2009).

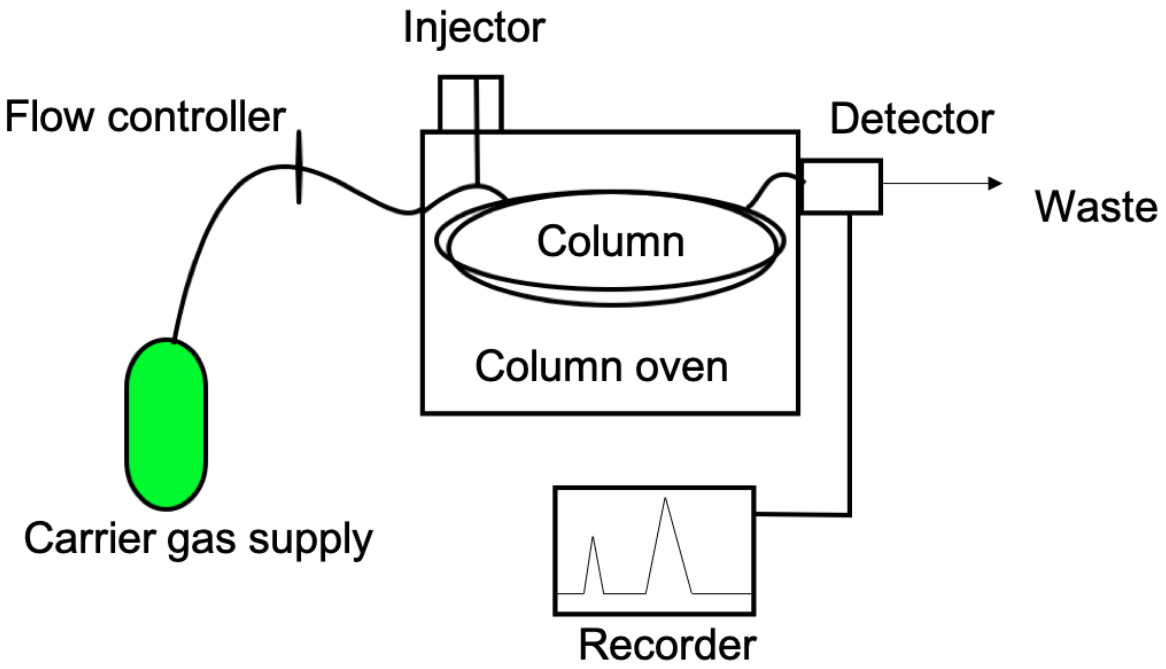


Figure 2.7: *General representation of gas chromatography.*

The injector as the name suggests is where a sample is introduced to the GC. A split/splitless injector is most commonly used, which allows for two different modes of sample introduction. Split injection involves vaporising usually 1 μL of the sample, containing a mixture of carrier gas, solvents, analytes and other solutes, where only a fraction of the vapour will enter the column due to the presence of an open valve (Grob, 2007; Miller, 2009). Split ratios may vary from 1:1 and up to 1:1000, where accordingly a higher split results in less amounts of the sample vapour entering the column (Miller, 2009). Splitless injection will on the contrary introduce most of the vapour to the column. Split injection has its benefits especially with regards to concentrated samples, whilst splitless injection oppositely is beneficial when analysing trace amounts of compounds (Grob, 2007). It should however be elucidated that splitless injection with volatile compounds may result in a phenomenon known as band broadening, which is a widening of the chromatographical peak and may ultimately result in a lower S/N (Grob, 1985; Harvey, 2013). Other examples of injection include classical vaporizing injection, direct injection, programmed temperature vaporising injection, solvent splitting and on-column injection (Grob, 2007; Miller, 2009). The constituents of a split/splitless injector are represented in Figure 2.8.

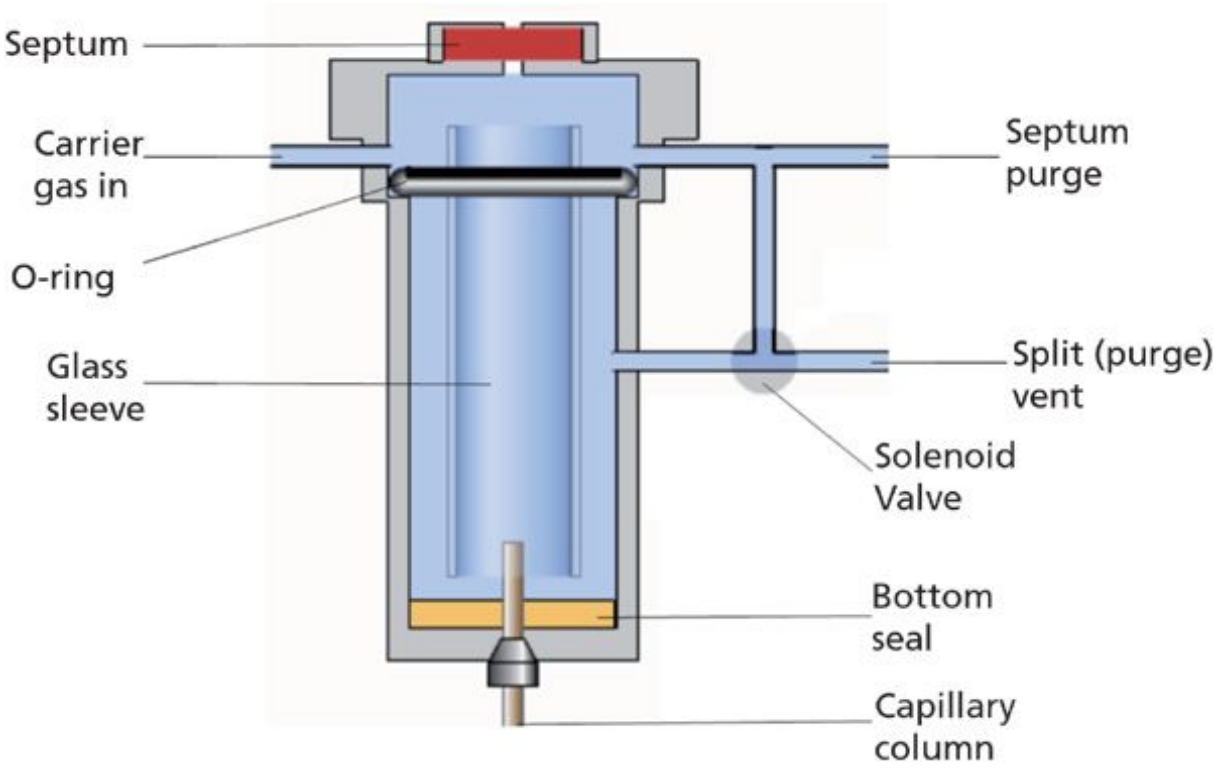


Figure 2.8: *Overview of a split/splitless injector. Redistributed from Snow (2018).*

Following injection, the sample enters an oven-heated column. Heating the column has its purpose in maintaining the compounds in the gaseous phase and adjusting the temperature gradient during the vapours' course through the column assists in separation (Engewald & Dettmer-Wilde, 2014). Columns may be separated into packed columns and capillary columns. Packed columns generally inhabit the stationary phase directly in the column (Rahman et al., 2015). Capillary columns on the other hand will have the stationary phase coated on the inner wall of the column (often referred to as open tubular), where three varieties are prevalent: wall-coated open tubular (WCOT), support-coated open tubular (SCOT) and porous layer open tubular (PLOT), visualised in Figure 2.9 (Buntinx et al., 2020). A WCOT column possesses a thin layer of liquid stationary phase coating the inner wall, a SCOT column inhabits a liquid covered porous layer and finally a PLOT column has its interior surface coated with a porous layer solid (Miller, 2009). Of the abovementioned varieties, the WCOT column is predominantly utilised.

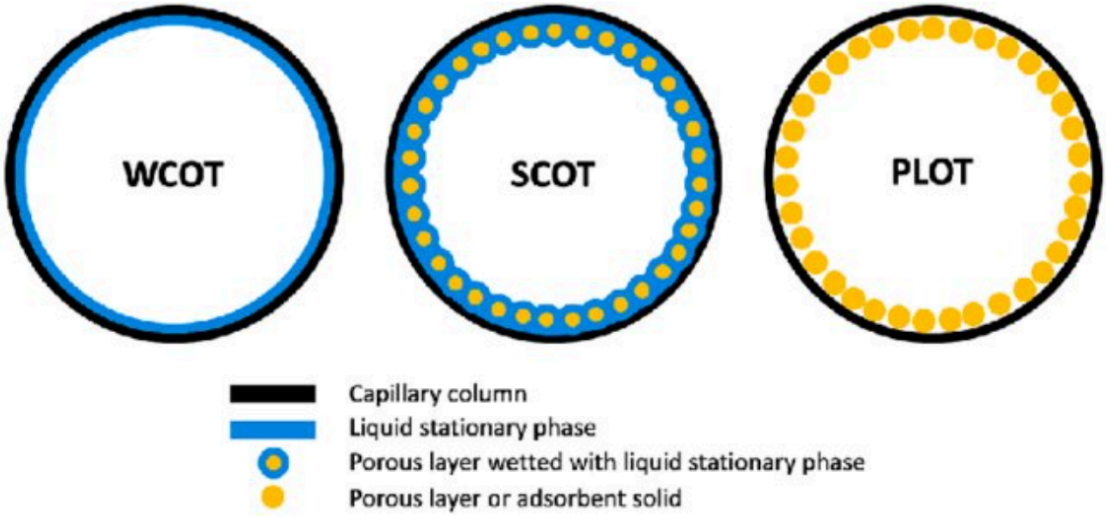


Figure 2.9: *Illustration of the three main capillary columns utilised in the GC. From Buntinx et al., (2020) unmodified and redistributed under license CC BY-NC-ND 4.0.*

Three different carrier gases comprise the main choices of mobile phases in GC, namely hydrogen, H_2, helium, He and nitrogen, N_2. The Van Deemter plot, illustrated in Figure 2.10, visualises the relationship between height equivalent of theoretical plates (HETP) and the flow rate of each of these gases, which is an important aspect with regards to the choice of carrier gas (Miller, 2009; van Deemter et al., 1956). Helium is favoured for its efficiency and inertness, but is less available and expensive in many countries (Bartram & Froehlich, 2010). As a result of this, it is presently more favoured to utilise hydrogen gas, which according to the van Deemter plot yields an even lower HETP at faster flow rates. However, this unfortunately poses a great explosion hazard risk (Bartram & Froehlich, 2010; Christie, 1989; Miller, 2009). Nitrogen gas is also commonly applied in GC due to its cost-effectiveness, inertness and safeness (relative to hydrogen), even though the HEPT inevitably will be elevated at higher velocities (Christie, 1989; Miller, 2009). It should be elucidated that the longer duration of analysis with N_2, as a result of the relatively low velocity, may be compensated for with for example decreasing the width and length of the column (Watson, 2017a).

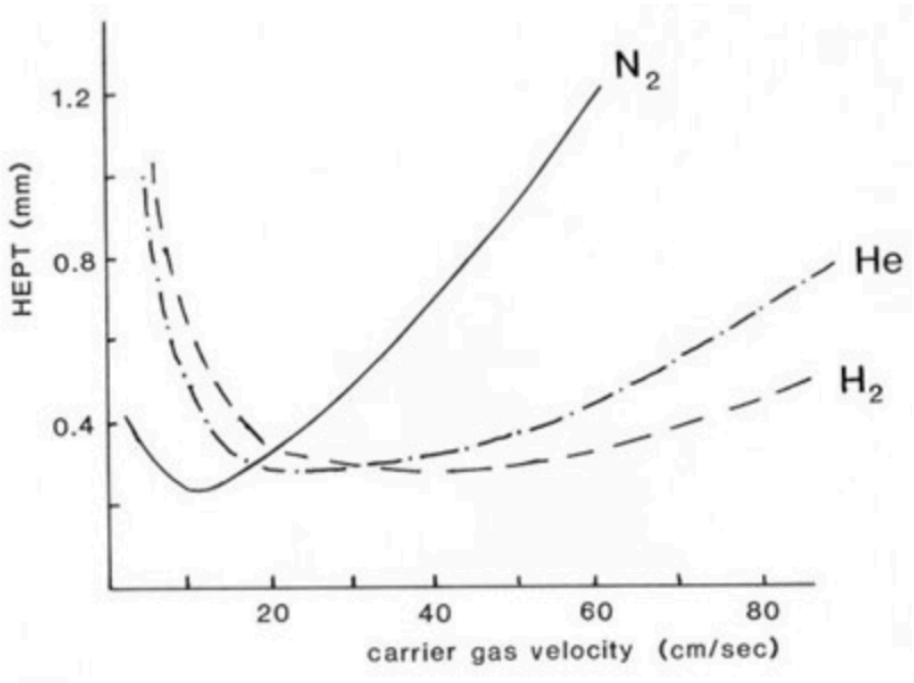


Figure 2.10: *Representation of a van Deemter plot with respect to common carrier gas choices associated with gas chromatography (Christie, 1989).*

As previously mentioned, MS is commonly coupled with GC as its detector and will be discussed further in the next chapter. Other common detectors for GC include FID and thermal conductivity detector, but will not be further discussed in this thesis (Miller, 2009).

2.2.3 Mass spectrometry

Mass spectrometry is a common detection method coupled with GC. In general, MS revolves around the production and analysis of ions and more specifically the mass to charge ratio, *m/z*, of ions, which is the mass of the ion *m* divided by the charge *z* (Hoffmann & Stroobant, 2007). Accordingly, the *m/z* ratio depends on the charge of the fragment ions. Most commonly with regards to smaller organic molecules, the charge of the ions is equal to 1, thus resulting in the *m/z* value directly reflecting the mass of the ion. An MS instrument is composed of an ion source, where ions are created, a mass analyser, which separates said ions according to their *m/z* and finally a detector, which detects and converts ions into a digital output (Hoffmann & Stroobant, 2007). The conversion of a molecule into ion fragments and the determination of their molecular mass from *m/z* is what makes MS a powerful technique with regards to

determination and elucidation of an analyte. In most cases with the commonly used electron ionisation technique in the ion source, a molecular ion M^+ is formed, which is the ionised form of the molecule analysed, corresponding directly to the molecular mass of the analyte (Dass, 2007). Figure 2.11 shows a general representation of MS.

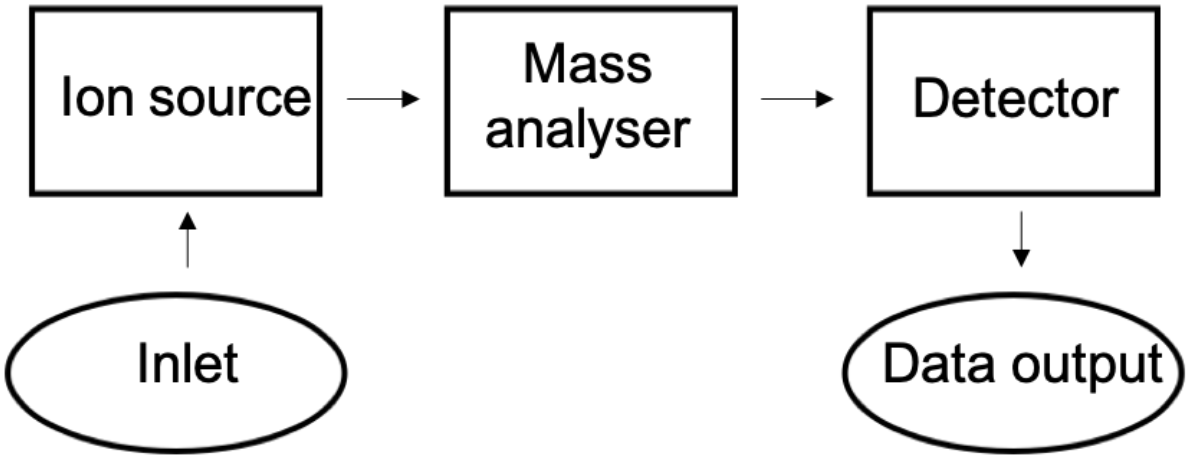


Figure 2.11: *A general representation of mass spectrometry.*

There are different ionisation techniques which may be utilised in the ion source depending on the analyte. Electron ionisation (EI) is the most popular ionisation method with regards to analysis of smaller organic molecules, often recognised with a molecular weight of less than 600 Da (Dass, 2007; Van Berkel, 2003). The EI ion source, as seen in Figure 2.12, converts gaseous analyte molecules in a high vacuum into ions by bombarding them with electrons emitted from a heated filament and accelerated towards an anode (Hoffmann & Stroobant, 2007). Emitted electrons are accelerated to 70 eV, which is a widely accepted plateau of electron energy in EI. The main reasons for this specific energy level are 1: less production of fragments occurring below 70 eV and 2: a decrease in M^+ production above 70 eV, as more radical species are formed, inducing a higher fragmentation occurrence (Dass, 2007; Margolin Eren et al., 2020). Ions are ejected out of the ion source by a positively charged repeller and directed towards a mass analyser. Other ionisation techniques are also commonly utilised, where chemical ionisation (CI), electron spray ionisation (ESI) and matrix assisted laser desorption ionisation (MALDI) are common examples (Hoffmann & Stroobant, 2007). The distinctive mass spectra obtained with EI and available online

libraries for comparison do however make EI the most prominent ionisation technique utilised for smaller organic molecules. The concept is illustrated in Figure 2.12.

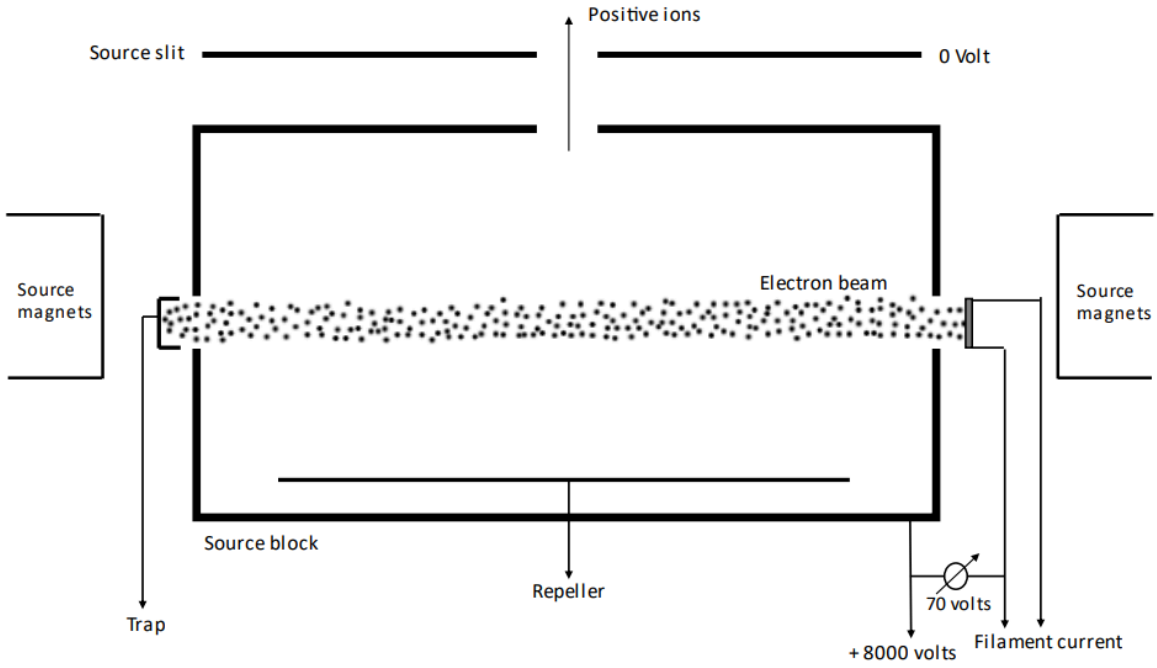


Figure 2.12: *Illustration of an electron ionisation source.*

Ions are sorted according to their *m/z* values in a mass analyser and converted to mass spectra in a detector. There are several different approaches to mass analysis which have been developed, where common examples are quadrupole, time of flight, ion trap, ion cyclotron resonance, magnetic sector and electrostatic sector mass analysers (Hoffmann & Stroobant, 2007). There are benefits and shortcomings of each mass analyser and a mass analyser is accordingly selected based on the analyte(s) of interest. Following mass analysis is detection, where the separated ions are converted into a quantifiable signal and ultimately into a spectrum (Dass, 2007). The most common detection method is electron multiplication, whereas faraday cups, photomultipliers and array detection are examples of other approaches.

In this project, GSM and MIB analysis was achieved with an emerging mode of mass analysis called orbitrap, or orbital trapping. This is a powerful technique which allows for both mass analysation and ion detection. An orbitrap, as reflected in Figure 2.13, consists of three electrodes where two of the electrodes possess a curved shape facing one another, resulting in a barrel-like structure, enclosing a third inner, spindle

shaped electrode (Hecht et al., 2019). Applying a voltage between the enclosing electrodes and the inner electrode yields a linear electric field, which results in harmonic oscillations along the direction of this field and thus attraction of ions to the inner electrode (Zubarev & Makarov, 2013). As ions with different *m/z* enter the field between the outer and inner electrodes, they oscillate at different frequencies which allows for the separation of fragment ions and subsequently creation of mass spectra via image current detection, where the outer electrodes serve as receiver plates (Hecht et al., 2019; Zubarev & Makarov, 2013). The C-trap is shown in Figure 2.13, which in principle matches the kinetic energy of fragment ions with the voltage of the inner electrode, yielding a steady trajectory as ions are introduced to the orbitrap (Hecht et al., 2019). Ions enter the orbitrap from the C-trap in short time intervals to yield oscillation in narrow bands along the electric field, ultimately resulting in a more desirable S/N (Hecht et al., 2019).

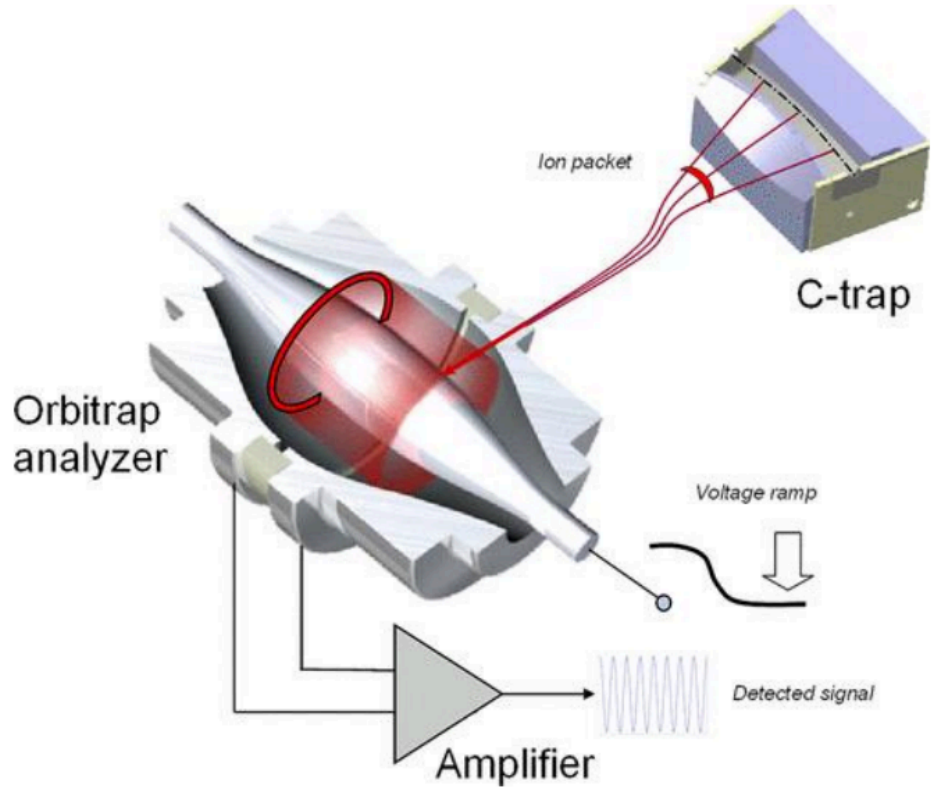


Figure 2.13: *Illustration of the orbitrap mass analyser. Redistributed from Zubarev and Makarov (2013).*

Current approaches utilise selected ion monitoring (SIM) for the analysis of GSM and MIB (Bristow et al., 2019). This approach involves programming the instrument to

detect specific *m/z* ion currents for the compounds of interest (Kitson et al., 1996). The GC Orbitrap utilised in this thesis was stated to possess similar sensitivities to SIM.

It is possible to obtain different chromatograms by utilising MS. Commonly, a total ion chromatogram (TIC) is obtained, which includes all peaks detected with MS in one scan (Stauffer et al., 2008). When quantifying specific compounds, extracted ion chromatograms (EIC) are commonly used, which exhibit intensified peaks related to specific, chosen fragment ions of the analyte (Murray et al., 2013; Stauffer et al., 2008) One sub-category of the EIC is a base peak chromatogram (BPC), where the chosen fragment ion is the base peak (highest intensity) fragment ion of the analyte (Murray et al., 2013).

2.3 Industrial and environmental aspects

GSM and MIB pose great environmental and industrial challenges. All though the compounds themselves cause minimal to no toxicological effects at such low concentrations, the consequences of GSM and MIB presence has been observed to result in huge financial losses and waste of water and seafood, where customer complaints and distrust have been named as central factors (McCrummen et al., 2018; Newcombe et al., 2010; Tucker, 2000). Additionally, the compounds tend to evade conventional water treatment procedures such as filtration, coagulation, chlorination and sedimentation, resulting in more costly treatment options being necessary (Kim & Park, 2021; Li et al., 2019; Xia et al., 2020). Off flavours caused by fatty tissue accumulation of the compounds in seafood may result in severe production and profit deficiencies, as products may be deemed undesired by consumers, in addition to livestock being purified for weeks in clean water following contamination (Abd El-Hack et al., 2022; Jüttner & Watson, 2007; Tucker, 2000).

3.0 Materials and Chemicals

3.1 Instruments and equipment

Compounds were identified via gas chromatography (GC) with high-resolution accurate-mass analysis (HRAM) by using an ExactiveTM GC Orbitrap GCMS, Thermo ScientificTM, Bremen, Germany. The software used for instrument control was ExcaliburTM, version 4.5. Acquired data were processed using Tracefinder 5.1, Thermo ScientificTM, Bremen, Germany. The scan range used for acquiring of data was *m/z* 50 - 600 with a resolution set to 60 000 FWHM, an electron energy of 70 eV and an ion source temperature set to 250 °C was used. Compound identity was confirmed via NIST20 (National institute of standards and technology, Gaithersburg, MS, USA) mass spectral library. The GC used was a Thermo ScientificTM TraceTM 1310 instrument equipped with a 60 m Restek column (Rtx® 2330) with 0.25 µm film thickness, 60 m long and ID of 0.25 mm. The stationary phase is highly polar and consists of 90 % biscyanopropyl and 10 % cyanopropylphenyl polysiloxane, Restek Corporation, Bellefonte, PA USA. Helium (99.99990% from Yara, Rjukan, Norway) was used as mobile phase at 1.5 mL/min at constant flow. The following temperature programming was utilised (file named geosmin.meth) unless informed otherwise in the thesis. The initial temperature was started at 50 °C, held for 5 min, increased to 140 °C (100 °/min), held for 15 min and increased to 260 (125 °/min), held for 5 min. The GC was equipped with a liquid autosampler, Thermo ScientificTM TriPlussTM 100LS, Thermo ScientificTM, Bremen, Germany. One microliter of sample was injected in a split/splitless injector operated in split mode with a split ratio set to 1:10.

Samples which required mechanical shaking were shaken with a Grant-bio PSU 20i Orbital Shaking Platform. SPE experiments were conducted with a Supelco Visiprep™ 24 port SPE Vacuum Manifold with attached Visiprep™ Large Volume Sampler adapters into Agilent Bond Elut 200 mg C18, 3 mL SPE cartridges. Extracted samples were centrifuged with a Hettich® EBA 20 Centrifuge AC/DC input 240 V AC. Vials were of the type ND11 0.2 mL crimp neck vials from VWR®. All water samples were created in pure water purified with a Milli-Q IQ 7000 Ultrapure Water System.

3.2 Chemicals

Chemicals utilised in these experiments, with respective suppliers and qualities are listed in the table below.

Table 3.0: Overview of chemicals utilised in thesis experiments.

Chemical	Quality	Supplier
(±)-Geosmin and 2-Methylisoborneol Solution 100 μg/mL	TraceCERT® certified reference material	Supelco
(±)-Geosmin 10 mg	≥ 97% (GC)	Sigma-Aldrich
Camphor 100 g	96 %	Sigma-Aldrich
1-Methylcyclohexanol	N/A	N/A
n-Heptane	HiPerSolv Chromanorm®	VWR Avantor
n-Hexane	HiPerSolv Chromanorm®	VWR Avantor
Dichloromethane	Pestinorm	VWR Avantor
Methanol	HiPerSolv Chromanorm®	VWR Avantor
Ethyl acetate	GPR Rectapur	VWR Avantor
$NaH_2PO_4 \bullet H_2O$	N/A	N/A
NaCl	N/A	N/A
Na_2SO_4 Anhydrous	N/A	N/A

4.0 Methods

The following chapters describe detailed methods of all conducted experiments. Table 4.0 represents the different aspects of optimisation emphasised in this project. The main modes of extraction from aqueous samples were LLE (including LLME) and SPE. These were investigated based on availability of equipment at the duration of the project. Internal standard quantification was tested within both extraction methods, while standard addition was investigated in SPE. With respect to LLE, evaporation of solvent was tested as a possible mode of concentrating extracts. The major

optimisation efforts with regards to SPE included removal of contaminants in the SPE column, in addition to aspects of analyte recovery; namely flow rate effect, in addition to solvent composition and extraction volume. Finally, temperature and split/splitless programming in the GC Orbitrap was investigated.

Table 4.0: Overview of optimisation experiments conducted in this thesis.

Liquid-liquid extraction	Solid-phase extraction	GC Orbitrap
Internal standard	Conditioning	TIC, EIC, BPC
Solvent evaporation	Optimisation - *Flow rate* - *Solvent composition* - *Eluent volume*	Temperature programming
Liquid-liquid microextraction		Split ratio
		Splitless
	Quantification - *Internal standard* - *Standard addition*	

The table lists an overview of the different optimisation efforts made in this thesis. Descriptions of each aspect of optimisation is found in the following chapters.

4.1 Liquid-liquid extraction

Some experiments were performed preliminary with regards to liquid-liquid extraction. One analysis involved LLE of 100 mL and 200 mL aqueous solutions containing various concentrations of GSM and MIB in the range 100 ng/L – 100 μg/L. To increase water polarity, 30 g of NaCl was added and the samples were extracted with an equal volume of a 50/50 mixture of ethyl acetate and heptane in a separatory funnel. This was followed by GC Orbitrap analysis of the collected organic phase. Another preliminary experiment involved evaporating solvents in concentrated heptane solutions. 3 mL samples with GSM concentrations ranging from 0.5 to 50 mg/L were completely evaporated with N_2(g) in both room temperature and in ice baths. The samples were redissolved in 1000 μL heptane and subsequently analysed with GC Orbitrap. The compound 1-methylcyclohexanol was investigated as a possible internal standard for LLE analysis. Various amounts of the compound were added to aqueous solutions containing GSM and MIB, extracted and analysed with LLE and GC Orbitrap.

Experiments involving liquid-liquid microextraction were also conducted. 3 x 200 mL aqueous samples (*n* = 3) containing 100 ng/L of both GSM and MIB were created. The solutions were added to 500 mL ISO bottles together with 8 g of $NaH_2PO_4 \bullet H_2O$, 40 g of NaCl and 1.0 mL of heptane. The bottles were shaken mechanically at 200 rpm for 5 minutes and added to 250 mL separatory funnels and left to allow separation for 5 minutes. Collection of the organic phase was achieved by discarding most of the aqueous layer and collecting the organic layer in a test tube. The samples were subsequently dried with 1 g of anhydrous Na_2SO_4, centrifuged at 4000 rpm for 10 minutes, added to GC vials and analysed with GC Orbitrap in split (1:10) and splitless mode.

4.2 Solid-phase extraction

The following chapters involve SPE as a mode of analyte extraction. Experiments were conducted in order to 1: optimise conditioning, 2: optimise extraction and 3: quantification by use of internal standards and standard addition. Figure 4.0 is a photo of a general SPE set up, utilised for experiments in this thesis, with minor adjustments depending on the experiment. The large volume sampler adapters allow for efficient and convenient aspiration of larger aqueous samples into the SPE cartridges. Benefits include easier control of sample flow rate through the column and automaticity.

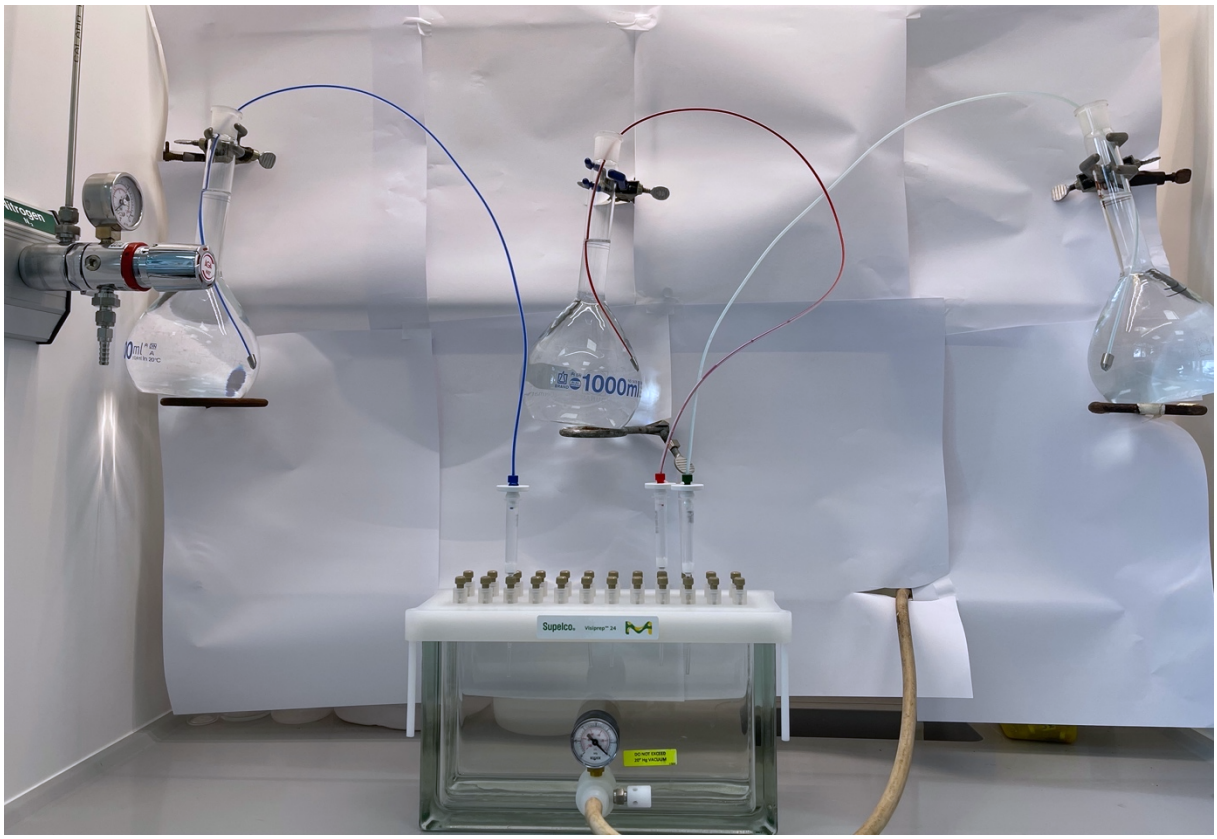


Figure 4.0: *A set up for SPE analysis demonstrating three sample replicates being aspirated through SPE cartridges through a vacuum manifold with adapters suited for large sample analysis. The adapters allow for easier control of factors such as flow rate and elevate the efficiency and automaticity of the extraction.*

4.2.1 Conditioning

9 mL of MilliQ water was run through the column under vacuum and was subsequently discarded. Following this, a 1 mL aliquot of a 50/50 mixture of ethyl acetate and heptane was run through the column and collected in a test tube containing 1 g of anhydrous $NaSO_4$ to remove any remaining water. The test tube was centrifuged at 4000 rpm for 10 minutes to further separate any remaining suspended water from the non-polar solvent and remove solid salt particles from the solution. 300 μL of the sample was then transferred to a GC vial. A control sample was created by adding 300 μL of the 50/50 ethyl acetate/heptane mixture to a GC vial. The two samples were analysed via GC Orbitrap.

Subsequent investigations were conducted with regards to further removal of contaminants. Firstly, two sets (n = 3) of 20 mL samples of 10 μg/L GSM and 10 μg/L

MIB were created. The first set was run with vacuum through SPE cartridges, which had not been conditioned with a non-polar solvent and instead approximately 9 mL of water, to observe contaminant effects on the chromatographical peaks. The second set was run through cartridges which had been conditioned with approximately 9 mL of a 50/50 ethyl acetate/heptane mixture prior to sample application. Samples were aspirated through the SPE columns with a flow rate of approximately 5 mL/min and eluted with 1 mL of the 50/50 ethyl acetate/heptane into test tubes containing approximately 0.5 g of anhydrous $NaSO_4$ powder, centrifuged 4000 rpm for 10 minutes and analysed with GC Orbitrap. Each sample was analysed three times yielding a total of nine injection replicates over three sample replicates for each conditioning method.

Further experiments were conducted to optimise conditioning. Emphasis was laid on removing remaining non-polar solvents from the column after the column was conditioned. Similar to the experiment conditioning with just the 50/50 ethyl acetate/heptane mixture, two sets (n = 3) of 20 mL solutions containing 10 μg/L GSM and 10 μg/L MIB were made. The first set was aspirated through SPE columns conditioned with just water, identical to the previous experiment, while the second set was pulled through SPE columns first conditioned with 9 mL heptane, then 3 mL DCM, to remove residual heptane and finally 3 mL of MeOH to remove residual DCM. The columns were then filled with water before the samples were applied. All samples were extracted, centrifuged and analysed with GC Orbitrap as with the former experiment, again yielding a total of nine injection replicates over three sample replicates for each conditioning method.

4.2.2 Optimising extraction

One of the main aspects of achieving optimal analyte recovery was by investigating the effect of flow rate through the SPE column. Five sets (n = 3) of 50 mL samples, with concentrations of each GSM and MIB equal to 20 μg/L, were passed through conditioned SPE columns. Each of the columns were conditioned with the following sequence of solvents: 9 mL heptane, 3 mL DCM, 3 mL MeOH and 3 mL H_2O. Samples were extracted with different flow rates, ranging from 1.1 mL/min to 14.9 mL/min. Table 4.1 reflects the average different flow rates of the replicates (*n* = 3).

Table 4.1: Flow rates of 20 μg/L SPE extracted GSM and MIB solutions. (*n* = 3)

Sample number	Average measured flow rate [mL / min]
1	1.1
2	2.3
3	5.2
4	10.4
5	14.9

Flow rates were calculated by measuring the time it took to for each sample to run through the column. The displayed values in mL/min reflects the average flow rates from the three replicates. Each sample was then eluted with 1 mL of a 50/50 ethyl acetate/heptane solution and analysed with GC Orbitrap.

Another aspect of analyte recovery from SPE was the elution solvent. Firstly, the optimal composition of the solvent was determined by testing various mixtures of heptane and a 50:50 solution of ethyl acetate and heptane, the two solvents being chosen based on preliminary experiments. Six sets (n = 3) of 20 mL solutions containing 20 μg/L each of GSM and MIB were created. The samples were applied to the SPE column with a flow rate of approximately 5 mL/min and eluted as follows for each set of replicates:

1) Eluted with 1000 μL ethyl acetate/heptane
2) Eluted with 800 μL ethyl acetate/heptane, followed by 200 μL heptane
3) Eluted with 500 μL ethyl acetate/heptane, followed by 500 μL heptane
4) Eluted with 400 μL ethyl acetate/heptane, followed by 600 μL heptane
5) Eluted with 300 μL ethyl acetate/heptane, followed by 700 μL heptane
6) Eluted with 1000 μL heptane.

Further experiments included determining an optimal eluent volume. Three sets (n = 3) of 20 mL solutions containing 20 μg/L each of GSM and MIB were aspirated to the SPE column with a flow rate of approximately 5 mL/min and initially extracted with 700, 1000 and 1300 μL 50:50 ethyl acetate/heptane. Subsequently, a second extraction

was made of each sample with 700 μL 50:50 ethyl acetate/heptane. All extractions were dried with anhydrous Na_2SO_4, centrifuged and analysed with GC Orbitrap.

A final experiment with respect to analyte recovery involved adding a salt solution to the samples to investigate possible effects of increased aqueous polarity. Two sets (n = 3) of 25 mL solutions containing 8.0 μg/L each of GSM and MIB were created. To the first set of replicates, no salt solution was added and the sample was applied to the SPE column, extracted and analysed as the previous experiment. To the second set of replicates, 3.0 mL of a saturated NaCl(aq) solution was added and the resulting solution was aspirated, extracted and analysed as the previous experiment.

4.2.3 Internal standards

Quantification via the use of internal standards was investigated. The compounds camphor and 1-methylcyclohexanol (depicted in Figure 4.1) were chosen as possible internal standards. Three replicates of 20 mL aqueous samples containing 2.5 μg/L each of GSM and MIB, in addition to 120 μg/L camphor and 255 μg/L 1-methylcyclohexanol were created and extracted with SPE, by aspirating with a flow rate of approximately 6 mL/min in cartridges conditioned with 9 mL heptane, 3 mL DCM, 3 mL MeOH and then 3 mL H_2O, followed by extraction with 1.0 mL of a 50/50 ethyl acetate/heptane solution into separate reagent glasses and dried with approximately 1 g of anhydrous Na_2SO_4. Test tubes were centrifuged at 4000 rpm for 10 minutes and analysed with GC Orbitrap, with three injection replicates for each sample, yielding n = 9. The procedure was repeated with samples containing 1.5 μg/L GSM and MIB, 60 μg/L camphor and 127 μg/L 1-methylcyclohexanol.

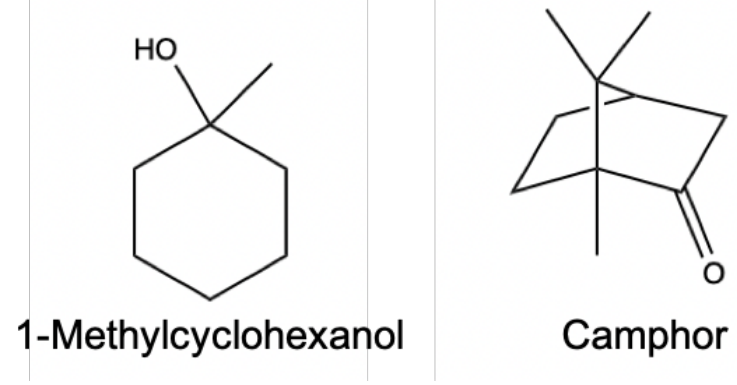


Figure 4.1: *1-Methylcyclohexanol (left) and camphor (right).*

4.2.4 Standard addition

Standard addition as a possible mode of quantification was also evaluated. Three aqueous 5.0 L solutions containing 5 ng/L each of GSM and MIB were created. Each replicate was divided into five 1.0 L aliquots and GSM and MIB were added to each of the five aliquots in an increasing series of 40, 50, 60, 70 and 80 ng. Each sample was aspirated with a flow rate of approximately 6 mL/min through SPE columns conditioned with 9 mL heptane, 3 mL DCM, 3 mL MeOH and then 3 mL H_2O, extracted with 1.0 mL of a 50/50 ethyl acetate/heptane solution into separate reagent glasses and dried with approximately 1 g of anhydrous Na_2SO_4. The replicates were centrifuged at 4000 rpm for 10 minutes and analysed with GC Orbitrap, with three injection replicates for each sample, yielding n = 9 for each concentration.

4.3 Total ion, extracted ion and base peak chromatograms

The chromatography of GSM and MIB was investigated. This was conducted in order to evaluate the use of TIC, EIC or BPC for quantification. A sample containing both GSM and MIB was created by combining approximately 100 μL of a 50 mg/L GSM solution in heptane, in addition to 100 μL of a 50 mg/L MIB in heptane solution, as well as approximately 150 μL of heptane in a GC vial. The sample was then analysed with GC Orbitrap (n = 3) and TICs, EICs and BPCs were acquired, the two latter by utilising 112 *m/z* and 95 *m/z* as quantitative ions for GSM and MIB respectively.

4.4 Temperature and split/splitless conditions for GC Orbitrap

The optimal temperature gradient was determined to yield S/N values as high as possible. Prior to these experiments, a temperature gradient had been developed by Professor Dag Ekeberg and senior engineer Hanne Devle and will be referred to as geosmin.meth (illustrated in Figure 4.2), which is the file name of this chromatographical method. Different factors of this temperature gradient were modified, namely the initial temperature, first temperature, hold times and temperature change rates. A series of 13 different temperature gradients were tested and compared with geosmin.meth and were named by numeration, e.g. geosmin2.meth and geosmin3.meth, continuing up to geosmin14.meth. The different temperature gradients are elucidated in Table A.1 in the appendix. All 14 programmes were run in 1:10 split mode.

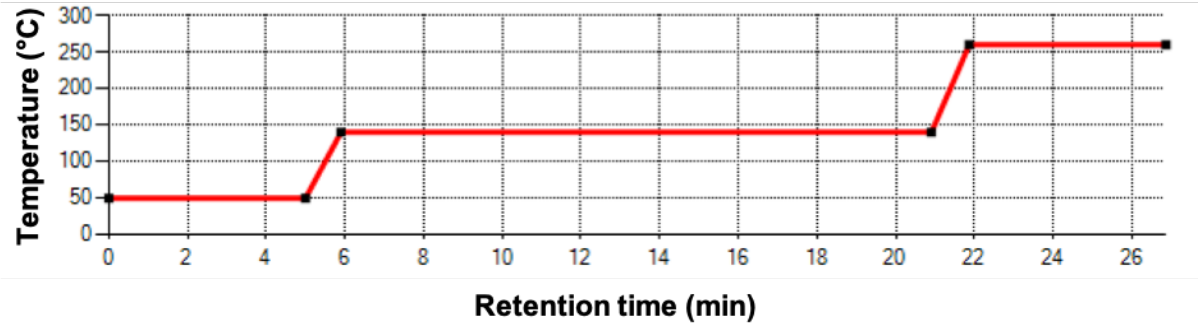


Figure 4.2: *GC Orbitrap temperature programming named geosmin.meth. The graph illustrates an initial temperature at 50 °C held 5 minutes, elevated 100 °C/min up to 140 °C, held 15 minutes and finally elevated 125°C/min up to 260 °C and held for 5 minutes.*

A sample containing both GSM and MIB was created to compare the different temperature gradients. Approximately 100 μL of a 50 mg/L GSM in heptane solution was transferred to a GC sample vial, in addition to 100 μL of a 50 mg/L MIB in heptane solution, as well as approximately 150 μL of heptane. The vial was capped and turned 10 times to homogenise the solution. Subsequently, the sample was subjected to GC Orbitrap analysis with the beforementioned 14 temperature gradients, each performed three times to yield three injection replicates.

Optimal split/splitless conditions were also evaluated. Three 1.0 L aqueous solutions containing 50 ng/L of both GSM and MIB were initially aspirated through a conditioned (with 9 mL heptane, 3 mL DCM, 3 mL MeOH and then 3 mL H_2O) SPE column and extracted with 1.0 mL of a 50/50 ethyl acetate/heptane solution into separate reagent glasses containing approximately 1 g of anhydrous Na_2SO_4 to remove excess water remnants. The three replicates were centrifuged at 4000 rpm for 10 minutes and 300 μL of each extraction was placed in GC vials. The sample replicates were each analysed three times with 1: the abovementioned geosmin.meth programme, 2: geosmin.meth in 1:5 split mode and 3: geosmin.meth in splitless mode.

5.0 Results and Discussion

Figures 5.0 and 5.1 display the obtained mass spectra of GSM and MIB respectively. The compounds were identified with library comparison of EI from NIST20. It should be noted that the M^+ of GSM at *m/z* 182 yields a low peak, but is indeed present. The peak has therefore been elucidated.

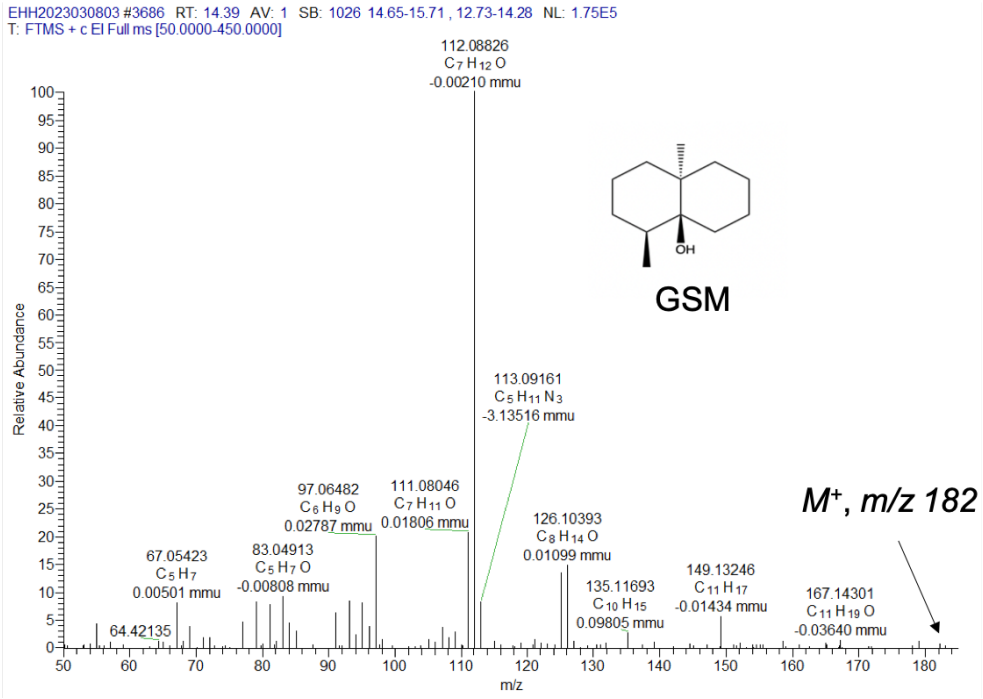


Figure 5.0: *Obtained mass spectrum of GSM.*

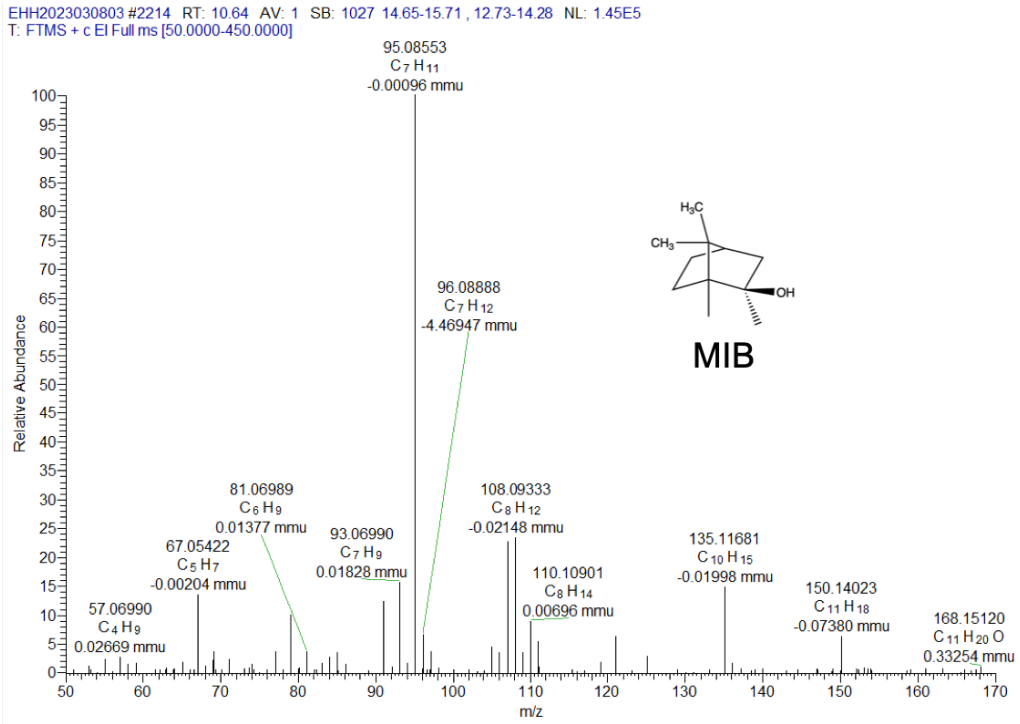


Figure 5.1: *Obtained mass spectrum of MIB.*

5.1 Liquid-liquid extraction

Results from the preliminary experiments assisted in subsequent analyses. Firstly, it was determined that ordinary LLE extractions applying an equal volume of organic solvent as the water sample were not applicable to detect and quantify GSM and MIB concentrations below the μg/L level. This was believed to be a cause of insufficient concentration levels of analytes inadequate for GC Orbitrap analysis. With regards to the evaporation experiments, all samples, regardless of concentration, did not yield peaks of GSM in the chromatograms (see Appendix Figure A.1). This significant loss of analyte is likely attributed to the semi-volatile nature of the compound and its vapour pressure. As MIB is even more volatile than GSM, it is therefore expected that similar observations would have been made with MIB. The volatile characteristics of these compounds evidently pose a grand disadvantage when attempting to evaporate off solvents, while being a clear benefit if utilised in headspace/P&T analysis as mentioned in chapter 2.2.1. Finally, 1-methylcyclohexanol did not express satisfactory similar properties to that of GSM, all though the compound was not compared with MIB. This compound was considered as an internal standard due to it being a tertiary alcohol like GSM and MIB, but the structure of the compound implies a higher polarity (one OH group to seven carbons) and thus a lower log K_{OW} of approximately 1.3 (NIH, 2023a). Further experimentation with regular LLE was discontinued due to the abovementioned results being inauspicious.

Results from the LLME experiments performed poorly against literature values. GSM and MIB at concentrations below 100 ng/L were not detected in two of the replicates in split mode (1:10), but were detected in splitless mode. Results were however not satisfying with regards to literature values. A chromatogram of the one 1:10 split replicate which yielded chromatographical peaks is available in the Appendix (Figure A.2). The methodology described in chapter 4.1 was mostly adapted from two experiments described in the literature, where the main difference in extraction was the utilisation of heptane as the organic solvent, as opposed to pentane or hexane (Ma et al., 2007; Shin & Ahn, 2004). Beforementioned experiments from the literature did also apply GC-MS analysis in SIM mode which was not chosen with the instrument used in experiments this thesis is based on. Shin and Ahn (2004) reported LOD values of 0.1 ng/L for both MIB and GSM with their method, while Ma et al. reported LOD values of 1.0 ng/L and 5.0 ng/L for GSM and MIB respectively.

Based on these results, it is proposed that LLME GC Orbitrap is not applicable for analysis of concentrations below 100 ng/L for GSM and MIB. Choosing heptane as an organic solvent was mainly based upon environmental and health purposes, but the experiment was as mentioned repeated with pentane and hexane to properly reflect the studies from Shin and Ahn (2004) and Ma et al. (2007). MIB was observed beneath LOQ, which may be attributed to its lower log K_{OW} value and the fact that it may possess stronger affinities to more polar solvents. Prior to this experiment, extraction with a 50/50 mixture of ethyl acetate was additionally attempted, but the organic solvent became completely suspended in the aqueous phase and was inseparable. The hydrophobicities of pentane, hexane and heptane were therefore preferred as a proper separation of aqueous and organic phase was possible without the use of large centrifugal equipment capable of handling 200 mL samples.

5.2 Solid-phase extraction

5.2.1 Conditioning

It was hypothesised that some contaminants may be present on the SPE column. To investigate this, deionised water was run through an SPE column to detect the presence of lipophilic contaminants. This was assumed to be relevant, since the SPE column consisted of a lipophilic C18 material and a non-polar solvent would be utilised for extraction and would thus dissolve any lipophilic contaminants. The chromatogram in Figure 5.2 represents one of the chromatograms obtained when solely conditioning SPE columns with water.

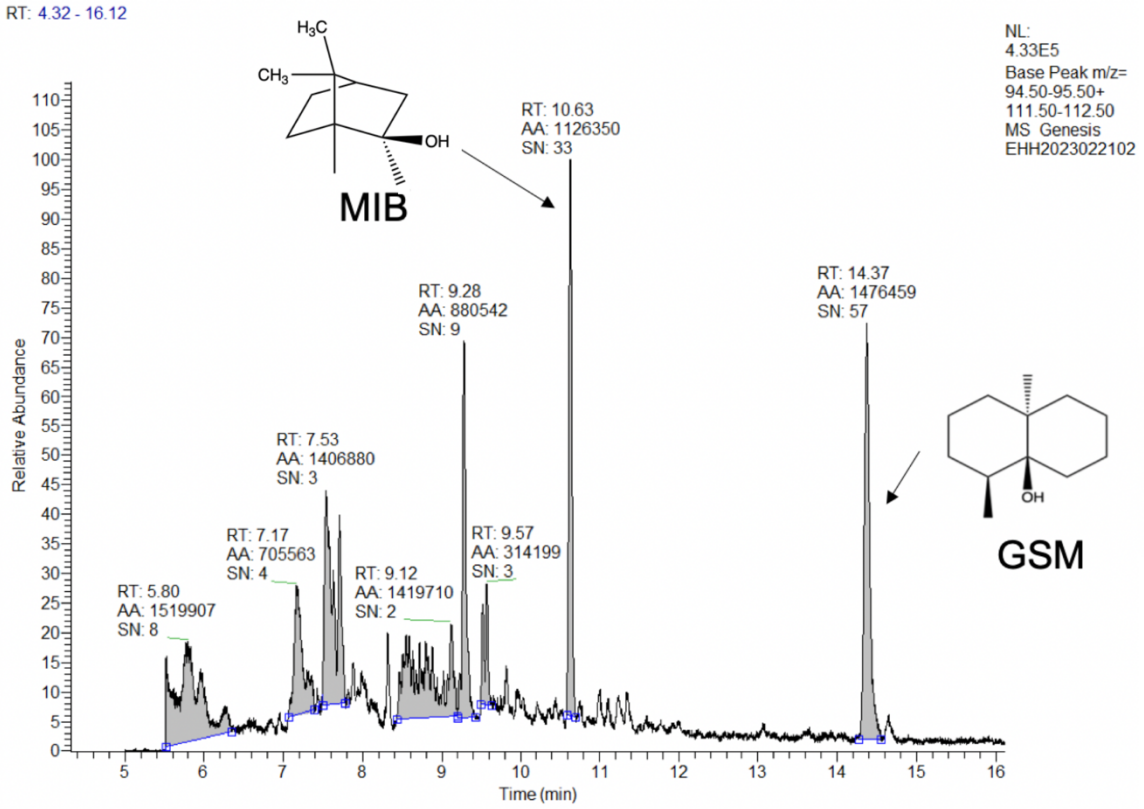


Figure 5.2: *Base peak chromatogram displaying MIB at 10.63 min and GSM at 14.37 min of a sample aspirated through an SPE column conditioned with water.*

Preliminary experiments revealed issues with noise especially with the elution of MIB. It was therefore hypothesised that this noise may significantly influence the S/N of MIB, which generally was lower than the S/N of GSM, eluting without much noise. Investigation of mass spectra of the noise peaks suggested that the noise likely was a result of hydrocarbon contaminants. It was therefore attempted to remove contaminants by starting the conditioning with heptane and the following chromatogram represents the results from this experiment. Additionally, it should be revealed that the S/N values calculated from the chromatographical peak integration are based on an automatic algorithm executed by the software. All further S/N values reported in this experiment are based on said algorithm for consistency.

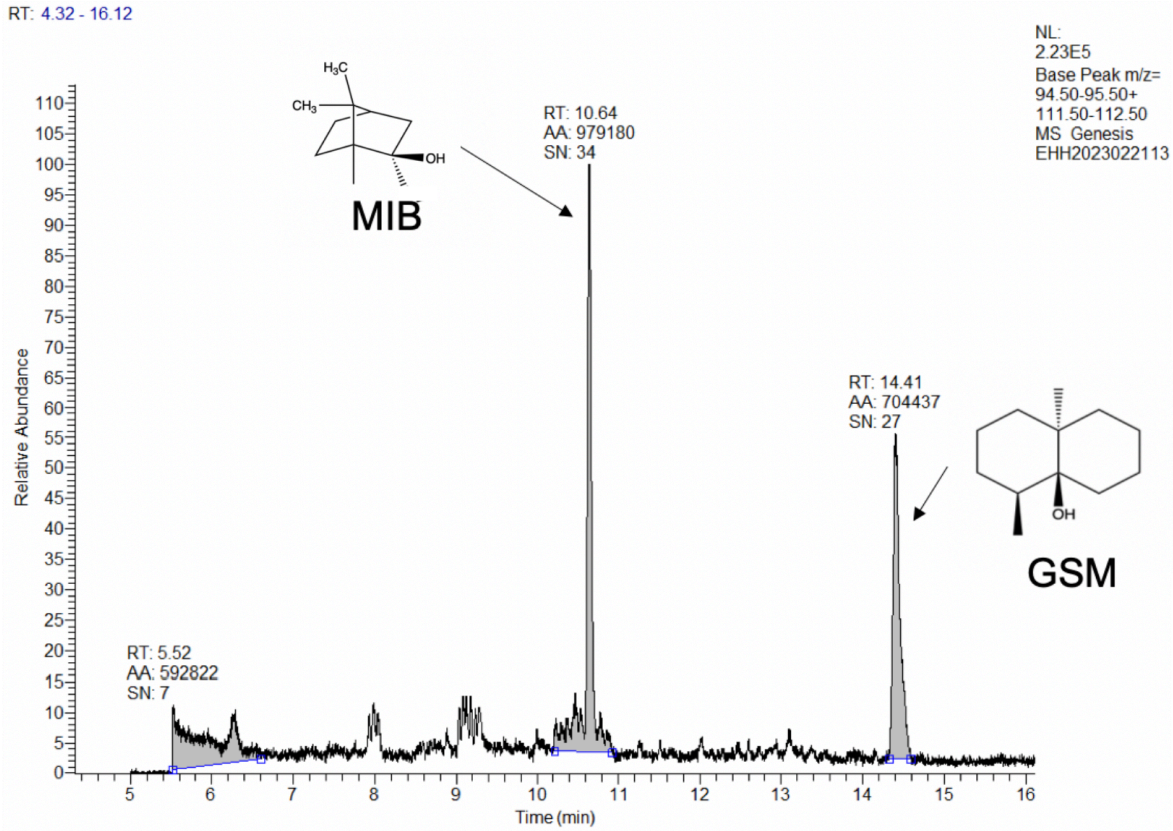


Figure 5.3: *Base peak chromatogram showing MIB at 10.64 min and GSM at 14.41 min of a sample aspirated through an SPE column conditioned with heptane.*

As visualised in Figure 5.3, contaminants were evidently removed by starting the conditioning with heptane. Interestingly, the GSM peak at 14.41 minutes decreased drastically both with regards to peak area and S/N. The peak area and S/N values from the nine injection replicates were averaged and presented in Figure 5.4 and 5.5.

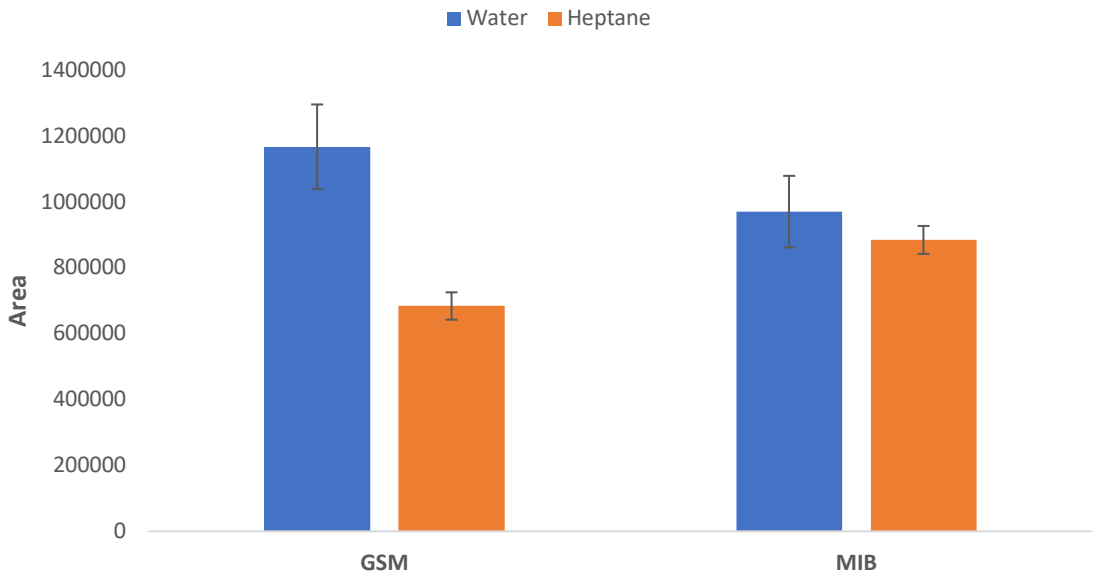


Figure 5.4: *Peak area of SPE aspirated aqueous GSM and MIB samples with different conditioning liquids; water (blue) and heptane (orange).*

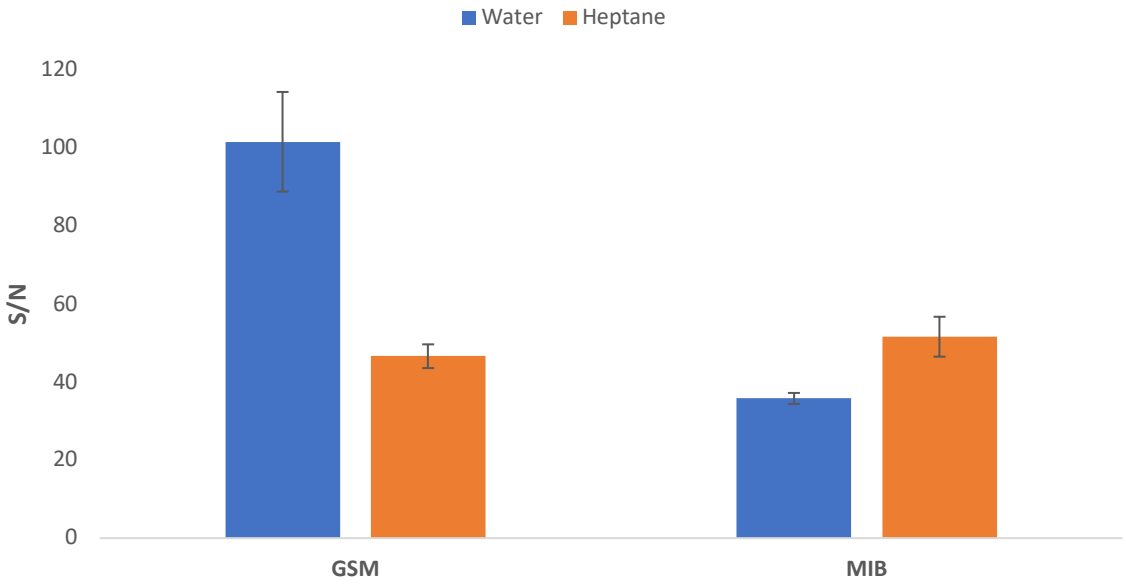


Figure 5.5: *S/N of aqueous GSM and MIB samples extracted with SPE, conditioned with different liquid; water (blue) and heptane (orange).*

All though contaminants were removed, it is evident that conditioning with heptane introduced challenges with the extraction of the analytes. Quite interestingly, both the peak area and S/N values of GSM were drastically depressed. On the contrary, the peak area of MIB decreased, with a remarkable increase in S/N. This supports the hypothesis that column contaminants were responsible for some of the S/N issues associated with MIB. As the extraction of GSM decreased relatively less than the

extraction of MIB, it was hypothesised that remnants of heptane on the SPE column may be the cause of this observation. The analytes may have partitioned into heptane residues and may further have been removed from the column mechanically by the flushing of the water sample. This is supported by the higher loss of GSM, as this compound is more lipophilic than MIB, explained by their respective log K_{OW} values of 3.570 and 2.931.

The following experiments were conducted to remove residual heptane. Conditioning with water was performed as a control and a new conditioning method was tested, involving a conditioning sequence of heptane, DCM and MeOH. These results are visualised in the chromatograms in Figures 5.6 and 5.7. For comparison of a pure sample containing only GSM and MIB, a chromatogram is available in the Appendix as Figure A.3.

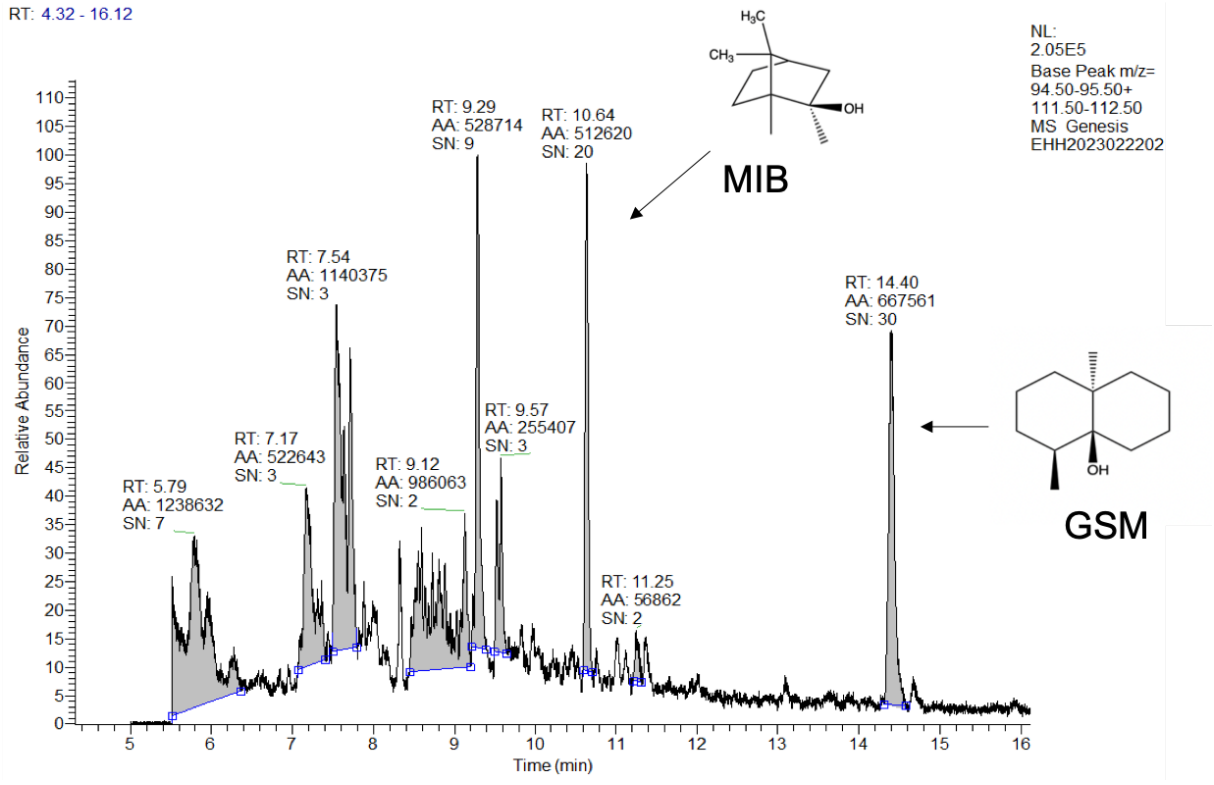


Figure 5.6: *Base peak chromatogram displaying MIB at 10.64 min and GSM at 14.40 min, of a sample aspirated through an SPE column conditioned with water.*

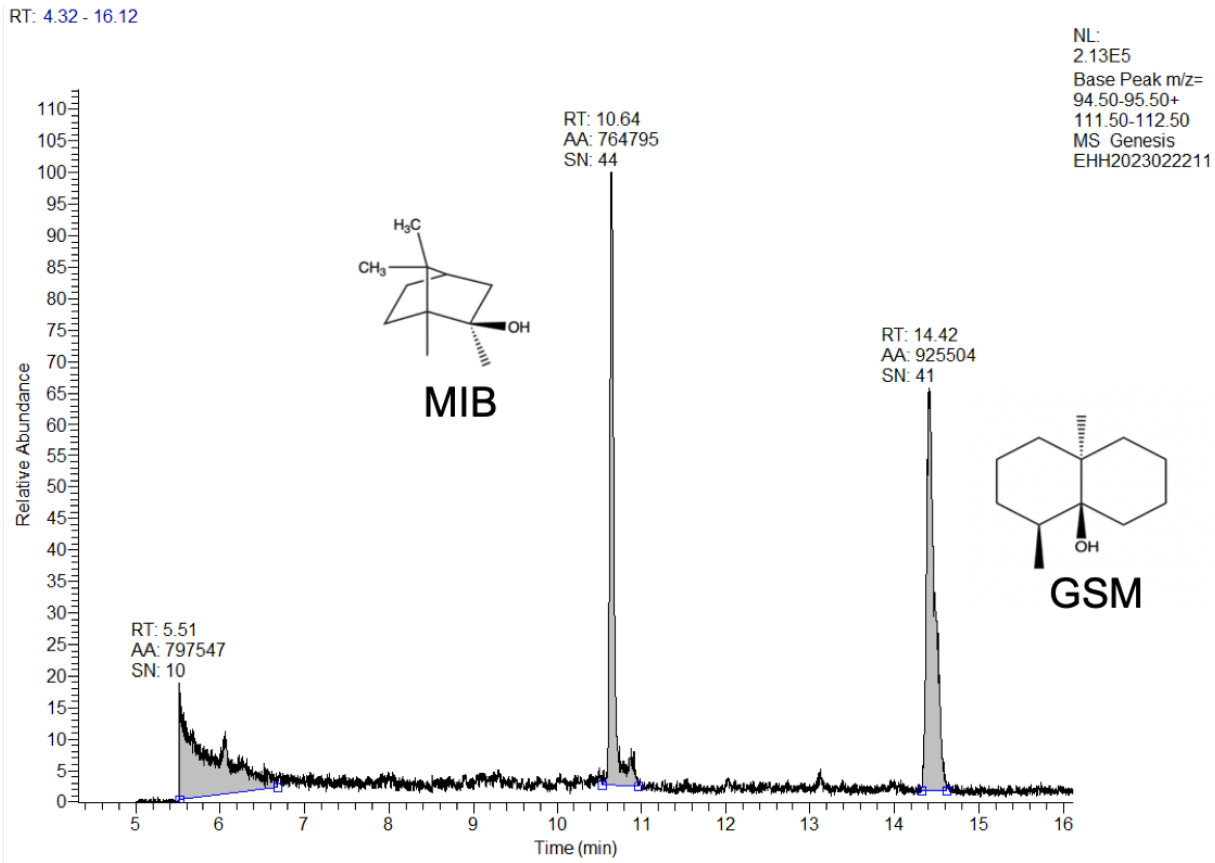


Figure 5.7: *Base peak chromatogram of MIB at 10.64 min and GSM at 14.42 min, of a sample aspirated through an SPE column conditioned with a sequence of heptane (9 mL), DCM (3 mL), MeOH (3 mL) and water (3 mL).*

It is evident that conditioning with heptane, DCM and MeOH removed contaminants even further. This resulted in a drastic elevation in the S/N of both GSM and MIB, further elucidating the importance of removing column contaminants. DCM, a solvent miscible with heptane, was chosen to remove residual heptane. DCM is miscible with MeOH and could therefore subsequently be removed. Adding water to the cartridge after this conditioning sequence finalised the conditioning. The average peak areas and S/N values from this experiment are visualised in Figures 5.8 and 5.9.

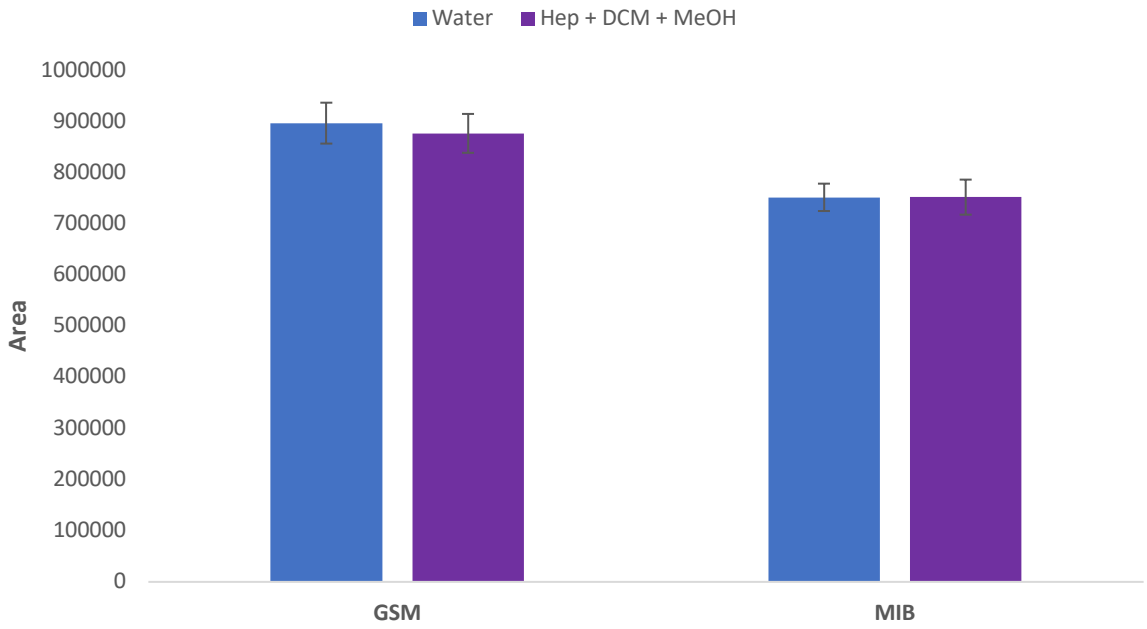


Figure 5.8: *Demonstration of peak areas for aqueous GSM and MIB samples extracted with SPE columns conditioned with water (blue) and a sequence of heptane, DCM, MeOH and water (violet).*

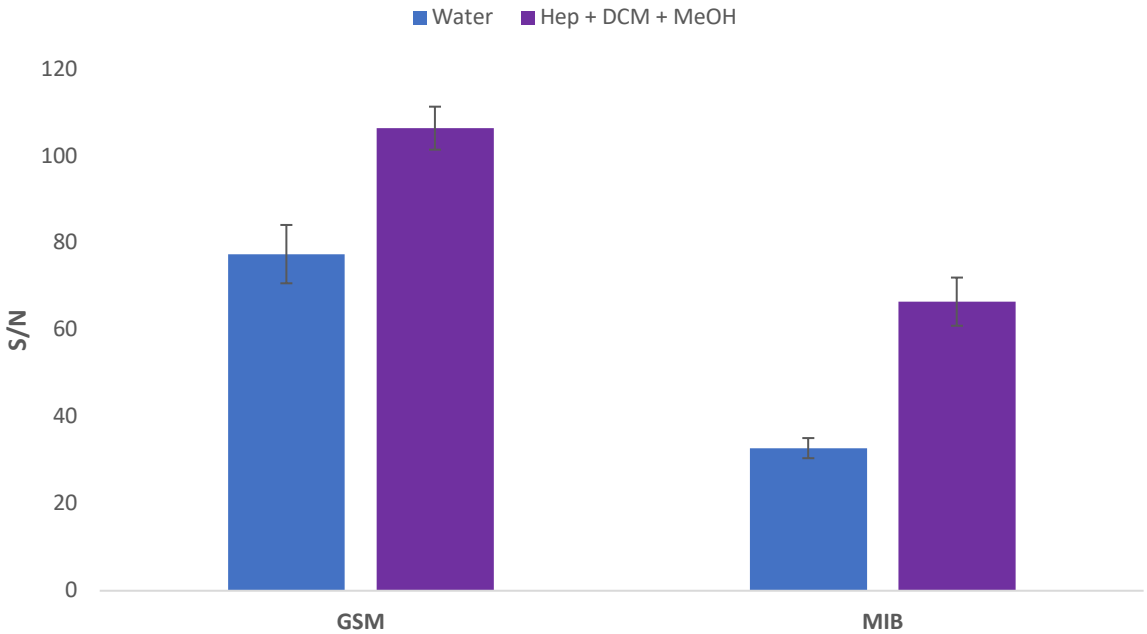


Figure 5.9: Elucidation *of S/N for aqueous GSM and MIB samples extracted with SPE columns conditioned with water (blue) and a sequence of heptane, DCM, MeOH and water (violet).*

Conditioning with heptane, DCM and MeOH appears to be beneficial. The peak area of GSM appears to somewhat decrease, while the peak area of MIB was retained with the new conditioning sequence. It should however be mentioned that the decrease in GSM peak area appears to be inconsequential, with respect to the overlapping error bars. On the other hand, it is revealed that the S/N of both GSM and MIB are elevated remarkably when this conditioning sequence is performed prior to sample application. In conclusion, a conditioning sequence such as the one developed in this experiment should be applied in order to achieve higher S/N values and thus improve the LOD and LOQ of both compounds.

5.2.2 Optimising extraction

Figure 5.10 illustrates the results obtained from the flow rate experiments described in chapter 4.2.2. Identical aqueous GSM and MIB solutions were aspirated through SPE columns with average flow rates of 1.1, 2.3, 5.2, 10.4 and 14.9 mL/min.

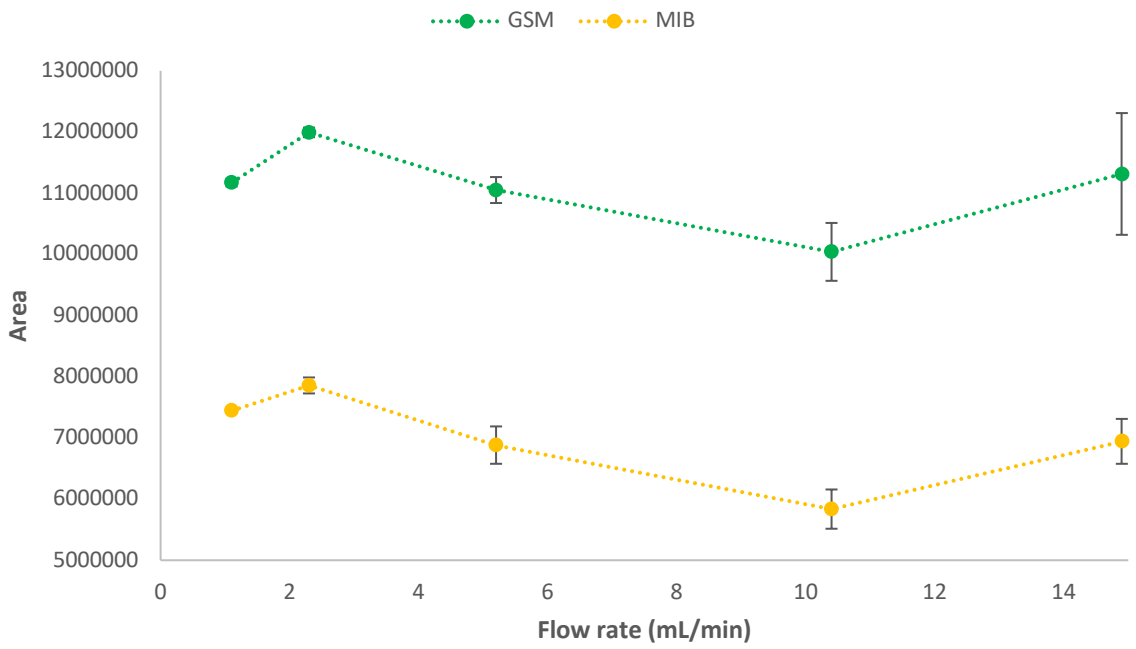


Figure 5.10: *Investigations in flow rate effect on SPE extractions (n = 9). The figure displays average area values on the y-axis of 5 identical solutions containing 20 µg/L of GSM (green) and MIB (yellow) aspirated through SPE columns at different flow rates on the x-axis.*

Figure 5.10 does not appear to indicate a consistent decrease in peak area with increasing flow rate through the SPE columns. From approximately 2 until 10 mL/min, the figure does express an apparent linear decrease in area, but this is however followed by a remarkable elevation with 15 mL/min. This observation somewhat contradicts current understandings of flow rate effect on SPE with respect to analyte recovery and that the maximum flow rate should not exceed 10 mL/min (Svahn & Björklund, 2019). Variations in recovery expressed in the figure may be a combination of several factors, such as loss of analyte through evaporation and unknown differences in conditioning. As n = 9 for each data point, in addition to the relative standard deviation (RSTD) values observed in the figure, it may also be suggested that the variation in recovery may be a result of general inconsistencies associated with SPE (Bristow et al., 2019). This observation poses important information with regards to SPE analysis of odorous compounds in water samples, as the difference in the total time of analysis can be reduced significantly in large quantity water samples. As it is apparent that at least GSM and MIB behave quite similarly with respect to flow rate, emphasis should instead be laid on managing a similar flow rate of each sample, to avoid potential sources of error.

Optimisation of solvent composition was carried out by mixing ethyl acetate and heptane at different ratios. Each extraction was conducted with 1 mL of solvent. Figure 5.11 reflects recorded peak areas of each extraction with various compositions of ethyl acetate and heptane.

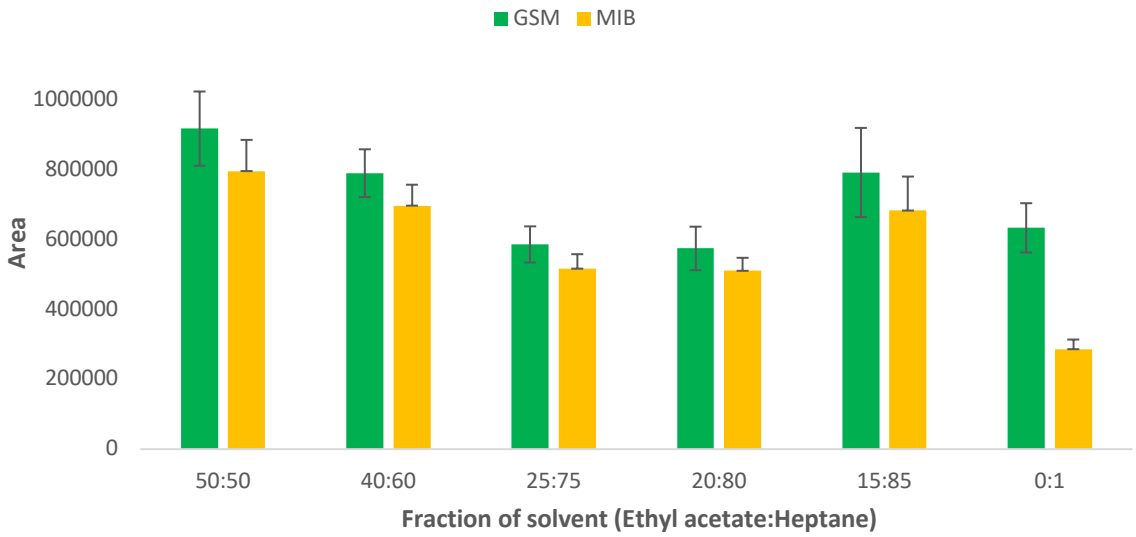


Figure 5.11: *Extractions of 20 mL solutions containing 20 μg/L each of GSM and MIB with 1.0 mL of different ethyl acetate/heptane compositions.*

The figure above suggests that the optimal solvent mixture is composed by equal amounts of ethyl acetate and heptane. Interestingly, contenders to this mixture were the 40/60 and 15/85 mixtures. Excluding the 15/85 mixture, elution of GSM and MIB appear to decrease as the fraction of heptane elevates, with the lowest peak area of MIB observed when pure heptane is utilised, attributed to its lower log K_{OW} and affinity to slightly higher polarities in solvents (Ikai et al., 2003; TGSC, N.d.-a). The 50/50 ethyl acetate/heptane mixture was therefore determined to be the optimal choice for extraction with SPE.

The volume of the eluent poses a significance on elution efficiency (Sharifi & Hadjmohammadi, 2006). Emphasis was in this thesis laid on finding the extraction volume which yielded a higher chromatographical peak, while avoiding loss of sample. The first figure below elucidates peak areas obtained with concentrated samples extracted with volumes of 700, 1000 and 1300 μL. Figure 5.12 represents peak areas obtained where a second extraction of 700 μL was conducted on the already extracted columns in order to compare the level of analyte remnants on the SPE columns. Figure 5.13 demonstrates peak areas following a second extraction, reflecting remnants of GSM and MIB.

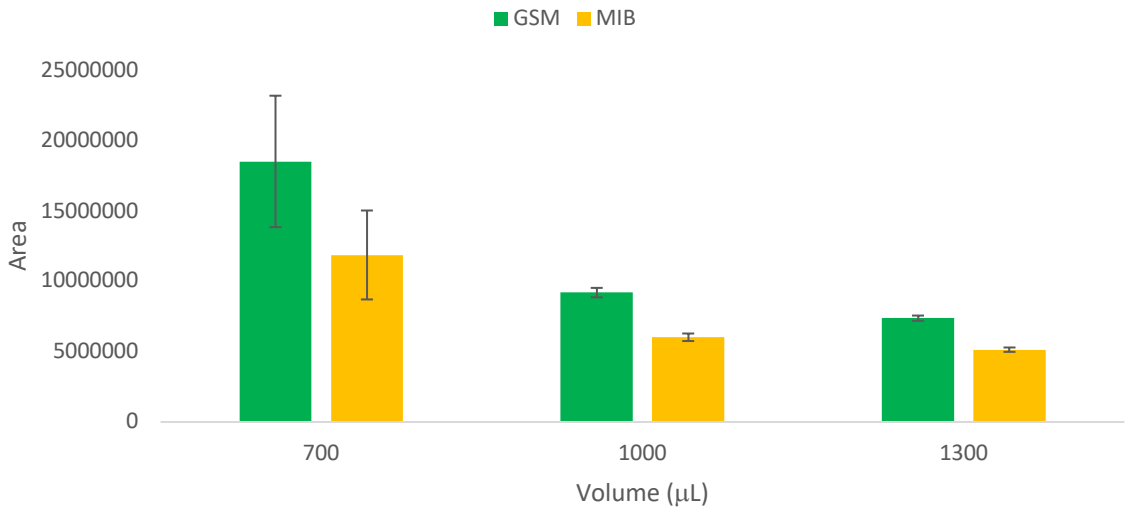


Figure 5.12: *Demonstration of peak areas of concentrated identical samples containing both GSM and MIB, extracted with various volumes of a 50/50 EA/heptane solution.*

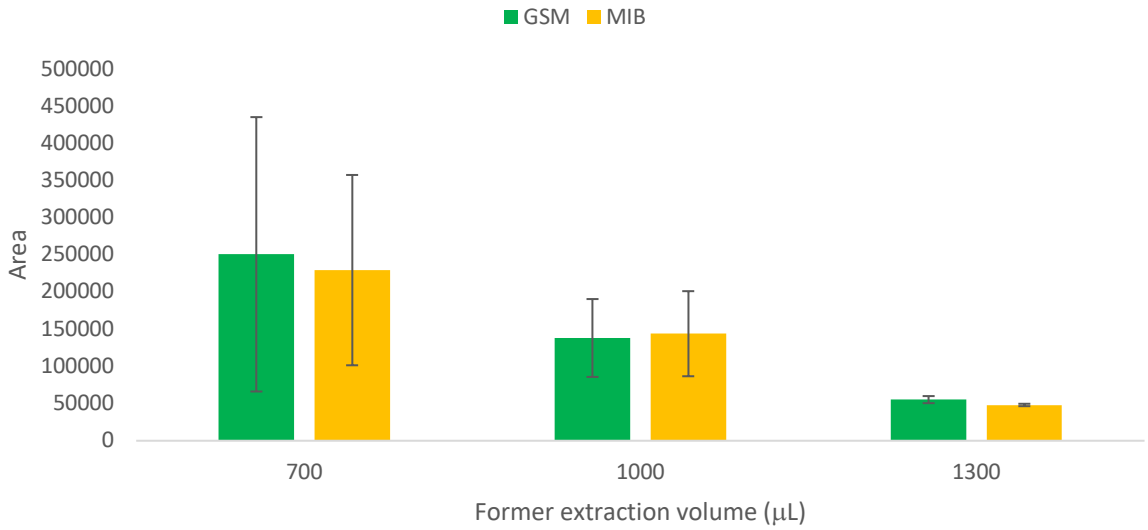


Figure 5.13: *Elucidation of peak areas of remnants in SPE columns following aspiration of identical concentrated samples containing both GSM and MIB, previously extracted with various volumes of a 50/50 ethyl acetate/heptane solution. Each sample was extracted with 700 µL of the 50/50 ethyl acetate/heptane solution.*

Peak areas were expectantly higher with the 700 µL second elution. This is explained by the fact that the concentrations of the elutions were higher as the solvent volume was lower, all though with inevitable remnants of analytes on the column as less solvent has forced elution of the compounds adsorbed to the C18 material. These

results do pose a challenging scenario, as a decision must be made with respect to both elution and a higher peak area. It was in this thesis decided that the 1000 μL solvent volume was optimal, being a medial choice sacrificing some peak area by yielding a higher elution efficiency than that of the 700 μL volume. It should as well be noted that the standard deviations (as elucidated by the error bars) are remarkably high following the 700 μL elution. Future experiments could involve further investigations in optimal elution volumes.

5.2.3 Internal standards

The internal standard candidates camphor and 1-methylcyclohexanol were evaluated by use of SPE. In order to be recognised as an appropriate internal standard, the two candidates were assessed based on linearity and consistent response factors to the analytes. Table 5.0 reflects results from these experiments, with determined response factors for each of the compounds and the two varying concentrations tested. Due to MIB and camphor possessing the same base peak ion, MIB did not express sufficient chromatographical peaks in this experiment as the concentration difference was too high between the two compounds. This resulted in only GSM being compared to the two compounds.

Table 5.0: Response factors of camphor and 1-methylcyclohexanol to geosmin.

Compound	Concentration [μg/L]	Response factor
Camphor	120	0.53
	60	0.56
1-methylcyclohexanol	255	0.40
	127	0.40

As demonstrated in the table, both camphor and 1-methylcyclohexanol appear to yield consistent response factors with GSM. On the other hand, camphor poses challenges with respect to the use of BPC and EIC as it produces the same base peak ion as MIB. This may be evaded if other quantitative ions are utilised, but may in turn sacrifice the S/N ratio of the chromatographical peak, due to less intensity relative to the base peak. Another option may be to utilise multiple reaction monitoring, which detects specific fragmentation reactions and will be specific to one compound (You et al., 2013).

Camphor as an internal standard has been utilised formerly in the literature with regards to quantification of GSM and MIB and consistency with SPE analysis is apparent as suggested with the results from this experiment (Wright et al., 2014). 1-Methylcyclohexanol has not been assessed in the literature and the results in Table 5.0 demonstrate that this compound may be applicable as an internal standard for SPE analysis. It should be revealed that the concentrations of both analytes and internal standards were relatively high when compared to the desired quantification limits at nanogram levels, and may therefore not properly reflect the suitability of the internal standards at lower concentrations. They do however impose some benefits with regards to health and environment, as opposed to current internal standards such as haloalkanes and halobenzenes (Churro et al., 2020; Yen et al., 2002).

5.2.4 Standard addition

Standard addition was also evaluated as a possible method for quantification. Spiking aliquots of samples with an increasing increment of analytes has significant benefits especially with respect to avoiding matrix effects in the analysis (Steliopoulos, 2015). Figure 5.14 displays an attempt of performing standard addition to a 5 ng/L water sample where each aliquot concentration was elevated with increments of 40, 50, 60, 70 and 80 ng/L.

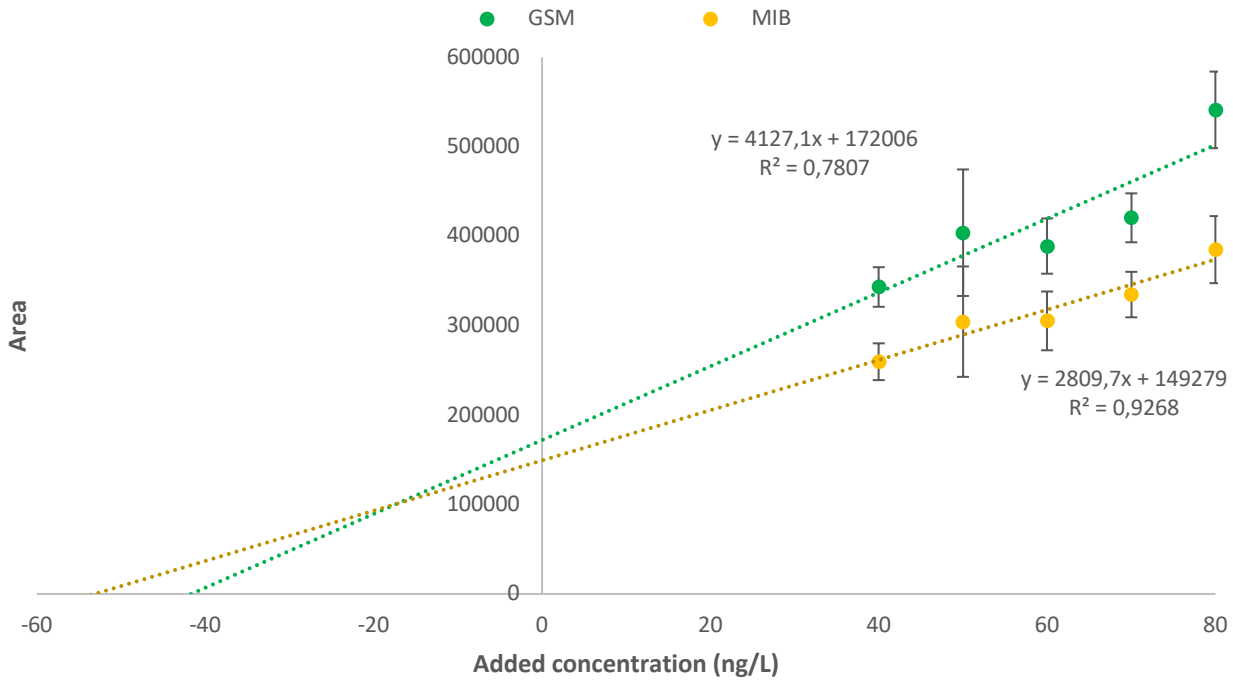


Figure 5.14: *Standard addition curves for GSM (green) and MIB (yellow). n = 9 for each data point from 40 ng/L – 70 ng/L and n = 6 for data points at 80 ng/L (due to experimental errors). GSM linear regression gave the equation y = 4127.1x + 172007 and an R^2 value of 0.7807. MIB linear regression gave the equation y = 4809.7x + 149279 and an R^2 value of 0.9268.*

The linear models created in Figure 5.12 allowed for the calculation of the proposed concentrations of GSM and MIB. As each aliquot has a spiked amount of analyte, standard addition reflects the sample concentration in its x-intercept value as y = 0, appearing on the negative side of the axis. With the linear models, the concentration of GSM was calculated to 41.7 ng/L and MIB to 53.1 ng/L, being eight-folds and ten-folds higher than the actual concentrations of these compounds, respectively.

There is strong evidence that standard addition is not applicable in SPE analysis of GSM and MIB. Firstly, surprisingly low goodness of fit was observed especially with GSM and there are therefore indications that the method's linearity is not satisfactory for quantification. Secondly, some of the data points expressed high RSTD values as visualised with the error bars. It should be revealed that some preliminary experiments yielded promising results with regards to standard addition, but as the experiments were repeated, extreme variations in calculated concentrations were observed and the method can therefore not be deemed reproducible. Matrix effects are less relevant

sources of error in this experiment, as all samples were created with the same stock solution. There may be other causes of these errors and such severe inconsistencies has in general been observed with larger sample sizes, with this one including samples of 1.0 L. Experiments have been conducted with lower volume samples that are more concentrated, yielding more consistent results. Additionally, inconsistencies associated with SPE is not an uncommon observation in the literature when used in GSM and MIB analysis (Bristow et al., 2019; Watson, 2017b).

5.3 Total ion, extracted ion and base peak chromatograms

The chromatography of GSM and MIB was investigated. Firstly, it was decided that TIC was not an applicable mode of quantifying these analytes, as little to no chromatographical peaks of GSM and MIB were observed in the chromatogram. They were however quantifiable in both BPC and EIC, illustrated in Figures 5.15 and 5.16. The base peak ions were *m/z* 95 for MIB and *m/z* 112 for GSM in BPC, with the same quantitative ions chosen for the EIC, as suggested in Ganegoda et al. (2020) and Tian et al. (2021).

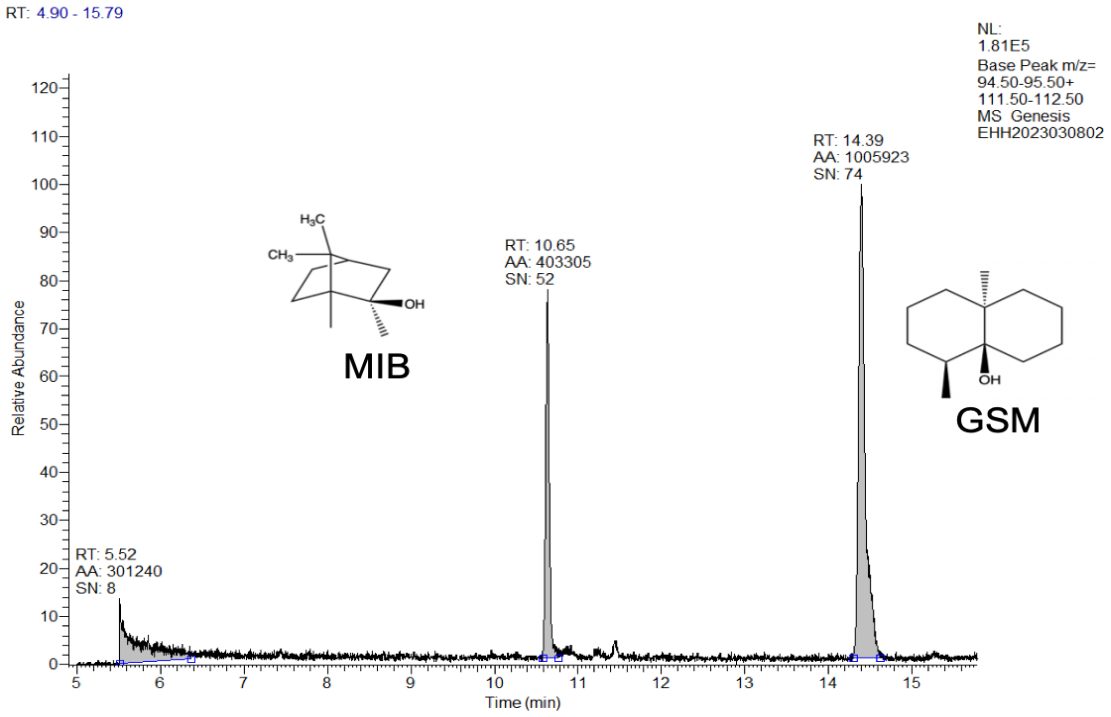


Figure 5.15: *Base peak chromatogram of MIB at 10.65 min with an S/N ratio of 52, while GSM appears at 14.39 min with an S/N ratio of 74. The BPC was constructed with m/z 95 (MIB) and m/z 112 (GSM) as base peak ions.*

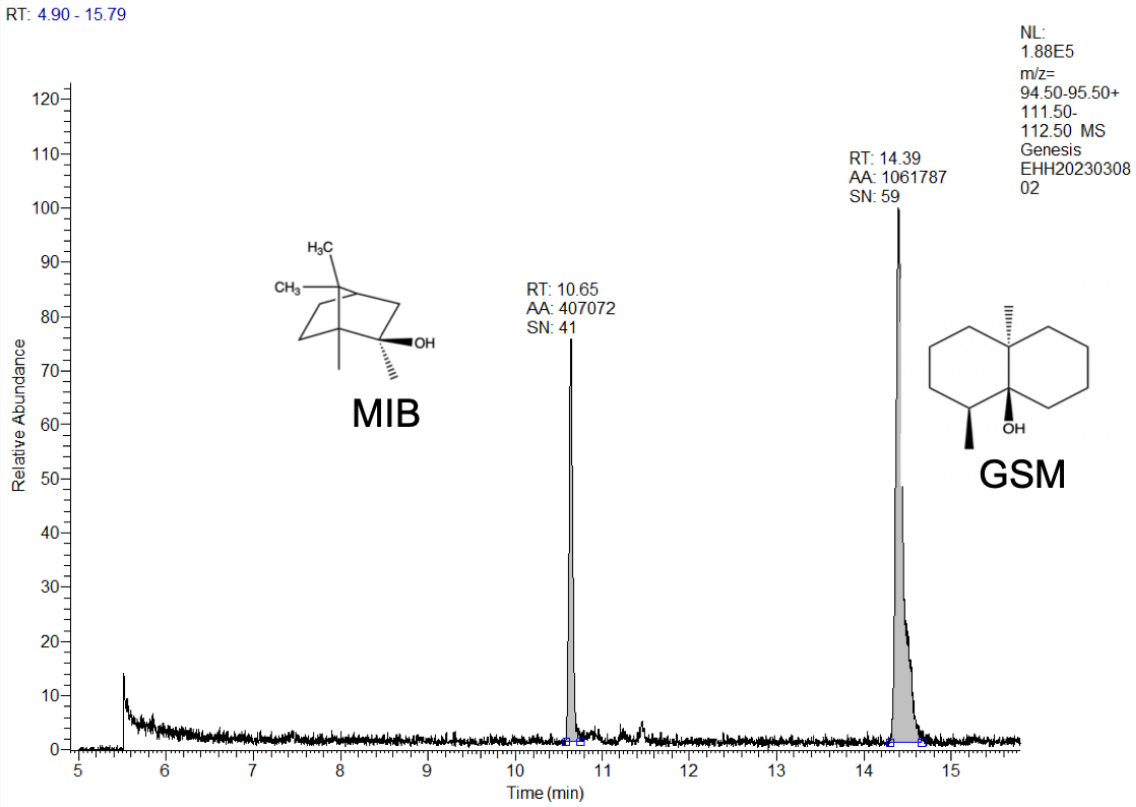


Figure 5.16: *Extracted ion chromatogram of MIB at 10.65 min with an S/N ratio of 41, while GSM appears at 14.39 min with an S/N ratio of 59. The EIC was constructed with m/z 95 (MIB) and m/z 112 (GSM) as quantitative ions.*

As seen in the figures, BPC yields the highest S/N for both compounds. On the contrary, a slightly higher peak area is observed in the EIC. As S/N ratio is a determining factor with respect to LOD and LOQ, it was decided that all chromatograms were to be analysed as BPC and not EIC.

5.4 Temperature and split/splitless conditions for GC Orbitrap

It may be proposed that the minimum amount of GSM and MIB applied to an SPE column should be approximately 20 ng and 30 ng respectively. This statement is based on S/N obtained and a summative deduction of the former observations and are rough estimates of LOQ values for the two compounds. This suggests that SPE coupled with GC Orbitrap may yield a maximum concentration of approximately 20 ng/L and 30 ng/L in 1.0 L samples if quantified with an appropriate internal standard. To decrease these values and improve the LOQ, experiments were conducted with respects to conditions in the GC Orbitrap instrumentation. Figure 5.17 illustrates S/N ratios of GSM and MIB obtained from 14 different temperature programmes where details of each programme

can be found in the appendix. Number 1 is the original temperature programme which has been utilised in nearly all the former experiments, while the following gradients are minor and major variations to this programme.

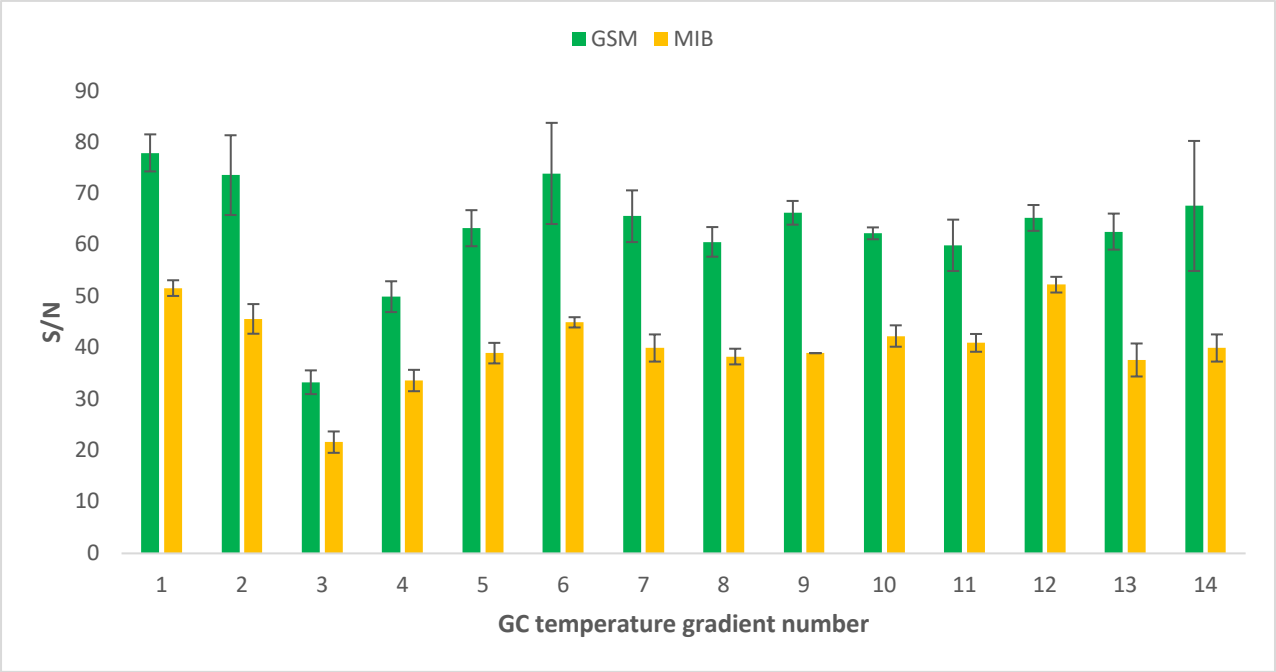


Figure 5.17: *Average S/N of the 14 different GC temperature gradient number (n = 3). An overview of the programming of each gradient is available in the Appendix.*

Figure 5.17 demonstrates that the original temperature gradient provides optimal S/N of GSM and the second highest of MIB. Temperature gradient number 12 expressed a slightly higher S/N for MIB, but by visually inspecting the bar chart, it can be observed that the relative standard deviations (STD) are overlapping, indicating that the difference may be inconsequential. It was therefore concluded that gradient number 1 was the optimal choice, with a temperature profile as visualised in Figure 4.2 in chapter 4.2.5. This also proposed that the LOQ of GSM and MIB in SPE analysis would not be improved by temperature programming alone, as this programme was utilised in the former experiments. It should also be mentioned that both the 50 mg/L solutions were more than 1.5 years old and may not reflect the actual concentrations due to degradation and/or loss of analytes. The solutions were still utilised due to this being a qualitative and not a quantitative analysis and the fact that all temperature gradients were compared by using the same sample.

Another aspect of improving LOD and LOQ values was hypothesised to involve split/splitless mode during the injection phase. As presented in the Methods section, samples injected to the GC Orbitrap were initially extracted with SPE, to possibly reflect how changes in the split and/or splitless mode may influence detection and quantification limits of the methodology. Figure 5.18 visualises S/N ratios of each split/splitless mode with identical samples.

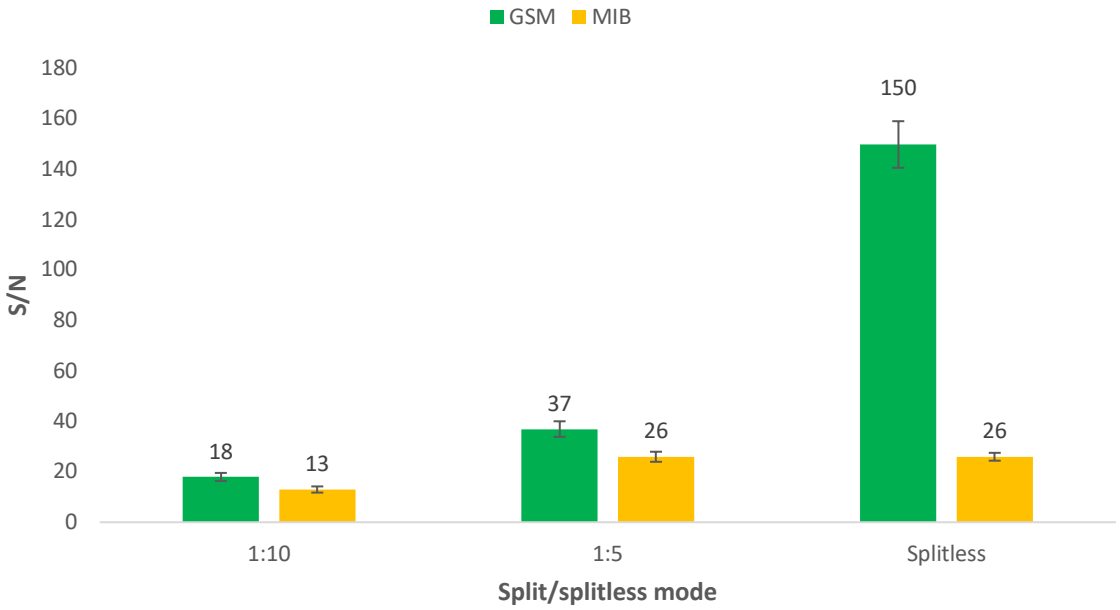


Figure 5.18: *Average S/N ratios of identical solutions run with different split/splitless modes (n = 9). Three 1.0 L solutions containing 50 ng/L of both GSM and MIB were extracted with SPE and run with temperature gradient number 1 in 1:10 split mode (left), 1:5 split mode (middle) and splitless (right).*

As seen in the Figure 5.18, decreasing the split increases the S/N of both GSM by the two-fold. The most significant increase is seen with GSM in splitless mode, with an S/N value of 150. Assuming this value to be proportional to the concentration of GSM, it may be roughly estimated that the LOQ of GSM in splitless mode in a 1.0 L sample is approximately 3 ng/L and LOD at approximately 1 ng/L, which now compares to some literature values (Bristow et al., 2019). Quantification and/or detection of GSM may therefore be achieved utilising a 1.0 L water sample and by the use of an appropriate internal standard. On the contrary, no increase is observed with respect to MIB. This may be attributed to several factors, such as band broadening, which is not uncommonly observed in splitless mode (Grob, 1985). Further inspection revealed

interesting observations with regards to the chromatographical peak areas, which are represented in Figure 5.19 in a bar chart.

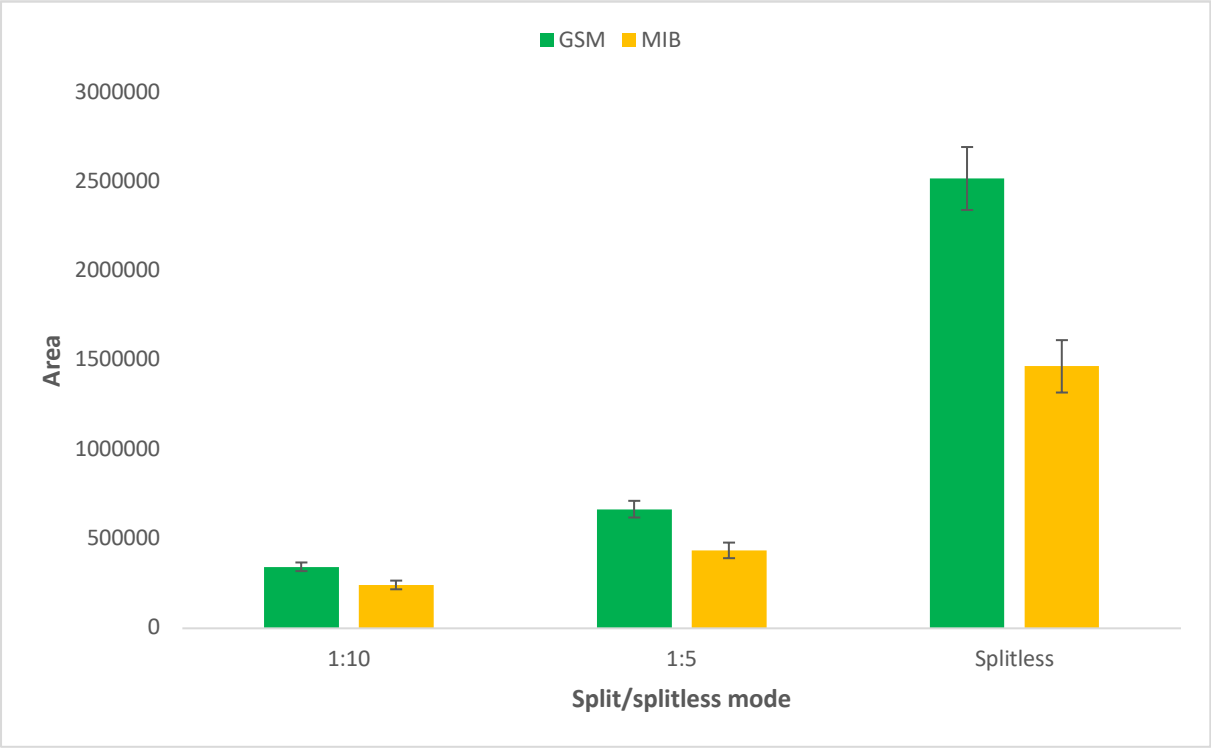


Figure 5.19: *Average peak areas (n = 9) of 1.0 L 50 ng/L GSM and MIB solutions extracted with SPE and run with different split/splitless modes (n = 9).*

The peak area does on the contrary to the S/N values follow a similar increasing trend with MIB as observed with GSM. This is expected, as reducing split and analysing in splitless mode allows a considerably larger amount of analytes into the GC column (Miller, 2009). These results indicate that MIB is subject to band broadening and/or other issues in splitless mode. In Figure 5.20, a BPC of MIB is presented as one of the injection replicates subjected to GC Orbitrap analysis in splitless mode.

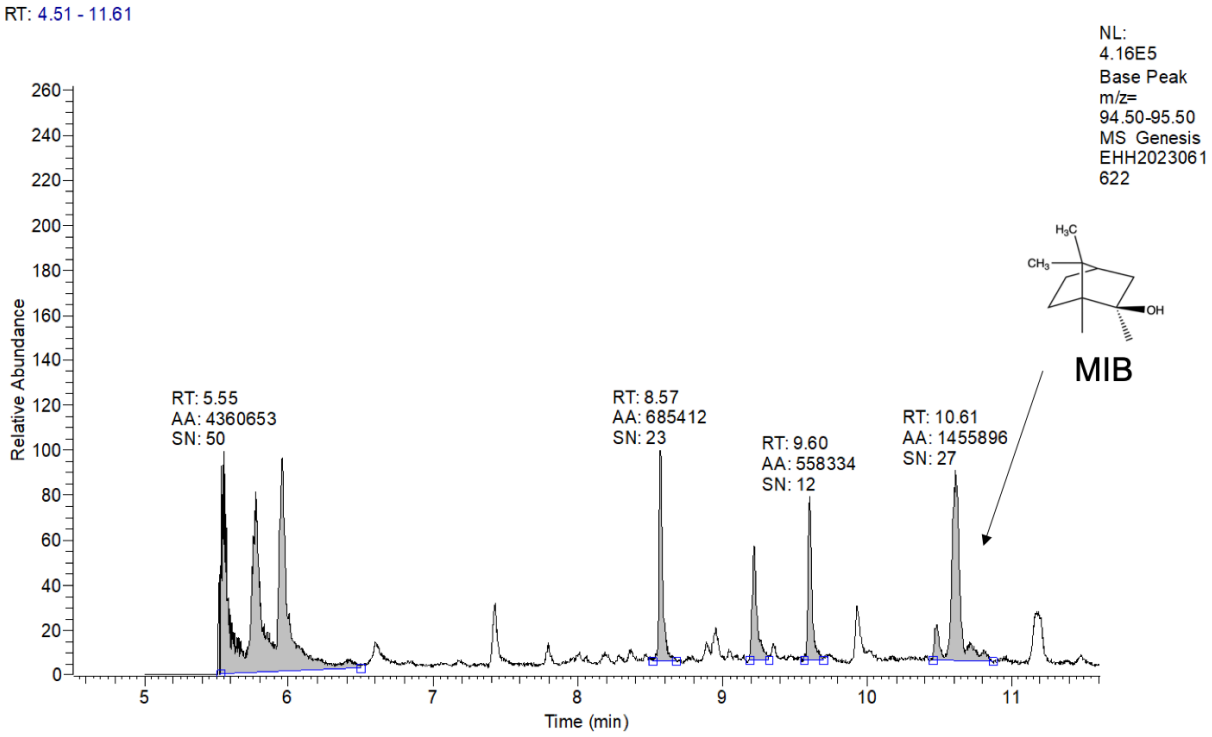


Figure 5.20: *Base peak chromatogram of MIB (10.61 min) in splitless mode.*

The chromatogram illustrates some interesting possible explanations of the low S/N ratio of MIB in splitless mode. Firstly, some contaminants initially disregarded in the conditioning experiments of chapter 5.2.1 reappear in splitless mode and may influence the S/N ratio. Secondly, as MIB expresses a significant peak area elevation in splitless mode, band broadening is most likely the second cause of the unexpected low S/N value, which may be deduced by investigating for example the contaminant peak at 8.57. The peak area of this contaminant is less than half than that of MIB, while the S/N only approximately 15 % less. Such observations may be indications of band broadening. Splitless mode may therefore not be appropriate for MIB analysis.

In summation, reducing the split in the GC Orbitrap programming increased the S/N and thus LOD and LOQ values of GSM and MIB. Exclusively for GSM, the LOQ and LOD was drastically improved in splitless mode as opposed to a 1:10 split. The greatest improvement of MIB was observed in the 1:5 split where the S/N doubled and thus the LOQ and LOD values were arguably halved to approximately 15 ng/L and 4.5 ng/L respectively in a 1.0 L sample with SPE analysis, which is higher than most literature values (Bristow et al., 2019). It should be mentioned that splitless mode demonstrated promising improvements in S/N ratios for GSM and could be utilised in trace analysis

of this compound. However, this may not be applicable for MIB as no improvement in S/N ratio was observed from 1:5 split to splitless mode. Further investigations could include even lower split ratios to improve the S/N of MIB without inducing band broadening.

6.0 Further Reflections and Considerations

Experiments in this thesis revealed that LLE and LLME were not appropriate extraction methods for GC Orbitrap with respect to GSM and MIB analysis. At best, LLME is assumed to achieve an LOQ and LOD of 20 and 6 ng/L respectively for GSM, while MIB may be estimated to have an LOQ value above 50 ng/L. This performs somewhat poorly to literature values and these extraction methods will therefore not be recommended for further analysis by GC Orbitrap analysis. SPE experiments ultimately yielded decent rough estimates of LOQ and LOD in splitless mode with GSM. However, for MIB using splitless mode, band broadening and poor chromatographical peaks were observed. One may therefore do injections both in reduced split mode for the purpose of detecting and quantifying MIB and in splitless mode in order to detect and quantify GSM. This however is more time consuming and less efficient. Overall, LLE and SPE coupled with GC Orbitrap do not compete well with current methods, especially the ones involving other extraction techniques.

The observations made with SPE conditioning and flow rate have proposed novel insight in the extraction of organic trace compounds from aqueous samples. Especially when analytes elute with similar retention times to column contaminants, the results discussed in chapter 5.2.1 can be valuable and the conditioning method may be applied to achieve cleaner chromatograms with reduced presence of contaminants. Additionally, results from the flow rate experiments in chapter 5.2.2 expressed that increasing the flow through the column does not sacrifice extraction efficiency as significantly as formerly thought and may allow for more efficiency. Additionally, camphor and 1-methylcyclohexanol did express promising results as internal standard candidates, all though the concentrations tested were at the microgram and not the nanogram level. Camphor has been tested in literature, but the experiments reveal 1-methylcyclohexanol as a novel internal standard candidate.

There were several extraction techniques which were not tested together with GC Orbitrap. Firstly, even though standard addition as a mode of quantification achieved poor results in SPE experiments involving large sample volumes, some more successful methods, such as headspace experiments and CLSA could be performed with standard addition. Several of these literature experiments may be executed with such low sample volumes that efficient standard addition methods possibly could be developed (Ikai et al., 2003; McGuire et al., 1981). There may be huge benefits to applying standard addition to these methodologies, namely 1: the certified standard solutions utilised in these experiments were relatively inexpensive, 2: avoidance of inaccuracies and matrix effects which may influence internal standards and 3: spiking water samples with GSM and MIB is less hazardous for the person performing the experiments and for the environment, as opposed to currently utilised internal standards such as haloalkanes and halobenzenes (Churro et al., 2020; Yen et al., 2002). Secondly, successful literature extraction methods are yet to be tested with GC Orbitrap and the sensitivity of the instrument may prove to be beneficial with other approaches.

7.0 Conclusions

This thesis involved efforts in optimisation of quantification of geosmin and 2-methylisoborneol utilising liquid-liquid extraction and solid-phase extraction coupled with GC Orbitrap. LLE, including LLME, did not yield results competing with literature values. With respect to SPE, a novel conditioning approach, involving a sequence of heptane, DCM, MeOH and water was developed, yielding improved extraction and S/N ratios. Other SPE efforts included investigating flow rate and solvent composition, where the experiments of the former concluded 1000 μL of a 50/50 mixture of ethyl acetate and heptane being optimal. Standard addition did not yield reproducible results with regards to quantification, but a novel internal standard candidate, 1-methylcyclohexanol, appeared to possess satisfactory response factors with GSM and likely MIB. Temperature programming was extensively tested and an optimal profile was determined. Split/splitless ratios were also investigated and it was observed that splitless was beneficial to GSM analysis, whilst a higher split ratio than 1:10 was optimal for MIB.

Bibliography

Abd El-Hack, M. E., El-Saadony, M. T., Elbestawy, A. R., Ellakany, H. F., Abaza, S. S., Geneedy, A. M., Salem, H. M., Taha, A. E., Swelum, A. A., Omer, F. A., AbuQamar, S. F., & El-Tarabily, K. A. (2022). Undesirable odour substances (geosmin and 2-methylisoborneol) in water environment: Sources, impacts and removal strategies. *Marine Pollution Bulletin*, *178*, 113579. https://doi.org/https://doi.org/10.1016/j.marpolbul.2022.113579

Alkarawi, D. (2016). *The Investigation of the Electrical Control of Hemimicelles and Admicelles on Gold for Analyte Preconcenctration* Western Kentucky University]. Bowling Green, Kentucky.

Assadi, Y., Farajzadeh, M. A., & Bidari, A. (2012). 2.10 - Dispersive Liquid–Liquid Microextraction. In J. Pawliszyn (Ed.), *Comprehensive Sampling and Sample Preparation* (pp. 181-212). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-381373-2.00051-X

Bartram, R. J., & Froehlich, P. (2010). *Considerations on Switching from Helium to Hydrogen*. Retrieved 13/02/23 from https://www.chromatographyonline.com/view/considerations-switching-helium-hydrogen

Berk, Z. (2018). Chapter 11 - Extraction. In Z. Berk (Ed.), *Food Process Engineering and Technology (Third Edition)* (pp. 289-310). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-812018-7.00011-7

Bristow, R. L., Young, I. S., Pemberton, A., Williams, J., & Maher, S. (2019). An extensive review of the extraction techniques and detection methods for the taste and odour compound geosmin (trans-1, 10-dimethyl-trans-9-decalol) in water. *TrAC Trends in Analytical Chemistry*, *110*, 233-248. https://doi.org/https://doi.org/10.1016/j.trac.2018.10.032

Brownlee, B., MacInnis, G., Charlton, M., Watson, S., Hamilton-Browne, S., & Milne, J. (2004). An analytical method for shipboard extraction of the odour compounds, 2-methylisoborneol and geosmin. *Water Science and Technology*, *49*(9), 121-127. https://doi.org/10.2166/wst.2004.0550

Bruchet, A. (2006). State of the art analytical methods for solving taste and odour episodes. *Water Science and Technology - WATER SCI TECHNOL*, *6*. https://doi.org/10.2166/ws.2006.799

Bruno, T., & Harries, M. (2019). Headspace Analysis: Purge and Trap. In: Encyclopedia of Analytical Science.

Buntinx, M., Maes, C., molder, m., Luyten, W., Herremans, G., Winckelmans, N., Peeters, R., & Carleer, R. (2020). Determination of the nitrogen gas transmission rate (N2GTR) of ethylene vinyl alcohol copolymer, using a newly developed permeation measurement system. *Polymer Testing*, *93*. https://doi.org/10.1016/j.polymertesting.2020.106979

Cane, D. E., He, X., Kobayashi, S., Ōmura, S., & Ikeda, H. (2006). Geosmin Biosynthesis in Streptomyces avermitilis. Molecular Cloning, Expression, and Mechanistic Study of the Germacradienol/Geosmin Synthase. *The Journal of Antibiotics*, *59*(8), 471-479. https://doi.org/10.1038/ja.2006.66

Cees, B., Zoeteman, J., & Piet, G. J. (1974). Cause and identification of taste and odour compounds in water. *Science of The Total Environment*, *3*(1), 103-115. https://doi.org/https://doi.org/10.1016/0048-9697(74)90042-4

Chen, L., Wang, X., Lu, W., Wu, X., & Li, J. (2016). Molecular imprinting: perspectives and applications [10.1039/C6CS00061D]. *Chemical Society Reviews*, *45*(8), 2137-2211. https://doi.org/10.1039/C6CS00061D

Christie, W. W. (1989). *Gas Chromatography and Lipids: A Practical Guide.* The Oily Press Ltd.

Churro, C., Semedo-Aguiar, A. P., Silva, A. D., Pereira-Leal, J. B., & Leite, R. B. (2020). A novel cyanobacterial geosmin producer, revising GeoA distribution and dispersion patterns in Bacteria. *Scientific Reports*, *10*(1), 8679. https://doi.org/10.1038/s41598-020-64774-y

Cook-Botelho, J. C., Bachmann, L. M., & French, D. (2017). Chapter 10 - Steroid hormones. In H. Nair & W. Clarke (Eds.), *Mass Spectrometry for the Clinical*

Laboratory (pp. 205-230). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-800871-3.00010-9

Dairi, T. (2010). 1.22 - Isoprenoid in Actinomycetes. In H.-W. Liu & L. Mander (Eds.), *Comprehensive Natural Products II* (pp. 789-814). Elsevier. https://doi.org/10.1016/B978-008045382-8.00011-3

Dass, C. (2007). *Fundamentals of Contemporary Mass Spectrometry* (1st ed.). Wiley-Interscience.

Dickschat, J. S., Wenzel, S. C., Bode, H. B., Müller, R., & Schulz, S. (2004). Biosynthesis of Volatiles by the Myxobacterium Myxococcus xanthus. *ChemBioChem*, *5*(6), 778-787. https://doi.org/https://doi.org/10.1002/cbic.200300813

Dionigi, C. P., Bett, K. L., Johnsen, P. B., McGillberry, J. H., Millie, D. E., & Vinyard, B. T. (1998). Variation in Channel Catfish Ictalurus punctatus Flavor Quality and its Quality Control Implications. *Journal of the World Aquaculture Society*, *29*(2), 140-154. https://doi.org/https://doi.org/10.1111/j.1749-7345.1998.tb00973.x

Engewald, W., & Dettmer-Wilde, K. (2014). Theory of Gas Chromatography. In K. Dettmer-Wilde & W. Engewald (Eds.), *Practical Gas Chromatography: A Comprehensive Reference* (pp. 21-57). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-54640-2_2

Engewald, W., Dettmer-Wilde, K., & Rotzsche, H. (2014). Columns and Stationary Phases. In K. Dettmer-Wilde & W. Engewald (Eds.), *Practical Gas Chromatography: A Comprehensive Reference* (pp. 59-116). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-54640-2_3

Ferguson, P., Cross, R., & Schad, G. (2022). Chapter 8 - Application of SFC for the characterization of formulated drug products. In M. Hicks & P. Ferguson (Eds.), *Separation Science and Technology* (Vol. 14, pp. 221-255). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-323-88487-7.00009-7

Ganegoda, S., Chinthaka, M., & Manage, P. (2020). Determination of Geosmin and 2-Methylisoborneol in water using Solid Phase Micro Extraction (SPME) and Gas Chromatography Mass Spectrometry (GC/MS). *Ecology*, *26*, 420-432.

Gerber, N. N., & Lechevalier, H. A. (1965). Geosmin, an Earthy-Smelling Substance Isolated from Actinomycetes. *Applied Microbiology*, *13*(6), 935-938. https://doi.org/doi:10.1128/am.13.6.935-938.1965

Giglio, S., Chou, W. K. W., Ikeda, H., Cane, D. E., & Monis, P. T. (2011). Biosynthesis of 2-Methylisoborneol in Cyanobacteria. *Environmental Science & Technology*, *45*(3), 992-998. https://doi.org/10.1021/es102992p

Grob, K. (1985). Band broadening in space in splitless injection. *Journal of Chromatography A*, *324*, 251-259. https://doi.org/https://doi.org/10.1016/S0021-9673(01)81324-8

Grob, K. (2007). *Split and Splitless Injection for Quantitative Gas Chromatography: Concepts, Processes, Practical Guidelines, Sources of Error: Fourth, Completely Revised Edition*. https://doi.org/10.1002/9783527612871

Harvey, D. (2013). *Contributions to Band Broadening in Chromatography*. Retrieved 05/04/23 from https://asdlib.org/imageandvideoexchangeforum/contributions-to-band-broadening-in-chromatography/

Hecht, E. S., Scigelova, M., Eliuk, S., & Makarov, A. (2019). Fundamentals and Advances of Orbitrap Mass Spectrometry. In *Encyclopedia of Analytical Chemistry* (pp. 1-40). https://doi.org/https://doi.org/10.1002/9780470027318.a9309.pub2

Hoffmann, E. d., & Stroobant, V. (2007). *Mass Spectrometry Principles and Applications* (Third ed.). John Wiley & Sons.

Howgate, P. (2004). Tainting of farmed fish by geosmin and 2-methyl-iso-borneol: a review of sensory aspects and of uptake/depuration. *Aquaculture*, *234*(1), 155-181. https://doi.org/https://doi.org/10.1016/j.aquaculture.2003.09.032

Hsieh, T. C.-Y., Tanchotikul, U., & Matiella, J. E. (1988). Identification of Geosmin as the Major Muddy Off-flavor of Louisiana Brackish Water Clam (Rangia cuneata). *Journal of Food Science*, *53*(4), 1228-1229. https://doi.org/https://doi.org/10.1111/j.1365-2621.1988.tb13571.x

Hwang, C. J., Krasner, S. W., McGuire, M. J., Moylan, M. S., & Dale, M. S. (1984). Determination of subnanogram-per-liter levels of earthy-musty odorants in water by the salted closed-loop stripping method. *Environmental Science & Technology*, *18*(7), 535-539. https://doi.org/10.1021/es00125a010

Ikai, Y., Honda, S., Yamada, N., Onuma, S., Tomita, B.-i., Kawamura, N., & Miyazaki, Y. (2003). Determination of Geosmin and 2-Methylisoborneol in Water using Solid Phase Extraction and Headspace-GC/MS. *Journal of the Mass Spectrometry Society of Japan*, *51*(1), 174-178. https://doi.org/10.5702/massspec.51.174

Izaguirre, G., & Taylor, W. D. (2004). A guide to geosmin- and MIB-producing cyanobacteria in the United States. *Water Science and Technology*, *49*(9), 19-24. https://doi.org/10.2166/wst.2004.0524

Jeleń, H. H., Majcher, M., Zawirska-Wojtasiak, R., Wiewiórowska, M., & Wąsowicz, E. (2003). Determination of Geosmin, 2-Methylisoborneol, and a Musty-Earthy Odor in Wheat Grain by SPME-GC-MS, Profiling Volatiles, and Sensory Analysis. *Journal of Agricultural and Food Chemistry*, *51*(24), 7079-7085. https://doi.org/10.1021/jf030228g

Jiang, J., He, X., & Cane, D. E. (2006). Geosmin Biosynthesis. Streptomyces coelicolor Germacradienol/Germacrene D Synthase Converts Farnesyl Diphosphate to Geosmin. *Journal of the American Chemical Society*, *128*(25), 8128-8129. https://doi.org/10.1021/ja062669x

Jiang, J., He, X., & Cane, D. E. (2007). Biosynthesis of the earthy odorant geosmin by a bifunctional Streptomyces coelicolor enzyme. *Nat Chem Biol*, *3*(11), 711-715. https://doi.org/10.1038/nchembio.2007.29

Johnsen, P. B., & Kuan, J.-C. W. (1987). Simplified method to quantify geosmin and 2-methylisoborneol concentrations in water and microbiological cultures.

Journal of Chromatography A, *409*, 337-342. https://doi.org/https://doi.org/10.1016/S0021-9673(01)86810-2

Jüttner, F., & Watson, S. B. (2007). Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters. *Appl Environ Microbiol*, *73*(14), 4395-4406. https://doi.org/10.1128/aem.02250-06

Kim, K. T., & Park, Y.-G. (2021). Geosmin and 2-MIB Removal by Full-Scale Drinking Water Treatment Processes in the Republic of Korea. *Water*, *13*(5), 628. https://www.mdpi.com/2073-4441/13/5/628

Kitson, F. G., Larsen, B. S., & McEwen, C. N. (1996). Chapter 1 - What Is GC/MS? In F. G. Kitson, B. S. Larsen, & C. N. McEwen (Eds.), *Gas Chromatography and Mass Spectrometry* (pp. 3-23). Academic Press. https://doi.org/https://doi.org/10.1016/B978-012483385-2/50002-6

Konieczka, P. (2012). 2.31 - Validation and Regulatory Issues for Sample Preparation. In J. Pawliszyn (Ed.), *Comprehensive Sampling and Sample Preparation* (pp. 699-711). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-381373-2.00064-8

Krasner, S. W., Hwang, C. J., & McGuire, M. J. (1983). A Standard Method for Quantification of Earthy-Musty Odorants in Water, Sediments, and Algal Cultures. *Water Science and Technology*, *15*(6-7), 127-138. https://doi.org/10.2166/wst.1983.0137

La Guerche, S., Chamont, S., Blancard, D., Dubourdieu, D., & Darriet, P. (2005). Origin of (−)-Geosmin on Grapes: On the Complementary Action of Two Fungi, Botrytis Cinerea and Penicillium Expansum. *Antonie van Leeuwenhoek*, *88*(2), 131-139. https://doi.org/10.1007/s10482-005-3872-4

Lehotay, S. J., & Schenck, F. J. (2000). Multiresidue Methods: Extraction. In I. D. Wilson (Ed.), *Encyclopedia of Separation Science* (pp. 3409-3415). Academic Press. https://doi.org/https://doi.org/10.1016/B0-12-226770-2/05621-0

Li, L., Yang, S., Yu, S., & Zhang, Y. (2019). Variation and removal of 2-MIB in full-scale treatment plants with source water from Lake Tai, China. *Water*

Research, *162*, 180-189. https://doi.org/https://doi.org/10.1016/j.watres.2019.06.066

Lin, T.-F., Watson, S., Dietrich, A. M., & Suffet, I. H. M. (2019). *Taste and Odour in Source and Drinking Water: Causes, Controls, and Consequences*. IWA Publishing.

Lindholm-Lehto, P. C., & Vielma, J. (2018). Controlling of geosmin and 2-methylisoborneol induced off-flavours in recirculating aquaculture system farmed fish—A review. *Aquaculture Research*.

Lovell, R. T., & Broce, D. (1985). Cause of musty flavor in pond-cultured penaeid shrimp. *Aquaculture*, *50*(1), 169-174. https://doi.org/https://doi.org/10.1016/0044-8486(85)90163-2

Lu, J., Wills, P. S., & Chris Wilson, P. (2016). Trace analysis of off-flavor/odor compounds in water using liquid-liquid microextraction coupled with gas chromatography—positive chemical ionization-tandem mass spectrometry. *Frontiers of Environmental Science & Engineering*, *10*(3), 477-481. https://doi.org/10.1007/s11783-015-0820-z

Ma, X., Gao, N., Chen, B., Li, Q., Zhang, Q., & Gu, G. (2007). Detection of geosmin and 2-methylisoborneol by liquid-liquid extraction-gas chromatograph mass spectrum (LLE-GCMS) and solid phase extraction-gas chromatograph mass spectrum (SPE-GCMS). *Frontiers of Environmental Science & Engineering in China*, *1*(3), 286-291. https://doi.org/10.1007/s11783-007-0048-7

Malleret, L., Bruchet, A., & Hennion, M. C. (2001). Picogram Determination of "Earthy-Musty" Odorous Compounds in Water Using Modified Closed Loop Stripping Analysis and Large Volume Injection GC/MS. *Analytical Chemistry*, *73*(7), 1485-1490. https://doi.org/10.1021/ac001010l

Maranata, G. J., Surya, N. O., & Hasanah, A. N. (2021). Optimising factors affecting solid phase extraction performances of molecular imprinted polymer as recent sample preparation technique. *Heliyon*, *7*(1), e05934. https://doi.org/https://doi.org/10.1016/j.heliyon.2021.e05934

Margolin Eren, K. J., Elkabets, O., & Amirav, A. (2020). A comparison of electron ionization mass spectra obtained at 70 eV, low electron energies, and with cold EI and their NIST library identification probabilities. *J Mass Spectrom*, *55*(12), e4646. https://doi.org/10.1002/jms.4646

McCrummen, S. T., Wang, Y., Hanson, T. R., Bott, L., & Liu, S. (2018). Culture environment and the odorous volatile compounds present in pond-raised channel catfish (Ictalurus punctatus). *Aquaculture International*, *26*(2), 685-694. https://doi.org/10.1007/s10499-018-0247-1

McGuire, M. J., Krasner, S. W., Hwang, C. J., & Izaguirre, G. (1981). Closed-loop stripping analysis as a tool for solving taste and odor problems. *Journal (American Water Works Association)*, *73*(10), 530-537. http://www.jstor.org/stable/41270578

Miller, J. M. (2009). *Chromatography: Concepts and Contrasts* (2nd ed.). John Wiley & Sons.

Murray, K. K., Boyd, R. K., Eberlin, M. N., Langley, G. J., Li, L., & Naito, Y. (2013). Definitions of terms relating to mass spectrometry (IUPAC Recommendations 2013). *Pure and Applied Chemistry*, *85*(7), 1515-1609. https://doi.org/doi:10.1351/PAC-REC-06-04-06

Newcombe, G., Ho, L., & Baker, P. (2010). *Management Strategies for Cyanobacteria (Blue-Green Algae): A Guide for Water Utilities*. WQRA. https://www.researchgate.net/publication/242740698_Management_Strategies_for_Cyanobacteria_Blue-Green_Algae_A_Guide_for_Water_Utilities

NIH. (2023a). *1-Methylcyclohexanol*. Retrieved 14/09/22 from https://pubchem.ncbi.nlm.nih.gov/compound/1-Methylcyclohexanol

NIH. (2023b). *2-Methylisoborneol*. Retrieved 16/03/23 from https://pubchem.ncbi.nlm.nih.gov/compound/2-Methylisoborneol

NIH. (2023c). *Geosmin*. Retrieved 16/03/23 from https://pubchem.ncbi.nlm.nih.gov/compound/Geosmin

Petersen, M., Alam, M., Rahman, M., Ali, M., Mahmud, S., Schluter, L., & Jørgensen, N. (2014). Geosmin off-flavour in pond-raised fish in Southern Bangladesh and occurrence of potential off-flavour producing organisms. *Aquaculture Environment Interactions*, *5*, 107-116. https://doi.org/10.3354/aei00100

Polak, E. H., & Provasi, J. (1992). Odor sensitivity to geosmin enantiomers. *Chemical Senses*, *17*(1), 23-26. https://doi.org/10.1093/chemse/17.1.23

Rahman, M. M., Abd El-Aty, A. M., Choi, J.-H., Shin, H.-C., Shin, S. C., & Shim, J.-H. (2015). Basic Overview on Gas Chromatography Columns. In *Analytical Separation Science* (pp. 823-834). https://doi.org/https://doi.org/10.1002/9783527678129.assep024

Raynie, D. E., & Watson, D. W. (2014). *Understanding and Improving Solid-Phase Extraction*. Retrieved 12/11/22 from https://www.chromatographyonline.com/view/understanding-and-improving-solid-phase-extraction-1

Romero, J., Manero, I., & Laso, J. (2007). Validation of Geosmin and 2-Methyl-i-Borneol Analysis by CLSA-GC-FID Method to Obtain ISO-17025 Accreditation. *Journal of Chromatographic Science*, *45*(7), 439-446. https://doi.org/10.1093/chromsci/45.7.439

Sharifi, V., & Hadjmohammadi, M. R. (2006). Use of Solid Phase Extraction for Sample Clean-up and Preconcentration of Vitamin B12 in Multivitamin Tablet before HPLC-UV, UV and Atomic Absorption Spectrophotometry. *Journal of Food and Drug Analysis*, *15*, 285. https://doi.org/10.38212/2224-6614.2410

Shellie, R. A. (2013). Gas Chromatography. In J. A. Siegel, P. J. Saukko, & M. M. Houck (Eds.), *Encyclopedia of Forensic Sciences (Second Edition)* (pp. 579-585). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-382165-2.00245-2

Shin, H.-S., & Ahn, H.-S. (2004). Simple, Rapid, and Sensitive Determination of Odorous Compounds in Water by GC–MS. *Chromatographia*, *59*(1), 107-113. https://doi.org/10.1365/s10337-003-0125-4

Snow, N. H. (2018). *Split, Splitless, and Beyond—Getting the Most from Your Inlet*. Retrieved 08/06/23 from https://www.chromatographyonline.com/view/split-splitless-and-beyond-getting-most-your-inlet-0

Stahl, P. D., & Parkin, T. B. (1994). Purge-and-Trap Extraction of Geosmin and 2-Methylisoborneol from Soil. *Soil Science Society of America Journal*, *58*(4), 1163-1167. https://doi.org/https://doi.org/10.2136/sssaj1994.03615995005800040022x

Stauffer, E., Dolan, J. A., & Newman, R. (2008). CHAPTER 8 - Gas Chromatography and Gas Chromatography—Mass Spectrometry. In E. Stauffer, J. A. Dolan, & R. Newman (Eds.), *Fire Debris Analysis* (pp. 235-293). Academic Press. https://doi.org/https://doi.org/10.1016/B978-012663971-1.50012-9

Steliopoulos, P. (2015). Extension of the standard addition method by blank addition. *MethodsX*, *2*, 353-359. https://doi.org/https://doi.org/10.1016/j.mex.2015.09.001

Svahn, O., & Björklund, E. (2019). High Flow-Rate Sample Loading in Large Volume Whole Water Organic Trace Analysis Using Positive Pressure and Finely Ground Sand as a SPE-Column In-Line Filter. *Molecules (Basel, Switzerland)*, *24*(7). https://doi.org/10.3390/molecules24071426

Targuma, S., Njobeh, P., & Ndungu, P. (2021). Current Applications of Magnetic Nanomaterials for Extraction of Mycotoxins, Pesticides, and Pharmaceuticals in Food Commodities. *Molecules (Basel, Switzerland)*, *26*, 4284. https://doi.org/10.3390/molecules26144284

TGSC. (N.d.-a). *2-methyl isoborneol*. Retrieved 16/03/23 from http://www.thegoodscentscompany.com/data/rw1384871.html

TGSC. (N.d.-b). *(+)-geosmin*. Retrieved 16/03/23 from http://www.thegoodscentscompany.com/data/rw1557971.html

Tian, L. L., Han, F., Fodjo, E. K., Zhai, W., Huang, X. Y., Kong, C., Shi, Y. F., & Cai, Y. Q. (2021). An Effective and Efficient Sample Preparation Method for 2-Methyl-Isoborneol and Geosmin in Fish and Their Analysis by Gas

Chromatography-Mass Spectrometry. *Int J Anal Chem*, *2021*, 9980212. https://doi.org/10.1155/2021/9980212

Tucker, C., & Martin, J. (1991). Environment-related off-flavors in fish. *Water Quality inAquaculture: World Aquaculture Soiety*, 133-179.

Tucker, C. S. (2000). Off-Flavor Problems in Aquaculture. *Reviews in Fisheries Science*, *8*(1), 45-88. https://doi.org/10.1080/10641260091129170

Tyler, L. D., Acree, T. E., Nelson, R. R., & Butts, R. M. (1978). Determination of geosmin in beet juice by gas chromatography. *Journal of Agricultural and Food Chemistry*, *26*(3), 774-775.

Van Berkel, G. J. (2003). An Overview of Some Recent Developments in Ionization Methods for Mass Spectrometry. *European Journal of Mass Spectrometry*, *9*(6), 539-562. https://doi.org/10.1255/ejms.586

van Deemter, J. J., Zuiderweg, F. J., & Klinkenberg, A. (1956). Longitudinal diffusion and resistance to mass transfer as causes of nonideality in chromatography. *Chemical Engineering Science*, *5*(6), 271-289. https://doi.org/https://doi.org/10.1016/0009-2509(56)80003-1

Watson, D. W. (2017a). *Advantages of Using Nitrogen in Capillary GC*. Retrieved 15/03/23 from https://www.chromatographyonline.com/view/advantages-using-nitrogen-capillary-gc

Watson, D. W. (2017b). *Three Common SPE Problems*. Retrieved 20/06/23 from https://www.chromatographyonline.com/view/three-common-spe-problems-0

Wilson, I. D., Adlard, E. R., Cooke, M., & Poole, C. F. (2000). *Encyclopedia of separation science*. Academic Press.

Wright, E., Daurie, H., & Gagnon, G. A. (2014). Development and validation of an SPE-GC-MS/MS taste and odour method for analysis in surface water. *International Journal of Environmental Analytical Chemistry*, *94*(13), 1302-1316. https://doi.org/10.1080/03067319.2014.974586

Xia, P., Zhang, S., Yu, J., Ye, H., Zhang, D., Jiang, L., Wang, Z., & Yin, D. (2020). Complex odor control based on ozonation/GAC advanced treatment: optimization and application in one full-scale water treatment plant. *Environmental Sciences Europe*, *32*(1), 50. https://doi.org/10.1186/s12302-020-00313-w

Xu, D., Yuan, B., Lv, H., & Lin, Q. (2010). *The Determination of Geosmin and 2-Methylisoborneol Using Stable Isotope Dilution Purge-and-Trap Gas Chromatography Mass Spectrometry.* Conference on Environmental Pollution and Public Health, Wuhan, China.

Yen, J.-H., Lin, K.-H., & Wang, Y.-S. (2002). Acute Lethal Toxicity of Environmental Pollutants to Aquatic Organisms. *Ecotoxicology and Environmental Safety*, *52*(2), 113-116. https://doi.org/https://doi.org/10.1006/eesa.2002.2167

You, J., Willcox, M. D., Madigan, M. C., Wasinger, V., Schiller, B., Walsh, B. J., Graham, P. H., Kearsley, J. H., & Li, Y. (2013). Chapter Four - Tear Fluid Protein Biomarkers. In G. S. Makowski (Ed.), *Advances in Clinical Chemistry* (Vol. 62, pp. 151-196). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-800096-0.00004-4

Young, W. F., Horth, H., Crane, R., Ogden, T., & Arnott, M. (1996). Taste and odour threshold concentrations of potential potable water contaminants. *Water Research*, *30*(2), 331-340. https://doi.org/https://doi.org/10.1016/0043-1354(95)00173-5

Zoeteman, B. C. J., & Piet, G. J. (1973). On the nature of odours in drinking water resources of The Netherlands. *Science of The Total Environment*, *1*(4), 399-410. https://doi.org/https://doi.org/10.1016/0048-9697(73)90027-2

Zubarev, R. A., & Makarov, A. (2013). Orbitrap Mass Spectrometry. *Analytical Chemistry*, *85*(11), 5288-5296. https://doi.org/10.1021/ac4001223

Appendix

Table A.1: Temperature programmes tested in GC Orbitrap analysis

Temp. prog.	#	Rate (°C/min)	Temp (°C)	Hold time (min)
geosmin.meth	Initial		50	5
	1	100	140	15
	2	125	260	5
geosmin2.meth	Initial		75	5
	1	100	140	15
	2	125	260	5
geosmin3.meth	Initial		90	5
	1	100	120	15
	2	125	260	5
geosmin4.meth	Initial		75	5
	1	100	130	15
	2	125	260	5
geosmin5.meth	Initial		80	5
	1	100	140	15
	2	125	260	5
geosmin6.meth	Initial		65	5
	1	100	140	15
	2	125	260	5
geosmin7.meth	Initial		50	5
	1	125	140	15
	2	125	260	5
geosmin8.meth	Initial		75	5
	1	125	140	15
	2	125	260	5
geosmin9.meth	Initial		75	5
	1	110	140	15
	2	125	260	5
geosmin10.meth	Initial		50	10
	1	100	140	15
	2	125	260	5
geosmin11.meth	Initial		50	5
	1	50	140	15
	2	125	260	5
geosmin12.meth	Initial		50	7
	1	100	160	15
	2	125	260	5
geosmin13.meth	Initial		75	10
	1	100	140	15
	2	125	260	5
geosmin14.meth	Initial		75	10
	1	50	140	15
	2	125	260	5

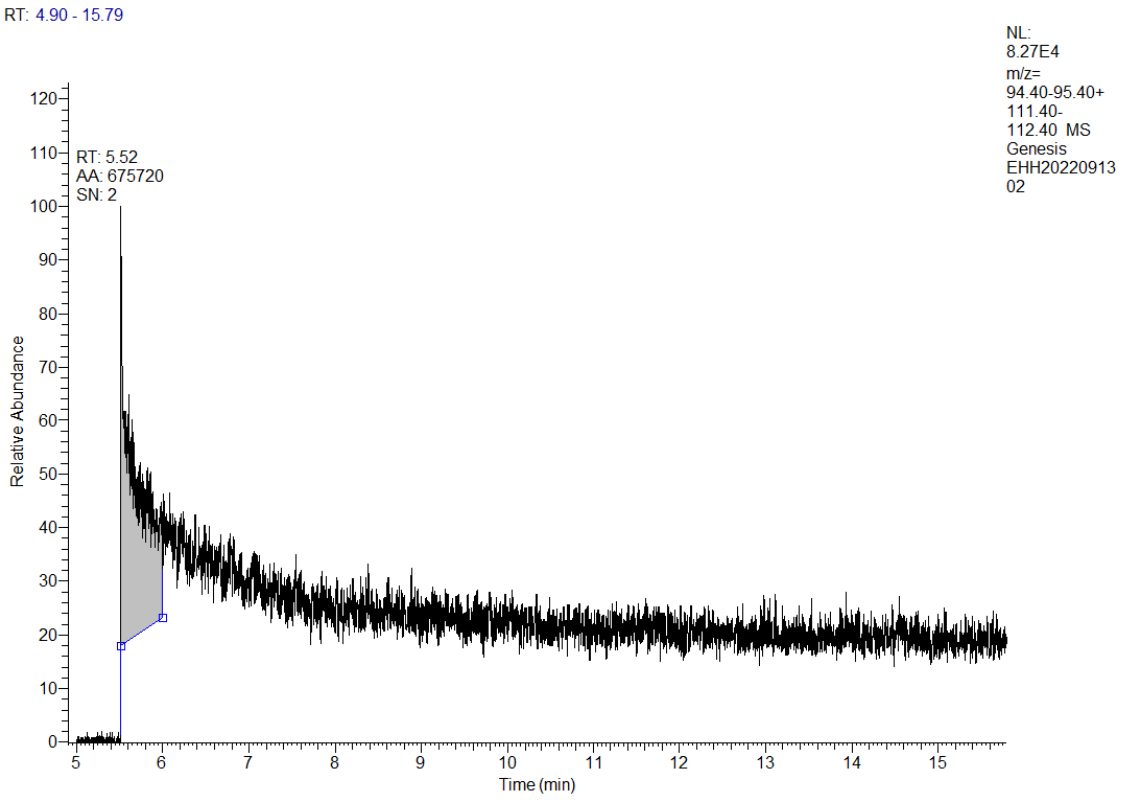


Figure A.1: *BPC of sample following an evaporation experiment.*

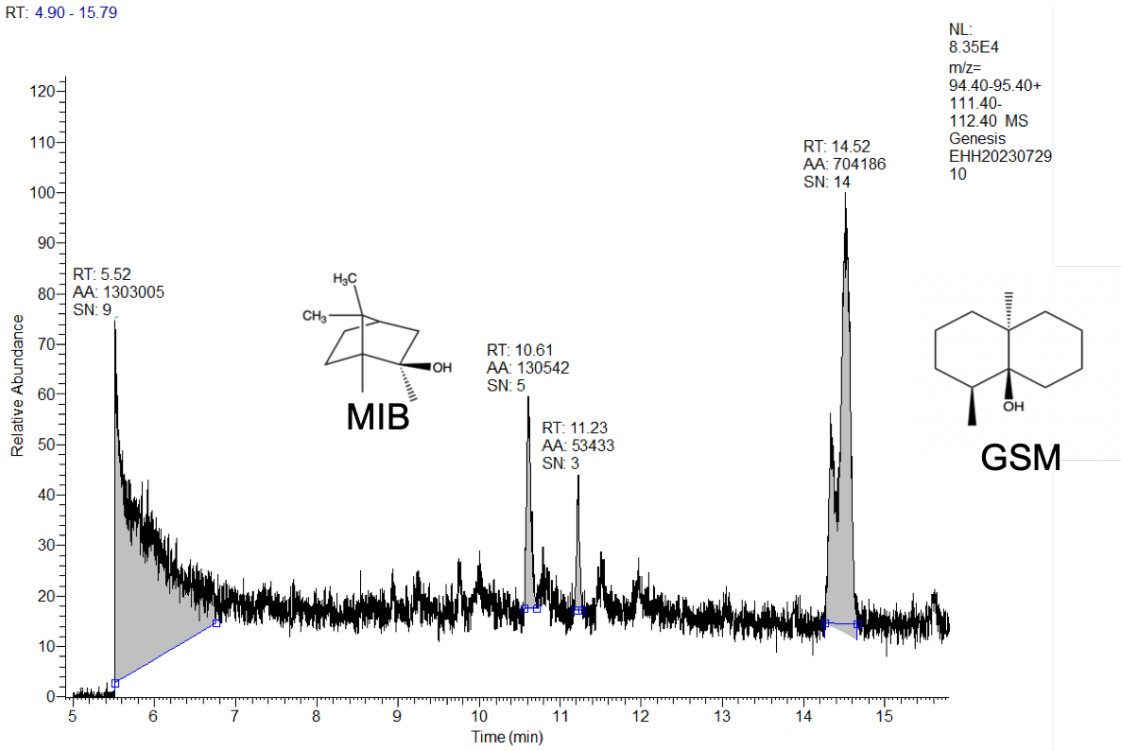


Figure A.2: *Base peak chromatogram showing MIB at 10.61 min and GSM at 14.52 min following liquid-liquid microextraction.*

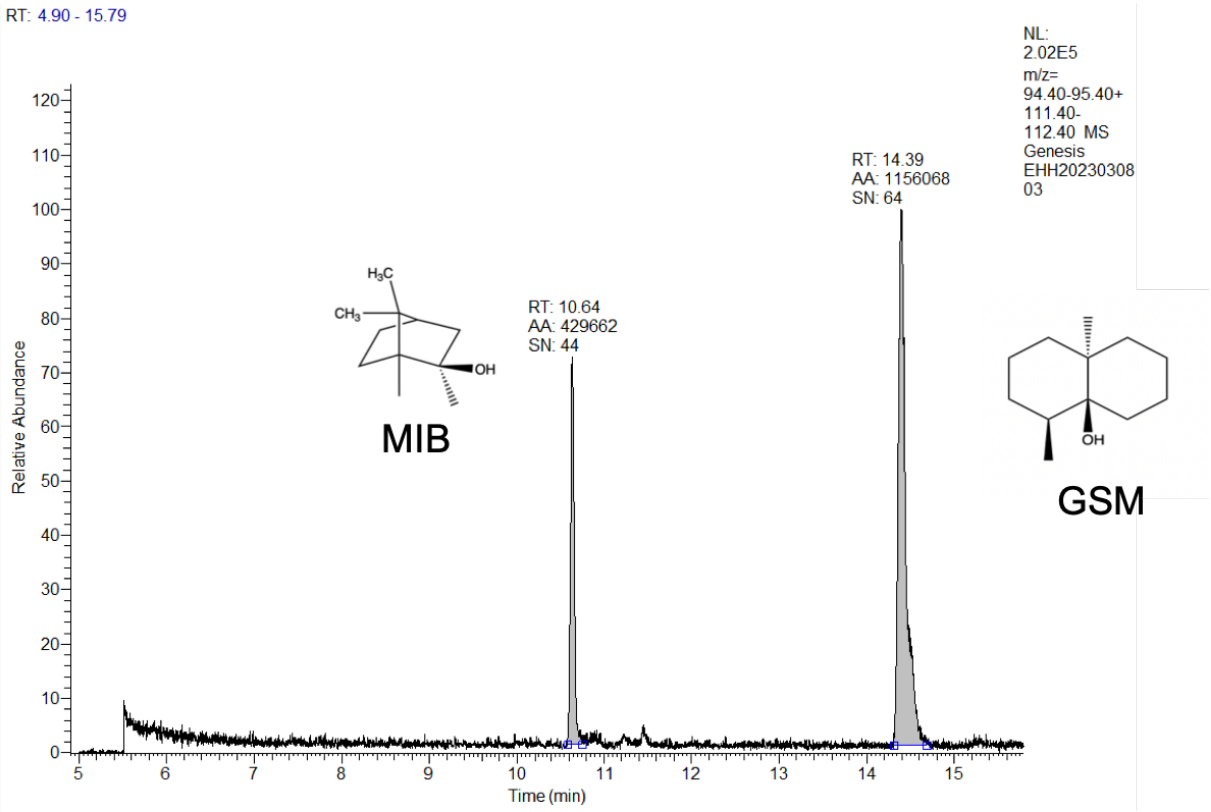


Figure A.3: *Base peak chromatogram showing MIB at 10.64 min and GSM at 14.39 min of a pure heptane sample containing only the two analytes.*

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway