A new method for the routine trace analysis of polychlorinated biphenyls in waste oil and contaminated soil

By

Reinardt Cromhout

Submitted in partial fulfilment of the requirements for the degree

Master of

Chemistry

in the faculty of Natural and Agricultural Science

University of Pretoria

July 2015

Declaration

I, Reinardt Cromhout , declare that the thesis/dissertation that I hereby submit for the degree of Master of Chemistry at the University of Pretoria is my own work and has not previously been submitted by me for degree purposes at any other university.

Signature:……. Date:

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Summary

Polychlorinated biphenyls (PCBs) were used in various industries and equipment such as transformers, paints and capacitors until 1979. PCBs enter the environment through improper disposal by users and manufacturers. The Stockholm Convention on persistent organic pollutants of which South Africa is a signatory states the identification and removal of PCB containing equipment with levels higher than 50 mg/l. PCB analysis in oils and oil contaminated soil is normally expensive in terms of time, instrumentation and uses large volumes of hazardous solvents which further increase cost per sample. Commercial laboratories are constantly under pressure to provide a faster turnaround time per sample at low cost and of an acceptable quality, therefore, a new method was developed to find a balance between these requirements. Numerous methods exist for the extraction and analysis of PCBs in waste oils and soils such as liquid-liquid extraction (LLE) and solid phase extraction (SPE) coupled with detection on GC-MS (gas chromatography- mass spectrometry). Most methods for the extraction and analysis of PCBs are laborious, time consuming and are of high cost per sample which is not suited for a commercial environment where sample throughput is also of cardinal importance.

The newly developed method utilizes the interaction of PCBs with dimethylsulphoxide (DMSO) for a fast liquid-liquid extraction that was combined with solid phase microextraction (SPME). For implementation in a commercial laboratory limited sample clean-up is a necessity and, therefore, the sensitivity and selectivity of GC-MS/MS (gas chromatography mass spectrometry/ mass spectrometry) was used. GC-MS/MS is able to filter out most spectral interferences and provides a high level of confidence in terms of identifiers.

This fast liquid-liquid extraction using small amount of solvent and automated submerged SPME GC-MS/MS makes this method ideal for commercial laboratories, low cost per sample, fast turnaround time and provides a quality result. Cost is further reduced by using GC-ECD (electron capture detector) for screening of samples, GC-ECD is extremely sensitive for halogenated compounds, ideal for PCB analysis.

Robustness of any method is important and more so for commercial laboratories. PCBs were successfully extracted from soil and various oils. Current regulatory limits for total PCBs are 50 mg/l in oil and 610 µg/kg in soil. This method offers a quantification limit of 5.35 mg/l in waste oil and 35 µg/kg in soil which is below the allowed maximum contamination levels (MCL) of these matrices.

Keywords: PCBs, submerged SPME, GC-MS/MS, robustness, waste oil, soil and commercial laboratory.

ACKNOWLEDGMENTS

I would like to offer my gratitude to:

Jan-Daniël Vorster and the entire team at UIS Organic laboratory for their support and guidance.

UIS Organic laboratory for funding my research and the opportunity to use their equipment.

Dr Willem Havenga, for his continuous scientific input and lending his wealth of knowledge.

Professor Egmont Rohwer, for his advice and scientific guidance in my research.

My wife Melissa for her love, support and encouragement through this long journey.

I would like to thank my Lord, Jesus Christ, without him none of this would be possible.

Thank you

Reinardt

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ABBREVIATIONS

ASE	-	Accelerated Solvent Extractor
BTEXMN	-	Benzene, Toluene, Xylenes , Methyl tert-butyl ether (MTBE) and Naphthalene
BZ Number	-	Balschmitter-Zell number
DIN	-	Deutsches Institut für Normung
DMSO	-	Dimethylsulphoxide
DRO	-	Diesel range organics
ECD	-	Electron capture detector
FID	-	Flame Ionization detector
GC	-	Gas chromatography
GC-HRMS	-	Gas chromatography high resolution mass spectrometry
GC-QMS	-	Gas chromatography quadruple mass spectrometry
GRO	-	Gasoline range organics
ITQ	-	Ion Trap Quadruple
IUPAC	-	International Union for Pure and Applied Chemistry
LLE	-	Liquid-liquid Extraction
MCL	-	Maximum contamination level
MIM	-	Multiple ion monitoring
MS	-	Mass spectrometer
MS/MS	-	Mass spectrometry/mass spectrometry
OCP	-	Organochloro pesticides
PAH	-	Polyaromatic Hydrocarbons
PCB	-	Polychlorinated biphenyls
PDMS	-	Polydimethyl siloxane
POPs	-	Persistent Organic Pollutants
PTFE	-	Polytetrafluoroethylene
PTV	-	Programmed Temperature Vaporization
SANAS	-	South African National Accreditation System
SANS	-	South African National Standards
SIM	-	Single ion monitoring
SPE	-	Solid phase Extraction
SPME	-	Solid phase microextraction
SQ	-	Single Quadruple
SVOC	-	Semi-volatile organic compounds
TEF	-	Toxic equivalency factor
TIM	-	Total ion monitor
TQ	-	Triple quadruple
UISOL	-	UIS Organic Laboratory
UNEP	-	United Nations Environmental Program
US EPA	-	United States Environmental Protection Agency
Volatile DRO	-	Volatile diesel range organics C10-C20
VOC	-	Volatile organic compounds

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Chapter 1

Introduction

1.1 Background

Polychlorinated biphenyls (PCBs) are a group of compounds once associated with countless applications. PCBs have the following physical properties [1, 2, 3]:

- odourless
- clear to pale yellow viscous liquids (depending on chlorination)
- lipophilic
- high thermal stability
- high dielectric constants
- resistance to degradation

Polychlorinated biphenyls were classified by the United Nations Environmental Program (UNEP) as one of the original 12 persistent organic pollutants (POPs) [4, 5].The production of PCBs and PCB-containing equipment has stopped since the late 1970s, but contaminated oil and soils are still found and form part of most countries' environmental screening/remediation legislations. The 50 mg/kg or 0.005% total PCB concentration is widely accepted as the maximum allowed contamination level for waste oils [6, 7]. South Africa is one of the countries that signed the Stockholm Convention on POPs in 2001. As signatories of the Stockholm Convention, South Africa agreed to reduce and eliminate the release of POPs for the protection of human health as well as the environment [4]. According to the objectives set by the Stockholm Convention all PCB equipment containing a total PCB concentration higher than 50 mg/kg are to be identified and removed by 2025 [8]. Some countries will allow the end of lifetime use of PCB-contaminated transformer oil lower than 500 mg/kg [7].

The screening levels regarding soil contaminated with PCBs may differ between countries depending on legislation. The maximum contamination level (MCL) of Australian EPA's is 2 mg/kg for soil that can be reused as fill material. Countries such as South Africa and many others provide a more detailed breakdown of allowable limits for PCB-contaminated soil that is governed by land use.

Land-use classification is beneficial for commercial/industrial areas where the impact on the environment and human health might be negligible with more stringent limits for areas that protect water resources (Table 1.1). This approach is more useful as it is not a general limit that is applicable to all land uses [9].

Table 1.1: South African National Norms and Standards (SANS) for the Remediation of Contaminated Land and Soil Quality for total PCBs [9]

Parameter	Units	All land-uses Protective of water resources	Informal residential	Standard residential	Commercial/ industrial
PCB	mg/kg	0.61	1.7	3.6	11

Extraction and analysis of compounds from extremely difficult matrices are common place in organic environmental laboratories. The selection of methods that have sufficient selectivity and acceptable sensitivity is of paramount importance for use and implementation. Commercial laboratories are confronted with high sample volumes, fast turnaround times and low cost per analysis requirements from customers conducting rapid assessments of contaminated areas/ pollution sites. The inventory of Eskom, South Africa's only electricity-generating company, states that 62% (17 086) of their equipment contain total PCB concentration greater than 50 mg/kg. As Eskom has to focus on their core business which is power generation, regulatory analysis are often outsourced to the private sector, and therefore, a large market and need exist for PCB analysis with the implementation of South African Norms and Standards (SANS) for the remediation of contaminated land and soil quality [9]. South Africa and many other countries also require classification of waste where PCBs are included before any waste is allowed at a disposal site [10]. PCBs are also unintentionally formed in industrial processes where extremely high

temperatures are used, such as waste incineration and metal production. PCBs are formed in the ambient air around these smelters and are released into the environment through the flue gas (exhaust gases) of waste incinerations [11, 12].

A commercial method has to minimise the use of skilled labour and increase the level of automation to reduce cost per analysis and increase sample throughput. Commercial laboratories are also focused on methods that make use of lower solvent volumes due to their high cost and develop greener methods that are more environmentally safe (less chlorinated solvents) as waste removal and incineration are extremely expensive. The UIS Organic Laboratory (UISOL) makes use of solid-phase microextraction (SPME), which is an extremely green method using little to no solvent for sample extraction and analysis.

The value triangle (Figure 1.1) demonstrates the dilemma that commercial laboratories face:

- A quality, inexpensive analysis will yield long turnaround times.
- A quality, fast analysis will be expensive.
- An inexpensive, fast analysis will not deliver a quality result.

The key for any method to be viable in a commercial laboratory is to find the balance between turnaround time, cost and quality of analysis (fit for purpose).

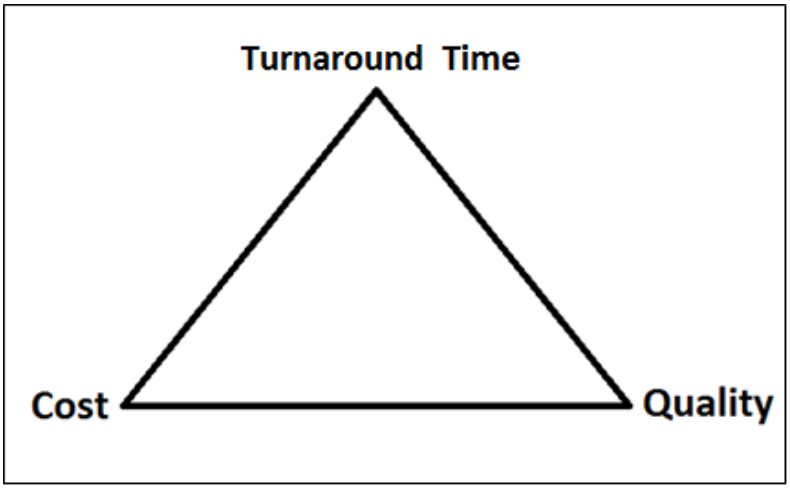


Figure 1.1: Value triangle [13]

1.2 Difficulty of PCB analysis

The analysis of PCBs in transformer and waste oil is not a trivial exercise due to the lipophilicity of PCBs and the complexity of transformer oil and other waste oil mixtures. Transformer oil consists mainly of mineral oil between carbon numbers 15 and 35. When waste or waste oils are found in the environment, PCBs are some of the compounds that require quantification to establish appropriate forms of disposal as per legislation [14]. Waste oils that may also be encountered in the environment are fish and vegetable oil [15, 16].

The selective extraction of PCBs from oil matrices has been the focus of many PCB analysis techniques. These extraction procedures involve numerous clean-up steps to remove all hydrocarbon interferences to accurately classify commercial PCB mixtures (Aroclors) as well as quantify total PCB concentrations. Figure 1.2 (a) clearly shows the complexity of the hydrocarbon matrix from which potential PCBs are to be extracted. Methods need to selectively extract PCBs (Figure 1.2 (b)) from the hydrocarbon matrix for accurate identification and quantification.

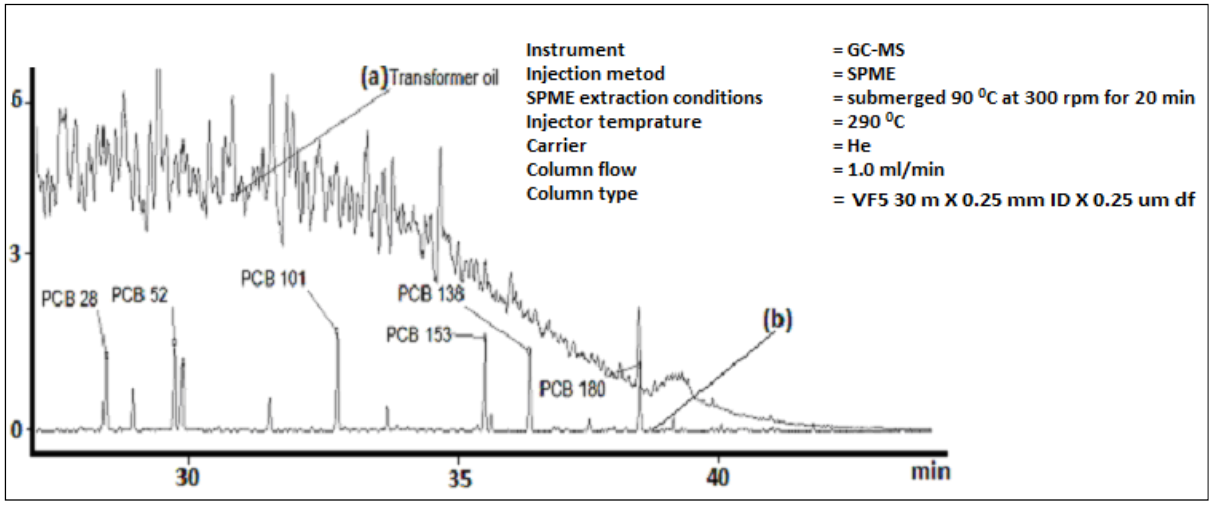


Figure 1.2: Total ion monitor-monitoring (TIM) of transformer oil (a) and (b) TIM of indicator PCBs. *Overlaid chromatogram of co-eluting transformer oil and PCBs. SPME extraction followed by GC-MS detection as discussed in chapter 5.*

Extensive sample clean-up procedures are usually required, which are time consuming and use large volumes of solvent. The clean-up procedures are necessary for the selective extraction of PCBs for the accurate identification and quantifications on low-cost detectors such as an electron capture detector (ECD). GC-ECD is often used by commercial laboratories due to its sensitivity for halogenated organic compounds and for its low operating costs.

The complexity of real PCB sample analysis does not only lie with the matrix, but also with the multiple PCB congeners found in commercial mixtures and environmental samples, as seen in figure 1.3. Commercial PCB mixtures consist of countless overlapping PCBs obscuring lower concentration PCBs and therefore an experienced operator and optimal instrumental conditions are needed for accurate quantification.

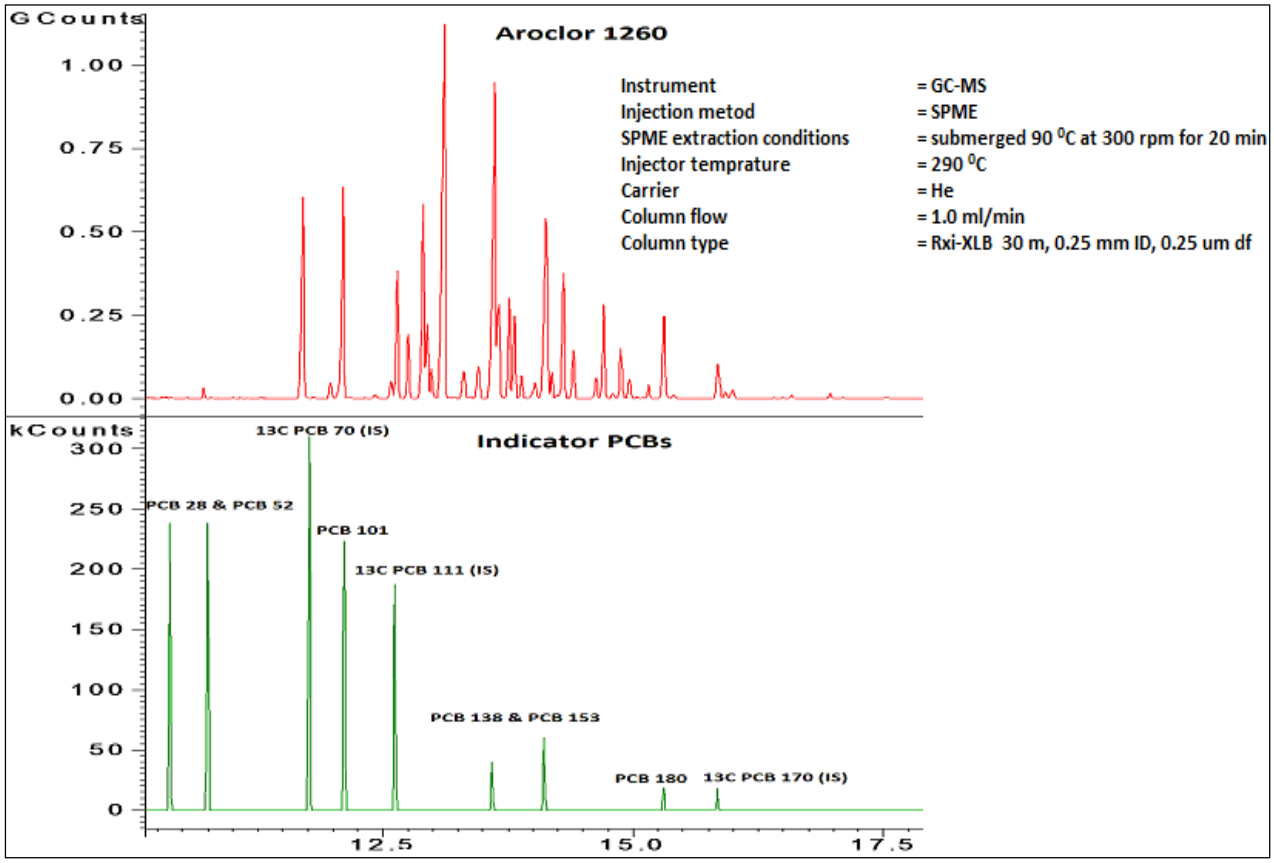


Figure 1.3: Gas chromatogram of Aroclor 1260 as discussed in chapters 2 and 5.

1.3 Aims and approach

Methods currently being used in our laboratory are not competitive on the international market, since international competition laboratories offer faster analysis at our cost per sample, and therefore a new approach for PCB analysis is required. The aim of this project is to develop a new method for the fast, uncomplicated, economical, robust extraction and analysis of PCBs in waste oils and contaminated soils for a commercial laboratory application (UIS Organic Laboratory).

My approach is to develop a new method for the analysis of PCBs from waste oil and soil and to critically evaluate traditional extraction methods such as liquid-liquid extraction (LLE), solid-phase extraction (SPE) as well as headspace solid-phase microextraction (SPME). This critical evaluation will shed some light on aspects of each method that could potentially be used in the development of a new method that is better suited to our commercial laboratory.

UIS Organic Laboratory is predominantly a SPME laboratory and therefore a SPME-based method will be best suited to our laboratory in terms of implementation and integration into our current systems. Optimisations of the LLE and SPME procedure will be investigated to enhance selectivity and sensitivity.

UIS Organic Laboratory has a wide variety of gas chromatographic (GC) instruments with different detectors, such as:

- single quadruple (SQ)
- triple quadruple (TQ)
- ion trap quadruple (ITQ)
- electron capture detector (ECD)
- flame ionisation detector (FID)

These abovementioned systems will also be optimised to reduce sample preparation and increase sample throughput. The critical evaluation of these systems will determine which system is best fit for purpose for the analyses of PCBs in waste oil and soil using SPME. UIS Organic Laboratory has stringent acceptance criteria for sample analysis. Every batch of ten samples requires verification at the start and end of each

batch, blank, duplicate as well as a complete calibration. Therefore, aspects such as simplicity of procedure, robustness, speed, quality and repeatability will form an integral part of the development of this method. Accreditation of this method will be conducted by the South African National Accreditation Service (SANAS). As it is required of most environmental laws, such as the South African waste classification and management regulations, improper disposal of wastes will most probably end up in court [10].

1.4 Structure of thesis

- Chapter 2: PCBs – a historical perspective
- Chapter 3 : Literature review of methods for PCB analysis
- Chapter 4 : Solid-phase microextraction (SPME)
- Chapter 5: Description and evaluation of extraction procedures
- Chapter 6: Description and evaluation of total analytical systems
- Chapter 7: Conclusions

Chapter 2

PCBs- a historical perspective

2.1 PCB structure and nomenclature

PCBs consist of two bonded phenyl rings with hydrogen and chlorine atoms substituted in various combinations from carbon 2-6 and 2'-6' (Figure 2.1). The two phenyl rings can rotate around the 1-1' bond depending on the position of *ortho* chlorine and hydrogen atoms, which also governs the toxicity of a congener. The International Union of Pure and Applied Chemistry (IUPAC) system of naming PCBs is extremely laborious to use and has potential for typographical errors with 2, 2', 5, 5'-tetrachlorobiphenyl as an example. Ballschmiter et al. proposed a shorthand system in 1980 which allocates a 'PCB' number for each of the 209 congeners following an ascending numerical order with chlorine substitution and sub indexed via the IUPAC naming system (refer to appendix A [17, 18]). The proposed and widely used PCB naming system of Ballschmiter et al. is known as *'BZ (*Ballschmiter-Zell) numbers' [18].

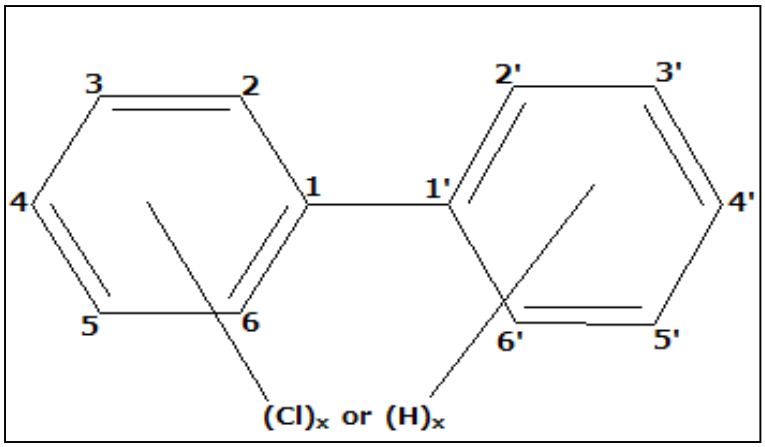


Figure 2.1: Different position (2-6; 2'-6') of chlorine atoms around two bonded phenyl rings (209 congeners)

Commercial PCB mixtures are known by numerous trade names such as Aroclors (USA), Soval (Russia) and Chlorofen (Poland). The commercial mixtures consist of various congeners, for example in Aroclor 1242 the first two digits indicate that the compound contains biphenyls and the last two digits refer to the

weight percentage of chlorine in the mixture [2, 19]. Aroclor 1016 is an exception to the percentage contribution rule of Aroclor numbering and Aroclor nomenclature. Aroclor 1016 consists of 42 % chlorine atoms by weight. Aroclor 1016 has the lowest toxic equivalency factor (TEF) of $1.9x10^{-6}$ of all the Aroclor mixtures, in comparison to a TEF of $2.6X10^{-3}$ for Aroclor 1254 and $5.0X10^{-4}$ for Aroclor 1260 [3, 19].

2.2 PCB toxicity

The toxicity of PCBs to humans and the environment differs vastly when the position of chlorine atoms around the bonded benzene rings are in positions 2-6 and 2'-6' (Figure 2.1). The ability to rotate into a planar configuration with non- and mono-*ortho* substituted chlorine atoms, for example PCB 77 and PCB 126, allow these PCBs to bind/interact with the aryl-hydrocarbon receptor (AhR) [18]. Coplanar PCBs and 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (2, 3, 7, 8 – TCDD) show similar interaction with the Ah receptor, and therefore classifies coplanar PCBs as 'dioxin-like' in their toxicity towards living organisms. Through this similar biological activity of 2, 3, 7, 8 – TCDD and coplanar PCBs, toxic equivalence factors (TEF) can be used to evaluate/determine the toxicity of dioxin-like PCBs when conducting risk assessments of contaminations.

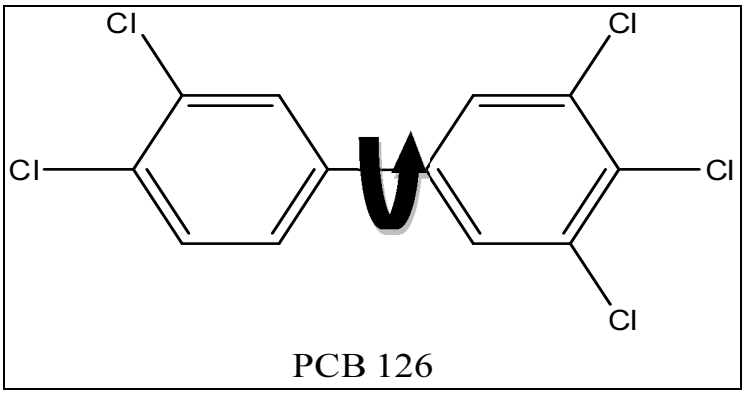


Figure 2.2: PCB 126 rotating into planar configurations (dioxin-like PCB)

The interactions of coplanar PCBs with the Ah receptor induced glucose and insulin resistance in laboratory mice with PCB-induced diabetes as a result [20]. This is just one explanation of the various biological activities of coplanar PCBs through the Ah receptor. Studies support the finding that PCBs are bioaccumulative. When these mice were obese, the negative effects of PCBs were lost, but the same

glucose-impaired homeostasis was observed as in lean mice upon drastic weight loss of the obese mice [20]. PCB 126, PCB 169 and PCB 77 are the most toxic 'dioxin-like' PCBs when evaluating TEF values, with PCB 126 being ten times less toxic than 2, 3, 7, 8 – TCDD (Table 2.1) [21]. Non- and mono-substituted PCBs are normally considered to be the most toxic PCBs due to their interaction with the Ah receptor, but recent studies indicate that non-planar PCBs also show signs of toxicity to humans and the environment [22].

Table 2.1: TEF of dioxin-like PCBs [21]

Type	BZ number	TEF
Non-*ortho*	77	0.0005
	126	0.1
	169	0.01
Mono-*ortho*	114	0.0005
	118	0.0001
	123	0.0001
	156	0.0005
	157	0.0005
Di-*ortho*	170	0.0001
	180	0.00001

Originally it was thought non-coplanar PCBs have little to no biological activity. A dioxin-like PCB can be defined as a PCB with at least four chlorine atoms in any of the six lateral positions (3, 3' 4, 4', 5, 5'), which includes none or one *ortho* chlorine substituents [23]. Table 2.2 indicates that only a few dioxin-like PCBs form part of the major contributors in Aroclor mixtures, clearly showing the importance of toxicity inclusion of 'non-coplanar/non-dioxin'-like PCBs to conduct accurate ecological risk assessment. Recent studies have indicated that di-*ortho* chloro-substituted PCBs have biological activity. Non-planar PCBs

interact with the ryanodine receptor type 1 (RyR1), and this receptor can be seen as a calcium release channel [22].

The interaction of non-coplanar PCBs with RyR1 enhances the release of Ca^{2+}, which leads to damage and cell death (excitotoxcity). Research shows that coplanar PCBs (PCB 77) have no activity towards the RyR1 receptor. Different *ortho*/ para /meta positions of chlorine, hydroxyl and methyl-sulfonyl metabolites substituents have different potencies. Hydroxyl and bulky methyl-sulfonyl substituents in para position showed decreased biological activity when compared to the parent PCB. Hydroxyl substituents found in a para position without any deactivating groups promote higher biological activity than its parent PCB (Figure 2.3) [22].

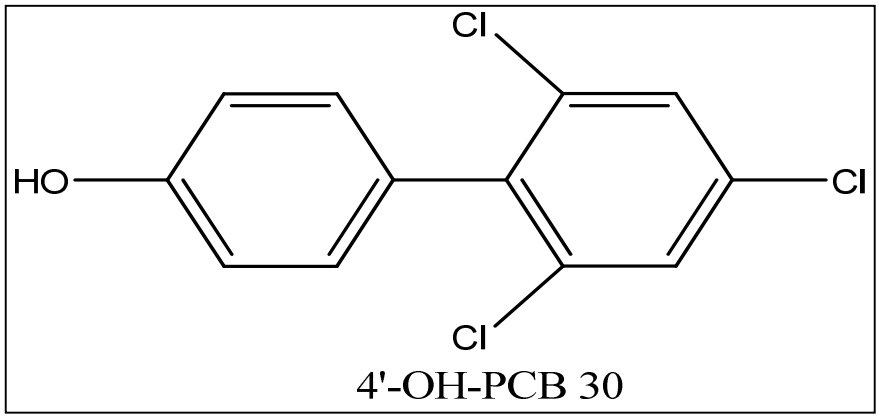


Figure 2.3: Metabolic hydroxylation of PCB 30

2.3 Uses and occurrence in the environment

The manufacture and use of technical Aroclor (PCB) mixtures changed over time:

- Aroclor 1254 and Aroclor 1260 were used prior to the 1950s in the USA.
- 1950s–1960s: Aroclor 1242 was predominantly used and was replaced by Aroclor 1016 in the 1970s.

PCBs were used in two types of systems, namely open and closed systems. PCBs were used in open systems such as paints, fire retardants, plastics and rubbers. Examples of closed systems are transformers, hydraulic systems and capacitors [3]. PCBs were widely used for their thermal insulating and dielectric properties in transformers and capacitors. PCBs are man-made compounds and do not occur naturally. PCB contamination in soil, rivers/dams and sewage drainage systems occur due to manufacturers and users like the Monsanto Corporation polluting a town such as Anniston in the United States of America (USA) [24-27].

The solubility of PCBs in water decreases with the increasing degree of chlorination, PCB 28 (Log $_{O/W}$ 5.55) and PCB 180 (Log $_{O/W}$ 7.29), for example [27, 28]. Log $_{O/W}$ is a coefficient expressed in a logarithmic scale of the solubility of a compound in octanol (non-polar solvent) compared to water (polar solvent). As the Log $_{O/W}$ value increases so does the solubility in non-polar solvents. The heavier and less water-soluble PCBs are more readily found in soil sediment [25, 26]. Technical PCB mixtures like Aroclors consist of a variety of PCB congeners (Table 2.2) with different volatilities and solubilities that are found in air, water and soil [19].

Table 2.2: Composition (%) of PCBs in comparison to the total concentrations in Aroclor formulations [19]

Homologue	Ar-1016	Ar-1221	Ar-1232	Ar-1242	Ar-1248	Ar-1254	Ar-1260	Ar-1262
Major PCBs (%)								
D2CB-8/5	*8.8000*	*13.0000*	*7.9000*	*8.9000*	*0.5200*	*0.1300*	*0.1200*	*0.1600*
T3CB-18	*6.9000*	*0.5900*	*2.8000*	*5.2000*	*2.5000*	*0.1000*	*0.0560*	*0.1600*
T3CB-31	*9.3000*	*0.6000*	*3.5000*	*6.9000*	*5.5000*	*0.1500*	*0.0750*	*0.2000*
T3CB-28	*9.7000*	*0.7700*	*3.5000*	*7.1000*	*5.5000*	*0.1300*	*0.0720*	*0.2200*
T3CB-33/20	*6.7000*	*0.5900*	*2.8000*	*5.0000*	*2.5000*	*0.1000*	*0.0610*	*0.1600*
T4CB-52	*6.0000*	*0.1600*	*2.3000*	*3.5000*	*5.6000*	*4.8000*	*0.3100*	*0.2100*
T4CB-44	*4.6000*	*0.1100*	*1.7000*	*2.8000*	*4.2000*	*1.4000*	*0.0390*	*0.1000*
T4CB-70	*0.9000*	*0.1700*	*2.9000*	*5.2000*	*9.1000*	*3.4000*	*0.0800*	*0.2100*
T4CB-66	*0.5800*	*0.1400*	*2.4000*	*4.5000*	*6.8000*	*1.2000*	*0.1400*	*0.1600*
P5CB-95	*0.3600*	*0.0280*	*0.5400*	*0.6800*	*1.5000*	*6.3000*	*2.1000*	*0.6700*
P5CB-101	*0.0570*	*0.0250*	*0.7400*	*0.8400*	*2.5000*	*9.7000*	*2.8000*	*0.8800*
P5CB-110	*0.0100*	*0.0300*	*0.2000*	*1.0000*	*2.7000*	*12.0000*	*1.5000*	*0.3400*
P5CB-118	*0.0029*	*0.0150*	*0.3800*	*0.5900*	*1.7000*	*6.9000*	*0.5600*	*0.2000*
P5CB-105	*0.0031*	*0.0100*	*0.2300*	*0.4100*	*0.9900*	*2.6000*	*LRL*	*0.0670*
H6CB-149	*LRL*	*0.0026*	*0.1800*	*0.0750*	*0.1300*	*3.6000*	*7.7000*	*5.3000*
H6CB-153	*0.0021*	*0.0024*	*0.1800*	*0.0580*	*0.1800*	*4.6000*	*9.5000*	*6.4000*
H6CB-138	*LRL*	*0.0043*	*0.2300*	*0.0600*	*0.2100*	*7.0000*	*8.3000*	*3.4000*
H7CB-187	*LRL*	*LRL*	*0.0680*	*0.0090*	*0.1200*	*0.5000*	*5.4000*	*11.0000*
H7CB-174	*LRL*	*LRL*	*0.0700*	*0.0073*	*0.1100*	*0.5100*	*4.9000*	*6.5000*
H7CB-180	*LRL*	*LRL*	*0.1300*	*0.0110*	*0.3500*	*0.9500*	*13.0000*	*15.0000*
H7CB-170	*LRL*	*LRL*	*0.0720*	*0.0048*	*0.1100*	*0.5600*	*4.8000*	*2.5000*
O8CB-199	*LRL*	*LRL*	*0.0020*	*0.0030*	*0.0500*	*0.1000*	*1.4000*	*4.9000*
O8CB-203/196	*LRL*	*LRL*	*0.0030*	*0.0049*	*0.0660*	*0.1200*	*2.0000*	*4.9000*
O8CB-194	*LRL*	*LRL*	*0.0030*	*0.0036*	*0.0920*	*0.0920*	*1.7000*	*3.2000*

Table key

LRL= less than reporting limit (<0.0001%)

D2;T3;T4;P5;H6;H7 and O8 = Di;Tri;Tetra;Penta;Hexa;Hepta and Octa

CB = Chlorinated biphenyl

Number (eg. 28) = PCB 28 -Ballschmitter-Zell number (BZ)

The lipophillicity and resistance to degradation of PCBs results in the bioaccumulation of PCBs higher up in the food chain. If humans continuously consume fish that are contaminated with PCBs, the PCB concentration will bioaccumulate (increase) in fatty tissue due to the lipophillicity of PCBs. Transfer of higher chlorinated biphenyls with higher Log $_{O/W}$ occurs more readily in the food chain [29]. A study conducted on a coastal environment in Canada clearly showed the relationships between PCB concentrations in sediment and lower trophic biota [29].

2.4 Indicator PCBs

The use of the six indicator PCBs (PCB 28; PCB 52; PCB 101; PCB 138; PCB 153 and PCB 180) to measure PCB levels in oil, soil, humans, animals and produce is common practice, and PCB 118 is also included in some instances [30-34]. Concentrations of individual indicator PCBs may vary throughout different Aroclor mixtures, such as Aroclor 1260 which consists of more highly chlorinated biphenyls. Physical properties of indicator PCBs also play an important role in their detection in real samples. Heavily chlorinated PCBs (PCBs 138, PCB 153 and PCB 180) are less volatile and are more bio-accumulative and , therefore, most likely to be detected in higher concentrations in soils and biological samples [31, 32, 34].

It is not economically viable for commercial laboratories to analyse all 209 PCB congeners to determine the total concentrations of PCBs in waste oil and soils. Deutsches Institut für Normung or the German Institute for Standardisation (DIN) 12766 describes a method to estimate the total PCB concentration. This method entails the sum concentration of the six indicator PCBs multiplied by a factor 5 to calculate an approximate total PCB concentration present in the sample [35]. In proficiency testing, this method has shown to overestimate the results, but it was still within the acceptable limits [36]. DIN 12766 is extremely useful for commercial laboratories because it only considers indicator PCBs that:

- simplify instrumental conditions;
- eliminate minor PCB contributors;
- simplify chromatographic separation;

- decrease cost of analysis;
- decrease sample turnaround time; and
- have acceptable quality.

The UIS Organic Laboratory uses the value triangle (Figure 1.1) to evaluate a method potential for implementation into routine analysis techniques. The DIN 12766 calculation method is in the middle of the value triangle, which is perfect for implementation in our commercial laboratory.

Chapter 3

Literature review of methods for PCB analysis

3.1 Canadian Environmental Protection Series method for the extraction and clean-up of PCBs from soils and waste oils [37]

3.1.1 Summary

The Canadians' method for the extraction and analysis of PCBs from transformer oil and soil involves multiple sample clean-up procedures with the use of larger quantities of solvent. This method extracts the PCB from soils using a Soxhlet/accelerated solvent extraction (ASE) and a liquid-liquid extraction (LLE) for PCBs in waste oil. Multiple column clean-ups are required to remove potential hydrocarbon interferences as well as instrumental analysis to estimate PCB contamination. The final analysis for the presence of PCBs is determined using GC-MS.

3.1.1.1 Method

3.1.1.1.2 Materials and equipment

Table 3.1: Materials and equipment required for extracting and analysing PCBs in soil and waste oil using the Canadian Environmental Protection series method [37]

Glassware	Materials	Instrumentation
Buchner funnels	Sodium sulphate	Soxhlet/ASE
Separation funnels	Silica	GC-ECD
Glass columns	Basic alumina	GC-FID
Glass EPA vials	DMSO/water (97.5/2.5) v/v	GC- MS
	44% (w/w) Sulphuric acid on silica	
	33% (w/w) of 1M sodium hydroxide on silica	
	10% (w/w) of silver nitrate on silica	

3.1.1.1.3 Liquid-liquid extraction – PCBs partitioning into DMSO

The extraction of PCBs from waste oil is extremely difficult due to the lipophilic nature of PCBs. Numerous techniques make use of the aromaticity of the two phenyl rings in PCBs to selectively extract PCBs from aliphatic matrices using aprotic solvents. Aprotic solvents can be described as solvents without a hydrogen atom attached to an electronegative element of a molecule [38]. Dimethylsulphoxide (DMSO) is an aprotic solvent that is regularly used to selectively extract PCBs from aliphatic matrices, such as transformer oil. An electrostatic complex forms between the polarized sulphur atoms in DMSO with the π-electrons of the bonded phenyl rings in PCBs (Figure 3.1) [39].

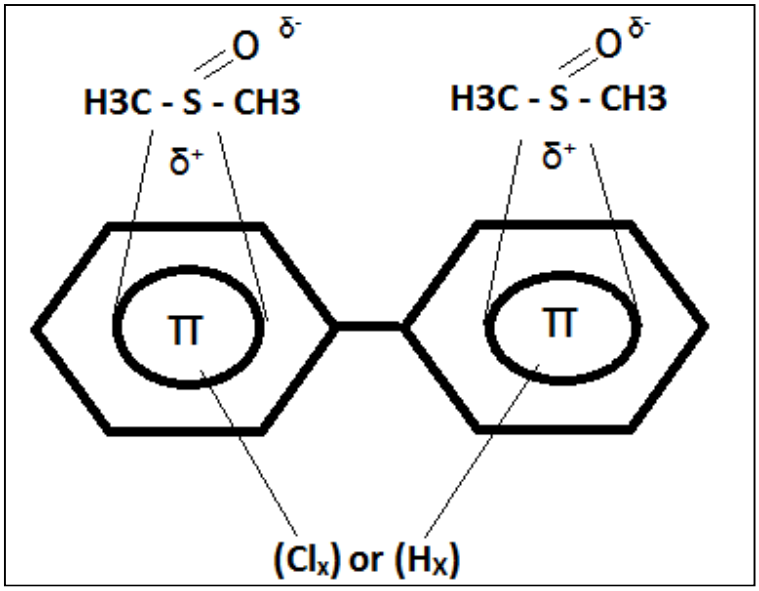


Figure 3.1: Interaction of DMSO with aromatic moiety [39]

3.1.1.1.4 Soil extraction procedure

Soil samples need to be air dried before extraction can proceed. The extraction can either be done with a Soxhlet extraction or faster extraction techniques such as laboratory microwave apparatus and accelerated solvent extraction (ASE). ASE allows for rapid and complete extraction of solid matrices at higher temperatures under high pressure to prevent boiling of solvents used. The extract is dried with sodium sulphate and concentrated to a few millilitres. The extract is now ready for clean-up [37].

3.1.1.1.5 Waste oils extraction procedure

According to the Canadian method (0.5 g) of oil sis diluted with 5 ml of hexane and transferred to a separation funnel. The container is rinsed several times with hexane until the final volume in the separation funnel is 20 ml. Twenty millilitres (20 ml) of concentrated sulphuric acid (H_2SO_4) is added to a separation funnel and shaken vigorously for 2 minutes. Acid treatment of oils oxidizes most Polyaromatic hydrocarbons (PAH) and other organic double bonds, PCBs and aliphatic compounds are not prone to oxidation with acid treatment. The mixture is allowed to separate for 15 minutes and the excess acid is drained. Any remaining acid is removed by washing the extract with deionised water (5 ml) [37].

Seventy five millilitres (75 ml) of DMSO/water (pre-prepared) is added to a separation funnel and shaken vigorously for 2 minutes, after 10 minutes the phases should be separated. This process must be repeated at least twice. The DMSO/water extract must be washed with hexane for 1 minute and left to separate for 10 minutes to remove any residual oil present in the DMSO/water extract. Hexane used to wash the DMSO/water extract also needs to be extracted twice with DMSO and should be combined with the initial DMSO/water extract [37].

Seven hundred and fifty millilitres (750 ml) of deionised is added to the DMSO/water extract and extracted with 100 ml of hexane, followed by 2 x 50 ml with hexane. Sodium sulphate should be used to dry the extract and should be concentrated to a few millilitres. This fraction contains the PCBs and is now ready for clean-up [37].

3.1.1.1.6 Sample clean-up procedures

When working with PCB concentrations in soils higher than 500 mg/kg it will be pointless to spike with surrogate PCBs as there will be a large dilution to reduce the PCB concentration to fit the linear working range limitations. The approximate PCB concentration in the soil will need to be determined using a GC-ECD to apply the appropriate dilution of the PCBs before the additions of surrogate PCBs. The same sample procedure will need to be followed when working with waste oils from transformers and capacitors as PCB concentration could be high when working with these types of matrices [37].

Sample clean-up is required to remove all interferences to reach lowest possible detection limits. An Acid/base/silver nitrate/silica column is the first clean-up step in this method. The column should be

packed in the following order: 10% silver nitrate/silica (1.5 g), silica (1.0 g), 33% 1M sodium hydroxide/silica (2.0 g), 44% sulphuric acid/silica (4.0 g), silica (2.0 g) and sodium sulphate (1.0 g) (Figure 3.2). Condition the column with 30 ml of 3% dichloromethane in hexane (v/v). The sample extract should be introduced onto the column and the sample container should be washed three times with hexane and added to the column. When the third wash has drained, 50 ml of 3% dichloromethane in hexane should be added (v/v). The acid/silica needs to be assessed for saturation through the indication of colour after the 50 ml has drained [37].

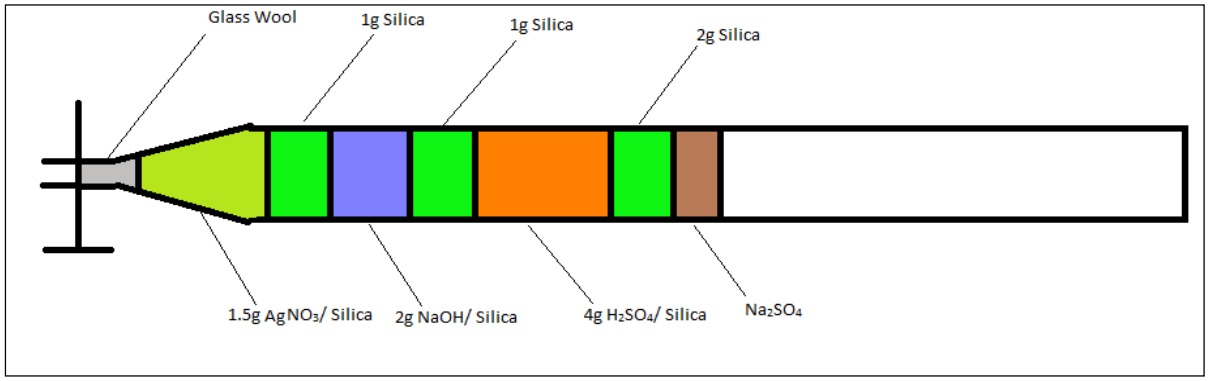


Figure 3.2: Cross section of basic clean-up column [37]

If the acid/silica layer in the column has shown signs of saturation (colour), indicative of PAH concentrated sulphuric acid should be used to wash the sample extract multiple times (four maximum) in a separation funnel until no colour is present in the acid layer. The extract and acid should be separated and washed with 1M sodium hydroxide and a final wash with deionised water. The remaining aqueous liquid should be removed with sodium sulphate (Na_2SO_4) [37].

If the silver nitrate/silica layer shows signs of saturation, indicative of sulphur compounds an additional column should be run. The concentrated extract must run through a prewashed 10% silver nitrate/silica column (2.5 g) with 30 ml of 3% dichloromethane in hexane and then concentrated [37].

The method recommends that the extract be assessed for hydrocarbon presence through analysis using GC-FID. If a hydrocarbon hump is present on the chromatogram (Figure 3.3) an alumina column is needed to remove this hydrocarbon interference. A 2.5 g packed alumina column is required with the sample added after the column has been prewashed (2 x 15 ml). PCBs are eluted with 20 ml of 5% dichloromethane in hexane. The sample is now ready for GC-MS analysis [37].

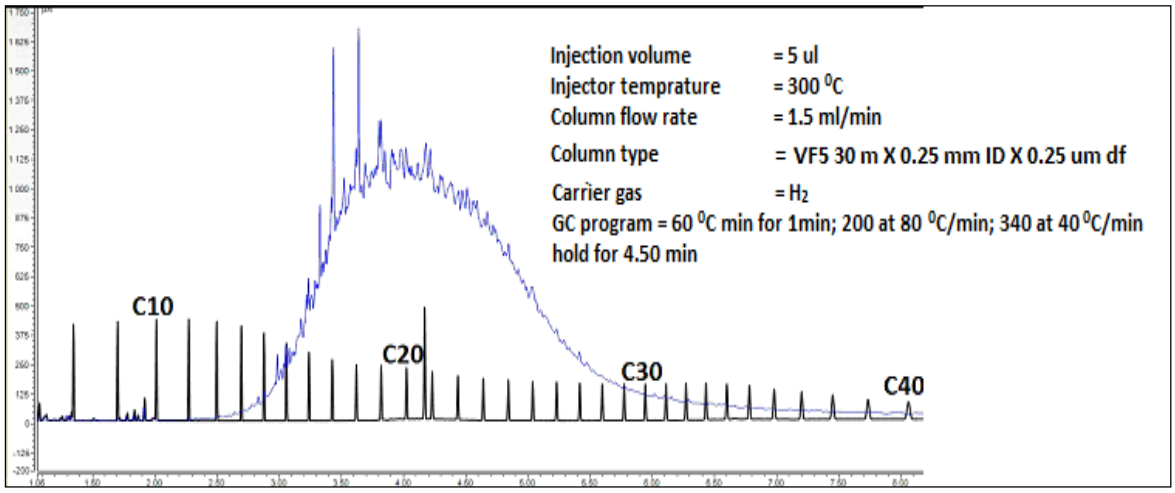


Figure 3.3: Chromatogram of transformer oil (hydrocarbon hump) and alkanes C8-C40 on GC-FID. Chromatogram generated from own work with UIS Organic equipment

3.1.1.1.7 Flow diagram of Canadian method extraction procedure

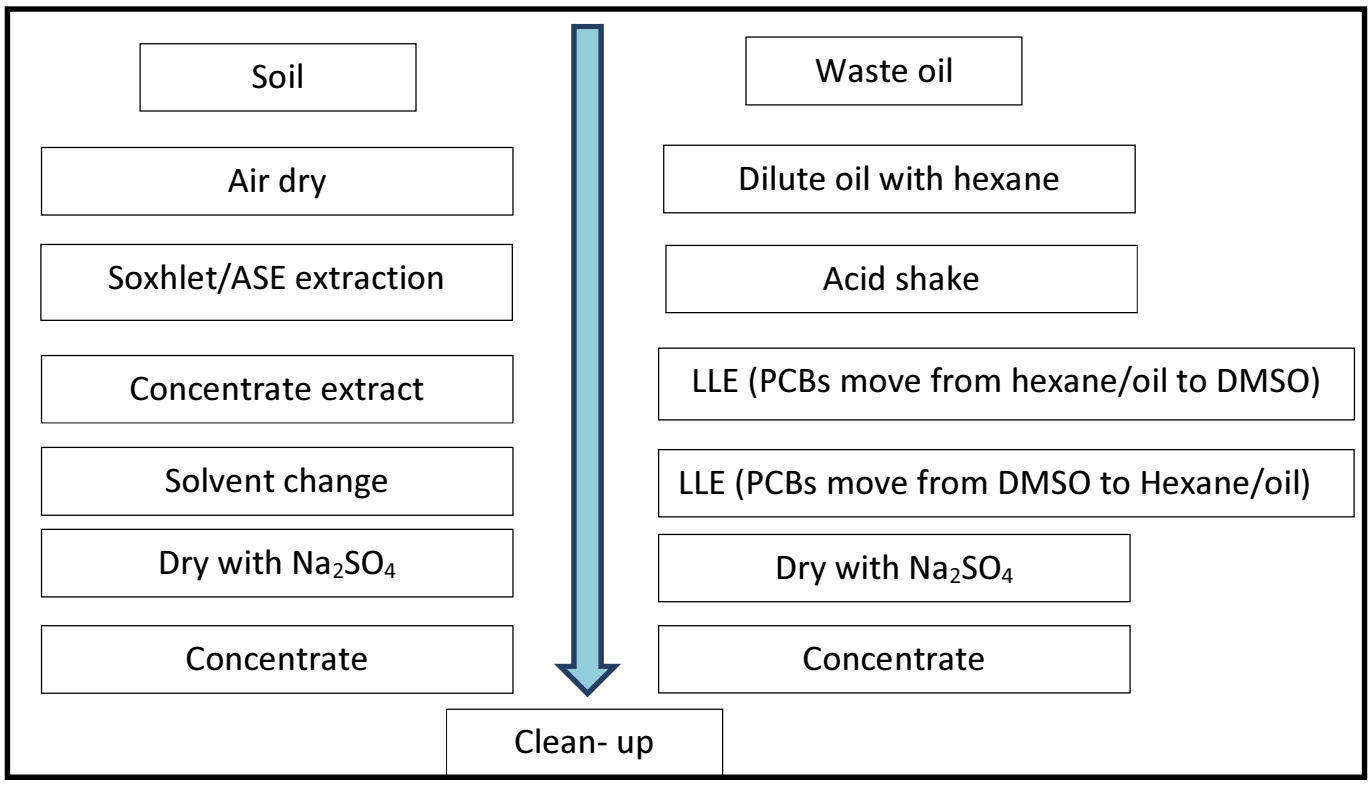


Figure 3.4: Flow diagram of extraction method for soils and waste oils [37]

3.1.2 Advantages of the method

The extensive clean-up procedure ensures limited matrix interference with larger signal to noise ratios of potential PCBs in the sample. Concentration of the solvent enables the quantitative determination of PCBs in waste oils at low concentrations. Cleaner chromatograms also provide better confidence in qualitative determinations and lowers false negative identification of PCBs. The injection of cleaner sample matrices:

- lowers cost and time spent on instrument maintenance;
- involves less routine replacement of injector liners that can severely influence the systems performance; and
- Involves less gas chromatographic column maintenance.

3.1.3 Disadvantages of the method

The Canadian method requires an array of different glassware as well as preparation of numerous materials used in the clean-up of samples for quantitative determination (see Table 3.1). A large volume of solvent is used for the extraction and elution of PCBs from the hydrocarbon matrices. Waste generated by the Canadian method is immense and will increase the cost of analysis as waste disposal can become costly for a commercial laboratory. Samples may require up to four clean-up procedures, which is not ideal for a commercial method due to faster and more cost-effective requirements from commercial laboratories. In a best-case scenario, a sample will only need one clean-up procedure, but will require three instrumental determinations:

- Determine approximate PCB concentration with GC-ECD
- Determine if hydrocarbons are present with GC-FID
- Quantitative determination of PCBs with GC-MS

Purchasing three instruments for a single test method is not cost-effective for a commercial laboratory and these instruments require some level of expertise to maintain and operate, which is not commercially viable.

Every sample clean-up requires a time-consuming concentration step and the continuous transfer of samples could result in the loss of PCBs if great care is not taken. This method also generates large volumes of data that need to be stored and transferred from systems to servers, which in itself is a tedious exercise. Every system also requires quality control and control charts that need to be completed and maintained.

When taking all the above mentioned factors into account, this method is not fit for a commercial laboratory as the test method provides:

- a quality result; 
- low cost of analysis; and 
- short turnaround times. 

3.2 Supelco ®- PCB extraction from waste oils using sulphoxide-bonded solid-phase extraction (SPE) [40-42]

3.2.1 Summary

Supelco® developed a method for the extraction of PCBs using solid-phase extraction (SPE). The sample extract is introduced onto the SPE with aliphatic hydrocarbons eluting faster than the PCBs, which are retarded by the sulphoxide SPE phase. The aliphatic-containing fraction is discarded and the second PCB fraction is concentrated and analysed using gas chromatography high-resolution mass spectrometry (GC-HRMS) or gas chromatography quadruple mass spectrometry (GC-QMS).

3.2.1.1 Method

3.2.1.1.1 Materials and equipment

Table 3.2: Materials and equipment required for PCB extraction from soils and waste oils using sulphoxide-bonded SPE phase [40]

Glassware	Materials	Instrumentation
EPA glass vials	Supelclean sulphoxide phase (3 g/ 6 g)	ASE
Glass columns	Acetone	GC-HRMS/GC-QMS
Soxhlet	Hexane	

3.2.1.1.2 Extraction of PCBs from oil-contaminated soil and waste oils

This method does not describe the extraction of PCBs from oil-contaminated soils, but only describes oils. Soil samples contaminated with oil can be extracted using an ASE or Soxhlet extraction. Supelclean® prepacked SPE (6 g) commercial product or your own packed Supelclean® column can be used for separation of PCBs from the hydrocarbon matrix. The SPE phase should be washed with 20 ml of acetone and conditioned with 40 ml of hexane in a column. The oil sample is diluted 1:1 (v/v) to a final volume of 250 µl or one can use the soil sample extract in hexane. Sample/extract is introduced onto the cartridge with the walls of the cartridge washed (2 X 0.5 ml) using hexane. When the sample has cleared the upper frit of the SPE cartridge, the oil is eluted with 11 to 12 ml of hexane. As the hexane of the oil elution step

clears the upper frit of the SPE cartridge, the retarded PCBs are be eluted with 25 ml of hexane and concentrated for analysis using GC-HRMS or GC-QMS [40-42].

3.2.1.1.3 Flow diagram of Supelclean® SPE extraction method

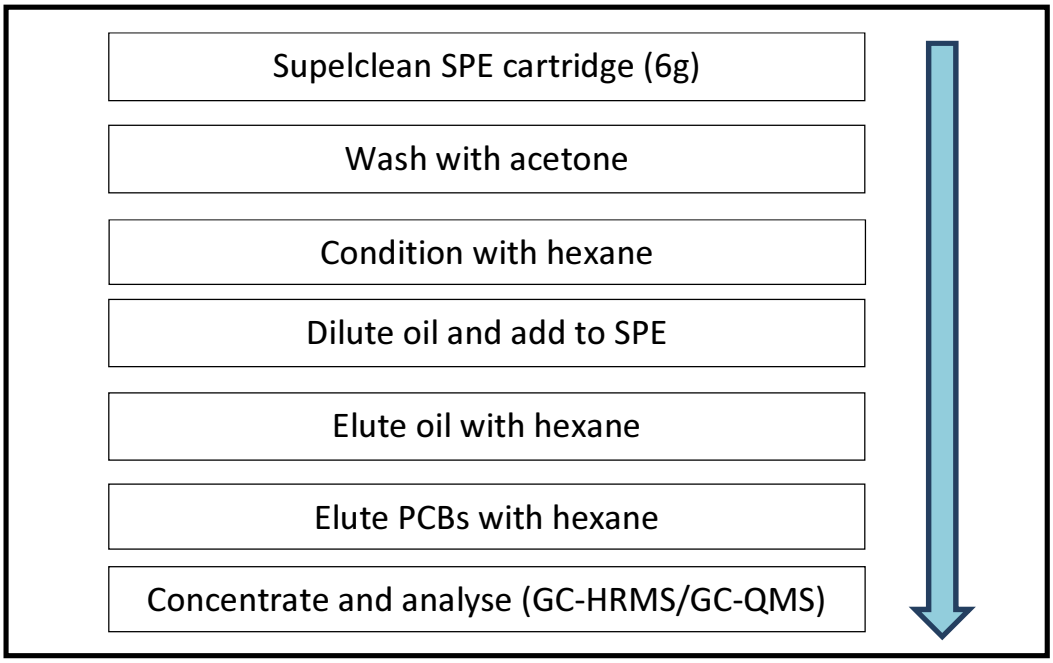


Figure 3.5: Flow diagram of SPE Supelclean ®SPE extraction method [42]

3.2.2 Advantages of this method

The separation of PCBs and waste oils using SPE Supelclean® is conducted with a simple five- to six-step extraction procedure (Diagram 3.2). Supelclean® uses fewer solvents than the Canadian method for example, and multiple samples can be separated simultaneously, which in turn will lower the cost per analysis and decrease turnaround times. The interaction of PCBs with the SPE phase results in the faster elution of waste oils and separates most hydrocarbon co-elution from the PCBs (Figure 3.6) in one procedure. The Supelclean SPE method has a low detection limit of 0.33 ng/g on GC-high-resolution mass spectrometry and 6.34 ng/g on GC- quadruple mass analyser (average DL of 5 of the Indicator PCBs) [40].

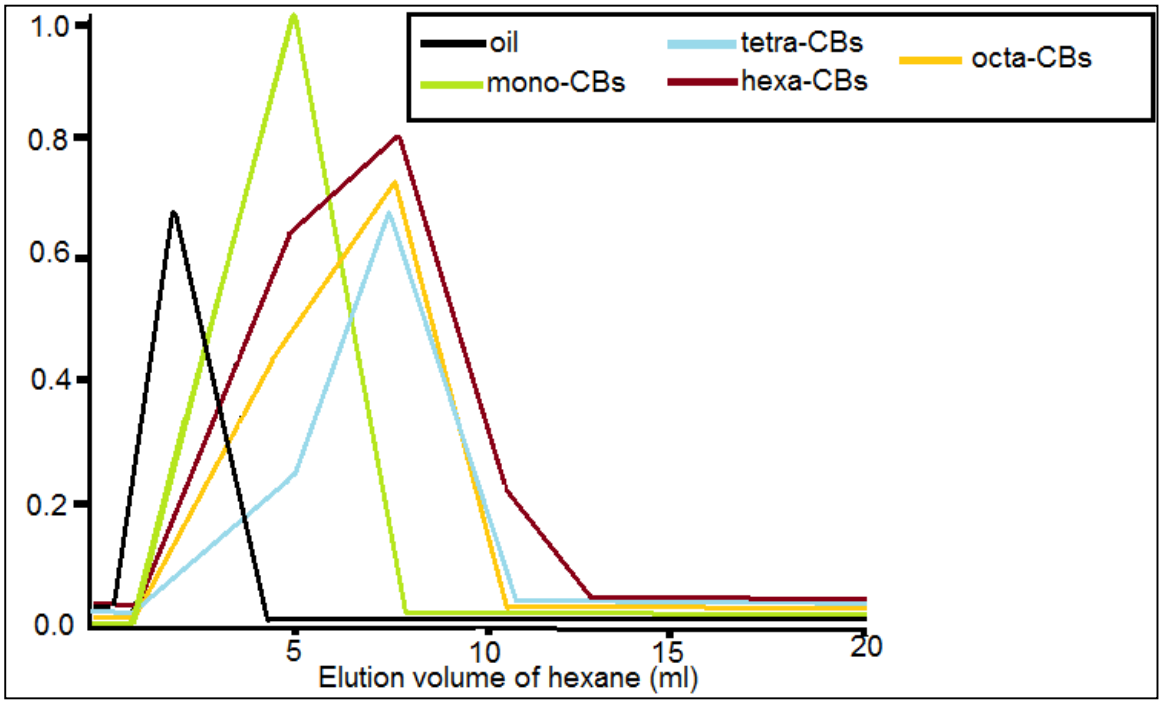


Figure 3.6: Elution volumes and recoveries of PCBs and oil of Supelclean® SPE separation method. Sketch based on information supplied by Supelco® presentation and application note [41, 42]. *Note the incomplete separation of PCBs and oil at the 5 ml cut off. Monochlorinated biphenyls (Mono-CBs) are not important when determining the total PCB concentration using the DIN12776 method (chapter 5).*

3.2.3 Disadvantages of this method

When using prepacked SPE cartridges, it can become an expensive separation procedure, but the bulk SPE phase is available and this lowers the cost significantly. The introduction of sample onto the SPE and repeatable sample separation requires some level of skill and experience, which will influence the cost as well as turnaround time per sample. Figure3.1 shows the elution of oil and PCBs using the Supelclean® SPE method. There is a level of co-elution that will introduce aliphatic hydrocarbons into the GC-MS system and will influence the detection limit of PCBs and the potential use of a less expensive detector such as GC-ECD.

This method requires an initial wash with acetone to remove moisture from the SPE, a necessary but tedious step. Moisture decreases the retention of PCBs in the SPE and it is ,therefore, an essential step, followed by a hexane-conditioning step. Each sample requires a concentration step, which is also time consuming and not ideal for commercial laboratories. The Supelclean® SPE method is simple but time consuming and has a high cost per analysis.

This method is not fit for a commercial laboratory as the test method provides a:

- quality result; 
- low cost of analysis; and 
- short turnaround times. 

3.3 Liquid-liquid extraction of PCBs in waste oils coupled with headspace solid-phase microextraction and gas chromatography atomic emission detection (AED) [43]

3.3.1 Summary

Sample/extract undergoes an acid treatment followed by an LLE with DMSO to extract the PCBs from the aliphatic matrix. The PCB-containing DMSO is washed with hexane, diluted with brine (salt water) and analysed with headspace solid-phase microextraction (SPME) and gas chromatography atomic emission detection (GC-AED) or GC-MS.

3.3.1.1 Method

3.3.1.1.1 Materials and equipment

Table 3.3: Materials and equipment required for extracting and analysing PCBs from soils and waste oils using liquid-liquid extraction (LLE) coupled with headspace SPME GC-AED

Glassware	Materials	Instrumentation
Separation funnels	Water (300 mg NaCl per ml)	ASE
Soxhlet apparatus	Hexane	GC-AED/MS
	DMSO	
	65 µm Polydimethylsiloxane-divinylbenzene (PDMS-DVB) SPME	

3.3.1.1.2 Soil and waste oil extraction procedure

This method does not describe the extraction of PCBs from soils contaminated with waste oils, but this can be done using accelerated solvent extraction (ASE) or Soxhlet extraction, as described and used in the Canadian method [37].

Waste oil must be diluted with 6 ml of hexane or alternatively the soil sample extract can be used. The diluted waste oil/ extract must be treated with concentrated H_2SO_4 for 10 minutes and the two phases

should be separated (acid treatment oxidises interferences). PCBs are extracted from the hexane layer with 7 ml DMSO using an LLE. Any hydrocarbons present in the DMSO extract are removed by washing with hexane. The DMSO extract is diluted with H_2O containing 300 mg of NaCl per millilitre (1:4 DMSO: H_2O). PCBs are headspace extracted from the diluted sample with a 65 µm PDMS-DVB fibre for 50 min at 100 °C followed by desorption in GC inlet and AED detection [43].

3.3.2 Advantages of this method

This method only uses two clean-up procedures to limit interferences that are relatively simple without any complicated steps and can easily be followed by laboratory technicians. No specialised phases, glassware or solvents need to be purchased to introduce this method in a commercial laboratory. This method does not use any back extraction procedures for liquid injection; PCBs are immediately extracted with headspace SPME from the DMSO extract. The SPME eliminates time-consuming concentration of samples on a rotary evaporator or turbo evaporator, which saves a significant amount of time and will decrease turnaround times, which is ideal for commercial laboratories. This method uses headspace SPME which reduces interferences in terms of non-volatile compounds. The handling of sample extracts has also been reduced when referring to the abovementioned methods, and will lead to improved repeatability as well as long-term reproducibility.

Using SPME to extract PCBs directly from the DMSO with an automated sampling system makes it attractive for use in commercial laboratories.

3.3.3 Disadvantages of this method

The clean-up procedures employed remove most interference from the sample matrix, but the high detection limits prove that there are still interferences present (detection limit of 0.5 to 1.0 µg/g). Interferences are further limited by using headspace SPME, but sacrificing detection limit due to the low volatility of PCBs. The low volatility of PCBs as well as using headspace SPME extraction increase extraction time to 50 minutes per sample before any gas chromatographic analysis has started. The time saved on the extraction and clean-up steps is wasted on instrument time, which severely influences sample

throughput, which is one of the most import factors that are considered when a method is evaluated for commercial laboratory implementation.This method is not fit for a commercial laboratory as the test method provides a:

- quality result; 
- cost of analysis; and 
- short turnaround time. 

3.4 Conclusion

Each of the abovementioned methods has advantages and disadvantages in the analysis of PCBs present in waste oil and oil-contaminated soil, and some methods are better designed for commercial purposes than others. Three factors are important for the introduction of a new method into a commercial laboratory:

1. quality;
2. cost; and
3. turnaround time.

The required detection limits for total PCBs in waste oil and soil are relatively high, from 0.61 mg/kg (soil) to 50 mg/kg (waste oils), and therefore detection limits can be sacrificed for increased speed and lower cost [6, 9]. The detection limit requirements are the biggest drivers governing method selection. Detection limits will determine the level of sample clean-up required and will in turn influence the speed of analysis and operator involvement that determines the cost per analysis. Commercial laboratories are continuously under pressure to lower the cost, increase the speed of analysis as well as sample throughput.

All of the above mentioned methods can be used to determine PCBs in waste oils and soils, but all of them have factors that make them unattractive for introduction into commercial laboratories.

We were independently investigating a similar strategy that is described in method 3 [43]. We decided to modify the 3rd method to overcome some of its deficiencies described in chapters 5 and 6:

- is the acid wash necessary ;
- is the hexane wash necessary ;
- is 65 µm PDMS-DVB the only SPME fiber that can be used;
- can the method be simplified ;
- can the method be modified for commercial application;

Chapter 4
Solid-phase microextraction (SPME)

4.1 Design of SPME

SPME was developed by Janusz Pawliszyn to save time on sample preparation and extraction through a solventless liquid extraction [44]. SPME is used commercially in automated samplers that are capable to heat and agitate samples for optimal extraction of analytes. SPME fibre assemblies consist of a 1- 2cm phase, polydimethylsiloxane (PDMS) for example, that house the fibre/phase in a hollow syringe (Figure 4.1). This assembly is extremely effective for the purpose it was designed for.

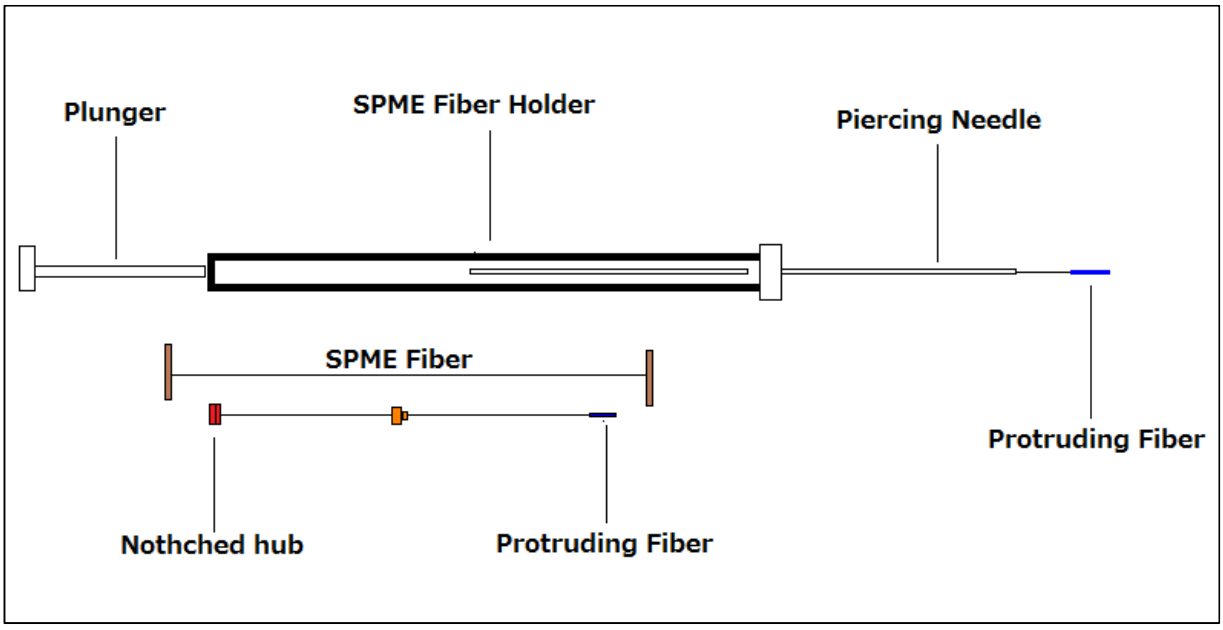


Figure 4.1: Commercial SPME device and fibre assembly from Supelco® [45]. *Sketch to illustrate a SPME fiber housed in a commercial holder that would be used in an automated sampler. The SPME holder is essential due to the fragility of the SPME fiber.*

4.2 SPME theory and optimizations

Analytes adsorb/absorb onto the SPME phase, this adsorption/absorption can occur either by immersing the fibre into the sample or through headspace sampling [46, 47]. Volatility of analytes and detection limits play a major role in selecting a type of extraction and SPME fibre phase. The fibre is then thermally desorbed when placed in a GC injector [48].

The amount of analyte extracted onto the SPME can be expressed by equation (4.1) and (4.2):

$$n = \frac{K_{fs} V_f C_0 V_s}{K_{fs} V_f + V_s} \quad (4.1)$$

Where,

$$K_{fs} = C_f^{\infty} / C_s^{\infty} \quad (4.2)$$

n= moles of analyte adsorbed by the SPME phase

C_0= initial concentration of the analyte in the sample

K_{fs}= partition coefficient for analyte between SPME phase and sample

V_f = volume of coating

V_s= volume of sample

C_f= equilibrium concentration of analyte in the SPME phase

C_s= equilibrium concentration of analyte in sample matrix

SPME fibres are designed to extract organic compounds and, therefore, the C_f will be large when sampling from an aqueous solution, this is directly proportional to the partitioning coefficient K_{fs}. The large K_{fs} value results in good sensitivity as well as high level of concentration for low detection limits [48].

Equation 4.1 can further be simplified when working with a large sample volume.

$$n = K_{fs} V_f C_0 \qquad (4.3)$$

The simplification of the equation allows the use of SPME for field applications. Samples can now be concentrated onto fibres in remote areas where access is limited and will save money on sample transport costs [48]. SPME extraction optimization is essential for fully utilizing the advantages that SPME offer above other extraction techniques such as LLE and SPE.

The following factors have an impact on extraction efficiency of analytes:

- extraction temperature;
- ionic strength of aqueous sample matrix;
- pH;
- agitation ;
- derivatising of analytes; and
- fiber selection.

At equilibrium (fibre and analyte) the extraction process using SPME is complete but long equilibration times might not be commercially viable [49]. Internal standards can be used to compensate for incomplete, kinetically limited extractions and widely used at UIS Organic laboratory. Internal standards can only be used for quantification of analytes if their chemical and physical properties are similar to those of the analytes.

Increasing the extraction temperature can increase the non-equilibrium extraction efficiency of less volatile compounds like polyaromatic hydrocarbons (PAH), figure 4.2, that have long equilibration times [50]. Increasing the extraction temperature does increase the rate transfer of analyte to the fibre but the extraction process is exothermic. Therefore at equilibrium and if the extraction was done at high temperature less analyte would be extracted by the SPME fibre [49]. The reduction in extraction efficiency cannot only be attributed to exothermic processes. The higher extraction conditions result in atmospheric

release of analytes and unwanted desorption of analytes, figure 4.3 illustrates more dramatic loss of volatile analytes at increased extraction temperatures. Commercial laboratories cannot afford to work at equilibrium due to fast turnaround demands; therefore increased SPME extraction temperature is essential for less volatile analytes.

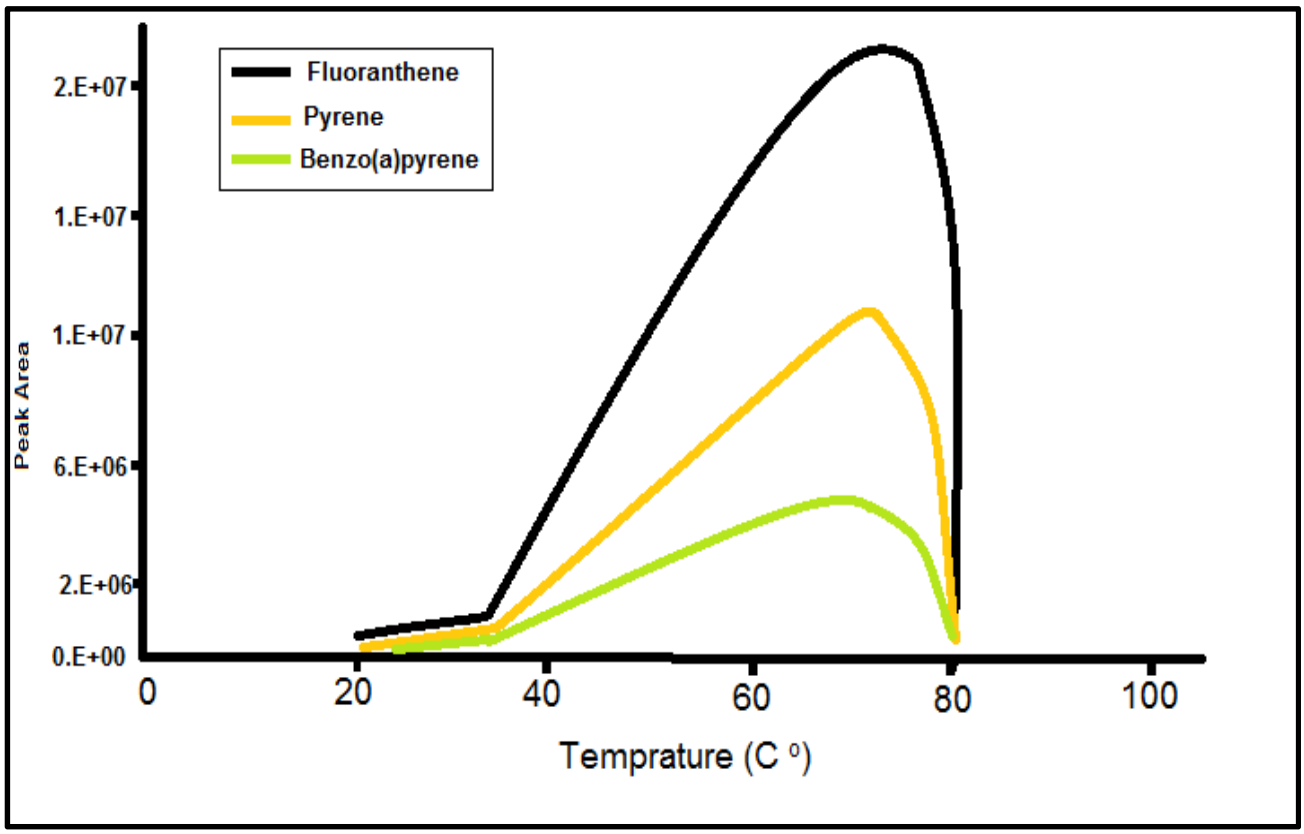
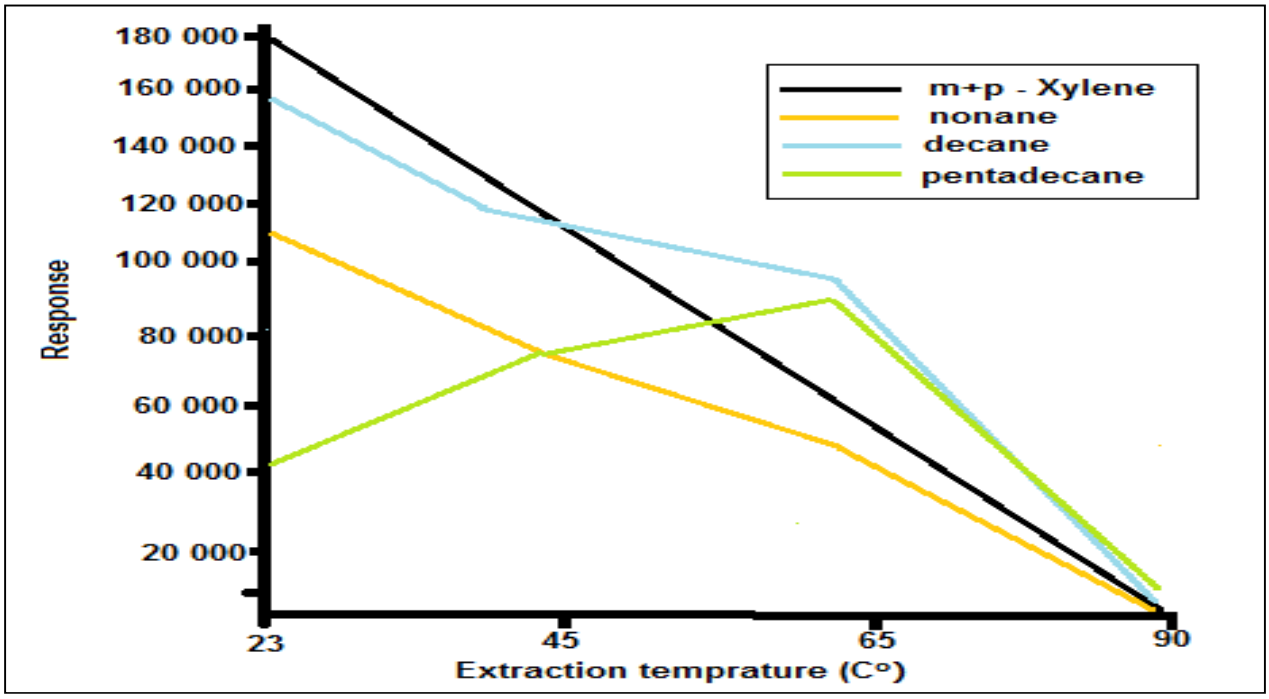


Figure 4.2: Graph based on information from literature review [50].Effect of temperature on extraction efficiency of low volatile compounds. *Note the dramatic temperature decrease at after 70˚C which is attributed to exothermic SPME extraction process, desorption of analytes and atmospheric losses upon sample vial piercing*



Graph 4.2: Graph based on information obtained in literature review. Extraction of selected petrochemical analytes at increased temperature [47]. *After 45 min extraction time the temperature was gradually increased until 90˚C. A gradual decrease in extraction efficiency was observed for m+p-Xyles until decane (volatile compounds). An increase for less volatile pentadecane was observed until 65˚C followed by gradual decrease until 90˚C. The rapid decline of extraction efficiency can be attributed to the volatility of the compounds, exothermic and atmospheric processes.*

The addition of salts to the sample matrix can also increase extraction efficiency. Salts decrease the solubility of dissolved organic analytes in water and, therefore increase the SPME extraction efficiency (K_{fs} increases), known as the "salting out" effect [49]. As the solubility of analytes decrease the effect of increased ionic strength of the aqueous sample also diminishes [51].

Extraction efficiencies of some compounds can also be enhanced by adjusting the pH of the solution/sample. SPME can only extract neutral analytes and therefore acidic compounds will have higher extraction efficiencies at lower pH and inversely for compounds of a basic nature [49, 53].

Derivatising agents can also be used to enhance the extraction efficiency of analytes and also change physical properties like volatility for example to allow headspace analysis that will ensure less matrix interference as opposed to direct immersion [49].

Derivatising agents can increase interferences and lower extraction efficiency depending on the method of derivatising:

- derivitising before SPME extraction;
- during SPME extraction ;
- post SPME extraction ;

Derivatising agents could also contribute to better chromatography of specific analytes [49].

Fibre selection plays a vital role in extraction efficiency of analytes. Commercially available SPME fibres extract analytes through absorption and adsorption. Absorption through SPME is when the analytes partition in liquid like SPME phase and adsorption is where analytes are physisorbed or chemisorbed onto the surface of the phase. Adsorption in micro-and meso- pores of high surface area materials can be complicated by preferential adsorption of non-analyte molecules leading to a vastly reduced capacity as well as sensitivity towards certain analytes [52].

The manufacturer of SPME fibres states that decreasing fibre thickness increases the extraction efficiency of heavier less volatile compounds. This increase in response is due to the faster migration into thinner SPME phases [52]. This explanation is questionable, thicker fibres will only increase the retention of analtyes at a given temperature. According to equation 4.3 the possible reason why this trend is observed is due to incomplete desorption at maximum temperature.

Fibre selection is one of the most important factors when optimizing methods for commercial application. Commercial laboratories cannot afford to stop sample sequences to change fibres, therefore, selecting an all-round fibre that can be used for multiple applications is essential. Recently more expensive automated sampling systems are able to change fibres automatically increasing the flexibility of SPME and enhance its status as an alternative for LLE and SPE.

4.3 Application

SPME has been applied in various fields for the quantitative and qualitative identification of organic contaminants in aqueous, biological and solid matrices. Contaminants include PAHs, BTEXMN, PCBs, pesticides, drugs, explosives and detection of accelerants in arson investigations [49]. Contaminants in aqueous medium using submerged and headspace techniques are widely documented as well as headspace analysis of solids/soil for volatile compounds. The analysis of heavier less volatile compounds involves the addition of a pre-extraction of the solid sample with a SPME "friendly" solvent like methanol or DMSO [43].

The flexibility of SPME is highlighted in the detection of licit and illicit drugs in human hair samples. This analytical analysis technique only involves one preparation step followed by headspace analysis [53]. Almost all analytical analysis techniques involving SPME are faster, easier and obtain the same or lower detection limits (Table 4.1) than conventional liquid injection techniques [49]. Lower detection limits are obtained through complete injection of all extracted analytes through splitless injection.

The solventless extraction of analytes from aqueous samples and limited use of solvent in solid-sample extraction are some of the biggest advantages of SPME. SPME decreases turnaround times by lowering sample preparation time, lowers cost per analysis due to solventless preparation and will provide a quality result if proper validation and optimisations are done. SPME is fit for use in a commercial laboratory.

Table 4.1: Summary for SPME and EPA detection limits of various classes of analytes [49]

Analyte	SPME limit of detection (LOD) (mg/l)	EPA limit of detection (mg/l)
BTEX	0.001-0.015	0.030-0.090
PAH	0.001-0.020	0.040
PCB	0.003	0.060-0.100

Chapter 5

Experimental

5.1 Chemicals, standards and materials

Materials used in all experiments are tabulated in table 5.2. PCB standard in isooctane (10 µg/ml) was supplied by Sigma Aldrich© (Riedel-de Häen) containing a mixture of 7 PCBs: PCB 28 (2,4,4'-trichlorobiphenyl);PCB 52 (2,2',5,5'-tetrachlorobiphenyl);PCB 101 (2,2'4,5,5'-pentachlorobiphenyl); PCB 138 (2,2',3,4,4',5'-Hexachlorobipehnyl) ; PCB 153 (2,2'4,4',5,5'-hexachlorobiphenyl) ; PCB 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl); PCB 209 (decachlorobiphenyl).

The PCB internal standard used was supplied by Wellington Laboratories (WP-ISS) in nonane/toluene containing four $^{13}C_{12}$-PCB congeners: 70L (2,3',4',5-tetrachloro[$^{13}C_{12}$]biphenyl); 111L (2,3,3',5,5'-pentachloro[$^{13}C_{12}$]biphenyl); 138L (2,2',3,4,4',5'-hexachloro[$^{13}C_{12}$]biphenyl); 170L (2,2',3,3',4,4',5-heptachloro[$^{13}C_{12}$]biphenyl). Dimethylsulfoxide (DMSO) was supplied by Labscan (Poland) 99, 5%; hexane (UV) by Honeywell (Burdick & Jackson USA) and sodium chloride (NaCl) was supplied by Merck (Darmstadt, Germany). The transformer oil used in all experimentation was obtained from previous work conducted for an environmental consultant, which was collected from transformers in use. Oils also used in experimentation: Varian vacuum-lubricating pump oil (Bruker, USA), motor oil (own vehicle), olive oil (Woolworths, RSA) and salmon oil (Vital Health Foods, RSA).

Table 5.1: Materials used to extract PCBs from soils and waste oils for the developed method

Materials
40 ml US EPA vials with PTFE lined septa
GlassCo 50 ml separation funnels
Eppendorf pipette (1 ml & 5 ml)
Hamilton micro-syringes (10 µl, 100 µl, 1000 µl)

5.2 Instrumentation

5.2.1

Chromatographic separations were achieved on a Varian 450GC with a 1079 programmable temperature vaporisation (PTV) injector using a VF5 (5% phenyl 95% polydimethylsiloxane) 30 m x 0.25 mm (0.25 d_f) column by Agilent Technologies with helium as carrier. Mass spectral data was collected by Varian 300msTQ mass spectrometer with Argon (Ar) as collision gas for MS/MS analysis. Automation was done with a Combi-Pal auto sampler (CTC Analytics/Leap Technologies) equipped with a heating/agitation attachment as well as a fibre bake-out station. Solid-phase microextraction (SPME) fibres from Supelco® were used to extract PCBs from the aqueous medium, followed by desorption in the injector port (290˚C) for 10 minutes and separated by the following Varian 450GC programme: column temperature started at 40˚C and held for 2 minutes; heated to 130˚C at 7˚C/min; heated to 200 at 5˚C/min ; heated to 260˚C at 6˚C/min and heated to 290˚C at 90˚C/min and held for 5 minutes. The SPME fibre is conditioned for 10 min at 250˚C before continuing with “prep-ahead” for the next injection. Prep-ahead is a function of the autosampler which is useful and essential for commercial laboratories. The autosampler will continue and extract the next sample while the previous sample is being separated and analysed by the GC-MS.

5.2.2

Chromatographic separations were also achieved on a Varian CP 3800 with a 1077 injector using a VF5 30 x 0.25 mm (0.25 d_f) column by Agilent Technologies and VF1 (100% polydimethylsiloxane) 15 m X 0.25 mm (0.25 d_f) with nitrogen as carrier for GC-ECD (electron capture detector) detection. Automation was done with Combi- Pal auto sampler (CTC Analytics/Leap technologies) equipped with a heating/agitation attachment as well as a fibre bake-out station. Solid-phase microextraction (SPME) fibres from Supelco® were used to extract PCBs from the aqueous medium followed by desorption in injector port (290˚C) for 10 minutes and separated by the following Varian CP 3800 GC program: column temperature started at 50˚C and heated to 300˚C at 10˚C/min.

5.2.3

Soil samples were extracted using a Dionex ASE 2000 accelerated solvent extractor (ASE). Hexane was used as solvent for extraction with the following programming: pre-heat for 1 minute; heat for 5 minutes; flush solvent for 60 seconds; purge for 90 seconds (two cycles); pressure of 2000 psi and heating of 100˚C.

5.3 Column selection

Column selection is extremely important when working with complex analytes such as PCBs and commercial PCB mixtures. Due to similar chemical and physical properties some PCBs will co-elute when using certain columns. In this case, we used a 5% phenyl 95% polydimethylsiloxane column (column phase most commonly used at UIS Organic laboratory). This type of column is an excellent all-round column that can be used for numerous chromatographic separations, but it has limitations when separating PCB congeners.

Commercial Aroclors for example, do not consist of all possible 209 congeners and only have selected PCBs as major contributors to the total PCB concentration in commercial mixtures (Table 2.2). PCB 52 normally has no co-eluting PCBs, but PCBs 84 and 90 co-elute with PCB 101. PCB 163 and PCB 158 co-elute with PCB 138 [6, 24 and 37] on 5 % phenyl phases. These co-elutions do not have any major contributions to the total commercial PCB mixture and, therefore, their concentrations are negligible.

However, PCB 28 and PCB 31 as well as PCB 153 and PCB 105 co-elute and they all form part of the major contributors of Aroclors (Table 2.2) [6, 24 and 37]. This is a major limitation when using a 5% phenyl 95% polydimethylsiloxane column set for PCB separation using gas chromatography. Co-elutions will, therefore, severely affect the concentration per PCB as well as the total PCB content when using DIN 12766 to calculate the total PCB concentration. This overestimation can be seen in Table 5.2 where a BP-5 type capillary column (5% phenyl 95% polydimethylsiloxane) was used for gas chromatographic separation. PCB 28 concentration is 50 % higher than the certified concentration and PCB 31 accounts for this overestimation (Table 5.2 & 5.3). The same can be seen for PCB 153, which was overestimated by approximately 40 %, which was attributed to the presence of PCB 105 (co-elution).

Table 5.2: Indicator PCB concentrations in certified Aroclor 1254 [43]

BZ number	Found (mg/kg) concentration	Certified concentration (mg/kg)
28	1.6 ± 0.2	0.8 ± 0.07
52	33 ± 3	31.4 ± 1.8
101	56 ± 5	57.2 ± 1.9
153	66 ± 6	39.0 ± 1.7

Table 5.3: Percentage contribution to co-elution concentration in commercial Aroclor mixtures [17]

Aroclor	1016 (%)	1221 (%)	1232 (%)	1242 (%)	1248 (%)	1254 (%)	1260 (%)
PCB 28	49	44	50	49	50	54	48
PCB 31	51	56	50	51	50	46	52
PCB 153	40	71	44	12	15	64	99
PCB 105	60	29	56	88	85	36	1

An alternative column set that can be considered is the Rxi-XLB (low polarity phase), 30 m X 0.25 mm IDX 0.25 µm d_f (Restek) column which is able to prevent co-elutions between PCBs mentioned in Table 5.3 as well as shorten sample run time dramatically and by doing so eliminating the overestimation of PCB concentrations. The GC programme is as follows: 120 ˚C for 1 minute; increase temperature to 200 ˚C at 30 ˚C/min to 320 ˚C at 6 ˚C/min with a He as carrier gas at 1.38 ml/min (Chromatogram 5.1).

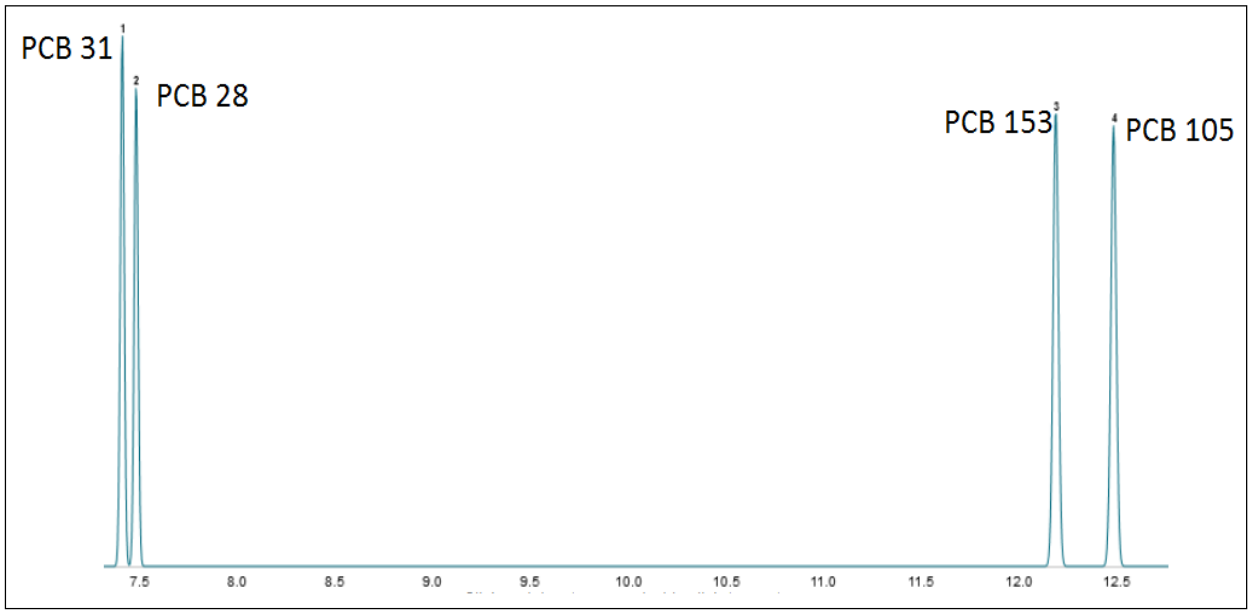


Figure 5.1: Selected PCBs generated by Restek EZGC modeller and reproduced with permission from Restek [54]. *This computer generated chromatogram was generated from a free online GC modeller created by Restek and accessed on their website,* www.restek.com.

5.4 Extraction procedure for used for the extraction of PCBs from soil and waste oil

Small modifications of the 3rd literature method were required for better integration into a commercial laboratory. Areas of method simplification were determining if the acid and hexane washing steps were necessary and if better detection limits could be achieved with less clean-up steps using more sophisticated detection equipment. In trying to address the deficiencies of method 3 the following procedure was developed.

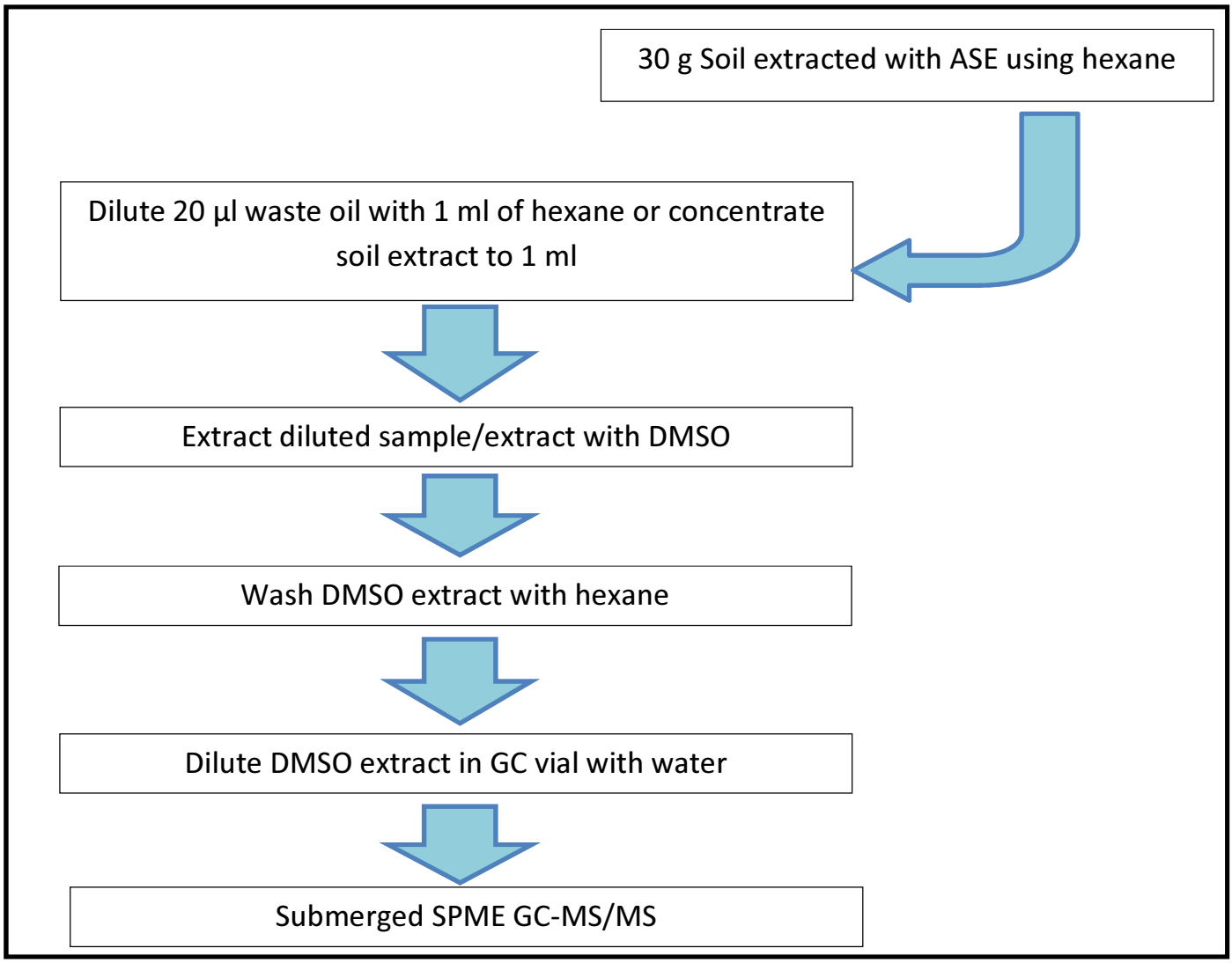


Figure 5.2: Flow diagram of PCB extraction from waste oils and soils using liquid-liquid extraction

5.5 Extraction of PCBs from soil and waste oils

Soil (30 g soil contaminated with spiked transformer oil) was extracted using an accelerated solvent extraction with hexane as solvent. The hexane extract was dried with sodium sulfate (Na_2SO_4) and concentrated to 200 µl (if the sample contamination allows it). Soil contaminated with transformer oil may contain more than 200 µl oil per 30 g of soil. If the volume oil extracted is > 200 µl, the extract volume will influence the detection limit of this procedure. Twenty (20) µl of waste oil/soil extract was diluted with 1000 µl of hexane in a glass EPA vial and 10.5 µl $^{13}C_{12}$-PCB internal standard was added. The extract was added to a 50 ml separation funnel. Extraction was done by adding 1750 µl DMSO (X2) and shaking vigorously for 60 seconds. The layers were separated.

One thousand (1000) µl DMSO extract (PCB containing) was added to a 50 ml separation funnel and washed with 625 µl hexane by shaking vigorously for 60 seconds. Sodium chloride (NaCl) was weighed (0.5 g) on a mass balance in a 10 ml GC vial. The DMSO extract (PCB-containing) was diluted 1:5 (DMSO: water) and capped with bimetallic cap. The submerged SPME was completed at 90˚C, 300 rpm for 20min followed by injection in GC-MS/MS or GC-ECD.

5.6 Experiments

5.6.1 Liquid-liquid extraction (LLE)

5.6.1.1 Initial investigation using a simple liquid-liquid extraction step without a washing step

This investigation was done to limit the amount of steps in the extraction process as this influence sample throughput, cost per analysis and reproducibility by laboratory technicians.

5.6.1.1.1 Experimental conditions

(a) Two hundred (200) µl transformer oil was diluted with 10 ml of hexane and extracted with 35 ml DMSO. The DMSO and hexane layers were separated. Dimethyl sulfoxide (DMSO) extract was diluted with water (1:4 DMSO: water) and spiked with 1 µl PCB standard (10 µg/ml) and analysed by submerged SPME GC-MS. No internal standard was added at this point as I as no quantification was required at this point.

(b) Transformer oil (100 µl, 200 µl, 300 µl and 400 µl) was diluted with 10 ml of hexane (spiked with 35 µl PCB standard, concentration of 35 µg/l per PCB) and extracted with 35 ml DMSO. The DMSO and hexane layers were separated. The DMSO extract was diluted with water (1:4 DMSO: water) and analysed by submerged SPME-fiber submerged in the extract GC-MS.

5.6.1.1.2 Results and discussion using a simple liquid-liquid extraction step without a wash step

Significant chromatographic peak shifts were observed compared to control retention times when only using a simple LLE, figure 5.3. It was also noted that when the transformer oil volumes were increased, peak shift also increased (figure 5.4). A total oil and grease analysis was conducted after the first extraction procedure (200 µl transformer oil) and 6% transformer oil (60 000 mg/l) was found to be co-extracted with the PCBs in DMSO, and one can assume that more oil will be co-extracted as the transformer oil volume increases (Figure 5.4). The selectivity of the LLE was lower than expected as DMSO and hexane/oil is immiscible.

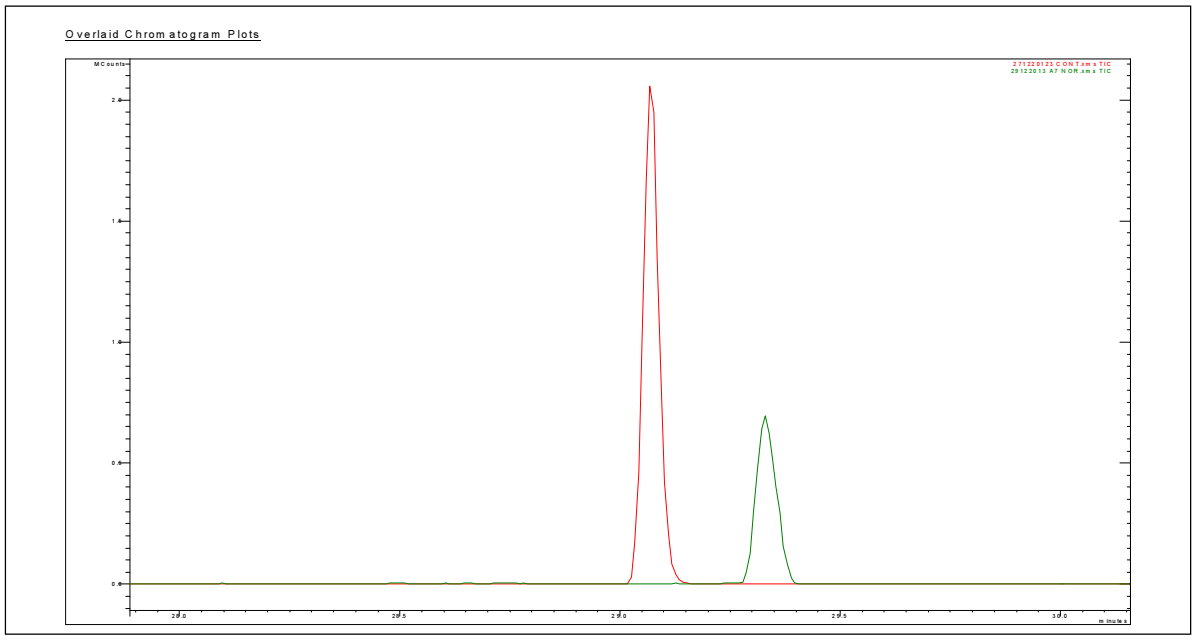


Figure 5.3: Overlay chromatogram of PCB 28 control (red) and PCB 28 extracted in the presence of transformer oil (green)

	PCB 28 - Control		PCB 28 - 200 µl transformer oil

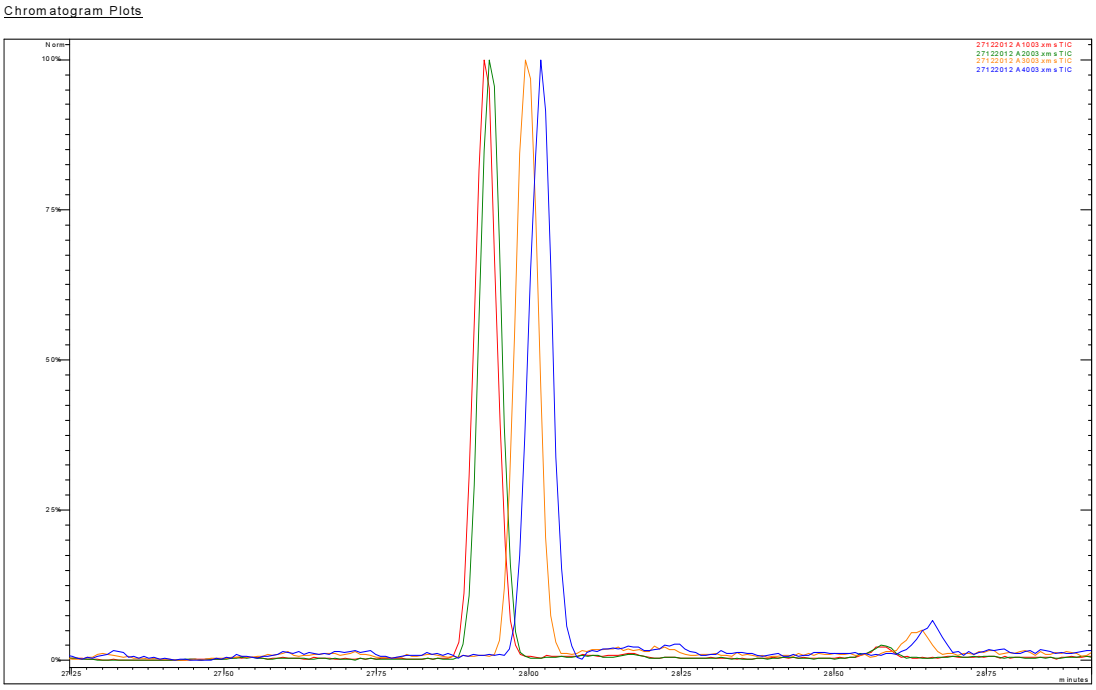


Figure 5.4: PCB 28 Chromatograms extracted in the presence of different volumes of transformer oil extractions

	PCB 28 - 100 µl transformer oil		PCB -28 -200 µl transformer oil
	PCB -28 -300 µl transformer oil		PCB -28 -400 µl transformer oil

The high concentration of transformer oil present on the column increased the film thickness and, therefore, the retention times when compared to control without any oil. Figure 5.5 and 5.6 show that peak shift which decreased as the heavier PCBs were eluted from the GC column. The heavier PCBs (PCB 138 – PCB 180) start to elute when the concentration of transformer oil starts to decline and this can clearly be seen on figure 1.2. It can be observed from the chromatograms that the decline in film thickness (oil) gives rise to reduction in peak shift.

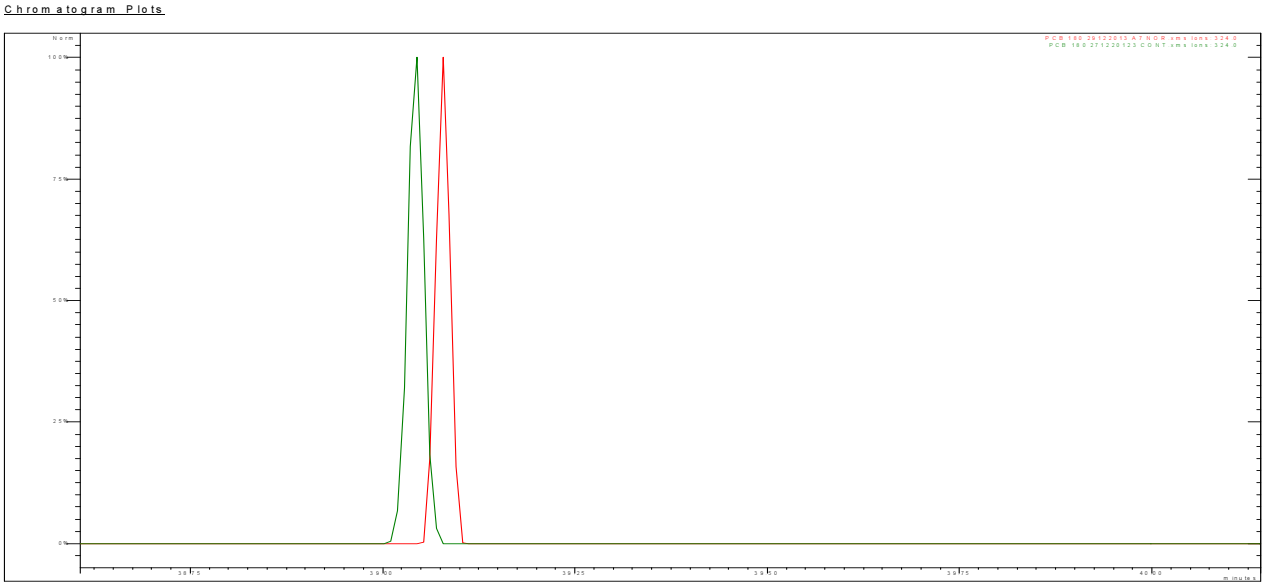


Figure 5.5: Overlay chromatogram of PCB 180 control (green) and PCB 180 extracted in the presence of 6% transformer oil (red)

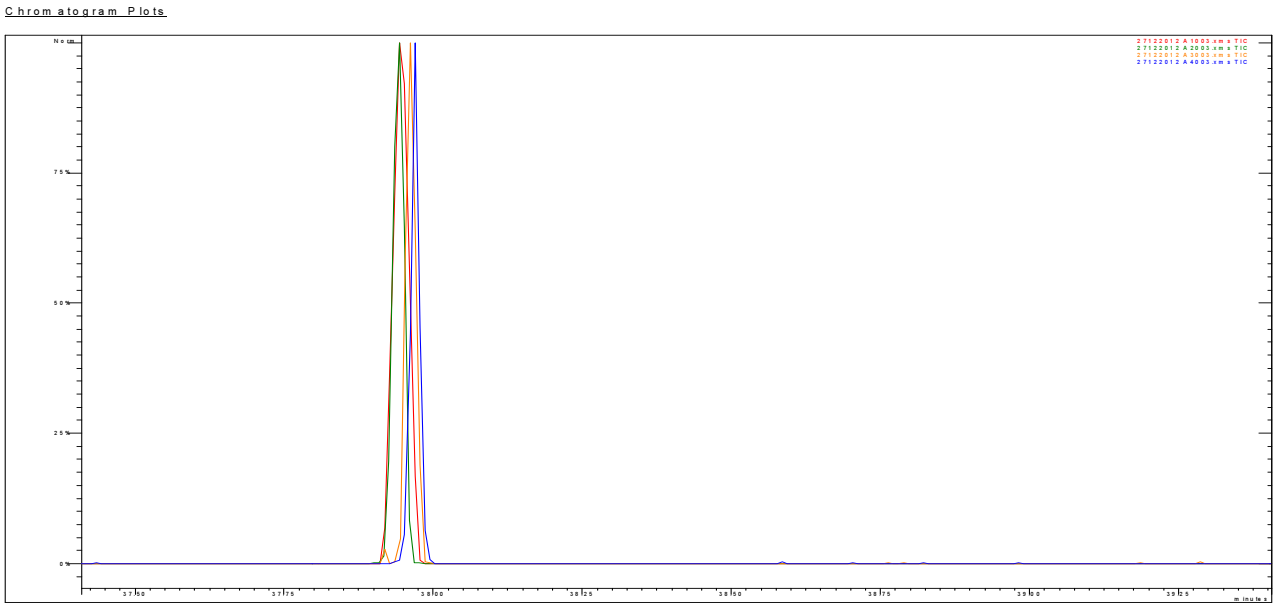


Figure 5.6: PCB 180 chromatograms extracted in the presence of different volumes of transformer oil extractions

5.6.2 Rate of partitioning

5.6.2.1 Experimental conditions

(a) Ten (10) ml of hexane was spiked with 35 µl of PCB standard (10 µg/ml) and was extracted with 35 ml of DMSO for 1 minute, 5 minutes and 10 minutes.

(b) Two hundred (200) µl of transformer oil was diluted with 10 ml of hexane and spiked with 35 µl of PCB standard (10 µg/ml), extracted with 35 ml of DMSO for 1 min. The hexane layer was concentrated to 1 ml, 200 µl of sample was then cleaned using the Supelclean ® method and analysed with submerged SPME GC-MS.

5.6.2.2 Results and discussion of rate of partitioning of first extraction

(a) The relatively fast partitioning of PCBs into the DMSO makes this liquid-liquid extraction attractive for implementation into a commercial laboratory. No substantial increase in extraction efficiency was observed when the extraction time is increased by factor 10 (Figure 5.7).

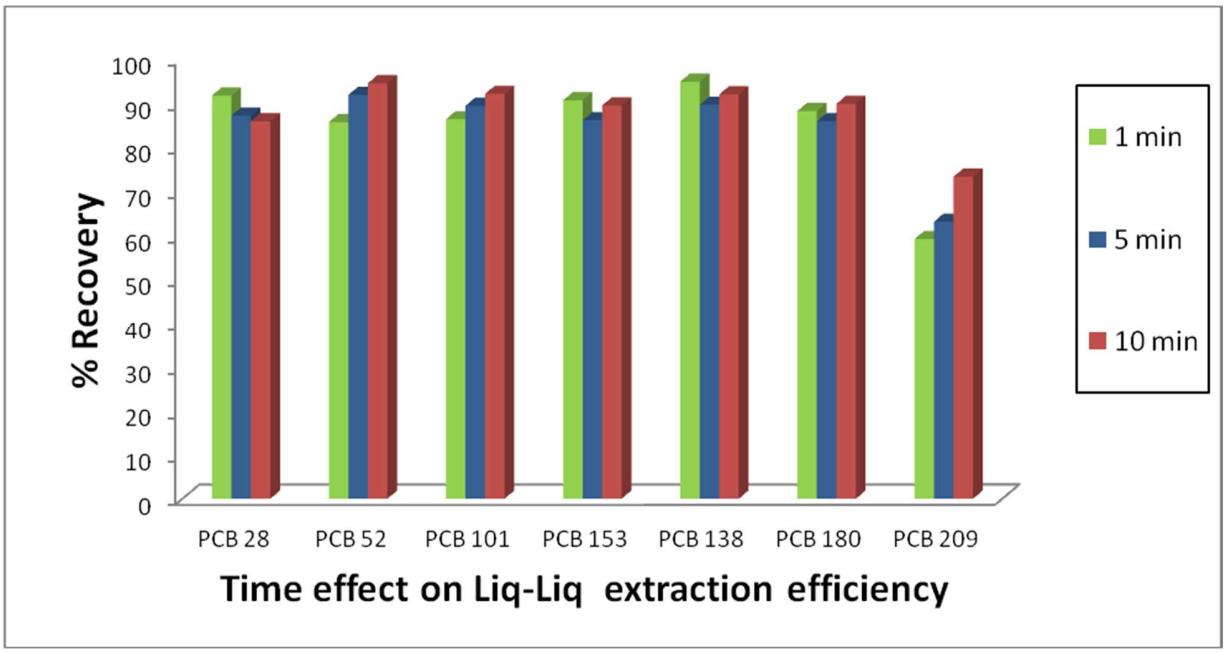


Figure 5.7: Extraction efficiency of liquid-liquid extractions at increasing extraction times

(b) An average of 87% indictor PCBSs were extracted from waste oil using LLE. Only one liquid-liquid extraction will be sufficient to determine PCB concentrations in waste oils (Table 5.4).

Table 5.4: PCB extraction efficiency of liquid-liquid extraction

BZ number	Recovery of PCBs extracted from waste oil (%)
28	85
52	89
101	89
153	88
138	88
180	81

5.6.3 Clean-up procedure

During the initial investigation (5.6.1) it was found that the presence of co-extracted waste oil leads to the following four problems:

1. Increased SPME fibre (non-polar) deterioration due to high oil (non-polar) concentration present.
2. Frequent system maintenance will be required more often as one introduces large concentrations of hydrocarbons into the GC-MS system. This will increase the systems baseline and warrant a system clean-up.
3. Difficulty in identifying PCBs due to peak shift and poor separation due to the high concentration of hydrocarbons present.
4. Saturation of the SPME fiber can therefore result in poor extraction efficiencies

A reduction in transformer oil introduced onto SPME fibre and gas chromatogram is therefore required. This would be studied by:

(a) using a less DMSO soluble non-polar diluting solvent

(b) dilution of sample

(c) introducing a washing step

5.6.3.1 Experimental conditions

(a) Two hundred (200) µl transformer oil was diluted with 10 ml of hexane spiked with 35 µl PCB (10 µg/ml) standard and extracted with 35 ml DMSO for 60 seconds. Two hundred (200) µl transformer oil was diluted to 10 ml of pentane spiked with 35 µl PCB (10 µg/ml) and extracted with 35 ml DMSO for 60 seconds. The hexane and pentane fractions were dried with Na_2SO_4. Fractions were evaporated and oil residues were weighed to determine the percentage (%) of oil recovered (total oil and grease).

(b) Two hundred (200) µl transformer oil was diluted with 10 ml of hexane spiked with 35 µl PCB (10 µg/ml) standard and extracted with 35 ml DMSO for 60 seconds. These extracts were diluted with DMSO before subjecting to SPME GC-MS analysis.

(c) Twenty (20) µl transformer oil was diluted with 10 ml of hexane spiked with 3.5 µl PCB (10 µg/ml) standard and extracted with 3.5 ml DMSO for 60 seconds. The extracts were washed with 5 ml, 2.5 ml, 1.25 ml and 0.625 ml hexane for 60 seconds and analysed with submerged SPME GC-MS.

5.6.3.2 Results and discussion of clean-up procedure

(a) Ninety two percent (92%) of transformer oil was recovered after the first extraction with DMSO using hexane to dilute the oil and 90% of transformer oil was recovered using pentane to dilute the oil. Hexane solubility in DMSO is 2.9 g/100 ml compared to pentane's lower solubility of 0.35 g/100ml [53]. This experiment was conducted to reduce the oil co-extracted with DMSO and to reduce the hydrocarbons on column to prevent PCB peak shift. Lower pentane solubility in DMSO will ensure better partitioning of oil in the aliphatic phase. Similar results were obtained due to the fact that layer separation was achieved

immediately after the 60 second extraction. Long separation times are not desirable as this method needs to be fast, 10 minute separation times per sample would not be acceptable. It was also noted that the pentane is difficult to work with having a low boiling point of 36.1^{0}C at 760 mm Hg (we are working at 710 mm Hg). Hexane has a boiling point of 69 ^{0}C at 760 mm Hg, is ,therefore, a lot easier to work with in a commercial laboratory and is also less expensive per litre.

(b) Reduction of hydrocarbons is essential to prevent peak shift. This was done through diluting the DMSO extract with DMSO before GC-MS analysis. This worked well to reinstate retention times with dilutions of 2:8 (DMSO: water) and 1:9 (DMSO: water) illustrated in figure 5.8. The estimated detection limits without any dilution was 100 µg/l to 200 µg/l per PCB, the quantification limit of total PCBs are just under 30 mg/l if the 1:9 dilution (lower estimate) and 60 mg/l (upper estimate) is used with the DIN 12766 method.

The best-case dilution will, therefore, place the quantification limit extremely close or over the accepted 50 mg/l limit used by most countries when testing for PCBs in waste oils. Diluting the DMSO extract to prevent/reduce peak shift and reduce hydrocarbons in the system will have to be abandoned. This process saves time and reduces peak shift, but sacrifices detection limit to the extent that it cannot be applied in a commercial laboratory. The required limits for total PCB concentration oil waste oil may change and could render the method obsolete if this procedure was implemented.

Chromatogram Plots

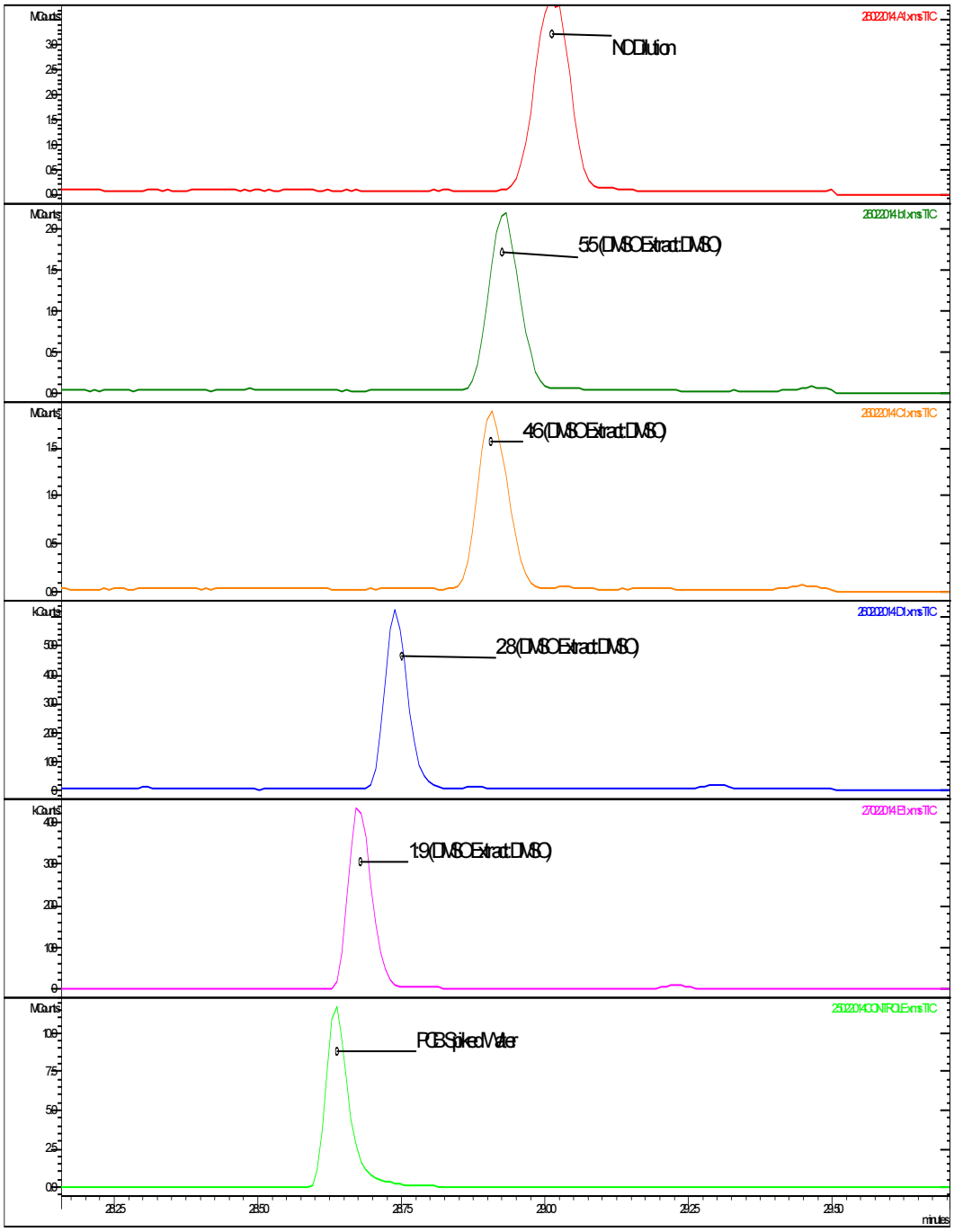


Figure 5.8: Chromatogram of PCB 28 with various dilutions to reinstate retention times

(C) In various methods, washing the extract with hexane is used to reduce/extract hydrocarbon interferences after the first extraction has been conducted. This experiment was conducted to determine the minimum hexane (volume) washing needed to prevent PCB peak shift. Slight peak shifts were observed when the extract was washed with 5 ml of hexane. Washing the DMSO extract with 0.625 ml of hexane resulted in an acceptable reduction in the peak shift observed (Table 5.5 and Figure 5.9). The amount of co-extracted waste oil reduced from 6 % to 1 % by the washing (0.625 ml) procedure, a reduction of 83 % (10 00 mg/l) which supports the reduction in peak shift observed.

Table 5.5: Retention time movement in seconds with various wash volumes

BZ number	RT (s) movement with no wash (es)	RT (s) movement with 5 ml wash(es)	RT (s) movement with 2.5 ml wash(es)	RT (s) movement with 1.25 ml wash(es)	RT (s) movement with 0.625 ml wash(es)
28	19.26	1.5	2.04	1.98	4.02
52	18.18	0.48	2.04	1.5	4.02
101	16.74	0.48	1.5	0.96	3.06
153	14.16	-0.06	0.96	-0.12	2.04
138	13.2	0	1.02	-0.06	1.56
180	9.72	0	0.48	-0.6	1.02

Washing the DMSO extract with hexane has proven to be the best method to reduce peak shift without sacrificing detection limits and only adding 2 to 3 minutes of extraction time per sample. Using 0.625 ml as opposed to 5 ml of hexane is not as effective, but a Δt (s) time window ≤ 5 seconds is still acceptable (0.625 ml) and limits the use of solvent which is paramount for commercial laboratories. The peak shift is unwanted, but acceptable at the UIS Organic Laboratory, resulting in a small reduction in the quality of the result for a large gain in sample throughput and cost.

Chromatogram Plots

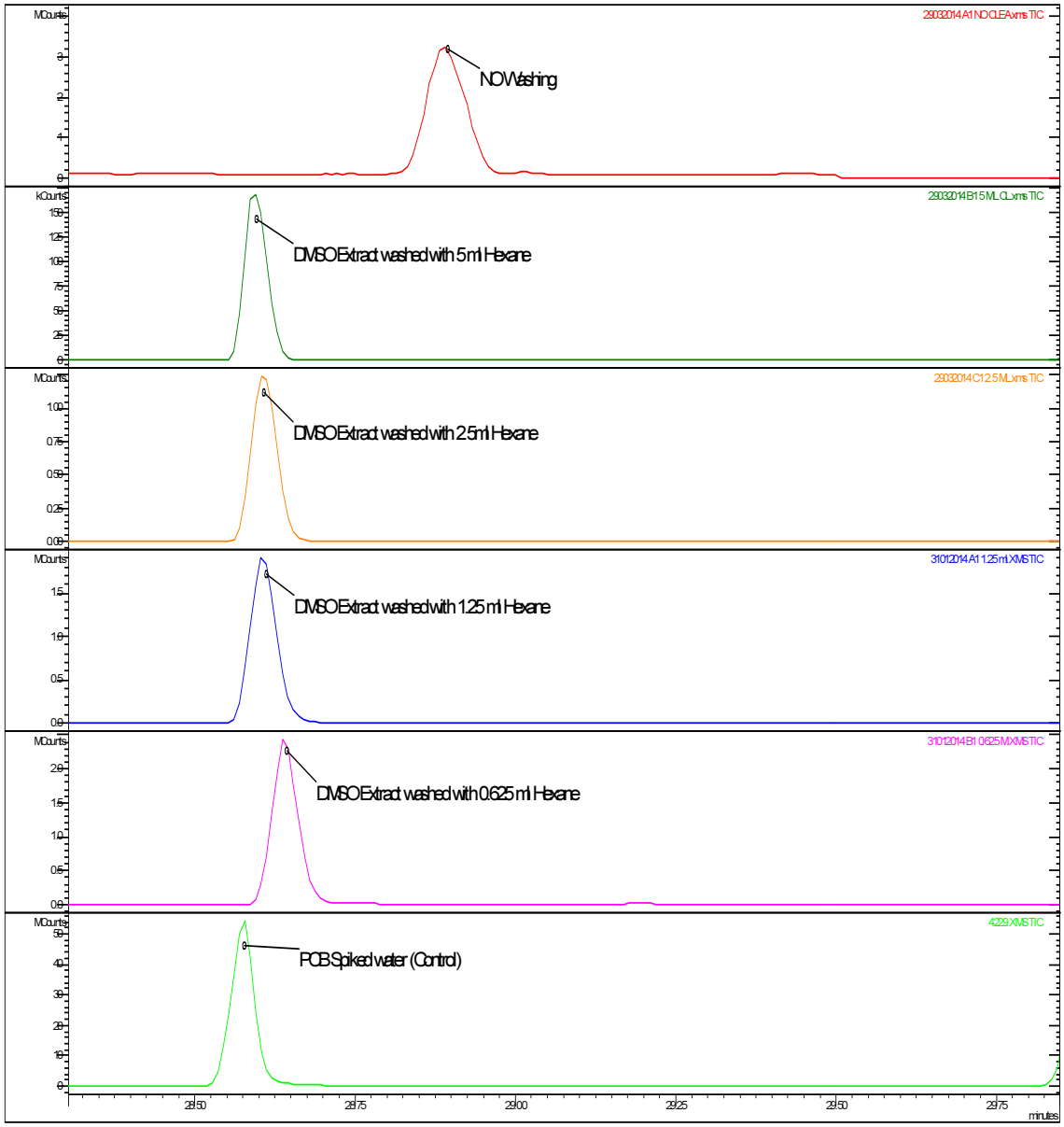


Figure 5.9: Chromatogram of PCB 28 using various washing volumes

5.6.4 Robustness of extraction procedure

Robustness can be described as a methods ability to deal with changes in conditions such as matrix to obtain a reproducible result.

5.6.4.1 Experimental conditions

(a) Five types of oils were used to test the robustness of this technique:

- transformer oil
- motor engine oil
- olive oil
- fish oil
- vacuum pump oil

Twenty micro litre of each oil was diluted with 1 ml of hexane spiked with 3.5 µl PCB (10 µg/ml) standard and 10.5 µl $^{13}C_{12}$-PCB internal standard (1 µg/ml) was added. The diluted sample was then extracted with 3.5 ml of DMSO and washed with 0.625 ml hexane for 60 seconds followed by submerged SPME (100 µm PDMS) at 90°C, 300 rpm for 20min and injection in GC-MS/MS.

(b) One hundred µl of transformer oil was diluted with 5 ml of hexane spiked with 17.5 µl PCB (10 µg/ml) standard and added to 30 g of soil collected from the laboratory garden. This soil mixture was extracted using an accelerated solvent extractor (ASE) and was dried using Na_2SO_4. The ASE extract was concentrated to 200 µl and a 20 µl sub-sample was diluted with 1 ml of hexane, and 10.5 µl of $^{13}C_{12}$-PCB internal standard was added. The diluted sample was then extracted with 3.5 ml of DMSO, washed with 0.625 ml hexane for 60 seconds followed by submerged SPME (100 µm PDMS) at 90°C, 300 rpm for 20 minutes and injection in GC-MS/MS.

5.6.4.2 Results and discussion of the robustness of the extraction procedure

(a) These oils all have different uses, viscosities and chemical compositions. The lipophilic PCBs were extracted with acceptable recoveries of between 82 and 103 % (expected value of 1750 µg/l) from different matrices (Table 5.6).

Table 5.6: Recoveries from different oil matrices of indicator PCBs (PCB 28, 52, 101, 153, 138 and 180) expressed as an average of all indicator PCBs extracted

Matrix	Composition	Average of extracted indicator PCB (ug/l)	Recovery (%)
Fish oil	Triacylglycerols	1611	92
Motor oil	PAH/Aliphatic hydrocarbons	1750	100
Transformer oil	PAH/Aliphatic hydrocarbons	1804	103
Olive oil	Triacylglycerols	1729	99
Vacuum pump oil	Solvent-dewaxed heavy paraffinic	1435	82

(b) The expected concentration of PCBs in the soil sample spiked with transformer oil was 5.8 µg/kg per PCB. The determination of PCBs in soil was also successful (Table 5.7). Accelerated solvent extraction forms a key role in the extraction of PCBs from soil samples. There are two additional steps added to the sample extraction procedure: (1) an ASE extraction, as well as (2) a concentration step. The quantification limit in soil is dependent on the amount of waste oil extracted from the soil. Working with small volumes of oil or any matrix for that matter can be difficult and sample losses can become a problem. This extraction procedure, therefore, requires a minimum sample concentration volume of 200 µl from the 30 g of soil sample. A 20 µl sub-sample is taken representing a fraction of the soil and if the soil sample extract cannot be concentrated to 200 µl (high oil content).The 20 µl sub-sample will, therefore, represent a smaller fraction of the soil. When applying this method for the determination of total PCB, the user must document and determine whether the quantification limit is acceptable in terms of allowable MCL levels. The volume of oil extracted from the sample is directly proportional to the quantification limit of total PCBs using DIN 12766 (graph 5.2) in the soil. Figure 5.10 can, therefore, assist to determine whether the method can be used for the analysis of total PCBs in the soil sample. This method has a quantification limit of 35 µg/kg for total PCBs in 30 g of soil if the sample can be concentrated to 200 µl. When the 200 µl

concentration volume cannot be achieved due to higher total oil content refer to graph 5.2 for quantification limits.

Table 5.7: Recoveries from soil contaminated with transformer oil spiked with indicator PCBs (PCB 28, 52, 101, 153, 138 and 180) expressed as an average of all indicator PCBs extracted

Matrix	Composition	Average extracted indicator PCBs (µg/kg)	Recovery (%)
Soil	Transformer oil and organic matter	5.13	88

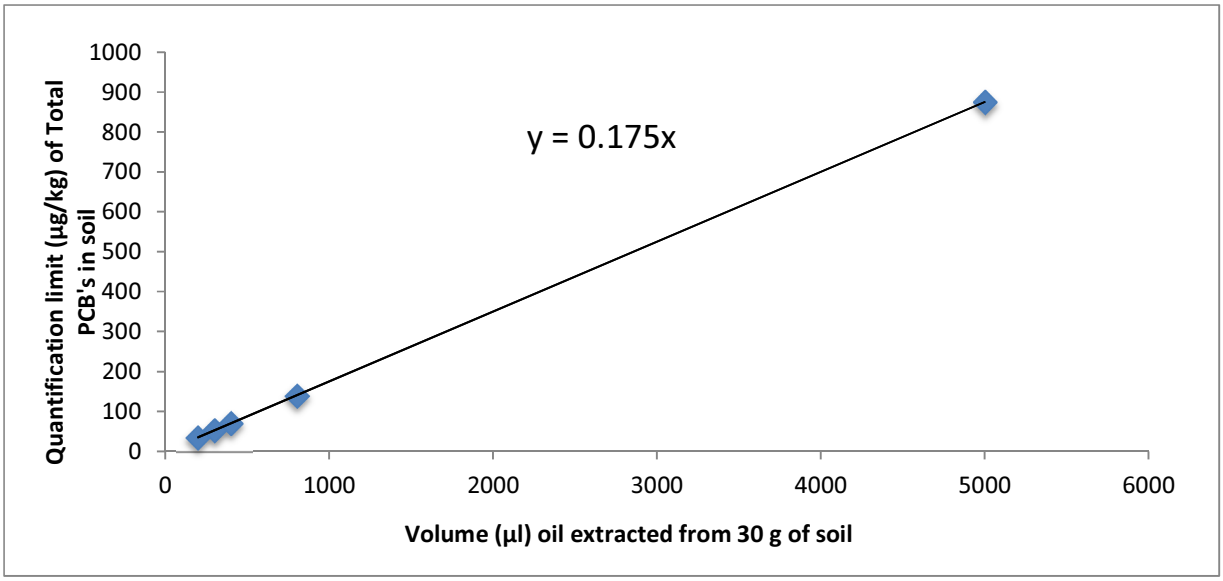


Figure 5.10: Quantification limit of total PCB concentration from different volumes of oil extracted from soil samples. *Quantification limits of total PCBs in soil are governed by the concentration of the hexane extract. The extraction method and clean-up procedures use a 20 µl sub-sample of ≥ 200 µl concentrated hexane extract. The 20 µl sub-sample of the ≥ 200 µl extract represents a fraction of the PCBs present in the 30 g soil sample. Figure 5.10 can therefore be used to determine if this extraction method is fit for purpose in terms of the MCL as stated in table 1.1.*

The abovementioned results show that the developed method is able to handle changes in matrix and interferences. This method can easily be introduced into commercial laboratories that receive samples of unknown waste matrices that need classification in terms of total PCB concentration.

5.6.5 SPME selection and SPME phase thickness

SPME selection plays an important role in order to obtain desired selectivity as well as sensitivity. A factor also influencing SPME selection in a commercial laboratory is the selection of fibres that can be used for a multitude of applications. Film thickness forms an integral part of the capacity of a SPME fiber and is therefore extremely important when selecting a fiber. Commercial laboratories analyse highly contaminated samples and this, therefore, results in numerous repeat analyses to dilute sample concentration to within the linear range of the fibre. Normally SPME analysis is used under splitless GC injector conditions, overloading of the GC capillary column is, therefore, also a reality when using thicker fiber phases as all analytes are injected into the GC. In our laboratory SPME forms a major part of our daily sample analysis, using polydimethylsiloxane (PDMS) SPME fibres. The PDMS phase is extremely versatile with applications in SVOC, PCB, VOC, OC pesticides, GRO, volatile DRO and arson samples. There are three PDMS phase thicknesses that are commercially available: 7 µm, 30 µm and 100 µm with applications to specific types of compounds and conditions.

5.6.5.1 Experimental conditions

PCB (0.5 µl) standard (200 µg/l) was spiked into 5000 µl H_2O and extracted with 7 µm, 30 µm and 100 µm PDMS fibres (submerged) and analysed using GC-ECD. A 1:4 (DMSO:water) was not used at this stage as the fiber thickness was the only parameter being investigated. The samples were agitated at 300 (revolutions per minute) rpm for 10 minutes at 60 ˚C, followed by splitless injection with injector heated to 290˚C.

5.6.5.2 Results and discussion

Figure 5.11 shows the 7 µm PDMS has no selectivity for the lighter PCBs with increased extraction efficiency for heavier PCBs. Yellow 30 µm PDMS showed a good all-round extraction for PCBs, but the lower extraction efficiency of PCB 28 compared to the 100 µm is problematic. Matrix effects are at a maximum (Figure 1.2) in the region of PCB 28 and PCB 52, which will therefore influence the detection

limits of these PCBs. An equivalence point is observed at PCB 180 for all PDMS fibres and at PCB 209 the 100 µm shows the worst extraction efficiency.

The low extraction efficiency of the thinner PDMS fibres (7 µm and 30 µm) for the lower molecular weight PCBs could be attributed to desorption before injection as the agitation temperature was at 60 °C, which is the UIS Organics' standard extraction temperature for submerged analysis. The decrease in extraction efficiency for PCB 209 using 100 µm PDMS can be attributed to the incomplete desorption of the X 3 thicker 100 µm PDMS fibre. The incomplete desorption of heavier analytes occur due to the maximum operating temperature (280 ^{0}C) limitations of the 100 µm PDMS fibre. PCBs are being extracted under non-equilibrium conditions, according to equation 4.3 fiber thickness is directly proportional to the amount of analytes extracted, and therefore, I would expect an increase in extraction efficiency if the fibre could be desorbed at higher temperatures. Currently the fibre desorption temperature is 10 ^{0}C above the advisable limit and will have an impact on the lifetime of the fibre. Due to the limited extraction temperature it is clear the figure 5.11 is misleading as the heavier PCBs (PCB 180 & 209) are not being fully desorbed and showing a non-linear relationship which is contradictory to equation 4.3.

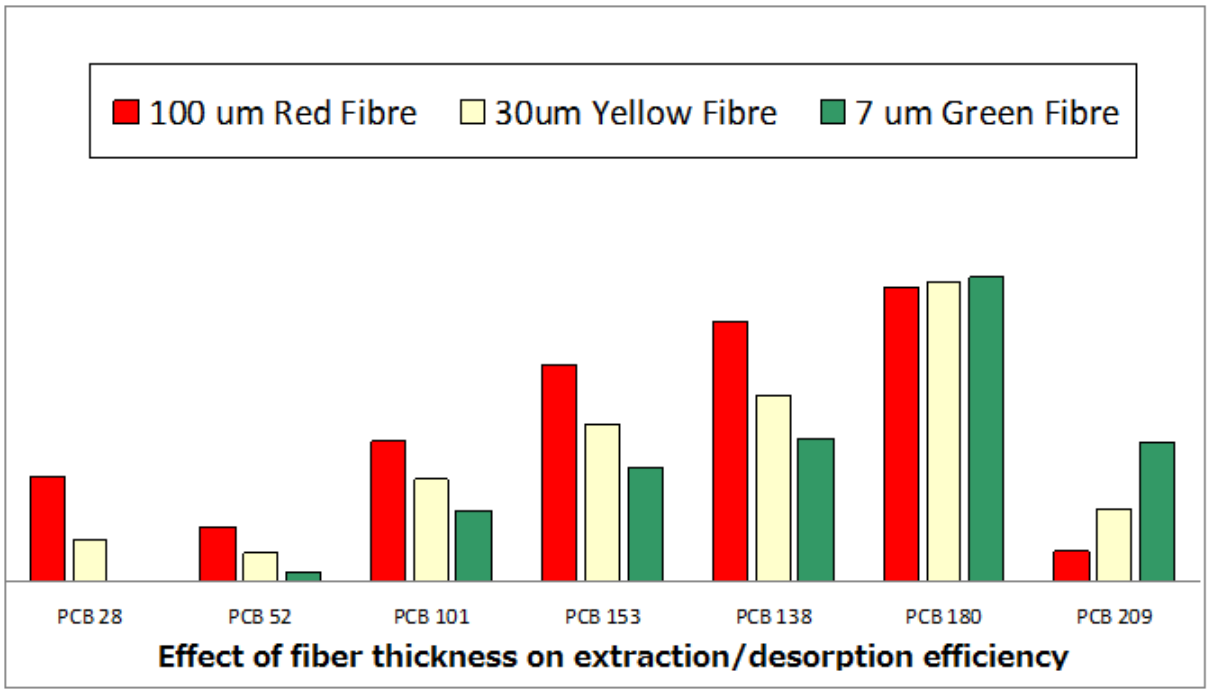


Figure 5.11: Extraction/desorption efficiencies of different PDMS phases under (time constrained) non-equilibrium conditions

The DIN 12766 method only deals with PCBs 28, 52, 101, 138, 153 and PCB 180, for which the 100 µm PDMS fibre performed the best in terms of extraction/desorption efficiency. Matrix effects are severe with the 10 000 mg/l hydrocarbons co-extracted with the PCBs, and therefore fibre capacity is of cardinal importance with 100 µm PDMS offering the highest capacity as well. Fibre longevity also plays an important role in fibre selection as well as a larger role in commercial laboratories. The 100 µm fibre is able to do more samples before fibre degradation starts to influence repeatability and sensitivity of the fibre extraction. From the above results it is clear that the 7 µm and 30 µm PDMS fibres are not suited for these extraction conditions and therefore not suitable for this method.

5.6.6 Influence of SPME extraction temperature on extraction efficiency

5.6.6.1 Experimental conditions

PCB (0.5 µl) standard (200 µg/l) was spiked into 5000 µl H_2O, was concentrated using 100 µm PDMS fibre (submerged) and analysed using GC-ECD. A 1:4 (DMSO: water) was not used at this stage as the fiber thickness was the only parameter being investigated. The samples was subjected to temperatures of 30 ^{o}C, 60 ^{o}C and 90 ^{o}C for 20 minutes at 350 rpm.

5.6.6.2 Results and discussion

The equilibrium of the extraction is clearly not reached at 30 ^{0} C (Figure 5.12). A drastic increase in extraction efficiency was observed when the extraction temperature of the SPME extraction was increased. Faster partitioning of PCBs into the 100 µm PDMS is due to increased analyte diffusion coefficients at higher temperatures, which is especially visible with increased extraction efficiency of heavier PCBs with slower diffusion [49]. The 90 ^{0} C extraction temperature, therefore, increases the extraction efficiency of all PCBs and most importantly for PCB 28 and PCB 52, which co-elute with the majority of waste oil (Figure 1.2). Due to the fact that PCB 180 is more chlorinated than PCB 28, a higher relative response was observed on the GC-ECD, which is extremely sensitive for chlorinated compounds.

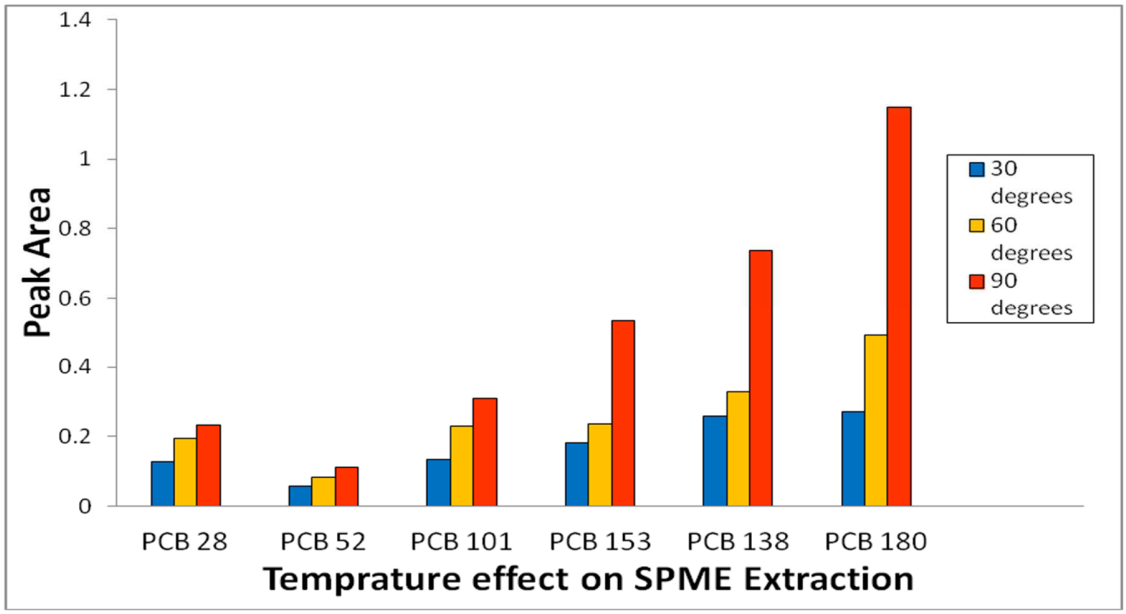


Figure 5.12: Effect of SPME extraction temperature on extraction efficiencies under time constrained non-equilibrium partitioning conditions

5.6.7 Effect of increased agitation speed on SPME extraction

The use of SPME in commercial laboratories are normally done under non- equilibrium, kinetically determined extraction conditions. It is, therefore, obvious that the transport of molecules from the aqueous sample to the fiber will be increased by forced convection, not only by increased diffusivities. We thus had to investigate sample agitation as a parameter that could influence sample extraction efficiency.

5.6.7.1 Experimental conditions

PCB (1 µl) standard (10 µg/ml) was spiked into 5 ml of water: DMSO (4:1). The submerged extraction was done with 100 µm PDMS SPME for 20 min at 90 ^{0}C with varying agitation speeds.

5.6.7.2 Results and discussion

Increasing the agitation speed reduces the equilibration time of analytes by assisting in the mass transport of analytes from the sample matrix into the SPME [49]. By increasing the agitation speed of extraction, the equilibration time is reduced. The effect is more profound on heavier PCBs due to slow diffusion (mass transfer) into the thick 100 µm fibre (Figure 5.13 & 5.14). It was also found that at a high agitation speed

(>400 rpm) fibre damage increased due to fibre contact with vial walls. This effect is more evident on used fibres, which become more brittle and lose flexibility due to degradation of the PDMS operating at high desorption temperatures. The costs of SPME fibres outweigh the gain in minor response of PCBs and therefore a lower 300 rpm was used to limit fibre damage.

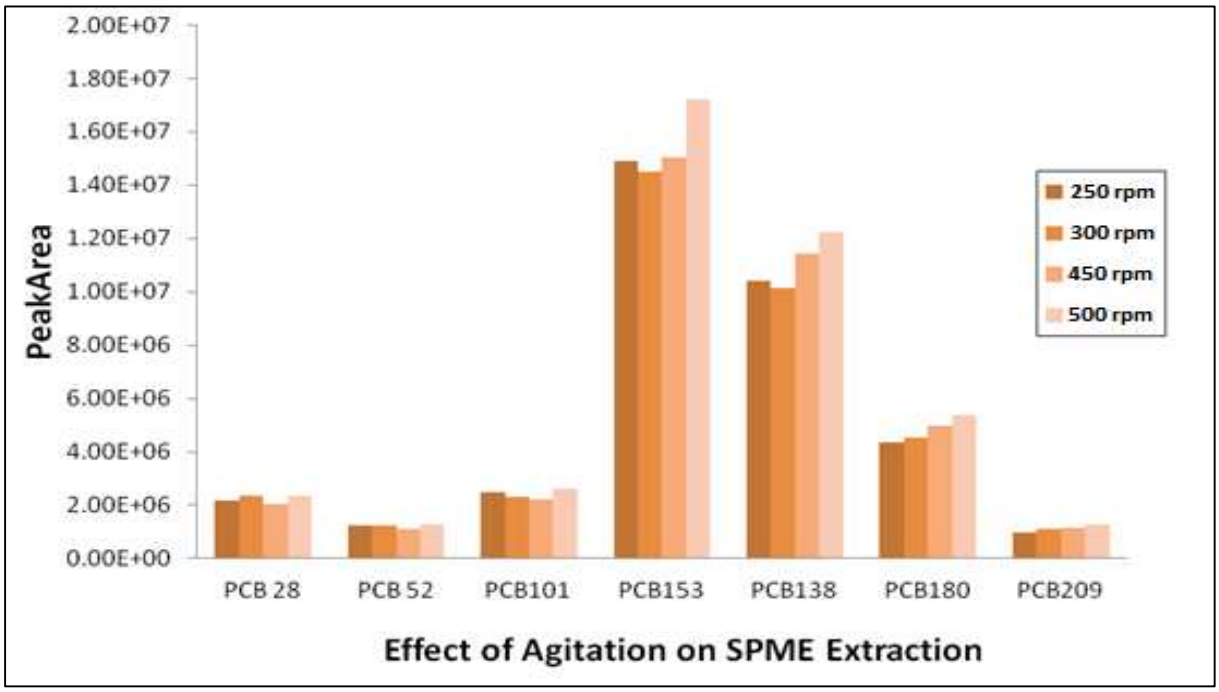


Figure 5.13: Effect of varying agitations speeds on extraction efficiency of PCB using submerged SPME

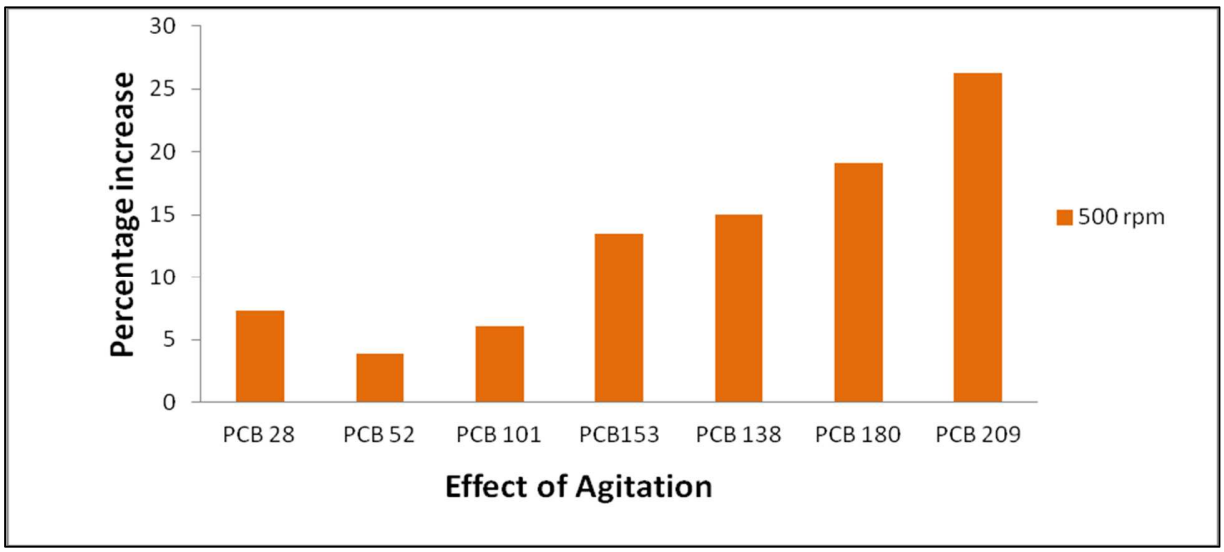


Figure 5.14: Percentage increase in extraction efficiency between 250 rpm and 500 rpm

5.6.8 Effect of NaCl addition on SPME extraction efficiency

The addition of NaCl (salt) increases the ionic strength of the aqueous sample and decreases the solubility of the dissolved organic analytes. Salting out was investigated because of the noise created by the co-eluting transformer oil with regards to the more water soluble PCBs 28 & 52. Salting out was therefore investigated possibly generate more stable ions and increase reproducibility in terms of linear responses.

5.6.8.1 Experimental conditions

PCB (1 µl) standard (10 µg/ml) was spiked into 5 ml of water: DMSO (4:1). Submerged extraction was done with 100 µm PDMS SPME for 20 minutes at 90 ^{0}C. NaCl was added in increasing increments.

5.6.8.2 Results and discussions

The increase in ionic strength of an aqueous solution to improve extraction efficiencies is common practice and was successfully applied in this instance. PCB 28 has a $Log_{o/w}$ 5.55 and PCB 180 a $log_{o/w}$ 7.29; thus PCB 28 is more soluble in water than PCB 180 [28]. The addition of 0.5 g of NaCl showed the best overall increase in PCB SPME submerged extraction efficiencies, with decreasing effectiveness as the water solubility of the PCBs declines (Figure 5.15).

A negative effect was observed with the addition of high NaCl additions (2 g of NaCl per 5 ml). The ionic strength of water will reach a point where extraction efficiency will start to decline. At high water ionic strengths, the PCBs will start to precipitate and adsorb to the glass vial walls [55, 56]. Lower soluble PCBs (PCB 180) are more effected than higher water-soluble PCBs (PCB 28), which could explain the drastic reduction in extraction efficiency for PCB 180 at 2.0 g NaCl [56, 57].

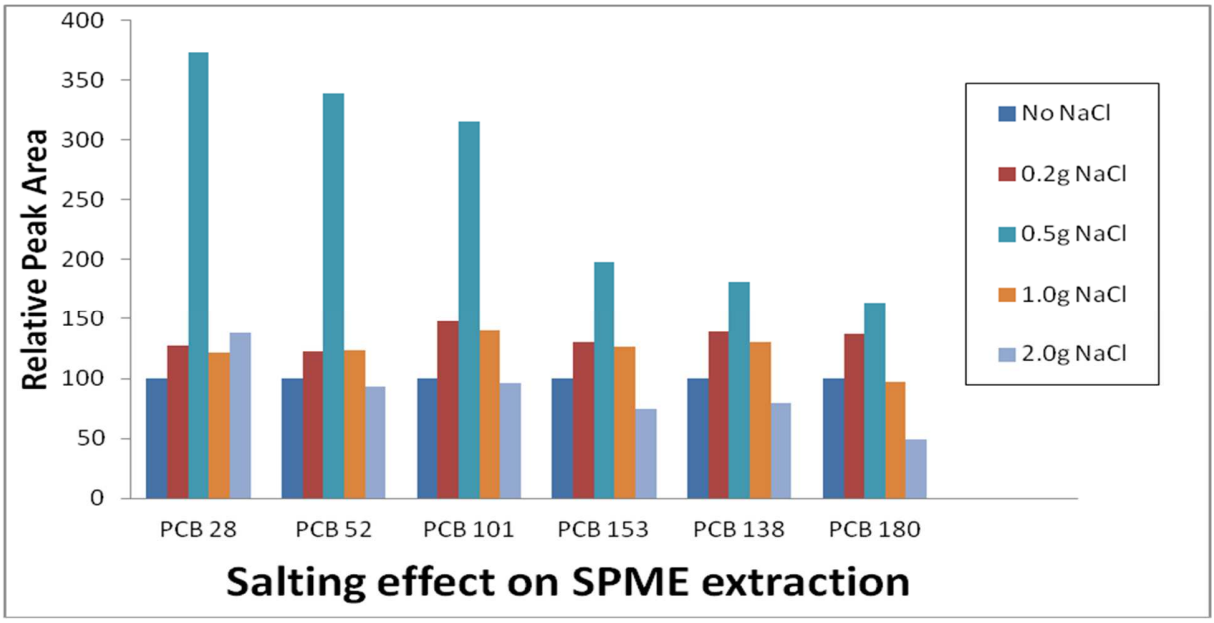


Figure 5.15: Effect of salt addition on SPME submerged extraction efficiency. *Note that the responses are relative to the response of PCB extraction with no addition of NaCl*

5.6.9 Method performance

5.6.9.1 Typical Indicator PCB chromatogram

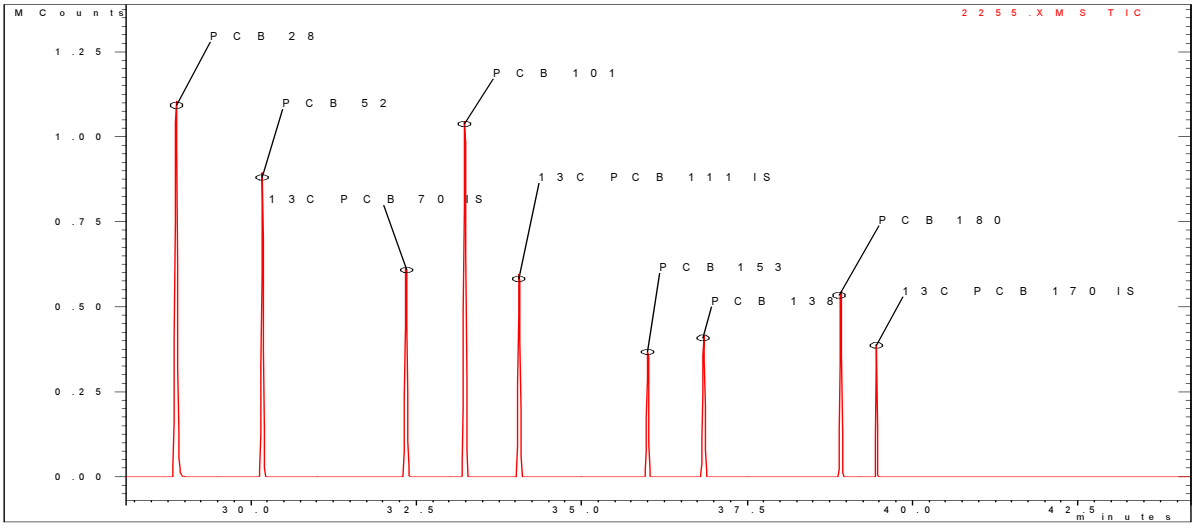


Figure 5.16: Typical indicator PCB and internal standards chromatogram

Instrument conditions:

Instrument	= GC-MS/MS
Injection metod	= SPME
SPME extraction conditions	= submerged 90 ^{0}C at 300 rpm for 20 min
Injector temprature	= 290 ^{0}C
Carrier	= He
Column flow	= 1.0 ml/min
Column type	= VF5 30 m X 0.25 mm ID X 0.25 um df

5.6.9.2 Typical indicator PCB calibration curve

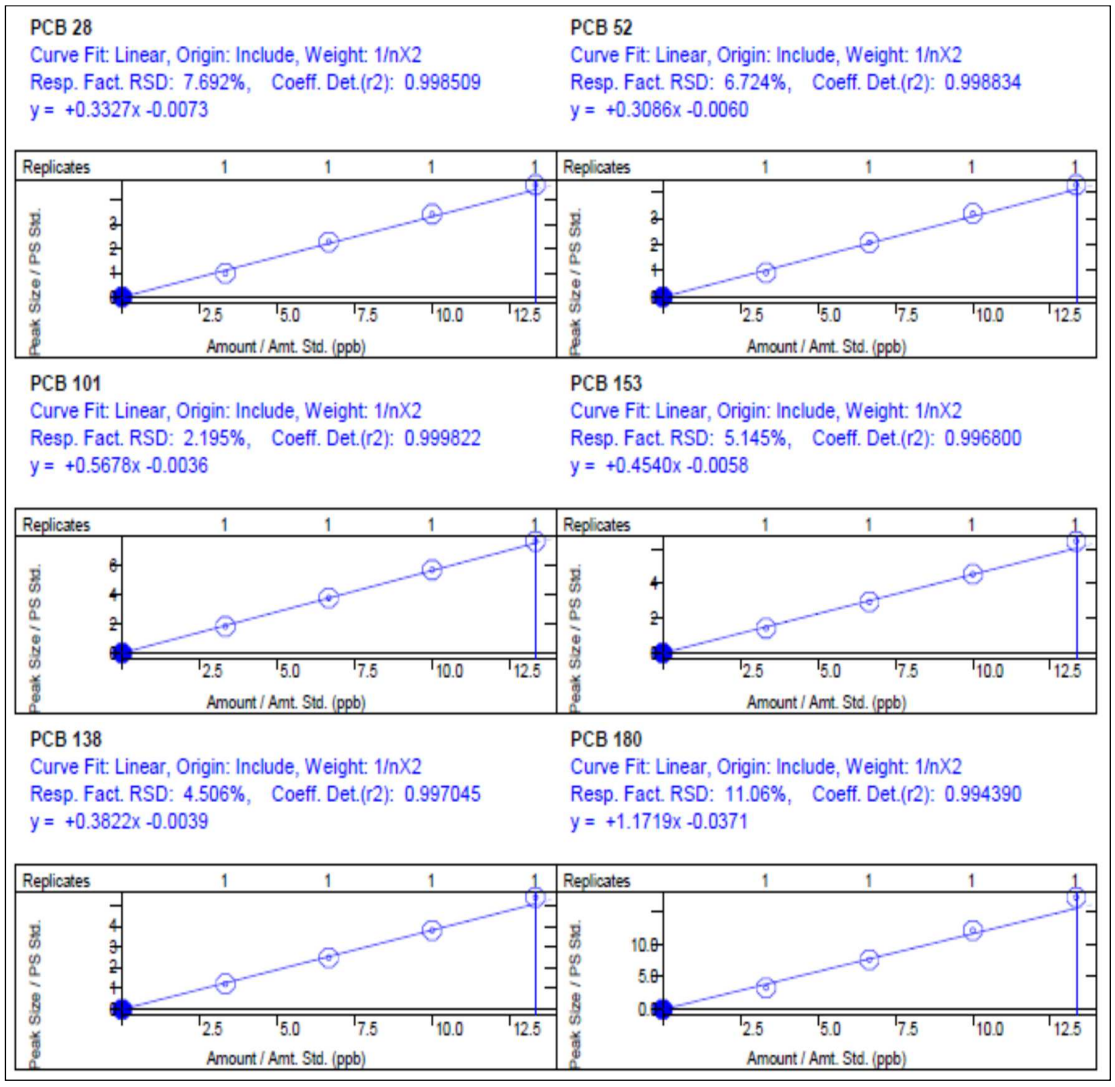


Figure 5.17: Typical four-level calibaration curve of indicator PCBs

5.6.9.5 Quantification

All the spiked standards were used dierctly from the primary standards as they were provided by the manufactures as described in section 5.1 (chemicals,standards and materials). Internal standard method was used for calibration and quantification which is essential when using SPME. All calibrations were conducted using the same condions as samples, 0.5 g of NaCl and 1:4 (DMSO:water) as matrix, spiked with PCB calibration standard and internal standards. The only element that is different from the sample preperation is that no oil was added as the $^{13}C_{12}$-PCB internal standards are chemically and physically similar than the PCBs tested, the realtive response between the internal standard and compounds are measured for calibration and quantification.

5.6.9.4 Statistical evaluation

Table 5.8 clearly shows the repeatability of the extraction/desorption procedure, which has good overall recoveries. PCB 180 has lower recoveries when compared to the rest of the PCBs. PCB 180 is more lipophilic than the other PCBs and is better retained by the aliphatic oil matrix; therefore lower recoveries are observed. Incomplete desorption of PCB 180 also decreased recoveries.

Table 5.8: Statistical evaluation of extraction method from waste oils (1750 µg/l expected concentration)

BZ number	Run 1 (µg/l)	Run 2 (µg/l)	Run 3 (µg/l)	Average (µg/l)	Standard deviation (µg/l)	% RSD	Percentage recovery (%)
28	1733	1723	1852	1769	58	3	101
52	2005	1624	1763	1797	157.	9	103
101	2246	1699	2161	2036	240	12	116
138	2009	1756	1391	1719	254	15	98
153	2233	1893	1538	1888	284	15	108
180	1565	1248	1467	1426	132	9	82

5.7 Conclusion

The above experiments demonstrate the complexity of the sample matrix and the ability of the current liquid-liquid extraction and clean-up procedure to remove most interference. Using small volume of solvents and the simplicity of a two-step extraction procedure coupled with automated SPME makes this method extremely useful for commercial laboratories. The above has also proven the robustness of the method. This method is able to cope with changes in matrix and has shown good repeatability as well as recoveries for the extraction of PCBs from waste oils and oil-contaminated soil.

Chapter 6

Qualitative and quantitative analysis using different instrumentation

6.1 Introduction

Selectivity and sensitivity are the most important factors for commercial laboratories in terms of instrument selection. Correct instrument selection can save a laboratory time and money in terms of sample clean-up. This method, described in Chapter 5, is based on minimal sample clean-up. Coupling this method to instrumentation that is both selective and sensitive towards PCBs is crucial for integration into a commercial setting.

6.2 Electron capture detector (ECD)

6.2.1 Instrument performance

6.2.1.1 Experimental

(a) Twenty micro litre transformer oil was added to 1 ml of hexane spiked with 7 µl PCB (1 µg/ml). $^{13}C_{12}$-PCB internal standards (10.5 µl) were also added and extracted with 3.5 ml DMSO for 60 seconds. The extract was washed with 0.625 ml hexane and separated. The DMSO extract was diluted with water: DMSO (4:1) and 0.5 g of NaCl was added. The PCBs were extracted with submerged SPME at 60 ^{o}C and analysed on a GC-ECD.

6.2.1.2 Results and discussion

PCB 138 was not analysed due to co-elution with $^{13}C_{12}$-PCB 138 internal standards, and an ECD is not capable of filtering compounds by masses such as a mass spectrometer. An ECD is extremely sensitive for halogenated compounds like PCBs and this is evident in figure 6.1. As expected, the sensitivity of PCBs increased with the level of chlorination. PCB 28 and PCB 52 had the worst recoveries of all the PCBs when using a 30% relative standard deviation (350 ± 105 µg/l) (Table 6.1).

The unacceptable recoveries of PCB 28 and PCB 52 were due to co-elution being present as well as a high level of interferences in their elution window. The ECD response is matrix dependant, clear indication of negative movement of baseline is visible indicating interference. Interferences co-eluting with the internal standard ($^{13}C_{12}$-PCB 70) also contributed to the low recovery of PCB 28 and PCB 52 (Figure 6.2). The GC-ECD analysis was not intended to be used for quantification, but rather as a system for screening purposes; therefore more co-elution was observed with shorter run times.

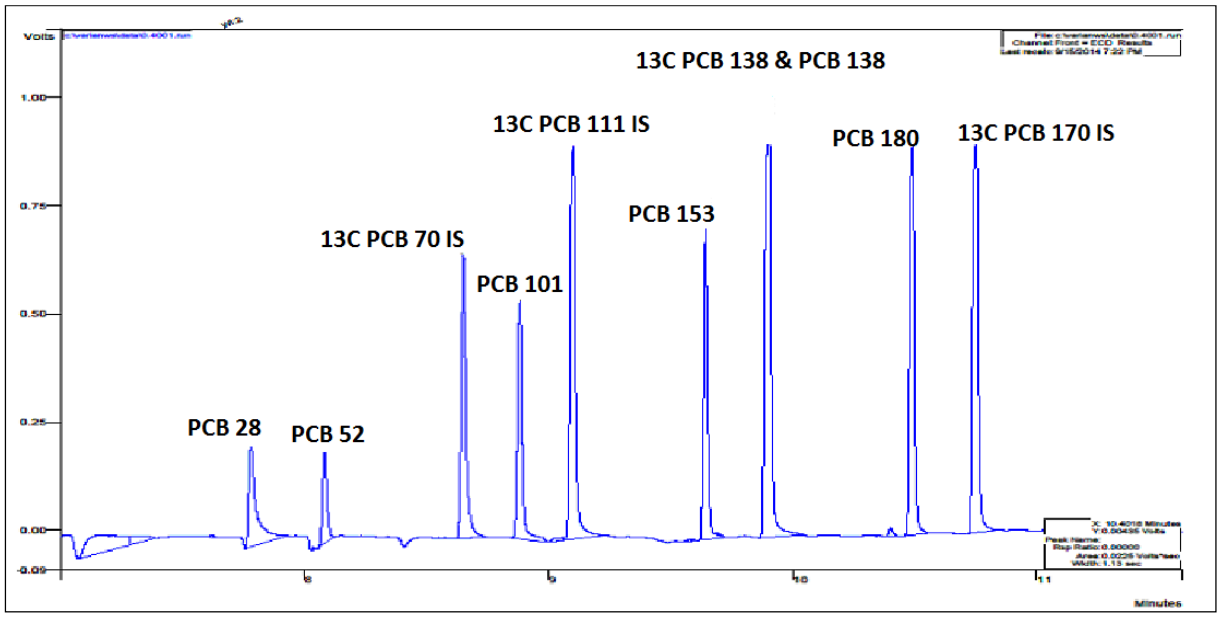


Figure 6.1: Indicator PCBs at 0.4 µg/l extracted with SPME and analysed with GC-ECD (control)

Table 6.1: PCBs extracted with developed method and analysed using SPME GC-ECD

BZ number	Expected concentration(µg/l)	Concentration found (µg/l)	Recovery (%)
28	350	197	56
52	350	228	65
101	350	397	113
153	350	352	101
138	350	X	X
180	350	450	129

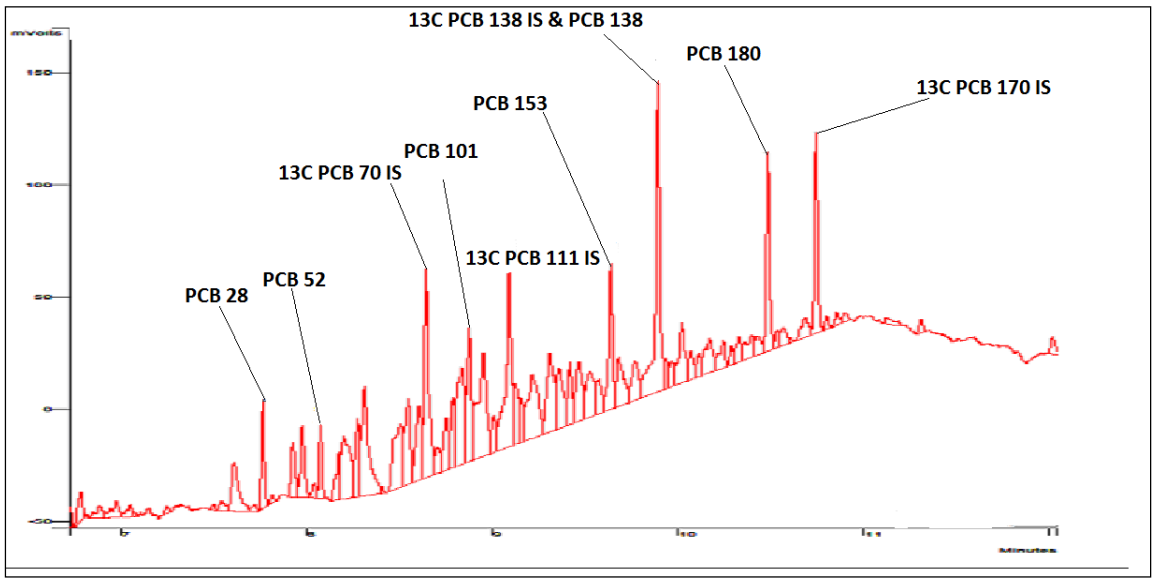


Figure 6.2: Indicator PCBs at 0.4 µg/l extracted with SPME and analysed with GC-ECD

6.2.1.3 Conclusion

Using a GC-ECD for PCBs analysis from waste oils by means of the developed method is not acceptable for the quantitative determination of PCBs, but rather for the qualitative determination of PCBs. The system's inability to filter matrix interference and obtain mass spectral data for confidence in quantification renders the GC-ECD unsuitable for quantification. However, GC-ECD was able to detect all PCBs with total PCBs at 10.5 mg/l using the DIN 12766 method. This is almost ten times lower than the MCL level of total PCBs in oil. GC-ECD can, therefore, be used to screen samples for PCB contamination and save cost per analysis by using a less expensive system with lower running costs.

6.3 Ion trap mass analyser (ITQ)

6.3.1 Instrument performance

6.3.1.1 Experimental

The following trap conditions were optimised to obtain the best possible signals from the complex samples matrix using an ion trap mass analyser:

- maximum ionization time
- scan time
- prescan ionisation time
- ionization storage levels

Background mass selection and rf dump value were also set to ensure unwanted masses were ejected from the ITQ. A narrow mass scanning strategy was also adopted [57].

(a) Twenty micro litre transformer oil was added to 1 ml of hexane spiked with 3.5 µl PCB (10 µg/ml). 10.5 µl $^{13}C_{12}$-PCB internal standards were also added and extracted with 3.5 ml DMSO for 60 seconds. The extract was washed with 0.625 ml hexane and separated. DMSO extract was diluted with water: DMSO (4:1) and 0.5 g of NaCl was added in a 5 ml GC vial. PCBs were extracted with submerged SPME and analysed on a GC-ITQ at 90 ^{o}C and 300 rpm.

6.3.1.2 Results and discussion

Sample recoveries were extremely poor for all PCBs, except PCB 180. The ITQ was unable to filter out noise from the sample matrix and low S/N internal standard peaks were generated, which inflated the PCB concentrations. PCB 180 showed acceptable recoveries due to the fact that most interference was eluted as stated before. I was unable to obtain similar results found in literature indicating that further and better optimisation is required.

Positive identification of PCBs was also a problem because narrow mass range scanning was used as proposed by literature. Ions generated from the calibration and samples were different, which could indicate signs of space-charge effects and matrix interference (Figure 6.3). Most PCB integrations were missed by software integration due to mass spectral differences.

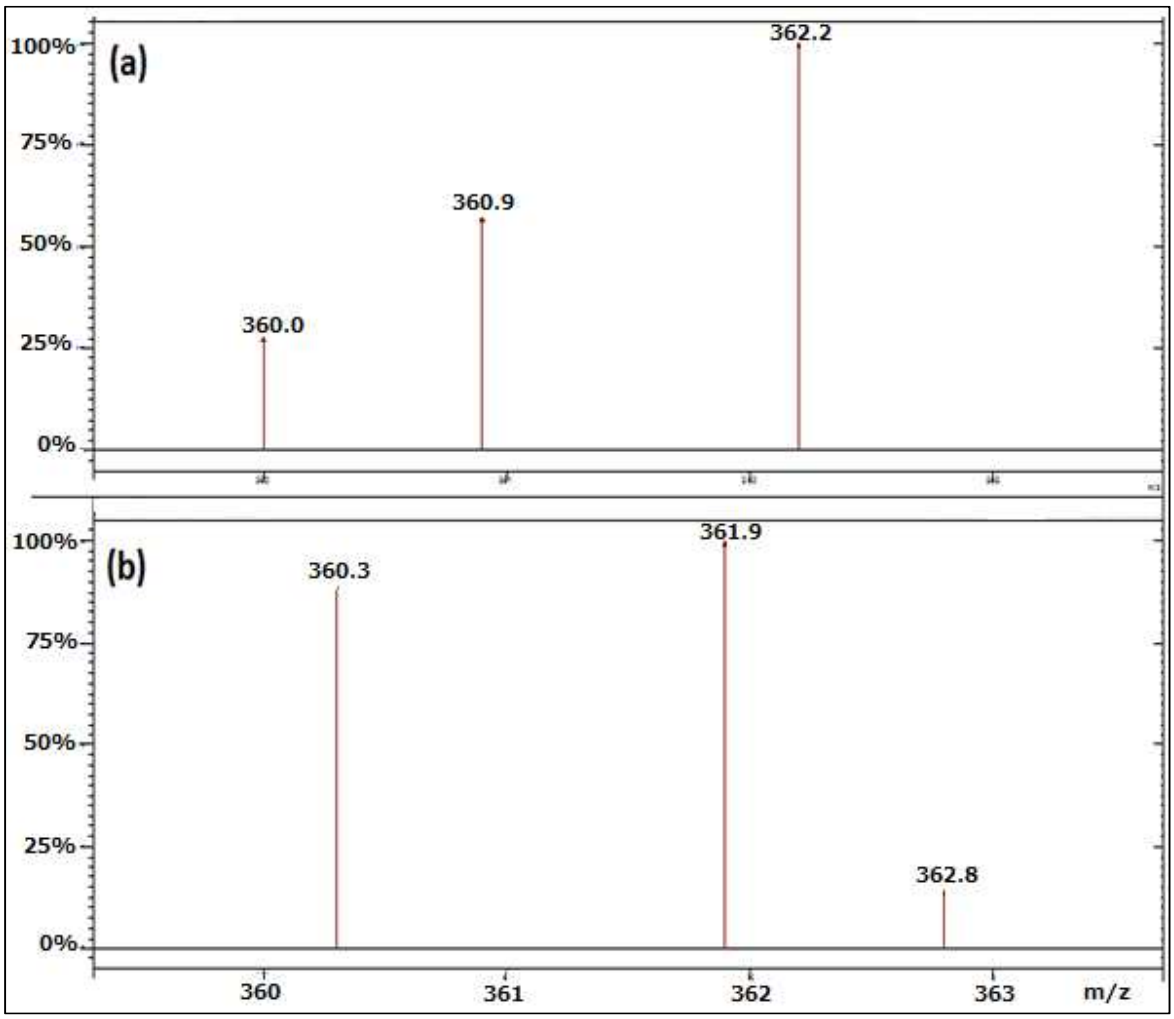


Figure 6.3: Mass spectrum of PCB 153 from a (a) control with no interfering matrix and (b) spiked a sample

6.3.1.3 Conclusion

From the above it is clear that more optimisation is required. Narrow mass scanning limits the positive identification of PCBs from transformer oil, and limited information is obtained from the narrow mass spectral data. Using the ITQ in MS/MS mode would enhance the detection of PCBs, but this functionality was not available with the ITQ used. In a commercial setting, ITQ mass detection may also not be viable using the developed method. The ITQ tends to become 'dirty' when analysing highly contaminated samples, which will increase maintenance on the system. Analysis of PCBs from the DMSO extract using an ITQ (narrow mass scanning) as detector is therefore not fit for the purpose.

6.4 Quadruple mass analyser operated in single ion monitoring (SIM) and multiple ion monitoring (MIM) mode

6.4.1 Instrument performance

6.4.1.1 Experimental

Twenty micro litre transformer oil was added to 1 ml of hexane spiked with 3.5 µl PCB (1 µg/ml). $^{13}C_{12}$-PCB internal standards (10.5 µl) were also added and extracted with 3.5 ml DMSO for 60 seconds. The extract was washed with 0.625 ml hexane and separated. DMSO extract was diluted to water: DMSO (4:1) and 0.5 g of NaCl was added. PCBs were extracted with submerged SPME at 90 ^{o}C and 300 rpm and analysed on a GC-MS (quadruple mass analyser).

6.4.1.2 Results and discussion

Operating the quadruple in multiple ion monitoring (MIM) mode proved successful in detecting all PCBs with a quantification limit of 175 µg/l (table 6.2) per PCB. The overall S/N ratios were low due to matrix interferences. The only drawback in operating in MIM mode for the analysis of PCBs is the level of confidence in terms of mass spectral data. All mass spectral data is generated from only one mass spectrum which lowers confidence and could result in false positive detection of PCBs.

Table 6.2: Quantification limit of PCBs in waste oil analysed using a quadruple in MIM mode

BZ number	Expected concentration(µg/l)	Concentration found (µg/l)	Recovery (%)
28	175	212	121
52	175	208	119
101	175	221	126
153	175	213	122
138	175	210	120
180	175	186	106

6.4.1.3 Conclusion

The quantification limit of total PCBs is almost factor 10 below the regulatory limit, but the low level of identifiers is a drawback using SIM. Multiple m/e ions can be selected from one mass spectrum, but it has its limitations and false negative identification is, therefore, a reality when analysing PCBs in waste oil. MIM using a quadruple can be used, but MS/MS will perhaps be more suited as better selectivity and detectability is obtained for the identification of PCBs in complex matrices through the filtering of chemical noise.

6.5 Quadruple mass analyser operated in MS/MS mode

6.5.1 Instrument performance

6.5.1.1 Experimental

Twenty micro litre transformer oil was added to 1 ml of hexane spiked with 3.5 µl PCB (1 µg/ml). $^{13}C_{12}$-PCB internal standards (10.5 µl) were also added and extracted with 3.5 ml DMSO for 60 seconds. The extract was washed with 0.625 ml hexane and separated. DMSO extract was diluted to water: DMSO (4:1) and 0.5 g of NaCl was added. PCBs were extracted with submerged SPME at 90 ^{o}C and 300 rpm and analysed on a GC-MS/MS (quadruple mass analyser).

6.5.1.2 Results and discussion

Table 6.3 shows the same quantification limit when operating in (MIM) mode at 175 µg/l per PCB. The S/N ratios are dramatically increased due to higher level of filtering when analysing using MS/MS. Increased S/N ratios, however, did not translate into decreased detection limits, indicating a limitation in the extraction method and not instrumental conditions. No repeatable analysis could be conducted at a lower concentration level.

Table 6.3: Detection limit of PCBs in waste oil analysed using a quadruple in MS/MS mode

BZ number	Expected concentration(µg/l)	Concentration found (µg/l)	Recovery (%)
28	175	149	85
52	175	174	100
101	175	221	126
153	175	226	129
138	175	200	114
180	175	189	108

MS/MS fragments are generated through the collision with collision gas (argon for example). The secondary fragmentation event generates daughter ions from parent ions and therefore adds a level of confidence, which limits false negative determination of PCBs (Table 6.4). Each PCB has 5 identifiers, namely retention time, parent (multiple) and daughter ions (multiple).

Table 6.4: Multiple identifiers using MS/MS

BZ number	Retention time(min)	Parent ions (m/z)			Daughter ions (m/z)			Collision energies (ev) for daughter ions		
		1	2	3	1	2	3	1	2	3
PCB 28	28.577	256	258		186	188		25	25	
PCB 52	29.874	292			220	222	257	25	25	5
PCB 101	32.924	324	326		254	256		28	22	
PCB 153	35.676	359	360	372	289	290	372	28	25	5
PCB 138	36.522	359	360	372	289	290	372	*28*	25	5
PCB 180	38.584	394	396		324	326		25	30	

MS/MS adds selectivity to the analysis by filtering non-specific ions (chemical noise). The system is able to identify and quantify PCBs more accurately and generates 'clean' chromatograms through mass filtering (Figure 6.4). The expected decrease in sensitivity is clear when referring to figure 6.4 but also note the increase in the S/N ratio through the filtering of chemical (hydrocarbon) noise.

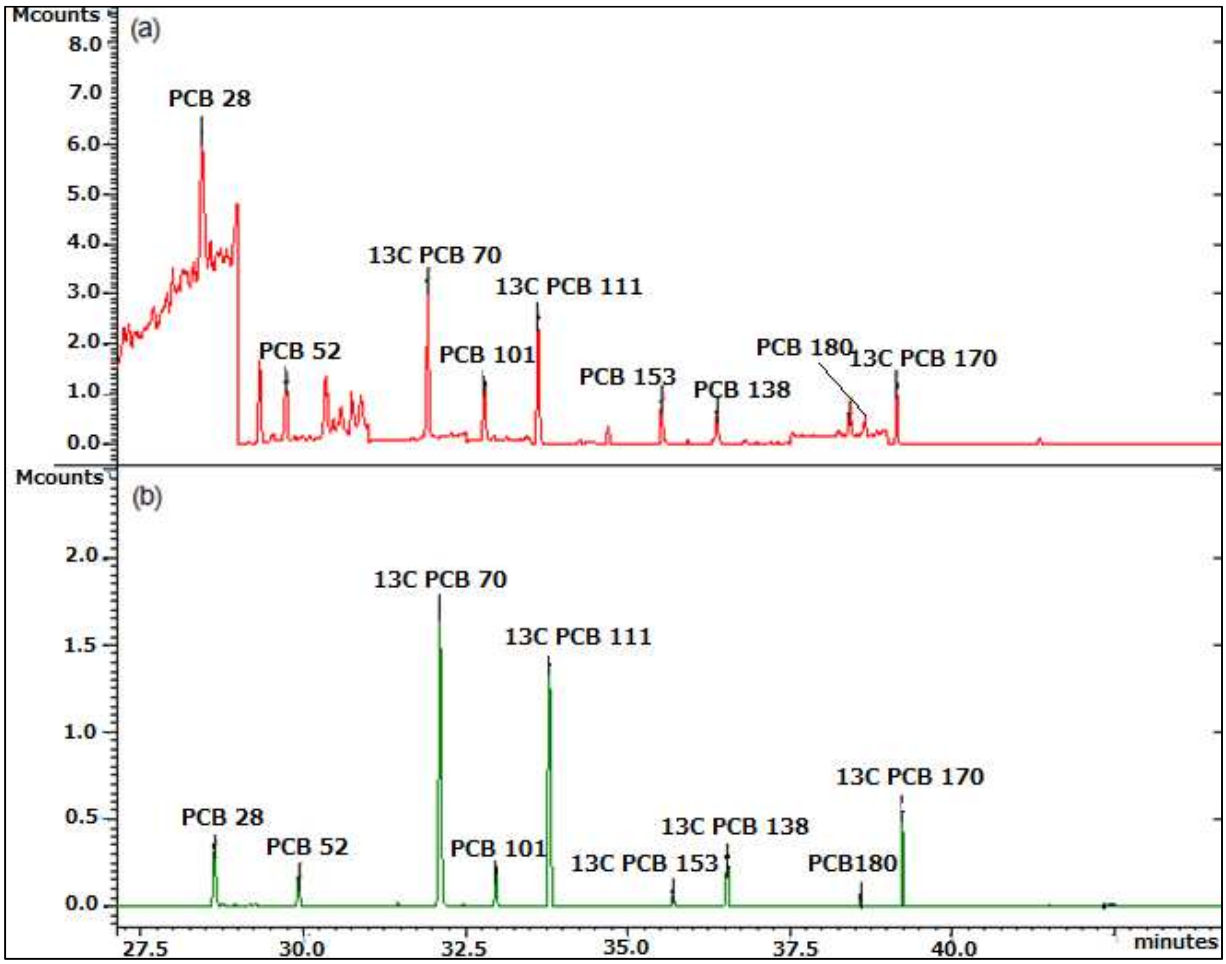


Figure 6.4: Chromatogram of indicator PCBs using (a) MIM and (b) MS/MS (multiple ions table 6.4) for mass detection at the same concentration from a spiked oil sample

4.5.1.3 Conclusion

Mass detection using MS/MS is best suited for PCB analysis using the abovementioned method with limited sample clean-up. Triple quadruples are also commercially viable as they have multiple applications and are easy to maintain – the system is ready to use within three hours of start-up. The only drawback of using triple quadruple mass analysers is the relative large cost of acquisition.

6.6 Conclusion

The extraction procedure of extracting PCBs from waste oil involves limited sample clean-up. Numerous systems exist for PCBs analysis, but only a GC-MS/MS offers the selectivity and sensitivity required for PCB analysis in difficult matrices. A triple quadruple is an expensive instrument to buy, but saves time in terms of sample preparation. Sample preparation is one of the most time-consuming elements in commercial analysis. Cost can further be reduced by using a GC-EμCD for screening of samples.

A GC-ECD is:

- inexpensive to buy;
- inexpensive to maintain;
- inexpensive to operate, using limited electricity and N_2 as carrier gas; and
- extremely sensitive for halogenated organic compounds (PCBs).

Most samples submitted, environmental (soil and waste) and waste oils, do not contain any PCBs as a result of a halt in production in 1979. At current legislations levels a GC-ECD can be used to screen samples, providing results either above or below legislation levels. Only when a sample is positive should a triple quadruple be used for conformation of PCB presence and quantification.

Chapter 7

Conclusion

7.1 Conclusion

The aim of this study was to develop a method for the analysis of PCBs in waste oil and soil samples. This method is intended for introduction into a commercial laboratory and therefore needs to be:

- fast;
- uncomplicated;
- economical; and
- robust.

Partitioning of PCBs into DMSO through liquid-liquid extraction occurs at a rapid rate, with 87% indicator PCBs extracted in 60 seconds. Only one 60-second wash step is used to remove 83% of the residual interfering matrices (oil) that are co-extracted into the DMSO. No concentration or phase transfers are required for oil samples and an additional ASE step is required for soil samples. Submerged SPME is used to extract PCBs from SPME-compatible polar and aprotic DMSO.

This method is extremely robust and was successful in extracting lipophillic PCBs from various high lipophilic content matrices:

- soil
- transformer oil
- motor oil
- fish oil
- vacuum pump oil

A quality result is obtained through detection using GC-MS/MS. This instrument has good selectivity and detectability towards PCBs. GC-MS/MS is able to filter matrix interference, which is essential for accurate quantification of PCBs in a sample that has undergone limited sample clean-up.

Cost per analysis is kept at a minimum through the use of a simple liquid-liquid extraction of PCBs, only using 5.125 ml of solvents. Partitioning of PCBs is fast using liquid-liquid extraction coupled with submerged SPME at non-equilibrium conditions, cost per analysis is therefore low due to fast sample preparation and extraction. The simplicity of the extraction procedure (LLE and SPME) allows the commercial laboratories to use inexperienced technicians to conduct extractions and keep cost per analysis low. Employing a GC-ECD to screen samples for PCB contamination further lowers the cost per analysis as the majority of samples can be expected to be of low PCB content below the 50 mg/l threshold specified for waste oils. An SPME compatible autosampler is required for this method but working second-hand autosamplers can be bought at costs as low as R50 000 but can be as high as R300 000 for a new system. This method also has a quantification limit of 5.25 mg/l for total PCBs, which is almost ten times lower than the allowable MCL in waste oil. A detection limit of 35 µg/kg in 30 g of soil for total PCBs is 17 times lower than the allowable MCL for soil that is protective of water resources (Table 1.1).

Table 7.1 compares the Supelco® SPE method (routinely used by UIS Organic laboratory) with the newly developed method and clearly shows the advantage of the new method in a commercial laboratory. The new method is 39% faster than the Supelco® method, profit margins are higher per sample with lower expenditure on consumables as well as a decreased quoted cost to customers.

Table 7.1: Comparison of Supelco® SPE and newly developed method

	Supelco ® SPE	New method
Time spent on sample prep before extraction (per 10 samples)	40 min	40 min
Time spent on extraction (10 samples)	80 min	40 min
Time spent on sample preparation for instrumental analysis	60 min	30 min
Cost of extraction consumables per analysis	Proprietary information	14 times less
Quoted cost to customer	Proprietary information	27% less

Extraction consumables consist of the cost of hexane, DMSO, Supelclean ®SPE phase, SPME fibers and technician/laboratory assistant time

Normally a commercial laboratory would need to sacrifice one element of the value triangle (Figure 7.1). The quality of the result is usually sacrificed to obtain a faster analysis at low cost in a commercial laboratory. The newly developed method can be placed in the middle of this value triangle:

- quality result; ✔
- fast analysis; and ✔
- low cost per sample. ✔

Table 7.1 clearly shows how the new method will increase sample throughput and lower cost of analysis which give laboratories using this method in a commercial laboratory the competitive edge against other laboratories. The developed method is therefore fit for purpose in a commercial environment.

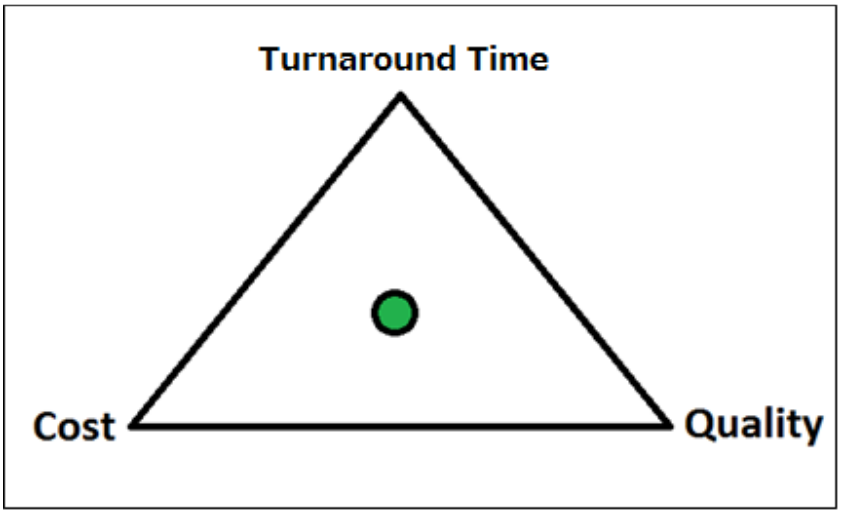


Figure 7.1: Value triangle for developed method [58]

7.2 Future works

The accreditation and participation in proficiency testing will have to be completed as it is a requirement of most South African environmental law as stated before [9].This method can potentially be used in the analysis of biological samples (fish) for PCB contamination, and the same DMSO LLE methodology coupled with submerged SPME GC-MS/MS in industrial areas can be applied. Extraction efficiency would be similar due to the method's proven robustness. Areas of contamination can therefore be mapped and possible sources can be identified.

In commercial laboratories the combination of tests saves time in terms of sample preparation and analysis costs. Dioxin, PCB, furan and PAH analysis are traditionally expensive and extremely difficult in high hydrocarbon concentration matrices. These compounds can possibly be extracted and analysed with the same LLE SPME procedure in one analytical run, reducing the cost of analysis for commercial laboratories. This would only be achievable using longer columns for separations of co-eluting compounds coupled with GC-MS/MS.

References

1 Afful, S., Awudza, J.A.M., Twumasi, S.K., Osae, S., 2013. Determination of Indicator Polychlorinated Biphenyls (PCBs) by Gas Chromatography-Electron Capture Detector. *Chemos*, 93, 1556-1560

2 O'Neil, M.J., Heckelman, P.E., Koch C.B., Roman, K.J., 2006. *The Merck Index*, 14th ed., Merck Research Laboratories, Whitehouse Station, NJ. ISBN 9780911910001.

3 Fiedler, H. Polychlorinated Biphenyls: Uses and Environmental Releases, URL: http://www.chem.unep.ch/pops/pops_inc/proceedings/bangkok/fiedler1.html. [Accessed 02 June 2014.]

4 UNEP., 2011. Stockholm convention on Persistent Organic Pollutants – Draft Revised Guidance on the Global Monitoring Plan for Persistent Organic Pollutants. Geneva, UNEP/POPs/COP.5/INF/27.

5 UNEP., 1999. Guidelines for the Identification of PCBs and Materials Containing PCBs. United Nations Environmental Programme, Geneva, Switzerland.

6 Basel Convention Series: Technical Guidelines. Technical Guidelines for the Environmentally Sound Management of Wastes Consisting of, Containing or Contaminated with Polychlorinated Biphenyls (PCBs), Polychlorinated Terphenyls (PCTs) or Polybrominated Biphenyls (PBBs). *BASEL Convention Series*. SBC.Nr.2005/2.

7 Christan, E., Janse, J., 2005. EuroPCB: Inventory PCB Enforcement in Member States. Part 1.

8 South African Department of Environmental Affairs., 2012. National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants.

9 *Government Gazette*., 2014. National Norms and Standards for the Remediation of Contaminated Land and Soil Quality. Pretoria. Vol. 587, 37603.

10 *Government Gazette*., 2013. WASTE CLASSIFICATION AND MANAGEMENT REGULATIONS. Pretoria. Vol. 578, 36784.

11 Hu, J., Zheng, M., Liu, W., Nie, Z., Li, C., Liu, G., Xiao, K., 2014. Characterization of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans, Dioxin-like Polychlorinated Biphenyls, and Polychlorinated Naphthalenes in the Environment Surrounding Secondary Copper and Aluminium Metallurgical Facility in China. *Environmental Pollution*, 193, 6-12.

12 Chen, T., Li, X., Yan, J., Jin, Y., 2009. Polychlorinated Biphenyls Emission from a Medical Waste Incinerator in China. *Journal of Hazardous Material*, 172, 1339-1343.

13 McGee, P., McAliney, P., 2007. Painless Project Management, a Step-by-step Guide for Planning, Executing and Managing Projects. John Wiley and Sons, Inc., Hoboken, NJ. ISBN 97880470117217.

14 http://powerlinkoil.com/knowledge-base-transformer-oil-insulating-oil/ [Accessed on 20/07/2014.]

15 Hamilton, M.C., Hites, R.A., Schwager, S.J., Foran, J.A., Knuth, B.A., Carpenter, D.O., 2005. Lipid Composition and Contaminants in Farmed and Wild Salmon. *Environ.Sci.Technol*, 39, 8622-8629.

16 http://lipidlibrary.aocs.org/Lipids/tag1/index.htm [Accessed on 20/07/2014.]

17 Ballschmitter, K., Zell, M., 1980. Analysis of Polychlorinated biphenyls (PCB) by Glass Capillary Gas Chromatography. Frensenius Z*J. Anal. Chem*, 302, 20-31.

18 Guitart, R., Puig, P., Gomez-Catalan., 1993. Requirement for Standardized Nomenclature Criterium for PCBs: Computer-Assisted Assignment of Correct Denomination and Numbering. *Chemos*, 27, No.8, 1451-1459

19 Takasuga, T., Senthilkumar, K., Matsumura, T., Shiozaki, K., Sakai, S., 2006. Isotope Dilution Analysis of Polychlorinated Biphenyls (PCBs) in Transformer Oil and Global Commercial PCB Formulations by High Resolution Gas Chromatography-high Resolution mass spectrometry. *Chemos*, 62, 469-484

20 Baker, N.A., 2013. Polychlorinated Biphenyl Ligands of the Aryl Hydrocarbon Receptor Promote Adipocyte-Mediated Diabetes. University of Kentucky, Thesis and Dissertations-Nutritional sciences. Paper7

21 Ahlborg, U.G., Becking, G.C., Birnbaum, L.S., Brouwer, A., Derks, H.J.G.M., Golor, G., Hanberg, A., Larsen, J.C., Liem, A.K.D., Safe, S.H., Waern, F., Younes, M., Yrjanheikki, E., 1994. Toxic Equivalency Factors for Dioxin-Like PCBs. *Chemos, Vol*. 28. No. 6, 1049-1067

22 Pessah, N.I., Hansen, G.L., Albertson, T.E., Garner, C.E., Ta, T.A., Do, Z., Kim, K.H., Wong, P.W., 2006. Structure-Activity Relationship for Noncoplanar Polychlorinated Biphenyl Congeners towards the Ryanodine Receprot-Ca^{2+} Channel Complex Type 1 (RyR1). *Chem.Res.Toxicol*, 19, 92-101

23 Henry, T.R., DeVito, M.J. 2003. Non-Dioxin-like PCBs: Effects and Considerations in Ecological Risk Assessments. Ecological Risk Assessments Support Centre. ERASC-003

24 The Chemical Industry Archives., 9 January 2002. The Inside Story: Anniston, AL in-depth. http://www.chemicalindustryarchives.org/dirtysecrets/annistonindepth/indepth.pdf. [Accessed on 14 July 2014.]

25 EPA. Technical Fact Sheet on: Polychlorinated biphenyls (PCBs).

26 Berdard, D.L., May, R.J., 1996. Characterization of Polychlorinated Biphenyls on the Sediments of Woods Pond: Evidence for Microbial Dechlorination of Aroclor 1260 in Situ. *Environ.Sci.Technol*, 30, 237-245.

27 U.S Department of Health and Humans Services.,2000. Toxicological Profile for Polychlorinated Biphenyls (PCBs).

28 Li,N., Wania, F., Lei, Y. D., Daly , G.L., 2003. A Comprehensive and Critical Compilation, Evaluation, and Selection of Physical-Chemical Property Data for Selected Polychlorinated Biphenyls. *J.Phys.Chem.Ref.Data, Vol*. 32, No 4

29 Kuzyk, Z.Z.A., 2000. Bioaccumulation of PCBs from Contaminated Sediments in a Coastal Marine Ecosystem of Northern Labrador. MSc thesis, Queen's University Kingston, Ontario, Canada.

30 EFSA., 2005. The Opinion of the Scientific Panel on Contaminates in the Food Chain on a Request from the Commission Related to the Presence of Non-Dioxin-like Polychlorinated Biphenyls (PCBs) in Feed and Food. *The EFSA Journal, 284*, 1-137.

31 Duc, V.T., Viet, H.S., 2013. Residue and Temporal Trend of Polychlorinated Biphenyls (PCBs) in Surface Soils from Bacbibh, Vietnam. *International Journal of Environmental, Ecological, Geological and Mining Engineering*, Vol 7, No 7.

32 Domotorova, M., Sejakova, Z.S., Kocan, A., Conka, K., Chovancova, J., Fabisikova, A., 2012. PCDDs, PCDFs, Dioxin-like and Indicator PCBs in Soil from 5 Selected Areas in Slovakia. *Chemos*, 89, 480-485.

33 Kim, M., Kim, S., Yun, S., Lee, M., Cho, B., Park, J., Son, S., Kim, O., 2004. Comparison of Seven Indicator PCBs and Three Coplanar PCBs in Beef, Pork and Chicken Fat. *Chemos*, 54, 1533-1538.

34 Skrbic, B., Szyrwinska, K., Durisic-Mladenovic, N., Nowicki, P., Lulek, J., 2010. Principal Component Analysis of Indicator PCB Profiles in Breast Milk from Poland. *Environment International*, 36, 862-872

35 DIN EN 12766-2., 2001. Determination of PCBs and Related Products, Part 2: Calculation of Polychlorinated (PCB Content).

36 Institute for Interlaboratory Sudies Spijkenisse, the Netherlands., 2012. Results of Proficiency Test PCB in (Mineral Oil), November 2011. Report iis11Lo5.

37 Environmental Protection Series.,1997. Reference Method for the Analysis of Polychlorinated Biphenyls (PCBs). Canada. Report EPS 1/RM/31 March 1997

38 Solomons, T.W.G., Fryhle, C.B., 2008. *Organic Chemistry*, 9th Edition, John Wiley & Sons Inc. USA. ISBN-13: 97804716684961

39 Larsen, R. Tilio, R. Kapila, S., 1991. A DMSO-Based Cleanup Procedure for Determination of PCBs in Waste Oil. *Chemos*, 3, 1077-1084

40 Numata, M., Kaneko, T., Mi, Q., Ye, M., Kawamata, S., Matsuo, M., Yartita, T., 2008. Preparation of a Sulfoxide Group and Ammonium-salt Bonded Silica Stationary Phase for the Separation of Polychlorinated Biphenyls from Mineral Oils. *J. Chromatogr. A*, 1210, 68-75.

41 SUPELCO. Supelclean Sulfoxide SPE Product Line. T707009.

42 Mi, C., Kaneko, T., Ye, M., Numata, M. Analysis of PCBs in Transformer Oil with Sulfoxide - bonded SPE Phase and GC-MS. Supleco, Div. of Sigma-Aldrich. T408040

43 Cariado, M.R., Pereiro, I.R., Torrijos, R.C., 2004. Selective Determination of Polychlorinated Biphenyls in Waste Oils Using Liquid-liquid Partition Followed by Headspace Solid Phase Microextraction and Gas Chromatography with Atomic Emission detection. *J. Chromatography .A*, 1056, 263-266.

44 Pawliszyn, J., 1997. Solid Phase Microextraction – Theory and Practice. Wiley-VCH, Canada. ISBN 0471190349.

45 Lord, H., Pawliszyn, J., 2000. Evolution of Solid-phase Microextraction Technology. *J. Chromatography . A,* 885, 153-193.

46 De Perre, C., Le Menach, K., Ibalot, F., Parlanti, E., Budzinski, H., 2014. Development of Solid-phase Microextraction to Study Dissolved Organic Matter – Polyaromatic Hydrocarbon Interactions in Aquatic Environment. *Analytica Chimica Acta*, 807, 51-60.

47 Tankiewicz, M.,Morrison, C., Biziuk, M., 2013. Application and Optimization of Headspace Solid-phase Microextraction (HS-SPME) Coupled with Gas Chromatography – Flame-Ionization Detector (GC-FID) to Determine Products of the Petroleum Industry in Aqueous Samples. *Microchemical Journal*, 108, 117-123.

48 ET AL., 1998. Supelco Bulletin 923 – Solid Phase Microextraction: Theory and Optimization of Conditions. Sigma-Aldrich Co. T198923.

49 Pawliszyn, J., 2012. Handbook of Solid Phase Microextraction. Elsevier, Electronic Edition.DOI: 10.1016/B978-0-12-416017-0.00016-4

50 Rainawati, E., Balasubramanian, R., 2009. Optimization and Validation of Solid Phase Micro-extraction (SPME) Method for Analysis of Polycyclic Aromatic Hydrocarbons in Rainwater and Stormwater. *Physics and Chemistry of the Earth*, 34, 857-865.

51 Lambropoulou, D.A., Albanis, T.A., 2001. Optimization of Headspace Solid-phase Microextraction Conditions for the Determination of Organophosphorus Insecticides in Natural Waters. J. *Chromatography. A*, 992, 243-255.

52 Shirey, R.E., Mindrup, R.F., 1999. SPME-Adsorption Versus Absorption: Which is Best for Your Application?. *Supelco*, Sigma-Aldrich Co. T400011

53 Sporkert, F., Pragst, D., 2000. Use of Headspace Solid-phase Microextraction (HS-SPME) in Hair Analysis for Organic Compounds. *Forensic Science International*, 107, 129-148.

54 Restek. EZGC Chromatographic modeller. http://www.restek.com/ezgc-cm. [Accessed on 17/08/2014.]

55 Johansen, S.S., Pawliszyn, J., 1996. Trace Analysis of Hetro Aromatic Compounds (NSO) in Water and Polluted Ground Water by Solid Phase Micro-extraction (SPME). *J.High Resol. Chromatogr. Vol* 19, 627-632

56 Johansen, S.S., Pawliszyn, J., 1999. Applications of Solid Phase Microextraction. DOI:10.1039/9781847550149-00238

57 Salomon, K., Buttrill, S.E., 1993. Optimization of ion trap parameters for the analysis of dilute samples in the presence of an interfering matrix – Analysis of Polychlorinated Biphenyls in Transformer Oil. *J. Chromatogr.A*, 657, 139-145.

Appendix A

Table of 209 PCB congeners and corresponding BZ numbers [4]

BZ number	IUPAC name
1	2-Chlorobiphenyl
2	3-Chlorobiphenyl
3	4-Chlorobiphenyl
4	2,2'-Dichlorobiphenyl
5	2,3-Dichlorobiphenyl
6	2,3'-Dichlorobiphenyl
7	2,4-Dichlorobiphenyl
8	2,4'-Dichlorobiphenyl
9	2,5-Dichlorobiphenyl
10	2,6-Dichlorobiphenyl
11	3,3'-Dichlorobiphenyl
12	3,4-Dichlorobiphenyl
13	3,4'-Dichlorobiphenyl
14	3,5-Dichlorobiphenyl
15	4,4'-Dichlorobiphenyl
16	2,2',3-Trichlorobiphenyl
17	2,2',4-Trichlorobiphenyl
18	2,2',5-Trichlorobiphenyl
19	2,2',6-Trichlorobiphenyl
20	2,3,3'-Trichlorobiphenyl
21	2,3,4-Trichlorobiphenyl
22	2,3,4'-Trichlorobiphenyl
23	2,3,5-Trichlorobiphenyl
24	2,3,6-Trichlorobiphenyl
25	2,3',4-Trichlorobiphenyl
26	2,3',5-Trichlorobiphenyl
27	2,3',6-Trichlorobiphenyl
28	2,4,4'-Trichlorobiphenyl
29	2,4,5-Trichlorobiphenyl
30	2,4,6-Trichlorobiphenyl
31	2,4',5-Trichlorobiphenyl

32	2,4',6-Trichlorobiphenyl
33	2,3',4'-Trichlorobiphenyl
34	2,3',5'-Trichlorobiphenyl
35	3,3',4-Trichlorobiphenyl
36	3,3',5-Trichlorobiphenyl
37	3,4,4'-Trichlorobiphenyl
38	3,4,5-Trichlorobiphenyl
39	3,4',5-Trichlorobiphenyl
40	2,2',3,3'-Tetrachlorobiphenyl
41	2,2',3,4-Tetrachlorobiphenyl
42	2,2',3,4'-Tetrachlorobiphenyl
43	2,2',3,5-Tetrachlorobiphenyl
44	2,2',3,5'-Tetrachlorobiphenyl
45	2,2',3,6-Tetrachlorobiphenyl
46	2,2',3,6'-Tetrachlorobiphenyl
47	2,2',4,4'-Tetrachlorobiphenyl
48	2,2',4,5-Tetrachlorobiphenyl
49	2,2',4,5'-Tetrachlorobiphenyl
50	2,2',4,6-Tetrachlorobiphenyl
51	2,2',4,6'-Tetrachlorobiphenyl
52	2,2',5,5'-Tetrachlorobiphenyl
53	2,2',5,6'-Tetrachlorobiphenyl
54	2,2',6,6'-Tetrachlorobiphenyl
55	2,3,3',4-Tetrachlorobiphenyl
56	2,3,3',4'-Tetrachlorobiphenyl
57	2,3,3',5-Tetrachlorobiphenyl
58	2,3,3',5'-Tetrachlorobiphenyl
59	2,3,3',6-Tetrachlorobiphenyl
60	2,3,4,4'-Tetrachlorobiphenyl
61	2,3,4,5-Tetrachlorobiphenyl
62	2,3,4,6-Tetrachlorobiphenyl
63	2,3,4',5-Tetrachlorobiphenyl
64	2,3,4',6-Tetrachlorobiphenyl
65	2,3,5,6-Tetrachlorobiphenyl
66	2,3',4,4'-Tetrachlorobiphenyl
67	2,3',4,5-Tetrachlorobiphenyl
68	2,3',4,5'-Tetrachlorobiphenyl
69	2,3',4,6-Tetrachlorobiphenyl

70	2,3',4',5-Tetrachlorobiphenyl
71	2,3',4',6-Tetrachlorobiphenyl
72	2,3',5,5'-Tetrachlorobiphenyl
73	2,3',5',6-Tetrachlorobiphenyl
74	2,4,4',5-Tetrachlorobiphenyl
75	2,4,4',6-Tetrachlorobiphenyl
76	2,3',4',5'-Tetrachlorobiphenyl
77	3,3',4,4'-Tetrachlorobiphenyl
78	3,3',4,5-Tetrachlorobiphenyl
79	3,3',4,5'-Tetrachlorobiphenyl
80	3,3',5,5'-Tetrachlorobiphenyl
81	3,4,4',5-Tetrachlorobiphenyl
82	2,2',3,3',4-Pentachlorobiphenyl
83	2,2',3,3',5-Pentachlorobiphenyl
84	2,2',3,3',6-Pentachlorobiphenyl
85	2,2',3,4,4'-Pentachlorobiphenyl
86	2,2',3,4,5-Pentachlorobiphenyl
87	2,2',3,4,5'-Pentachlorobiphenyl
88	2,2',3,4,6-Pentachlorobiphenyl
89	2,2',3,4,6'-Pentachlorobiphenyl
90	2,2',3,4',5-Pentachlorobiphenyl
91	2,2',3,4',6-Pentachlorobiphenyl
92	2,2',3,5,5'-Pentachlorobiphenyl
93	2,2',3,5,6-Pentachlorobiphenyl
94	2,2',3,5,6'-Pentachlorobiphenyl
95	2,2',3,5',6-Pentachlorobiphenyl
96	2,2',3,6,6'-Pentachlorobiphenyl
97	2,2',3,4',5'-Pentachlorobiphenyl
98	2,2',3,4',6'-Pentachlorobiphenyl
99	2,2',4,4',5-Pentachlorobiphenyl
100	2,2',4,4',6-Pentachlorobiphenyl
101	2,2',4,5,5'-Pentachlorobiphenyl
102	2,2',4,5,6'-Pentachlorobiphenyl
103	2,2',4,5',6-Pentachlorobiphenyl
104	2,2',4,6,6'-Pentachlorobiphenyl
105	2,3,3',4,4'-Pentachlorobiphenyl
106	2,3,3',4,5-Pentachlorobiphenyl
107	2,3,3',4',5-Pentachlorobiphenyl

108	2,3,3',4,5'-Pentachlorobiphenyl
109	2,3,3',4,6-Pentachlorobiphenyl
110	2,3,3',4',6-Pentachlorobiphenyl
111	2,3,3',5,5'-Pentachlorobiphenyl
112	2,3,3',5,6-Pentachlorobiphenyl
113	2,3,3',5',6-Pentachlorobiphenyl
114	2,3,4,4',5-Pentachlorobiphenyl
115	2,3,4,4',6-Pentachlorobiphenyl
116	2,3,4,5,6-Pentachlorobiphenyl
117	2,3,4',5,6-Pentachlorobiphenyl
118	2,3',4,4',5-Pentachlorobiphenyl
119	2,3',4,4',6-Pentachlorobiphenyl
120	2,3',4,5,5'-Pentachlorobiphenyl
121	2,3',4,5',6-Pentachlorobiphenyl
122	2,3,3',4',5'-Pentachlorobiphenyl
123	2,3',4,4',5'-Pentachlorobiphenyl
124	2,3',4',5,5'-Pentachlorobiphenyl
125	2,3',4',5',6-Pentachlorobiphenyl
126	3,3',4,4',5-Pentachlorobiphenyl
127	3,3',4,5,5'-Pentachlorobiphenyl
128	2,2',3,3',4,4'-Hexachlorobiphenyl
129	2,2',3,3',4,5-Hexachlorobiphenyl
130	2,2',3,3',4,5'-Hexachlorobiphenyl
131	2,2',3,3',4,6-Hexachlorobiphenyl
132	2,2',3,3',4,6'-Hexachlorobiphenyl
133	2,2',3,3',5,5'-Hexachlorobiphenyl
134	2,2',3,3',5,6-Hexachlorobiphenyl
135	2,2',3,3',5,6'-Hexachlorobiphenyl
136	2,2',3,3',6,6'-Hexachlorobiphenyl
137	2,2',3,4,4',5-Hexachlorobiphenyl
138	2,2',3,4,4',5'-Hexachlorobiphenyl
139	2,2',3,4,4',6-Hexachlorobiphenyl
140	2,2',3,4,4',6'-Hexachlorobiphenyl
141	2,2',3,4,5,5'-Hexachlorobiphenyl
142	2,2',3,4,5,6-Hexachlorobiphenyl
143	2,2',3,4,5,6'-Hexachlorobiphenyl
144	2,2',3,4,5',6-Hexachlorobiphenyl
145	2,2',3,4,6,6'-Hexachlorobiphenyl

146	2,2',3,4',5,5'-Hexachlorobiphenyl
147	2,2',3,4',5,6-Hexachlorobiphenyl
148	2,2',3,4',5,6'-Hexachlorobiphenyl
149	2,2',3,4',5',6-Hexachlorobiphenyl
150	2,2',3,4',6,6'-Hexachlorobiphenyl
151	2,2',3,5,5',6-Hexachlorobiphenyl
152	2,2',3,5,6,6'-Hexachlorobiphenyl
153	2,2',4,4',5,5'-Hexachlorobiphenyl
154	2,2',4,4',5,6'-Hexachlorobiphenyl
155	2,2',4,4',6,6'-Hexachlorobiphenyl
156	2,3,3',4,4',5-Hexachlorobiphenyl
157	2,3,3',4,4',5'-Hexachlorobiphenyl
158	2,3,3',4,4',6-Hexachlorobiphenyl
159	2,3,3',4,5,5'-Hexachlorobiphenyl
160	2,3,3',4,5,6-Hexachlorobiphenyl
161	2,3,3',4,5',6-Hexachlorobiphenyl
162	2,3,3',4',5,5'-Hexachlorobiphenyl
163	2,3,3',4',5,6-Hexachlorobiphenyl
164	2,3,3',4',5',6-Hexachlorobiphenyl
165	2,3,3',5,5',6-Hexachlorobiphenyl
166	2,3,4,4',5,6-Hexachlorobiphenyl
167	2,3',4,4',5,5'-Hexachlorobiphenyl
168	2,3',4,4',5',6-Hexachlorobiphenyl
169	3,3',4,4',5,5'-Hexachlorobiphenyl
170	2,2',3,3',4,4',5-Heptachlorobiphenyl
171	2,2',3,3',4,4',6-Heptachlorobiphenyl
172	2,2',3,3',4,5,5'-Heptachlorobiphenyl
173	2,2',3,3',4,5,6-Heptachlorobiphenyl
174	2,2',3,3',4,5,6'-Heptachlorobiphenyl
175	2,2',3,3',4,5',6-Heptachlorobiphenyl
176	2,2',3,3',4,6,6'-Heptachlorobiphenyl
177	2,2',3,3',4,5',6'-Heptachlorobiphenyl
178	2,2',3,3',5,5',6-Heptachlorobiphenyl
179	2,2',3,3',5,6,6'-Heptachlorobiphenyl
180	2,2',3,4,4',5,5'-Heptachlorobiphenyl
181	2,2',3,4,4',5,6-Heptachlorobiphenyl
182	2,2',3,4,4',5,6'-Heptachlorobiphenyl
183	2,2',3,4,4',5',6-Heptachlorobiphenyl

184	2,2',3,4,4',6,6'-Heptachlorobiphenyl
185	2,2',3,4,5,5',6-Heptachlorobiphenyl
186	2,2',3,4,5,6,6'-Heptachlorobiphenyl
187	2,2',3,4',5,5',6-Heptachlorobiphenyl
188	2,2',3,4',5,6,6'-Heptachlorobiphenyl
189	2,3,3',4,4',5,5'-Heptachlorobiphenyl
190	2,3,3',4,4',5,6-Heptachlorobiphenyl
191	2,3,3',4,4',5',6-Heptachlorobiphenyl
192	2,3,3',4,5,5',6-Heptachlorobiphenyl
193	2,3,3',4',5,5',6-Heptachlorobiphenyl
194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl
195	2,2',3,3',4,4',5,6-Octachlorobiphenyl
196	2,2',3,3',4,4',5,6'-Octachlorobiphenyl
197	2,2',3,3',4,4',6,6'-Octachlorobiphenyl
198	2,2',3,3',4,5,5',6-Octachlorobiphenyl
199	2,2',3,3',4,5,5',6'-Octachlorobiphenyl
200	2,2',3,3',4,5,6,6'-Octachlorobiphenyl
201	2,2',3,3',4,5',6,6'-Octachlorobiphenyl
202	2,2',3,3',5,5',6,6'-Octachlorobiphenyl
203	2,2',3,4,4',5,5',6-Octachlorobiphenyl
204	2,2',3,4,4',5,6,6'-Octachlorobiphenyl
205	2,3,3',4,4',5,5',6-Octachlorobiphenyl
206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl
207	2,2',3,3',4,4',5,6,6'-Nonachlorobiphenyl
208	2,2',3,3',4,5,5',6,6'-Nonachlorobiphenyl
209	Decachlorobiphenyl