Development of Solid Phase Microextraction Methodologies for the Determination of Anthropogenic Contaminants from Fatty Food Matrices

by

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of Contributions

A portion of the materials presented in Chapter 2, specifically section 2.2.3 entitled Previous Work has previously been submitted to the University of Waterloo as a project report by the author of this thesis, directed by Emanuela Gionfriddo and supervised by Prof. Janusz Pawliszyn. All experimental work conducted in the laboratory as well as data analysis was performed by the author of this thesis; experimental design and data interpretation were done jointly with Prof. Emanuela Gionfriddo. Furthermore, a portion of the materials in Chapter 2 is content from a manuscript in preparation entitled "Direct immersion SPME in soymilk for pesticide analysis at trace levels by means of a matrix compatible coating" co-authored by Prof. Emanuela Gionfriddo, Dr. Xiujuan Li, and the author of this thesis, supervised by Prof. Janusz Pawliszyn. Remaining unpublished experimental work conducted in the laboratory, experimental design, data analysis and interpretation was performed by the author of this thesis; writing was done jointly with Prof. Emanuela Gionfriddo.

Chapter 3 consists of unpublished work from a project entitled "Determination of Polychlorinated n-Alkanes in Cod Liver Oil using SPME" carried out in collaboration with Dr. Jonathan Grandy, Dr. Varoon Singh, Prof. Emanuela Gionfriddo, Abir Khaled and Virginia Galpin, supervised by Prof. Janusz Pawliszyn. In most cases, experimental design and planning, experimental work conducted in the laboratory, data analysis and interpretation, as well as writing were done by the author of this thesis. Previous research described in section 3.2.1 was done solely by Prof. Emanuela Gionfriddo and is included briefly in this thesis for clarity and discussion of experimental design improvements done in later sections. Previous research described in section 3.2.2 was done by Prof. Emanuela Gionfriddo and Abir Khaled and is included in this thesis briefly

for clarity in future experimental design improvements. Section 3.4.2.1 experimental design was done under the supervision of Prof. Emanuela Gionfriddo, all laboratory experiments, data analysis and interpretation as well as writing were done by the author of this thesis. The entirety of the work shown in Section 3.4.2.2 was performed by the author of this thesis. Work shown in sections 3.4.3.3, 3.4.3.4 and 3.4.3.5 was performed by the author of this thesis with materials developed in-house and obtained from Dr. Jonathan Grandy and Dr. Varoon Singh. The procedure to make membrane slurry was previously published by Jonathan Grandy in "Development of a Carbon Mesh Supported Thin Film Microextraction Membrane As a Means to Lower the Detection Limits of Benchtop and Portable GC/MS Instrumentation". Ready-made carbon mesh membranes and the development procedure and calculation methods for unsupported membranes were provided by Dr. Jonathan Grandy. Methacrylic acid – co – ethylene glycol dimethacrylate particles, both magnetic and non-magnetic, were synthesized in-house by Dr. Varoon Singh. Experimental design and planning, a portion of experimental work conducted in the laboratory, all data analysis and interpretation, as well as writing of section 3.4.3.6 was done by the author of this thesis; a portion of the experimental work was performed by Virginia Galpin under the supervision of the author.

Abstract

Anthropogenic contaminants have been on the rise worldwide due to global industrialization. The world is now faced with the consequences of the pollution caused by the exposure of man-made chemicals in the environment. Testing of foodstuff for these pollutants has become extremely important as various fields of science continuously demonstrate the dangers posed by such contaminants to environmental, and, ultimately human health. Pesticides and machining oils contaminating soil and river water are of particular note as their use is widespread and they have a tendency to bioaccumulate in adipose tissue. Moreover, bioaccumulated compounds may be further biomagnified up the food chain until they are consumed by humans. Currently, methods for screening bioaccumulants in fatty foods are time-consuming and resource-intensive resulting in significant environmental waste. Hence, this thesis presents the development of eco-friendly methods to test for pesticides and polychlorinated n-alkanes (PCAs) using solid phase microextraction (SPME) techniques in fatty food matrices.

The determination of pesticides was performed from soymilk samples using matrix-compatible polydimethylsiloxane (PDMS) overcoated divinylbenzene/PDMS (DVB/PDMS) SPME fibers on GC-MS instrumentation. Targets for a broad-spectrum representation of commonly used pesticides included trifuralin, dimethoate, diazinon, malathion, chlorpyrifos, thiabendazole, phosalone, λ-cyhalothrin, α-β-cyfluthrin, and esfenvalerate. The method exhibited good figures of merit indicated by low limits of detection at 1ppb for all but trifuralin at 2.5ppb, well below maximum residue levels set by both the Canadian and United States government agencies raging from 0.01 to 8 ppm. The linear range for each compound spanned from the limits of detection up to 1000ppb. Validation of the method was accomplished according to the FDA

guidelines; precision and accuracy from back-calculated results reached targets within 70 – 130% of known values with less than 20% RSD. In two other milk brands sampled, Chlorpyrifos and Malathion were each found below MRL for both Canada and the USA, however Dimethoate was found at 742 and 745 ppb in each respectively.

PCAs are another example of potent environmental bioaccumulants. Many industrial oils find their way into natural waterways and bioaccumulate in fatty tissues such as fish livers which are harvested to produce oil supplements used for their omega-3 and omega-6 fatty acid content. Screening PCAs from cod liver oil difficult due to the hydrophobic properties of the matrix; a hydrophilic lipophilic balance particle (HLB/PDMS) coated aluminum thin film solid phase microextraction (TF-SPME) blade was used for the extraction. Eight PCA standards ranging from chlorodecane to 1,1,1,3,14,15 - hexachloropentadecane were used as proxies for this work encompassing short and medium chain PCAs with varying degrees of chlorination. Previously unreported Kovats retention indices for two PCAs: 1,1,1,3 – tetrachlorodecane and 1,2,9,10 – tetrachlorodecane were found to be 1649 and 1786, respectively. Calibration of the method was performed with a linear range of 0.075 to 0.75ppm on a conventional GC-MS with electron impact ionization and a single quadrupole. Method limits of quantitation (MLOQ) were determined by multiplying the standard deviation of the lowest calibration point by 10, then dividing by the slope; the MLOQ ranged from 0.217 to 0.07ppm. Standard addition was performed in a second oil to validate method recovery; quantities of PCAs in the second oil were below MLOQ for each compound found. Spiked samples of the second oil had good inter-matrix accuracy for many analytes as the slopes of the two curves were comparable. Four compounds were found at concentrations above MLOQ in other oil brands sampled, the highest at 1.228ppm. The method fits the testing range quoted by the Stockholm Convention on POP, ranging between 0.7-5.5 ppm.[57]

Overall this thesis presents two inexpensive methods with reusable materials for sampling persistent organic pollutants in fatty food matrices and can serve as a benchmark for modification for future use in other foodstuffs.

Acknowledgments

I would like to thank Dr. Pawliszyn for the opportunity to study as a part of this lab, as well as Dr. Smith and Dr. Palmer for kindly agreeing to be on my advisory committee. I would like to also thank Dr. Chong the graduate coordinator at my proposal, Dr. Deichmann, the Graduate Studies and Research Associate Chair and Dr. Power, the Department Chair.

This project would not be possible without the support of Apotex Pharmachem, Dr. Horne and Dr. Smerek. Furthermore, the assistance and advice provided by the Science Technical Services group at the University of Waterloo is much appreciated.

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List of Abbreviations

ACE	acephenanthrene
ACN	acetonitrile
ACY	acenaphthylene
ANT	anthracene
B(a)A	benz[a]anthracene
Car	carboxen
CHR	chrysene
CIS	cooled injection system
DI	direct immersion
DMSO	dimethyl sulfoxide
DVB	divinylbenzene
ECD	electron capture detector
EI	electron ionization
EM	electron multiplier
ETP	extraction time profile
FID	flame ionizing detector
FLE	fluorene
FLT	fluoranthene
GC	gas chromatograph
GPC	gel permeation chromatography
HLB	hydrophobic lipophilic balance
HS	headspace
IARC	International Agency for Research on Cancer
IMO	International Maritime Organization
IT	ion trap
LLE	liquid-liquid extraction
MAA-co-EGDMA	methacrylic acid - co - ethylene glycol dimethacrylate
MLOQ	method limit of quantitation
MPS	multipurpose sampler
MRL	maximum residue level
MS	mass spectrometer/spectrometry
NAP	naphthalene
PAH	polycyclic aromatic hydrocarbons
PAN	polyacrylonitrile
PCA	polychlorinated n-alkanes
PDMS	polydimethylsiloxane
PHE	phenanthrene
PY	pyrene
QuEChERS	quick, easy, cheap, effective rugged safe

RSD	relative standard deviation
SPE	solid phase extraction
SPME	solid phase microextraction
TDU	thermal desorption unit
TFM	thin film membrane
TFME	thin film membrane extraction
TIC	total ion chromatogram
ToF	time of flight
VOC	volatile organic component

1. Chapter 1: Introduction

1.1. Sample Preparation

Sample preparation is normally an enrichment process which removes matrix components are removed from a sample, ensuring that the cleanest, and most compatible extract is introduced into the instrument.[1] This process generally involves an extraction step to either collect the analyte of interest out of the matrix, or remove components that could interfere with analyte detection from the sample. Further concentration or enrichment steps ensure that the analytes are present at a suitable concentration to be detected by the instrument. This improves the signal to noise ratio, or sensitivity, of the method to the target analytes and prevents the instrument from accumulating debris from dirty samples.

In most cases, sample preparation is often the most time-consuming step before analysis.[2] Common sample preparation methods include preparative chromatography, solid phase extractions (SPE), Soxhlet extraction, and QuEChERS (portmanteau for quick, easy, cheap, effective, rugged, and safe).[3–6] These methods can be expensive due to the large quantity of materials and specialized instrumentation required. Many of these methods involve large volumes of solvents requiring sample concentration steps as the unmodified extractant is too dilute to detect. This often takes large amounts of time and can introduce significant error. Sample preparation always involves a tradeoff between reduction of co-extractants and reducing the total steps required, as each modification can introduce error.

1.1.1. Solid Phase Microextraction (SPME)

Solid phase microextraction (SPME) is a versatile, reusable sampling technique that combines sampling and sample preparation such as preconcentration and cleanup into a single step and can often be adapted to multiple instruments.[1] The commercial fiber design used in this work includes a 1cm, 100µm diameter coating on a silica support, housed in a protective needle that is fixed into a plunger device.[1] The most common absorptive coating used for gas chromatography (GC) is polydimethylsiloxane (PDMS) due to it's thermal stability and extraction capabilities. Other adsorptive polymers can be used as well, such as divinylbenzene (DVB) or carboxen (Car) which are suspended in PDMS and adhered to the support.

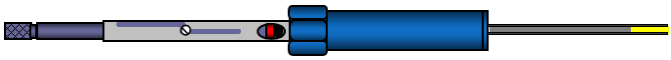


Figure 1: A diagram of the typical commercially available SPME device

Fundamentally, two modes of sampling can be used for a typical GC fiber: direct immersion (DI) and headspace (HS) extraction.[1,7,8] The needle is introduced into the sample in the DI format, and the fiber is exposed to the matrix.[9] Alternatively, if the matrix is complex and DI sampling could result in significant interferences due to fiber fouling or contaminant coextraction, HS extraction is an effective alternative sampling format. HS extractions are particularly suited for volatile analytes capable of partitioning into the headspace, denoted by a large Henry's constant.[10] In this method, the fiber is exposed

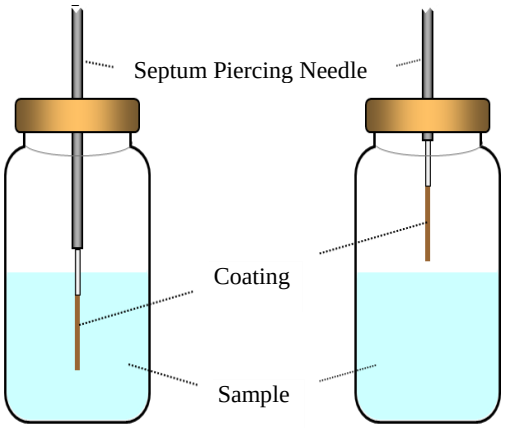


Figure 2: Two sampling modes of fiber SPME, direct immersion (DI) shown on the left and headspace extraction (HS) shown on the right.

in the gas phase above the sample, facilitating extraction of the volatile compounds while avoiding contact with the matrix.

Analyte partition between the sample and the coating on the fiber governs SPME.[1,8] When the fiber is exposed to the sample matrix, analytes move from the matrix into the coating; subsequently, the extracted analytes can be desorbed using heat or solvent.[1] The partitioning of the analytes occurs either by absorption or adsorption onto the fiber depending on the type of sorbent used; absorption occurring when using liquid like coatings and adsorption occurring onto active sites of solid materials.[7] The most common fiber type, made with absorptive PDMS, is used for volatiles and non-polar semi-volatiles.[11]

An extraction phase specifically designed for direct immersion in complex matrices is a PDMS/DVB/PDMS fiber.[12] DVB particles are suspended in PDMS and the mixture is fixated onto the support, making a regular PDMS/DVB fiber; afterwards, an additional layer of PDMS is deposited on top of the DVB functioning as a semipermeable protective layer.[12] The main benefit of the additional PDMS is ensuring a smooth surface on the outermost portion of the fiber preventing the matrix adhesion seen on the rougher, non-overcoated, DVB surface. For this reason it is significantly easier to clean than PDMS/DVB alone, resulting in a longer life span and more accurate results.[12]

Alternatively, to improve headspace extraction for samples in the laboratory, vacuum can be introduced to the system. While the equilibrium concentrations are independent of the total pressure of the system, for some analytes a change in pressure may impact the rate of extraction.[13]

A device used more frequently on-site rather than the typical SPME fiber is the thin film membrane (TFM). Thin film membrane extraction (TFME) functions the same as an SPME fiber, but due its larger surface area it can extract more analyte, increasing sample enrichment. The commercially available TFM are supported on carbon mesh which is coated with the selected polymer on both sides. Membranes can be cut to custom sizes, however standard ones are 0.45 cm by 2.00 cm, which optimally fit into empty sorbent tubes made for thermal desorption, as well as the Gerstel™ thermal desorption unit sample tubes.

1.1.2. Solid Phase Extraction (SPE)

Solid phase extraction is a sample preparation technique often used for separating target analytes from complex matrices and to concentrate the analytes into a small volume of solvent compatible with the analysing instrumentation of choice.[14] The use of this technique may be necessary, for example, with a large volume of wastewater which is to be analysed for trace amounts of hormones.[15] The analytes in this example are very dilute while the matrix contains many other components which can interfere with the analysis and damage the instrument.[14] The general workflow requires the entire sample to be miscible in solvents and the target analytes must have an affinity to an extraction phase. The sample is run into a cartridge prepared with a solid phase extractant; this is usually powdered silica previously treated with a hydrophobic organic compound serving as the functionalized extraction phase. This cartridge can then be rinsed to remove undesired components of the matrix and then the analytes are eluted using a small volume of solvent. Using this method, a large volume sample can be reduced to only a few millilitres of elution solvent, preconcentrating the target analytes into an instrument-compatible solvent.

Due to the simplistic workflow as well as the preconcentration and sample cleanup SPE offers it is a popular choice for trace analysis. While SPE has its place in an analytical chemist's toolbox, the purpose of this thesis is to develop methods using SPME which can provide the same benefits of sample cleanup and preconcentration while also being reusable and permitting thermal – and therefore solventless – desorptions.

1.1.3. Gel Permeation Chromatography (GPC) and Size Exclusion Chromatography

Another common choice for separating nonpolar compounds is size exclusion chromatography involving hydrophobic packing material, termed gel permeation chromatography or GPC.[14] Unlike other types of chromatography or sample preparation, there is no extraction phase the analytes bind to; the process of separation happens solely due to molecular size. Components that are much larger than the pores will elute with the mobile phase, since they are not retained in the pores, while very small particles will be the last to elute since they fit into all pores and will therefore have the longest path length. Between these two extremes, particles of similar size to the pores will be separated by size; this can be particularly useful when working with mixtures of compounds which have similar physio-chemical properties but differ by chain length or molecular weight. GPC is often used as one step in the sample cleanup for extracting PCAs from complex matrices for this reason; removal of other components of the matrix is simplified and groups of PCAs can be separated based on chain length.

Unfortunately, like other forms of preparative chromatography, this technique is rather solvent-heavy, in that large volumes must be used for each sample, and the sample becomes diluted

through the elution process. To combat this, SPE is often used following this technique to further separate and preconcentrate the samples.

1.1.4. QuEChERS

Currently, the most common method for pesticide detection is QuEChERS, an acronym of "Quick Easy Cheap Effective Rugged Safe"[16]. As the gold standard of analytical testing for complex matrices, it was designed to account for a wide range of complications that arise from non-optimal samples. All samples are weighed in order to avoid inconsistencies in volume due to differences in density or the propensity of foaming. Solvent is used to dilute the sample; this can reduce issues with viscosity and can act as a liquid-liquid extraction (LLE) or cause precipitation of interfering components. Desiccant salts and buffers are added to remove water from the sample and adjust the pH to a neutral level. The sample is centrifuged, and the supernatant is then mixed with a sorbent to purify it further, then an internal standard can be added, and the sample is introduced to the analytical instrumentation. This process nearly guarantees all aspects of any sample become normalized and safe to injection into an instrument, however the process is very analyst-dependent, and the large number of steps makes the method prone to errors. Furthermore, it is difficult to automate due to the equipment required, preventing error reduction using automation. Reducing these steps for the same result is therefore the target of this work, facilitated using SPME

1.2. Instrumentation

1.2.1. Gas Chromatography

Gas Chromatography (GC) is an analytical technique used to separate mixtures of volatile compounds.[17] A GC instrument consists of 4 main parts: a heated injector, a column, a housing oven, and a detector. The injector is heated generally to a temperature 50°C above the boiling point of the target analytes, allowing the sample to vaporize and be quickly introduced into the column with a stream of inert gas.[14] This inert gas is known as the carrier gas, and functions as the mobile phase in the separation. Common carrier gases include helium, hydrogen and nitrogen, where helium is the most popular. Once the analytes have been transported to the column containing the stationary phase, they are separated via their physiochemical properties and relative affinities for the stationary phase. The stationary phase is often comprised of a thin cross-linked polymer resulting in analyte separation by polarity or boiling point.[17] To aid in this separation, the column is temperature-controlled by the column oven. The temperature may be kept at a ertain level above the boiling point of the target analytes or it may be ramped over time. This ramp allows for better separation of closely eluting compounds. On the other hand, with analytes that are widely separated in their elution, ramping the temperature will accelerate the elution of the tardier analyte and thus improve throughput. After elution, the separated analytes travel to the detector at the end of the column providing retention time and peak area.

Gas chromatography is often chosen over other chromatographic methods due to the ease of use and upkeep of the equipment. While it does require the target analytes to be volatile or semivolatile, and thermally stable up to 300°C, it is very useful if compounds are not soluble in liquid chromatography systems.

1.2.2. Detectors

Many detectors are available for gas chromatography. Electron capture detectors (ECD) are very sensitive to halogenated compounds and can be beneficial to the detection of chlorinated environmental pollutants. A mass spectrometer (MS) can be used to obtain additional mass analysis and identification if separation by GC is difficult. Different mass spectrometer types include the quadrupole (single or triple), time of flight (ToF) and ion trap, among others. The method of ionization used for analytes exiting the GC column depend on the mass analyser used. Hard and soft ionization methods impact the fragmentation in the MS differently based on the amount of energy transferred to the ions.

The ECD has been used extensively in literature; it is particularly sensitive to highly electronegative compounds such as halogens, nitriles, organometallics, and oxygen-containing compounds.[6,14,18] A beta emitter produces electrons that collide with a makeup gas; this forms more free electrons until sufficient amounts are created to produce a measurable current after colliding with an anode on the opposite side of the detector producing a current.[19] The changes in current due to collisions with other molecules lead to analyte detection and quantification. This makes the ECD a good choice for the detection of halogens, as they will be detected easily due to their electronegativity. A limitation observed when using an ECD is that contaminants in the matrix could also produce conflicting peaks resulting in complex chromatograms. Furthermore, the ECD does not offer identification, therefore all target analytes must be determined solely by retention time in the chromatogram.

In contrast to an ECD, the MS is an analyzer that can provide ion mass information in addition to measuring retention time and peak area.[17] The MS generally consists of three parts, the ionization source, the mass analyzer and a detector.[14] Ionization sources can be grouped into two types – hard and soft; hard ionization sources generate electrons which impact the molecules and cause them to ionize and later fragment.[14] Soft ionization methods are used if extensive fragmentation should be avoided; in those methods the energy of the colliding particle is reduced such that fragmentation is limited. Ideal ionization sources vary greatly depending on the type of analyte being studied the method of introduction into the MS, and the mass analyser. Two methods of sample introduction exist – batch, which involves direct introduction of the sample to the MS, and chromatographic, where the sample undergoes separation prior to introduction to the MS.[20]

When using chromatographic methods, a common ionization source is electron impact ionization (EI), a hard ionization source; here a heated filament expels electrons into the stream of incoming separated analytes.[17] This ionization type can be paired with a single quadrupole mass analyser. Ions travel to the quadrupole and are directed by the combined radio frequency voltage and direct offset voltage applied to the poles. Separation within the mass analyser is based on mass to charge ratio, as ions of a certain mass will have a given velocity, and the movement of the molecule, governed by the applied fields, will be dependent on the charge. Due to this process, only those ions for which the user selects will make it through the mass analyser to the detector. A common detector for these instruments is an electron multiplier (EM); acting similarly to the previously used photomultiplier tubes, it involves ions striking a dynode resulting in a cascade of electrons being sent to a current-to-voltage converting amplifier allowing a computer readout.[21] Advancements to the EM detector, such as a continuous dynode or multiple channel EMs have

improved the electronic amplification; this is particularly important for higher sensitivity mass spectrometry devices such as a Time of Flight Mass Spectrometer (ToF-MS).[21]

1.2.3. Automation

As stated previously, sample preparation and analysis are often extremely time consuming; more sample handling also increases chances of introducing error. To improve this, automation is often used to remove the human aspect from the analysis. Autosamplers can be used for many steps in sample preparation such as adding standards or derivatization agents, heating and stirring, cleaning of equipment, and consistent sampling using a variety of tools. The benefits of using one can be seen clearly by improved results, and higher throughput with more samples being processed. For gas chromatography, a prominent name for autosamplers is CTC Analytics with their PAL Autosamplers.

Automatic samplers have improved significantly over the past twenty years from the first gas and liquid chromatography PAL System Autosampler Platform in 1998.[22] Improvements in motor stability, materials, and computer technology have changed the capabilities and consistency of autosamplers. New models now have more options such as automatically changing tools (e.g. from a syringe to an SPME device), advanced cleaning procedures, and improved injection systems. Gerstel, another prominent manufacturer of autosamplers for GC, developed a thermal desorption unit (TDU) and a cooled injection system (CIS). These allow for the thermal desorption of irregularly sized items. A thermally stable item can be placed into a thermal desorption tube for transfer into the TDU where it is heated to similar desorption temperatures as a regular GC would use, ranging from 200°C to 280°C. As the TDU is heating, a cooled injection system sits below

and focuses the desorbed sample into a cryogenically cooled analyte plug. Once desorption is complete, the CIS rapidly heats, and the analytes are introduced into the column. This cryo-trap is necessary, because larger items placed in the TDU tubes will require a longer desorption time; however, significant band broadening would be seen without using this cooling trap. Furthermore, when not using atypically sized items, an injector head can be mounted onto the CIS, allowing it to act effectively like a regular injector. The benefit to the CIS injector style is that it operates on a spring-loaded o-ring piece that moves out of the way as a sample is injected. This type of system replaces disposable septa.

1.3. Applications

1.3.1. Pesticides in Soymilk

Soymilk is the most popular plant based dairy alternative milk product used to replace regular milk consumption. Similar to dairy milk, it is a stable emulsion of oil, water and proteins and is often fortified with vitamins, minerals and emulsifying agents, in addition to containing natural fibers and sugars occurring in plant matter. In the context of sample preparation, soymilk is a challenging matrix considering the type and number of analytical procedures typically needed to isolate the compounds of interest in clean extracts while also avoiding interfering co-extracted components. Since the sample matrix is an emulsion, the analytical techniques must be designed to consider contaminants that can exist in both the hydrophobic and hydrophilic phases.

In literature, various methods have been reported for contaminants analysis of soybeans, however, only few reports are devoted to pesticides residue analysis in soymilk products. The

sample preparation techniques of choice for soy milk analysis are usually QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe)[23–25], solid phase extraction (SPE)[26].

1.3.2. Polychlorinated n-Alkanes in Cod Oil

Polychlorinated n-alkanes (PCAs) have been an environmental concern in North America since 1993, when the Government of Canada added the class of compounds to the Priority Substances List.[27] Due to their high boiling points and thermal stability, this class of compounds was used in industry as a machining lubricant and as an additive in plasticizers and flame retardants.[27] Environment Canada has emphasized monitoring these compounds since they were classified by the International Agency for Research on Cancer (IARC) as potentially carcinogenic to humans. They are also classified as a severe marine pollutant by the International Maritime Organization (IMO) due to their propensity to bioaccumulate in fish.[28]

Sample preparation methods for extracting these compounds from various samples must be improved and expanded on. The most common combination of techniques includes liquid-liquid extraction, fat hydrolysis using an acid wash, GPC and Florisil or silica columns.[18,29–32]The most notable matrices of interest are fish and fish oil products as well as breastmilk, as bioaccumulation of PCAs in these foodstuffs place them into the human food chain.[5,29,31,33] Having an extremely high fat content, these samples have very complex matrices; therefore, a single technique is rarely used standalone. Often many separation techniques are needed to ensure adequate matrix removal, without which instrumentation may become damaged and chromatographic separation of analytes compromised.

The complex matrices also result in significant background noise due to other hydrophobic components that are likely to co-extract. These include fatty acids, cholesterol, retinoids and vitamins, all of which can compete for extraction space on sorbents and hinder separation. Other pollutants such as alkanes or heavier aromatic compounds such as polycyclic aromatic hydrocarbons may also be present and co-extract due to having similar properties. Reduction of the background is critical for accurate and sensitive quantitation; to optimize cleanup, many researchers use a combination of some or all of the aforementioned techniques to isolate the PCAs from other coextractants.[18,29–32]While this generally ensures sufficient sensitivity in detection, it also generates significant amounts of waste, has long turnaround times, and introduces error with each step that is added to the process.

1.4. Thesis Objective

The objective of these projects is to develop new methods of detecting anthropogenic compounds in fatty matrices made for human consumption, notably soymilk and fish oil products. Current methods for pesticide and polychlorinated n-alkane (PCA) detection in food products are time consuming, multi step – therefore error prone, and have significant negative environmental impacts due to solvent and acid use. New methods are therefore being investigated in order to transfer commercial testing to more sustainable practices. SPME should meet this requirement for a cleaner, cheaper, reusable and more eco-friendly analytical method. The objective of this thesis is the development of these methods targeting future implementation at the industrial level.

2. Chapter 2: Analysis of Pesticides from Soymilk Using Direct Immersion SPME

Preamble

A portion of this chapter has been previously presented to the University of Waterloo as an Honours Research Project and is included as background to support the finalized methods and validation results that are part of this thesis. A portion of the written material contained in this chapter also appears in a manuscript in preparation entitled “Direct immersion SPME in soymilk for pesticide analysis at trace levels by means of a matrix compatible coating”; some material is co-authored by Emanuela Gionfriddo.

2.1. Introduction

The targeted pesticide group for soymilk analysis includes trifuralin, dimethoate, diazinon, malathion, chlorpyrifos, thiabendazole, phosalone, λ-cyhalothrin, α-β-cyfluthrin, and esfenvalerate. Seen from the logP values in Figure 3, the compounds comprise both hydrophobic and hydrophilic substances, and in this respect are representative of industrially applied pesticides.

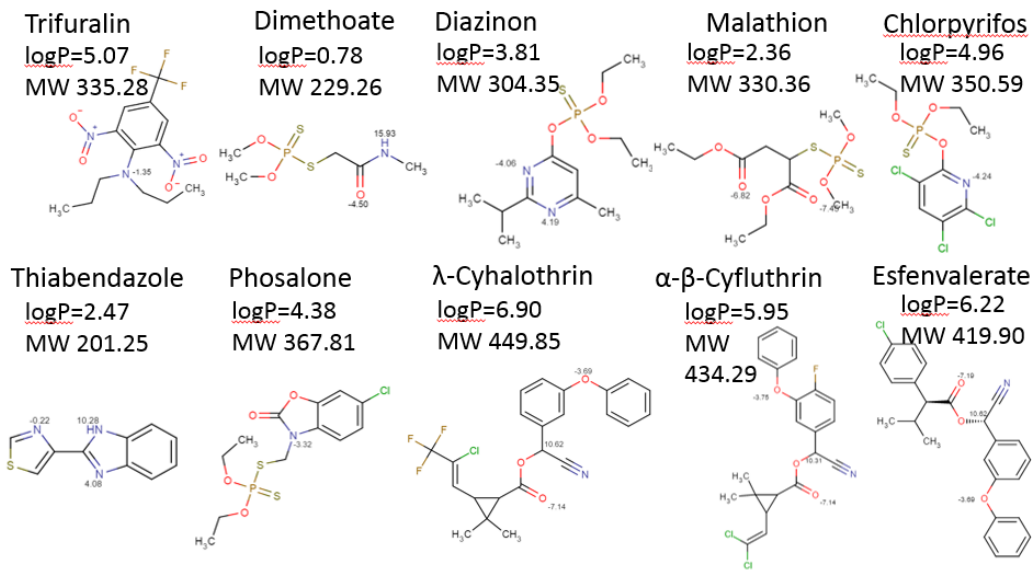


Figure 3:Pesticides used in this study and their structure, logP and molecular weight (abbr. MW, g/mol), in the order of boiling point.

Maximum residue levels (MRLs) regulated for contaminants in food are usually applied to raw materials and not always to their derivatives; in this case, soybeans as opposed to the milk, tofu or miso. Values shown in Table 1 are for whole beans; the Health Canada guidelines state that if a residue limit is not explicitly given, the value for industry must be less than 0.1ppm.[27,34,35]

Table 1: MRL values for the target analytes for both Canada and USA in ppm.

	USA	*CAN*	
Chlorpyrifos	0.3	NA	
Cyfluthrin	0.03	NA	
Diazinon	NA	NA	
Dimethoate	0.05	NA	
Esfenvalerate	0.05	NA	
Lambda-Cyhalothrin	0.01		0.2
Malathion	8	NA	
Phosalone	NA	NA	
Thiabendazole	0.1	NA	
Trifluralin	0.05		0.05

As stated in the introduction, current methods heavily rely on using QuEChERS and SPE to perform cleanup and concentration; the goals of this work is to reduce the waste associated with those methods by applying a microextraction technique.

2.2. Experimental

2.2.1. Reagents and Supplies

Table 2: Solvents used for each pesticide to ensure solvation at storage temperatures.

Compound	***Solvent***
Trifuralin	Acetonitrile
Dimethoate	Acetonitrile
Diazinon	Acetonitrile
Diazinon D10	Acetonitrile
Malathion	Acetonitrile
Malathion D6	Acetonitrile
Chlorpyrifos	Acetonitrile
Thiabendazole	Methanol
Thiabendazole D4	Methanol
Phosalone	Acetonitrile
λ-Cyhalothrin	Methanol
Cyfluthrin	Dichloromethane
Esfenvalerate	Methanol

The pesticides trifuralin, dimethoate, diazinon, malathion, chlorpyrifos, thiabendazole, phosalone, λ-cyhalothrin, α-β-cyfluthrin, and esfenvalerate were Pestanal grade and purchased from Sigma Aldrich (Oakville, ON, Canada). Internal standards Diazinon D10, Thiabendazole D4 and Malathion D6 were purchased from Sigma Aldrich (Oakville, ON, Canada). Pure standards were kept in dark conditions and refrigerated in their original packaging at 4°C. Stock solutions of each compound were prepared at concentrations ranging from 1ppb to 1000ppm using the solvent specified in Table 2.

Solutions were stored in the dark and chilled to -30°C. Working mixtures were prepared in methanol, monthly or more often as needed, and kept in dark conditions and refrigerated to -30°C. HPLC grade solvents methanol, ethanol, acetone, dichloromethane and acetonitrile as well as sodium chloride salt, were purchased from Sigma Aldrich (Oakville, ON, Canada). Water was purified using Milli-Q systems (Etobicoke, ON, Canada).

SPME fibers PDMS/DVB (65 μm) and PDMS (100 μm) were purchased from Millipore Sigma (Bellefonte, PA, USA). Matrix compatible PDMS/DVB/PDMS fibers were initially prepared in the laboratory according to the procedure described by Souza-Silva et. al and

subsequently purchased from Millipore Sigma (Bellefonte, PA, USA) upon their release into the market.[12]

Unsweetened organic soymilk was purchased from local markets in Waterloo (ON, Canada) and treated as follows: all milk was stored in the dark under refrigeration at 4°C and kept from freezing when not in use. Soymilk was divided in aliquots for transfer, in order to avoid warming and cooling cycles which might damage the texture integrity of the milk. Milk cartons were replaced upon reaching the expiry date on the package. All solutions were equilibrated to room temperature prior to handling.

2.2.2. Instrumentation

For coating lifetime evaluation and initial optimization, a Varian 3800/4000 GC-IT/MS (Mississauga, ON, Canada) equipped with a splitless/split injector and an ion trap MS detector was used for all analyses. A Combi-Pal autosampler was used and controlled by Cycle Composer Software (version1.4.0). MS operational conditions were: EI at 70 eV; ion source temperature, 240°C; transfer line temperature, 260°C. The ion trap was operated in full scan mode within an m/z range of 35-450.

For the method development, a Pegasus III 4D GC-ToF/MS (LECO, Saint Joseph, MI, USA) was used. The instrument was controlled by ChromaTOF software from LECO. The GC-ToF/MS was equipped with an MPS autosampler (Gerstel Inc., Linthicum, MD, USA) for automated SPME sampling and desorption. The autosampler was controlled by Maestro software (Gerstel Inc. Linthicum, MD, USA). MS operational conditions were: electron ionization (EI) at 70 eV; ion

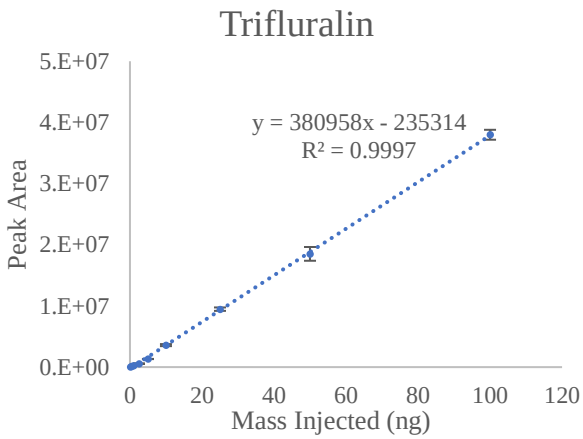
source temperature, 200°C; transfer line temperature 25°C, the mass range acquired was 35-600 m/z.

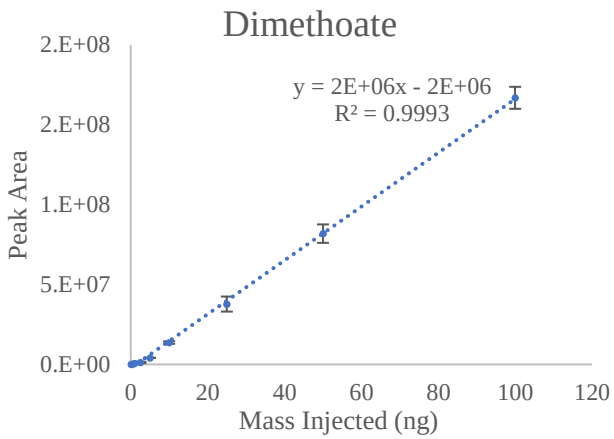
For method validation, an Agilent 7890-5977A GC-MS (Agilent Technologies Santa Clara, CA, United States) was used. The instrument was equipped with MPS Robotic Pro autosampler (Gerstel Inc., Linthicum, MD, USA). The mass spectrometer was operated in selected ion monitoring (SIM) mode. The transfer line temperature was 250°C, MS source 230°C, and MS quadrupole 150°C, electron ionization (EI) was performed at 70 eV. The instrument was controlled by MassHunter workstation software (Agilent Technologies Santa Clara, CA, United States) with embedded Maestro software (Gerstel Inc., Linthicum, MD, USA)

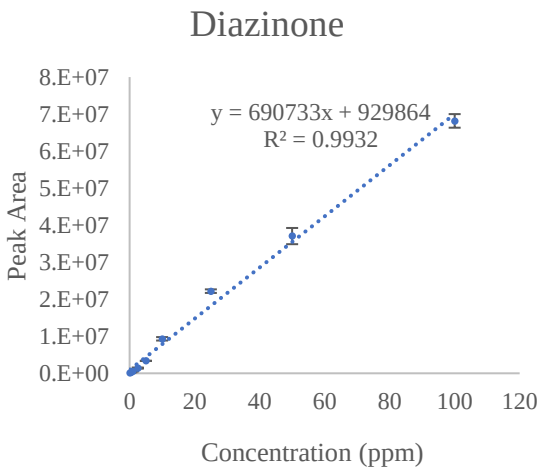
Gas chromatographic method parameters for all the instruments used were: starting oven temperature of 80°C, held for 2 minutes, ramp at 6°C/min to 280°C and held for 4 minutes, resulting in a 40 min run time. The carrier gas was helium, with a flow rate of 1.5 ml/min. Chromatographic separation was carried out using ultra-high purity helium provided by Praxair (Danbury, CT, USA) and a HP-5MS capillary column (30mx 0.25mm x0.25µm) (Agilent Technologies Santa Clara, CA, United States).

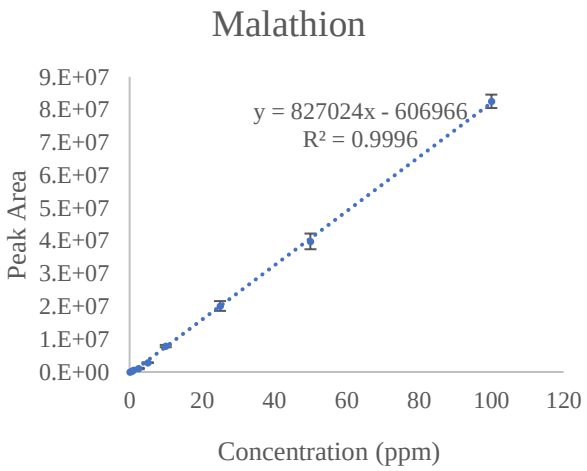
2.2.3. Previous Work

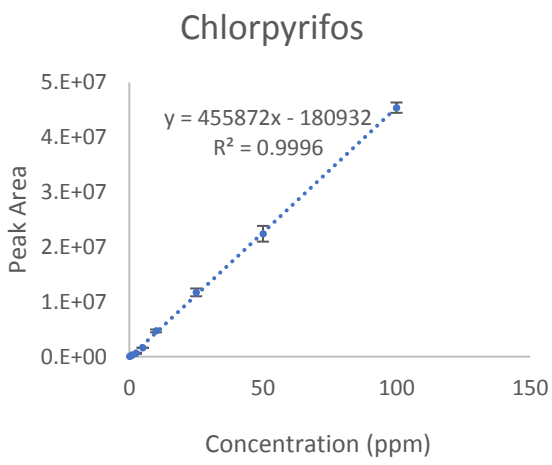
Linear range of the instrument was determined by a liquid injection calibration; the range injected was 1ng to 100ng.

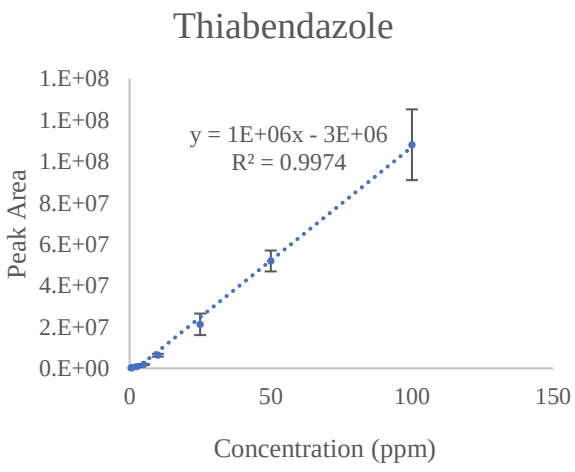

Trifluralin
y = 380958x - 235314
R² = 0.9997
Peak Area
Mass Injected (ng)
5.E+07
4.E+07
3.E+07
2.E+07
1.E+07
0.E+00
0
20
40
60
80
100
120


Dimethoate
y = 2E+06x - 2E+06
R² = 0.9993
Peak Area
Mass Injected (ng)
2.E+08
2.E+08
1.E+08
5.E+07
0.E+00
0
20
40
60
80
100
120


Diazinone
y = 690733x + 929864
R² = 0.9932
Peak Area
Concentration (ppm)
8.E+07
7.E+07
6.E+07
5.E+07
4.E+07
3.E+07
2.E+07
1.E+07
0.E+00
0
20
40
60
80
100
120


Malathion
y = 827024x - 606966
R² = 0.9996
Peak Area
Concentration (ppm)
9.E+07
8.E+07
7.E+07
6.E+07
5.E+07
4.E+07
3.E+07
2.E+07
1.E+07
0.E+00
0
20
40
60
80
100
120


Chlorpyrifos
y = 455872x - 180932
R² = 0.9996
Peak Area
Concentration (ppm)
5.E+07
4.E+07
3.E+07
2.E+07
1.E+07
0.E+00
0
50
100
150


Thiabendazole
y = 1E+06x - 3E+06
R² = 0.9974
Peak Area
Concentration (ppm)
1.E+08
1.E+08
1.E+08
8.E+07
6.E+07
4.E+07
2.E+07
0.E+00
0
50
100
150

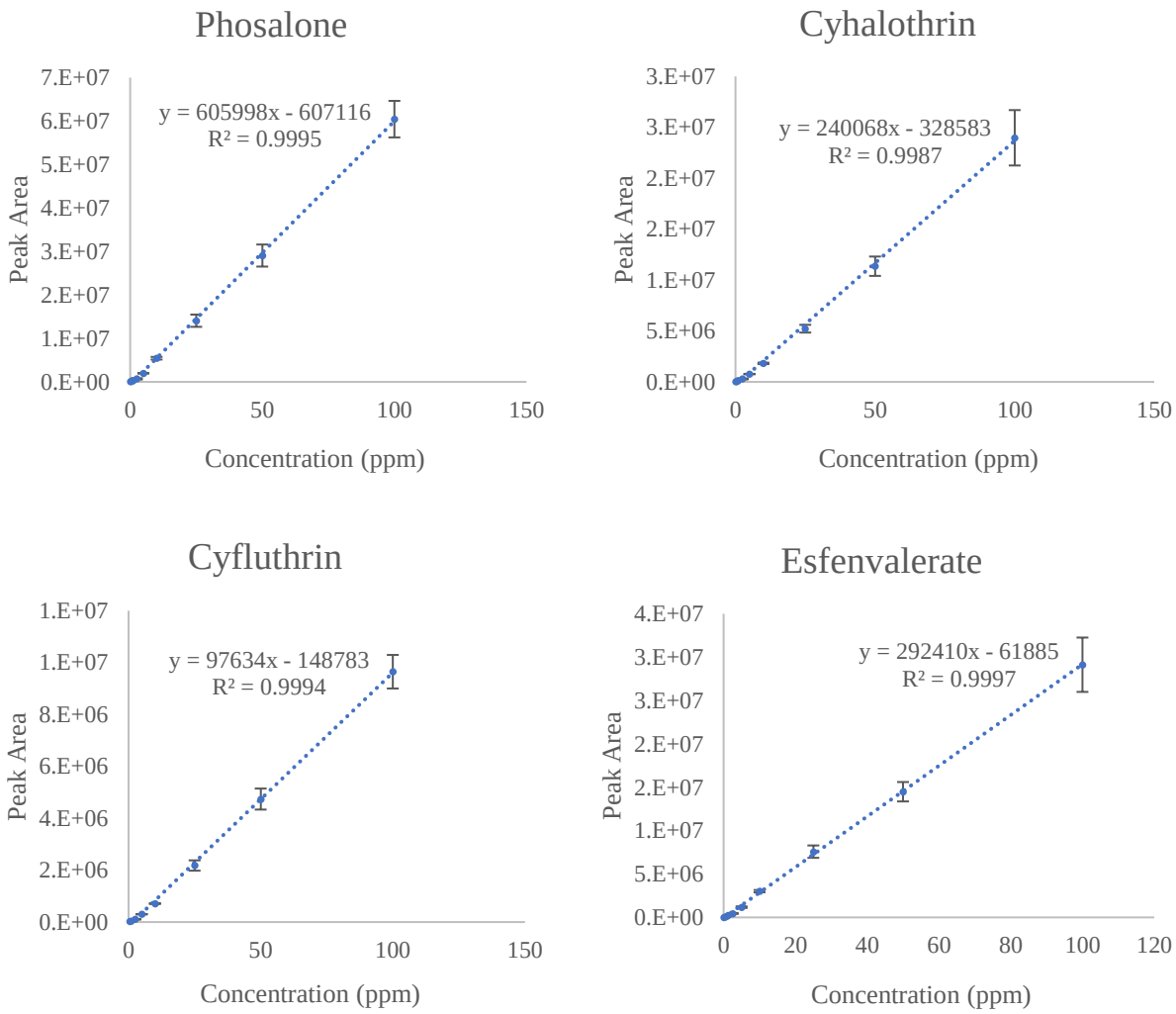


Figure 4:Instrumental calibration for all compounds performed on GC-MS in SIM mode.

Direct immersion was used for sampling the soymilk. Due to the complexity of the matrix, an overcoated fiber was used for this study. This style of fiber is comprised of a regular DVB/PDMS 65µm diameter fiber, coated with an extra layer of PDMS, resulting in a smooth surface that prevents matrix adhesion. Previous studies have shown that the addition of this coating significantly reduces the

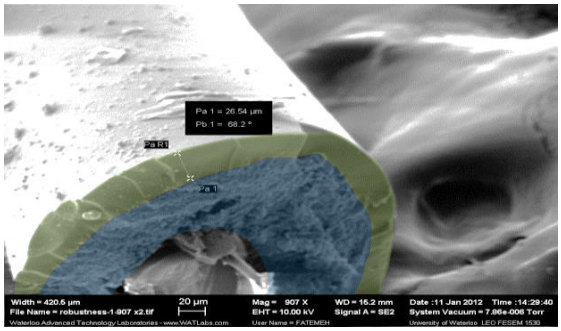


Figure 5: A microscope image of the overcoated fiber showing the two layers, PDMS in green and DVB/PDMS in blue

amount of matrix attachment to the fiber thus increasing its lifetime. [12] In other studies, the matrix in question was heavily populated with fiber and sugars that burn quickly in a GC injector port resulting in permanent adhesion of the caramelized sugars onto the fiber.[12] A similar concern exists with soymilk, due to the complexity of the matrix, particularly the fat and protein content. A maillard reaction on either the fiber on the sides of the injector port liner would cause a permanent elevated background or reduce extraction efficiency, resulting in skewed data.

To begin optimization, a commonly used technique to improve extraction is the addition of salt. In complex matrices, this can help increase the amount extracted by the fiber by increasing the ionic strength of the solution. At the same time, higher ionic strength promotes partitioning of nonpolar analytes into the fiber as they become less soluble in the solution as more salt is added. For the soymilk samples, it can be seen from Figure 6 that the addition of salt had statistically no effect on the extraction. This may be due to the complexity of the matrix, as the analytes may also partition into the suspended fat before the fiber is introduced.

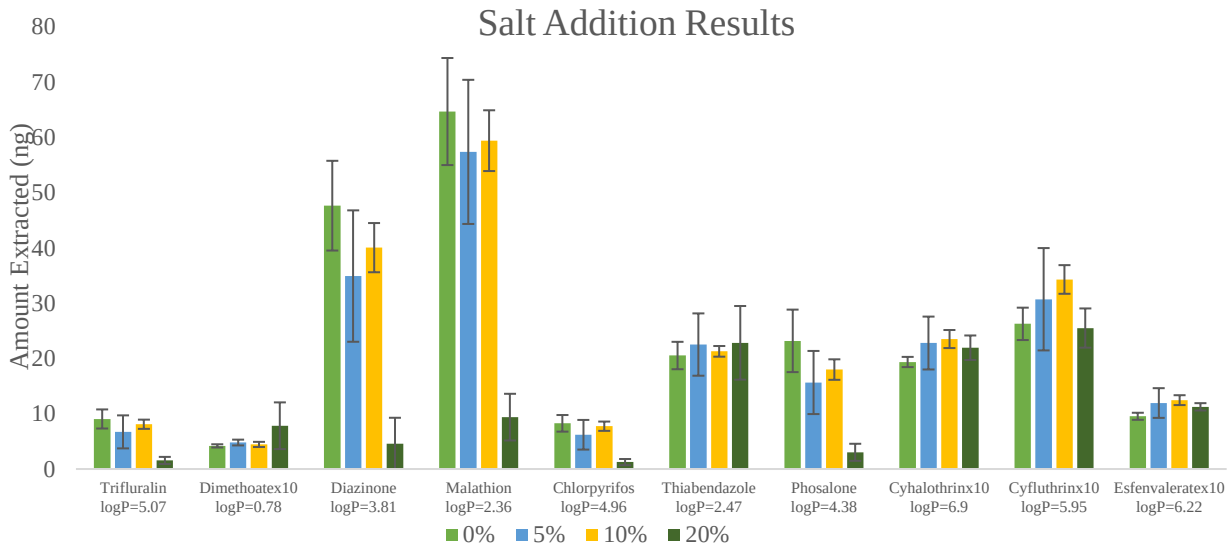


Figure 6:Results from addition of sodium chloride salt to the soymilk matrix in 5, 10, and 20 percent by weight.

Since salting out the analytes was not effective, and possibly due to the fatty nature of the matrix, the addition of solvent was also tested in order to promote extraction from the suspended fat in the matrix. Each sample was diluted by half with a mixture of water and solvent, ranging from zero to 100% solvent. These samples were vortexed for 10 minutes at 1500 rpm after dilution to ensure thorough mixing.

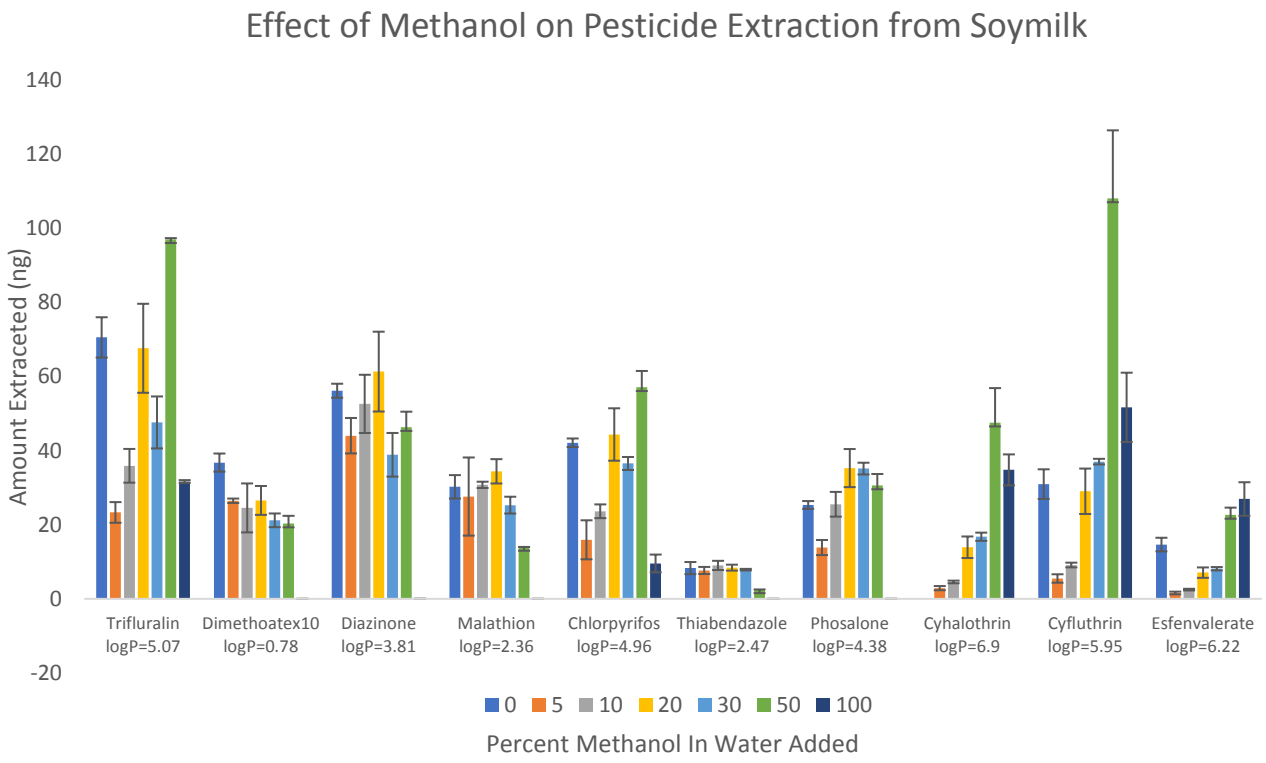
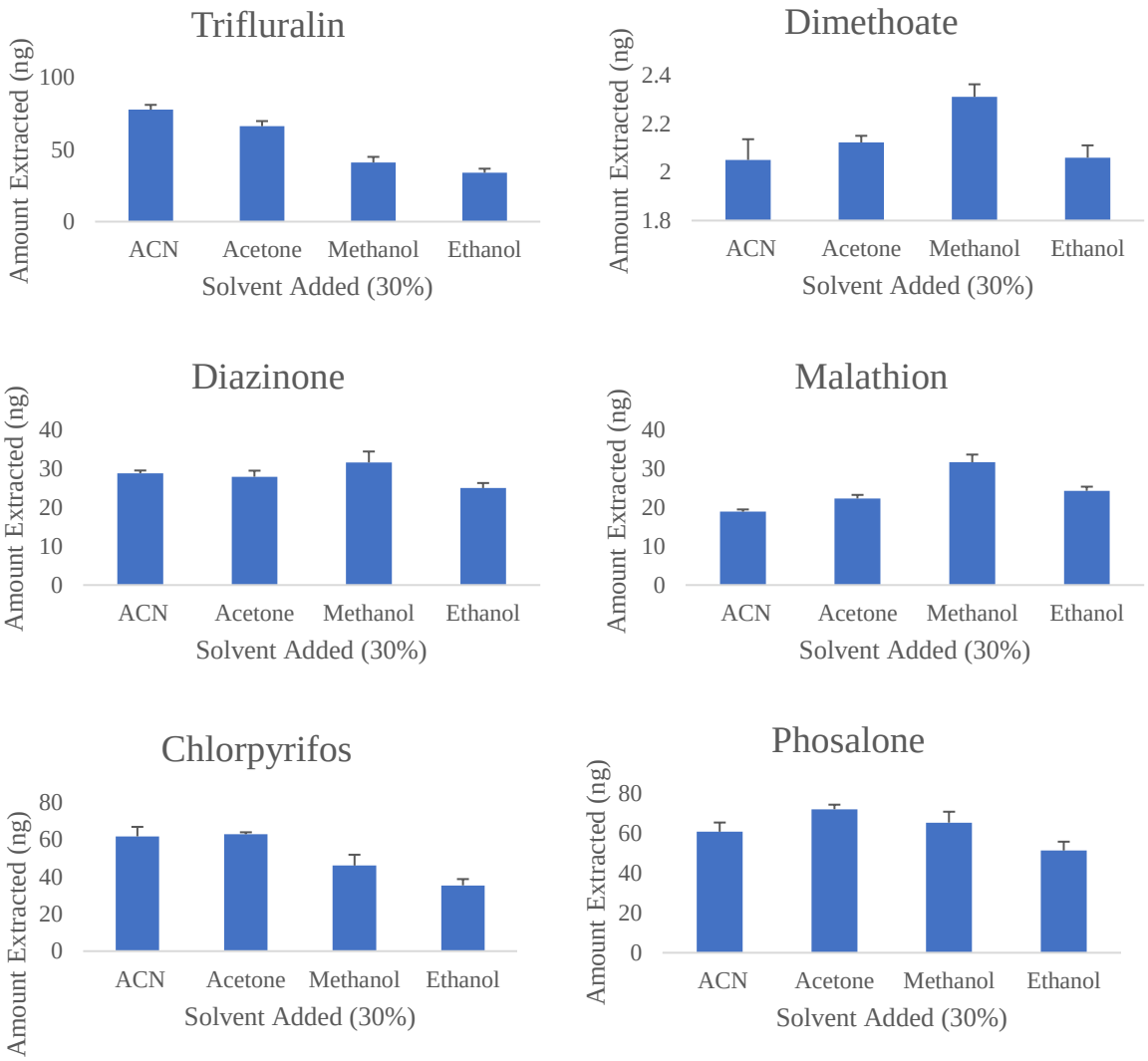


Figure 7:The effects of different concentrations of methanol on the extraction performance of the system; concentrations of methanol ranging from 0% (or 100% water) to 100% methanol.

Results show that for the compounds with lower log P, the addition of solvent made no impact on the extraction performance, however for those with higher log P the added solvent improved the amount extracted with increasing volumes of solvent. Accordingly, further solvent use parameters were optimized as it was clear that it would improve results for some compounds while

providing no hindrance to others. The optimal concentration was found to be 30%, as it was the highest concentration at which the emulsion would remain stable. At 50% solvent, coagulation occurred within 20 hours of dilution, disallowing overnight sampling. Using 100% solvent resulted in immediate coagulation of the sample, hindering extraction for many compounds. Different solvents were tested at 30%, as seen in figure 8.



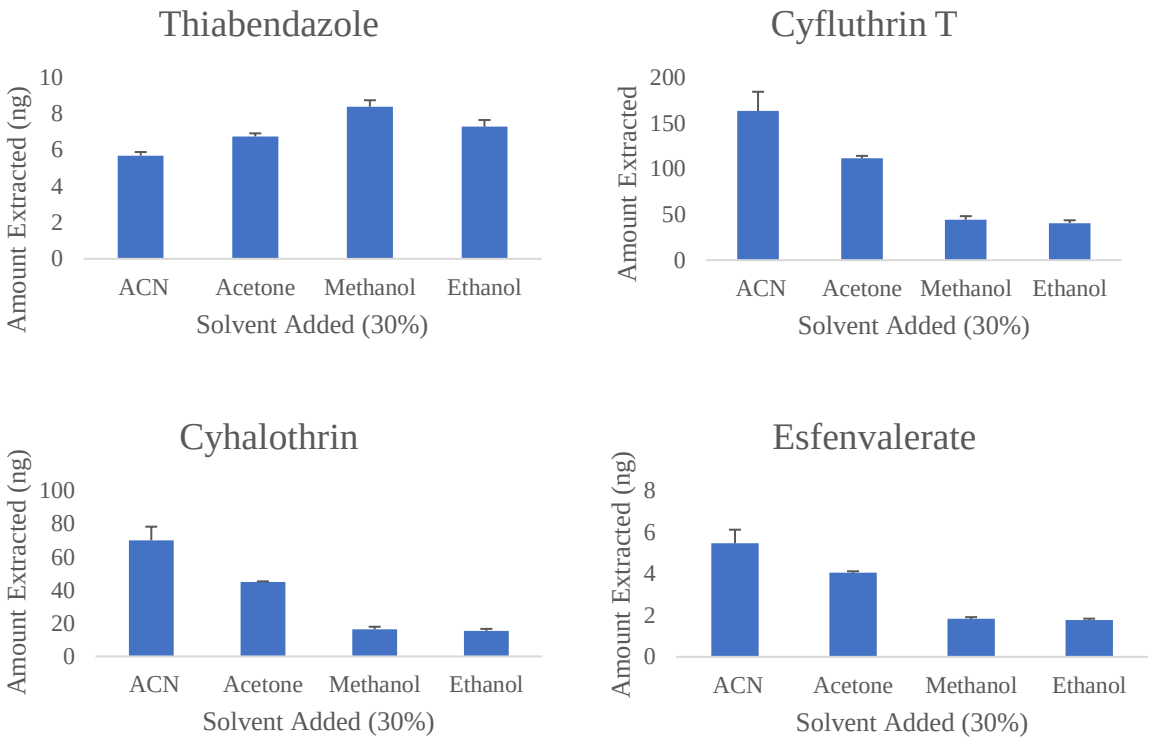
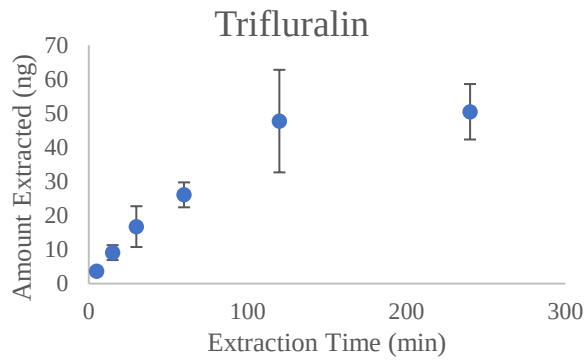
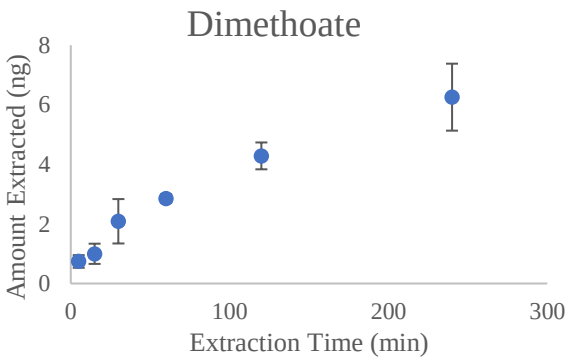


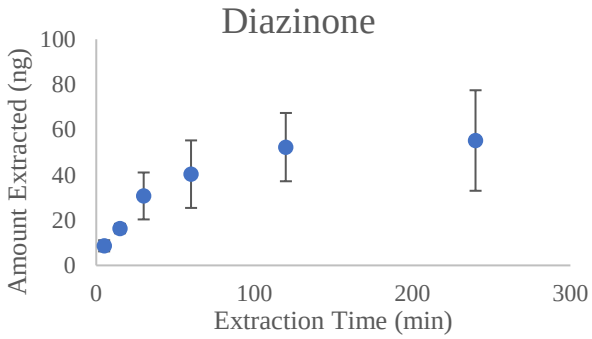
Figure 8:Solvent addition was tested over different solvents after 30% was determined to be the optimal concentration in the dilution mixture, n=3, performed on GC-MS in SIM mode.

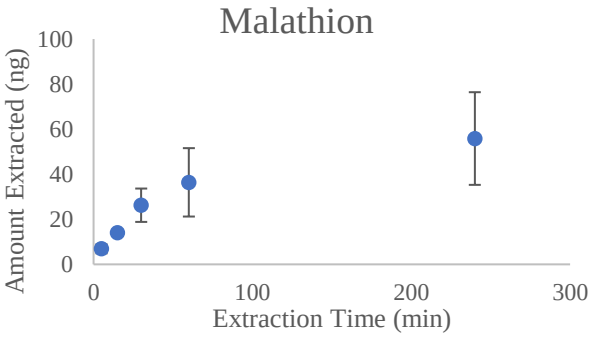
For the majority of analytes, acetone had a positive effect on extraction, compared to other solvents, and did not cause a lapse in precision. Furthermore, of the four solvents sampled, acetone is safer for use and for the environment when compared to ACN or methanol. It was therefore chosen as the optimal solvent moving forward.

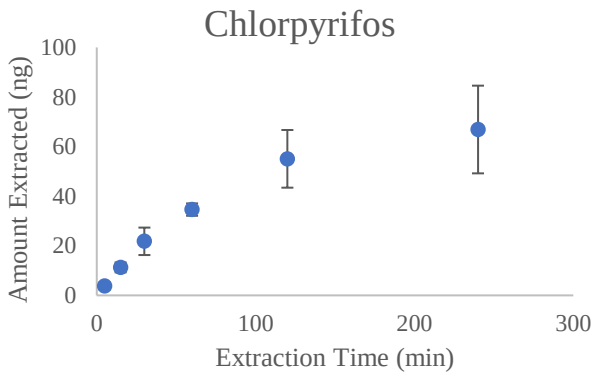
An extraction time profile was done using the abovementioned optimized parameters. This determines the linear range for extraction of each analyte as well as the equilibration point. Sampling at equilibrium obviates the need to precisely control extraction time. In contrast, if an extraction is performed in the linear regime, the smallest deviation in the extraction time may prove to have a big difference in amount extracted.

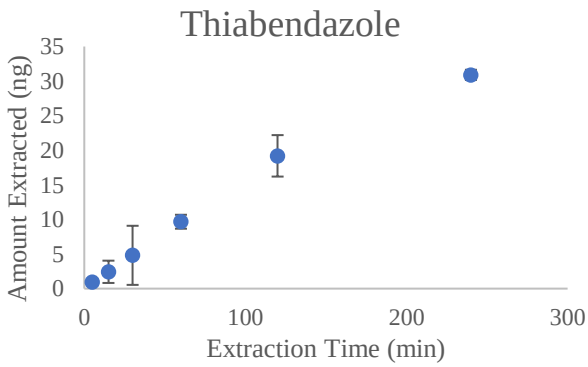

Trifluralin
Amount Extracted (ng)
70
60
50
40
30
20
10
0
0
100
200
300
Extraction Time (min)

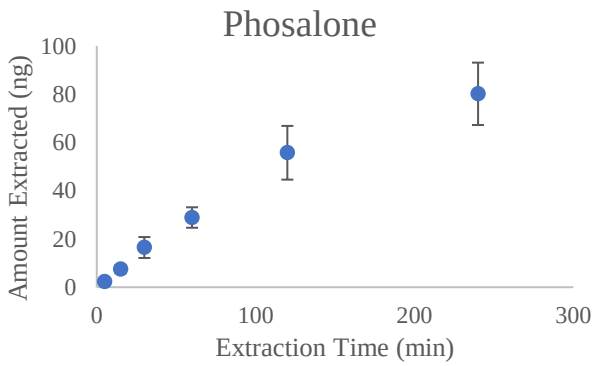

Dimethoate
Amount Extracted (ng)
8
6
4
2
0
0
100
200
300
Extraction Time (min)

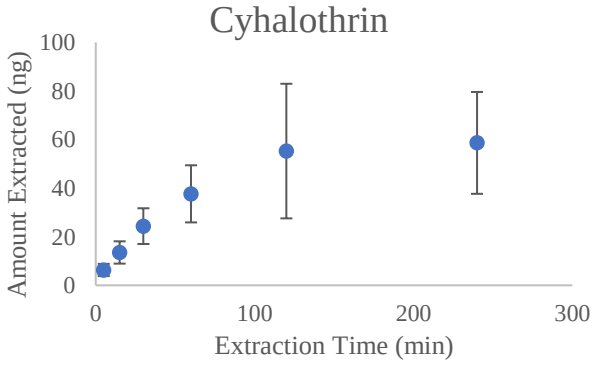

Diazinone
Amount Extracted (ng)
100
80
60
40
20
0
0
100
200
300
Extraction Time (min)


Malathion
Amount Extracted (ng)
100
80
60
40
20
0
0
100
200
300
Extraction Time (min)


Chlorpyrifos
Amount Extracted (ng)
100
80
60
40
20
0
0
100
200
300
Extraction Time (min)


Thiabendazole
Amount Extracted (ng)
35
30
25
20
15
10
5
0
0
100
200
300
Extraction Time (min)


Phosalone
Amount Extracted (ng)
100
80
60
40
20
0
0
100
200
300
Extraction Time (min)


Cyhalothrin
Amount Extracted (ng)
100
80
60
40
20
0
0
100
200
300
Extraction Time (min)

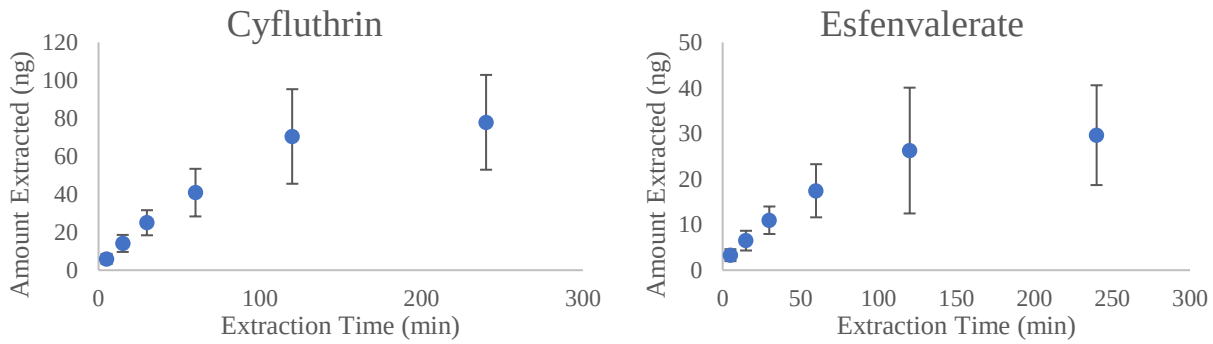


Figure 9:Extraction time profiles for each of the compounds, n=3, performed on GC-MS in SIM mode.

The results of the extraction time profiles show that the analytes will not reach equilibrium until well into the 3 hours mark. This is not a reasonable turnaround time for a sample, as such a compromise must be made, and some analytes must be sampled in the linear regime. This is no longer as much of a concern as in the past due to the advent of GC-SPME amenable autosamplers. The difference in extraction time from one sample to the next once the system is automated is near negligible, even in a linear regime of an extraction time profile.

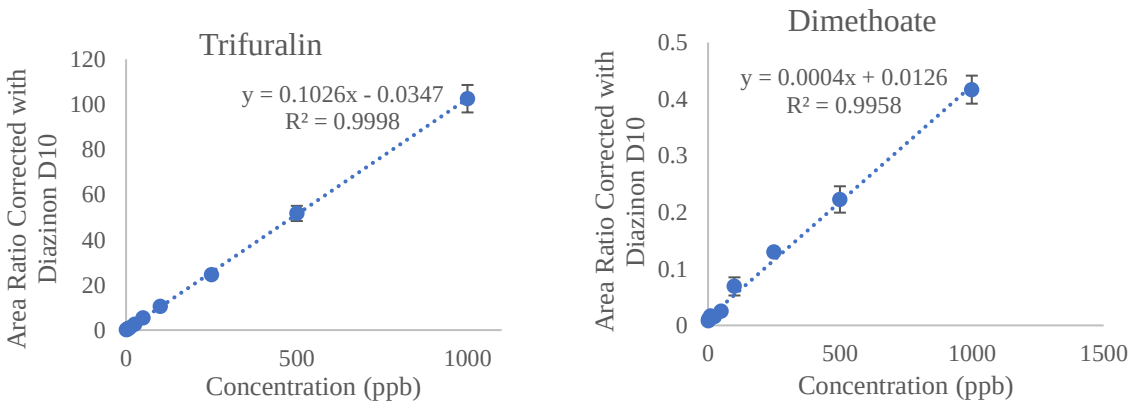
2.2.4. Finalized Methods

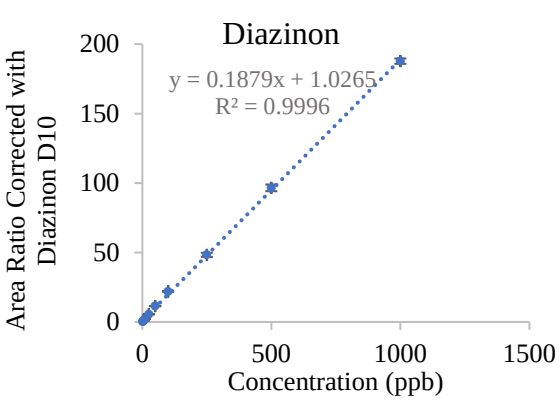
Organic and unsweetened soymilk was stored at +4°C. Prior analysis, the soymilk was brought to room temperature and weighed in batches daily for spiking and sample distribution. Each sample contained 4.5g of soymilk, weighed and spiked using 45ul of the working solution. For method validation 45ul deuterated internal standards solution were also spiked into the sample. The spiked soymilk was placed to vortex in a benchtop mixer at room temperature and 1500rpm for 1 hour. The solution was then mixed with 4.5g of an acetone: water solution (3:7 v:v). The vials were capped and placed into a benchtop mixer and vortexed again at 1500rpm for 10 minutes. The prepared samples were then placed into the autosampler Gerstel MPS Robotic (Gerstel Inc. Linthicum, MD, USA) for extraction.

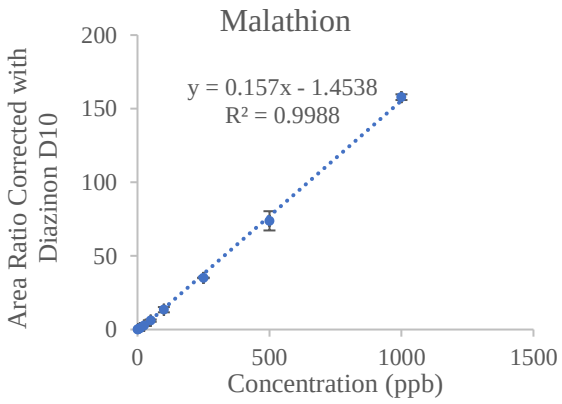
The extraction of each sample took place for 40 minutes at 40°C and 500 rpm stirring after a 1 min sample incubation at the same pre-sets. Extraction was followed by a rinsing step of 10 sec in an acetone: water solution (1:9 v:v) at 500 rpm. The desorption was carried out for 15 min at 270 °C and a wash step was included afterwards in an acetone: water solution (1:1 v:v) for 1 min at 40°C at 500 rpm. At the desorption conditions used no analyte carry over was found. For the fiber lifetime assessment, batches of 10 samples were prepared according to the procedure described above. Prior to and after extraction of each sample batch, quality control analyses were carried out in order to establish instrumental signal stability by extraction of the targeted analytes spiked in water with a PDMS 100 µm SPME fiber. After analysis of each batch of samples the SPME fibers (PDMS/DVB/PDMS and PDMS/DVB) were inspected under a microscope and cleaned with a Kimwipe soaked with acetone.

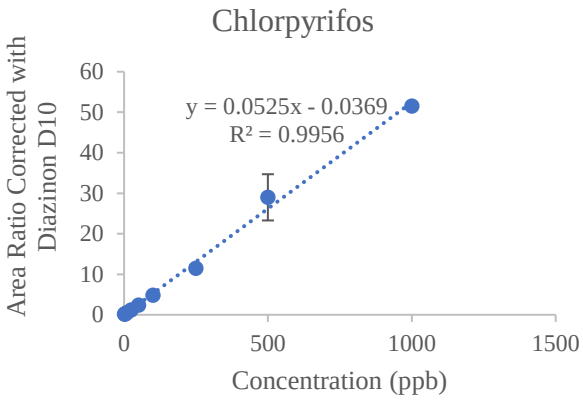
2.3. Validation Results and Discussion

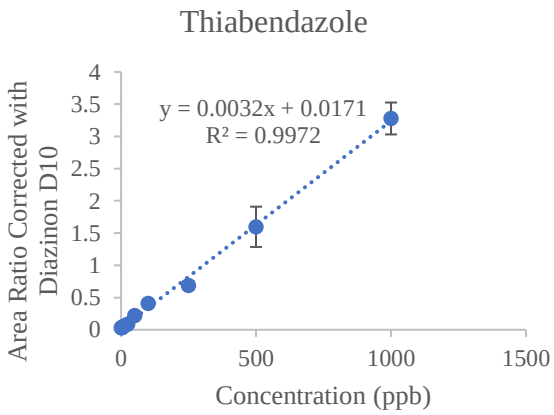
The finalized procedure was used to perform a matrix-matched calibration using organic unsweetened soymilk. Previous matrix blanks showed no residue in the original soymilk used for this study. The results shown here have been corrected with the internal standard diazinon D10.

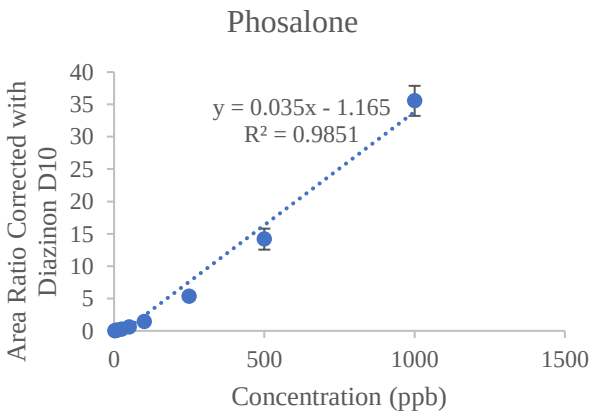


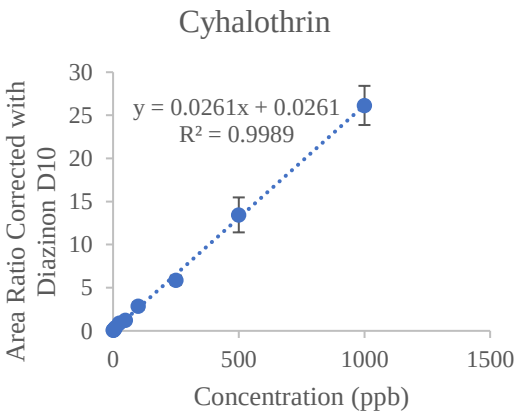

Diazinon
y = 0.1879x + 1.0265
R² = 0.9996
Area Ratio Corrected with Diazinon D10
Concentration (ppb)
0
50
100
150
200
0
500
1000
1500


Malathion
y = 0.157x - 1.4538
R² = 0.9988
Area Ratio Corrected with Diazinon D10
Concentration (ppb)
0
50
100
150
200
0
500
1000
1500


Chlorpyrifos
y = 0.0525x - 0.0369
R² = 0.9956
Area Ratio Corrected with Diazinon D10
Concentration (ppb)
0
10
20
30
40
50
60
0
500
1000
1500


Thiabendazole
y = 0.0032x + 0.0171
R² = 0.9972
Area Ratio Corrected with Diazinon D10
Concentration (ppb)
0
0.5
1
1.5
2
2.5
3
3.5
4
0
500
1000
1500


Phosalone
y = 0.035x - 1.165
R² = 0.9851
Area Ratio Corrected with Diazinon D10
Concentration (ppb)
0
5
10
15
20
25
30
35
40
0
500
1000
1500


Cyhalothrin
y = 0.0261x + 0.0261
R² = 0.9989
Area Ratio Corrected with Diazinon D10
Concentration (ppb)
0
5
10
15
20
25
30
0
500
1000
1500

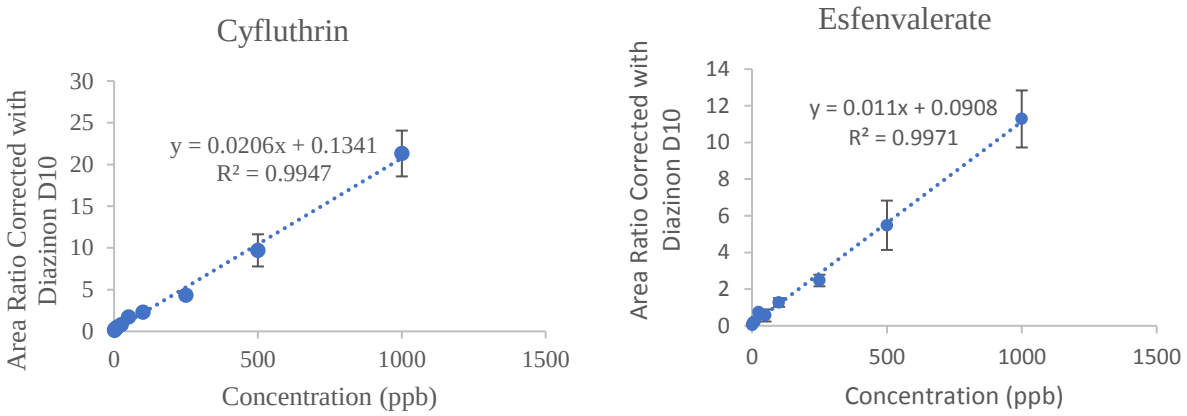


Figure 10:Matrix matched calibration of all standards, each corrected with diazinon D10, n=3, performed on GC-MS using SIM mode.

The same procedure was used for the validation; where three different concentrations were used to validate the method. Inter-and intra-day reproducibility was shown to be good for all compounds based on published method development guidelines.

Table 3: Inter- and Intra day validation accuracy results using the optimized procedure outlined above, using three levels of validation 15, 75 and 200 ppb, n=4. Accuracy was back calculated from the method and compared to known values as a percentage.

Compounds	***Accuracy (%) Day 1***			***Accuracy (%) Day 2***			***Accuracy (%) Day 3***		
Level (ppb):	**15**	**75**	**200**	**15**	**75**	**200**	**15**	**75**	**200**
Trifuralin	88	94	103	80	91	92	74	58	77
Dimethoate	94	72	96	102	89	114	128	120	118
Diazinon	99	102	109	107	102	102	102	119	130
Malathion	111	78	81	117	85	82	119	100	106
Chlorpyrifos	87	94	102	96	94	91	85	93	99
Thiabendazole	106	129	81	81	90	119	82	121	124
Phosalone	118	104	104	116	105	111	123	122	124
Cyhalothrin	111	99	80	101	74	77	87	80	71
Cyfluthrin	114	97	88	89	77	94	89	89	95
Esfenvalerate	89	78	79	104	74	124	97	99	112

Table 4: Precision results for the Inter- and intra day validation, using three levels of validation 15, 75 and 200 ppb, n=4. Percent relative standard deviation was used to assess method precision.

Compounds	***Precision (%) Day 1***			***Precision (%) Day 2***			***Precision (%) Day 3***		
Level (ppb)	**15**	**75**	**200**	**15**	**75**	**200**	**15**	**75**	**200**
Trifuralin	2	4	5	2	2	5	8	4	12
Dimethoate	14	6	3	6	15	16	1	11	23
Diazinon	6	1	2	8	1	2	9	3	3
Malathion	6	1	2	6	2	2	5	4	3
Chlorpyrifos	2	3	2	6	4	6	11	5	5
Thiabendazole	13	28	16	4	16	15	11	12	3
Phosalone	18	4	2	12	4	8	13	11	8
Cyhalothrin	4	14	4	13	7	5	11	21	9
Cyfluthrin	9	13	2	15	8	10	11	19	25
Esfenvalerate	9	14	3	16	9	10	13	10	11

The validation accuracy results shown have been back calculated using the calibration curves shown in Figure 10. The results were then compared against the known value spiked into each sample, resulting in an accuracy value; values of 100% demonstrate exact back calculation and are accepted to a range between 70 and 130%.[36] The precision values shown are the relative standard deviations for the results of the validation. The linear range of each compound is listed in Table 5.

Finally, two different unknown, non-spiked commercial soymilk samples were tested using this method shown in Table 5. Five compounds were found, Dimethoate, Malathion, Chlorpyrifos., Phosalone and Cyfluthrin. While the values of most pesticides are below MRLs for the soybeans, the value for dimethoate in one sample exceeds both the Canadian MRL (100ppb) and the U.S. value (50ppb).[34,37]

Table 5: Unknown Sample results, U.S. maximum residue levels, and linear range.

Concentration/ Compound	*Sample 1 Concentration (ppb)*	*Sample 2 Concentration (ppb)*	*U.S. MRL (ppb)*	*Method Linear Range*

Trifuralin	BQL	BQL	50	1-1000
Dimethoate	118.86	6.5	50	1-1000
Diazinon	BQL	BQL	N/A	1-1000
Malathion	27.38	28.2	8000	1-1000
Chlorpyrifos	7.40	7.7	300	1-1000
Thiabendazole	BQL	BQL	20	1-1000
Phosalone	40.13	33.6	N/A	2.5-1000
Cyhalothrin	BQL	BQL	10	1-1000
Cyfluthrin	20.50	BQL	30	1-1000
Esfenvalerate	BQL	BQL	50	1-1000

2.4. Conclusions

Soymilk pesticide analysis is a time-consuming process in industry when using QuEChERS or SPE procedures followed by a chromatography step. In contrast, the method developed and validated here shows a turnaround time of an hour and a half per sample, with the entire extraction, rinsing, desorption and washing process done by a compact autosampler. The samples, after dilution with the solvent mixture, were shown to be shelf stable at room temperature for 72 hours, allowing for streamlined preparations to optimize the workflow. Overall, this method meets the thresholds of MRLs imposed by both Canadian and American regulatory agencies while being an eco-friendly and cost-effective alternative to traditional sample preparation technologies.

3. Chapter 3: Polychlorinated n-Alkane Determination from Cod Liver Oil

Preamble

Previous research in section 3.2 was performed by Emanuela Gionfriddo and Abir Khaled and is included in this thesis as background to support the development of experimental design. Work performed by both contributors was considered for project advancement and further plans were developed based on the obtained results.

Vacuum SPME experimental design was done under the guidance of Emanuela Gionfriddo. Classical TFME carbon mesh membranes were made in-house and supplied by Jonathan Grandy. MAA-co-EGDMA particles, both magnetic and non-magnetic, as well as HLB particles, were synthesized by Varoon Singh; procedures for magnetic particle use were also provided by Varoon Singh. Procedures for membrane coating were provided by Jonathan Grandy. A portion of the laboratory experiments done for the calibration were performed by Ginny Galpin under the supervision of the author of this thesis.

3.1. Introduction

The primary goal of this project was to find a clean, quick and reusable method to sample PCAs from cod oil. Currently, sampling involves many preparatory steps involving long wait times, with some methods lasting up to days due to evaporation steps; many also use disposable cartridges and large volumes of both sample and solvents.[29]

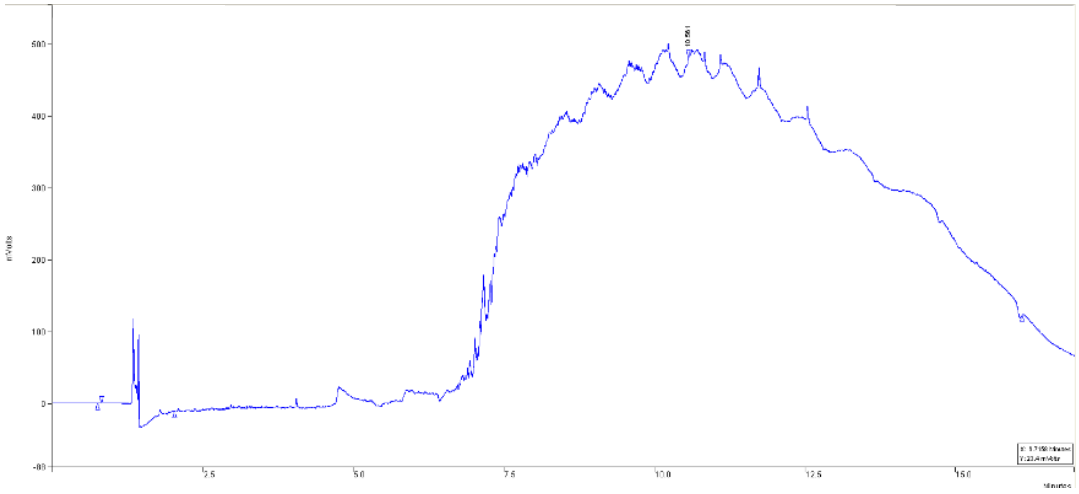


Figure 11: A single 10ppm 1ul liquid injection of a commercially available short chain PCA standard mixture; GC-ECD

A key difficulty in this study is the sample itself. PCAs can be separated into three groups, short, medium, and long chain, and the members of each group will vary in chlorination level and therefore by mass. An example of a clean stock PCA mixture is shown below in Figure 11.

The low resolution illustrates the difficulty in separating the PCAs from each other. They are made by reacting chlorine gas with alkanes of varying chain lengths, resulting in the formation of a mixture. To obtain individual standards, they must be distilled and fractionated, making the individual standards both rare and expensive. No method in the literature has been able to chromatographically separate the PCAs within their groupings; as such, the generally accepted method of assessing the response for these compounds is a total baseline-to-baseline integration of the merged peak seen in Figure 11. In addition to the poorly resolved standards, the matrix itself is cod liver oil which is primarily made up of organic components that dissolve these hydrophobic analytes very well. The effective and clean extraction of these compounds will require a careful selection of techniques to avoid coextracting the cod liver oil and its components.

3.2. Previous Research

3.2.1. Fiber SPME

Preliminary research for this project began with sampling PCA mixtures from pure water using SPME. PDMS and PDMS/DVB/PDMS coatings were chosen for their matrix compatibility and hydrophobic nature. Two classes of PCAs were chosen for this test, short chain (C_{10}-C_{13}) and medium chain (C_{14}-C_{17}) PCAs. They were spiked to 60 ng/mL and 300 ng/mL respectively into 9ml of ultrapure water. As seen in Figure 12, both fiber types demonstrated comparable extraction rates, however the PDMS fiber displayed greater reproducibility; and for this reason, it was chosen for subsequent experiments.

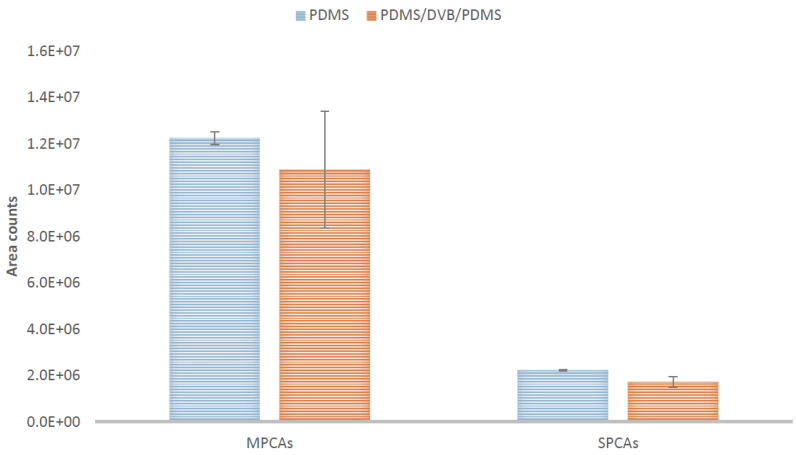


Figure 12: Comparison of PDMS versus PDMS/DVB/PDMS fiber coatings for extraction capabilities of medium chain PCAs and short chain PCAs spiked in water at 300 ng/mL and 60 ng/mL respectively.

Another fiber of hydrophobic nature, C18/PAN was also tested. It is not compatible with the thermal desorption used in GC, so the fiber was desorbed in solvent and the solution was injected directly. Neither mixture of compounds could be seen in the chromatographs using multiple desorption solutions, therefore, experiments returned to the PDMS coating with thermal desorption.

The detection range of the GC-ECD was found using liquid syringe injections of standard solutions within the range of 0.25 µg/L to 300 µg/L and were followed by a calibration plot using DI-SPME using PDMS with a PCA mixture in water.

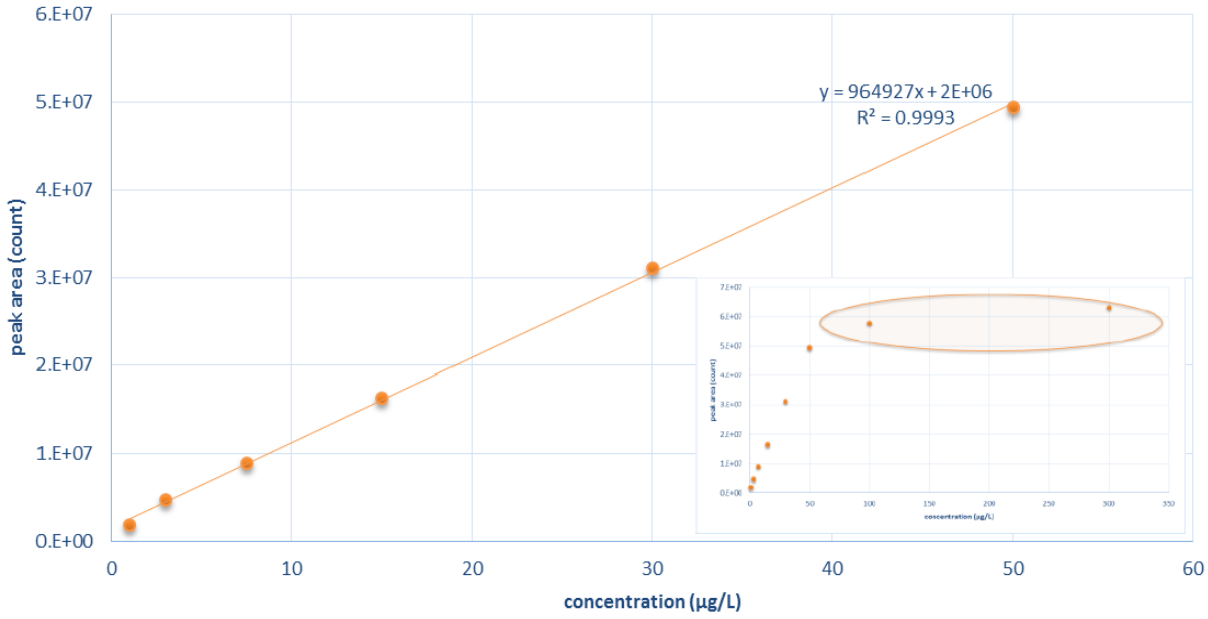


Figure 13: Calibration plot of medium chain PCAs in water extracted with DI-SPME.

**Note the loss of linearity in the expanded version in the insert. This was due to the hydrophobicity of the analytes which caused adhesion to the vial surface at high concentrations.*

The calibration showed significant deviation from linearity above 50 µg/L, likely due to the immiscibility of the analytes in water at high concentrations. Similar tests were successfully done with short chain and long chain PCAs with similar results. Sensitivity of the ECD to the PCAs using DI-SPME from water was markedly better than that of the MS instruments previously tested, as such, the ECD was used moving forward.

To prepare for the fish oil sample matrix, a complex, and dirty sample, cleaning steps were initially tested to ensure adequate retention of analytes while still using pure water as the matrix.

Rinsing was done in 5, 15 and 30 second intervals using pure water, 9:1 and 1:1 ratios of acetone and water, as well as the same ratios of isopropanol and water. The results can be seen in Figure 14 below; the rinse solutions providing the smallest loss in analytes are water and 1:1 acetone and water mixtures over all three time intervals. Due to the hydrophobic nature of the oil matrix, the water alone will likely not provide sufficient cleaning, and therefore the acetone-water mixture at 1:1 was chosen moving forward.

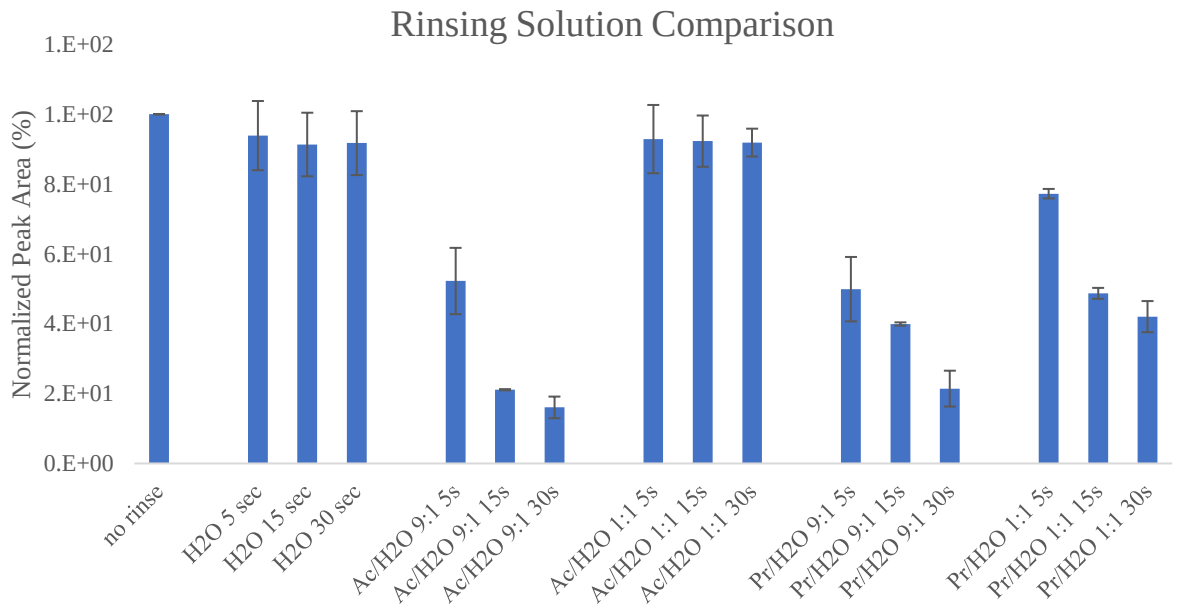


Figure 14: Five rinsing solutions were tested with three time intervals of 5, 15 and 30s. Solutions were water, 9:1 or 1:1 solutions of acetone (Ac) or propanol (Pr) and water.

Following the rinsing optimization, a similar DI-SPME test was done in oil, samples (7g) of medium chain PCAs were spiked to 10mg/mL (shown by the blue line in Figure 15 below). The spiked sample was compared to a fiber blank (green), a clean oil sample (magenta), and the result of the DI-SPME test of 5 µg/L water sample with the same PCA mixture (red) as seen in Figure 15. Similar tests were also done using different oils and individual standards instead of PCA

mixtures. All iterations showed similar results: while water extractions showed clear peaks with low background, the oil samples displayed extremely high background, limiting sensitivity and peak identification.

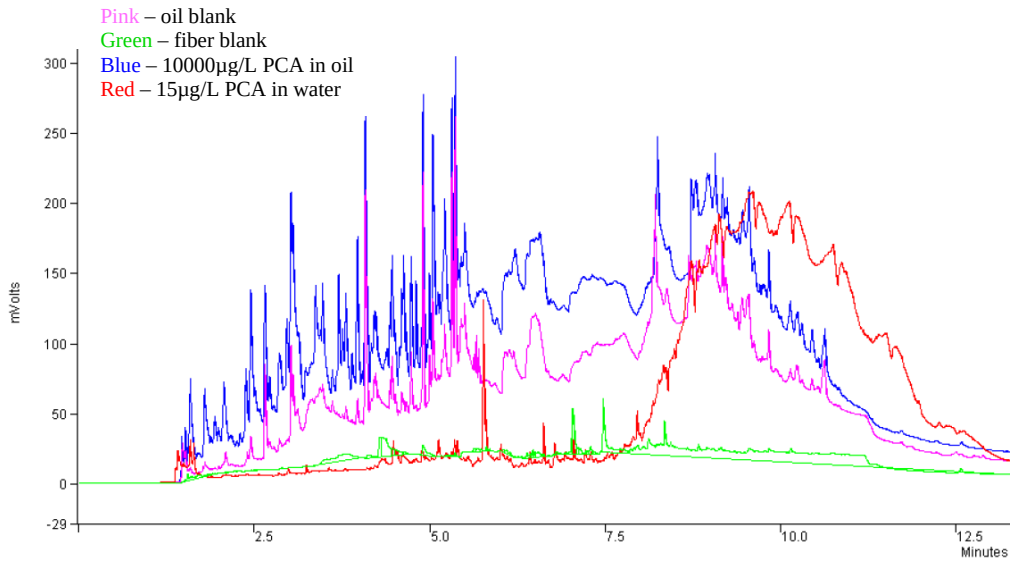


Figure 15: DI-SPME sampling of 10mg/L medium PCA spiked cod liver oil (blue), oil blank (magenta), 5 µg/L water sample with the same PCA mixture (red) and a fiber blank (green). Performed using GC-ECD.

The extremely high background noise obtained from the oil samples is likely due to contamination of the oil with other halogenated, oxygenated or nitrogen-containing compounds for which the ECD is particularly sensitive. Due to this, modifications to the matrix or the use of a different detector may be necessary to obtain acceptable sensitivity. Considering that PDMS has been selected as the most appropriate extraction material for the fiber, different matrix modification methodologies that are compatible with PDMS were explored.

3.2.2. Matrix Modification Using Saponification

Saponification was first tested with three methods. In the first (1) method, 0.2g of oil were dissolved 5ml of an acetone-hexane mixture of 2:8 ratio and then saponified with 5ml of 1% (w/v)

sodium hydroxide solution for 10 minutes under 80°C with agitation. After cooling, the supernatant was taken and dried, then reconstituted in water and sampled using DI-SPME. The second method (2) involved similar steps but involved 1g oil, 8ml of the same sodium hydroxide solution and no addition of organic phase. The aqueous phase was collected and neutralized, sodium chloride was added to induce a salting out effect. In the third method (3) 0.6g of oil were dissolved in 5ml hexane, and then saponified using an ethanolic 1% sodium hydroxide solution. The third method did not show phase separation, therefore the PCAs could not be isolated. None of the methods showed significant background noise reduction, and only method 2 isolated one of the three representative standards.

Another method tested was lipid hydrolysis using sulfuric acid. This involved the use of 0.3g oil dissolved in 5ml cyclohexane to which 1ml concentrated acid was added. After stirring, the phases were allowed to separate, and the upper organic phase was collected and treated again with the acid. The organic phase was collected and evaporated at 45°C under nitrogen, and reconstituted with water, then sampled using DI-SPME. The extraction was done for 1 hour after a 5-minute incubation, with all steps occurring at 60°C. While this method was effective at reducing the background noise and allowing some improved detection of the PCAs, it was not reproducible and could not be reliably used.

As the attempted methods have yet to provide a solution to all three issues of sensitivity, noise and reproducibility, different approaches are pursued.

3.3. Calculations and Fundamentals

3.3.1. Partition Coefficients

Due to the high number of congeners of PCAs and the difficulty in their separation using chromatographic methods, a representative set of individual standards was selected based on those used by Yuan et al. for their use in PCA signal deconvolution using a ToF-MS.[38] The selected standards range from short chain polychlorinated alkanes to medium chain, and are listed in the table below. The use of individual standards was used to develop the method with a simplified system into which complexity can be built as the development progresses.

Table 6: Representative individual PCA standards to be used for development

PCA Name	*Molecular Formula*	*Monoisotopic Mass (g/mol)*	*Chlorine % (wt)*	*log P*	*Henry's Constant (atm m^3 mol^{-1})*
1-chlorodecane	$C_{10}H_{21}Cl_1$	176.1332	19	5.7	2.26×10^{-2}‡
1,10-dichlorodecane	$C_{10}H_{20}Cl_2$	210.0942	33	5.6	6.69×10^{-3}‡
1,2,5,6,9,10-hexachlorodecane	$C_{10}H_{16}Cl_6$	345.9380	60	4.8	2.08×10^{-5}†
1,1,1,3,10,11-hexachloroundecane	$C_{11}H_{18}Cl_6$	359.9540	58	6.6	1.22×10^{-5}†
1,1,1,3-tetrachlorodecane	$C_{10}H_{18}Cl_4$	278.0162	50	6.5	5.67×10^{-4}†
1,2,9,10-tetrachlorodecane	$C_{10}H_{18}Cl_4$	278.0162	50	5.20	9.25×10^{-5}†
1,1,1,3,12,13-hexachlorotridecane	$C_{13}H_{22}Cl_6$	387.9853	54	7.9	1.10×10^{-5}†
1,2,13,14-tetrachlorotetradecane	$C_{14}H_{26}Cl_4$	334.0789	41	7.4	9.34×10^{-6}†

Predicted values obtained from: †US Environmental Protection Agency ‡Royal Society of Chemistry [37,39]

Having obtained the standards, their log K_{ow}, or log P values were found; this value is generally used to estimate the interactions the analytes have with the fiber when sampling from an aqueous matrix. In this estimation, the octanol phase is equated with the fiber extraction phase, while water serves as a proxy for the matrix. In the case of this project however, since the matrix is not aqueous, the log K_{ow} was used to determine the log K_{oa} which is the log of the partition

coefficient between octanol and air, this would estimate the ability of the analytes to partition into the headspace from the oil. To do this, the Henry's constant was found for each compound and the following formula was employed.

Equation 1

$$K_{oa} = \frac{K_{ow}(RT)}{H}$$

Where K_{oa} and K_{ow} are the partition coefficients for octanol-air and octanol-water, respectively, R is the ideal gas constant, T is temperature – room temperature was used in the calculation, and H is the Henry's constant for the specific analyte. This provided the results in the following table.

Table 7: calculated octanol-air partition coefficients, and vapour pressures for the selected analytes

PCA Name	*log K_{oa}*	*Vapour Pressure (25°C) (atm)*
1-chlorodecane	5.7	9.84×10^{-3}‡
1,10-dichlorodecane	6.2	1.11×10^{-2}‡
1,2,5,6,9,10- hexachlorodecane	7.9	7.50×10^{-3}†
1,1,1,3,10,11-hexachloroundecane	9.9	3.72×10^{-4}†
1,1,1,3-tetrachlorodecane	7.2	7.94×10^{-6}†
1,2,9,10-tetrachlorodecane	7.4	1.76×10^{-5}†
1,1,1,3,12,13-hexachlorotridecane	11.2	5.48×10^{-5}†
1,2,13,14-tetrachlorotetradecane	10.8	1.29×10^{-4}†

Predicted values obtained from: †US Environmental Protection Agency ‡Royal Society of Chemistry [37,39]

The vapour pressures were used to better predict how the analytes would interact with the oil-air barrier. The vapour pressure of the oil was also experimentally determined by increasing the temperature of a vial sealed with oil and measuring the pressure in the system. Two data points were then used in Equation 2 to obtain the enthalpy of vaporization. All data point combinations were used, and the values averaged, with an RSD of 7%.

Equation 2

$$\Delta H_{vap} = ln\left(\frac{P_1}{P_2}\right) R \left(\frac{1}{T_2} - \frac{1}{T_1}\right)^{-1}$$

The enthalpy of vaporization was calculated with a result of 0.176 m^3 atm/mol. This was then used to determine the vapour pressure using the same equation. Assuming a value of 150°C as the distillation temperature, as provided by a purification laboratory, and assuming a similar distillation setup is used to that described in Dinamarca et al. with a vacuum of 2×10^{-2} mmHg, the vapour pressure of the oil used was determined to be 3.33×10^{-6} atm.[40] This is less than even the lowest vapour pressure of any of the standards, suggesting that headspace SPME extraction could be effective for this project.

3.3.2. Solubility Parameters

Furthermore, the solubility parameter was calculated using the summation method recommended by Dunkel:

Equation 3

$$\delta = \left(\frac{\sum_i \Delta h_i}{V} - \frac{RT}{V}\right)^{\frac{1}{2}}$$

using published tables for Δh_i values for contributions from each atom or group.[41]

For the oil, assuming it was largely long chain fatty acids, the solubility parameter was found to be 8.5 $(cal/cm^3)^{1/2}$. Literature values of the solubility constant for PDMS were found to be on average 15.1 $(cal/cm^3)^{1/2}$.[42]

The value of the oil was found to be about half that of the PDMS fiber, suggesting that the fiber will not swell due to interactions with the oil. Furthermore, literature reports indicate that fiber swelling is pronounced with solvents whose solubility constants are close to those of the fibers; on the other hand, if the difference between the two constants is greater than 4 values or more the solvents are known to not cause damage to the fiber.[43]

3.3.3. Vacuum SPME

Since Henry's Constants are only affected by high pressures exceeding 500 kPa, partial pressures and concentrations at equilibrium are effectively independent of total pressure for systems at or below atmospheric pressure. Thus, the total amount of analyte extracted by the fiber in an experiment with varying sample vessel pressure will remain constant. While introducing vacuum will not lead to more analyte being extracted, the benefit of using vacuum assisted SPME is changing the rate of the extraction. In HS-SPME, the analyte partitions first from the sample phase to the headspace phase, and then from gas phase into the coating. The partition between the sample and the gas phase is considered rate-limiting.

The tendency of an organic solute to partition into the headspace is governed by its vaporization pressure and solubility in the matrix. Provided that the propensity of the analyte to move to the headspace is greater than its solubility in the matrix, vacuum SPME can improve the rate of that transfer, effectively reducing the time required to reach equilibrium.

3.4. Experimental

3.4.1. Reagents and Supplies

Individual standards of chlorinated alkanes chlorodecane and 1, 10-dichlorodecane were purchased as from Sigma Aldrich (Oakville, ON, Canada). 1,1,1,3-Tetrachlorodecane , 1,2,9,10-Tetrachlorodecane, 1,1,1,3,10,11-Hexachloroundecane, 1,2,13,14-Tetrachlorotetradecane, 1,1,1,3,12,13-Hexachlorotridecane, and 1,1,1,3,14,15-Hexachloropentadecane were procured from Caledon Laboratories (Georgetown, ON, Canada) as a custom order to the highest purities possible (ranging from 96% to 99%) in isooctane. The highest available concentrations were procured at 1000ppm for 1,2,13,14-Tetrachlorotetradecane and 1,1,1,3,14,15-Hexachloropentadecane and 100ppm for the remaining 4. Standards were transferred from glass ampules to silanized 2mL amber vials and kept refrigerated in the dark at 4°C. Working solutions were prepared in concentrations ranging from 10ppb to 10ppm in isooctane and stored with the standards. ISO 17034 Chloroparaffin C10-C13, 55,5% Cl 100 µg/mL in Cyclohexane from Dr. EhrenstorferTM brand were purchased from LGC (Manchester, NH, USA) and were kept refrigerated at 4°C in their original container. Working solutions were prepared in 2ml amber silanized vials.

HPLC grade solvents acetone, dichloromethane, toluene and isooctane as well as sulfuric acid and a silanizing solution of 5% dimethyldichlorosilane in toluene were purchased from Millipore Sigma (Oakville, ON, Canada).

Membrane holders and thermal desorption tubes were obtained from Gerstel. (Linthicum, MD, USA).

SPME fibers PDMS/DVB/PDMS OC (100 μm) and HLB/PDMS (100 μm) as well as DVB particles (5μm) were purchased from Supelco (Bellefonte, PA, USA).

HLB and MAA-co-EGDMA were synthesized, and the latter was made magnetic, in-house by Dr. Singh as described elsewhere.[44] Heavy duty aluminum foil and aluminum sheeting (0.2mm thickness) was purchased from local markets. 500 and 800 grit sandpaper sheets were purchased from local hardware stores. DVB and HLB membranes were made in-house using carbon mesh support by Dr. Jonathan Grandy, following the procedure published previously.[45] MAA-co-EGDMA membranes were made following the previously published procedure by Grandy et. al. however the carbon mesh support was substituted for heavy duty aluminum foil; the same procedure was performed for HLB membranes using the foil support.

HLB, DVB and PDMS membranes were made in-house following the previously described method with substituting sanded thick aluminum sheeting in place of the carbon mesh. The rolled aluminum was straightened then sanded using 800 grit sandpaper using an orbital motion, then rinsed and wiped down using aliquots of each methanol, acetone and hexane, in the order written. Sheets were allowed to air dry, then were coated as described by Grandy et. al. previously.

The carbon fiber mesh weave (Panex 30) was provided by Zoltec Co. (Bridgeton, MO, USA). Liquid nitrogen was supplied by Praxair (Kitchener, ON, Canada); while ultrapure helium as supplied by Air Liquide Canada via VitalAire Canada Inc. (Mississauga, ON, Canada).

Unflavoured free flowing cod liver oil was used for sampling and was purchased locally from a health food market in Waterloo, Ontario. Additional cod liver oil, unflavoured types in both free

flowing form and capsules, as well as flavoured mixed fish oil were purchased from health food stores and pharmacies in Waterloo, Ontario.

3.4.2. Instrumentation

Gas chromatography was selected for this project due to the properties of the target analytes. The analytes are semivolatile and thermally stable, but more importantly, they are not amenable to reverse phase liquid chromatography, barring that technique from use. Early in this work, a GC-ECD, Varian 3800, was used for this project due to the chlorine on the compounds as it is a selective detector for electrophilic compounds. Nitrogen was used as the makeup gas, set to 25 mL/min, with the detector temperature set to 300°C.

While it is selective for halogenated compounds, it was also extremely sensitive to other components in the matrix which were not trivial to remove. To facilitate cleanup and preconcentration of the analytes, unconventional sampling methods were tested, many of which were not compatible with regular GC injectors. For these unconventional methods, a thermal desorption unit (TDU) was needed to house the samplers. This unit was permanently fixed to a GC-MS in the laboratory; as such, the project was moved to a single quadrupole GC-MS using an EI source.

Upon switching to the GC-MS, the primary goal was to demonstrate using scan mode that the developed method had a low baseline from effective sample cleanup. The added selectivity of the mass analyser allowed for further method development using individual representative standards in selected ion mode. Ultimately, the target of such research would be to combine it with detection and deconvolution methods as described by Yuan and colleagues, or high resolution mass

spectrometry methods as described by Thomas and colleagues for comprehensive PCA detection from various matrices.[31,38]

Mass spectrometer methods were made in both SIM and Scan modes. Scan methods had a solvent delay of 3.6 minutes, the quadrupole temperature was set to 150°C and the ion source temperature was 230°C. Masses were scanned between m/z 40 to 550. SIM methods contained 9 groups with ions selected based on the presence within the target analyte peaks and absence in blank oil samples. Ion groups were as follows: group 1, start time 4.5 minutes, contained chlorodecane and 1,10-dichlorodecane, ion m/z 91; group 2, start time 6.38 minutes, contained 1,1,1,3-tetrachlorodecane, ions m/z 135 and 187; group 3, start time 7.6 minutes, contained 1,2,9,10-tetrachlorodecane, ion m/z 139; group 4, start time 8.3 minutes, contained 1,1,1,3,10,11-hexachloroundecane, ions m/z 253 and 255; group 5, start time 9.1 minutes, contained 1,2,13,14-tetrachlorotetradecane, ions m/z 103, 139, 141 and 355; group 6, start time 9.6 minutes, contained 1,1,1,3,12,13-hexachlorotridecane, ion m/z 245; group 7, start time 10.0, considered mass spectrometer off time, ion m/z 301, no response at this m/z; group 8, start time 10.2 minutes, contained 1,1,1,3,14,15-hexachloropentadecane, ions m/z 109, 185, 187. Group 9 with a start time of 10.7 minutes was used to elute remaining oil components from the column at high temperature, selecting ion m/z 301 as a blank mass.

The oven method for this project used a starting column oven temperature of 50°C, followed by a 25°C/min ramp up to 300, at which it was held for 2 minutes. Such a high temperature was important in order to fully clean the column in case of any oil matrix being introduced into it. Run in splitless, the Gerstel Cooled Injection System (CIS) was set to -80°C for an initial time of 0.4 min followed by a 12.00°C/min ramp to 270°C where it was held for 5 minutes. Liquid nitrogen

was used as a coolant for the CIS. Prior to this, the desorption took place in TDU mounted on the top of the CIS. The TDU desorbs the membrane, starting at a temperature of 50°C for a hold time of 0.5min and ramps to 250°C at 700°C/min. This was held for 1 minute at the final temperature, at which point the CIS rapidly increased in temperature to inject the cryo-cooled analyte plug. The TDU transfer temperature was kept at 250°C to maintain the seals and drive out moisture and prevent it from contaminating the system. The CIS module was operated in solvent vent mode, with a pressure of 14.88 psi, both the vent flow and purge flow were 80 mL/min; the purge began after 5.4 minutes to effectively run the instrument in a splitless mode. Total column flow was 1.5 mL/min. The same GC method was used with the Varian GC-ECD, with the regular injector set to 270°C.

3.4.3. Methods

Cod liver oil was kept sealed in its original green-tinted container, covered in foil and refrigerated at 4°C to prevent oxidation. The oil was warmed to room temperature, weighed out and spiked in batches, reducing transfer error due to oil viscosity. Spiked oil was vortexed at 1500rpm for 1 hour at room temperature to thoroughly distribute the spiked compounds and allowed to equilibrate for an additional hour.

Four individual standards were obtained at higher concentrations. These included chlorodecane, and 1,10-dichlorodecane, obtained in pure form, as well as 1,2,13,14-tetrachlorotetradecane and 1,1,1,3,14,15-hexachloropentadecane obtained at 1000ppm in 1mL quantities in isooctane. These four compounds encompass the short chain, low chlorine content PCAs as well as medium chain high chlorine content PCAs, with two standards in between the

extremes. Optimization using these four was assumed to be applicable to the remainder of the selected individual standards as they fit within the window set by the lowest and highest molecular weight standard. The use of these 4 standards for optimization, over the use of all 8, was done primarily due to costs, as each standard was expensive and could only be purchased at a lower concentration in single millilitre quantities. Working solutions of this mixture were labelled as Mix-4, while those of the full set were labelled Mix-8. This full set was used primarily for calibration and validation; due to the limited amount of each standard.

3.4.3.1. Vacuum with HS SPME

Fiber SPME was revisited in order to test the headspace vacuum SPME system. Previous studies had only used direct immersion SPME and had generated a significant amount of noise in the sample due to the oil and other co-extractants. Often, these co-extractants are avoided in complex matrices by avoiding the matrix altogether and using headspace analysis.

As the compounds are sufficiently volatile for use in gas chromatography, introducing vacuum could accelerate the kinetics of the headspace extraction. Since the PCAs are high molecular weight compounds, this was seen as a potential benefit, as they would have long equilibration times.

A vacuum cap was designed by Dr. Gionfriddo and Mr. Dvorski in Science Technical Services (STS). This allows for a gastight fitting of an SPME fiber into a sample vial after it has been evacuated.

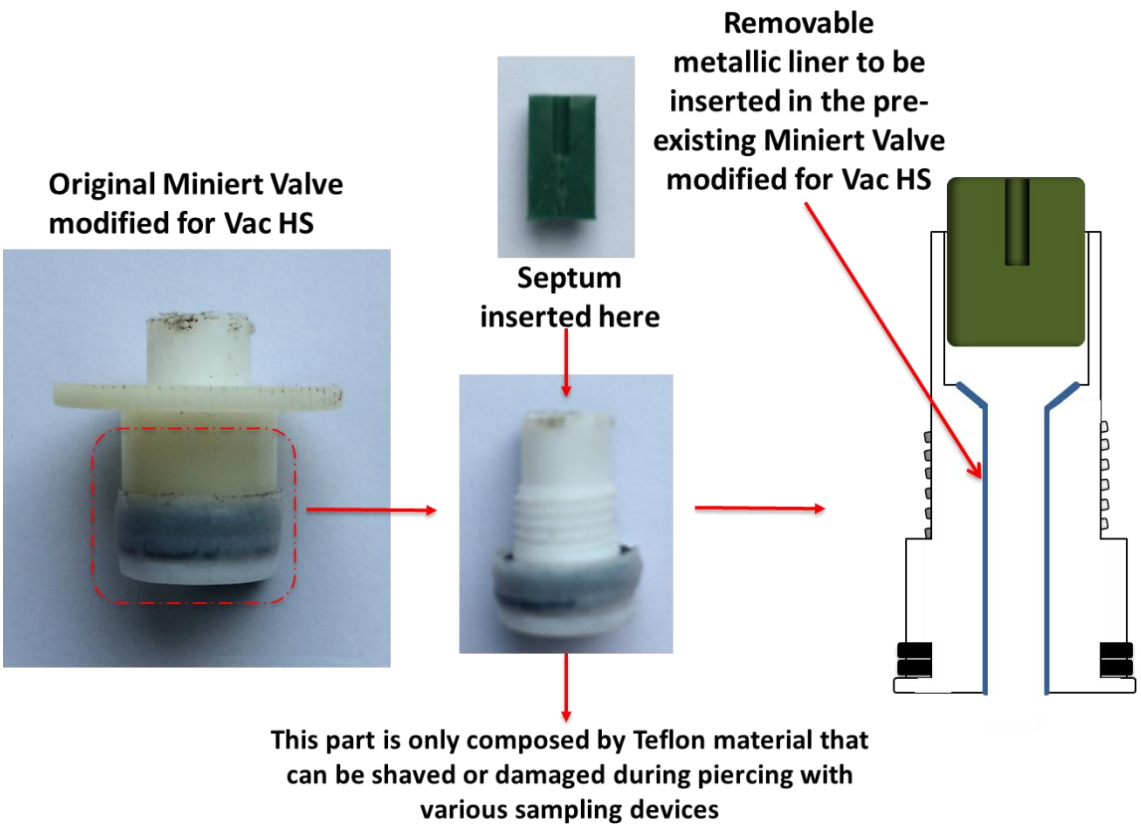


Figure 16: Miniert valves, modified with Teflon and a self-sealing pressure o-ring, fitted with a thermogreen septum to facilitate injection

To test the method, without using the costly PCA mixtures, a set of polyaromatic hydrocarbon (PAH) standards was used as a stand-in for the PCAs. The logP values of the PAH, especially of the heavier ones such as Pyrene resemble those of the PCAs.

Table 8:PAHs used as a stand-in set of analytes, their molecular weight, formula an their logP

	Molecular Formula	Molecular Weight (g/mol)	*logP*
Naphthalene (NAP)	$C_{10}H_{18}$	128	3.45
Acenaphthylene (ACY)	$C_{12}H_{8}$	152	4.26
Acephenanthrene (ACE)	$C_{16}H_{10}$	204	5.50
Fluorene (FLE)	$C_{13}H_{10}$	166	4.16
Phenanthrene (PHE)	$C_{14}H_{10}$	178	4.68
Anthracene (ANT)	$C_{14}H_{10}$	178	4.68
Fluoranthene (FLT)	$C_{16}H_{10}$	202	5.17
Pyrene (PY)	$C_{16}H_{10}$	202	5.17
Benz[a]anthracene (B(a)A)	$C_{18}H_{12}$	228	5.91
Chrysene (CHR)	$C_{18}H_{12}$	228	5.91

To test the theory of vacuum HS-SPME, the PAHs were spiked at 1ppm into water; after equilibrating for 30 minutes under 600rpm stirring, the samples were transferred to 20 mL vials to facilitate vacuuming. These vials were fitted with the modified miniert valves as seen in Figure 16 and vacuum was applied via a syringe through the thermogreen septum for 10 minutes while stirring at 600 rpm at room temperature. After vacuum was removed, the vial headspace was allowed to re-equilibrate with the sample for 10 minutes, followed by extraction using HS-SPME for 30 minutes. The same setup was used for non-vacuumed samples for comparison, the only difference was the lack of vacuum time; all else remained constant.

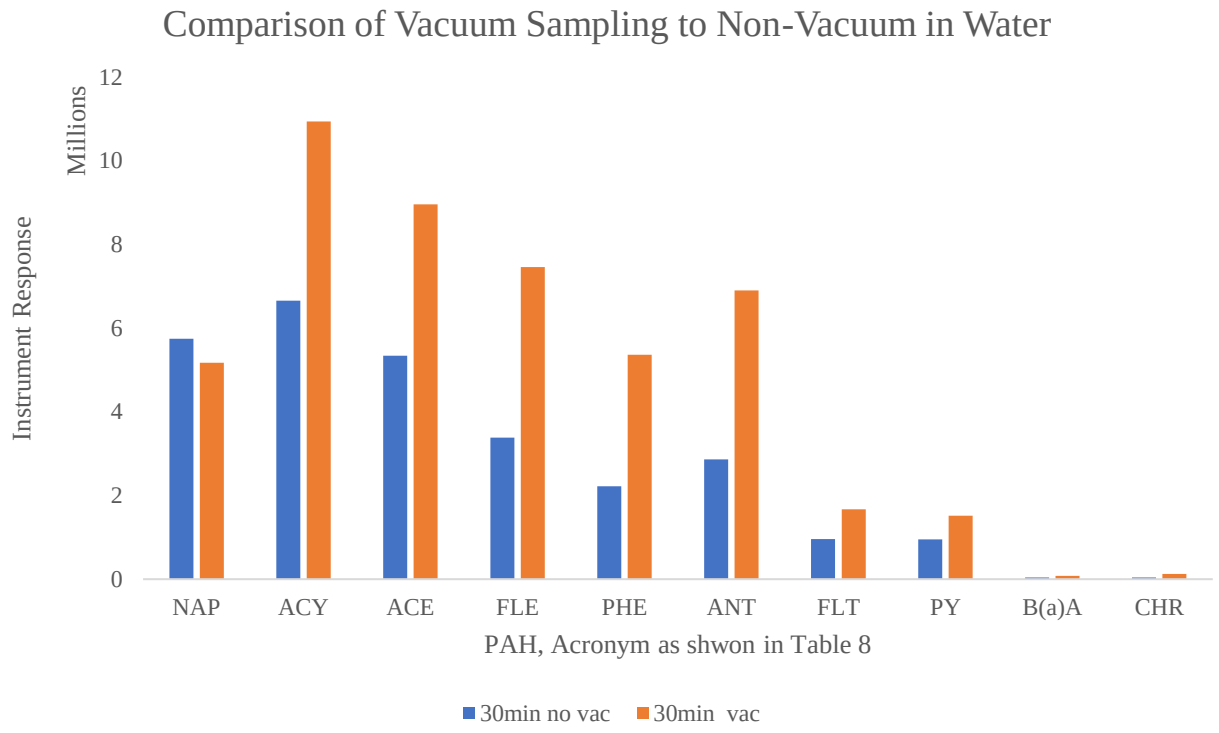


Figure 17:Comparison of vacuum SPME versus regular headspace SPME using PAHs in water. Samples were spiked to1ppm and processed with either regular HS-SPME, shown in blue, (10 min incubation, 30 min extraction at room temperature) or Vac-HS-SPME (10 min evacuation, 10 min incubation, 30 min extraction at room temperature) in grey.

Figure 17 demonstrates that, at constant extraction time, the application of vacuum increased the amounts of PAHs extracted. With these results, the same procedure was done with oil samples, adjusting the incubation time to 30 minutes from 10 minutes to improve the amount extracted. Vacuum and extraction time remained the same. 4g were used per vial for the spiked oil and the same comparison was made.

Upon applying vacuum to the vial, bubble formation occurred on the surface. This was hypothesized to be dissolved air in the oil that was bubbling out. Care was taken to watch for this foam to dissipate prior to removal of the vacuum line. This occurred around 7-8 minutes of the 10-minute vacuum application time. Figure 19 shows the results from the oil samples.

Figure 18: A foam layer on the oil upon vacuuming. The needle of the vacuum line can be seen in the top of the vial.

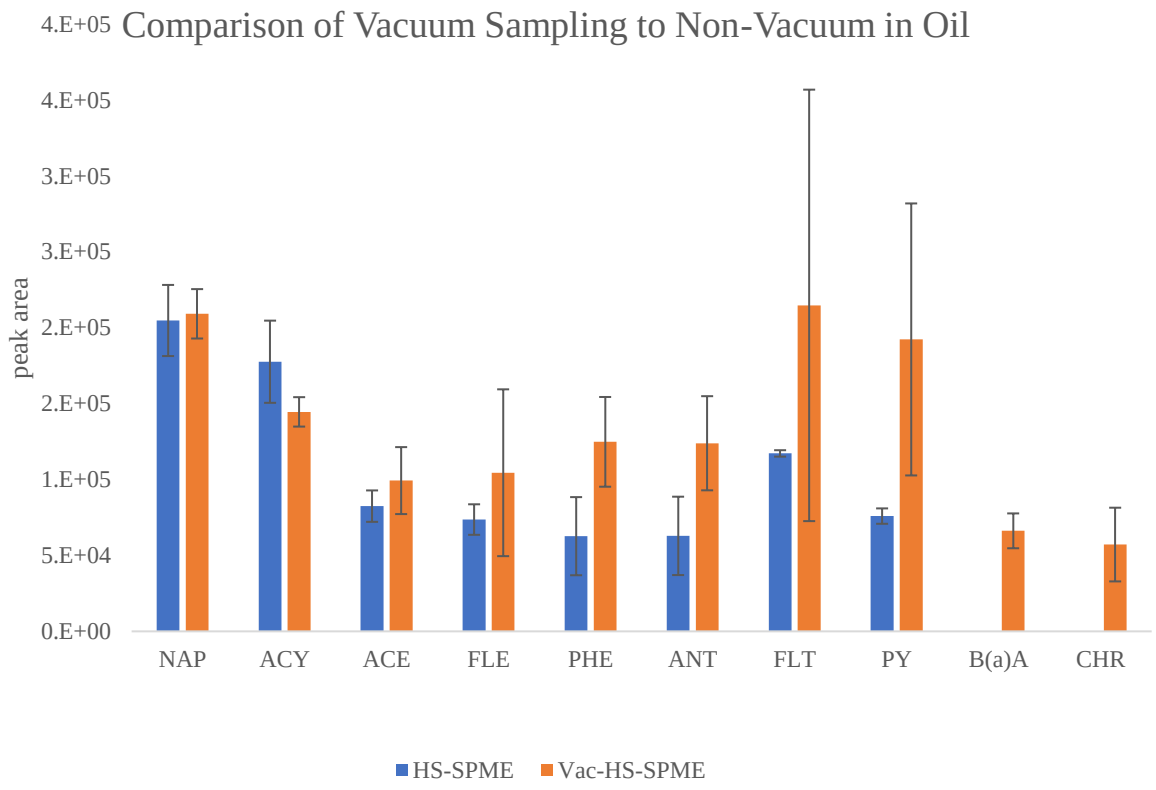


Figure 19: Sampling of PAH's in cod oil at 1ppm by HS-SPME (30 min incubation, 30 min extraction) and Vac-HS-SPME (10 min evacuation, 30 min incubation, 30 min extraction), GC-MS SIM mode, n=3, SD shown by error bars.

Noting that the vacuum SPME significantly improves the extraction capabilities of the system, a comparison was done at high temperatures with the oil to promote the partitioning of the heaviest PAHs to the headspace. It can be seen in Figure 20 that Pyrene is barely present in regular HS-SPME testing even at 85°C, while it doubles in extraction amount when the sample is vacuumed. As expected, all analytes show improved extraction at higher temperatures, however the vacuum results show a significant improvement in the extraction of the heaviest analytes – this was the effect sought after for application to the PCAs.

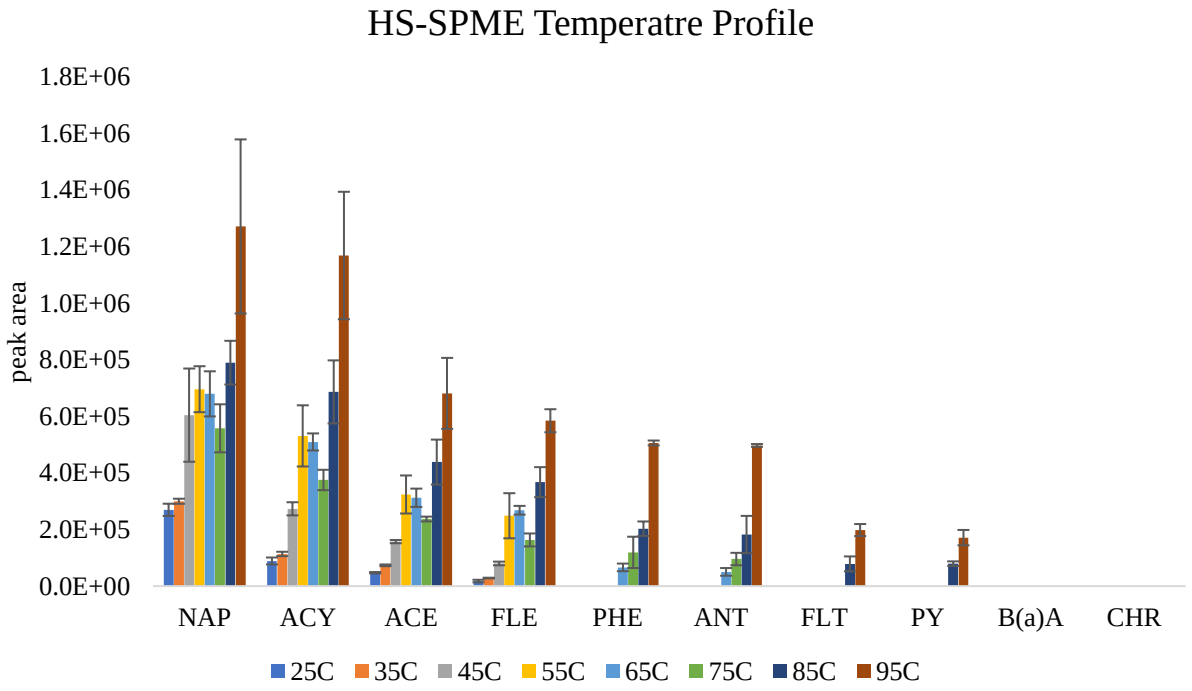


Figure 20:The temperature profile for regular HS-SPME with a 30 min incubation and 30 minute extraction, demonstrating the improvement in extraction ability at higher temperature, yet still insufficient to extract the heaviest compounds in the mixture. GC-MS, SIM mode, n=3, SD shown on graph.

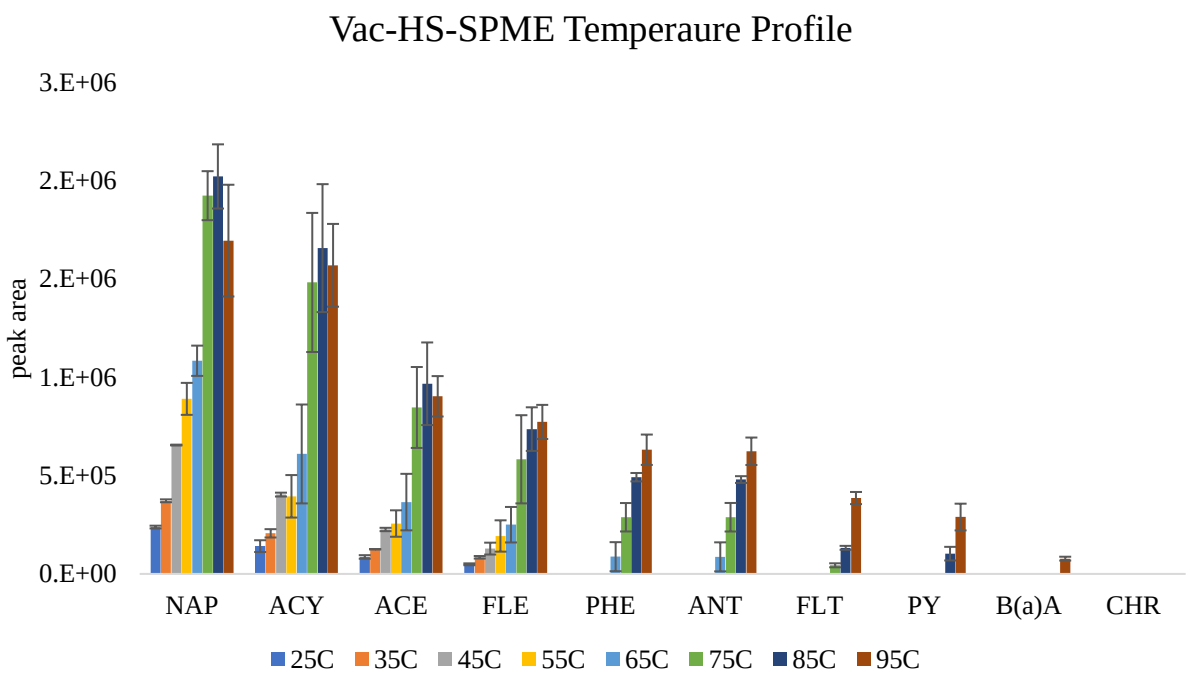


Figure 21: temperature profile for vacuumed HS-SPME with a 10-minute vacuum followed by 30 min incubation and 30-minute extraction. Not only are the heavier compounds extracted at a lower temperature, the amount extracted for each is nearly twice that obtained without vacuum. GC-MS, SIM mode, n=3, SD shown on graph.

The same method was applied to the PCAs in oil and analysed using the GC-ECD. It was noticed that upon using the vacuum method on the short chain PCAs, the sensitivity of the method decreased when comparing to that of a non-vacuumed trial. It is hypothesized that this may be due to the PCA's transitioning to the headspace and then being removed by the vacuum prior to sampling. Alternatively, this occurrence may be due to the vacuum pulling other VOC's out of the solution, causing a higher background, and thus making it more difficult to view the target compounds. In either case, the non-vacuumed sampled showed a clearer response to the PCA's, which can be seen below, as the non-vacuum sample (pink) shows the characteristic pattern of PCA's, while the vacuumed sample (red) response is barely distinguishable from the oil blank (blue). For reference, the fiber blank is in green at the bottom; this confirms the oil is causing the high background.

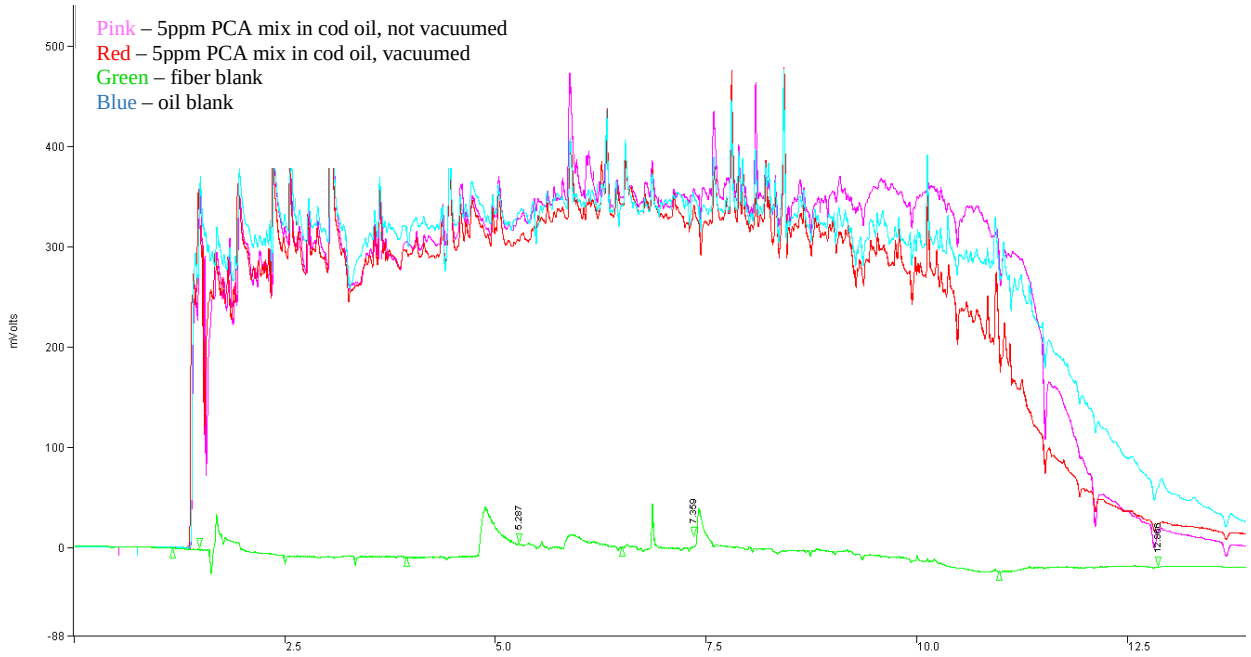


Figure 22: 5ppm PCA mixture spiked oil samples, tested with the vacuum method (red) and the non-vacuum method (pink); oil blank (blue) and fiber blank (green) for reference, performed on GC-ECD.

To confirm that the poor response of the PCAs was not due to a lack of fiber affinity, and that the method works using other matrices, the same tests were redone in water. 10 ppb samples of PCAs in water were sampled using the same method as described previously. The vacuum sample (red) showed significantly higher response than the non-vacuum (blue) for the PCAs, even at the very low concentration.

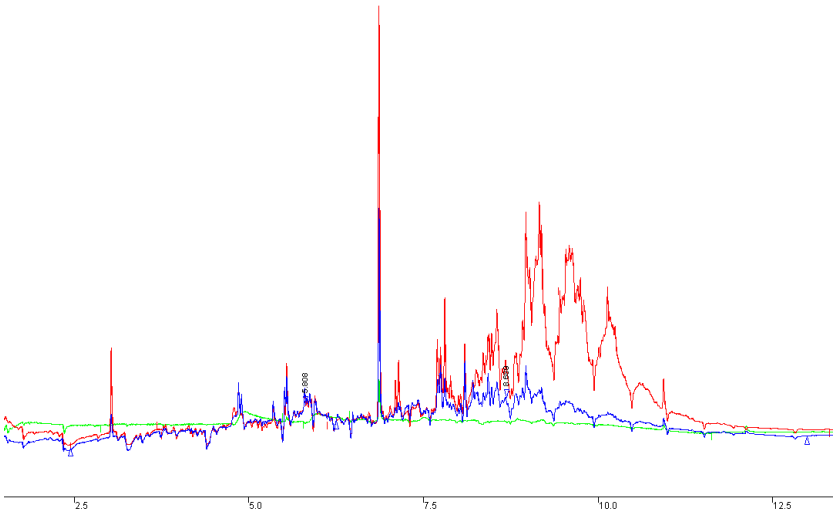


Figure 23: Samples of 10ppb PCAs spiked in water, tested with vacuum (red) and non-vacuum (blue) methods; fiber blank (green) for reference, performed on GC-ECD.

This set of data confirms the responses that were seen using PAHs, demonstrating the method with vacuum HS SPME may not by compatible with the PCAs in an oily matrix in which the PCAs are so heavily soluble. It appears that the vacuum SPME method is not specific to the PCAs within the oil, but also draws out other interfering species into the headspace which results in significant noise.

Since the headspace analysis was met with significant noise from VOC's in the oil, and the target compounds are only semi-volatile, it may be possible for direct immersion SPME to show better results. This issue of a complex matrix is seemingly further exacerbated in headspace, as the oil blank is significantly higher than the fiber blank alone, nearing the upper end of the detector capabilities. It is possible there are simply too many co-extracting volatile components in cod oil which must be avoided.

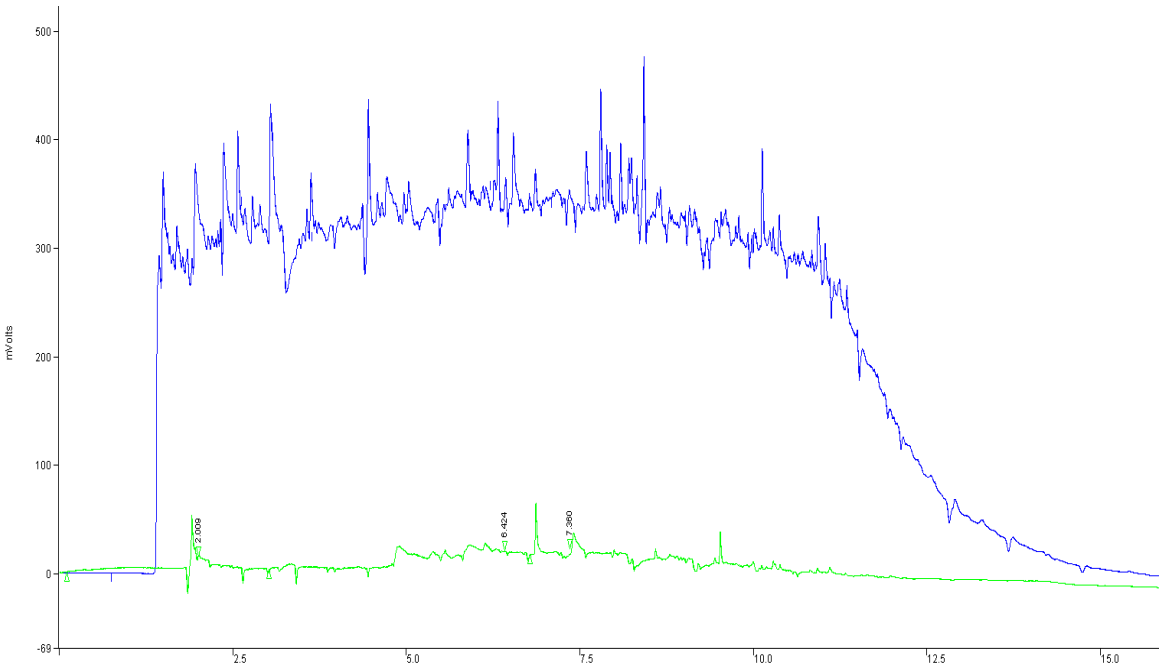


Figure 24: Oil blank when sampled with headspace SPME, performed on GC-ECD

Looking back on previous work done with DI SPME for this project, Figure 14 from section 3.2.1 showed a significantly lower baseline for the oil (around 200 mVolts) when compared to the responses using HS SPME (around 350 mVolts). For this reason, it may be worth revisiting the DI SPME approach for this matrix.

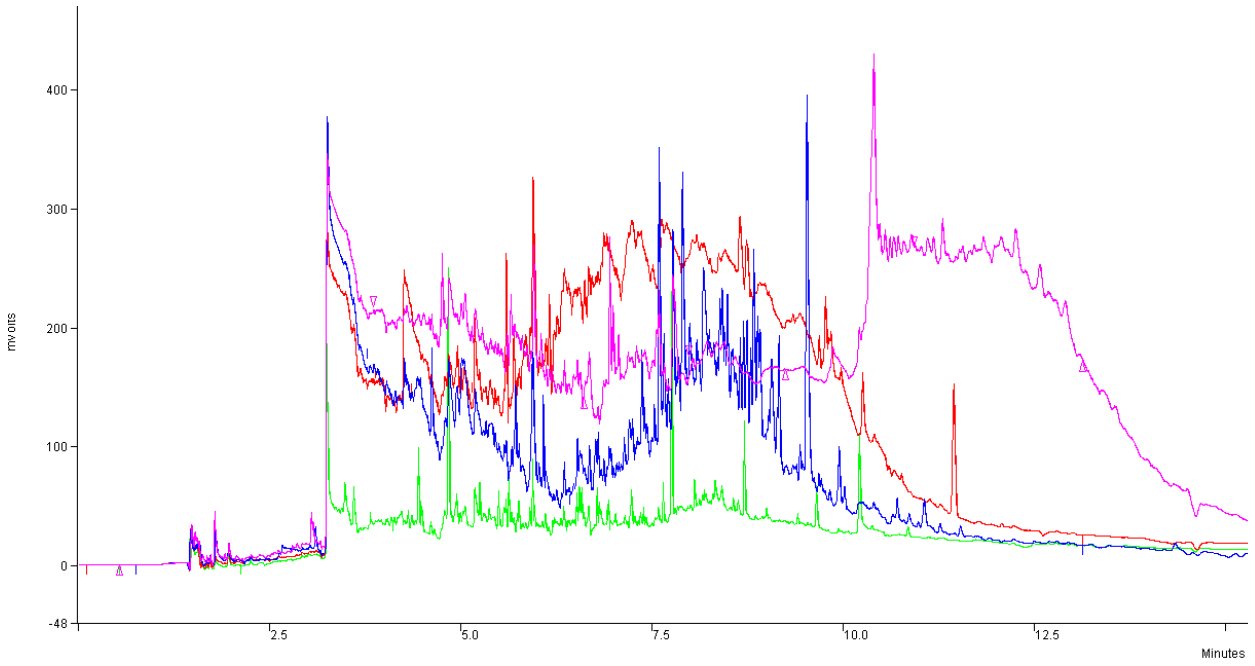


Figure 25: Figure 14 from section 3.2.1 – comparison of different oils for their noise response by direct immersion SPME; the blue line is the same oil as used for the headspace analysis, showing a much lower baseline. Performed on GC-ECD.

3.4.3.2. Direct Immersion SPME

A PDMS fiber was used to perform a 30-minute extraction as a starting point for optimization at 400 rpm via vortex and 50°C for a 100ppb sample in oil. It was noted that the fiber was under considerable strain due to the viscosity of the oil, as the vortex motion caused the SPME holder device to begin to unscrew. The holder was secured for the duration of the extraction however a different extraction phase may be brought into consideration to prevent breakages. This fiber was first rinsed in a 50% acetone water solution for 10 seconds in order to wash the oil off

the fiber prior to desorption. The desorption was performed at 270°C for 10 minutes and followed by a 1-minute wash in pure acetone to further strip away any residual matrix components on the fiber to prepare for the next use. Both the rinsing and washing steps were also done at 400 rpm. The use of this washing procedure has been suggested in prior works using fatty matrices such as

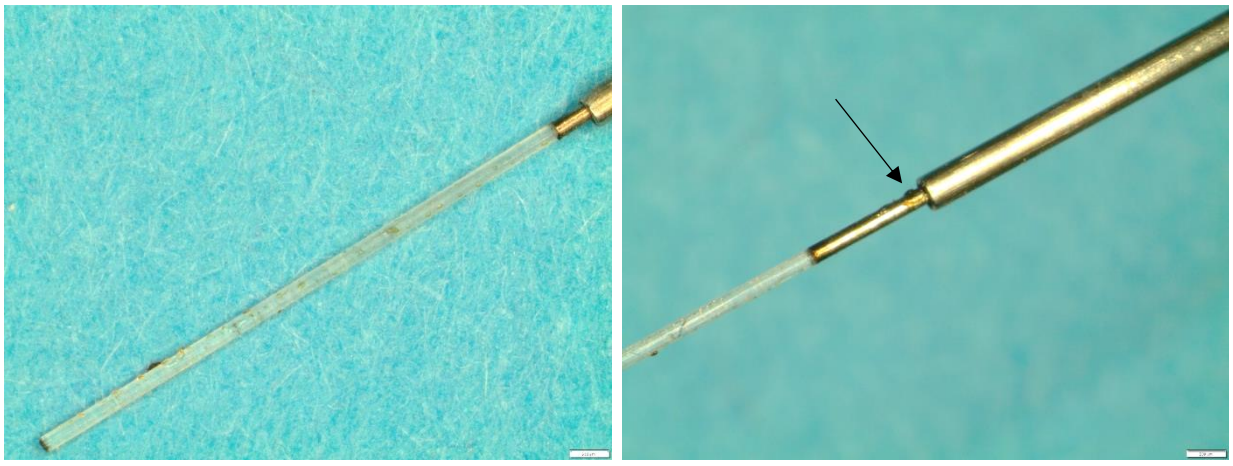


Figure 26: PDMS fiber after 30 minutes extraction in oil, followed by 10 second rinse in 50% acetone, desorption for 10 minutes and wash in 100% acetone for 1 minute.

avocado, and has been shown to extend the lifetime of PDMS coated fibers.[46] Unfortunately, the rinsing and washing procedures did not prove as effective as expected for this matrix. Microscope images of the fiber were taken after desorption to assess any damages, and it was clear that residual oil remained on the fiber through both the rinsing and washing, as seen in Figure 26. Furthermore, due to the viscosity of the oil, upon fiber withdrawal back into the protective needle, the oil also collected inside the needle, causing subsequent exposure and withdrawals to dirty the fiber, even after rinsing and washing. This can be seen in Figure 26 where the oil is caught on the metal part of the fiber support. This also led to contamination of the instrument liner. These experiments were also analysed using a GC-MS to rule out the ECD responding selectively to co-extractants and contaminants. In GC-MS, no peaks were isolated at the proper retention times in the ion-extracted chromatograms.

Hypotheses for lack of identification include too short of an extraction time, too small of an extraction surface, analyte loss during rising, and shifting in retention time due to the oil. This shift in retention time is further discussed below.

To assess method sensitivity and excess extraction time, the experiment was redone using a 1 ppm spike in oil and extracted overnight at room temperature. The fiber was rinsed in acetone at 400rpm for 1 minute at room temperature. When viewed under a microscope, the fiber remained coated with oil, and was therefore rinsed again with the same parameters. Target peaks were not seen in either TIC or extracted ion mode. The washing acetone was taken and injected into a GC-FID to confirm analytes were not being stripped away. No peaks were found at the target retention times to suggest that they were extracted into the solvent.

Based on literature by Holadova et. al. solvent can be added to promote extraction from oils.[47] This may work similarly to dilution or salt additions for aqueous media. In this case for DI sampling it would also serve double purpose as it would also decrease the viscosity of the matrix, reducing strain on the fiber. Table 9 shows some parameters of commonly used solvents to determine their eligibility for use with the oil. Solvents that are compatible with the fiber and will solubilize the PCAs were considered eligible, regardless of miscibility. If immiscible, the solvent could be used to form a suspension, similar to the soymilk sample from chapter 2, which would be both less viscous and may allow for the analytes to partition into the solvent phase to be in an effective free concentration.

Table 9:Assessment of common solvents for use in diluting the oil for sampling.

Solvent	*Miscibility*[48]	*PCA Solubility*	*Fiber Compatibility*	*Density g/ml*	*Eligibility*
Acetonitrile	×	✓	✓	**0.786**	✓
Acetic Anhydride	×	✓	✓	1.08	✓
Diacetin	×	✓	✓	1.17	✓
DMSO	×	✓	✓	**1.100**	✓
Ethylacetoacetate	×	✓	✓	1.02	✓
Ethylene glycol	×	×	✓	1.113	×
Glycerol	×	~	~	1.26	×
Methyl alcohol	×	×	✓	0.792	×
IPA	×	×	✓	0.786	×
Acetone	✓	✓	~	-	✓
Acetophenone	✓	✓	✓	-	✓
Diphenyl ether	✓	✓	✓	-	✓
Ethyl acetate	✓	✓	✓	-	✓
Toluene	✓	✓	✓	-	✓

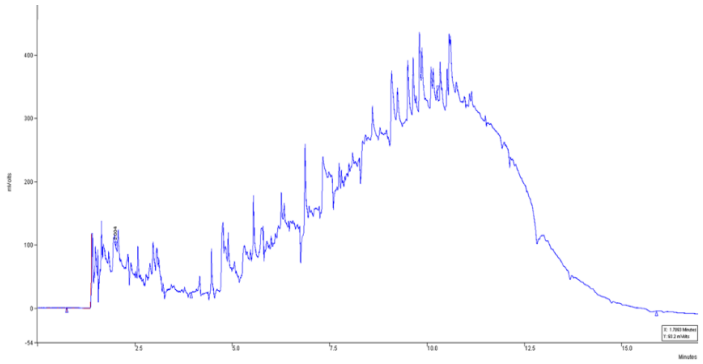


Figure 27: Chromatographic results of DI sampling using 4ml ACN with 4g of spiked cod oil.

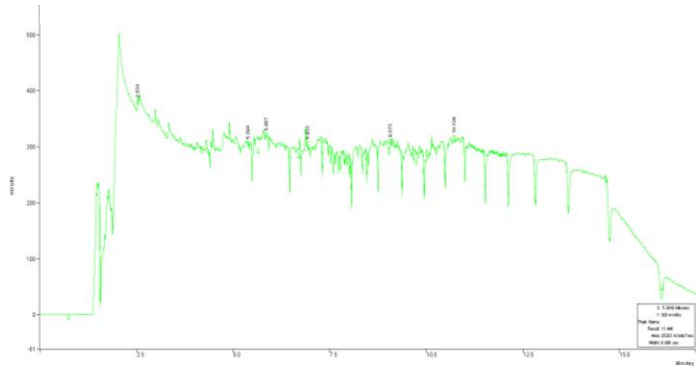


Figure 28: Results of DMSO addition

Acetonitrile (ACN) DMSO, toluene, and ethyl acetate were each used as matrix modifiers. 4mL of solvent were added to each sample of 4g spiked oil.

Figure 27 illustrates the results from the addition of ACN, demonstrating some but still insufficient background reduction, as the target PCA mixture was indistinguishable from noise. ACN belongs to the immiscible group of solvents and is less dense than the oil, so formed an additional phase between the oil and the headspace.

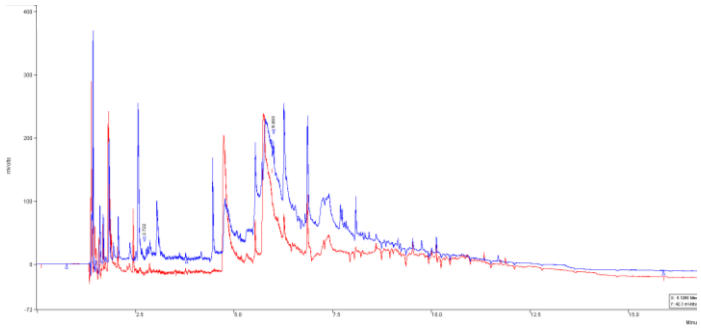


Figure 29: Results of Ethyl Acetate addition 2ml(red) 4ml(blue)

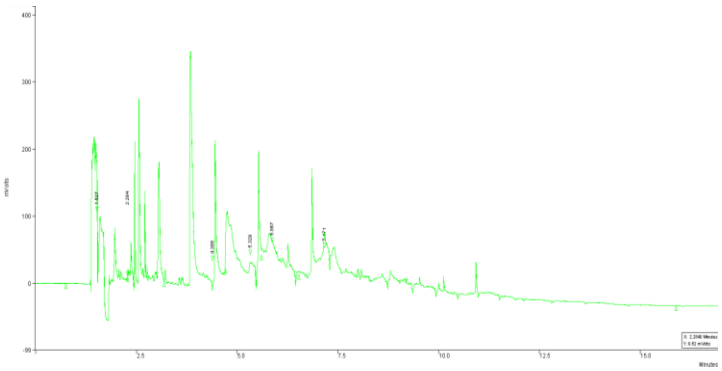


Figure 30: Results of Toluene addition

Agitation resulted in the formation of an emulsion that settled back within 5 minutes.

Figure 28 shows the results of adding DMSO, which also belonged to the immiscible group, but has a higher density compared to the oil, and formed a layer underneath. The solvent addition here did not appear to cause any beneficial effects to the target region.

Figure 29 and Figure 30 show the results of adding ethyl acetate and toluene, both belonging to the miscible group. The solvents showed significant noise reduction effects, however with that, they also removed the ability to extract the target analytes. These solvents will be avoided or used with extreme care in future work.

Sampling in cod oil alone was adjusted to use the upper extremes of optimizable parameters in order to test whether further optimization would be fruitful. An overnight extraction was performed at 65°C and 500rpm. Upon retrieval of the SPME device the next day, it was found that the prolonged agitation in the same position resulted in erosion of the protective needle due to friction against the vial cap, seen in Figure 31. This created a hole in the in the needle, which caused issues upon desorption, as the carrier gas at the inlet would be able to escape. These issues lead to the use of a different extraction phase configuration – thin film membrane extraction (TFME).

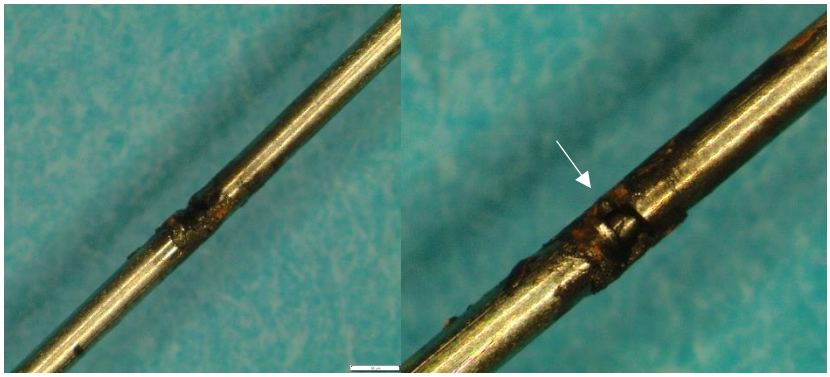


Figure 31: Fiber protective needle, eroded due to continuous friction against the vial cap. The image on the right shows where the metal was eroded to the point of breaking through the needle.

3.4.3.3. Classical TFME

As fiber direct immersion was shown by previous work to have poor cleanup and recovery, and new approaches ended with damaged fibers, classical thin film membrane extraction was tried since the membranes are more robust and do not have a breakable protective housing. Standard commercially available DVB-PDMS membranes were used. Due to the size of the membranes, the Gerstel TDU was needed; as such this work was limited to the GC-MS on which this inlet was mounted. Once the project was transferred to a GC-MS, the target of the project was to find a method that reduces the background in scan mode; however once a low baseline was achieved, individual analytes could be used with a SIM method, resulting in an overall more sensitive method.

Enough oil was required per sample to completely cover the membrane supported in a cotter pin clip, as irregularities in coverage would lead to poor reproducibility. As such, 6 grams of oil were used per sample, spiked to 1ppm using the 4 individual standards which were purchased at higher concentrations, specifically chlorodecane, 1,10-dichlorodeccane, 1,2,13,14-tetrachlorotetradecane and 1,1,1,3,14,15-hexachloropentadecane. As seen previously in Table 9 acetone was one of few commonly used solvents that is miscible with the cod oil, it was therefore used as a washing solution for the membranes prior to desorption. Rising was performed in 7ml of acetone, enough to cover the membranes, and vortexed at 1500rpm for 10s as a starting point. Due to evidence of oil still present in the membranes after 10s, longer washings were attempted. Cooling the acetone by placing it in an ice bath prior to longer washing periods, up to 30 seconds, was also tested in order to prevent analyte desorption into the washing solution. Membranes were blotted dry and placed into Gerstel TDU tubes fitted with a glass ball stopper and thermally extracted for 1 minute at 250°C with the TDU/CIS system. Remaining instrument parameters were as described in the instrumentation section.

While extraction of target compounds may have been improved, selectivity was poor due to the porous nature of the support resulting in very busy chromatograms. Being a carbon fiber mesh, the membrane effectively acted as a fabric which wicked the oil and retained it through multiple washings. This resulted in high background, even using a GC-MS as opposed to the ECD. As removing the oil was difficult, there was also some concern over contamination of the injection system and column. While preventative maintenance was done regularly

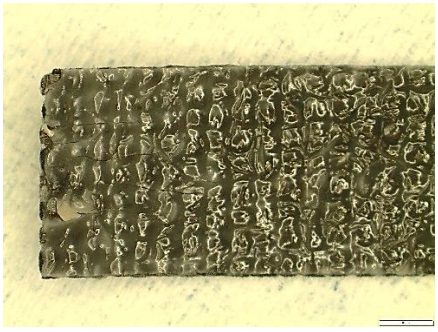


Figure 32: Degrading edge of carbon mesh supported PDMS membrane

and instrument blanks consistently returned to baseline, evidence of oil deposit inside the replaceable CIS liner were a cause for concern.

Furthermore, after only two extractions, the membranes began to degrade. As the same result was not seen when using the silica fiber support, it was hypothesized that this was likely due to the oil being present within the membrane support after the first sampling since it could not be effectively washed away. The constant exposure could have had a solubilizing or softening effect on the PDMS. To move away from this support and increase extraction phase surface area further, magnetic microspheres were used instead.

3.4.3.4. Magnetic Microspheres

Magnetic microspheres made with methacrylic acid – co – ethylene glycol dimethacrylate (MAA-co-EGDMA) were used for the functional groups this coating provided. We reasoned that the alkane portion of the PCAs would interact with the carbon chains within the coating, the esters in the structure could provide noncovalent halogen-oxygen bonding for the chlorine found on the ends of the PCAs.[49]

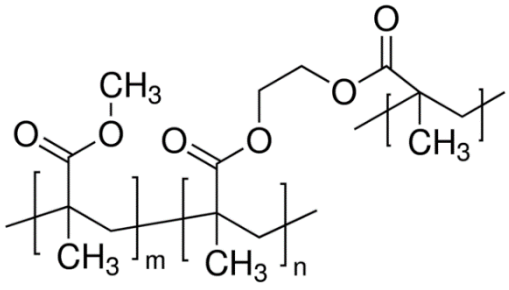


Figure 33: Methacrylic acid - co - ethylene glycol dimethacrylate coating composition

The microspheres were suspended in a transfer solution miscible with the target matrix and transferred by pipette; in this case, the solvent used was acetone. 500 µL of a 5µg/µl suspension was added to obtain 500 µg of particles per extraction. The sample containing 4g cod oil was then vortexed to allow for suspension of the particles in the oil, then after 10 minutes the microspheres were pulled to the bottom of the vial to concentrate them prior to isolating them on a metal blade. This was necessary since the oil was so viscous that the magnetic field around the blade itself was insufficient to collect particles out of the suspension in an practical time.

Figure 34: Image 1 shows the suspension of particles in cod oil after vortexing; this remained in suspension for 10 minutes as the particles extracted the analyte. Image 2 and 3 demonstrate the process for recollection of the particles to one location to facilitate collection with a blade.

Particles were collected using a blade exposed to a magnetic field for 5 minutes. The blade was then placed into a 2ml vial containing 1.5ml acetone to facilitate cleaning. A 2s vortex of the vial removed the particles from the blade and dispersed them throughout the cleaning solvent. A fresh blade was used to recover the particles and the blade was placed into a fritted TDU tube packed with glass wool for thermal extraction. The same parameters for the CIS and TDU were used as for the TFME.

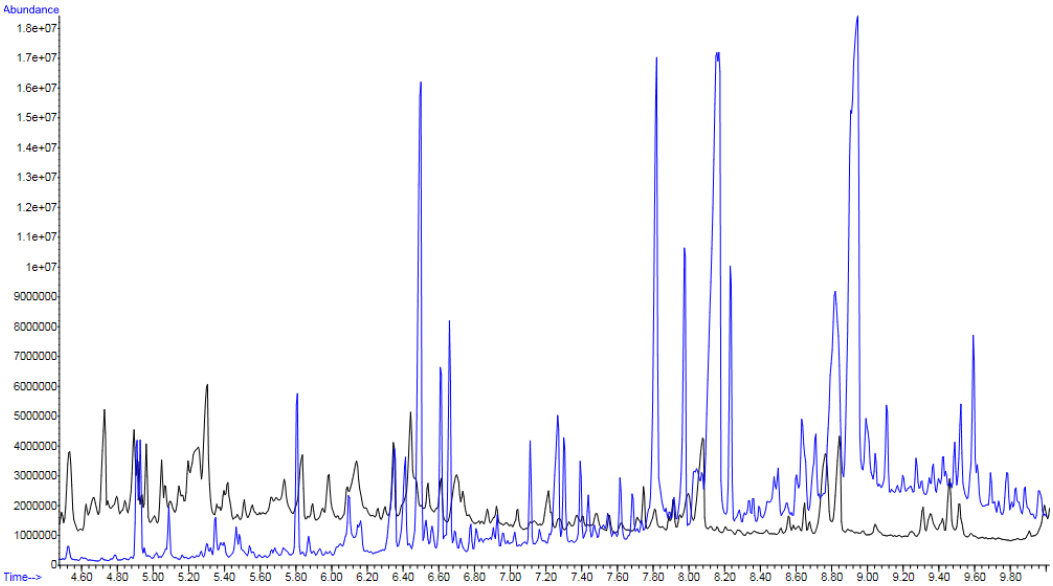


Figure 35: A comparison of chromatography results from carbon mesh supported extractions (blue) in comparison to extractions performed with the magnetic microspheres (black) from non-spiked oil matrix. Performed on GC-MS, full scan.

As seen in Figure 35, the background noise in the chromatogram due to co-extracted components or insufficiently washed oil is reduced significantly when using the microspheres. Unfortunately however, limited improvement was seen in the extraction of analytes when spiked. The signal to noise ratio increased from 1:1, or indistinguishable, using the carbon mesh membranes to approximately 2:1 using the microspheres. While the particles were no longer extracting as many other components of the oil, the extraction of the analytes suffered alongside this reduction in background. It was considered that this may have been due to losses in particles during the collection and washing process. Due to the viscosity of the oil, not all particles used for the extraction could be collected by the magnet. Further losses of particles occurred during the washing step due to re-dispersion of the particles in acetone in order to remove trapped oil. As a solution, the same particles were made into unsupported membranes using PDMS.

3.4.3.5. Unsupported and Foil Supported TFME

These had the same benefit as the magnetic microspheres – no porous support to collect oil, but also had the advantage of being easy to remove from the oil, with no losses of particles to the matrix. Spread coating methods were followed as described by Grandy and colleagues, however instead of coating onto a carbon mesh support, the membranes were spread onto a Teflon sheet.[50] After curing, these membranes could be easily peeled off the Teflon and secured onto needle pins for ease of use.

Extraction using these membranes followed the procedure described for TFME, however rinsing was reduced to 3 seconds in 1.5mL acetone. Similarly to what was seen with the microspheres, the background of the chromatograms from these membranes was near baseline, and the signal to noise ratio improved tenfold. Since the sorbent was embedded in the membranes, it was much easier to remove residual oil without losses of particles or much analyte. A total ion chromatogram is shown in Figure 36 demonstrating the reduced background obtained using the membranes; this is largely due to ease of cleaning the membranes before analysis. Furthermore, the membranes allow a significantly larger number of the particles to be exposed to the sample and no particles are lost upon retrieval of the membrane nor during rinsing. Table 10 shows a comparison between the peak areas obtained using both methods from cod oil

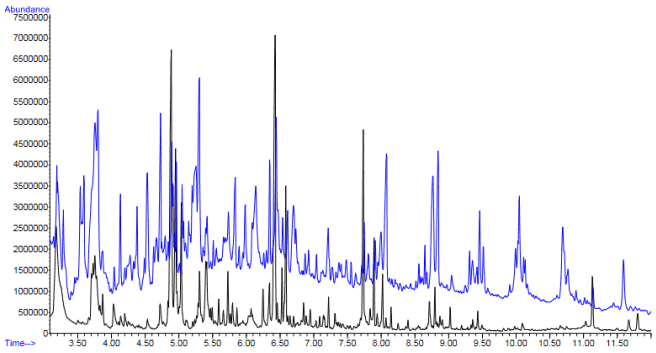


Figure 36: Total ion chromatogram comparison for microspheres (in blue) and unsupported membranes using the same particle sorbent (in black).

samples spiked with Mix-4. The ratio column demonstrates the impact of the membrane, with up to a 64-fold improvement in amount extracted over microspheres.

Table 10: Comparison of peak areas for a concentrated 10ppm sample of Mix-4 in cod liver oil by both unsupported membranes and microsphere particles of the same chemical composition.

Compound	*Unsupported Membrane Average (%RSD)*	*Microsphere Average (%RSD)*	*Ratio*
chlorodecane	2781107 (6)	43534 (4)	64
1,10-dichlorodecane	2388791 (11)	82562 (20)	29
1,2,13,14-tetrachlorotetradecane	2008510 (9)	141188 (18)	14
1,1,1,3,14,15-Hexachloropentadecane	66029 (12)	10739 (18)	6

Unfortunately, unsupported membranes are extremely fragile and prone to breaking, tearing away from the support pin, and moving with the flow caused by vortexing of the sample. All of these issues were exacerbated by the viscosity of the oil as well as the rinsing and washing requirements, adding more manual handling to the workflow. To combat the last issue, the membranes were sonicated at the lowest setting available on a Branson 3800 sonicator instead of vortexing. Even with careful handling, membranes degraded quickly with use.

In order to prevent breakage, a newer version was made more durable by spread coating the membrane slurry onto a sheet of thick aluminum foil instead of Teflon. This was much easier to handle, as it was coated single-sided and therefore no longer adhered to surfaces and could be easily manipulated with tweezers without ripping. The aluminum backing also provided enough support that the membrane no longer needed a pin to hold its shape without folding and reducing exposed surface area.

Foil-supported HLB membranes were made in the same way to compare the coatings as this is an effective adsorbent commonly used for lipophilic compounds in complex aqueous matrices.[50]

The only sample compounds tested were chlorodecane and 1,10-dichlorodecane; each was used at 10ppm.

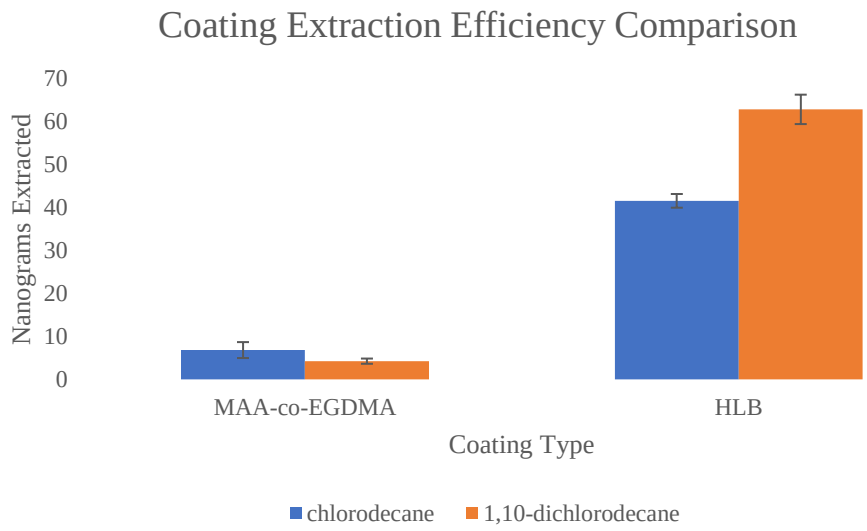


Figure 37: Membrane effectiveness comparison using 10ppm spiked cod oil samples. HLB and MAA-co-EGDMA membranes were used for extraction as described in section 3.4.3.3

Figure 37 shows the significant increase in uptake of the analyte by the HLB coating when compared to the MAA-co-EGDMA. As such, the HLB coating was selected in subsequent experiments.

3.4.3.6. Aluminum Blade TFME

To further improve robustness over the previous membranes on foil, membranes were made on a thicker sheet of sanded aluminum support. It was important to use a sheet of soft metal since membranes must be trimmed to size after coating, and difficulties in cutting could cause problems such as coating stripping, cutting irregularities and contamination. The 0.2mm thick sheet could be cut with relative ease using a sharp pair of regular scissors. This did not show any evidence of membrane stripping or other damage to the coating. Membranes were cut to strips 4.5mm wide

and 20.00mm long in order to optimally fit into the TDU tubes. The greater robustness of the support allowed coating on both sides. The same procedure was used as described by Grandy et. al..[45]

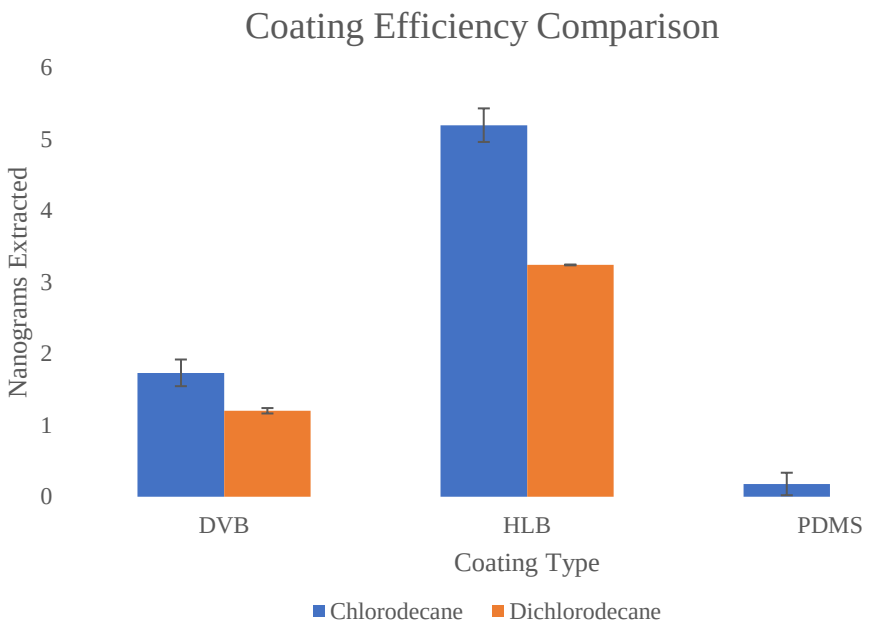


Figure 38: Comparison of DVB, HLB and PDMS membrane coatings on 0.2mm aluminum sheeting using 100ppb oil samples.

HLB was the target coating for this project, however DVB and pure PDMS membranes were also made using this support for comparison.[50] Using these new coatings, the concentration could be reduced by 2 orders of magnitude, from 10ppm to 100ppb with good recovery, as shown in Figure 38.

It was noted that PDMS on its own was not an effective extraction phase for the target analytes, and HLB extracted over two times more than DVB.

Furthermore, scans of oil samples were performed using both the foil and the 0.2mm support to compare the background obtained. Both demonstrated similar levels of background co-extractants from the oil. HLB (on solid support) and MAA-co-EGDMA (on foil) are depicted in Figure 39.

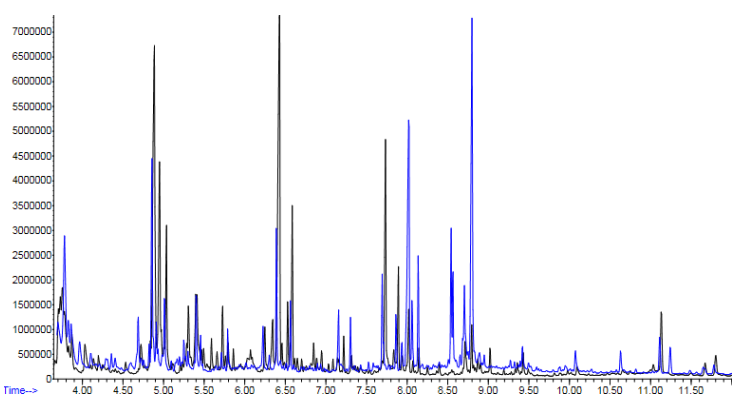


Figure 39: Full scan response of MAA-co-EGDMA membrane on foil (black) and HLB on solid aluminum (blue)

3.5. Results and Discussion

3.5.1. Kovats Retention Index

An alkane ladder gas generating vial with incrementally increasing n-alkanes previously made by Jonathan Grandy was sampled using a thin film membrane.[51] The same PCA method was used to desorb the ladder in order to calculate retention indices for compounds that fit within the ladder windows. Table 11 shows the retention times taken from Figure 40 used for retention index calculations.

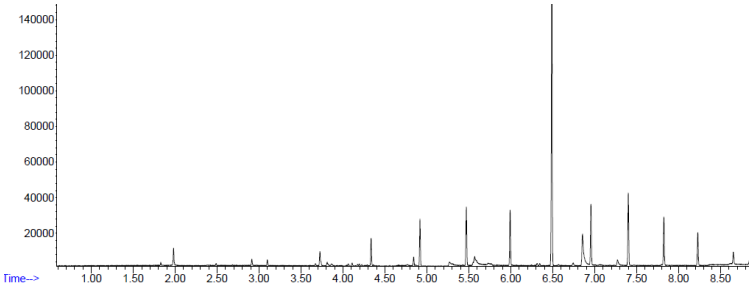


Figure 40: Alkane ladder, sampled using an aluminum supported HLB coated blade from a gas generating vial previously prepared by Jonathan Grandy.

Table 11: Retention times of the alkane ladder peaks compared to the number of carbons in the alkane.

Number of Carbons	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Retention Time	1.546	1.829	2.487	3.097	3.725	4.337	4.923
Number of Carbons	14	15	16	17	18	19	20
Retention Time	5.474	5.989	6.493	6.955	7.822	8.225	8.644

Table 12 below shows the retention times of each compound, with a known and calculated retention index available for each compound that fit within the ladder.

Table 12: Kovats retention indices for each compound, with their retention times and boiling points

Compound	*Retention Index*	*Retention Times*	*Calculated Retention Index*	*Boiling Point (°C)*
isooctane	691[52]	1.54	698	99[53]
chlorodecane	1264[54]	4.728	1267	223[55]
1,10-dichlorodecane	1545[56]	6.276	1557	251[37‡]
1,1,1,3-tetrachlorodecane		6.51	1649	317[37‡]
1,2,9,10-tetrachlorodecane		7.701	1786	289[37‡]
1,1,1,3,10,11-hexachloroundecane		9.022		360[37‡]
1,2,13,14-tetrachlorotetradecane		9.402		361[37‡]
1,1,1,3,12,13-hexachlorotridecane		9.788		359[37‡]
1,1,1,3,14,15-hexachloropentadecane		10.520		unknown

‡Boiling points obtained from the US Environmental Protection Agency are calculated estimates.

3.5.2. Optimization

Optimization of the SPME device and coating type led to a significant decrease in co-extracted background and resulting difficulties in sample detection. As depicted in Figure 41, a comparison of the methods in decreasing order of background matrix effects shows the improvements in method optimization from carbon mesh supported membranes to coated thermally stable aluminum blades.

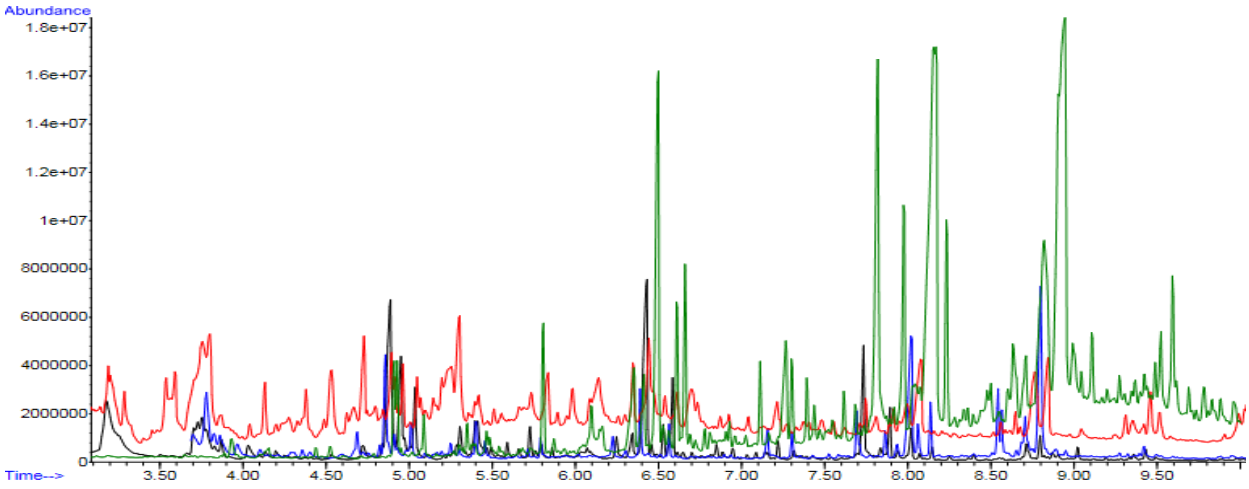


Figure 41: Comparison of non-spiked oil as a background from each style of sampling performed in scan mode. Carbon mesh in green, microspheres in red, unsupported membranes in black, and aluminum sheet supported membranes in blue.

As the blades have been most successful, they were used to complete the project and the protocol for their use was optimized further. As blades were more durable, Gerstel membrane clips were used to secure them in place to the top of the caps of each vial. This required an increase in volume to 8.5g as the membrane was now suspended, instead of seated against the bottom of the vial. The clips are shown in Figure 42 alongside the membrane.

Figure 42: Gerstel clip (left) and the 0.2mm aluminum supported membrane (right)

Primarily, the effects of temperature were investigated using this extraction phase, as generally an increased temperature leads to a larger amount of analyte extracted. Previous research with the PAHs also suggests that the increase in temperature would be especially effective for such hydrophobic compounds in a complex matrix. Chlorodecane and 1,10-dichlorodecane were used to spike the oil to 100ppb and the extraction was performed at 500rpm for 1 hour; four temperatures were tested, 35°C, 50°C, 65°C and 80°C. A 2s rinse was performed in 1.8mL acetone and the membranes were blotted dry prior to return to the TDU tube.

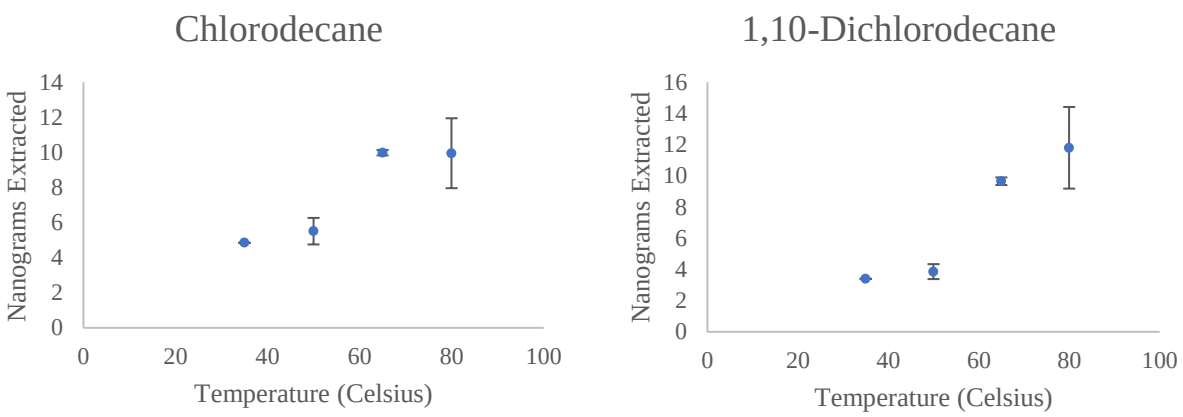


Figure 43: Temperature profiles of 2 compounds

As expected, an increase in temperature improved analyte recovery. Large errors associated with the highest temperature point, 80°C, were thought to be due to fluctuations in the heater block system of the autosampler, 65°C was therefore used moving forward.

Upon the change to a more durable and rigid coating support, larger agitation speeds could be used. Increasing the agitation speed improves extraction by reducing the boundary layer, however this is not effective if the support moves with the flow of the matrix.

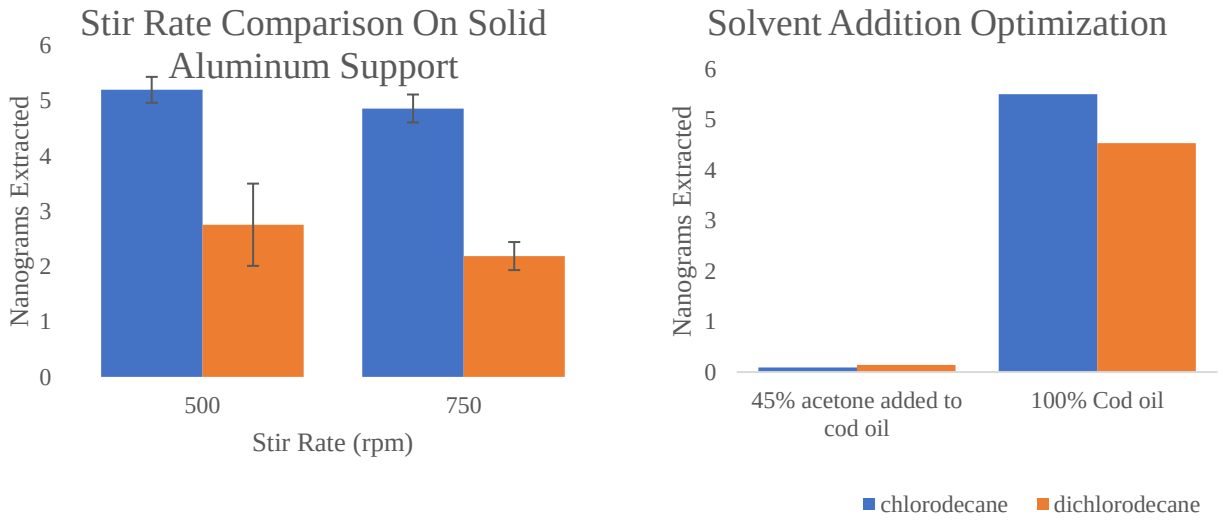
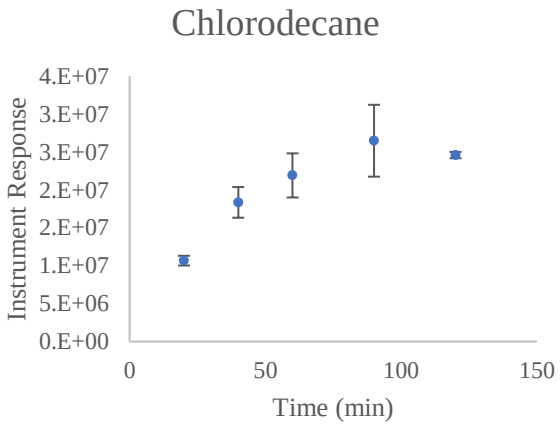


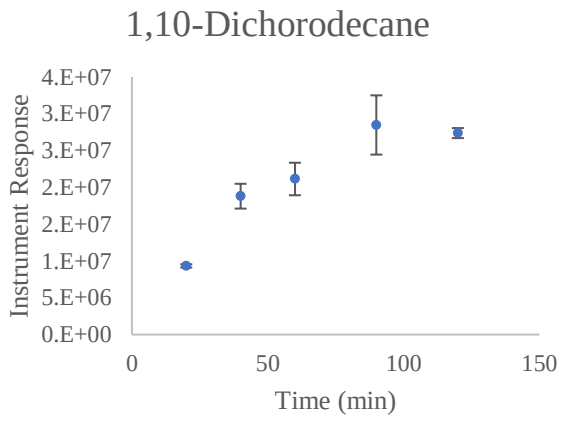
Figure 44: Effects of stirring rate and solvent addition on the efficiency of the extractions.

It was found that the stirring rate made no significant difference when the upper limit of the autosampler agitator was tested against the average rate. To preserve the lifetime of the membranes, the average rate was used.

The addition of solvent had previously been used in complex matrices to improve extraction efficiency, evidenced both in Chapter 2 listed above as well as previously in literature.[47] The use of acetone at 45% volume was used as it is miscible with the matrix. The sample with added solvent was incubated in a vortex at 1500 rpm for 1 minute to combine the solvent; as results from the extraction were so poor, replicates were not performed to avoid wasting oil stock.

The extraction time was testing using oil spiked with 100ppb mix-4 in order to determine if the heaviest compound was reaching equilibrium. Oil was spiked in a batch for all trials in order to prevent errors from pipetting influencing the standard deviations. Times of 30, 45, 60, 90 and 120 minutes per extraction were used at 65°C. Post extraction, membranes were blotted dry to remove oil and rinsed for 2s in acetone then blotted dry again using a fresh kimwipe.





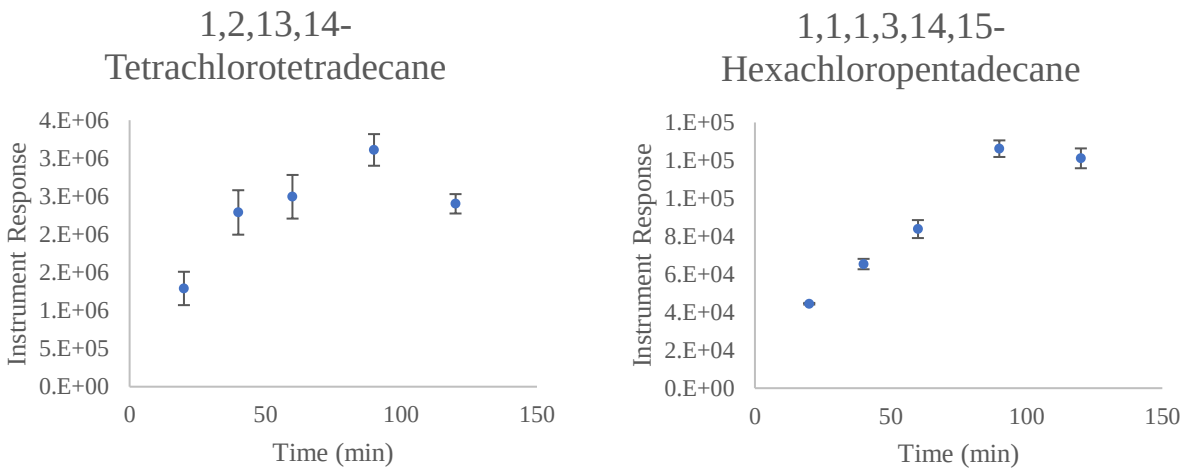


Figure 45: Extraction time profiles for mix-4 standards in cod liver oil. Optimal time shown in each is 90 min.

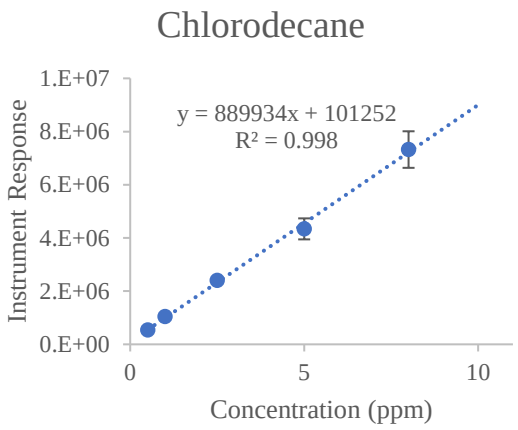
Extraction time profiles shown in Figure 45 show a plateau is reached by all compounds by 90 minutes. The equilibrium time was taken for this project as the extraction is not governed by an autosampler, as such human error is more prevalent and can be reduced by working at or beyond equilibrium times. Since extractions are performed on a multi-position vortex-heater block, throughput can be instead improved by extracting 6 samples simultaneously, then placing them onto the TDU sampling tray to be sampled automatically in a sequence.

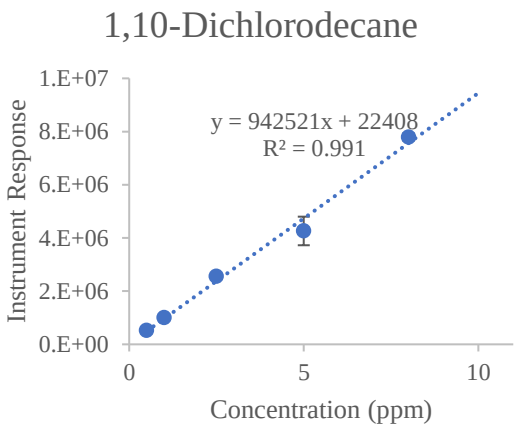
The finalized method uses 8.5g oil per sample and extraction at 65°C for 90 minutes at 500 rpm in a vortex-heater block. Membranes are blotted dry then agitated in 1.8mL acetone for 2s to strip away surface level oil and blotted dry with a kimwipe. The desorption was automated as escribed in the instrumentation section.

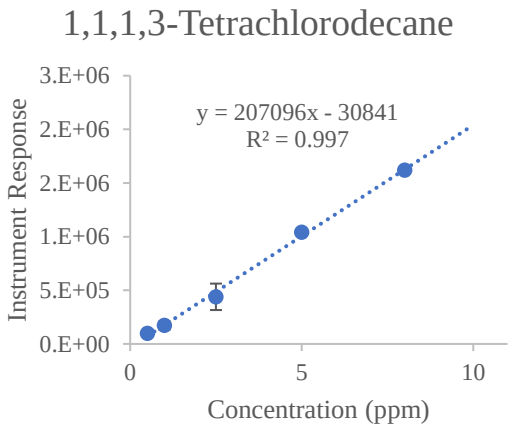
3.5.3. Calibration and Validation

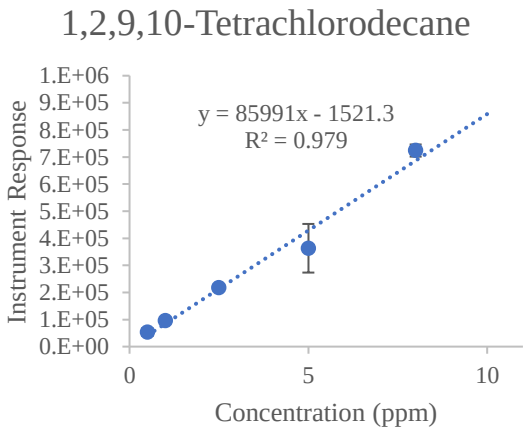
The instrument was calibrated using on membrane liquid injection, which permitted the use of the same TDU system as for regular sampling rather than replacing it for the septumless head

injector for needle injections. Membranes had 1 µL of standard solution deposited onto them at varying concentrations, then desorbed as normal. Membranes were recapped into the tubes immediately after spiking to prevent losses due to evaporation.









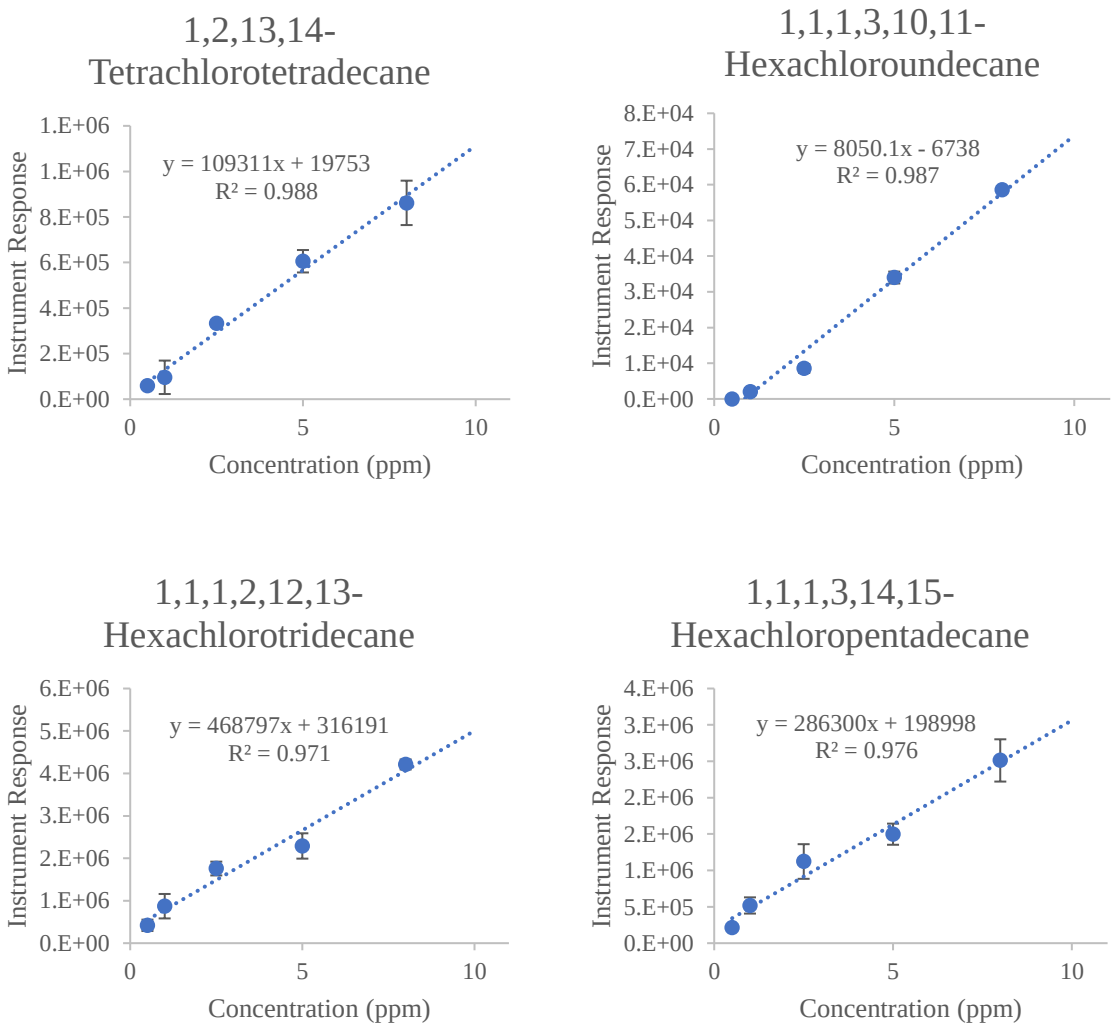
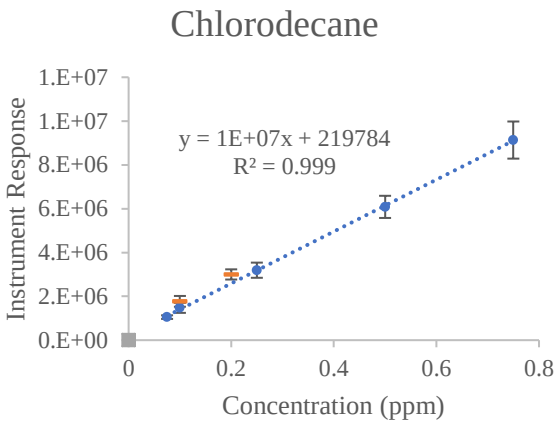
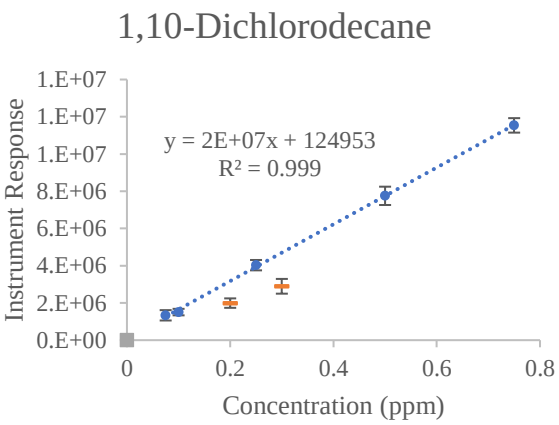
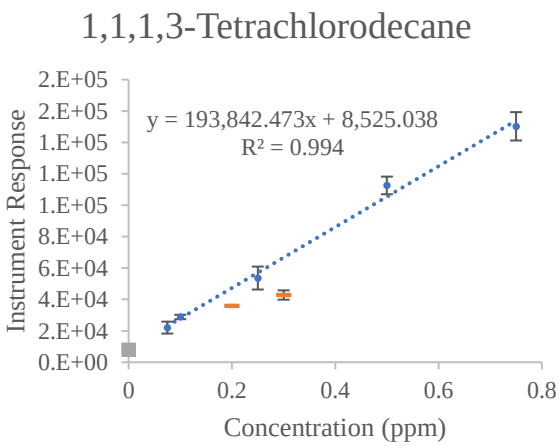


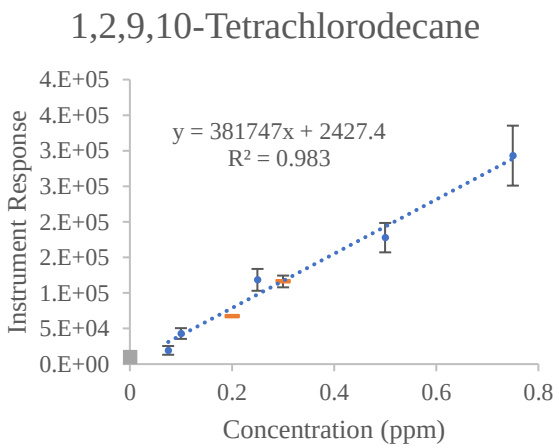
Figure 46: Instrument calibration made by on-membrane liquid injections

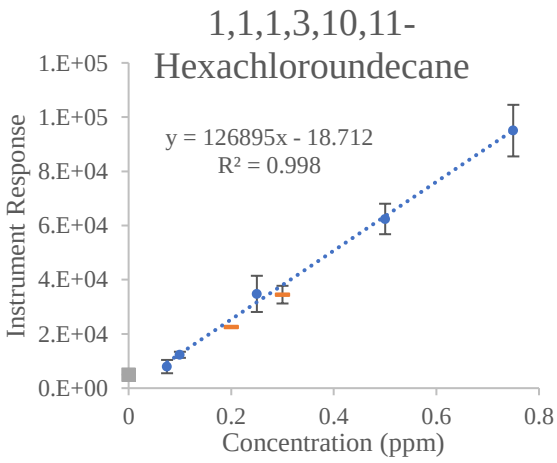
The method was calibrated using the mix-8 standard mixture; samples were spiked to form a calibration curve with the following points: 0.75, 0.5, 0.25, 0.1, 0.075 in ppm.

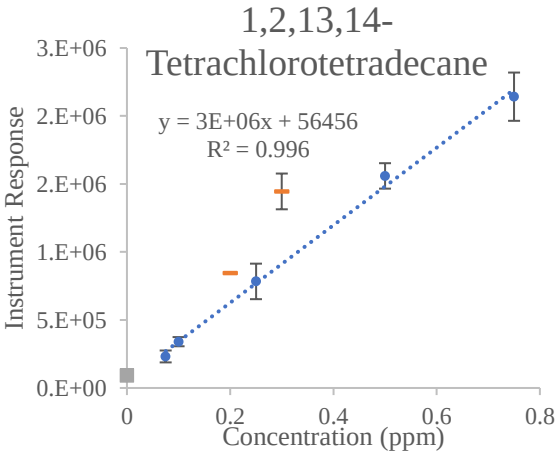

Chlorodecane
y = 1E+07x + 219784
R² = 0.999
Instrument Response
Concentration (ppm)
1.E+07
1.E+07
8.E+06
6.E+06
4.E+06
2.E+06
0.E+00
0
0.2
0.4
0.6
0.8


1,10-Dichlorodecane
y = 2E+07x + 124953
R² = 0.999
Instrument Response
Concentration (ppm)
1.E+07
1.E+07
1.E+07
8.E+06
6.E+06
4.E+06
2.E+06
0.E+00
0
0.2
0.4
0.6
0.8


1,1,1,3-Tetrachlorodecane
y = 193,842.473x + 8,525.038
R² = 0.994
Instrument Response
Concentration (ppm)
2.E+05
2.E+05
1.E+05
1.E+05
1.E+05
8.E+04
6.E+04
4.E+04
2.E+04
0.E+00
0
0.2
0.4
0.6
0.8


1,2,9,10-Tetrachlorodecane
y = 381747x + 2427.4
R² = 0.983
Instrument Response
Concentration (ppm)
4.E+05
4.E+05
3.E+05
3.E+05
2.E+05
2.E+05
1.E+05
5.E+04
0.E+00
0
0.2
0.4
0.6
0.8


1,1,1,3,10,11-
Hexachloroundecane
y = 126895x - 18.712
R² = 0.998
Instrument Response
Concentration (ppm)
1.E+05
1.E+05
8.E+04
6.E+04
4.E+04
2.E+04
0.E+00
0
0.2
0.4
0.6
0.8


1,2,13,14-
Tetrachlorotetradecane
y = 3E+06x + 56456
R² = 0.996
Instrument Response
Concentration (ppm)
3.E+06
2.E+06
2.E+06
1.E+06
5.E+05
0.E+00
0
0.2
0.4
0.6
0.8

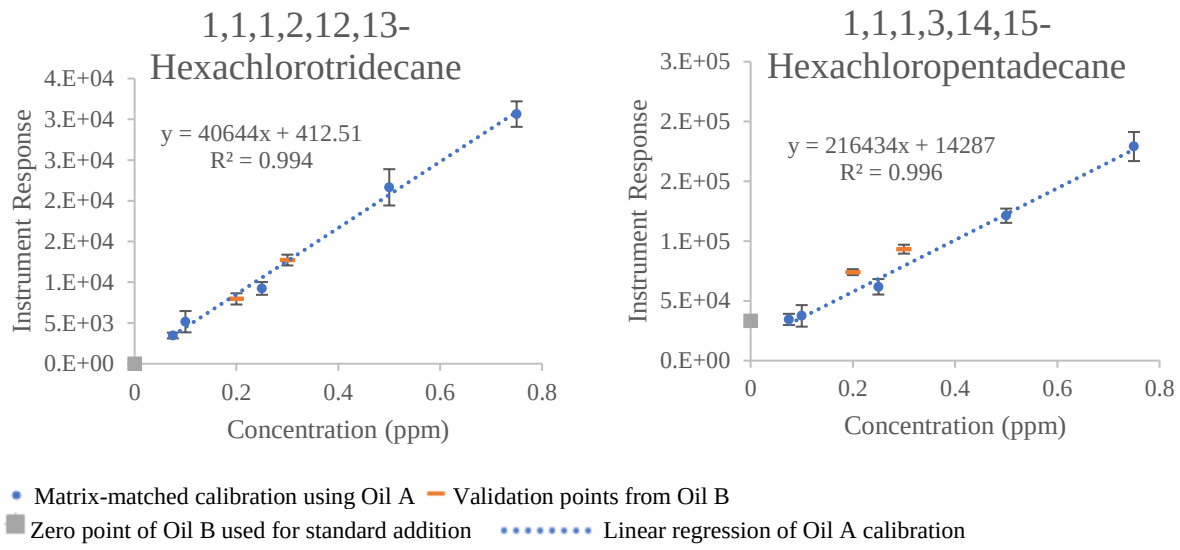
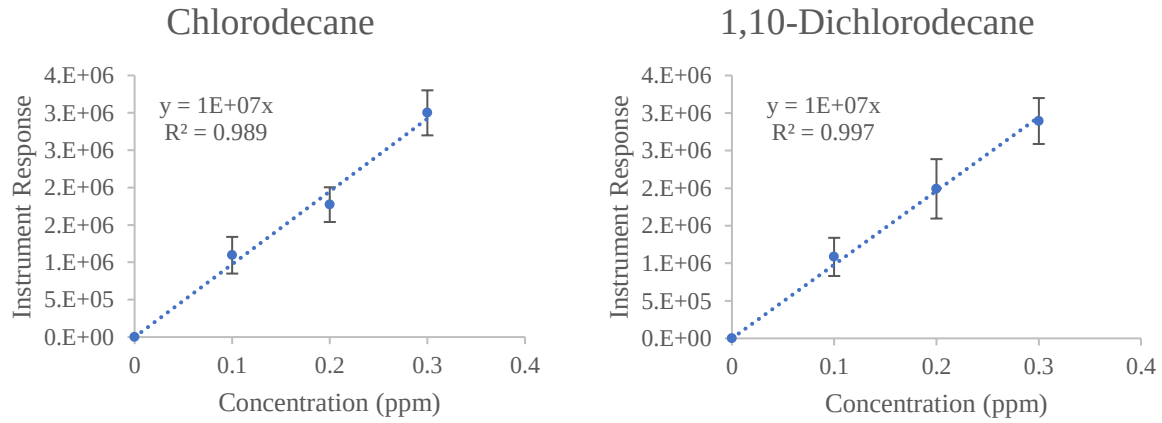


Figure 47:Matrix-matched calibration in cod liver oil A (blue) with selected standard addition points from Oil B acting as validation points (orange); grey is a blank of Oil B as a true unknown, non-spiked sample.

Samples of other oils were processed using the same procedure. A standard addition was performed with Oil B to validate the matrix calibration. Fortunately for the consumers of Oil B, but unfortunate for the purpose of validation, no significant levels of any of the target contaminants were found. Table 13 shows the results from both the calculation using the matrix matched calibration curve as well as the back-calculated values from the standard addition shown in in Figure 48.



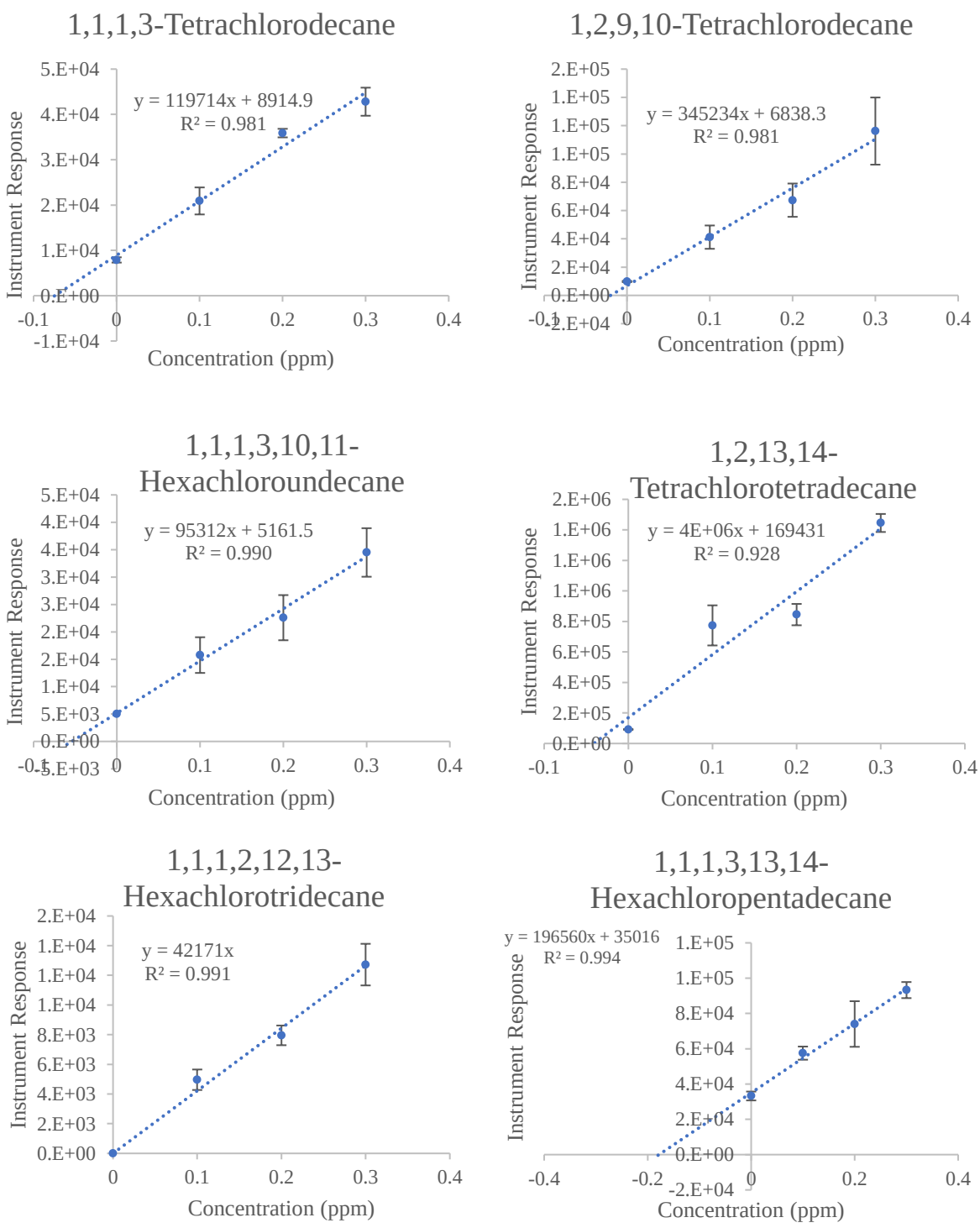


Figure 48: Standard addition curves using Oil B, for compounds where peaks were detected in the blank, the curve was extrapolated to show the intercept. Performed on GC-MS, showing response on a SIM method.

Table 13: Comparison of calculated values of Oil B blanks and standard addition using Oil B

Compound	*Calculated from Oil B blank (ppm)*	*Standard Addition (ppm)*	*MLOQ (ppm)*	*%RSD*
chlorodecane	-	0	0.069	-
1,10-dichlorodecane	-	0	0.183	-
1,1,1,3-tetrachlorodecane	0	0.074	0.196	7
1,2,9,10-tetrachlorodecane	0.019	0.020	0.161	5
1,1,1,3,10,11-hexachloroundecane	-	0	0.193	-
1,2,13,14-tetrachlorotetradecane	0.012	0.041	0.154	2
1,1,1,3,12,13-hexachlorotridecane	-	0	0.086	-
1,1,1,3,14,15-hexachloropentadecane	0.182	0.178	0.217	12

The method's limit of quantitation (MLOQ) was calculated as ten times the standard deviation of the lowest point divided by slope. It was found that the quantities of the three compounds which were detected in the native oil samples were below MLOQ. In order to validate the method, points from the standard addition were used as shown in the calibration curves in Figure 47 in orange.

Table 14: Comparison of slopes for the matrix-matched calibration against the slopes of the standard addition

Compound	*Slope of Calibration*	*Slope of Standard Addition*
chlorodecane	11843748	9677291
1,10-dichlorodecane	15244774	9587209
1,1,1,3-tetrachlorodecane	193842	119714
1,2,9,10-tetrachlorodecane	381747	345234
1,1,1,3,10,11-hexachloroundecane	126895	95312
1,2,13,14-tetrachlorotetradecane	2850679	4130857
1,1,1,3,12,13-hexachlorotridecane	40644	41160
1,1,1,3,14,15-hexachloropentadecane	216434	196560

Three other oils were sampled; detected compounds were recorded and back-calculated using the matrix-matched calibration curve; results are summarized in Table 15. 1,1,1,3-tetrachlorodecane was found in two oils, 1,2,9,10-tetrachlorodecane was found in oil E and 1,1,1,3,14,15-hexachloropentadecane was found in oil C, all above the MLOQ.

Table 15: Samples of other oil blanks, values were calculated using the matrix-matched calibration.

Compound	*Oil B*	*% RSD*	*Oil C*	*% RSD*	*Oil D*	*% RSD*	*Oil E*	*% RSD*
chlorodecane								
1,10-dichlorodecane		2	0.011					
1,1,1,3-tetrachlorodecane	0	5	0.045	7	0.487	8	0.286	6
1,2,9,10-tetrachlorodecane	0.019			5			1.228	13
1,1,1,3,10,11-hexachloroundecane								
1,2,13,14-tetrachlorotetradecane	0.012	24	0.147	2	0.446	10		
1,1,1,3,12,13-hexachlorotridecane								
1,1,1,3,14,15-hexachloropentadecane	0.182	11	0.472	12				

3.6. Conclusions and Recommendations

Overall, this method allowed for the detection of individual standards representing the range of short and medium chain polychlorinated alkanes at varying levels of chlorination by weight between 0.069 – 0.22ppm MLOQ. The method reduced sampling time to 90 minutes, with a chromatography time of 12 minutes, plus desorption at 5 minutes, totalling approximately two hours per batch of samples, much of which does not require analyst attention. This can be compared to current methods that require an estimate of 3 hours per sample involving tedious GPC an SPE cleanup, without considering the time required for GC-MS acquisition.[29] Apart from 1,2,9,10-tetrachlorodecane, detected amounts were below the lowest observed effect concentration (LOEC) found by the Stockholm Convention on POP, ranging between 0.7-5.5 ppm.[57] MLOQs were comparable to those obtained for deconvolution of short chain PCAs by Bogdal et. al. using bulk PCA standards on APCI-qTOF-HRMS where LODs ranged from 0.1-1.2ppm. While the instrumentation used in that study was significantly more powerful than the single quadrupole MS used here, Bogdal et. al. used bulk PCA mixtures, adding complexity to the system.

Recommended next steps for this project are to apply the same method in scan mode to a broad spectrum PCA sample. This would be more representative of true samples found in the environment, which can range in chain length and chlorination degree. Isolation of individual PCAs is not often done, in favour of determining total concentration.

A limitation on the throughput for this method is the number of positions on commercial heater-agitator blocks; only 6 positions are available and the chromatography methods per 6 samples do not exceed preparation time. This can be remedied by the use of a multi-position sampler brush to further improve efficiency.[1]

Furthermore, this sampling method could be combined with previously assessed deconvolution methods performed with high resolution mass spectrometry for more comprehensive PCA detection.[38] As demonstrated by Yuan et. al. characterization and quantification of PCAs in large groups was made possible from clean matrices. Further application of both these novel methods could improve environmental detection well beyond the current limitations.

4. Chapter 4: Summary

Industrialization worldwide has resulted in the rise of anthropogenic contaminants found in the environment leading to contamination of the food chain. Screening has become increasingly important to determine if foodstuffs have been contaminated or if cleaning procedures are effective. This thesis offers two inexpensive and reusable methods to perform that screening in very complex fatty food matrices.

Pesticides were extracted from soymilk using a PDMS/DVB/PDMS overcoated fiber that has been previously shown to be matrix compatible. This allowed for the direct immersion of the fiber into the sample to facilitate the extraction of semivolatile compounds. The resulting loaded fiber was then rinsed and desorbed thermally into a GC-MS for separation and detection. Limits of detection using this method were well below the MRLs published by both the Canadian and American environmental agencies, reaching the 1 ppb level; it was validated according to the FDA Bioanalytical Method Validation Guidance for Industry. The method was validated well below maximum residue levels set by both the Canadian and United States government agencies raging from 0.01 to 8 ppm. Precision and accuracy from back-calculated results reached targets within 70 – 130% of known values with less than 20% RSD. Of other brands sampled, Chlorpyrifos and Malathion were each found below MRL. Dimethoate was found 742 and 745 ppb in another soymilk. This method, while demonstrated on soymilk, has the potential to be applied to broader applications of foodstuffs, as allowed by the matrix-compatible fiber.

A method was developed to test for individual PCAs representing components of a broad band of both medium and short chain PCAs of varying chlorine content. This was accomplished by using an HLB coated aluminum blade extracting via direct immersion in the oil, followed by a rinse before thermal desorption and analysis by GC-MS. The method demonstrated linearity and MLOQ up to 0.2ppm levels, showing promise for further applications. Calibration of the method was performed with a linear range of 0.075 to 0.75ppm with MLOQ ranging from 0.217 to 0.069ppm. The method fit within the targeted testing range for the lowest observed effect concentration (LOEC) dictated by the Stockholm Convention on POP, ranging between 0.7-5.5 ppm.[57] A second oil was used to validate the method; quantities of PCAs assessed by standard

addition in the second oil were below MLOQ for each compound found. Inter-matrix accuracy for many analytes was demonstrated by comparing the slopes of the two curves. Other oil brands were sampled; four compounds were found at concentrations above MLOQ, the highest at 1.228ppm. Kovats retention indices for two PCAs: 1,1,1,3 – tetrachlorodecane and 1,2,9,10 – tetrachlorodecane were found to be 1649 and 1786 respectively.

In summary, both methods developed in this thesis have been effective in cleanup and preconcentration of environmental pollutants. The methods are inexpensive, and reusable, allowing for screening of the pesticides and PCAs respectively, while limiting the impact they impart on the environment.

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