DECLARATION

Analysis of selected organic pollutants in water using various concentration techniques

Sayjil Rohith Ramphal

I hereby declare that the dissertation herewith represents my own work. It has not been submitted before for any diplome/degree or examination at any other Technicon or University

______________________ ______________________

Sayjil Rohith Ramphal Date

I hereby approve the submission of the following dissertation.

______________________ ______________________

Prof K.G. Moodley Mr. D.K.Chetty

This __________ of __________ 2014, at the Durban University of Technology

DEDICATION

This thesis is dedicated to

LORD SRI KRISHNA

‘*Hare Krishna Hare Krishna Krishna Krishna Hare Hare*

Hare Rama Hare Rama Rama Rama Hare Hare’

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PRESENTATIONS AND PUBLICATIONS

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Conference presentations

- K G MOODLEY, D K CHETTY, S R CHETTY, S R RAMPHAL AND S CHELLAKOOTTY. Pre-concentration techniques for the analysis of trace levels of nitrosamines, dichlorobenzenes and butylated hydroxytoluene in water samples. Oral Presentration. 4th World Aqua Congress, India Habitat Centre, New Delhi, India, 8th – 10th December 2010

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- K G MOODLEY, D K CHETTY, S R CHETTY, S R RAMPHAL AND G GERICKE. Determination of trace chlorinated benzenes in Dam Water. Poster Presentation. Singapore International Water Week, Suntec Singapore International Convention & Exhibition Centre, Singapore, 4th – 8th July 2011

ABSTRACT

Among persistent organic pollutants, chlorobenzenes are some of the most frequently encountered compounds in aqueous systems. These compounds can enter the environment via natural and anthropogenic sources, and are ubiquitous due to their extensive use over the past several decades. Several chlorobenzene compounds, once in the environment, can biologically accumulate, and are reputed to be carcinogens and extremely hazardous to health. Several chlorobenzenes are listed as priority pollutants by the United States Environmental Protection Agency. Excessive exposure to these compounds affects the central nervous system, irritates skin and upper respiratory tract, hardens skin and leads to haematological disorders including anaemia. In spite of these harmful effects, chlorobenzenes are still used widely as process solvents and raw materials in the manufacture of pesticides, chlorinated phenols, lubricants, disinfectants, pigments and dyes. In the light of the above, it is imperative to monitor the levels of chlorinated benzenes in all types of surface waters, using low-cost but sensitive methods of preconcentration and detection.

In this study, a simple and relatively cheap preconcentration method using direct immersion solid phase microextraction (DI-SPME) followed by gas chromatography equipped with a flame ionisation detector (GC-FID) was developed for the analysis of 7 chlorinated benzenes in dam water. Experimental parameters affecting the extraction efficiency of the selected chlorobenzenes, such as fibre type, sample size, rate of agitation, salting-out effect and extraction time, were optimised and applied to the Grootdraai Dam water samples. The optimised method comprises the use of a 100 µm polydimethylsiloxane (PDMS) fibre coating; 5 ml sample size; 700 revolutions per minute rate of agitation and an extraction time of 30 minutes. The calibration curves were linear with correlation coefficients ranging from 0.9957–0.9995 for a concentration range of 1–100 ng/mℓ. The respective limits of detection and quantification for each analyte was as follows: 1,3-dichlorobenzene, 0.02 and 0.2 ng/mℓ; 1,4-dichlorobenzene, 0.04 and 0.4 ng/mℓ; 1,2-dichlorobenzene, 0.02 and 0.2 ng/mℓ; 1,2,4-trichlorobenzene, 0.3 and 2.7 ng/mℓ; 1,2,4,5-tetrachlorobenzene, 0.09 and 0.9 ng/mℓ; 1,2,3,4-tetrachlorobenzene, 0.07 and 0.7 ng/mℓ; pentachlorobenzene, 0.07 and 0.7 ng/mℓ. Recoveries ranged from 83.6–107.2% with relative standard deviation of less than 9%, indicating that the method has good precision, is reliable and free of matrix interferences. Water samples collected from the Grootdraai Dam were analysed using the optimised conditions to assess the potential of the method for trace level screening and quantification of chlorobenzenes. The method proved to be efficient, as 1,3 dichlorobenzene, 1,4-dichlorobenzene and pentachlorobenzene were detected at concentrations of 0.4 ng/mℓ, 1.7 ng/mℓ and 1.4 ng/mℓ, respectively.

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LIST OF ABBREVIATIONS

CW	Carbowax
DC	Direct current
DCB	Dichlorobenzene
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DI	Direct immersion
DVB	Divinlybenzene
ECD	Electron capture detector
FID	Flame ionization detector
GC	Gas chromatography
HCB	Hexachlorobenzene
HS	Headspace
IL	Ionic liquid
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification

LPME	Liquid phase microextraction
MAE	Microwave assisted extraction
MCB	Monochlorobenzene
MS	Mass spectroscopy
MWNT	Multi-walled carbon nanotubes
PA	Polyacrylate
PCB	Pentachlorobenzene
PDMS	Poly-dimethylsiloxane
POP	Persistent priority pollutants
PPM	Parts per million
RO	Reverse osmosis
RSD	Relative standard deviation
SANS	South African National Standard
SBSE	Stir-bar sorptive extraction
SDME	Single drop microextraction
SIM	Selected ion mode
SPE	Solid phase extraction

SPME	Solid phase microextraction
TCB	Trichlorobenzene
TD	Thermal desorption
TeCB	Tetrachlorobenzene
UK	United Kingdom
USA	United States of America
USEPA	United States Environmental Protection Agency
WBAE	Water bath assisted extraction
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background

Among persistent organic pollutants (POPs), chlorobenzenes are some of the most frequently encountered compounds in aqueous systems. These compounds can enter the environment via natural and anthropogenic sources, and are ubiquitous due to their extensive use over the past several decades (Noche et al., 2013; Soonthorntantikul et al., 2009). Several chlorobenzene compounds, once in the environment, can biologically accumulate. They are reputed to be carcinogens and extremely hazardous to health and several chlorobenzene compounds are listed as POPs by the United States Environmental Protection Agency (USEPA) (Noche et al., 2013; Esrafili et al., 2011; He et al., 2000). Excessive exposure to these compounds can affect the central nervous system, irritate and harden skin, irritate the upper respiratory tract, and give rise to haematological disorders including anaemia (Esrafili et al., 2012; Kozani et al., 2007; Khajeh et al., 2006).

However, despite the known toxicity of these compounds, they are widely used as process solvents, as precursors in the manufacture of pesticides, chlorinated phenols, lubricants, disinfectants, pigments and dyes (Noche et al., 2013; Esrafili et al., 2012; Grosjean, 1991; Vidal et al., 2007). There is a strong possibility that chlorobenzenes may be introduced into the environment in industrial regions in which they are used. However, due to their low solubility in water, chlorobenzenes may be present in very low concentrations in the aquatic environment

(Esrafili et al., 2012). Therefore, the sample preparation protocol needs to take these low concentration levels into account (Noche et al., 2013).

In this case, sample preparation requires pre-concentration of analytes and sample matrix clean-up to remove any interfering compounds (Chisvert et al., 2009). Various pre-concentration techniques have been used for the isolation of chlorobenzenes from water. These include traditional liquid-liquid extraction (LLE) (Ormad et al., 1996); solid phase extraction (SPE) (Wang and Lee, 1998; Liu et al., 2004); liquid phase microextraction (LPME) (Esrafili et al., 2011; Tor, 2006; Khajeh et al., 2006; Vidal et al., 2005); stir bar sorptive extraction (SBSE) (Montero et al., 2004) and solid phase microextraction (SPME) (He et al., 2000; Wang et al., 2003; Huang et al., 2007).

LLE is one of the oldest and most widely used concentration techniques in analytical chemistry (Bonansea et al., 2013; Rezaee et al., 2006). This technique suffers from several disadvantages such as being time consuming and using large amounts of expensive and toxic organic solvents (Noche et al., 2013). The latter will require additional costs for proper disposal, making it undesirable for modern day analysis. Since its introduction, SPE has been used as a successful alternative to LLE in environmental studies (Baltussen et al., 1997; Dean, 1998; Zwir-Ferenc and Biziuk, 2006). This technique involves passing aqueous sample through an appropriate sorbent and subsequent stripping of the analytes from the membrane using an suitable organic solvent. SPE is advantageous over LLE in the sense that low volumes of toxic solvents are used, thus eliminating a further pre-concentration step, and extraction times are greatly reduced (Bonansea et al., 2013; Iglesias, 2007).

SBSE, which is a simple and solventless technique, has been successfully applied to the extraction and enrichment of organic pollutants in environmental samples. It consists of a magnetic rod, incorporated into a glass jacket, which is coated with a polymeric layer, such as polydimethylsiloxane (PDMS) (Montero et al., 2004). With constant stirring, the coated stir bar extracts organic analytes from aqueous samples. The analytes are then thermally desorbed in a thermal desorption unit. This thermal desorption unit is expensive making it unaffordable to insufficiently funded laboratories. In contrast, SPME is a simple method of sample preparation which has received huge success since its advent (He et al., 2000). SPME consists of a quartz fibre coated with a polymeric layer, commonly PDMS, which is retractable inside the needle of a syringe-like devise (Montero et al., 2004). The fibre is immersed into aqueous samples to adsorb analytes which are then desorbed by exposing the fibre to the thermal conditions of the injector port of a gas chromatography (GC) instrument. SPME is advantageous over other preconcentration techniques as it is cost effective, rapid, does not utilize toxic solvents, incorporates sample preparation and GC injection in one step, does not require additional desorption equipment or modification of the GC injector port and has a high enrichment factor (Mitra, 2003; Wang et al., 2003; Wang, 1997).

Sample preparation utilizing one of the preconcentrationtechniques highlighted above is generally followed by GC analysis for analyte separation and detection. GC- mass spectroscopy (MS), due to its superior sensitivity, accuracy and resolution, is an excellent detector for chlorobenzenes. However, this detector requires large capital investment with high maintenance costs; therefore insufficiently funded laboratories require an alternate mode of detection without compromising factors such as detection limits and accuracy. The analysis of electronegative compounds is commonly achieved by GC- electron capture detector (ECD). This detector offers high resolution, good sensitivity within the nanogram range, and has a lower cost of operation (Fatoki and Awofolu, 2003). However, the enhanced sensitivity of both GC-MS and GC-ECD often leads to detector contamination which results in service breakdown, increased costs in maintenance, technician call out fees, loss of throughput and in some cases detector replacement. It is therefore necessary to test alternatives which could attain comparable detection limits at a relatively low cost.

1.2. Motivation for study

Qualitative organic analysis performed on Grootdraai Dam water samples using GC-MS at the Chemistry Department within the Durban University of Technology detected the presence of dichlorobenzene isomers. Several major industries abstract water from this impoundment; particularly the power utility Eskom (Thuthuka Power Station), which uses water from the Grootdraai Dam for use in power generation as well as in their cooling water system.

The presence of organic compounds during power generation is a nuisance. Organic species are decomposed into halogenated acids and acetic acid by the high operating pressure and temperature of the boilers. The formation of these acids reduces the pH of the boiler water, which in turn leads to corrosion of the turbines (Sithole, 2008). Understanding the threat of organic compounds, Eskom has an elaborate water treatment regimen consisting of conventional clarification coupled to reverse osmosis (RO) membranes.

RO membranes, with their extremely minute pore size, provide an absolute barrier to all (with the exception of dissolved gases) impurities present in feed water. As a result, it appears that Eskom is well equipped to mitigate the undesirable infrastructure effects of organic compounds. However, while treated water produced by RO is used by Eskom, the reject waste stream is disposed in an underground compartment of the New Denmark Colliery known as compartment 321 (Golder Associates, 2010). As a result is critical to analyse the organic determinants within the waste stream, so that a toxicity profile can be created and the environmental impacts of the waste stream can be assessed.

Furthermore, small communities along the Grootdraai Dam use water directly from the impoundment for domestic use. Several chlorobenzene compounds have been regulated by the World Health Organization (WHO) and USEPA as posing a threat to human health. To determine the environmental as well as human health risk of the Grootdraai Dam, it is critical to first detect and quantify, amongst others, all chlorobenzene compounds present within this embankment.

1.3. Project objective

The objective of this project was to develop a validated and reasonably cheap pre-concentration method for the detection and quantification of seven chlorobenzene compounds within the Grootdraai Dam. This study is based on use of a GC-Flame Ionization Detector (FID) as an alternative since it is a universal detector and is available in most analytical laboratories. Further it is robust and relatively easy to use. In order to use GC-FID to determine trace concentration of chlorobenzenes, direct immersion-solid phase micro extraction (DI-SPME) was selected as the pre-concentration technique. The various experimental parameters affecting the extraction efficiency of the selected chlorobenzenes was optimized and applied to Grootdraai Dam water samples for the detection and quantification of chlorobenzenes.

1.4. Specific objectives

The specific objectives of this study are as follows:

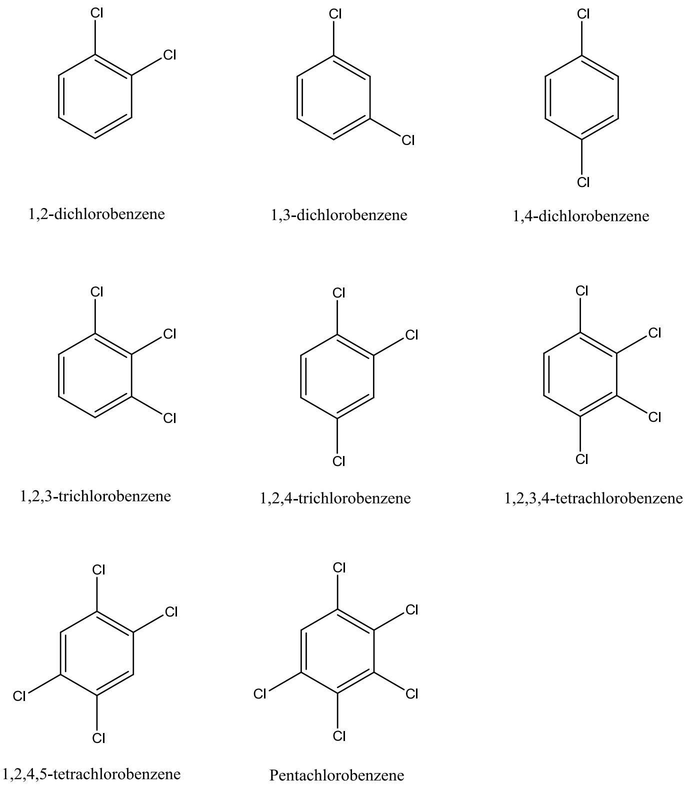
1. Determine the suitability of DI-SPME coupled to GC-FID for the detection of selected chlorobenzene compounds
2. Develop a validated DI-SPME pre-concentration method for the analysis of the seven dichlorobenzene compounds within the Grootdraai Dam
3. Perform a qualitative and quantitative analysis of the chlorobenzene compounds within the Grootdraai Dam
4. Determine the likely source of the detected chlorobezene compounds within the Grootdraai Dam

CHAPTER TWO

LITERATURE REVIEW

2.1. Identity and physical and chemical properties

Chlorinated benzenes are a group of aromatic compounds having the generic molecular formula $C_6H_{6-n}Cl_n$, (where n = 1-6). A chlorine can be substituted for a hydrogen to form 12 different chlorobenzene derivatives, namely monochlorobenzene (MCB), dichlorobenzene (DCB) (3 isomers), trichlorobenzene (TCB) (3 isomers), tetrachlorobenzene (TeCB) (3 isomers), pentachlorobenzene (PCB) and hexachlorobenzene (HCB). The structures of selected chlorobenzene compounds used in this study are shown in Figure 1.



1,2-dichlorobenzene 1,3-dichlorobenzene 1,4-dichlorobenzene

1,2,3-trichlorobenzene 1,2,4-trichlorobenzene 1,2,3,4-tetrachlorobenzene

1,2,4,5-tetrachlorobenzene Pentachlorobenzene

Figure 1: Molecular structure of selected chlorobenzene compounds

Once chlorobenzenes are introduced into the environment, their physical and chemical properties determine their fate and distribution. Each chlorobenzene compound has its own unique set of physical and chemical properties (Table 1). The mobility of chlorobenzenes is determined by their water solubility, fat solubility and volatility. Chlorobenzenes which exhibit high water solubility are highly mobile, while those which strongly adsorb onto soil are less mobile and less volatile (Kin, 2008).

Table 1: Physical-chemico properties of the chlorobenzene compounds (Noche et al., 2013; Malcolm et al., 2004)

Chlorinated benzene	**Aqueous solubility at 25^{o}C (mg/ℓ)**	**Octanol/water partition coefficient (log K_{ow})**	**Vapour Pressure at 25^{o}C (Pa)**	**Henry's law constant (kPa.m^{3}/mol)**
MCB	293	2.98	1665	0.377
1,2-DCB	91.1	3.38	197	0.198
1,3-DCB	123	3.48	269	0.366
1,4-DCB	30.9	3.38	90	0.160
1,2,3-TCB	12.2	4.04	17.3	0.306
1,2,4-TCB	45.3	3.98	45.3	0.439
1,3,5-TCB	3.99	4.02	24.0	0.233
1,2,3,4-TeCB	12.1	4.55	5.2	0.261
1,2,3,5-TeCB	2.81	4.65	9.8	0.593
1,2,4,5-TeCB	2.16	4.51	0.72	0.261
PCB	0.83	5.03	133	0.977

2.1.1. Aqueous solubility

The solubility of chlorobenzenes in water influences many processes such as evaporation, absorption and bioaccumulation (Finizio and Guardo, 2001). Water solubility is temperature dependent and is generally expressed as milligrams per litre (mg/ℓ) or parts per million (ppm). Water solubility can be used to predict the fate and mobility of compounds in the environment. Chlorobenzenes which have high water solubility will have a tendency to be mobilized by run-offs, rainwater or can be leached through the soil by groundwater. Table 1 shows that there is a general trend of decreasing water solubility with increasing level of chlorination of the benzene ring.

2.1.2. Octanol-water partition coefficient

Similar to solubility, the octanol-water partition coefficient (K_{ow}) plays an important role in predicting the environmental fate of compounds. It indicates the distribution of a compound between the organic (octanol) phase and the aqueous (water) phase, as indicated by equation 1 (Kin, 2008).

$$K_{ow} = \frac{C_{octanol}}{C_{water}} \tag{1}$$

where,

K_{ow} - Octanol-water partition coefficient

C - molar concentration

The octanol-water partition coefficient gives direct information on the hydrophobicity of a compound, which is used to determine its potential to bind to lipids in the environment (Finizio and Guardo, 2001). A high coefficient value indicates a high potential for compounds to partition to organic phase. This indicates that the compound will adhere to organic matter in the soil and has a high potential to accumulate in fats (Jenkins and Thomson, 1999).

2.1.3. Vapour pressure

Among the various physico-chemical properties, vapour pressure is one of the most elementary and essential parameters for predicting the transport, distribution and long-term fate of chlorobenzenes in the environment (Nakajoh et al., 2009). It is a measure of the tendency of a compound to volatilize. The higher the vapour pressure, the higher the volatility (Kin, 2008). Compounds with low vapour pressure are considered to be harmful pollutants due to their persistence and bioaccumulation potential (Paschke et al., 2005). A compound will be classified in a specific category of volatility, depending on its vapour pressure at a specific temperature (Table 2).

Table 2: Volatility rating scale for vapour pressure (Jenkins and Thomson, 1999)

Volatility rating	**Vapour pressure at 20-30^{o}C (mm Hg)**
Non-volatile	$< 1.0 \times 10^{-7}$
Slightly volatile	$1.0 \times 10^{-7} - 1.0 \times 10^{-4}$
Volatile	$1.0 \times 10^{-4} - 1.0 \times 10^{-2}$
Highly volatile	$>1.0 \times 10^{-2}$

2.1.4. Henry’s Law constant

The Henry’s Law constant describes the mass transfer of a compound between water and air. It describes the potential of a specific compound to volatilize from water or moist soil (Gorgenyi et al., 2002). At equilibrium between water and vapour phases, the Henry’s Law constant can be described by Equation 2:

$$= \frac{C_{i,g}}{C_{i,w}} \qquad (2)$$

where,

H – Henry’s Law constant

$C_{i,g}$ - molar concentration of the compound in the gas phase

$C_{i,w}$ - molar concentration of the compound in the water phase

A high Henry’s Law constant value indicates a higher potential of a compound to volatilize from soil or water, while a lower value indicates a higher leaching potential of the compound. This constant can be used to categorise a compound into groups of volatility (Table 3).

Table 3: Volatility rating scale for Henry's Law constant values (Jenkins and Thomson, 1999)

Volatility rating	**Henry’s law constant (atm.m^3/mol**
Non-volatile	$< 3.0 \times 10^{-7}$
Slightly volatile	3.0×10^{-7} - 1.0×10^{-5}
Volatile	1.0×10^{-5} - 1.0×10^{-3}
Highly volatile	$>1.0 \times 10^{-3}$

2.2. Sources of chlorobenzenes

Chlorobenzenes can be released into the environment from sites at which they are manufactured or used as precursors for the production of other compounds, during their disposal or they can be formed via the metabolic breakdown of other chlorinated compounds (WHO, 2004). MCB is manufactured worldwide at the level of 700,000 metric tonnes per annum and the estimated release of MCB into air is 0.2% of its total production and use (Othmer, 2000; Shareefdeen and Singh, 2005). It is used extensively in the manufacture of pesticides (dichlorodiphenyltrichloroethane-DDT), phenol and aniline, as an intermediate in the manufacture of *ortho*- and *para*-nitrochlorobenzene, rubber polymers, antioxidants and isocyanates, and as a carrier for textile dyes (Meek et al., 1994a; Mackay et al., 1996; Mansville, 1990). MCB primarily enters the soil through pesticides and subsequently enters the hydrosphere and atmosphere via evaporation and leaching.

1,2-DCB is primarily used as a solvent for degreasing and the removal of carbon in the metal and automotive industries (Meek et al., 1994 b). It is also used in the production of several herbicides such as diuron, propanil and neburon, insecticides and fumigants, as well as in the manufacture of lubricants and disinfectants. It is also used as a process solvent in the synthesis of toluene diisocyanate (Grosjean, 1991). The degradation of lindane in soil has been reported to produce 1,2-DCB. Two of the most significant sources of 1,2-DCB in water results from its use as a deodorizer as well as leaching and run-offs from chemical dump sites (CEPA, 1993).

1,4-DCB is commonly used in air fresheners, urinal deodorizers, insecticides for moths and carpet beetles, wood preservatives, and as a repellent for birds (McPherson et al., 2002; Botitsi, 2006; Tsimeli et al., 2008). 1,4-DCB is the most widely detected of the three DCB isomers in the environment. The USEPA estimates that 44 metric tons of the compound has been cumulatively released into the air, surface water, land and ground water (Newhart, 2007).

TCBs can be formed during various combustion processes, industrial cracking as well as the degradation of hexachlorocyclohexane. The main routes by which TCBs can enter the hydrosphere and atmosphere are during its manufacture and usage. (ECR, 2002). 1,2,3 trichlorobenzene is used as process solvent, as an intermediate for the manufacture of pesticides and several other chemical products such as herbicides, pigments and dyes (ECR, 2002). 1,2,4-TCB is the most widely used TCB isomer. It is used as an oil additive, dye carrier, pesticide intermediate, heat transfer medium, degreaser, lubricant, dielectric fluid in transformers and as a solvent in chemical manufacturing (Grosjean, 1991; Shcroll et al., 2004). 1,3,5-TCB is used in very small quantities as a chemical intermediate (ECR,2002).

1,2,3,4-TeCB is used in dielectric fluids. 1,2,4,5-TeCB is used commercially as an intermediate in the production of fungicides, herbicides, insecticides, disinfectants, preservatives and defoliants. It is also used in the synthesis of chemicals such as 2,4,5-trichlorophenol and 2,4,5-trichlorophenoxyacetic acid (Gustafson, 1998).

PCB is used as an intermediate in the synthesis of several chemicals such as pentachloronitrobenzene and has been reported as being a by-product during the synthesis of organochlorine compounds in chlor-alkali plants (Carrizo et al., 2008). PCB is also a component of chlorobenzene mixtures used to reduce the viscosity in heat transfer applications (King et al., 2003). The use of PCB has been banned in many countries; hence its presence in the environment should decrease over time.

2.3. Environmental levels

2.3.1. Air

Chlorobenzenes are released into the environment primarily during their manufacture. The incineration of chlorobenzenes can also lead to the emission of other compounds such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Volatile chlorobenzenes are extensively used in aerosols and solvents. Consequently, large quantities of chlorobenzenes are released into the air. Chlorobenzenes that are released into the atmosphere are generally of very low concentrations, often much less than a few µg/m^3. In a study carried out on air around the Grube Antonie (Bitterfeld, Gemany) landfill site average HCB levels were found to be 63 ng/m^3 (Paschke et al., 2006). Extractions were performed at two different sampling heights, 20 cm and 150 cm above ground level. The Σ (sum) of the concentrations of the TeCBs and HCB were found to be 1495 ng/m^3 and 605 ng/m^3 at a sampling height of 20 cm and 150 cm respectively. Concentrations of 1,2,4,5- and 1,2,3,4-TeCB, PCB and HCB were determined in air at 3 different sites (Liepzig, Roitzsch and Greppin) in Germany (Popp et al., 2000). The average concentrations of 1,2,4,5- and 1,2,3,4-TeCB, PCB and HCB in the Liepzig region were 6.36 pg/m^3, 9.48 pg/m^3, 10.66 pg/m^3 and 77.06 pg/m^3 respectively; in the Roitzsch region were 5.70 pg/m^3, 6.80 pg/m^3, 10.30 pg/m^3 and 145.58 pg/m^3 respectively; and in the Greppin region were found to be 25.4 pg/m^3, 30.90 pg/m^3, 28.02 pg/m^3 and 280.6 pg/m^3 respectively.

2.3.2. Rainwater

Several studies have determined chlorobenzenes in rainwater. 1,4-DCB was detected in rain water in the United Kingdom (UK) at an average concentration of 10 ng/ℓ (Fielding et al., 1981) while 1,2-, 1,3- and 1,4-DCB and 1,2,4-TCB were found at levels of less than 10 ng/ℓ each in rainwater from California, United States of America (USA) (Pankow, 1983). A qualitative study on rain water from Sweden revealed the presence MCB (Laniewski et al., 1998).

2.3.3. Surface water

The concentrations of chlorobenzenes in surface waters are generally in the sub ng/ℓ - µg/ℓ range, although these levels can be elevated in surface waters that are contaminated or which are near industrial discharge zones. Mean concentrations of MCB, 1,4-DCB, 1,3,5-TCB and 1,2,4-TCB from the North Sea were 4.1 ng/ℓ, 22 ng/ℓ, 1.3 ng/ℓ and 2.2 ng/ℓ respectively (Huybrechts et al., 2005). Similar concentrations of MCB, 1,4-DCB and 1,3,5-TCB were found at an average concentration of 3.85 ng/ℓ, 16.0 ng/ℓ and 2.2 ng/ℓ respectively, between the Zollenspieker and Seemannshoft areas of the River Elbe. Other chlorobenzenes were present at average concentrations of 12.7 ng/ℓ for 1,2-DCB, 4.25 ng/ℓ for 1,3-DCB, 0.8 ng/ℓ for 1,2,3-TCB, 5.3 ng/ℓ for 1,2,4-TCB, 0.86 ng/ℓ for 1,2,3,4-TeCB, 0.38 ng/ℓ for 1,2,3,5-TeCB, 0.83 ng/ℓ for 1,2,4,5-TeCB, 0.33 ng/ℓ for PCB and 3.8 ng/ℓ for HCB (Gotz et al., 1998). Much higher concentrations of MCB, 1,3-,1,4- and 1,2-DCB were found in Caspian Sea water at levels of 300 ng/ℓ, 100 ng/ℓ, 700 ng/ℓ and 100 ng/ℓ respectively (Khajeh et al., 2006). Water from the River Cinca contained levels of 1,3-DCB at 20 ng/ℓ; 1,4-DCB at 25 ng/ℓ; 1,2-DCB at 8 ng/ℓ; 1,3,5-TCB at 11 ng/ℓ; 1,2,3-

TCB at 1 ng/ℓ and PCB at 2 ng/ℓ (Ormad et al., 1996) while water from the Ren-Ai River (Taiwan) contained 1,24-TCB and HCB at concentrations of 6.6 ng/ℓ and 3.4 ng/ℓ respectively (Huang et al., 2007). Extremely high levels of chlorobenzenes can occur in heavily polluted surface waters. Gomez Belinchon et al. (1991) reported elevated values for chlorobenzenes from industrially polluted water from the Besos River (Spain). Mean values were 260 ng/ℓ for MCB; 600 ng/ℓ for 1,4-DCB; 5000 ng/ℓ for 1,2-DCB; 1100 ng/ℓ for 1,2,3-TCB and 8100 ng/ℓ for 1,2,4-TCB.

2.3.4. Wastewater

Chlorobenzenes in wastewater may originate from various sources such as wet and dry deposition of atmosphere, urban run-off, domestic wastewater and industrial effluents (Cai et al., 2007). Wastewater from the sewer network of Paris indicated the presence of HCB, PCB and TCB at levels between 10-50 ng/ℓ (Gasperi et al., 2008). A similar concentration of PCB was reported by Rule et al. (2006) on wastewater in the UK. These low levels indicate that chlorobenzenes are being removed from the wastewater by external processes. Due to the limited number industries surrounding the sampling sites, these lower chlorobenzenes levels could be due to their lack of use in industrial processes. Conversely, Zhao et al. (2009) analyzed wastewater samples obtained from a dye company in Wuhan (China). Extremely high values of MCB, 1,4-DCB, and 1,2-DCB were obtained at levels of 5800 ng/ℓ, 2800 ng/ℓ and 380 ng/ℓ respectively. These elevated values are due to chlorobenzenes being extensively used as intermediates and carriers in the manufacture of dyes.

2.3.5. Sewage sludge

Sewage sludge is a by-product of wastewater treatment process, resulting from the sedimentation of suspended solids. Organic pollutants have a great tendency to sorb onto suspended solids; therefore sewage sludge can contain significant concentrations of organic pollutants. In twelve sewage sludge samples from China the concentrations of chlorobenzenes ranged from 0.016-0.083 mg/kg for 1,2-DCB, 0.037-0.550 mg/kg for 1,4-DCB, 0.012-2.9 mg/kg for 1,2,4-TCB and 0.009-3.2 mg/kg for HCB (Cai et al., 2007). In another study in the UK, analysis of twelve wastewater sludges revealed that eleven chlorobenzenes were present ranging from 0.01 mg/kg for PCB to 40.2 mg/kg for 1,3-DCB (Rogers et al., 1989). The dichlorobenzene isomers were the most abundant and there was a general trend of decreased concentration with increasing chlorination.

2.3.6. Sediment

Hydrophobic organic compounds tend to sorb onto sediments. The structure and extensive surface area of sediments allows it to act as repositories for a wide range of compounds. This leads to the accumulation of these compounds in sediments. These compounds are not considered as being fixed onto the sediments and can be mobilized by changes in geochemical parameters such as pH, therefore, they could pose as a secondary source of pollution, causing contamination for many years, even when all sources of primary contamination has been cut off (since (Wolska and Namiesnik, 2002; Zoumis et al., 2001).

These sediment-organic pollutant ‘complexes’ enter the aquatic environment as dissolved and suspended matter in which they contribute to apparent concentrations of these compounds in the aqueous phase (Londo et al., 1996). Mean chlorobenzene concentration of twelve sediments from Lake Ladoga (Russia) ranged from 0.002-0.004 mg/kg for 1,3,5-TCB, 0.002-0.007 mg/kg for 1,2,4-TCB and 0.004-0.015 mg/kg for HCB (Ristola et al., 1996). The sum of chlorobenzene compounds (PCB+HCB) was found to be between 0.0004-0.0007 mg/kg in surface sediments from the Arctic Lake (Canada) (Muir et al., 1995). The sum chlorobenzene concentrations in the Yukon Lake (Canada) ranged between 0.0002-0.003 mg/kg with HCB being the major congener, contributing between 65-100% of the total chlorobenzene concentration (Rawn et al., 2001). Three separate studies were carried out on the sediments from Taiwan. The highest mean concentrations of chlorobenzenes in sediments from the Tsen-wen Chi estuary were 0.021 mg/kg, 0.003 mg/kg and 0.011 mg/kg for 1,3-DCB, 1,2-DCB and 1,2,4-TCB respectively (Lee and Fang, 1997). The sediments from the Kaohsiung coast were found to contain mean chlorobenzene concentrations of 0.017 mg/kg for 1,3-DCB, 0.02 mg/kg for 1,4-DCB, 0.008 mg/kg for 1,2-DCB, 0.011 mg/kg for 1,3,5-TCB, 0.010 mg/kg for 1,2,4-TCB, 0.005 mg/kg for 1,2,3-TCB, 0.011 mg/kg for 1,2,3,5-TeCB and 1,2,4,5-TeCB, 0.005 mg/kg for 1,2,3,4-TeCB, 0.006 mg/kg for PCB and 0.021 mg/kg for HCB.(Lee et al., 2000). In a follow up study on the same set of sediments in 2005, there were no significant difference between mean chlorobenzene concentrations obtained in comparison to that obtained in 2000 (Lee et al., 2005).

2.3.7. Soil

Soil is a complex matrix which plays a pivotal role in the retention of organic pollutants. It can be polluted by various organic pollutants as a result of sludge disposal, particularly chlorobenzenes, as they are ubiquitous in sludge. Once in the soil, there are many factors which determine the fate of chlorobenzenes, the most important of which is sorption. Sorption and desorption processes determine the rate of volatilization, leaching and the bioavailability of chlorobenzenes (Wang and Jones 1994). Sorption of chlorobenzenes is affected by many factors and it has been reported that in the presence of high organic matter content, the sorption of chlorobenzenes increases (Barber et al., 1992). Other factors which effect sorption are depth, soil moisture, pH and the presence of various surfactants (Njoroge et al., 1998; Sheng et al., 1998; Divincenzo and Dentel, 1996; Thibaud et al., 1993). Studies have been performed to accurately monitor the chlorobenzenes concentration in soils, since these compounds within the soil matrix can contribute to other forms of pollution. The concentrations of chlorobenzenes in soils from Bitterfeld (Germany) ranged from 0.0007-0.004 mg/kg for 1,2,4,5-TeCB and 0.0006-0.002 mg/kg for PCB (Rodil and Popp, 2006). Chlorobenzenes in soil can leach and pollute groundwater; contaminate surface waters due to drainage and run-offs and pollute the air as a result of volatilization.

2.4. Extraction of chlorobenzenes from an aqueous phase

Sample preparation is an extremely crucial step in an analytical process as it includes, amongst others, the preconcentration of analytes and sample matrix clean-up to remove any interfering compounds (Chisvert et al., 2009). In the sections to follow, a review of the various preconcentration techniques that have been used for the preconcentration of chlorobenzenes from water is described.

2.4.1. Liquid Liquid Extraction

LLE is one of the oldest and most widely used techniques used for the preconcentration of volatile hydrophobic organic compounds from water, prior to GC analysis (Rezaee et al., 2006; Yrieix et al., 1996). It is a fairly simple technique involving the exhaustive extraction of analytes which is generally performed in a separation funnel (Bjergaard and Rasmussen, 2008). The distribution of the analytes between the aqueous and organic layers can be described by the Nernst distribution law (Equation 3), in which the distribution coefficient describes the analyte ratio in each phase at equilibrium.

$$K_D = \frac{[X]_B}{[X]_A} \qquad (3)$$

where,

K_D – Distribution coefficient

$[X]_A$ - Concentration of the analyte in phase A

$[X]_B$ - Concentration of the analyte in phase B

Ormad et al. (1996) isolated chlorobenzenes from the River Cinca using dichloromethane (DCM) as an extracting solvent. Sample preparation consisted of extracting one litre of sample with three aliquots of DCM, a drying step over anhydrous sodium sulphate, sample clean up using a column of Florisil, removal of elemental sulphur, a further drying step over anhydrous sodium sulphate and concentrating the extract over a stream of nitrogen. The authors reported recoveries ranging from 94 – 135 % and detection limits of the analytes ranged from 1.79 µg/ℓ – 3.11 µg/ℓ.

Laniewski et al. (1998) attempted to identify chloro-organics in rain and snow using LLE as the mode of extraction. Sample preparation consisted of the following steps: sample concentration using a rotary evaporator, pH adjustment, extraction with hexane fractions followed by extraction with diethyl ether fractions, and finally concentration of the extracted fractions to 100 μℓ under a stream of nitrogen. This study found 1,2-, 1,3- and 1,4-DCB, 1,2,4,5- and 1,2,3,5-TeCB as well as PCB to be present in all samples at undisclosed concentrations.

LLE suffers several disadvantages such as being time consuming; requiring large sample as well as expensive and toxic organic solvent volumes, which require additional costs for proper disposal, making it undesirable for modern day analysis. Further, the high number of preparative steps this technique also offers a high probability of sample contamination as well as analyte losses due to volatilization. The recoveries of polar analytes from aqueous samples using LLE are very poor due to their relatively high partial solubility in water (Pichon, 2000). LLE also suffers inadequate sensitivity or selectivity towards compounds that have low partition coefficients between the aqueous and organic phase (Yrieix, 1996).

2.4.2. Solid Phase Extraction

Since its introduction, SPE has been used as a successful alternative to LLE in environmental studies. SPE is a non-equilibrium technique involving the exhaustive removal of compounds from aqueous samples. SPE methodology (Figure 2), which is based on simple chromatographic theory, requires passing an aqueous sample (mobile phase 1) through a solid sorbent (stationary phase) which is packed in a cartridge or enmeshed in a membrane-based extraction disk. Compounds with high affinity for the sorbent do not elute and are adsorbed onto the sorbent. These adsorbed compounds are then eluted from the sorbent by using a suitable organic solvent (mobile phase 2) (Mitra, 2003).

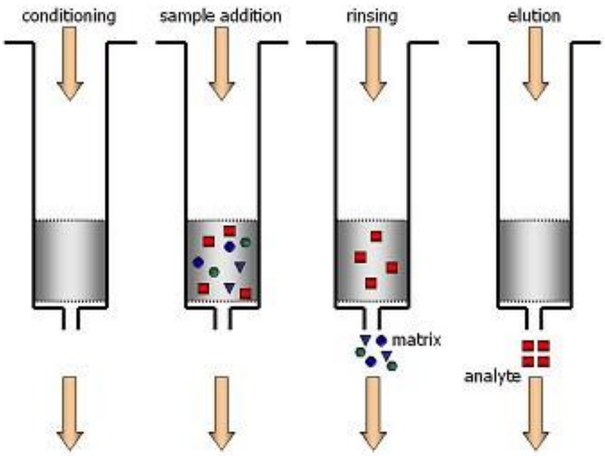


Figure 2: Schematic representation of a SPE extraction

One of the key procedures during the development of a SPE procedure is the selection of a suitable sorbent. Analytes which have a low affinity toward the sorbent can be lost during sample percolation, whereas, analytes that have a high retention (high affinity) on a sorbent can be difficult to desorb (Pichon, 2000). An ideal sorbent should adsorb compounds rapidly, be reproducible with the sorption process being completely reversible such that there is complete elution of adsorbed compounds from the sorbent (Mitra, 2003). The sorbent must also exhibit high porosity, large surface area, be free of leachable impurities and show high stability to the sample matrix as well as the elution solvents (Fritz and Macksa, 2000). There are a wide range of commercially available sorbents comprised of various polymeric materials. A review by Poole et al. (2000) systematically ranks the available sorbents as general purpose, class specific and compound specific.

Wang and Lee (1998) extracted ten chlorobenzenes from tap, river and ground water using a C_{18} cartridge packed with 200 mg of sorbent. After conditioning the cartridge with methanol, pre-filtered water samples were passed through the sorbent at a flow rate of 4 mℓ/min. The adsorbed compounds were then eluted with 2 aliquouts (1.5 mℓ) of ethyl acetate. The eluates were filtered through anhydrous sodium sulphate and evaporated under a gentle stream of nitrogen to 1 mℓ. Preliminary tests were carried out to assess the elution efficiency of several solvents. Hexane and methanol produced recoveries of between 50-70%, while DCM provided poor reproducibility. Toluene showed good elution efficiency, however, its high boiling point made it undesirable due to the possibility of volatilization of analytes during the preconcentration step. Ethyl acetate provided good recoveries and reproducibility as an elution solvent. The authors attributed this to its moderate polarity and good wettability of the SPE sorbent. The recoveries for the method ranged between 62.3% - 98.4% while the percentage relative standard deviation (%RSD) values were all below 10.6%. There were reported losses at the evaporation step of 11% for 1,3-DCB, while the losses for all other compounds were below 6 %. Although these losses were not significant, it can be magnified when lower concentrations are extracted.

To address the problems associated with the C_{18} sorbent toward the preconcentration of chlorobenzenes, Liu et al. (2004) suggested the use of multi-walled carbon nanotubes (MWNT) as a SPE sorbent for the preconcentration of DCB, 1,2,4-TCB, 1,2,4,5-TeCB and HCB from lake water. This study compared the extraction efficiency of a micro SPE column packed with 15 mg of MWNT material, a C_{18} bonded silica cartridge and an activated carbon cartridge. The columns were conditioned with methanol and 100 mℓ of sample was passed through the sorbent at 2 mℓ/min. The desorbed analytes were then eluted with 2 mℓ of methanol. The eluates were further dried with anhydrous sodium acetate and then made up to 2 mℓ with methanol. Optimization studies showed a decrease in recoveries when the mass of the MWNT sorbent was increased. This was due to the different column heights resulting in different retention of analytes onto the column. Recovery tests of water fortified with different concentrations (5, 10 and 20 ng/mℓ) of analytes showed that the MWNT sorbent provided the highest recoveries across all concentration levels. The adsorption capacity of the MWNT sorbent was found to be superior over the other two sorbents.

The advantages of SPE over LLE include reduced organic solvent consumption and waste solvent disposal costs; reduced potential for the formation of emulsions; and reduced cost, labour and extraction times. Additionally, SPE provides higher concentration factors than LLE, has a greater potential for selective extraction and can be automated with multiple extractions being carried out simultaneously (Iglesias, 2007; Mitra, 2003; Pichon, 2000). Despite the numerous advantages presented by SPE, the process does pose several drawbacks. The technique still utilizes toxic organic solvents. Particulate matter present in the sample causes plugging and channelling of the sorbents. The high retention of non-polar compounds on hydrophobic sorbents may pose difficulties in desorption. There have been reports of loss of analytes due to volatilization during the preconcentration step; non-polar compounds tend to adsorb onto the flask walls and connecting tubes leading to further losses, while polar analytes can be lost during the extraction due to their low affinity towards the sorbent. (Mitra, 2003; Pichon, 2000).

2.4.3. Solid Phase Microextraction

SPME was developed by Pauliszyn in 1989 to address the need for a rapid, solvent free and field compatible sample preparation technique and has been extensively used since its advent (He et al., 2000). SPME is a non-exhaustive equilibrium process which, according to Pawliszyn and Pedersen-Bjergaard (2006), is defined as a process that extracts only a portion of the analyte that is present in the sample.. The device consists of a quartz fibre coated with a polymeric layer, commonly PDMS, which is retractable inside the needle of a syringe-like devise (Montero et al., 2004).

There are seven types of SPME fibres commercially available, namely, polydimethylsiloxane (PDMS), PDMS-divinylbenzene (DVB), polyacrylate (PA), Carboxen-PDMS, Carbowax (CW)-DVB, CW-templated resin and DVB-Carboxen-PDMS (Stableflex) (Wang et al., 2003). The fibre is exposed to the sample either by directly immersing it into the aqueous sample or exposing it to the sample headspace to extract analytes. The fibres are then desorbed thermally by exposing the fibre to the injector port of the GC instrument. A typical SPME extraction is described in Figure 3.

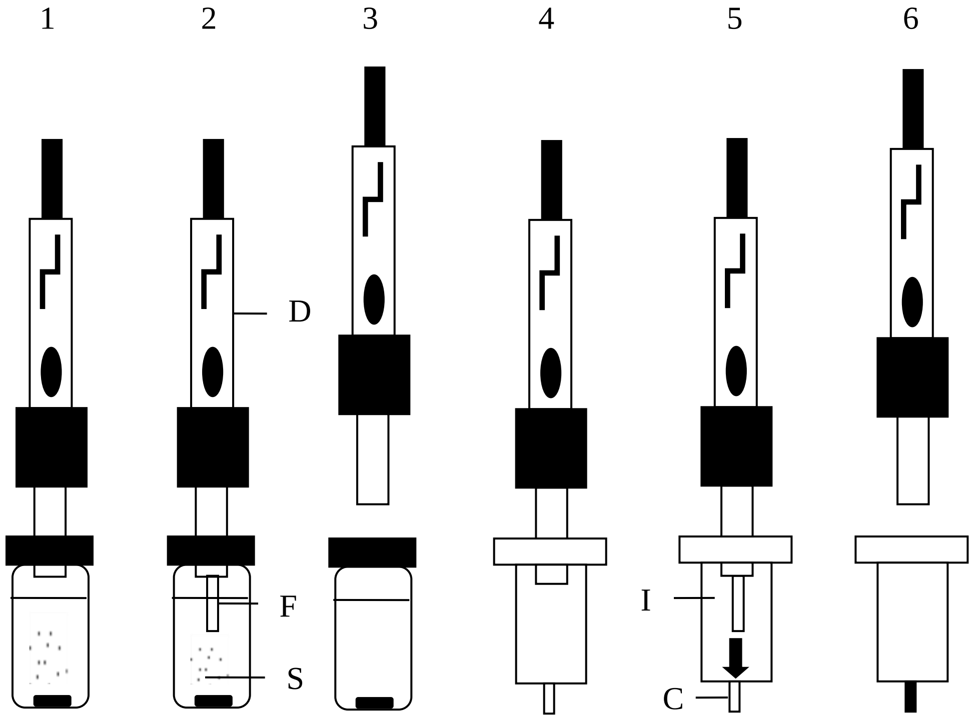


Figure 3: Steps taken in a typical SPME extraction 1) Pierce the septum of the sample vial; 2) Expose the fibre to adsorb analytes in the sample matrix for a specific time; 3) Retract the fibre after the specified time, 4) Pierce the septum of the injector port; 5) Expose fibre to the thermal conditions of the injector port to desorb the analytes; 6) Retract the fibre after desorption; D-SPME holder, F-fibre, S-sample, I-injector port, C-column.

An extraction is considered to be complete when the distribution of the analyte between the sample matrix and fibre has reached equilibrium. This equilibrium can be described by Equation 4:

$$n = \frac{K_{fs} V_f V_s C_0}{K_{fs} V_f + V_s} \tag{4}$$

where,

n - amount extracted by the coating

K_{fs} - distribution coefficient between the fibre coating and the sample matrix

V_f - volume of the fibre coating

V_s - volume of the sample

C_0 - initial concentration of the analyte in the sample

When the sample volume, relative to the fibre volume, is very large (i.e. $K_{fs}V_f <<< V_s$) then the amount extracted becomes independent of volume and Equation (4) simplifies to,

$$n = K_{fs}V_fC_0 \tag{5}$$

Upon the analysis of Equation 5, one can deduce that the amount extracted (n) is directly proportional to the concentration of analyte in the sample matrix. It is this attribute that has permitted SPME to have widespread application in field analysis (Mitra, 2003).

He et al. (2000) extracted ten chlorobenzenes from water using HS-SPME fitted with a 100 µm PDMS coated fibre. During the optimization of the method the authors reported that higher extraction times (30 minutes) were required for the higher chlorinated benzenes (TeCB, PCB and HCB) to reach equilibrium. The lower chlorinated benzenes (DCB and TCB) attained equilibrium in 10 minutes. The addition of NaCl at a concentration of 20% (m/v) also enhanced the extraction for most of the analytes except HCB. This is due to the direct relationship between the salting out effect and the solubility of the analytes. The salting out effect has a more profound effect on the extraction efficiency of analytes with higher water solubility. Since HCB has the lowest solubility of all the analytes, the effect of salting out effect was ineffective. Due to the low vapour pressure of both PCB and HCB, increasing the extraction temperature from 26°C to 40°C increased the extraction efficiency nearly two-fold. However, these elevated temperatures decreased the extraction efficiency of other analytes which have low vapour pressure. A similar problem was experienced by Huang et al. (2007). This occurred as the partition coefficient between the fibre coating and the headspace were decreased at elevated temperature. Stirring the sample at a high stir speed of 1500 revolutions per minute (rpm) produced higher extraction efficiencies for all analytes, especially those with lower vapour pressure (PCB and HCB). This phenomenon can be explained by the fact that stirring enhances the transfer of analytes from the sample to the headspace. The optimized method was linear over a range of 0.02 µg/ℓ - 20 µg/ℓ with correlation coefficients being greater than 0.991 for all compounds; except HCB (0.988). The method showed good repeatability with %RSD values of less than 9.4% for all compounds. The recoveries from reservoir and tap water ranged between 92.0-103.3% and 91.8-104.5% respectively.

Wang and co-workers developed a new dihydroxyl terminated benzo-15-crown-5 (DOH-B15C5) coated fibre by sol-gel technology and applied it to the extraction of four chlorobenzene compounds (Wang et al., 2003). Sol-gel technology involves the synthesis of an organic-inorganic hybrid polymer under very mild conditions. Polymers synthesized by sol-gel technology provide enhanced polymer stability for use as a stationary phase in chromatographic applications. Further advantages of sol-gel technology are described by Kumar et al. (2008). In this study, an 80 µm DOH-B15C5 was found to be thermally stable at a temperature of greater than 350°C, far superior to other commercially available fibres. The high thermal stability of the DOH-B15C5 coated fibre is due to the strong linkage between the coating and substrate through chemical bonding. Higher operating temperatures will allow for a great reduction in sample carryover between extractions.

Huang et al. (2007) used HS-SPME to extract five chlorobenzenes from river water using the PDMS-DVB fibre. This study compared the application of microwave assisted extraction (MAE) and water bath assisted extraction (WBAE) as heat sources towards improving the extraction efficiency of the method. The efficiency of all HS extractions depends on the transfer of the analyte from the sample matrix into the headspace. This can be accelerated by implementing the use of conventional heat sources. Conventional heat sources (such as WBAE) provide an inefficient way for energy transfer as thermal energy is transferred from the heat source to the analyte via conduction and convection hence making the extractions slower (Mitra, 2003). Microwave energy (such as MAE) provides a much more rapid and efficient source of heating. In MAE electromagnetic energy is transformed into heat energy via ionic conduction and dipole rotation (Mitra, 2003). This results in immediate localized superheating due to the direct coupling between microwaves and the analytes within the sample matrix. In general the authors found that MAE-SPME provided superior extraction over the WBAE-SPME method as MAE-SPME shortened the equilibrium times for most of the analytes. The extraction efficiency of MAE-SPME was between 1.4-3.9 times better than WBAE-SPME with MAE-SPME yielding higher signal responses for most analytes. The MAE-SPME proved to be linear with all correlation coefficients being greater than 0.997. The recoveries from river water ranged between 101%-116% with %RSD of less than 11%.

The advantages of SPME include the absence of solvent which reduces sample preparation steps, which in turn allows for rapid analysis thereby increasing throughput. The lack of toxic organic solvents makes this technology environmentally friendly and reduces cost associated with disposal of toxic waste. The SPME device is small thus making it portable and convenient for field work (Pawliszyn, 1997). It is also inexpensive and sensitive with high selectivity and concentrating ability. (Mitra, 2003; Wang et al., 2003). The only major disadvantage of SPME is possible matrix effects as any changes in the matrix will affect quantitative results since the distribution constant relative to that obtained in pure aqueous sample will be altered.

2.4.4. Liquid Phase Microextraction

LPME is a miniaturization of LLE, in which extraction takes place between a small volume of water immiscible organic solvent and an aqueous sample containing the compounds of interest (Xu et al., 2007). The volume of the organic solvent is typically between the microlitre and sub-microlitre range. Since LPME entails the use of a single drop as an extracting phase, it is termed single drop microextraction (SDME). SDME is a non-exhaustive equilibrium extraction technique performed by either directly immersing a micro-litre drop of organic solvent in an aqueous sample or by suspending the drop above the sample headspace (Figure 4) (Tor, 2006). After extraction, the droplet is withdrawn back into the syringe and then injected into an appropriate method of separation and detection.

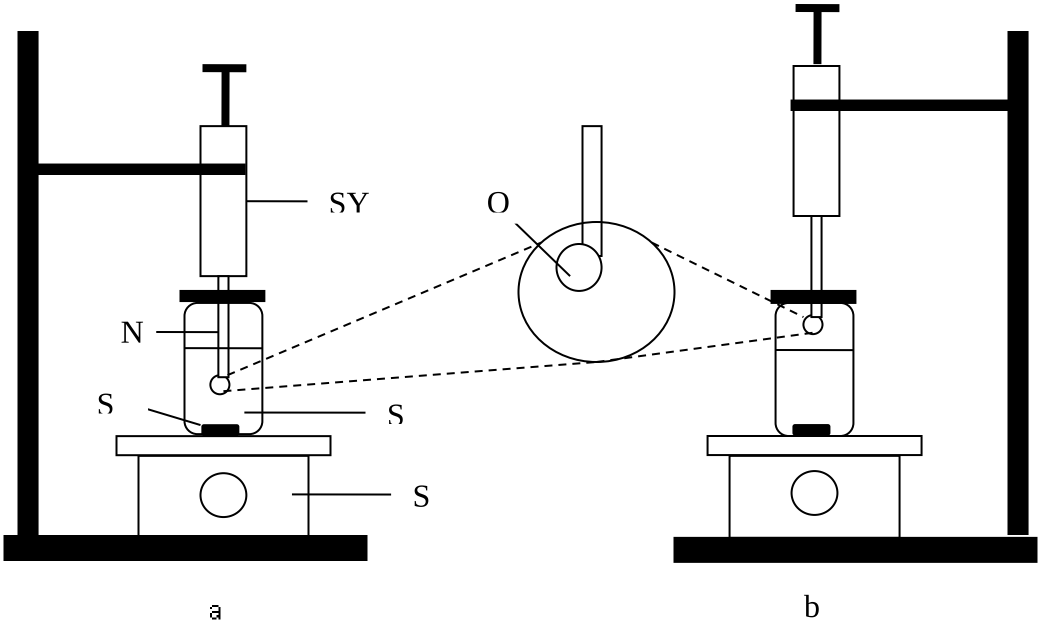


Figure 4: Schematic representation of the two modes utilized in a SDME extraction; a) direct immersion; b) headspace extraction, SY-syringe; N-needle; S-sample; SB-stir bar, ST-stirrer, O-organic drop

This technique is based on the distribution of analytes between the micro-drop of an organic solvent at the tip of a micro-syringe and the aqueous sample until a dynamic equilibrium of analyte concentration between the two phases is attained (Khajeh et al., 2006). The amount of analyte, *n*, extracted at equilibrium is described by the following equation:

$$n = \frac{k_{odw} V_d C_0 V_s}{k_{odw} V_d + k_{hs} V_{hS} + V_s} \qquad (5)$$

where:

k_{odw} - organic drop-water distribution constant

k_{hs} - headspace-water distribution constant

C_0 - initial concentration of the analyte in the matrix

V_d - volume of the drop

V_s - volume of the sample

V_{hs} - volume of the headspace

According to reports in literature, there are two modes in which SDME can be utilized namely, DI-SDME and headspace (HS)-SDME.

2.4.4.1. DI-SDME

Tor (2006) extracted MCB, 1,2-DCB, 1,3-DCB, 1,4-DCB and 1,2,3-TCB from tap and ground water using direct immersion SDME. The optimized method was as follows; 2 µℓ drop size; *n*-hexane extracting solvent; 5 minute extraction time; 600 rpm stir speed and 3 M ionic NaCl strength. The method was linear between the 1-50 µg range; correlation coefficient (R^2) values ranged between 0.993-0.996; and the recovery of all analytes ranged between 90% - 93% for tap water and 91% - 94% for ground water.

2.4.4.2. HS-SDME

HS-SDME is suitable for the extraction of volatile or semi-volatile compounds and due to its advantages; this mode has continued to gain wide consideration. HS analysis allows for rapid agitation of the sample solution without having any undesirable effects on the stability of the organic droplet (Xu et al., 2007). Agitation is an important parameter in the extraction process as it enhances the extraction and reduces the time to achieve thermodynamic equilibrium (Psillakis and Kalogerakis, 2002). The non-volatile component of the sample matrix is eliminated during HS extraction, hence, reducing interferences and enhancing reproducibility (Xu et al., 2007). These aforementioned advantages, coupled to the fact that chlorobenzenes are semi-volatile compounds has steered SDME research towards the use of the HS mode. A review of the application of HS-SDME towards the extraction of chlorobenzenes from water follows.

Vidal et al. (2005) extracted ten chlorobenzenes from tap and well water using HS-SDME. During the optimization of experimental parameters the authors noted a few problems. *n*- Hexane, due to its high vapour pressure, had a tendency to evaporate when exposed to air; hence, could not be used as an extracting solvent. The authors also observed an increase in extraction efficiency as the size of the extracting droplet was increased from 2 µℓ to 2.5 µℓ. However, the same trend was not observed when the droplet size was increased from 2.5 µℓ to 3 µℓ. This occurred as larger organic solvent drops require extended equilibration times. The optimized experimental parameters were as follows: toluene as an extracting solvent; 2.5 µℓ micro-drop; 10 mℓ sample size; 30% (w/v) NaCl; 1000 rpm stir speed and 5 minute extraction time. The method provided R^2 values ranging from 0.9886-0.9964, % RSD values below 13.2% for all compounds and the recoveries ranged between 84% - 99% for tap water and 82%-107% for well water. The enrichment factor (defined as the ratio of the final analyte concentration in the organic phase and the initial concentration of the analyte in the aqueous phase) ranged between 92 – 157 for most analytes, while PCB and HCB were found to be 31 and 17 respectively.

Khajeh et al. (2006) carried out a similar study; extracting MCB, 1,2-DCB, 1,3-DCB, 1,4-DCB, 1,2,3-TCB and 1,2,4-TCB from tap water, waste water and sea water. The difference in this study is that the authors optimized sample temperature, micro-syringe needle temperature and used a different extracting solvent and mode of detection. The optimized experimental parameters were as follows: dodecane as the extracting solvent; micro-syringe needle temperature of $0^{o}C$; 7 mℓ sample volume; 500 rpm stir speed; $45^{o}C$ sample temperature; 2 M NaCl and a 5 minute extraction time. The method performance data showed that the R^2 values ranged between 0.9936 - 0.9993 and %RSD values were below 12.6 % for all the analytes. The recoveries of spiked samples in tap water, waste water, waste water from a sugar factory and the Caspian Sea water for the studied analytes ranged between 102.5% - 106.2%, 101.9% - 106.0%, 101.9% - 105.7% and 101.5 - 105.6% respectively.

The use of organic solvents poses drawbacks such as limitation of drop size and evaporation of drop in the HS mode. There has been a recent trend of implementing an alternate extracting solvent, namely, ionic liquids (ILs) as the extracting solvent in SDME. ILs is a group of organic salts which are comprised of an organic cation and various anions. These liquids have high stability, extremely low vapour pressure and moderate miscibility of organic compounds. These properties allow for the application of longer extraction times, larger drop sizes, with minimal evaporation thereby enhancing the extraction efficiency of the method.

Vidal et al. (2007) extracted eight chlorobenzenes from tap water, river water and effluent water from a wastewater treatment plant using the IL 1-butyl-3-methyllimidazolium hexafluorophosphate [C_4MIM][PF_6] as the extracting solvent. The optimised method provided correlation coefficients and %RSD values ranging from 0.9981-0.9997 and 1.6%-5.1% respectively indicating that the method was linear and repeatable. The recovery of chlorobenzenes from tap water, river water and effluent water ranged between 94.1% - 100.9%, 99.3% - 106.6% and 60.8% - 120.6% respectively. The recoveries for tap water and river water are acceptable, whereas those for the effluent were rather low. The authors indicated that this could be due to the adsorption of analytes on to suspended solids, thereby decreasing the concentration of the analyte in solution.

Two other studies by Chisvert et al. (2009) and Zhao et al. (2009) extracted chlorobenzenes from water using the ILs 1-hexyl-3-methylimidazolium hexafluorophosphate [C_6MIM][PF_6] and 1-octyl-3-methylimidazolium hexafluorophosphate [C_8MIM][PF_6] respectively. Both methods produced excellent figures of merit with regard to method validation thus indicating their suitability towards the extraction and analysis of chlorobenzenes.

2.4.5. Stir Bar Sorptive Extraction

SBSE was developed at the Research Institute of Chromatography (Kortrijk, Belgium) in 1999 by Sandra and co-workers, with Gerstel (Mulheim, Germany) being the company responsible for commercialising the technique under the name of Twister (Sanchez-Rojas et al., 2009). It is a simple and solventless technique which is theoretically similar to SPME and has been successfully applied to the extraction and enrichment of organic pollutants in environmental samples (Mitra, 2003). Organic analytes are preconcentrated from aqueous samples by adsorption onto the PDMS coated surface of the stir bar. Extraction of aqueous samples usually transpires during constant stirring for a predetermined time. After the specified time, the stir bar is removed from the sample and transferred to a glass tube which is placed into a thermal desorption unit. This unit thermally desorbs analytes from the stir bar, cyrofocuses it in a pre-column and then transfers it to the GC by flash heating (Mitra, 2003).

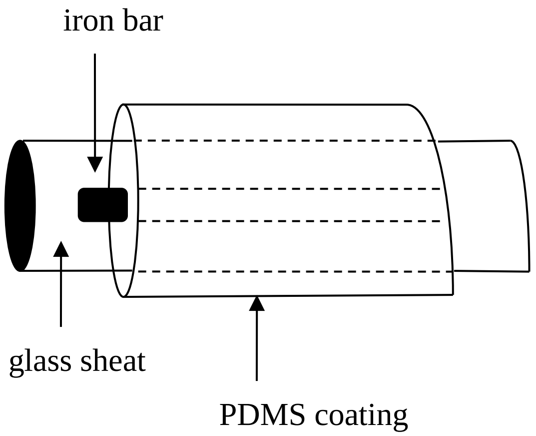


Figure 5: Components of a typical stir bar used for SBSE analysis

A typical stir bar (Figure 5) is 1-2 cm long and consists of a magnetic rod incorporated into a glass jacket which is coated with a 0.5 mm or 1 mm PDMS layer (Montero et al., 2004). Most applications are performed with pure PDMS coatings, although recently, other polymeric phases have been developed and tested. SBSE is an equilibrium technique and the extraction of analytes from aqueous phase is governed by the partitioning coefficient ($K_{PDMS/w}$) of the analyte between the PDMS phase and the aqueous phase (David and Sandra, 2007). This partitioning coefficient has been correlated to the octanol-water distribution coefficients (K_{ow}) which can give a good indication on the ability of PDMS toward the extraction of a particular analyte. Furthermore, the sorption equilibrium is governed by amount of PDMS used. This relationship is shown in Equation 6 (Mitra, 2003; David and Sandra, 2007):

$$K_{o/w} = K_{PDMS/w} = \frac{C_{PDMS}}{C_w}$$

$$= \frac{m_{PDMS}}{m_w} \frac{V_w}{V_{PDMS}}$$

$$= \frac{m_{PDMS}}{m_w} \beta \qquad (6)$$

where,

C_{PDMS} - concentration of the analyte in the PDMS phase at equilibrium

C_w - concentration of the analyte in water at equilibrium

m_{PDMS} - mass of analyte in the PDMS phase

m_w - mass of analyte in water

V_w - volume of water

V_{PDMS} - volume of PDMS

Montero et al. (2004) studied the applicability of SBSE with thermal desorption (TD) towards the extraction and analysis of chlorinated micropollutants (including PCB and HCB) in groundwater. The extraction time profile showed that equilibrium was reached for the analytes after 4 hours. The method was linear between a concentration range of 1-250 ng/ℓ. Recoveries from the groundwater samples were 81.4% and 79.8% for PCB and HCB respectively, while the

%RSD values were less than 3.5% indicating that the method is repeatable and void of any interfering compounds.

The key advantage of SBSE is that it uses a higher volume of PDMS coating (25-100 µℓ)PDMS coating than SPME (Leon et al., 2003). The larger PDMS volume leads to higher sample capacity, recoveries, extraction efficiency and sensitivity (Tan et al., 2008; Perez-Carrera et al., 2007). SBSE sensitivity is increased 50-250 fold as compared to SPME; hence, detection limits as low as the sub ng/ℓ – ng/ℓ levels having being reported (Rodil and Moeder, 2008). However, an increase in the PDMS coating leads to longer equilibration times which can be undesirable in laboratories that require high productivity and throughput. Unlike SPME, SBSE shows little opportunity towards automation. The major drawback of this technique lies in the desorption step. Firstly, a thermal desorption unit is expensive making it unattractive for insufficiently funded laboratories. Secondly, liquid desorption will add another step to the sample preparation process which could further hinder throughput, sensitivity and introduce other contaminants. Lastly, the manual transfer of the stir bar to the desorption unit may also lead to unwanted contamination or loss of analytes (Sanchez-Rojas, 2009).

2.5. Analysis of chlorobenzenes

The separation and trace analysis of chlorobenzenes; being semi-volatile in nature, is usually performed by chromatography. Chromatography is a physico-chemical process by which components in a mixture are separated. Separation is based on the concentration equilibrium of the component between two immiscible phases, namely a stationary phase and a mobile phase. The stationary phase is fixed and is immobilized within a column, while the mobile phase is forced through it. Separation of the compounds occurs due to differential partitioning between the mobile and stationary phases. GC and high performance liquid chromatography (HPLC) are the two most widely used chromatographic techniques in chlorobenzene analysis, with the former having superior popularity. This popularity stems from GC having good selectivity and resolution with good accuracy and precision (Kin, 2008; Rouessac and Rouessac, 2007). This section will emphasize on the principles of GC, as well as its application towards the separation and detection of chlorobenzenes from water systems.

2.5.1. Gas Chromatography

GC is a chromatographic separation instrument which is based on the differential migration of analytes between two immiscible phases in which the mobile phase is a gas passing through the stationary phase contained within a column (Kin, 2008). Separation of mixtures occurs while they are in the gaseous phase; therefore, solids and liquids need to be vaporized. This presents one of the major limitations of GC as its use is restricted to the study of thermostable compounds which have sufficiently high volatility.

A basic experimental layout of a GC comprises of the following components: a gas supply, a gas flow controller, an injector port, a column, an oven and a detector. Although the overall efficiency of the GC analysis is dependent on the combined optimal functioning of these components; this review will detail the function of just the column and detector.

2.5.1.1. GC columns

The column is arguably the most important element in the GC system. The quality of the resolution components can only be as good as the column (Kin, 2008). There are two types of columns, namely, packed and capillary columns. Packed columns have considerably large diameters ranging from 3.18 - 6.35 mm. Due to their unsuitability towards trace analysis, these columns are less frequently used today (Rouessac and Rouessac, 2007). Capillary columns have an internal diameter ranging between 100-530 μm, thickness of 50 μm and a length of between 12-100 meters. These columns are made from high purity fused silica and are flexible with high thermal stability. The stationary phase is impregnated as a film on the inner surface of the column and has an upper and lower operating temperature. At the lower temperature limit the three way concentration equilibria (equilibria between the analytes, mobile phase and stationary phase) does not occur while at the upper temperature limit degradation of the stationary phase occurs. Capillary column stationary phases can be categorized into three groups: 1) non-polar, 2) semi-polar and 3) polar. The non-polar phases are composed of methylpolysiloxanes, the semi-polar phases (50:50) (methylpolysiloxane/phenylpolysiloxane) and the polar phases contain polyethylene glycol. The structures of these phases are given in the Figure 6 below.

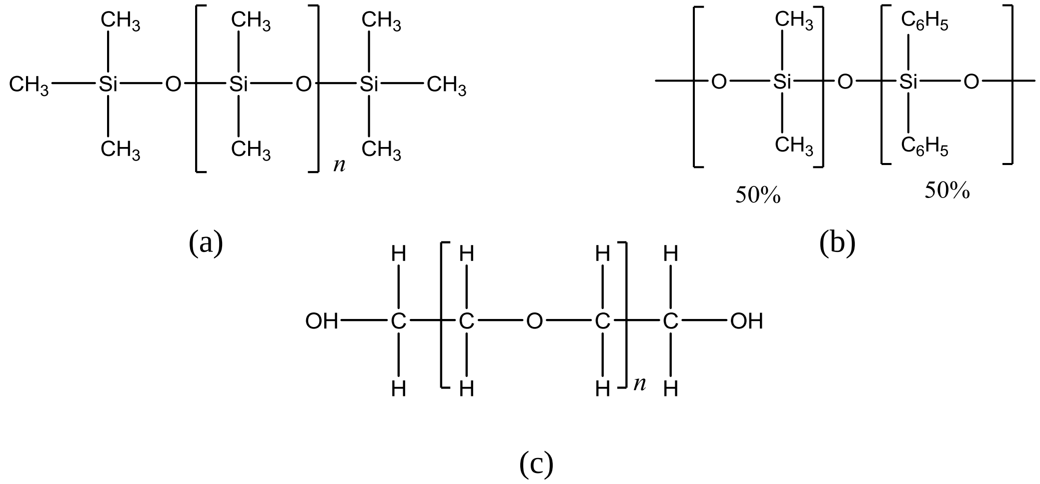

(a) (b) (c)

Figure 6: Structures of selected column stationary phases

Table 4 contains a summary of GC columns commonly used for chlorobenzene analysis. It is evident that researchers favoured the non-polar phase columns, particularly the 95% dimethylsiloxane:5% diphenylsiloxane composition column. This could stem from the column being readily available in most laboratories as it regarded as a universal column; suitable for the separation of most analytes.

Table 4: Summary of GC columns used for chlorobenzene analysis

Reference	Column	Composition	Polarity
He et al. (2000); Wang et al. (1998)	DB-1	100% dimethylsiloxane	Non-polar
Noche et al., 2013; Huang et al. (2007); Vidal et al. (2005); Montero et al. (2004); Wang et al. (2003); Ormad et al. (1996)	HP-5	95% dimethylsiloxane 5% diphenylsiloxane	Non-polar
Yrieix et al. (1996)	PTE-5		
Laniewski et al. (1998)	BPX-5		
Khajeh et al. (2006)	DB-5		
Chisvert et al. (2009); Tor (2006)	DB-624	94% dimethylpolysiloxane 6% cyanopropylphenyl	Intermediate

2.5.1.2. Detectors

The detector is housed in a thermally insulated unit and it is responsible for converting the interaction between analytes and the detector, into an electronic signal. There is a wide variety of detectors available, either for specialist applications or for more general use. A detector is generally operated isothermally at a temperature which is 20^{o}C higher than the maximum oven temperature. This prevents condensation of the sample which can cause considerable detector noise leading to decreased detector sensitivity, accuracy and precision of results (Kin, 2008).

There are several detectors which can be coupled to a GC. Some detectors are universal as they are sensitive to all components that elute from the column, while others are discriminatory as they are sensitive to specific components. The following criteria should be critically evaluated to judge the performance of a detector (Currell, 2000):

- Maximum operating temperature
- Sensitivity
- Dynamic linear range;
- Selectivity
- Noise.

Table 5: Comparison of the figures of merit for the various GC detectors

Detector	Selectivity	Sensitivity	Linear range	Operating Temperature (^oC)
Thermal conductivity detector	All compounds, besides carrier gas	5-20 ng	10^5-10^6	150-250
Flame ionization detector	Hydrocarbons	0.1-10 ng	10^5-10^7	250-450
Electron capture detector	Electronegative compounds (Halogens, nitrates and conjugated carbonyls)	0.1-10 pg	10^3-10^4	300-400
Nitrogen phosphorous detector	Nitrogen and phosphorous containing compounds	1-10 pg	10^4-10^6	250-300
Photo-ionization detector	Hydrocarbons and sulphur and phosphorous derivatives	25-200 pg	10^5-10^6	200
Mass spectrometer	Any compound that produces a fragment within the specified mass range	1-10 ng	10^5-10^6	250-300

Table 5 describes the sensitivity and selectivity of some of the common detectors used for GC analysis. Due to the electronegative character of chlorobenzenes compounds as well as their trace presence in aquatic systems, the detectors primarily used for their analysis have been the electron capture detector (ECD) and the mass spectrometer (MS). These detectors have high selectivity and far superior sensitivities for these compounds as compared to the other available detectors. Other studies have carried out the analysis of chlorobenzenes using alternate detectors such as the atomic emission detector (AED) (Laniewski, 1998) and flame ionization detector (FID) (Zhao, 2009; Wang, 2003; Yrieix, 1996). However, the detectors used in the above mentioned citations do not offer any degree of selectivity or enhanced sensitivity towards chlorobenzenes. In the sections to follow, a review will be limited to the detection of chlorobenzenes using GC-ECD, GC-MS and GC-FID.

Gas Chromatography – Electron Capture Detector

The ECD is a selective detector that is regarded as excellent for the trace analysis of electronegative compounds such as halogenated or nitro- compounds. A flow of carrier gas (nitrogen or argon) is ionized by electrons which have been emitted from a β-radioactive source (^{63}Ni foil). The ionized carrier gas passes between two electrodes maintained at a specific voltage to produce an equilibrium base current (I_0). If an electronegative species traverses the zone between the two electrodes, they capture thermally excited ions to form heavily negative ions which are less mobile thereby leading to a decrease in the signal (Currell, 2000; Rouessac and Rouessac, 2007). The ECD can be used in two different modes, namely, the direct current (DC) mode and the pulsed mode. The main difference between the two modes is that under the DC mode conditions the electrons in the detector have higher kinetic energy than the carrier gas, whereas under pulsed mode conditions, the electrons are in thermal equilibrium with the gas molecules (Maggs et al., 1971).

Studies carried out by Khajeh et al. (2006); Tor (2006) and Ormad et al. (1996) analyzed chlorobenzenes in aqueous systems using GC-ECD. These studies produced varying limits of detection (LOD) which were dependant on the type of preconcentration technique used (Table 6). The major advantage of this detector is its high sensitivity towards electronegative organic compounds. However, the high sensitivity also contributes to instrument downtime, as the electron capture detector is prone to contamination. Further disadvantages include sensitivity to aqueous solutions, narrow linearity range and the presence of a radioactive source, which may require a license prior to use and expensive disposal costs (Brandenberger and Maes, 1997).

Table 6: Comparison of the LOD's obtained in studies utilizing ECD

Compound	**Limit of detection (µg/ℓ)**			
	a	***b***	c^1	c^2
MCB	1.9	0.008	-	-
1,2-DCB	0.2	0.005	4.4	1.8
1,3-DCB	0.1	0.004	4.4	1.8
1,4-DCB	3.0	0.006	4.4	2.2
1,2,3-TCB	-	0.006	3.1	2.2
1,2,4-TCB	-	-	2.7	1.8
1,3,5-TCB	-	-	4.4	2.2
1,2,3,4-TeCB	-	-	4.0	3.1
PCB	-	-	2.7	3.1
HCB	-	-	1.3	3.1

a – HS-SDME preconcentration (Khajeh et al, 2006)

b - SDME preconcentration (Tor, 2006)

c^1- granular activated carbon (GAC) preconcentration (Ormad et al., 1996)

c^2- LLE preconcentration (Ormad et al., 1996)

Gas chromatography - mass spectrometry (GC-MS)

MS is used for the characterization of matter by determining the atomic masses of each constituent in the sample matrix (Rouessac and Rouessac, 2007). GC-MS is a technique which utilizes the benefits of both GC and MS to provide a higher degree of identification by providing structural information on the components within a sample matrix. Structural data can be used to confirm the presence of components in a sample mixture or it can be used alleviate any confusion brought about by complex chromatograms.

For any given test mixture, the GC column provides separation of components. These separated components have different retention times; eluting from the column separately. These components then traverse the interface between the GC and MS and enter the MS in which a minute amount of sample, which is present in the gas phase and devoid of any carrier gas, is ionized by chemical or electron ionization (Currell, 2000). In chemical ionization, a reactant gas (methane) is ionized by an electron beam. These ionized gas molecules then interact with the analyte molecules and in the process, a transfer of charge occurs. In electron ionization, ionization is attained by directing an electron beam across the input of analyte flow and is by far the most commonly used method of ionization.

Following ionization, the ions are then focussed and accelerated by a series of electronic lenses to increase their kinetic energy. The ions enter a mass analyzer in which they are separated according to their mass-charge ratio (*m/z*) (Rouessac and Rouessac, 2007). There are several different types of mass analyzers available, such as the quadrupole, ion trap and time-of-flight analyzers, with each having their own specific design to provide selective separation. Once separated, the ions collide with the sensor of the detector which measures electrical charge and amplifies the weak ionic current. The electronic signal is then converted and displayed as two-dimensional plot (mass spectrum) of signal intensity versus atomic mass (Da) (Rouessac and Rouessac, 2007; Currell, 2000).

The detection system of a mass spectrometer can be used in a number of modes, namely, the full scan mode, the selected ion mode (SIM), peak hopping mode or the total ion current. A full scan mode records several signals over a broad range of mass value; while the peak hopping mode records signals at a number of specific mass values and the total ion current mode gives a combined current across all masses (Currell, 2000).

GC-MS has extremely high sensitivity and has an option of using the SIM mode. The SIM mode scans a specific mass value range; therefore, it can eliminate any matrix interferences which are a huge detriment to trace analysis (Currell, 2000). Due to the aforementioned reasons, many studies involving the analysis of chlorobenzenes (Chisvert et al., 2009; Tan et al., 2008; Basheer et al., 2005; Vidal et al., 2005; Liu et al., 2004; Montero et al., 2004; Leon et al., 2003; He et al., 2000; Wang et al., 1998) has favoured the use of GC-MS. From Table 7, it can be seen that these studies produced extremely low LODs, indicating that this analytical instrument is highly suitable for trace analysis.

Table 7: Comparison of the LOD's obtained in studies utilizing MS detection

Compound	**Limit of detection (ng/ℓ)**						
	a	***b***	***c***	***d***	***e***	***f***	***g***
MCB	-	-	-	-	-	-	20
1,2-DCB	2.0	6.0	-	6.0	12.0	80	2
1,3-DCB	3.0	3.0	-	6.0	10.0	80	70
1,4-DCB	2.0	6.0	-	6.0	-	100	70
1,2,3-TCB	2.0	6.0	-	4.0	13.0	10	2
1,2,4-TCB	2.0	6.1	-	4.0	31.0	-	3
1,3,5-TCB	4.0	6.7	-	4.0	19.0	10	4
1,2,3,4-TeCB	2.0	3.0	-	3.0	28.0	-	0.3
1,2,4,5-TeCB	2.0	3.0	-	3.0	20.0	-	0.6
PCB	1.0	16.0	0.3	4.0	28.0	-	2
HCB	2.0	31.0	0.5	6.0	45.0	-	2

a – Chisvert et al. (2009), IL-HS-SDME preconcentration

b – Vidal et al. (2005), SDME preconcentration

c – Montero et al. (2004), SBSE preconcentration

d – He et al. (2000), SPME preconcentration

e – Wang and Lee (1998), SPE preconcentration *f* - Esrafili et al. (2012), Hollow fibre LPME preconcentration

g - Noche et al. (2013), Microextraction by packed sorbent preconcentration

The major disadvantages of GC-MS are that is time consuming and has high capital and operating costs (NRC, 1991). Similar to GC-ECD, the GC-MS system is prone to contamination due to high sensitivity. Furthermore, highly skilled labourers are required to perform the operation and maintenance of such the instrument; as well as to perform suitable sample clean up prior to analysis.

Gas chromatography – flame ionization detector

Due to its diverse and comprehensive response, the FID is considered as a universal detector. The FID offers excellent sensitivity towards the detection of hydrocarbons. Analytes which have been separated by the analytical column are delivered by the carrier gas into a flame which is fuelled by hydrogen and synthetic air. These analytes undergo a combustion process in which ions and free electrons are formed. A potential is applied across the flame and as ionic species pass through two electrodes within the detector they amass onto a charge collector, producing a detector signal in the form of a small current. The magnitude of the detector signal is proportional to the organic ions present in the flame (Poole, 2003).

A disadvantage of GC-FID technique is that it is considered to be a universal detector which lacks selectivity and has a broad sensitivity range. With the exception of a study performed by Zhou et al. (2009), the analysis of chlorobenzenes using GC-FID has not been the analytical instrument of choice. Advantages of the FID include low detection limits, long term stability, simplicity of operation and construction, low dead volume, fast signal response and excellent linear response range (Poole, 2003). Furthermore, the GC-FID has relatively low capital, operational and maintenance costs; is found is most analytical laboratories; and is a suitable method of quantification for poorly funded laboratories. Additionally, GC-FID has far less service interruptions due to cell and sample contamination than it more sensitive counterparts. As a result, if one can analyse specific organic determinants to trace levels, or to levels governed by regulatory standards by GC-FID, the economic and productivity challenges associated with the more sensitive GC detectors can be overcome.

CHAPTER THREE

EXPERIMENTAL

3.1. Chemicals

All chemicals used in this study were of the highest purity. Methanol (HPLC grade, 99.9%); 1,2,4-TCB (99%); 1,2,3,4-TeCB (98%); 1,2,4,5-TeCB (98 %) and PCB (98 %) were purchased from Sigma Aldrich (Milwaukee, WI, USA). 1,2-DCB (>98%); 1,3-DCB (>98%), 1,4-DCB (>98%) and reagent grade sodium chloride were purchased from Merck (Darmstadt, Germany). Water used in this study was obtained from a Millipore RO15 purification water system from Waters (Massachusetts, USA). All standards were prepared in methanol while the matrix used in the optimization study was Millipore water.

3.2. SPME material

The SPME components (manual syringe holder and fibers) were purchased from Supelco (Bellefonte, PA, USA). Two fiber types attached to a standard 24 gauge needle were used in this study; namely, 85 µm polyacrylate (PA) and 100 µm polydimethylsiloxane (PDMS). The properties of these two fibres are given in Table 8 below:

Table 8: Properties of SPME fibres used in this study

Fibre type	**Film thickness (µm)**	**Description**	**pH stability range**	**Maximum temperature (^{o}C)**	**Conditioning temperature (^{o}C)**	**Conditioning time (min)**
PDMS	100	Non-bonded	2-10	280	250	30
PA	85	Bonded	2-11	320	300	120

3.3. Instrumentation

The separation and detection of the analytes was carried out on a GC-2014 Shimadzu (Shimadzu Corporation, Kyoto, Japan) GC, fitted with a FID system. Helium was used as the carrier gas which was passed through an oxygen, moisture and hydrocarbon trap prior to entering the GC. All GC injections were done in the splitless mode and the injector port was equipped with a splitless mode compatible glass insert. The vent valve of the split outlet was closed for 30 seconds, following which it was opened to give a split ratio of 1:20. The injector port temperature was maintained at 250^{o}C.

The separation of the analytes was performed on a SBPX-5 column (30 m x 0.25mm internal diameter, 0.25 µm film thickness) supplied by SGE (Victoria, Australia). The column flow rate was maintained at 1 mℓ/min. To maximise resolution of the analytes the column temperature was controlled by a temperature programme. The following temperature programme was used: 60^{o}C held for 2 minutes; ramped at 10^{o}C/min from 60-70^{o}C and held for 4 minutes; ramped at 5^{o}C/min from 70-85^{o}C; ramped at 15^{o}C/min from 85-205^{o}C; ramped at

25^{o}C/min from 205-280^{o}C and held for 5 minutes. The total analysis time was 26 minutes. The FID temperature was maintained at 300^{o}C. All data was processed using the GC solution Post-run software (Shimadzu, version 2.30).

3.4. Preparation of standard solutions

1000 mg/ℓ single standard stock solutions of each analyte was prepared in methanol. From this a 1000 mg/ℓ multi-compound standard was prepared, from which a 0.1 mg/ℓ working solution was prepared by a series of dilutions. The working solution was prepared daily using deionized water and all solutions were stored in the dark at 4^{o}C.

3.5. Samples

Samples were collected from the Grootdraai Dam by Eskom personnel in 1 litre Schott bottles and couriered to the Durban University of Technology, Chemistry Department. The Grootdraai Dam is an impoundment on the Vaal River which is situated north-east of Standerton in the province of Mpumalanga, South Africa (Locality: 26^{o}54’33.6”S 29^{o}23’42.5”E) (Ginkel, 2001). The dam was established in 1981, has a gross storage capacity of 364 million m^{3}, surface area of 39 km^{2} and an average depth of 27 metres. The Grootdraai Dam is upstream of the Vaal Dam, forming part of the Usuthu – Vaal Scheme (Stauss, 2006; Jeleni and Mare, 2007).

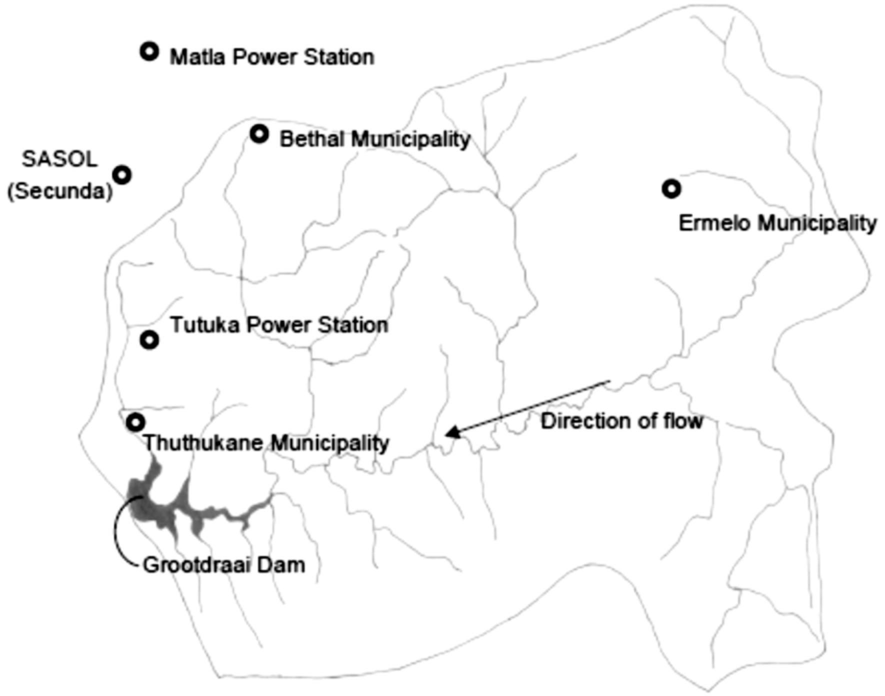


Figure 7: Grootdraai Dam catchment

With the exception of small local community, there is limited domestic use of water directly from the dam (Ginkel, 2001). The Grootdraai Dam catchment has a surface area of 7928 km^2 and the major water users are industries and municipalities (Figure 7) (Strauss, 2006; Ginkel, 2001).

3.6. Glassware

To minimize any contamination and interferences, glassware was meticulously cleaned. Firstly all glassware was washed with Teepol® soap and rinsed thoroughly with Millipore water. The glassware was then soaked overnight in cleaning preparation solution. After this, glassware was then rinsed with Millipore water, then acetone and dried in an oven set at 60^{o}C. The glassware was then capped to prevent the accumulation of dust particles and stored in a dark, cupboard in an air conditioned laboratory. Prior to use, glassware was rinsed three times with 5 mℓ aliquots of methanol.

3.7. SPME procedure

The optimization of the main parameters influencing an SPME extraction was performed using Millipore water fortified with the seven chlorobenzene compounds contained in 22 mℓ amber glass vials. For a typical experiment, 0.1 mℓ of the 100 ng/mℓ working standard was added to a vial containing 5 mℓ of Millipore water to yield a concentration of 2 ng/mℓ of each analyte. In the case of the experiments for salting out effect, 5 mℓ sodium chloride solutions of 5%, 10%, 15% and 20% (m/v) instead of pure millipore water was used. To enhance the transfer of analytes to the fibre, agitation was performed using a 2 cm Teflon stir bar, coupled to a magnetic stirrer. The vials were then sealed with screw type plastic caps containing a PTFE/silicon septum.

The SPME manual holder needle was then used to penetrate the septum, and the fibre was then directly immersed into the sample solution for a pre-determined time. To enhance exposure of the fibre to the analytes, precaution was taken to ensure that the fibre was exposed to the outside flow of the vortex rather than directly within the vortex. After extraction, the fibre was retracted back into the protective needle and removed from the sample vial. Lint free tissue paper was then used to remove any water present at the entrance of the protective needle.

The protective needle was then used to penetrate the septum of the GC, and the fibre was then exposed to thermal conditions of the injector port for a desorption time of 15 minutes. All samples were freshly prepared minutes before the extraction to reduce/eliminate any losses due to volatilization or contamination due to prolonged storage. SPME fibres were conditioned for 30 minutes every morning and blank fibre injections were performed to assess the presence of any contamination within the fibre.

3.8. Statistical analysis

All data obtained from the optimisation and method validation experiments were statistically analysed using Microsoft® Excel 2010. General statistics (mean, standard deviation, standard error and % relative standard deviation) was applied to all chromatographic data. In the case of the optimisation experiments, additional comparative statistical analysis (Student's t-test and ANOVA) was performed. The Student's t-test (two-tailed with equal variance, $\alpha = 0.05$) was applied to the data obtained from the fibre optimization experiment, while ANOVA (single factor, $\alpha = 0.05$) was applied to sample size, stir speed and salt addition experiments. Furthermore, if the ANOVA test indicated a significant difference between data sets, t-tests were done to identify precisely where the significant difference existed. The following hypotheses and conditions were used for the statistical evaluation.

3.8.1. Student’s t-test

A two-tailed Student’s t-test ($\alpha = 0.05$) was applied to the data sets obtained from the fibre optimization experiments. The hypothesis (H_0) and alternative hypothesis (H_a) were as follows:

$H_0 = \mu_{PA} = \mu_{PDMS}$

$H_a = \mu_{PA} \neq \mu_{PDMS}$

Conditions

If probability value (p-value) is less than alpha ($\alpha = 0.05$) then H_0 is rejected, with H_a being accepted. Alternatively, if t-calculated (t_{calc}) is greater than t-critical (t_{crit}) H_0 is rejected and H_a is accepted.

3.8.2. ANOVA

Single factor ANOVA data analysis was performed on the sample size, stir speed and salt addition experiments. The number of data sets for each of the above experiments was as follows: stir speed (4 - 300 rpm, 500 rpm, 700 rpm, 1100 rpm); sample size (3 – 5 mℓ, 10 mℓ, 15 mℓ); salt addition (5 – 0%, 5%, 10%, 15%, 20%). The hypothesis (H_0) and alternative hypothesis (H_a) for each experiment were as follows:

Stir speed

$H_0 = \mu_{300\text{rpm}} = \mu_{500\text{rpm}} = \mu_{700\text{ rpm}} = \mu_{1100\text{rpm}}$

$H_a = \mu_{300\text{rpm}} \neq \mu_{500\text{rpm}} \neq \mu_{700\text{ rpm}} \neq \mu_{1100\text{rpm}}$

Sample size

$H_0 = \mu_{5\text{m}\ell} = \mu_{10\text{m}\ell} = \mu_{15\text{m}\ell}$

$H_a = \mu_{\text{ m}\ell} \neq \mu_{1\text{ m}\ell} \neq \mu_{1\text{ m}\ell}$

Salt addition

$H_0 = \mu_{0\%} = \mu_{5\%} = \mu_{10\%} = \mu_{15\%} = \mu_{20\%}$

$H_a = \mu_{0\%} \neq \mu_{5\%} \neq \mu_{10\%} \neq \mu_{15\%} \neq \mu_{20\%}$

Conditions

If probability value (p-value) is less than alpha ($\alpha = 0.05$) then H_0 is rejected, with H_a being accepted. Alternatively, if F-calculated (F_{calc}) is greater than F-critical (F_{crit}) H_0 is rejected and H_a is accepted.

CHAPTER FOUR

DISCUSSION

4.1. Suitability of DI-SPME-GC-FID for the analysis of chlorobenzenes

Preliminary experiments were performed to assess the preconcentration efficiency of DI-SPME coupled to GC-FID. Direct injections of the standards were made at two different concentrations, 100 ng/mℓ and 10000 ng/mℓ. The 100 ng/mℓ injection did not yield any detectable peaks while the 10000 ng/mℓ injection produced peaks. However, these peak areas were minute. To enhance detectability, preconcentration was required. To assess the performance of the SPME technique, 10 mℓ of distilled water was spiked with 10 ng/mℓ of each analyte and extracted for 10 minutes with agitation (500 rpm), using the PDMS fibre (Figure 8).

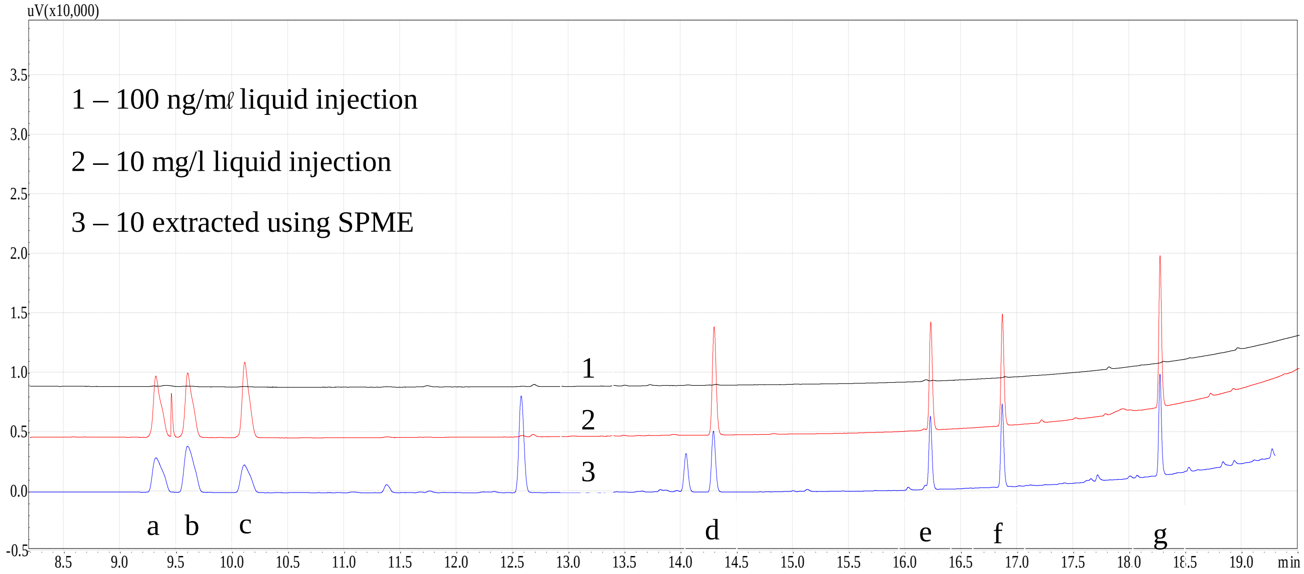


Figure 8: Suitability of SPME; (1) 100 ng/mℓ liquid injection; (2) 10 mg/l liquid injection; (3) 10 ng/mℓ extracted using SPME; (a) 1,3-DCB; (b) 1,4-DCB; (c) 1,2-DCB; (d) 1,2,4-TCB, (e) 1,2,4,5-TeCB; (f) 1,2,3,4-TeCB; (g) PCB

From Figure 8, one can ascertain that SPME significantly enhanced the level of detection of all analytes. The peak areas after SPME extraction were almost the same as that of the 10 mg/ℓ liquid injection. This indicates that SPME can offer an enrichment factor of approximately 1000 times. This indicates that DI-SPME coupled to GC-FID is a viable technique for the detection of chlorobenzenes at trace levels.

4.2. Optimization of the SPME procedure

Prior to one using SPME for the analysis of samples, the relevant parameters affecting the process need to be fully understood and optimized. In this study, five parameters were optimized, namely, fibre coating, agitation speed, sample size, salting out effect and extraction time. The results of these experiments will be discussed in the sub-sections to follow.

4.2.1. Fibre coating

Selecting an appropriate fibre coating is the most crucial step in an SPME process. The chemical nature of the target analytes provides a guideline as to which fibre coating should be utilized. Similar to that of column stationary phase, the general selection rule for fibre coating is ‘like attracts like.’ This study investigated the applicability of two fibres which were available, namely, polyacrylate (PA) and polydimethyl siloxane (PDMS). The adsorption efficiencies of these two fibres towards the extraction of chlorobenzenes spiked at 2 ng/mℓ each are shown in Figure 9 below.

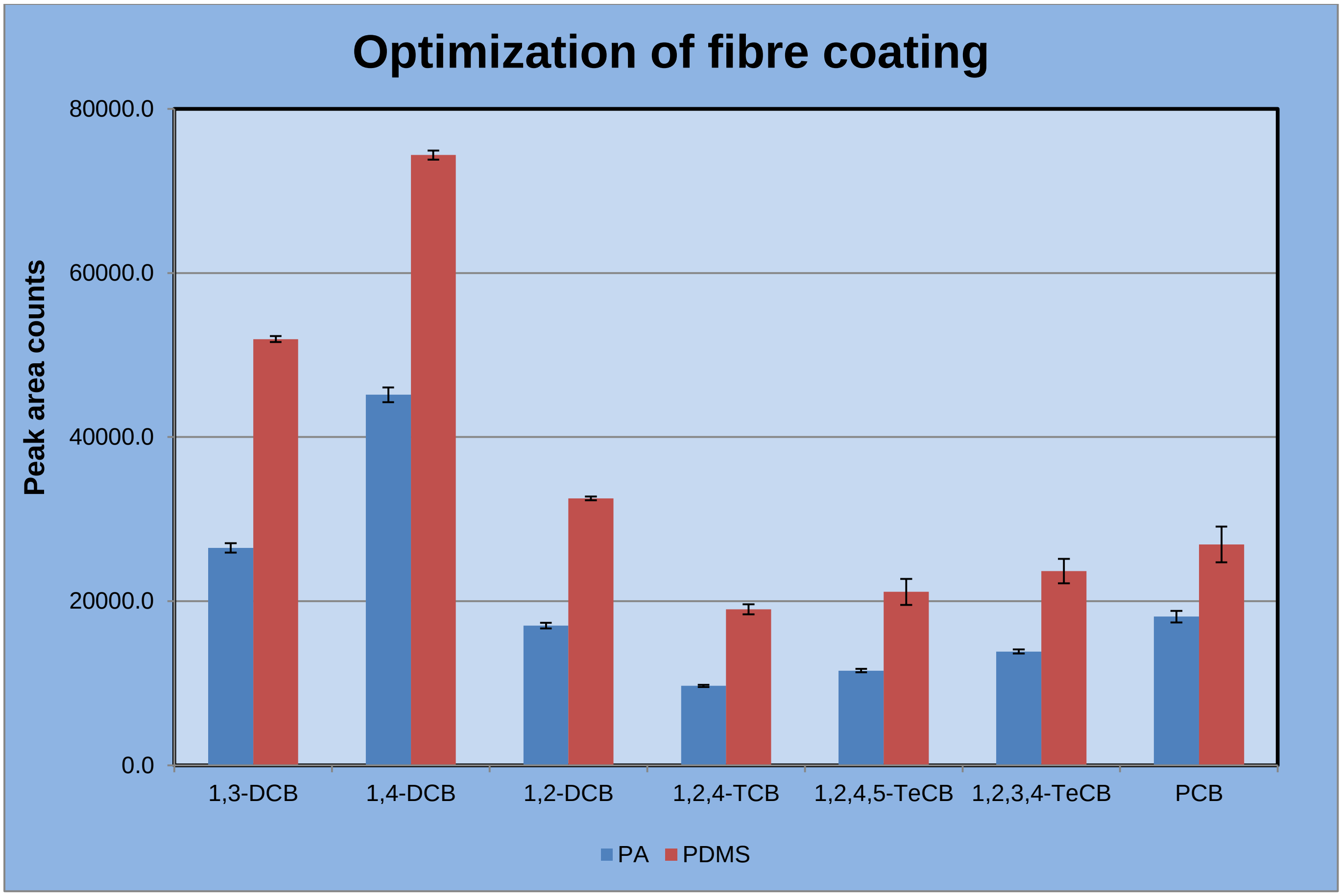
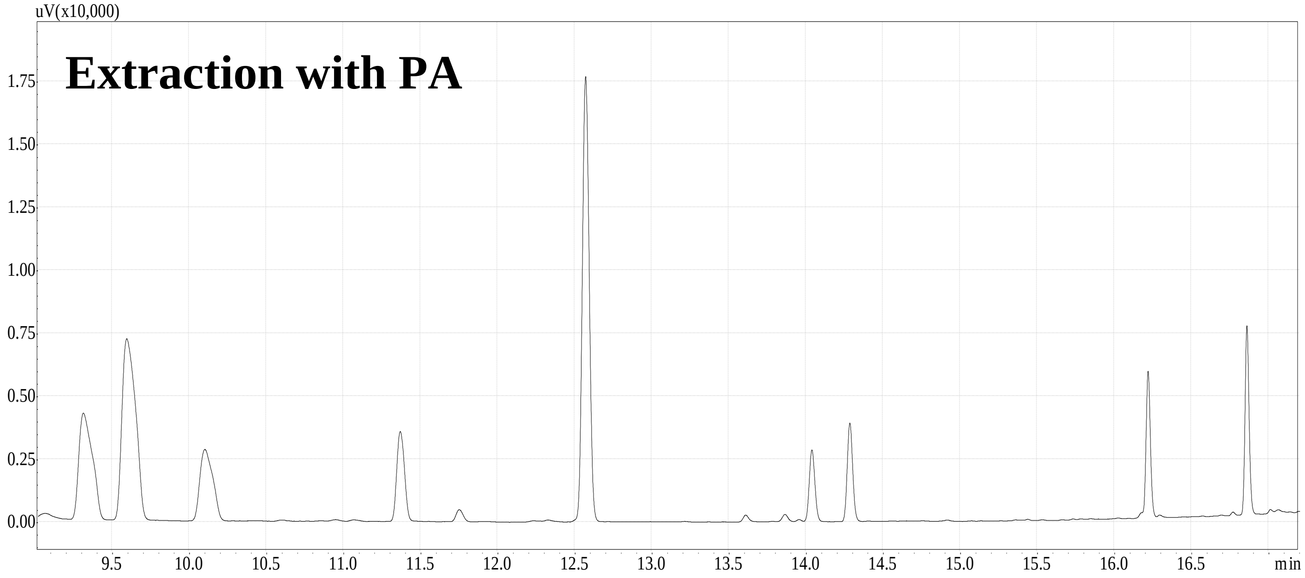
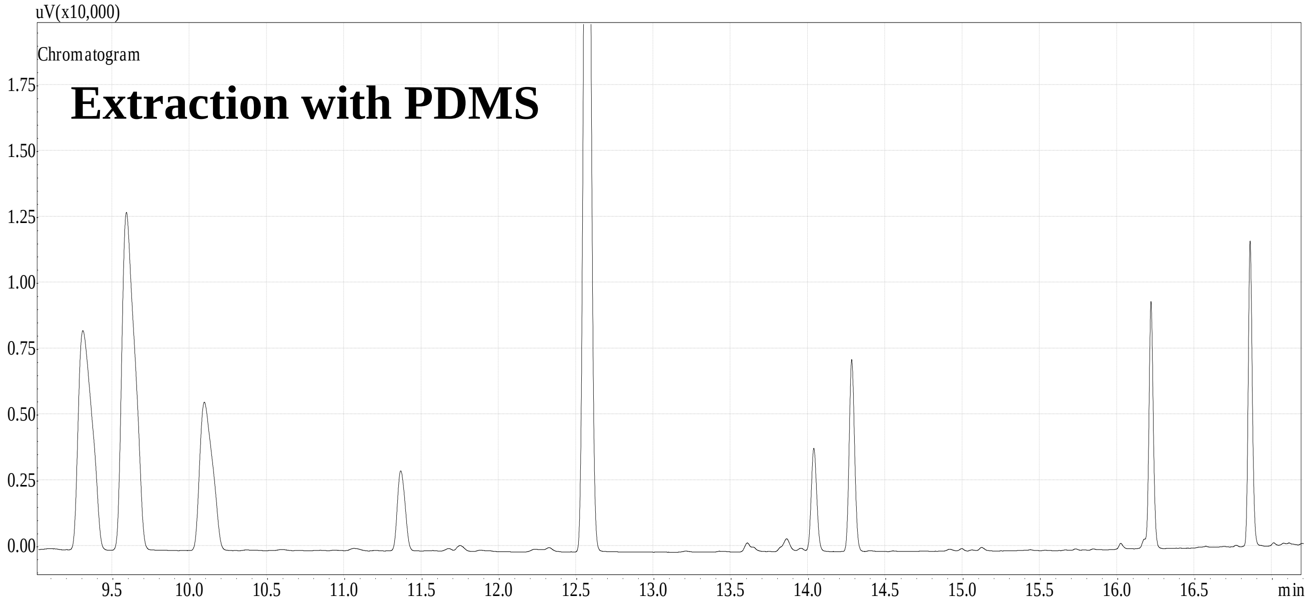


Figure 9: Efficiency of PA and PDMS coatings towards the extraction of chlorobenzenes (10 ng/mℓ concentration)

As shown in Figure 9 above, the PDMS fibre coating produced a higher sensitivity, as compared to the PA fibre coating towards the adsorption of chlorobenzenes. Based on peak area counts, extractions carried out with PDMS were between 1.3-1.9 times higher than that of PA. A comparison of the chromatograms obtained from extractions performed with the PA and PDMS fibres is shown in Figures 10 a and b below.



(a)



(b)

Figure 10: Comparison of chromatograms obtained from extraction with (a) PA and (b) PDMS fibres

A two-tailed Student's t-test ($\alpha = 0.05$) assuming equal variance was applied to the peak area counts obtained from the extractions with each of the two fibres. The hypothesis (H_0) and alternative hypothesis (H_a) for the statistical analysis were as follows:

$H_0 = \mu_{PDMS} = \mu_{PA}$ (accept if p-value > α)

$H_a = \mu_{PDMS} \neq \mu_{PA}$ (accept if p-value < α)

Table 9 below indicates that the probability values (p-values) obtained from the statistical analysis were less than α (0.05) for all the chlorobenzene compounds. Therefore, H_0 is rejected, and conclude that the PDMS and PA fibres significantly affect extraction efficiency.

Table 9: Statistical data for the selection of SPME fibre

Compound	t critical	t calculated	α	p-value
1,3-DCB	2.776445	-38.1845	0.05	0.00000281
1,4-DCB		-27.3769		0.00001059
1,2-DCB		-36.9179		0.00000321
1,2,4-TCB		-15.0026		0.000115
1,2,4,5-TeCB		-6.0216		0.0038317
1,2,3,4-TeCB		-6.55268		0.0028047
PCB		-3.83606		0.0185219

An analysis of the properties of both the analytes and the fibres provides an explanation for this difference in extraction efficiencies. PDMS is a non-polar fibre, thus suitable for non-polar analytes, while PA is a polar coating which is suitable for polar analytes. The chlorobenzene compounds used in this study are predominantly non-polar compounds, with an increase in polarity with increasing chlorination. Therefore, based on the ‘like attracts like’ principle the PDMS coating provides higher extraction efficiency. A supporting reason for the variance in adsorption efficiency is the difference in the volume of extracting phase; with PDMS and PA having extracting phase volumes of 100 µm and 85 µm respectively. In general a higher volume of extracting phase increases the capacity of a fibre to adsorb analytes, hence the higher adsorption efficiency for the PDMS fibre. Due to the discussion above, the PDMS coated fibre was selected for the rest of the study.

4.2.2. Rate of stirring

Equilibration times are dependent on the operational efficiency and the speed of stirring as it promotes the mass transfer of analytes between the bulk of the sample and fibre (Pawliszyn, 1997). There are two factors which greatly influence the rate of stirring, namely, the dimensions of the sample vessel and the dimension of the stir bar (Kin, 2008). In this experiment the optimum stir speed was determined by using four different rates (300 rpm, 500 rpm, 700 rpm and 1100 rpm) using a 2 cm stir bar and a 22 mℓ sample vessel (Figure 11).

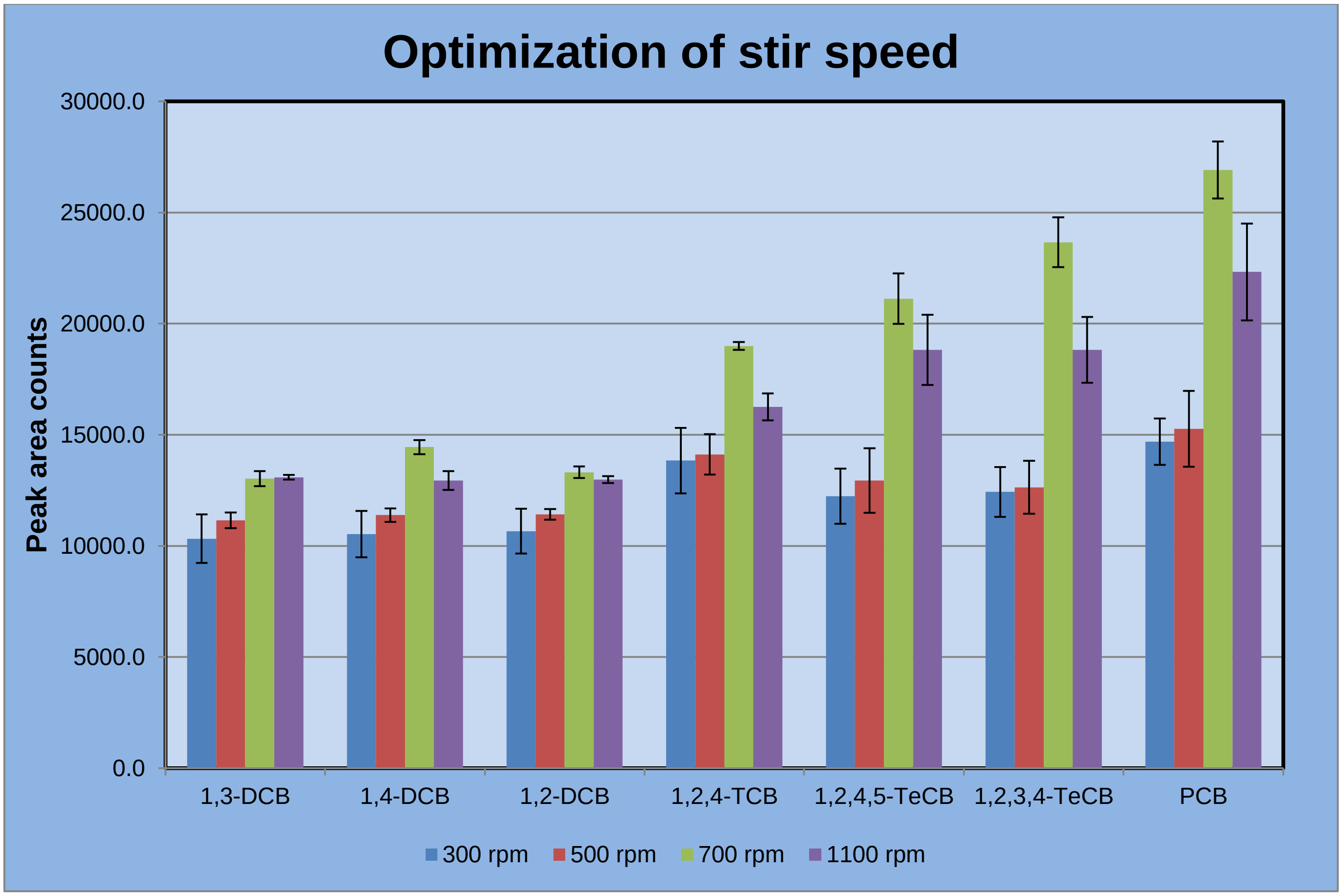


Figure 11: Influence of the rate of agitation on extraction efficiency

Figure 11 indicates that the response of each analyte increases with increasing stir speed. This was expected as SPME is an equilibrium process and an enhanced diffusion (increased stir rate) through the phases will lead to a decrease in equilibration times (Pawliszyn, 1997). Single factor ANOVA data analysis ($\alpha = 0.05$) was performed on the peak areas obtained as the stir speed was varied from 300 rpm – 1100 rpm. The H_0 and H_a for the statistical analysis were as follows:

$H_0 = \mu_{300rpm} = \mu_{500rpm} = \mu_{700\ rpm} = \mu_{1100rpm}$ (accept if p-value > α)

$H_a = \mu_{300rpm} \neq \mu_{500rpm} \neq \mu_{700\ rpm} \neq \mu_{1100rpm}$ (accept if p-value < α)

Table 10 below indicates that the p-values for each chlorobenzene compound were less than α (0.05), indicating that is a significant difference when the rate of agitation was altered.

Table 10: Statistical data for rate of agitation optimization

	F critical	F calculated	α	p-value
1,3-DCB	4.066181	5.28946	0.05	0.026552
1,4-DCB		8.253009		0.007849
1,2-DCB		5.520322		0.023804
1,2,4-TCB		8.533226		0.007114
1,2,4,5-TeCB		11.0496		0.00323
1,2,3,4-TeCB		20.46871		0.000414
PCB		13.7842		0.001585

It is evident that at a stir speed of 700 rpm resulted in the highest extraction efficiency while further increase to 1100 rpm resulted in a decrease in extraction efficiency. Kin (2008) experienced a similar phenomenon. This is attributed to the vibration of the stir bar and the irregular agitation of the sample. Furthermore, a stir rate of 1100 rpm resulted in splashing of sample onto walls of glass vials. This could have resulted in the loss of analytes and decreased extraction efficiency. Higher random errors were obtained at higher stir rates, indicating that experiments at this stir speed were a lot more difficult to reproduce, thereby decreasing method precision. From Figure 11, it is apparent that the rate of agitation had a minimum effect on the lower chlorinated benzenes (1,3-DCB, 1,2-DCB and 1,4-DCB, 1,2,4-TCB) while higher stir rates promoted an enhanced extraction efficiency for the higher chlorinated benzenes (1,2,4,5- and 1,2,3,4-TeCB and PCB).

Further statistical analysis was performed to assess the impact of agitation performed at 300 rpm and 700 rpm respectively. A two-tailed Student's t-test ($\alpha = 0.05$) assuming equal variance was applied to the peak area counts obtained from the extractions performed at 300 rpm and 700 rpm. The H_0 and H_a for the statistical analysis were as follows:

$H_0 = \mu_{300rpm} = \mu_{700rpm}$ (accept if p-value > α)

$H_a = \mu_{300rpm} \neq \mu_{700rpm}$ (accept if p-value < α)

Table 11: Statistical data for agitation at 300 rpm and 700 rpm

	t-critical	t-calculated	α	p-value
1,3-DCB	2.776445	-2.41511	0.05	0.073149138
1,4-DCB		-2.21835		0.090778906
1,2-DCB		-2.24108		0.08851545
1,2,4-TCB		-1.99817		0.1163597
1,2,4,5-TeCB		-4.3135		0.012511371
1,2,3,4-TeCB		-4.41096		0.011592007
PCB		-4.9668		0.007668906

Table 11 above indicates that the p-values for 1,3-DCB, 1,4-DCB, 1,2-DCB and 1,2,4-TCB were greater than α (0.05), while the p-values for 1,2,4,5-TeCB, 1,2,3,4-TeCB and PCB were less than α (0.05). This indicates that H_0 can be accepted for 1,3-DCB, 1,4-DCB, 1,2-DCB and 1,2,4-TCB and rejected for 1,2,4,5-TeCB, 1,2,3,4-TeCB and PCB. The statistical analysis confirms that there is no significant difference in the extraction efficiencies of the lower chlorobenzene compounds as the stir rate is changed from 300 rpm to 700 rpm. This suggests that the rate of mass transfer from the bulk of the sample to the fibre is sufficient for low molecular weight chlorobenzenes at low rates of agitation. The converse is true of high molecular weight chlorobenzenes; therefore a stir speed of 700 rpm was deemed optimum and was used for the rest of the study.

4.2.3. Sample size

In all studies carried out on water matrices using direct immersion-SPME (Filho et al., 2010; Lopez-Darias et al., 2010a; Lopez-Darias et al., 2010b; Meng and Anderson, 2010; Li et al. 2009; Luan et al., 2007; Junior and Re-Poppi, 2007) a constant sample size was maintained, while optimizing all other parameters. This study investigated the change in extraction efficiency with varying sample volumes to assess any slight improvements in extraction efficiency. The extraction efficiencies of three different sample volumes (5 mℓ, 10 mℓ and 15 mℓ) were investigated (Figure 12).

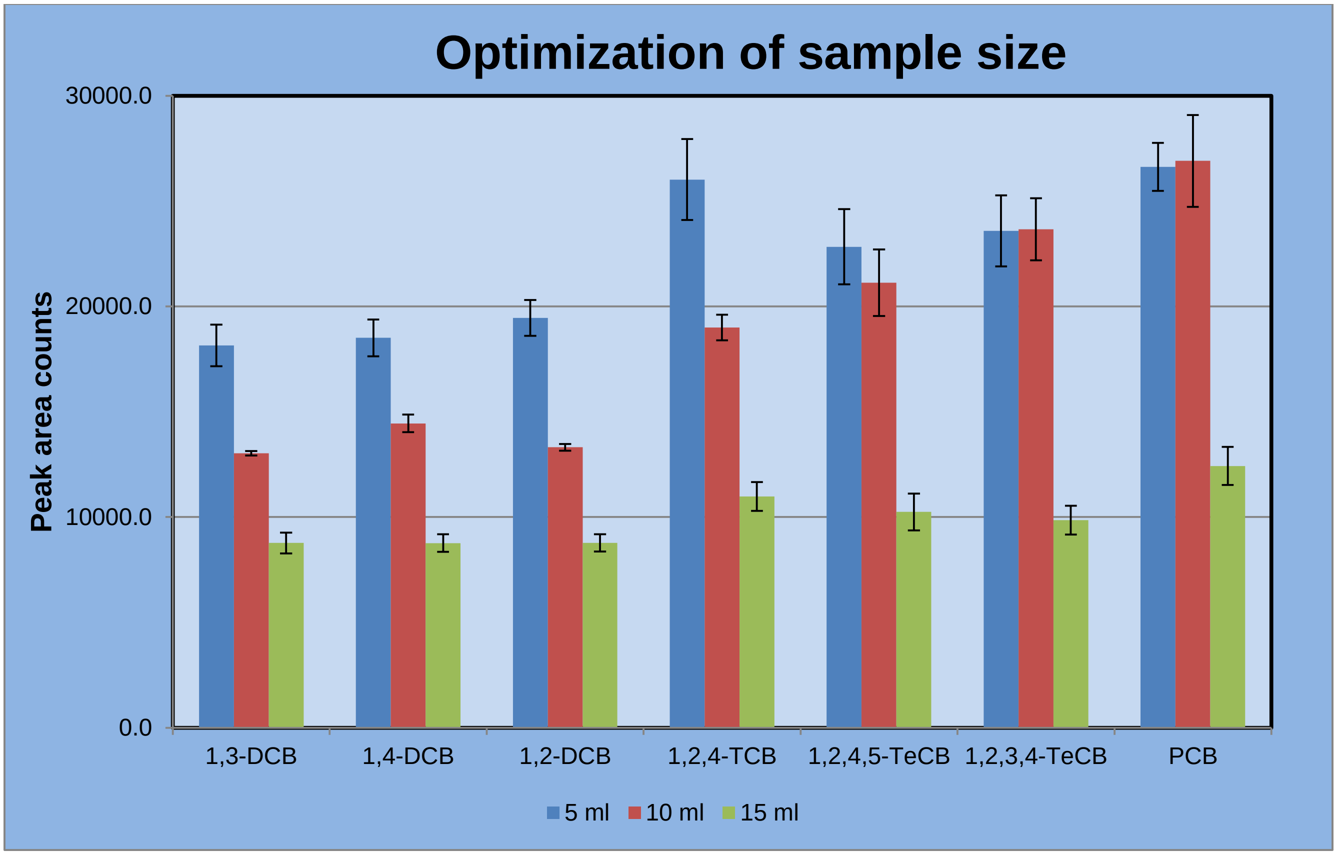


Figure 12: Influence of sample size on extraction efficiency

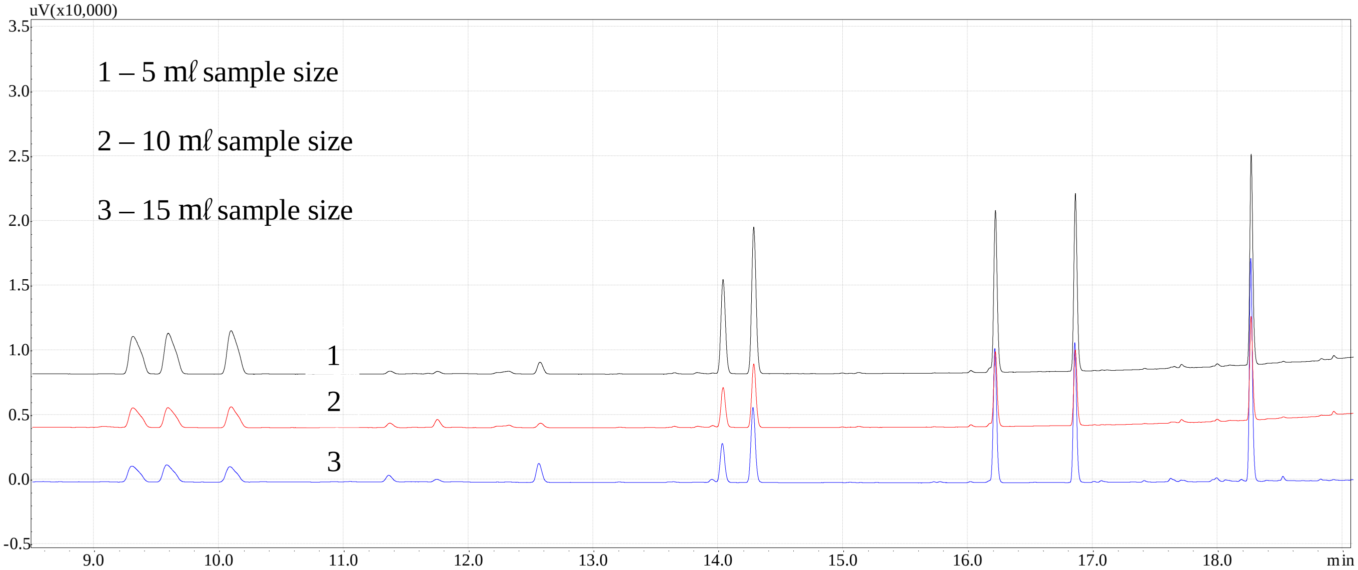


Figure 13: Optimization of sample size; (1) 5 mℓ; (2) 10 mℓ; (3) 15 mℓ

Figures 12 and 13 indicate that superior extraction efficiency was obtained using a sample volume of 5 mℓ. Further increases in sample volumes resulted in decreased extraction efficiency. Similar observations were reported by Zhu et al. (2003). Theoretically, the amount of analyte adsorbed by a SPME fibre can be described by Equation 8 (Louch et al., 1993) :

$$n_A = \frac{kV_f V_s C_s}{kV_f + V_s} \tag{8}$$

where K is the distribution coefficient, V_f is the volume of the fibre coating, V_s is the volume of the sample and C_s is the initial concentration of the analyte within the sample. The total amount of analyte is given by Equation 9:

$$n_{total} = V_s C_s \tag{9}$$

The recovery (R) of an analyte extracted can be defined by Equation 10:

$$R = \frac{n_A}{n_{total}} = \frac{kV_f}{(kV_f + V_s)} \tag{10}$$

Equation 10 shows that analyte recovery decreases with increasing sample volume. Equation 9 shows that n_{total} decreases with decreasing volume. However, according to Zhu et al. (2003), with decrease in V_s, the increasing rate of recovery compensates for the declining rate of n_{total}. This means that at lower sample volume, the analyte recovery is enhanced; although, less analyte will be adsorbed by the fibre.

The results obtained in this study are consistent with Equation 10. The enhanced extraction efficiency at lower sample volume is due to a larger phase ratio between volume of the fibre coating and sample. The phase ratio can be increased by increasing the volume of the fibre coating, or by decreasing the sample volume. The latter is a more appropriate in increasing the phase ratio as a major disadvantage of the former (slow mass transfer) makes it impractical (Zhu et al., 2003).

Single factor ANOVA data analysis ($\alpha = 0.05$) was performed on the peak areas obtained as the sample volume was varied from 5 mℓ – 15 mℓ. The H_0 and H_a for the statistical analysis were as follows:

$H_0 = \mu_{5m\ell} = \mu_{1\ m\ell} = \mu_{15m\ell}$ (accept if p-value > α)

$H_a = \mu_{5m\ell} \neq \mu_{10m\ell} \neq \mu_{15m\ell}$ (accept if p-value < α)

Table 12: Statistical analysis of sample size selection

Compound	f critical	f calculated	α	p-value
1,3-DCB	5.143253	53.80424	0.05	0.000147
1,4-DCB		64.27197		0.0000887
1,2-DCB		93.6663		0.0000299
1,2,4-TCB		28.74078		0.000844
1,2,4,5-TeCB		11.7087		0.008485
1,2,3,4-TeCB		15.79177		0.004069
PCB		8.332614		0.018551

Table 12 above indicates that the p-value for each chlorobenzene compound was less than α (0.05). This indicates that H_a can be accepted and that sample volume significantly affects extraction efficiency. However, Figure 12 indicates that changing the sample size from 5 mℓ to 10 mℓ had little impact on the extraction efficiencies of the higher chlorinated benzenes (1,2,4,5-TeCB, 1,2,3,4-TeCB and PCB).

As a result, further statistical analysis was performed on the extraction efficiency of these two sample volumes (5 mℓ and 10 mℓ) to assess if there is a significant difference between the two. A two-tailed Student's t-test ($\alpha = 0.05$) assuming equal variance was applied to the peak area counts obtained from the extractions performed at 300 rpm and 700 rpm. The H_0 and H_a for the statistical analysis were as follows:

$H_0 = \mu_{5m\ell} = \mu_{10m\ell}$ (accept if p-value > α)

$H_a = \mu_{5m\ell} \neq \mu_{10m\ell}$ (accept if p-value < α)

Table 13: Statistical analysis of extraction efficiency between 5 mℓ and 10 mℓ

	t critical	t calculated	α	p-value
1,3-DCB	2.776445	5.15479	0.05	0.006722
1,4-DCB		4.186003		0.013851
1,2-DCB		7.06421		0.002118
1,2,4-TCB		3.450274		0.026048
1,2,4,5-TeCB		1.304847		0.26195
1,2,3,4-TeCB		0.873089		0.431895
PCB		0.880856		0.428142

Table 13 above indicates that the p-values for the lower chlorobenzene compounds (1,3-DCB, 1,4-DCB, 1,2-DCB and 1,2,4-TCB) were less than α, while those of the higher chlorobenzene compounds (1,2,4,5-TeCB, 1,2,3,4-TeCB and PCB) were greater than α. This indicates that H_0 can be accepted for the lower chlorinated compounds and rejected for the higher chlorinated compounds and that sample volume has less impact on the extraction efficiency of higher chlorinated benzenes. In this study, all further experiments were conducted using a sample volume of 5 mℓ, as this volume produced a significant gain in extraction efficiency for the lower chlorinated benzenes (1,2-DCB, 1,3-DCB, 1,4-DCB, and 1,2,4-TCB).

4.2.4. Salting out effect

The salting out effect is extensively employed in SPME to modify the sample matrix using various salts. An increase in ionic strength reduces the solubility of some analytes, thereby increasing the extraction efficiency, since more of the analyte is available for adsorption onto the fibre (Kin, 2008). In this experiment, aqueous samples (containing 2 ng/mℓ of each analyte) with NaCl concentrations (m/v) ranging from 0-20% were studied and room temperature to determine the ionic strength at which the extraction was optimum (Figure 14).

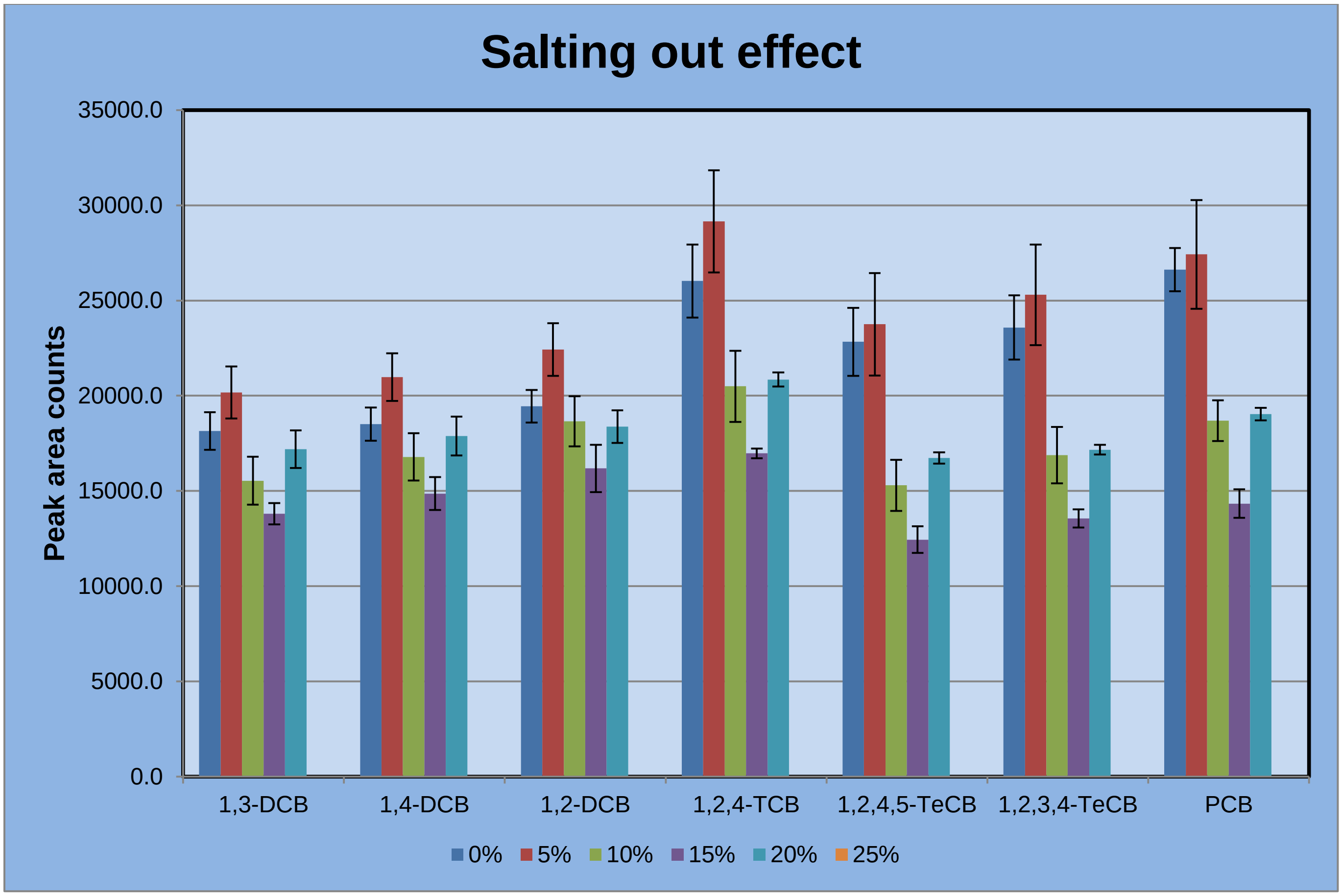


Figure 14: Results obtained from the study of salting out effect

Figure 14 indicates that 5% (m/v) salt concentration had a slight improvement on the extraction efficiency while a further increase in salt concentration resulted in a drastic decrease in extraction efficiency. Ionic strength has a greater effect on analytes which have high water solubility (Santos et al., 1996). Therefore, it is evident that the compounds with higher water solubility (1,2-DCB, 1,3-DCB, 1,4-DCB and 1,2,4-TCB) had an increase in extraction efficiency of between 9-13%. In contrast, ionic strength had a minimal influence on compounds with lower water solubility (1,2,3,4-TeCB, 1,2,4,5-TeCB and PCB) with an increase in extraction efficiency of between 2-6%. This phenomenon occurs as a result of water molecules forming hydration spheres around the salt molecules. These hydration spheres reduce the amount of water molecules available to dissolve the analytes which results in an increased mass transfer of certain analytes into the extraction phase (He et al., 2000; Psillakis, 2002).

He et al. (2000) reported an increase in extraction efficiency with ionic strength on the extraction of chlorobenzenes from river water using HS-SPME. However, the authors reported an optimum ionic strength of 20% (m/v). In contrast, the optimum ionic strength in this study is 5% (m/v) which can be explained in terms of the mode of extraction used. In the study performed by He et al. (2000), the headspace mode was used. Therefore, the fibre was not exposed to the ionic species and other interferences in the sample matrix. In this study the direct immersion mode was used. Thus the fibre was directly exposed to the ionic species and interferences in the sample matrix. Initially an increase in extraction efficiency will be experienced (salting out effect) at lower salt concentrations. However, further increases in salt concentration will result in a decrease in extraction efficiency. This is due to an increase in the interactions between the analytes and the ionic species which reduces the analytes ability to move into the extracting phase.

A two-tailed Student's t-test ($\alpha = 0.05$) assuming equal variance was applied to the peak area counts obtained from the extractions performed with salt concentrations of 0% and 5%. The H_0 and H_a for the statistical analysis were as follows:

$H_0 = \mu_{0\%} = \mu_{5\%}$ (accept if p-value > α)

$H_a = \mu_{0\%} \neq \mu_{5\%}$ (accept if p-value < α)

Table 14: Statistical analysis of extraction efficiency performed with salt concentrations of 0% and 5%

Compound	t-critical	t-calculated	α	p-value
1,3-DCB	2.776445	-1.19911	0.05	0.296662
1,4-DCB		-1.6246		0.179571
1,2-DCB		-1.83236		0.140841
1,2,4-TCB		-0.18569		0.861725
1,2,4,5-TeCB		0.584041		0.590544
1,2,3,4-TeCB		0.39584		0.712429
PCB		0.762298		0.488355

Table 14 above indicates that the p-value for each chlorobenzene isomer was less than α. As a result, H_0 can be accepted and it can be concluded that altering the salt concentration from 0% - 5% did not significantly enhance the extraction efficiency.

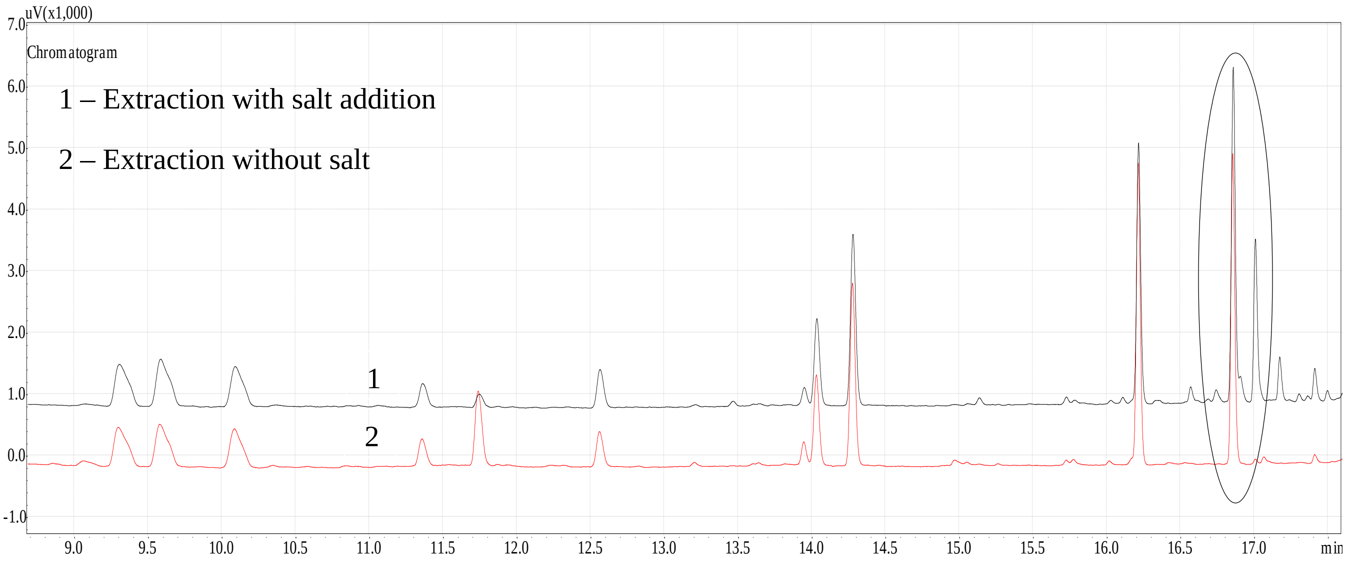


Figure 15: Interfering compound due to the addition of salt; (1) Extraction with the addition of 5% (m/v) salt; (2) Extraction with 0% (m/v) salt

Furthermore, the addition of salt resulted in interfering compounds being detected (Figure 15). These interferences would have resulted in the reduction of both the LOD and limit of quantification (LOQ) of PCB. Since the salting out effect had a minimal effect on the extraction efficiency, a compromise had to be reached, and it was decided to carry out further experiments without the addition of any salt.

4.2.5 Optimization of extraction time

SPME is an equilibrium process. Therefore it is imperative to determine the time required for each analyte to attain equilibrium with the fibre coating. The above optimized conditions were applied at extraction times ranging from 5 minutes to 120 minutes at room temperature. The results are depicted in the adsorption - time curve (Figure 16) below.

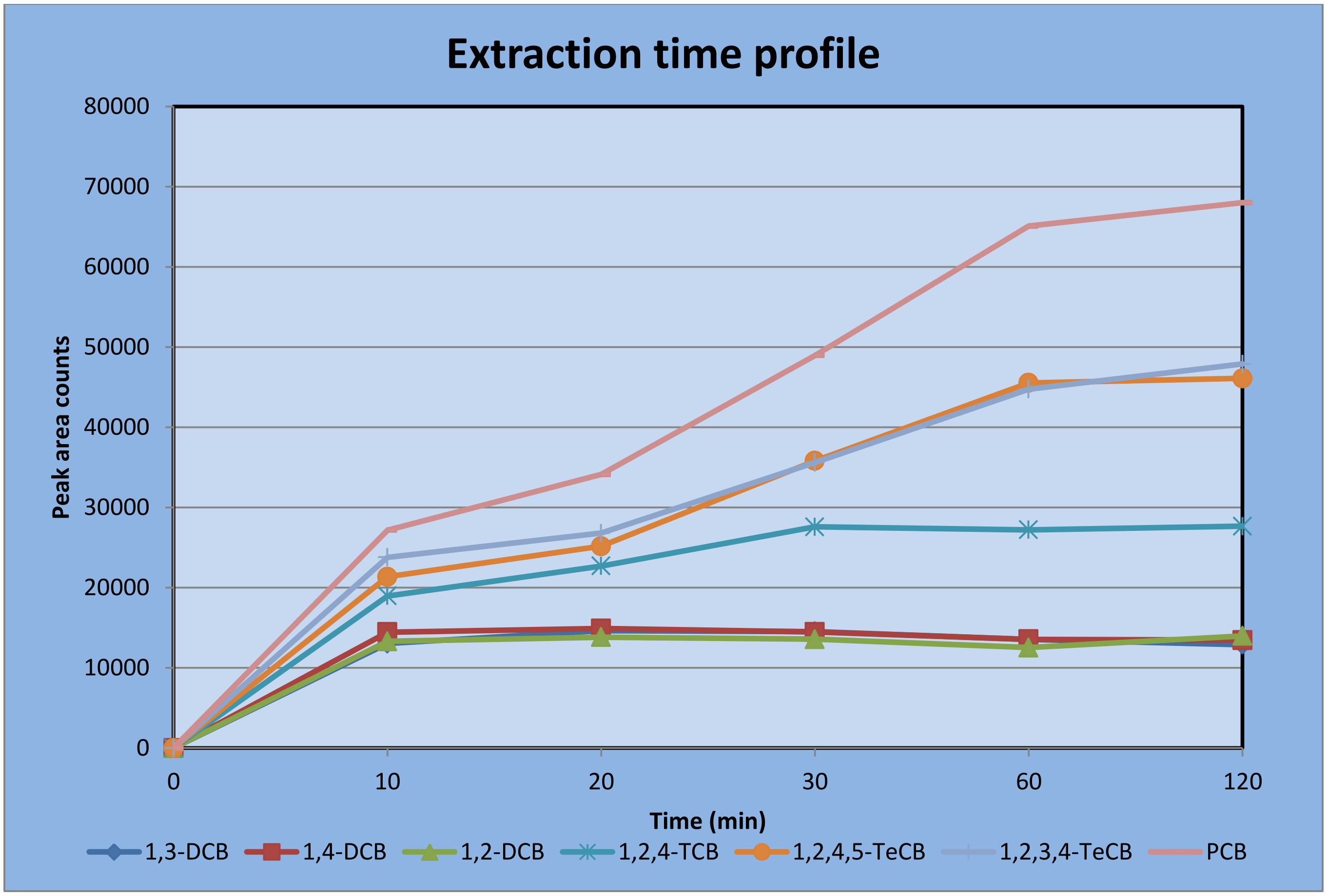


Figure 16: Adsorption time profile of the studied analytes

The equilibration time is defined as the time after which the amount of analyte extracted remains constant (Pawliszyn, 1997). Figure 16 above shows that the DCB isomers attained equilibrium rapidly. 1,2,4-TCB required 30 minutes to attain equilibrium while 1,2,3,4-TeCB, 1,2,4,5-TeCB and PCB required 60 minutes. The results indicate that equilibrium times are compound-dependent, having a trend of increasing times with increasing chlorination. These results are similar to that attained by He et al. (2000) who reported rapid equilibration times for chlorobenzenes for the lower chlorinated benzenes and increasing equilibration times with increasing chlorination The chlorobenzene compounds with higher vapour pressures and octanol-water partition coefficients as well as lower molecular mass attained equilibrium rapidly. To enhance throughput, a 30 minute extraction was selected for the rest of this study.

4.3. Validation of the analytical method

A newly developed analytical method needs to be validated to determine if it is suitable for its intended application. In this study, the validation of DI-SPME coupled to GC-FID was investigated. The validity of the analytical methods was investigated under the optimized DI-SPME conditions: 100µl PDMS fibre; 700 rpm rate of agitation; 5 mℓ sample size; 0% salt addition and 30 minutes extraction time.

4.3.1. Linearity

The linearity of an analytical method is critical as it shows that there is a mathematically verified linear relationship between instrumental response and analyte concentration within a well-defined range (NCCLS, 2003). This simple mathematical relationship allows for the linear interpolation of data, which is the underlying principle of a calibration curve. In this study, five point calibration curves were determined. These calibration curves were constructed as XY-scatter plots of analyte concentration versus analytical response. The relationship between the analyte concentration and analytical response is modelled by a process called linear regression. This statistical analysis tool inserts a trend line which best fits a given set of data. The equation of the trend line can then be used to determine unknown x and y values while the correlation coefficient (R^2) determines the linearity of the trend line. The correlation coefficient determines the strength of the linear relationship between a given set of points. A R^2 value close to 1 indicates a very strong positive linear relationship between the points and therefore indicates strong linearity of an analytical method. In this study the linearity of the analytical method was determined within an analyte mass range of 1 – 100 ng/mℓ. Figures 17 and 18 and Table 15 below indicates calibration curves and the correlation coefficients obtained in this study.

Table 15: Correlation coefficients for the optimized method

Compound	Correlation coefficient (R^2 value)
1,3-DCB	0.9987
1,4-DCB	0.9957
1,2-DCB	0.9995
1,2,4-TCB	0.9984
1,2,4,5-TeCB	0.9966
1,2,3,4-TeCB	0.9975
PCB	0.9996

The correlation coefficients of the analytes ranged from 0.9957 – 0.9995 at an analyte concentration range of 1 – 100 ng/mℓ. This indicates good linearity of the optimized method as there is a linear relationship between analyte mass and detector response within the range of trace concentration level. He et al. (2000) reported correlation coefficients of greater than 0.9910 for each analyte at a linearity range of 0.2 – 20 µg/ℓ using HS-SPME coupled to GC-MS. This study produced slightly improved correlation coefficients of greater than 0.9957, indicating that there is a more accurate mathematical relationship between detector response and analyte concentration.

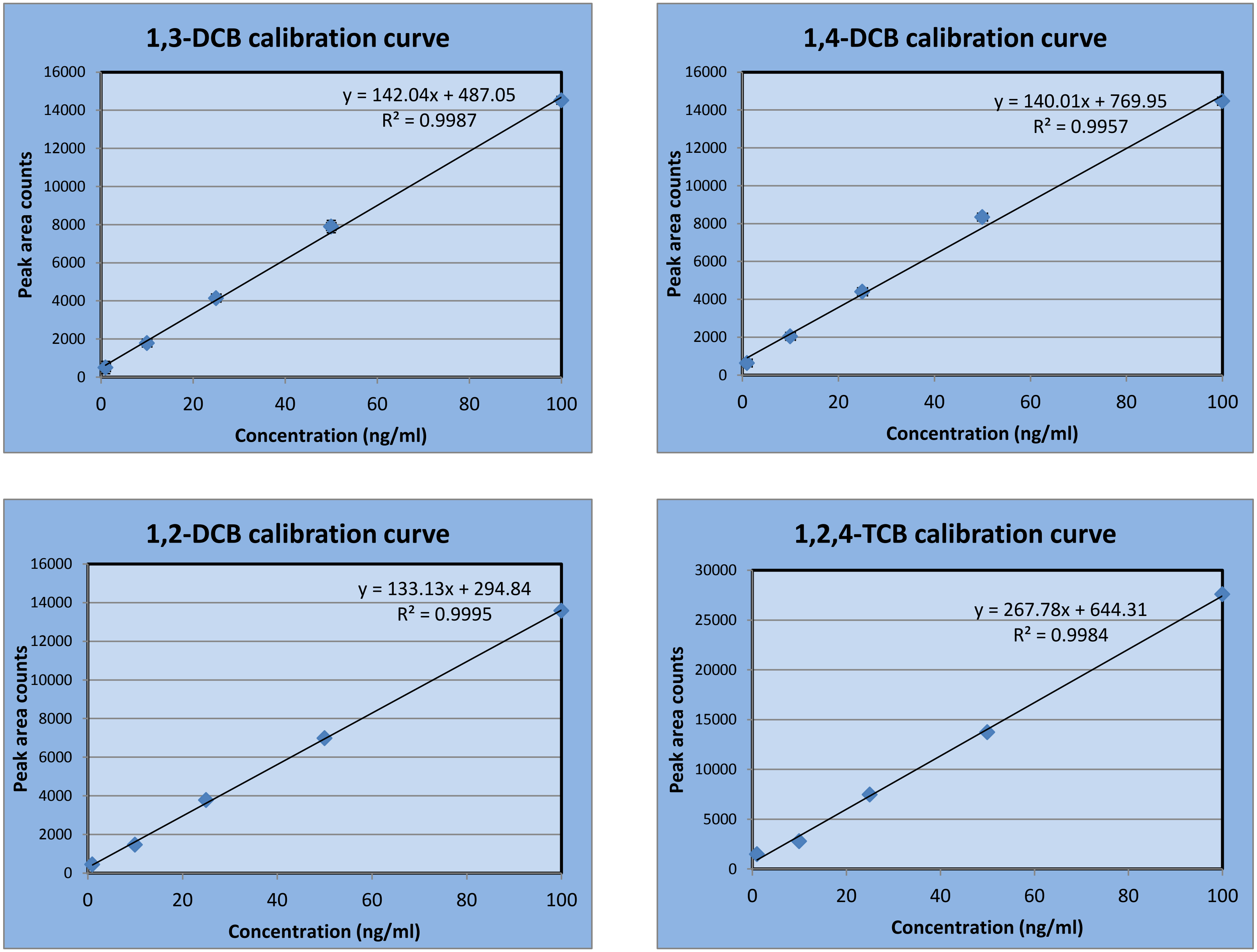


Figure 17: Calibration curves for 1,3-DCB; 1,4-DCB; 1,2-DCB and 1,2,4-TCB

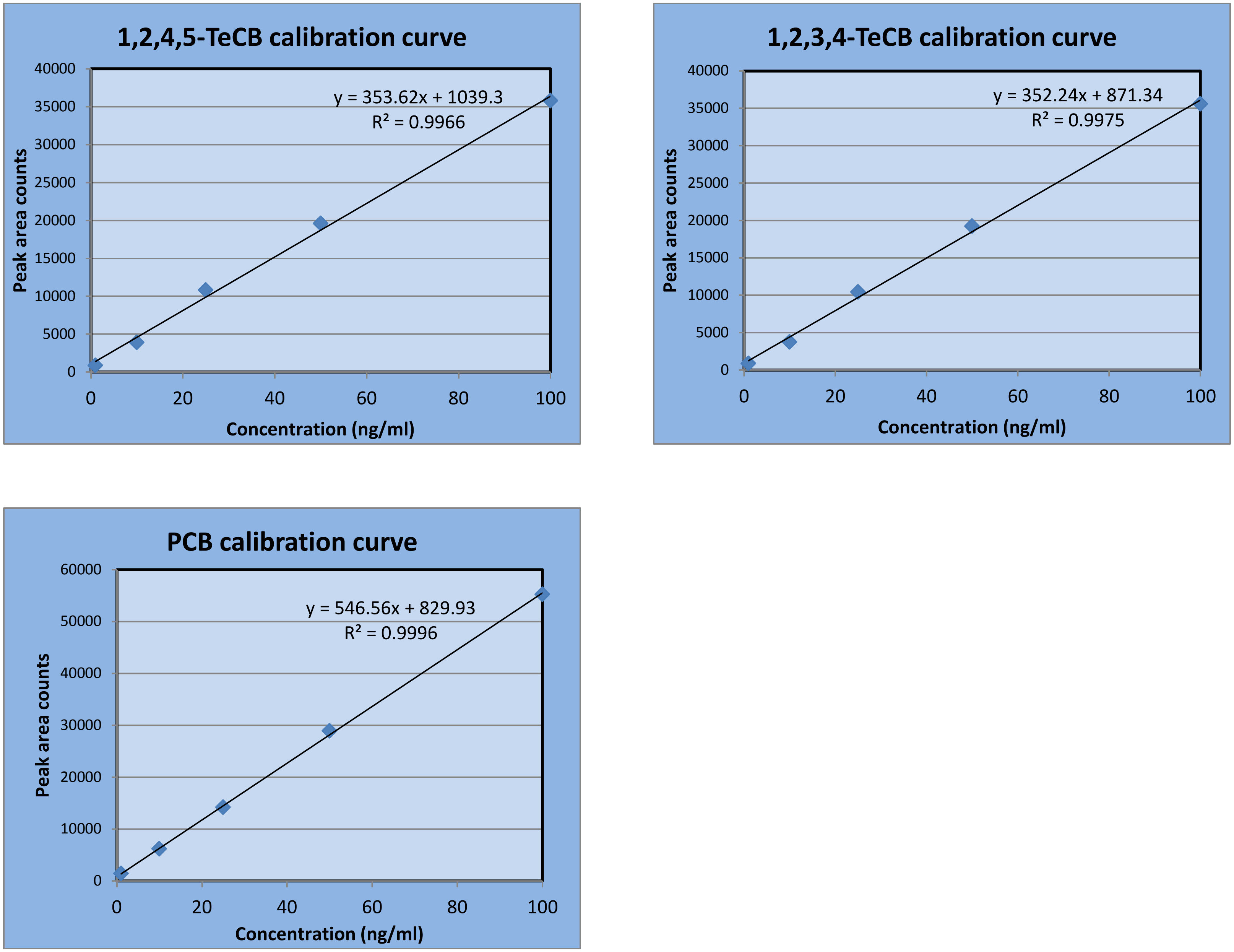


Figure 18: Calibration curves for 1,2,4,5-TeCB; 1,2,3,4-TeCB and PCB

4.3.2. Limits of detection (LOD) and limits of quantification (LOQ)

In this study, the LOD was defined as the concentration of analyte which yielded as signal to noise ratio of three. This represented the lowest detectable concentration of the analyte by the analytical instrument; however, it did not necessarily mean that it was quantifiable. The LOQ was defined as the concentration of analyte which yielded as signal to noise ratio of ten. This represented the lowest quantifiable analyte concentration by the analytical instrument.

Table 16: Limits of detection and quantification compared to WHO and USEPA limits

Compound	LOD	LOQ	LOD^a	WHO Limits	USEPA Limits
	ng /mℓ				
1,3-DCB	0.02	0.2	-	-	-
1,4-DCB	0.04	0.4	0.1	300	75
1,2-DCB	0.02	0.2	0.1	1000	600
1,2,4-TCB	0.3	2.7	0.5	-	70
1,2,4,5-TeCB	0.09	0.9	-	-	-
1,2,3,4-TeCB	0.07	0.7	-	-	-
PCB	0.07	0.7	-	-	-

a – Limits of detections obtained from Zhou et al. (2009)

Table 16 shows the LOD and LOQ obtained for each analyte. The LOD obtained for 1,4-DCB, 1,2-DCB and 1,2,4-DCB is 1.9 – 4.6 fold lower to those obtained by Zhou et al. (2009). These authors used HS-SDME coupled to GC-FID to analyse chlorobenzenes in water. This indicates that the DI-SPME method developed in this study is superior to the HS-SDME method of pre-concentration. Furthermore, the LOQ's obtained in this study are well below the regulatory limits of the WHO and USEPA drinking water guidelines. This indicates that the

LOQ obtained in this study is well within the levels required for trace concentration analysis and is suitable for the routine analysis of chlorobenzenes in drinking water.

4.3.3. Recovery experiments on dam water

Recovery experiments were performed to determine the efficiency of the method towards the extraction of chlorobenzene from dam water, as well to investigate the presence of any matrix interferences. An efficient method, free of any matrix interferences, should ideally offer recoveries of close to 100 %. In this study the optimized method was initially applied to 5 mℓ of water collected from the Grootdraai Dam. This is regarded as the blank experiment and was done to investigate the natural presence of the analytes in the dam water sample.

Figure 19 a below indicates the presence of both 1,3-DCB, 1,4-DCB and PCB in the dam water sample at concentrations of 0.429 ng/mℓ, 1.685 ng/mℓ and 1.433 ng/mℓ respectively. Subsequently, the optimized method was then applied to 5 mℓ of the Grootdraai Dam water which was fortified with 5 ng/mℓ of each analyte (Figure 19 b). To enhance the accuracy of recovery experiment, the peak areas for the specific analytes obtained from the blank experiment were subtracted from the peak areas of the analytes obtained from this experiment. The mean recovery data as well as the %RSD values are listed in Table 17 below.

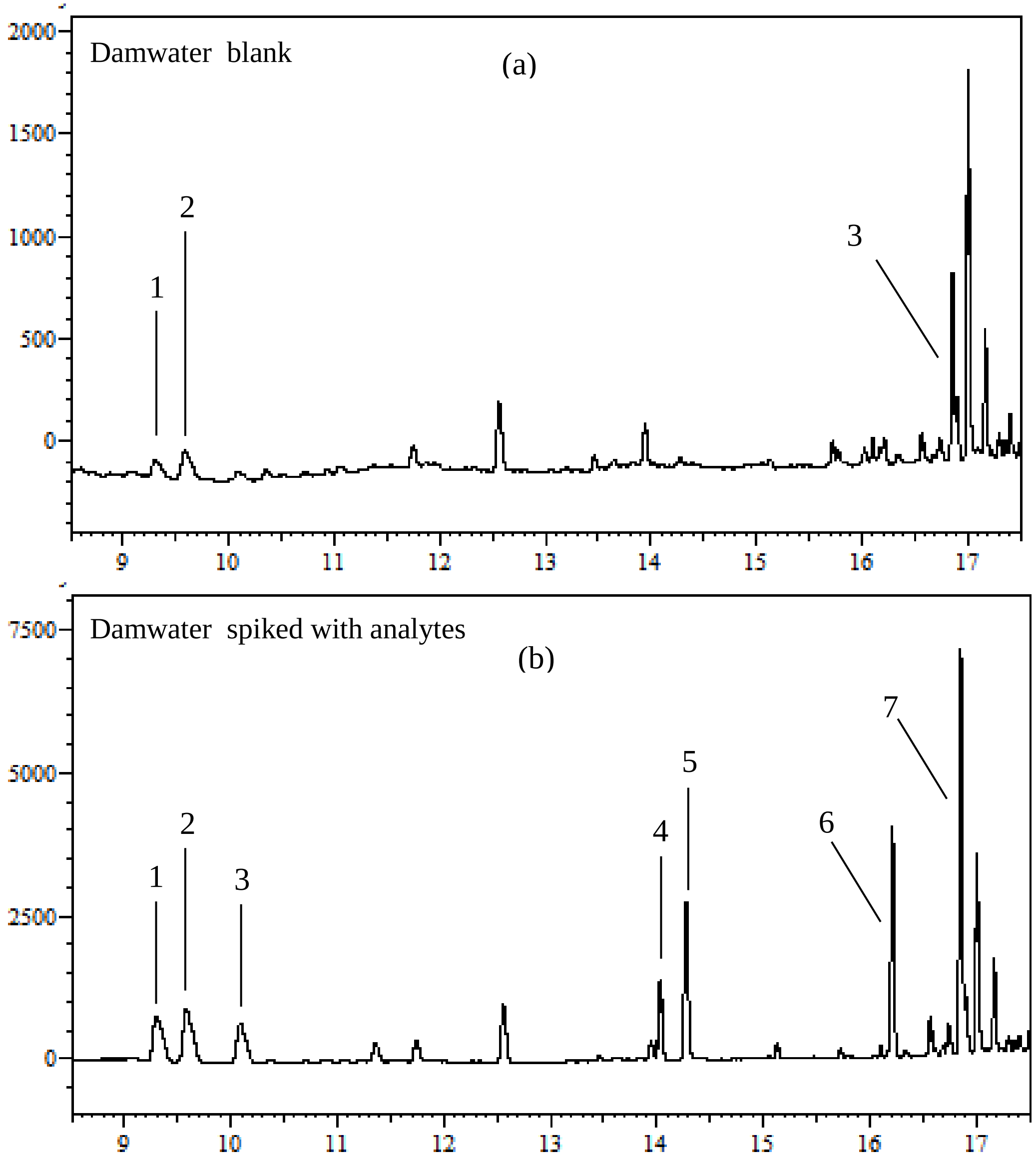


Figure 19: (a) Dam sample blank; (b) Dam sample fortified with 5ng/mℓ of each analyte; (1) 1,3-DCB; (2) 1,4-DCB; (3) 1,2-DCB; (4) 1,2,4-TCB, (5) 1,2,4,5-TeCB; (6) 1,2,3,4-TeCB; (7) PCB

Table 17: Recovery results and accuracy of the method

Compound	Presence in sample blank (ng/ml)	Average recoveries (ng/ml) (*n* = 3)	Average recoveries (%) (*n* = 3)	% RSD
1,3-DCB	0.429	5.28	105.6	2.2
1,4-DCB	1.685	5.12	102.4	1.9
1,2-DCB	Not detected	5.07	101.4	5.6
1,2,4-TCB	Not detected	4.67	93.4	0.6
1,2,4,5-TeCB	Not detected	4.18	83.6	9.2
1,2,3,4-TeCB	Not detected	5.14	102.8	0.5
PCB	1.433 ng/ml	5.36	107.2	7.2

The average recoveries obtained in this study were within acceptable limits ranging between 83.6% – 107.32% with RSD values of less than 10%. This indicates that the optimized method is quantitative, void of any matrix interferences and has good repeatability.

4.3.4. Comparisons to literature reports

The data from 5 reports published in journals have been tabulated in Table 18 to indicate the relative rank and value of the method reported herein compared with reputedly more sophisticated and sensitive methods. Although the LODs for the GC-FID method are much higher, relative to the studies listed Table 18 (Vidal et al., 2005; Vidal et al., 2007; Li et al., 2010; Bagheri and Aghakhani, 2011), it is worth noting that these LODs are adequate for monitoring

Table 18: Comparison of optimized method to other studies

Reference	**Sample preparation and detection method**	**Extraction time (min)**	**Number of analytes, Linear range (ng/mℓ), Range of recoveries, % Relative standard deviation Limit of detection (ng/mℓ)**
Vidal et al. (2005)	Single drop micro-extraction (SDME); GC-MS	5	10, 0.2–50, 97% mean, 2.1–13.2%, 0.003–0.031
Vidal et al. (2007)	Micro-wave assisted headspace (HS)-SDME; HPLC	10-20	8, not specified, 99% mean, 2.3–8.3%, 0.02–0.04
Li et al. (2010)	Novel SPME coating; GC-ECD	15	7, not specified , 91% mean, 2.1–4.9%, 0.0003–0.002
Bagheri and Aghakhani (2011)	Novel nanofibre coating – HS-SPME ; GC-MS	Not specified	not specified, 0.050–1, 94–102%, 3–8%, 0.01
Zhao et al. (2009)	SDME, FC-FID	20	5, 1–1 000, 88.9–110.9%, 7.73–12.39%, 0.1–0.5
This study (2012)	Direct immersion SPME GC-FID	30	7, 1–100 , 83.6–107.2%, 0.5–9.2 %, 0.02–0.3

samples to assess compliance with regulatory limits specified in international standards and guidelines (WHO in this case). In addition, the GC-FID is easy to use and is relatively inexpensive. However, upon comparison with a study using a similar detection system coupled to HS-SDME (Zhao et al., 2009), the LOD obtained in this study for 1,4-DCB, 1,2-DCB and 1,2,4-DCB is 1.9–4.6-fold lower. Additionally, the method used in this study gives a higher degree of precision. This indicates that the DI-SPME method developed in this study is superior to the HS-SDME method of pre-concentration.

4.3.5. Occurrence of chlorobenzenes within the Grootdraai Dam

There were three chlorobenzene compounds detected within the Grootdraai Dam. These included 1,3-DCB; 1,4-DCB and PCB at concentrations of 0.429 µg/ℓ, 1.685 µg/ℓ and 1.433 µg/ℓ respectively. The major water users of the Grootdraai Dam are industries and municipalities (Table 19).

Table 19: Water use trend of the Grootdraai Dam

User	**Demand (m^3/a)**	**Return (m^3/a)**
Irrigation	321 500	-
Tutuka Power Station	47 420 000	-
Matla Power Station	53 838 000	-
Sasol	91 250 000	4 015 000
Ermelo Municipality	3 600 000	1 982 124
Bethal Municipality	5 420 250	3 011 250
Thuthukane Township	1 427 556	642 400

The contamination of chlorobenzene compounds within the Grootdraai Dam could be due to agricultural chemical run-off, irresponsible discharge of effluent from chemical industries, air emissions during chemical manufacturing, return flow from wastewater treatment plants or disposal of materials containing these compounds within the catchment of this impoundment.

1,3-DCB and 1,4-DCB are commonly used in the manufacture of pesticides, indicating that agricultural activities within the catchment which use these pesticides as a possible source. Various environmental influences, particularly storm water run-off, can mobilize the chlorobenzes which will ultimately enter the waterways of the Grootdraai Dam through surface runoff.

The occurrence of PCB within the Grootdraai Dam is quite significant, as there is currently no large scale use and commercial production of the chemical (CIA). However, since PCB is produced in small quantities during the chlorination of benzenes, one can anticipate the presence of PCB when DCB is detected. Historically, PCB has been used in the manufacture of fungicides, and more recently, as a flame retardant. This could indicate past PCB contamination, which has been re-mobilized through environmental processes; and eventually entered and accumulated within the Grootdraai Dam.

Domestic and industrial discharge could also be a source for chlorobenzenes. These compounds can be introduced into the Grootdraai Dam via return flows from wastewater treatment plants within the catchment. 1,4-DCB is used in common household products such as air fresheners, moth balls and urinal deodorizers. These chlorobenzene compounds are also common components of industrial degreasing solvents. Negligent and poor waste disposal protocols at both domestic and industrial level can lead to contamination. The major industries on the Grootdraai catchment are Eskom’s Matla and Thuthuka Power Stations and Sasol Secunda Complex. Table 19 indicates that both Eskom Power Stations have a zero discharge policy; as a result, contamination is negligible. The Sasol Secunda Complex, as well as the Thuthukane Township, Bethal and Ermelo Municipalities are the main contributors to return flow into the Grootdraai Dam. As a result, one cannot rule out these as possible sources of chlorobenzenes contamination.

4.3.6. Comparison to drinking water guidelines

The USEPA's Clean Water Act is the cornerstone of surface water protection in the United States. The two dichlorobenzenes detected in this study (1,3-DCB and 1,4-DCB) are listed as toxic pollutants,

The South African Drinking Water Standard (SANS) 241:2011 for drinking water does not regulate any of the detected chlorobenzenes. The SANS 241:2011 is derived from the World Health Organisation (WHO) Guidelines for drinking water quality. Section 4.3.5 of the SANS 241:2011 standard indicates that for the organic determinands which are not listed, one should refer to the WHO Guidelines for drinking water for comparative purposes (DWAF, 2005).

The WHO has set a health based guideline value of 300 ng/mℓ for 1,4-DCB (WHO, 2011). This indicates that the level of 1,4-DCB within the Grootdraai Dam (1.685 ng/mℓ) is well within the WHO guideline and does not pose a significant health risk. The WHO has not derived for the other two compounds detected in this study.

CHAPTER 5

CONCLUSIONS

Since chlorobenzenes are widely used in various applications; they find their way into surface water through various mechanisms. These compounds pose a severe health risk; therefore, their presence within surface waters needs to be constantly monitored. Since these compounds naturally occur at very low concentrations; adequate sample preconcentration coupled to an appropriate analytical instrument is required for their quantification.

The application of DI-SPME coupled to GC-FID for the analysis of seven chlorobenzenes from dam water has been demonstrated in this study. To enhance the DI-SPME method, the effects of some analytical parameters, namely fibre type, sample size, rate of agitation, salting out effect and extraction time, were optimised. The proposed method is as follows: a 5 mℓ dam water sample is stirred at 700 rpm without the addition of any salt. A PDMS coated SPME fibre is then immersed into the sample solution for 30 minutes to adsorb chlorobenzene compounds.

The results of the study indicate that DI-SPME coupled to GC-FID is viable for routine monitoring of dam samples for the presences of chlorinated benzenes. Additionally, the method is adequate to detect below the limits set for two of the three chlorobenzenes regulated by the USEPA and one of the two chlorobenzenes listed by the WHO. Furthermore, the optimised DI-SPME method produced lower LODs than the HS-SDME. An added advantage of the method is that it utilizes a detector that is relatively cheap. It should therefore be of service to organisations which monitor their drinking water, as well as source water, for levels of chlorobenzenes, in order to ensure public health protection and safety of its customers.

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ADDENDUM I

RESULTS

SPME optimisation experiments

Table 20: Results for the selection of fibre coating

Compound	Average peak area counts (*n* = 3)		Standard deviation (σ)		Standard error	
	PA	PDMS	PA	PDMS	PA	PDMS
1,3-DCB	26481.4	51934.1	974.5	619.0	562.7	357.4
1,4-DCB	45149.9	74366.1	1574.4	968.4	909.0	559.1
1,2-DCB	17010.5	32521.7	613.3	391.7	354.1	226.1
1,2,4-TCB	9678.3	18995.3	231.2	1050.5	133.5	606.5
1,2,4,5-TeCB	11536.9	21124.7	339.8	2736.8	196.2	1580.1
1,2,3,4-TeCB	13864.3	23660.6	419.7	2555.2	242.3	1475.2
PCB	18118.0	26913.1	1227.6	3776.6	708.7	2180.4

PA = polyacrylate

PDMS = Polydimethyl siloxane

Table 21: Results for the optimization of stir speed

Compound	Average peak area counts ($n = 3$)				Standard deviation (σ)				Standard error			
	300 rpm	500 rpm	700 rpm	1100 rpm	300 rpm	500 rpm	700 rpm	1100 rpm	300 rpm	500 rpm	700 rpm	1100 rpm
1,3-DCB	10325.9	11149.9	13088.1	13029.0	1889.7	609.3	594.5	187.6	1091.0	351.8	343.2	108.3
1,4-DCB	10532.7	11389.3	12944.8	14447.3	1802.7	521.2	545.0	729.4	1040.8	300.9	314.7	421.1
1,2-DCB	10666.8	11416.1	12988.1	13313.3	1737.3	414.9	447.6	273.4	1003.0	239.5	258.4	157.8
1,2,4-TCB	13839.7	14118.5	16262.3	18995.3	2077.9	1580.9	304.0	1050.5	1469.3	912.8	175.5	606.5
1,2,4,5-TeCB	12239.4	12946.7	18825.1	21124.7	1760.5	2508.1	1973.3	2736.8	1244.8	1448.1	1139.3	1580.1
1,2,3,4-TeCB	12432.0	12639.7	18820.6	23660.6	1590.3	2068.5	1940.1	2555.2	1124.5	1194.2	1120.1	1475.2
PCB	14690.8	15272.3	22327.6	26913.1	1477.6	2950.4	2215.7	3776.6	1044.8	1703.4	1279.2	2180.4

Table 22: Results for the optimization of sample size

Compound	Average peak area counts (n = 3)			Standard deviation (σ)			Standard error		
	5 mℓ	10 mℓ	15 mℓ	5 mℓ	10 mℓ	15 mℓ	5 mℓ	10 mℓ	15 mℓ
1,3-DCB	18155.0	13029.0	8769.6	1712.1	187.6	852.9	988.5	108.3	492.4
1,4-DCB	18508.9	14447.3	8766.5	1514.0	729.4	727.3	874.1	421.1	419.9
1,2-DCB	19453.0	13313.3	8776.3	1480.3	273.4	701.9	854.7	157.8	405.2
1,2,4-TCB	26024.9	18995.3	10981.8	2716.1	1050.5	1181.9	1920.6	606.5	682.4
1,2,4,5-TeCB	22833.3	21124.7	10245.4	2528.8	2736.8	1513.4	1788.1	1580.1	873.8
1,2,3,4-TeCB	23585.4	23660.6	9849.3	2387.9	2555.2	1186.4	1688.5	1475.2	685.0
PCB	26627.8	26913.1	12423.4	1607.5	3776.6	1568.7	1136.7	2180.4	905.7

Table 23: Results for the salting out effect

Compound	Average peak area counts (n = 3)					Standard deviation (σ)					Standard error				
	0 %	5 %	10 %	15 %	20 %	0 %	5 %	10 %	15 %	20 %	0 %	5 %	10 %	15 %	20 %
1,3-DCB	18155.0	20171.7	15537.7	13804.7	17194.5	1712.1	2356.6	2171.1	971.3	1722.2	988.5	1360.6	1253.5	560.8	994.3
1,4-DCB	18508.9	20980.1	16787.1	14861.3	17884.7	1514.0	2156.2	2152.7	1491.6	1779.9	874.1	1244.9	1242.8	861.2	1027.6
1,2-DCB	19453.0	22428.5	18654.3	16183.5	18377.5	1480.3	2391.4	2274.9	2150.7	1492.2	854.7	1380.7	1313.4	1241.7	861.5
1,2,4-TCB	26024.9	29163.0	20494.8	16971.6	20855.1	2716.1	4649.0	3231.0	445.1	650.9	1920.6	2684.1	1865.4	257.0	375.8
1,2,4,5-TeCB	22833.3	23755.9	15292.6	12441.1	16735.8	2528.8	4662.7	2330.2	1213.6	505.2	1788.1	2692.0	1345.3	700.7	291.7
1,2,3,4-TeCB	23585.4	25303.7	16877.5	13555.6	17167.3	2387.9	4575.8	2564.9	815.2	453.0	1688.5	2641.9	1480.8	470.6	261.5
PCB	26627.8	27428.1	18686.1	14331.6	19030.9	1607.5	4945.8	1851.6	1297.6	569.7	1136.7	2855.5	1069.0	749.2	328.9

Statistical analysis

Table 24: Statistical results of fibre coating optimisation experiment

Compound	t critical	t calculated	α	p-value
1,3-DCB	2.776445	-38.1845	0.05	0.00000281
1,4-DCB		-27.3769		0.00001059
1,2-DCB		-36.9179		0.00000321
1,2,4-TCB		-15.0026		0.000115
1,2,4,5-TeCB		-6.0216		0.0038317
1,2,3,4-TeCB		-6.55268		0.0028047
PCB		-3.83606		0.0185219

Table 25: Statistical results of stir speed optimisation experiment

	F critical	F calculated	α	p-value
1,3-DCB	4.066181	5.28946	0.05	0.026552
1,4-DCB		8.253009		0.007849
1,2-DCB		5.520322		0.023804
1,2,4-TCB		8.533226		0.007114
1,2,4,5-TeCB		11.0496		0.00323
1,2,3,4-TeCB		20.46871		0.000414
PCB		13.7842		0.001585

Table 26: Statistical results from Student's ttest between 300 rpm and 500 rpm stir speed experiments

	t-critical	t-calculated	α	p-value
1,3-DCB	2.776445	-2.41511	0.05	0.073149138
1,4-DCB		-2.21835		0.090778906
1,2-DCB		-2.24108		0.08851545
1,2,4-TCB		-1.99817		0.1163597
1,2,4,5-TeCB		-4.3135		0.012511371
1,2,3,4-TeCB		-4.41096		0.011592007
PCB		-4.9668		0.007668906

Table 27: Statistical results of sample size optimisation experiment

Compound	f critical	f calculated	α	p-value
1,3-DCB	5.143253	53.80424	0.05	0.000147
1,4-DCB		64.27197		0.0000887
1,2-DCB		93.6663		0.0000299
1,2,4-TCB		28.74078		0.000844
1,2,4,5-TeCB		11.7087		0.008485
1,2,3,4-TeCB		15.79177		0.004069
PCB		8.332614		0.018551

Table 28: Statistical results from Student's t-test between 5 mℓ and 10 mℓ sample size experiments

	t critical	t calculated	α	p-value
1,3-DCB	2.776445	5.15479	0.05	0.006722
1,4-DCB		4.186003		0.013851
1,2-DCB		7.06421		0.002118
1,2,4-TCB		3.450274		0.026048
1,2,4,5-TeCB		1.304847		0.26195
1,2,3,4-TeCB		0.873089		0.431895
PCB		0.880856		0.428142

Table 29: Statistical results of salting out effect experiment

Compound	F-critical	F-calculated	α	Probability
1,3-DCB	3.47805	5.211484	0.05	0.015682
1,4-DCB		4.482846		0.024763
1,2-DCB		3.836401		0.038503
1,2,4-TCB		7.95319		0.003759
1,2,4,5-TeCB		7.339524		0.005008
1,2,3,4-TeCB		7.59954		0.004426
PCB		6.287141		0.008533

Table 30: Statistical results from Student's ttest between 0% and 5% salt concentration experiments

Compound	t-critical	t-calculated	α	p-value
1,3-DCB	2.776445	-1.19911	0.05	0.296662
1,4-DCB		-1.6246		0.179571
1,2-DCB		-1.83236		0.140841
1,2,4-TCB		-0.18569		0.861725
1,2,4,5-TeCB		0.584041		0.590544
1,2,3,4-TeCB		0.39584		0.712429
PCB		0.762298		0.488355

Validation of optimised SPME method

Table 31: Linearity of method

Compound	Linear ranges (ng)	Equation of trendline	Correlation coefficient (R^2 value)
1,3-DCB	1 – 100	$y = 142.04x + 485.05$	0.9987
1,4-DCB		$y = 140.01x + 769.95$	0.9957
1,2-DCB		$y = 133.13x + 294.84$	0.9995
1,2,4-TCB		$y = 267.78x + 644.31$	0.9984
1,2,4,5-TeCB		$y = 353.62x + 1039.3$	0.9966
1,2,3,4-TeCB		$y = 352.24x + 871.34$	0.9975
PCB		$y = 478.38x + 1927.7$	0.9966

Table 32: Results for the recovery tests on dam water

Compound	Average peak area counts (*n* = 3)	Average recoveries (ng/mℓ) (*n* = 3)	Average recoveries (%) (*n* = 3)	Standard deviation	Standard error	% RSD
1,3-DCB	4240.1	5.28	105.6	2.3	1.3	2.2
1,4-DCB	4352.8	5.12	102.4	2.0	1.2	1.9
1,2-DCB	3672.4	5.07	101.4	5.7	3.3	5.6
1,2,4-TCB	6894.7	4.67	93.4	0.5	0.3	0.6
1,2,4,5-TeCB	8438.3	4.18	83.6	7.4	4.3	9.2
1,2,3,4-TeCB	9939.4	5.14	102.8	0.5	0.3	0.5
PCB	14744.7	5.36	107.2	7.0	4.1	7.2

Table 33: Limit of detection (LOD) and limit of quantification (LOQ) of the optimised method

Compound	LOD (ng/mℓ)	LOQ (ng/mℓ)
1,3-DCB	0.0204	0.2048
1,4-DCB	0.0429	0.4293
1,2-DCB	0.0219	0.2193
1,2,4-TCB	0.2650	2.650
1,2,4,5-TeCB	0.0910	0.9105
1,2,3,4-TeCB	0.0730	0.7305
PCB	0.0720	0.7203

$$LOD = 3\left(\frac{S}{N}\right) \tag{1}$$

$$LOQ = 10\left(\frac{S}{N}\right) \tag{2}$$

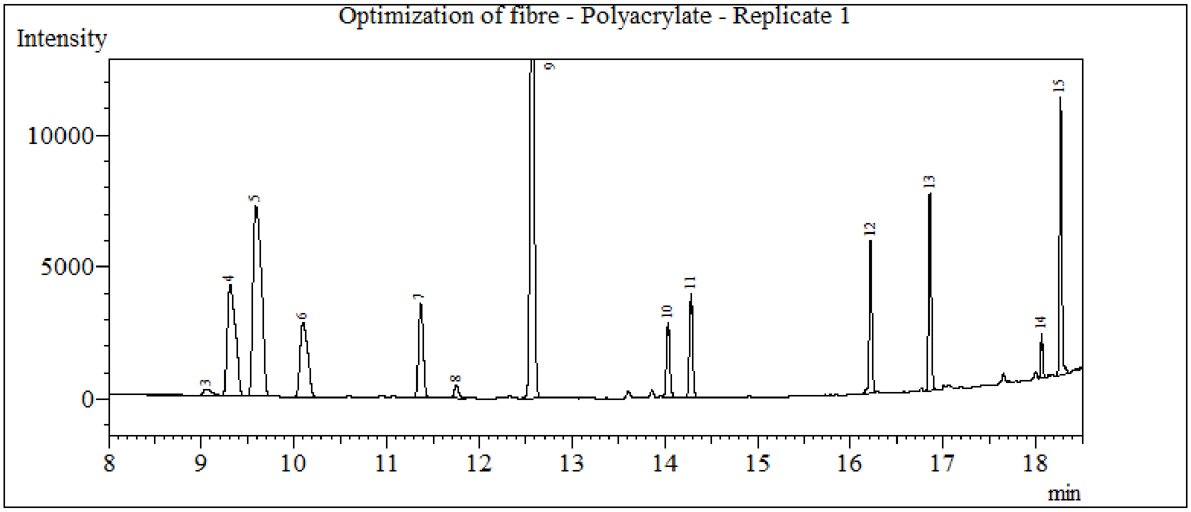
Where,

S = signal

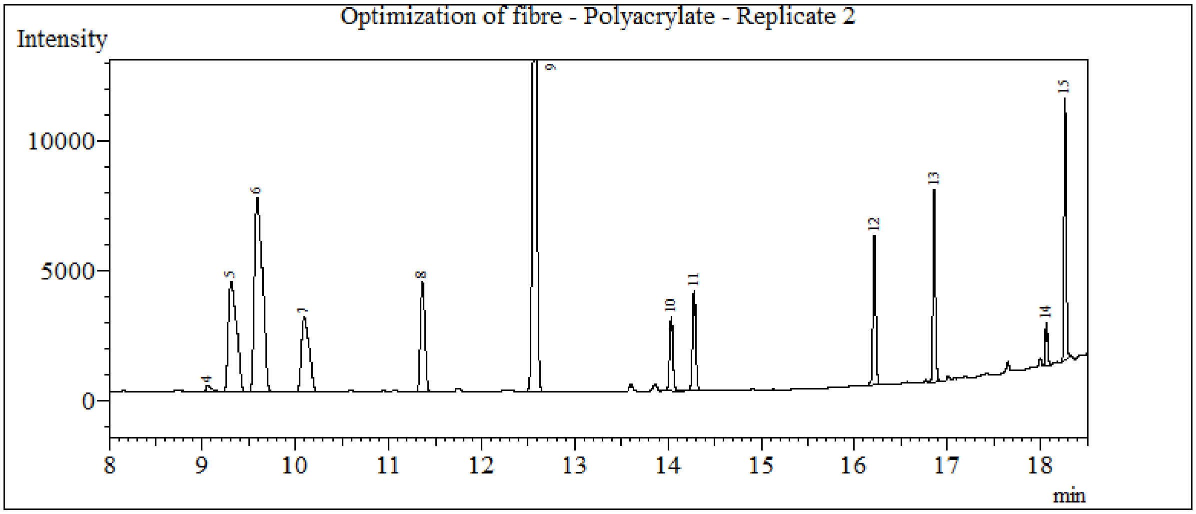
N = noise

ADDENDUM II

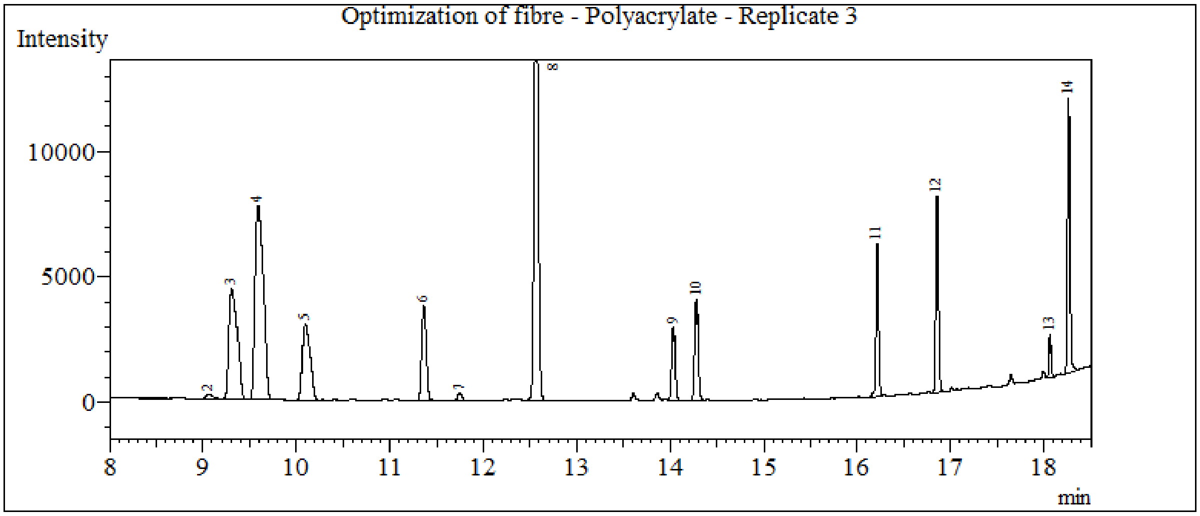
CHROMATOGRAMS



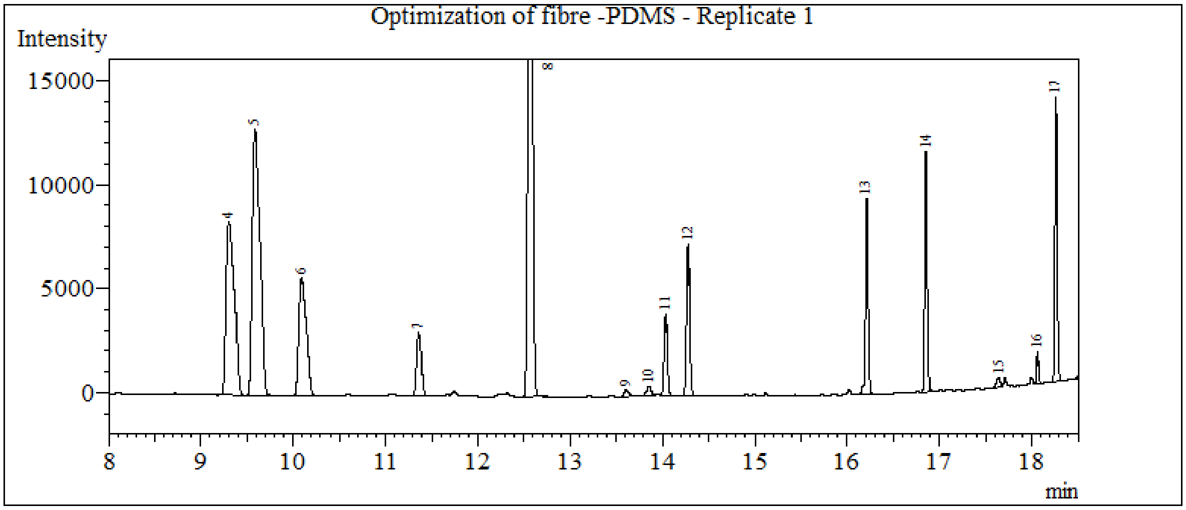
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.311	25696.5	4212.9
5	1,4-DCB	9.593	43994.2	7193.8
6	1,2-DCB	10.099	16531.4	2830.2
11	1,2,4-TCB	14.283	7169	3893.4
12	1,2,4,5-TeCB	16.217	9625.7	5698.5
13	1,2,3,4-TeCB	16.858	11552.9	7413.1
15	PCB	18.266	13747.5	10290.3



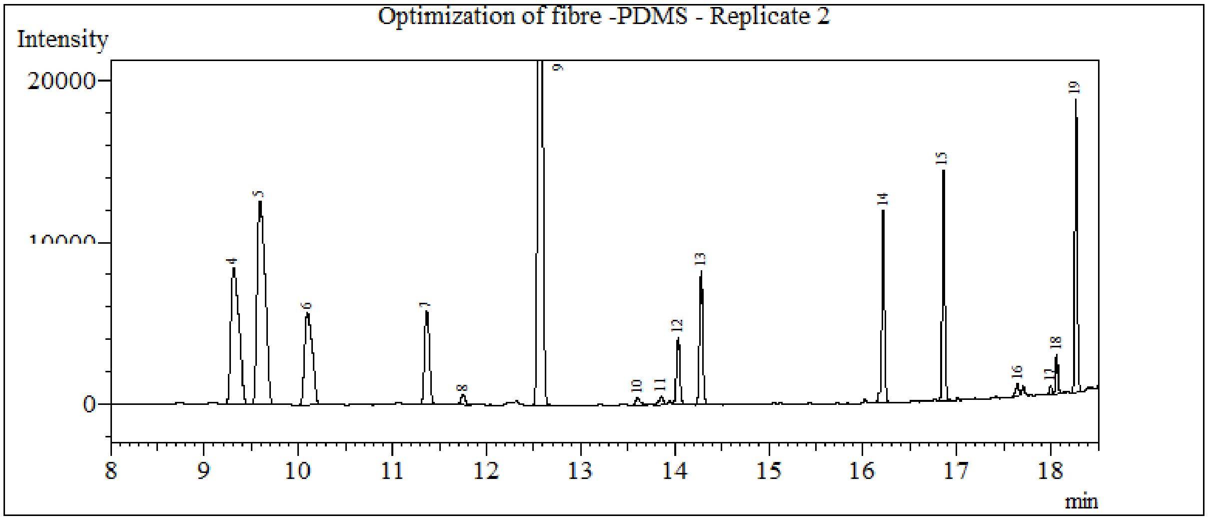
Peak #	Ret time	Retention time	Area	Height
5	1,3-DCB	9.311	26175.5	4226.1
6	1,4-DCB	9.591	44512.3	7463.4
7	1,2-DCB	10.098	16798.3	2881.4
11	1,2,4-TCB	14.281	7147	3814.8
12	1,2,4,5-TeCB	16.216	9477.9	5714.9
13	1,2,3,4-TeCB	16.857	11189.3	7198.9
15	PCB	18.265	13515.3	9963.9



Peak #	Ret time	Retention time	Area	Height
3	1,3-DCB	9.310	27572.2	4395.5
4	1,4-DCB	9.592	46943.1	7744.2
5	1,2-DCB	10.097	17701.7	3064.8
10	1,2,4-TCB	14.281	7398.7	3991.7
11	1,2,4,5-TeCB	16.215	9931.3	6022.2
12	1,2,3,4-TeCB	16.856	11868.4	7629.4
14	PCB	18.265	14330	10845.0



Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.306	51599.3	8315.8
5	1,4-DCB	9.588	74398.1	12818.0
6	1,2-DCB	10.092	32540.8	5639.6
12	1,2,4-TCB	14.280	9674.4	7246.8
13	1,2,4,5-TeCB	16.216	17920.6	9321.2
14	1,2,3,4-TeCB	16.857	18662.5	11351.7
17	PCB	18.265	21245.1	13466.7



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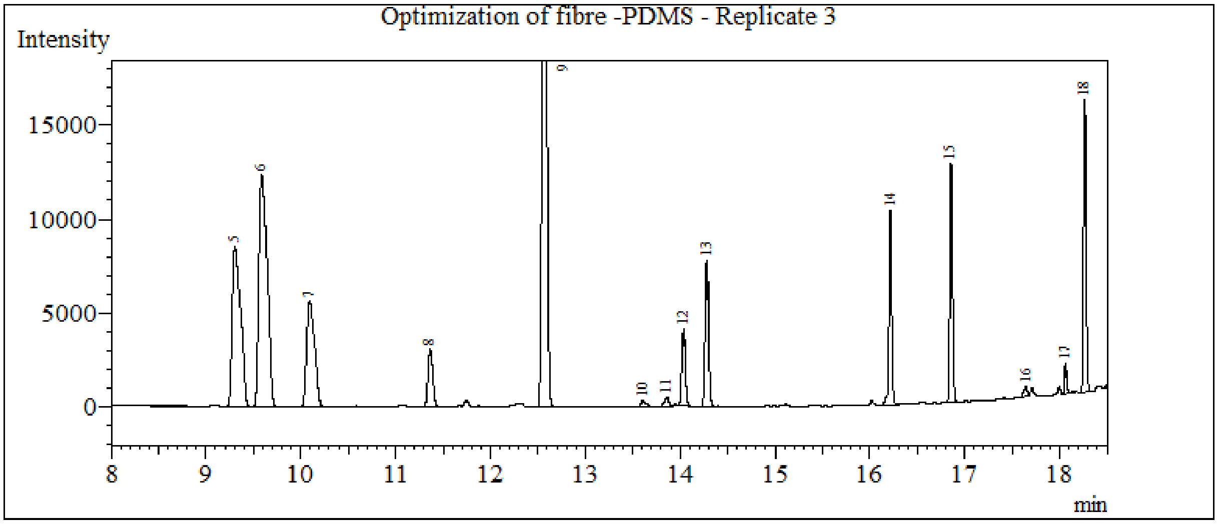
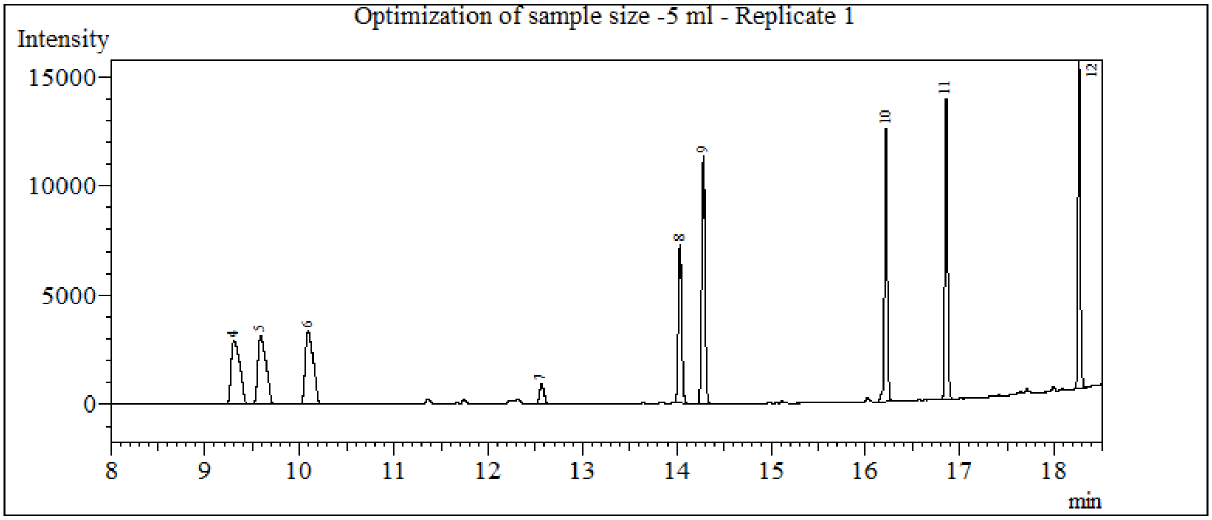
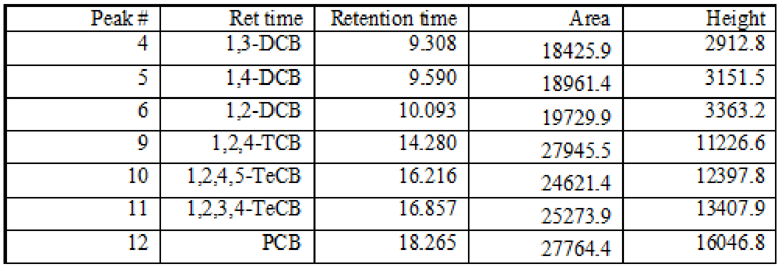
4	1,3-DCB	9.312	51554.5	8446.6
5	1,4-DCB	9.593	73382.1	12601.5
6	1,2-DCB	10.097	32120.8	5688.2
13	1,2,4-TCB	14.281	10175.8	8135.5
14	1,2,4,5-TeCB	16.216	20019.8	11806.8
15	1,2,3,4-TeCB	16.857	24071.4	13989.9
19	PCB	18.265	26335.7	17915.1

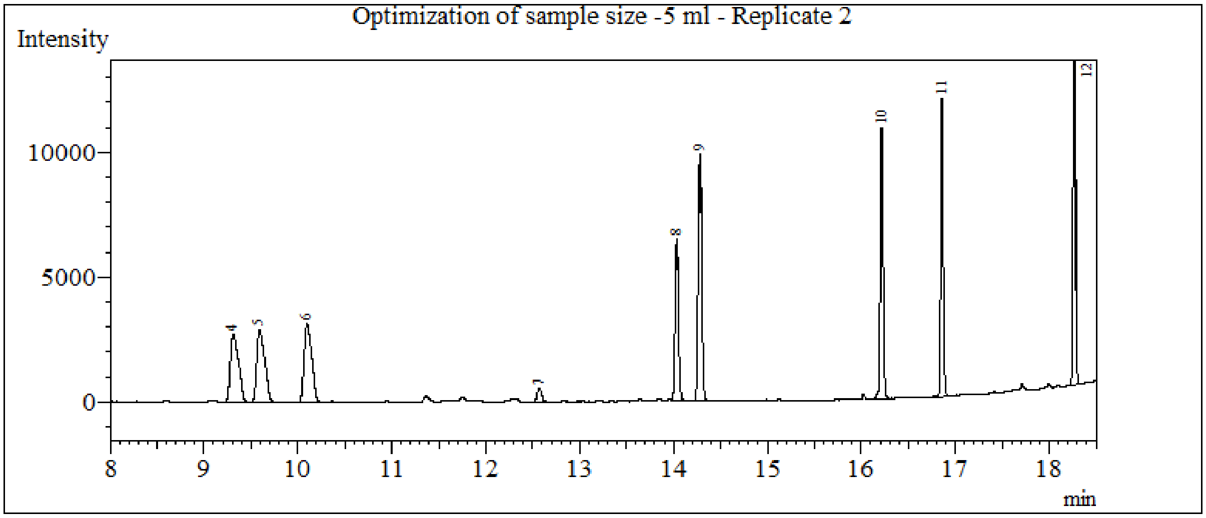


Peak #	Ret time	Retention time	Area	Height
5	1,3-DCB	9.306	52648.4	8493.0
6	1,4-DCB	9.590	75318.1	12314.8
7	1,2-DCB	10.094	32903.5	5661.3
13	1,2,4-TCB	14.280	10050.2	7683.7
14	1,2,4,5-TeCB	16.216	19045.6	10238.8
15	1,2,3,4-TeCB	16.857	20640.1	12384.5
18	PCB	18.265	23401.1	15335.5

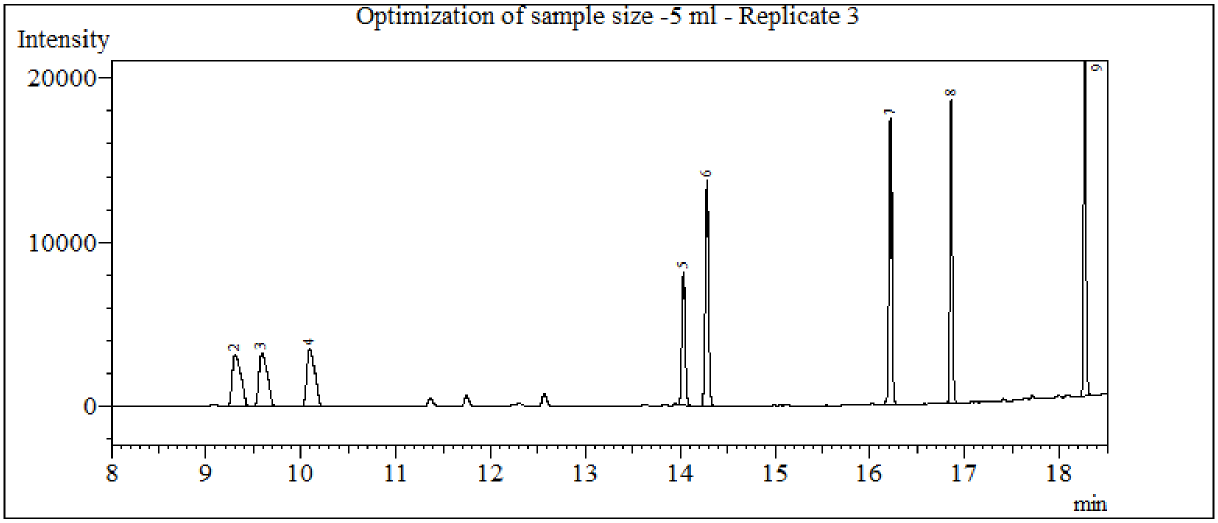




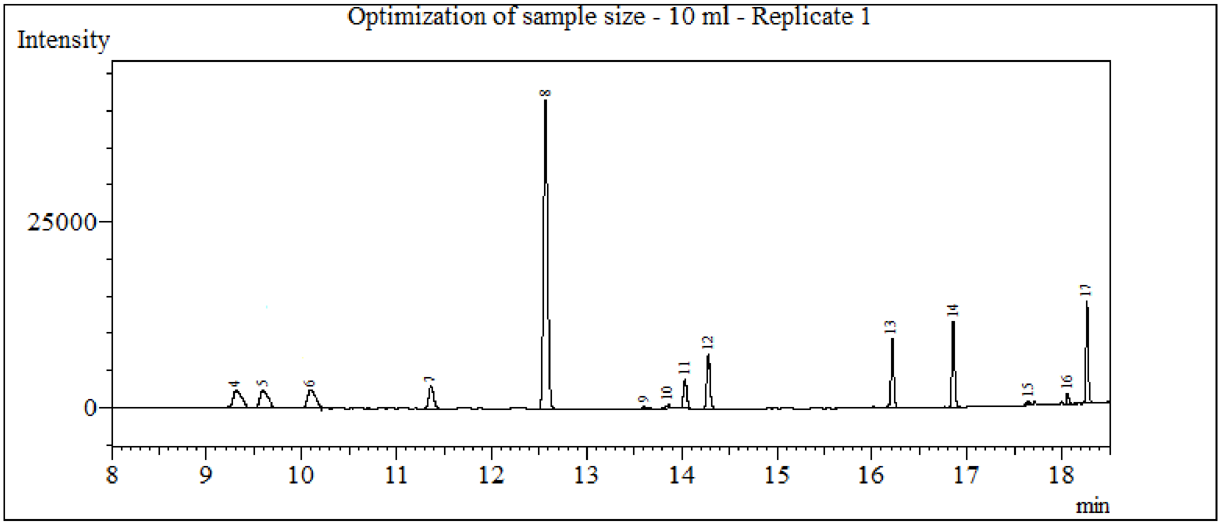
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.308	18425.9	2912.8
5	1,4-DCB	9.590	18961.4	3151.5
6	1,2-DCB	10.093	19729.9	3363.2
9	1,2,4-TCB	14.280	27945.5	11226.6
10	1,2,4,5-TeCB	16.216	24621.4	12397.8
11	1,2,3,4-TeCB	16.857	25273.9	13407.9
12	PCB	18.265	27764.4	16046.8



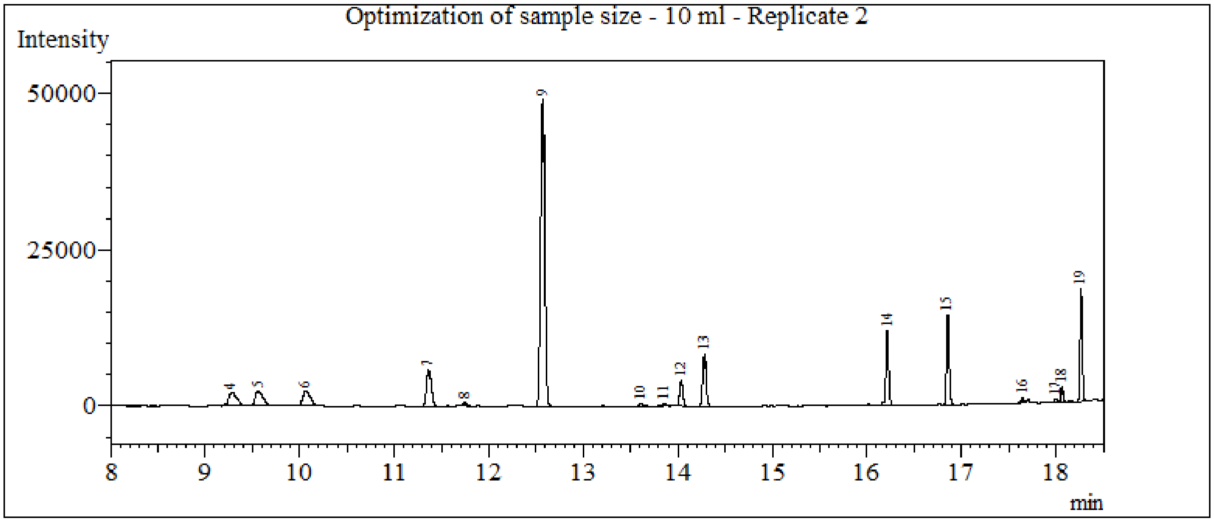
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.312	16323.6	2720.6
5	1,4-DCB	9.594	16820.2	2872.1
6	1,2-DCB	10.098	17853.8	3125.0
9	1,2,4-TCB	14.281	24104.3	9755.1
10	1,2,4,5-TeCB	16.217	21045.2	10716.3
11	1,2,3,4-TeCB	16.857	21896.9	11649.2
12	PCB	18.265	25491.1	14673.2



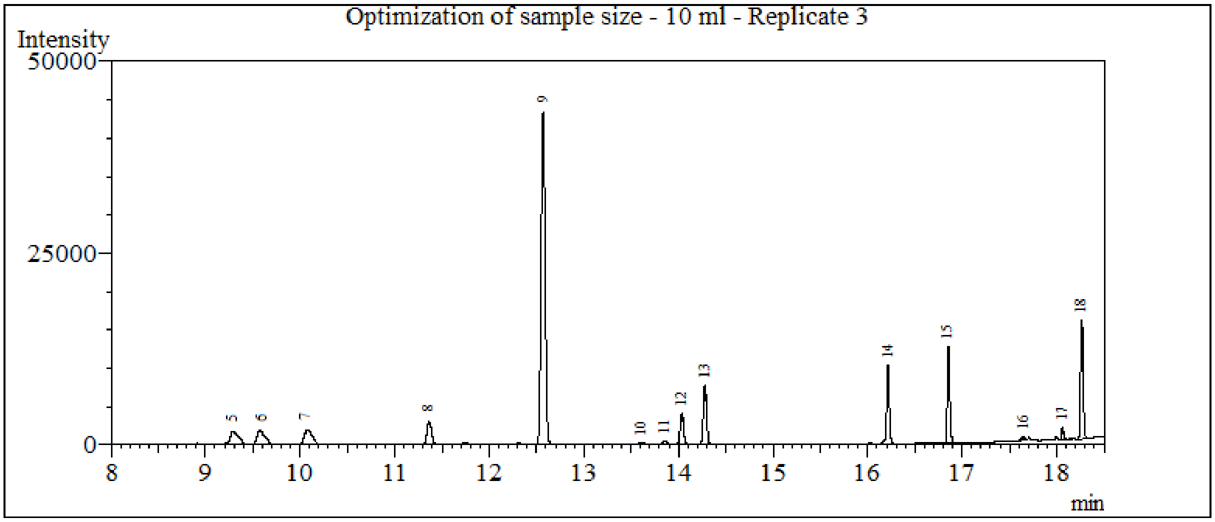
Peak #	Ret time	Retention time	Area	Height
2	1,3-DCB	9.308	19715.6	3102.7
3	1,4-DCB	9.589	19745	3227.0
4	1,2-DCB	10.095	20775.4	3529.3
6	1,2,4-TCB	14.281	33328.2	13519.1
7	1,2,4,5-TeCB	16.217	33723.5	17150.5
8	1,2,3,4-TeCB	16.858	34070.3	18244.6
9	PCB	18.267	43596.3	24943.6



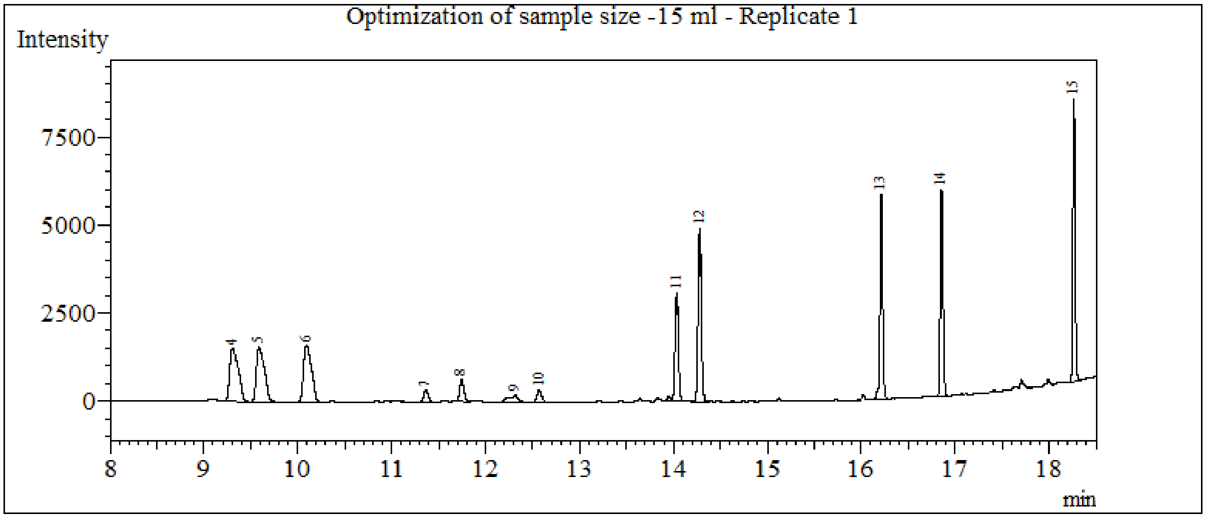
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.298	12987.4	1928.3
5	1,4-DCB	9.579	14563.7	2009.3
6	1,2-DCB	10.083	13483.1	2026.8
12	1,2,4-TCB	14.280	17920.6	7246.8
13	1,2,4,5-TeCB	16.216	18662.5	9321.2
14	1,2,3,4-TeCB	16.857	21245.1	11351.7
17	PCB	18.265	23430.8	13466.7



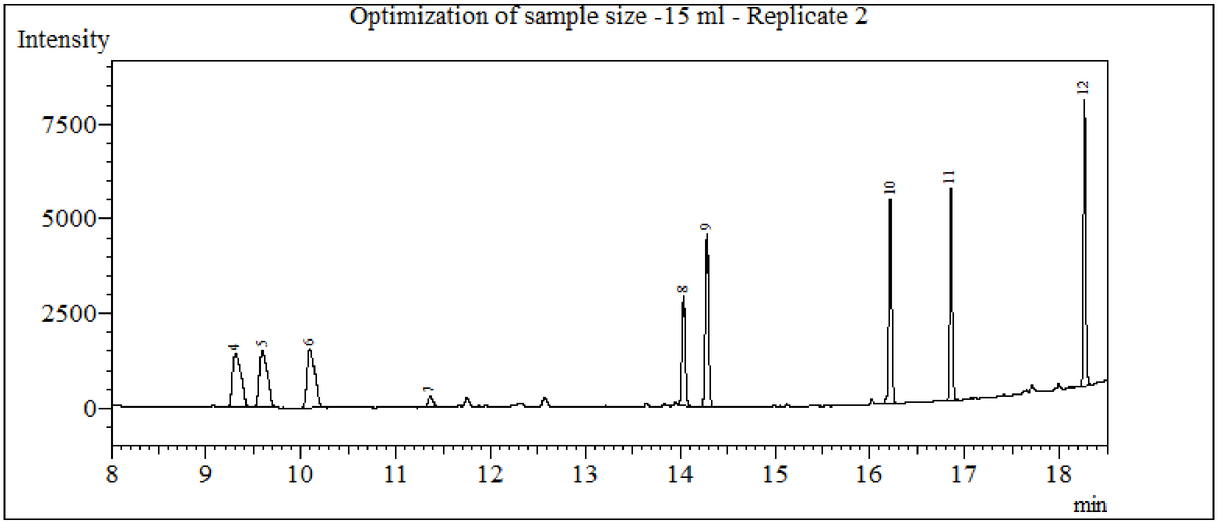
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.298	13234.3	1717.4
5	1,4-DCB	9.578	13667.1	1839.0
6	1,2-DCB	10.081	13459.4	1893.7
13	1,2,4-TCB	14.281	20019.8	8135.5
14	1,2,4,5-TeCB	16.216	24071.4	11806.8
15	1,2,3,4-TeCB	16.857	26335.7	13989.9
19	PCB	18.265	30927.6	17915.1



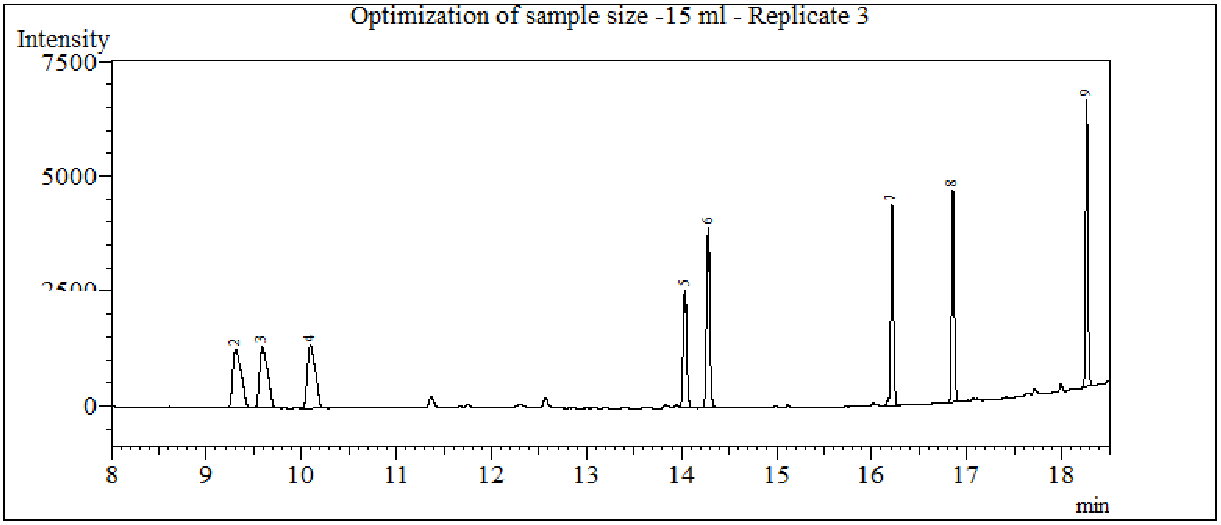
Peak #	Ret time	Retention time	Area	Height
5	1,3-DCB	9.294	12866.7	1755.9
6	1,4-DCB	9.575	15112.4	1847.0
7	1,2-DCB	10.080	12998.6	1859.0
13	1,2,4-TCB	14.280	19045.6	7683.7
14	1,2,4,5-TeCB	16.216	20640.1	10238.8
15	1,2,3,4-TeCB	16.857	23401.1	12384.5
18	PCB	18.265	26380.8	15335.5



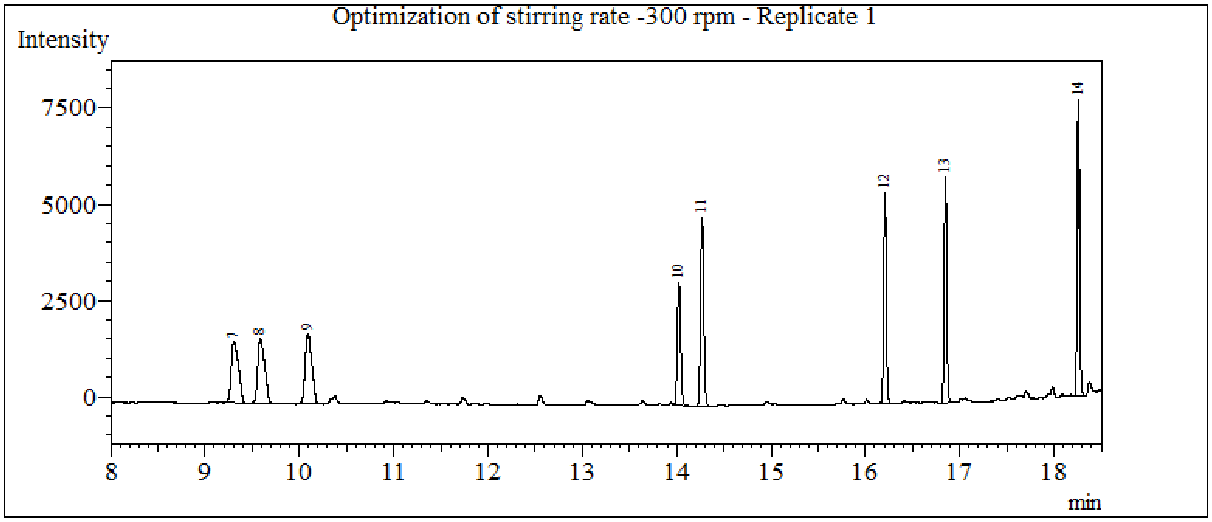
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.308	9530.8	1497.7
5	1,4-DCB	9.588	9453.6	1522.2
6	1,2-DCB	10.094	9428.2	1613.1
12	1,2,4-TCB	14.281	8109.9	4779.0
13	1,2,4,5-TeCB	16.216	11994.9	5479.1
14	1,2,3,4-TeCB	16.856	11577.7	5457.8
15	PCB	18.264	10824.6	7683.2



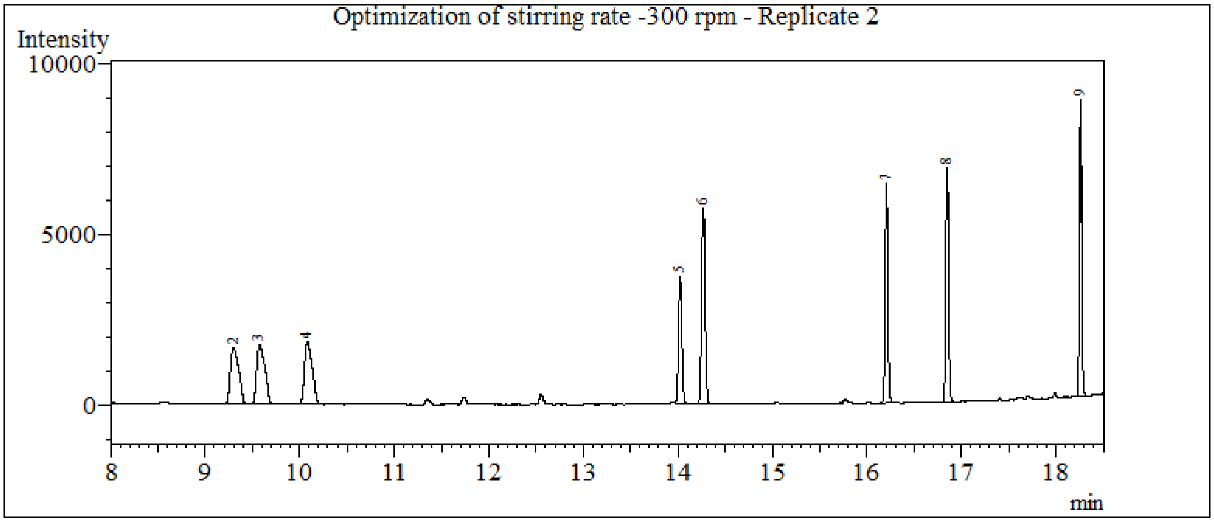
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.312	8930.2	1425.7
5	1,4-DCB	9.594	8841.3	1486.0
6	1,2-DCB	10.096	8867.5	1543.0
9	1,2,4-TCB	14.281	7318.5	4536.9
10	1,2,4,5-TeCB	16.216	11267.1	5323.6
11	1,2,3,4-TeCB	16.857	10558.6	5467.4
12	PCB	18.264	10194.8	7469.7


Optimization of sample size -15 ml - Replicate 3

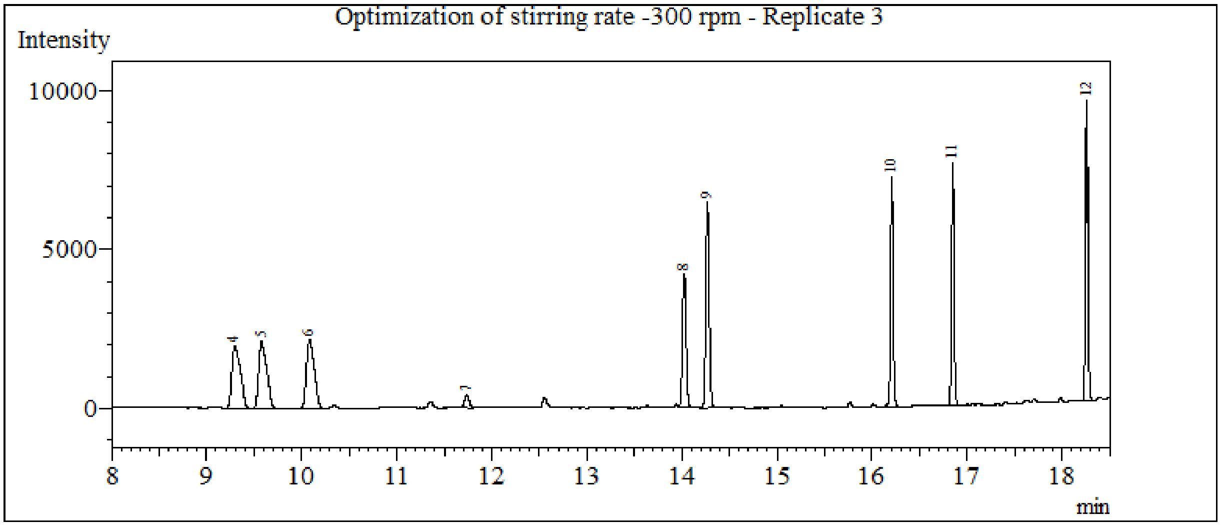
Peak #	Ret time	Retention time	Area	Height
2	1,3-DCB	9.310	7847.8	1258.5
3	1,4-DCB	9.591	8004.7	1325.1
4	1,2-DCB	10.096	8033.3	1380.4
6	1,2,4-TCB	14.281	6461.3	3870.4
7	1,2,4,5-TeCB	16.215	9683.4	4348.7
8	1,2,3,4-TeCB	16.856	8599.9	4551.1
9	PCB	18.264	8528.5	6212.1


Optimization of stirring rate -300 rpm - Replicate 1

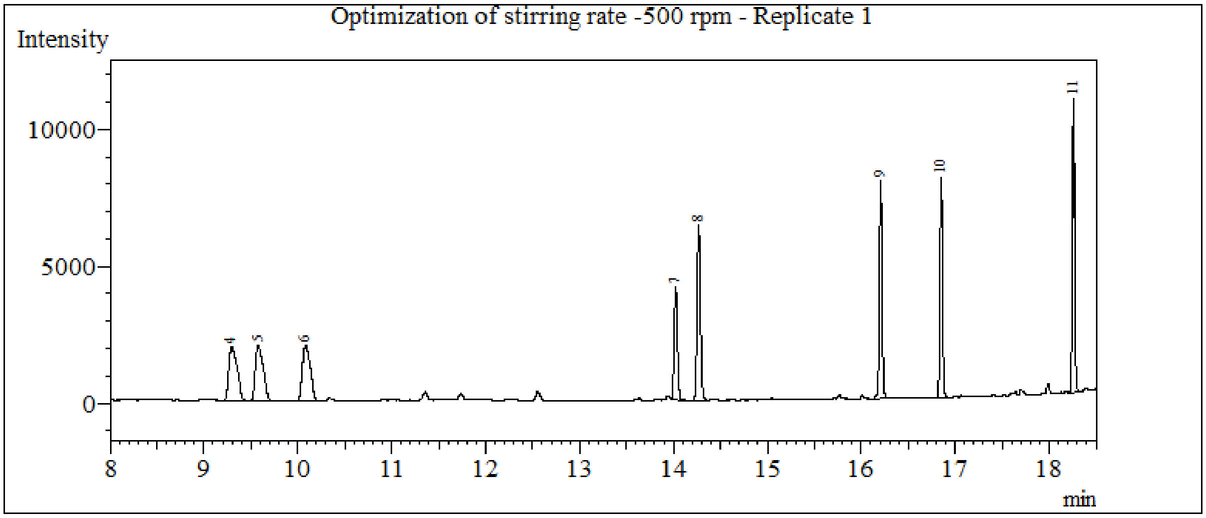
Peak #	Ret time	Retention time	Area	Height
7	1,3-DCB	9.308	8422.5	1594.4
8	1,4-DCB	9.588	8697.8	1696.4
9	1,2-DCB	10.092	8931.9	1823.7
11	1,2,4-TCB	14.270	11689	4891.3
12	1,2,4,5-TeCB	16.207	10429.8	5399.5
13	1,2,3,4-TeCB	16.849	10766.9	5777.2
14	PCB	18.257	13163.3	7521.0



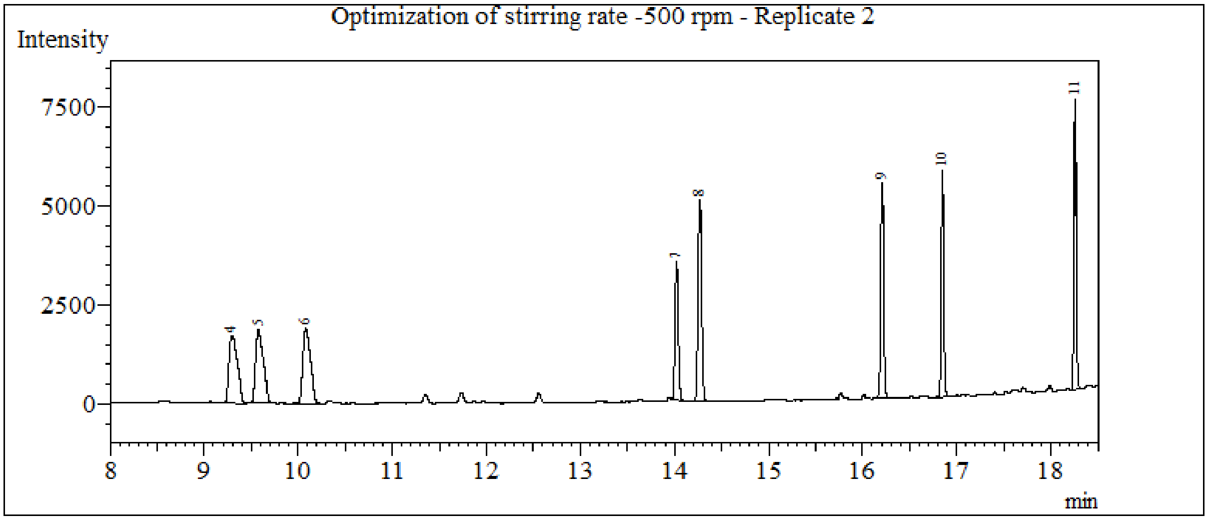
Peak #	Ret time	Retention time	Area	Height
2	1,3-DCB	9.299	10353.5	1665.8
3	1,4-DCB	9.576	10599	1751.0
4	1,2-DCB	10.082	10661.9	1846.9
6	1,2,4-TCB	14.270	13993.8	5723.6
7	1,2,4,5-TeCB	16.208	12342.2	6315.9
8	1,2,3,4-TeCB	16.849	12593.8	6787.7
9	PCB	18.258	14796.3	8557.0



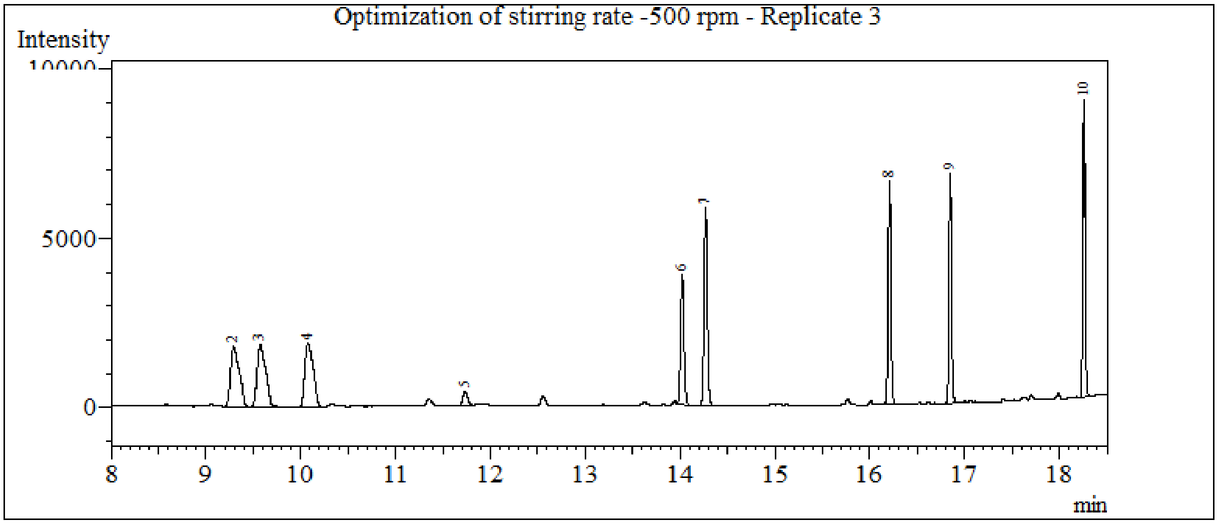
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.296	12201.6	1953.8
5	1,4-DCB	9.578	12301.4	2083.0
6	1,2-DCB	10.083	12406.5	2177.1
9	1,2,4-TCB	14.270	15836.2	6403.9
10	1,2,4,5-TeCB	16.208	13946.2	7124.4
11	1,2,3,4-TeCB	16.849	13935.2	7569.2
12	PCB	18.258	16112.8	9298.5



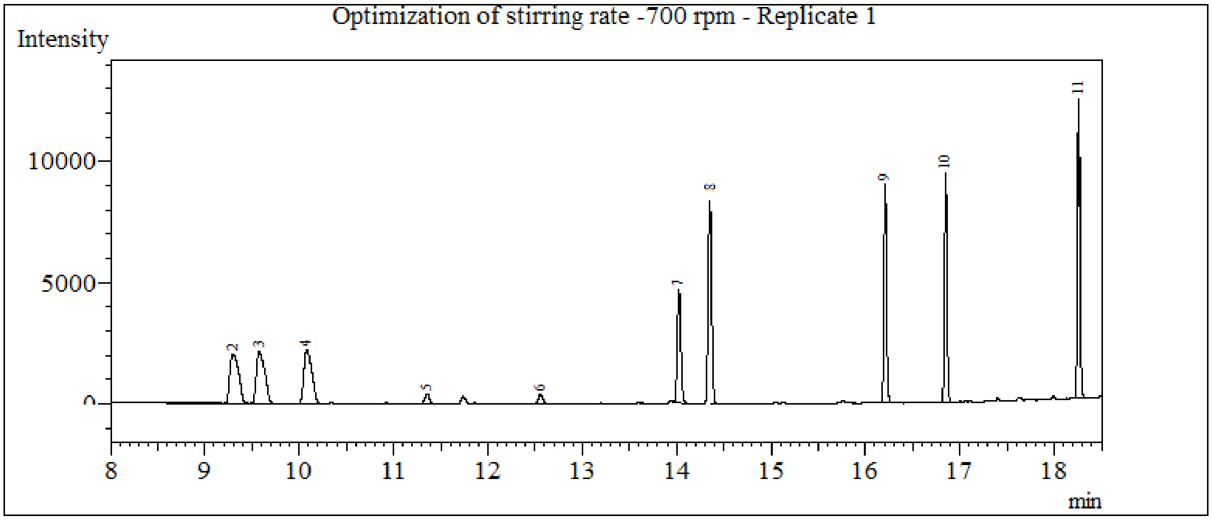
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.298	11745.9	1928.3
5	1,4-DCB	9.579	11891.7	2009.3
6	1,2-DCB	10.083	11796.8	2026.8
8	1,2,4-TCB	14.270	15564.8	6353.8
9	1,2,4,5-TeCB	16.208	15582.6	7830.0
10	1,2,3,4-TeCB	16.849	14710.7	7994.8
11	PCB	18.258	18356.4	10563.6



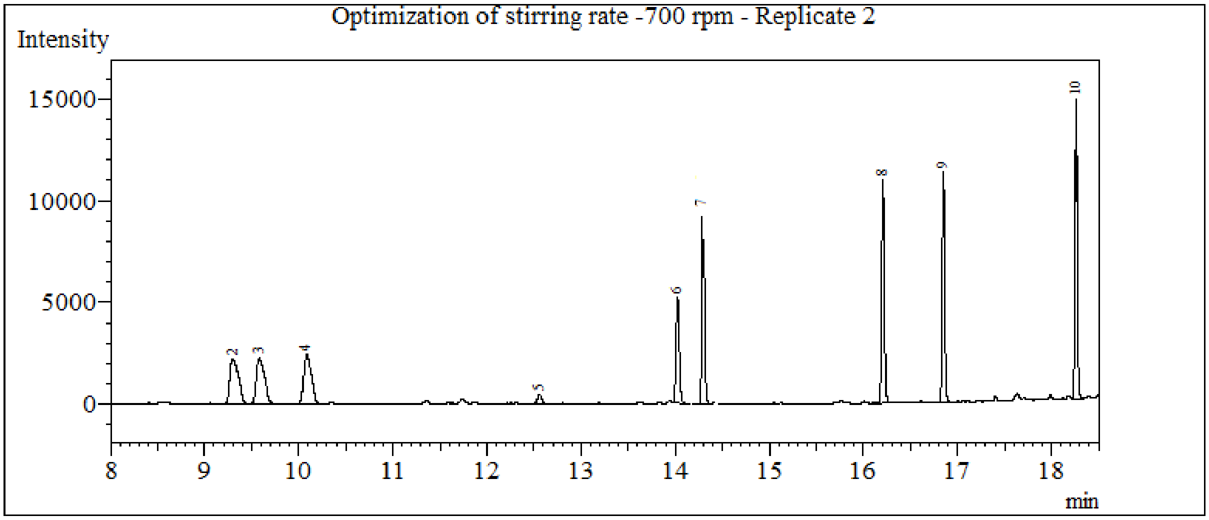
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.298	10528.2	1717.4
5	1,4-DCB	9.578	10851.2	1839.0
6	1,2-DCB	10.081	10973.9	1893.7
8	1,2,4-TCB	14.270	12430.7	5085.0
9	1,2,4,5-TeCB	16.208	10589.7	5382.9
10	1,2,3,4-TeCB	16.849	10573.8	5690.5
11	PCB	18.258	12476.9	7207.7



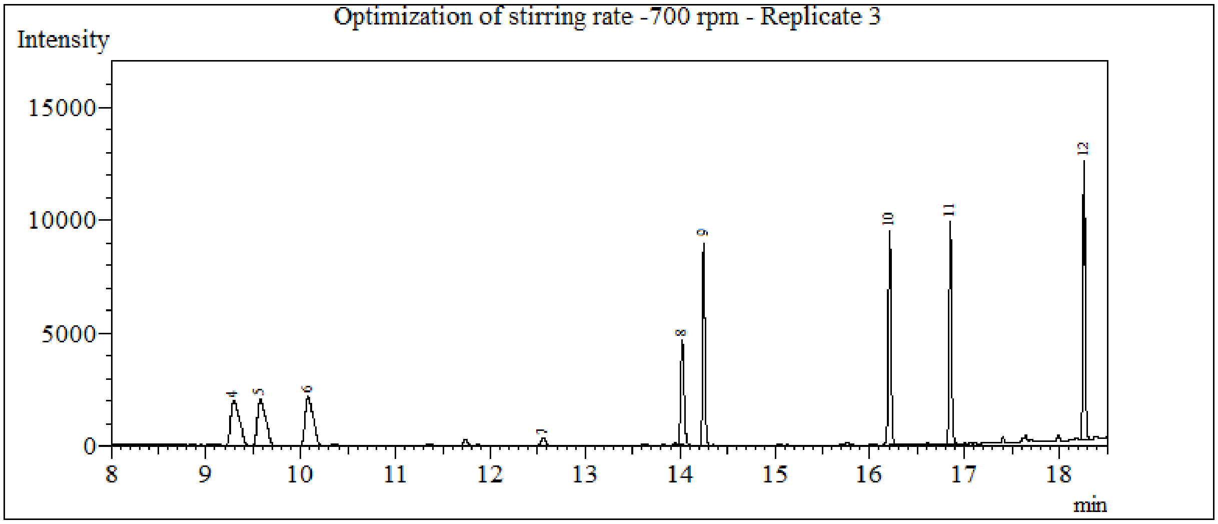
Peak #	Ret time	Retention time	Area	Height
2	1,3-DCB	9.294	11175.5	1755.9
3	1,4-DCB	9.575	11425	1847.0
4	1,2-DCB	10.080	11477.7	1859.0
7	1,2,4-TCB	14.270	14360	5801.1
8	1,2,4,5-TeCB	16.208	12667.9	6437.8
9	1,2,3,4-TeCB	16.848	12634.7	6728.0
10	PCB	18.258	14983.7	8681.5



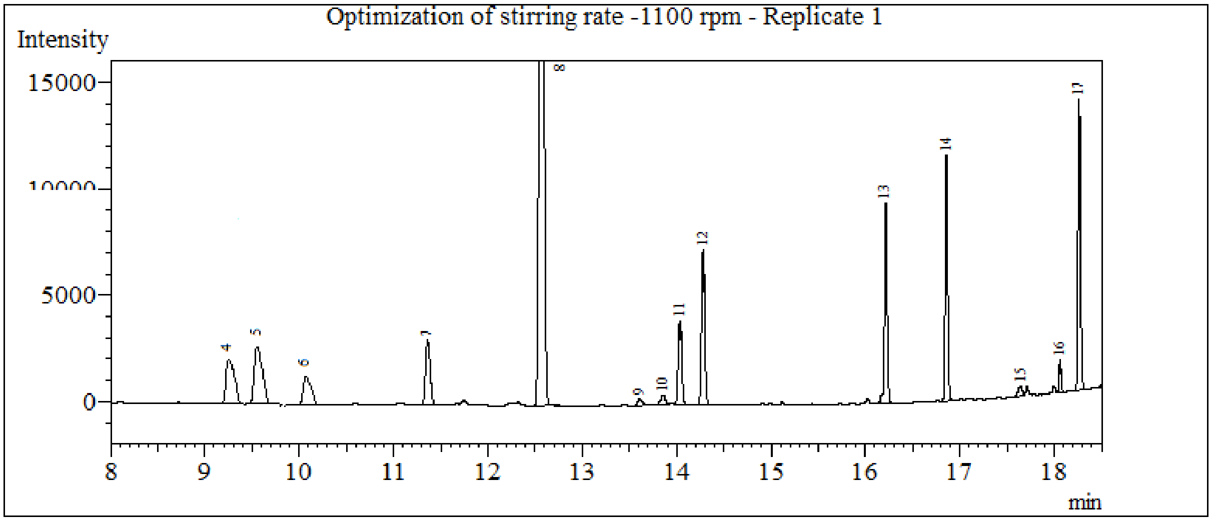
Peak #	Ret time	Retention time	Area	Height
2	1,3-DCB	9.301	12932.8	2030.9
3	1,4-DCB	9.579	12926.6	2136.3
4	1,2-DCB	10.084	12849.1	2238.9
8	1,2,4-TCB	14.271	15986.2	7390.7
9	1,2,4,5-TeCB	16.209	17288.7	8902.4
10	1,2,3,4-TeCB	16.849	17246.3	9376.8
11	PCB	18.259	21023.3	12230.3


Optimization of stirring rate -700 rpm - Replicate 2

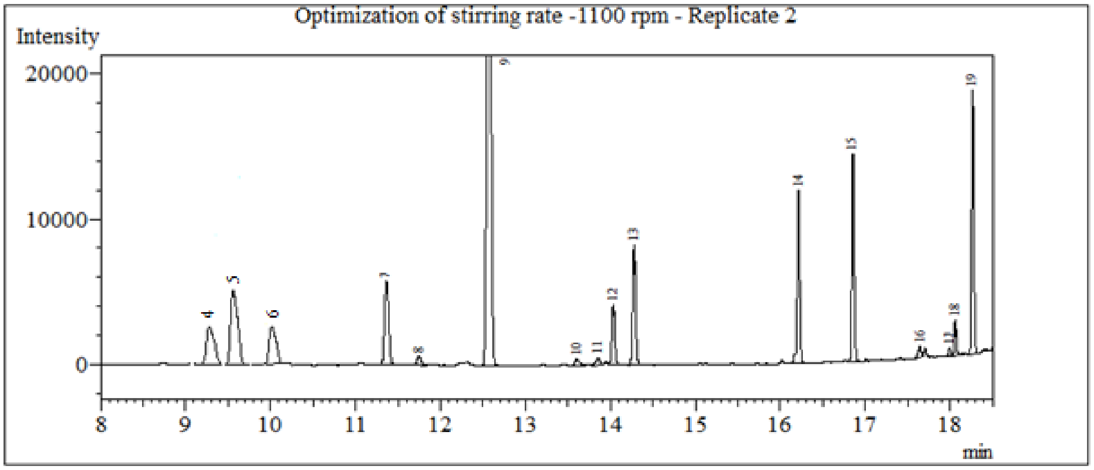
Peak #	Ret time	Retention time	Area	Height
2	1,3-DCB	9.300	13744.8	2197.3
3	1,4-DCB	9.580	13498.7	2219.1
4	1,2-DCB	10.087	13488.7	2380.0
7	1,2,4-TCB	14.272	16588.6	9298.5
8	1,2,4,5-TeCB	16.209	21050.5	10835.5
9	1,2,3,4-TeCB	16.850	20988	11241.0
10	PCB	18.259	24885.9	14568.1

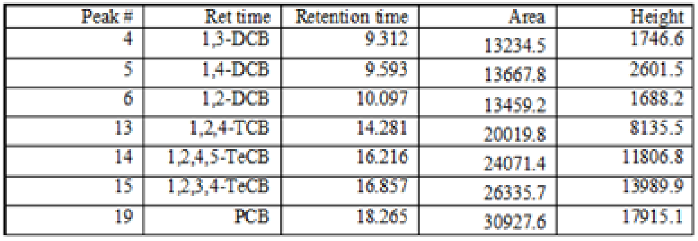


Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.295	12586.7	1987.8
5	1,4-DCB	9.575	12409.1	2034.9
6	1,2-DCB	10.080	12626.4	2184.1
9	1,2,4-TCB	14.272	16213.9	9567.3
10	1,2,4,5-TeCB	16.209	18136.1	9328.7
11	1,2,3,4-TeCB	16.850	18227.4	9777.4
12	PCB	18.259	21073.7	12255.9

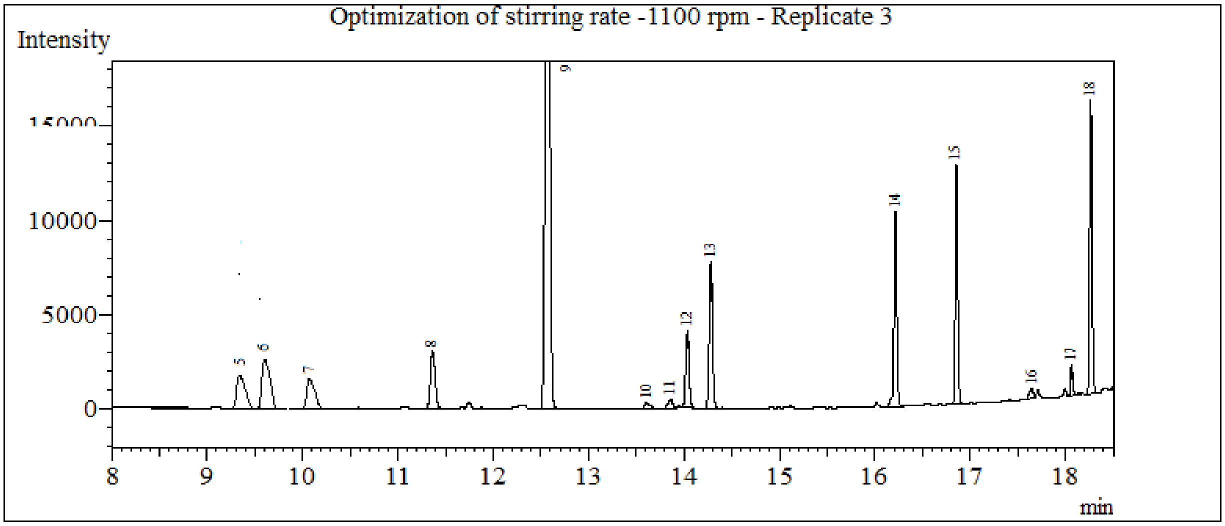


Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.306	12987.1	1915.8
5	1,4-DCB	9.588	14563.8	2818.0
6	1,2-DCB	10.092	13483.7	1639.6
12	1,2,4-TCB	14.280	17920.6	7246.8
13	1,2,4,5-TeCB	16.216	18662.5	9321.2
14	1,2,3,4-TeCB	16.857	21245.1	11351.7
17	PCB	18.265	23430.8	13466.7

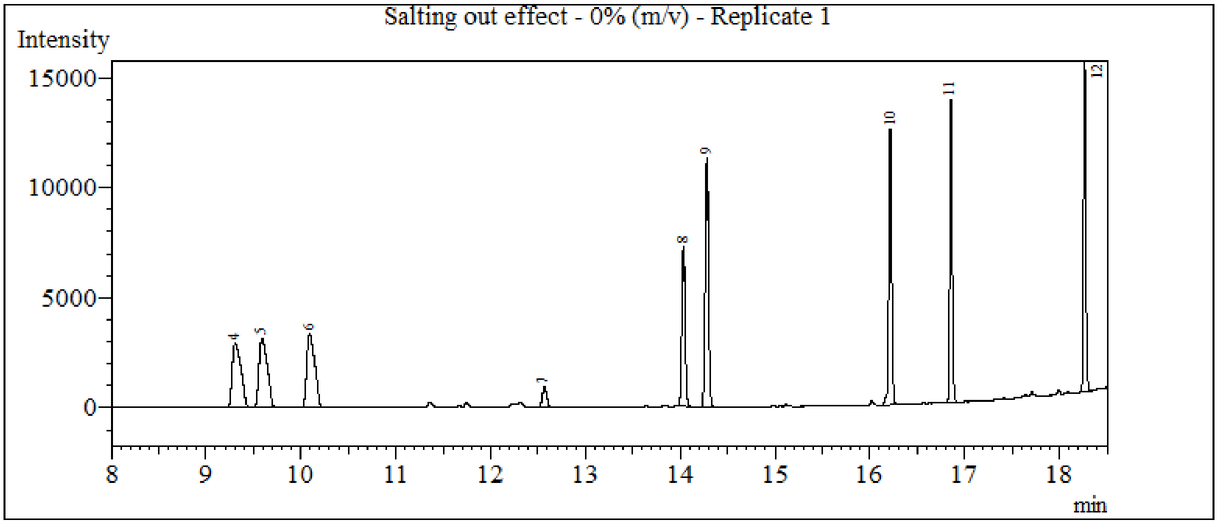




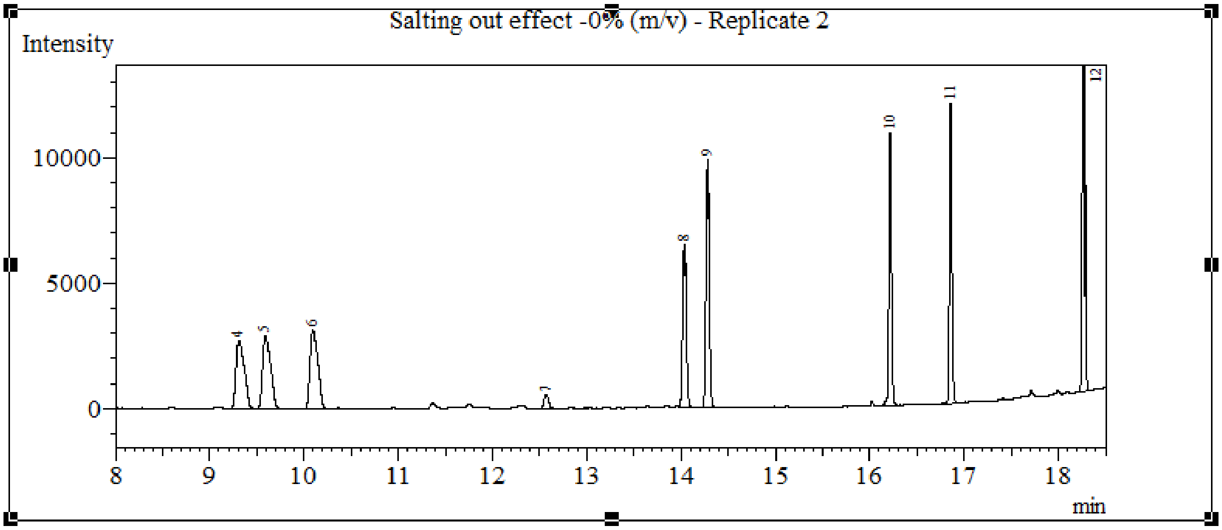
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.312	13234.5	1746.6
5	1,4-DCB	9.593	13667.8	2601.5
6	1,2-DCB	10.097	13459.2	1688.2
13	1,2,4-TCB	14.281	20019.8	8135.5
14	1,2,4,5-TeCB	16.216	24071.4	11806.8
15	1,2,3,4-TeCB	16.857	26335.7	13989.9
19	PCB	18.265	30927.6	17915.1



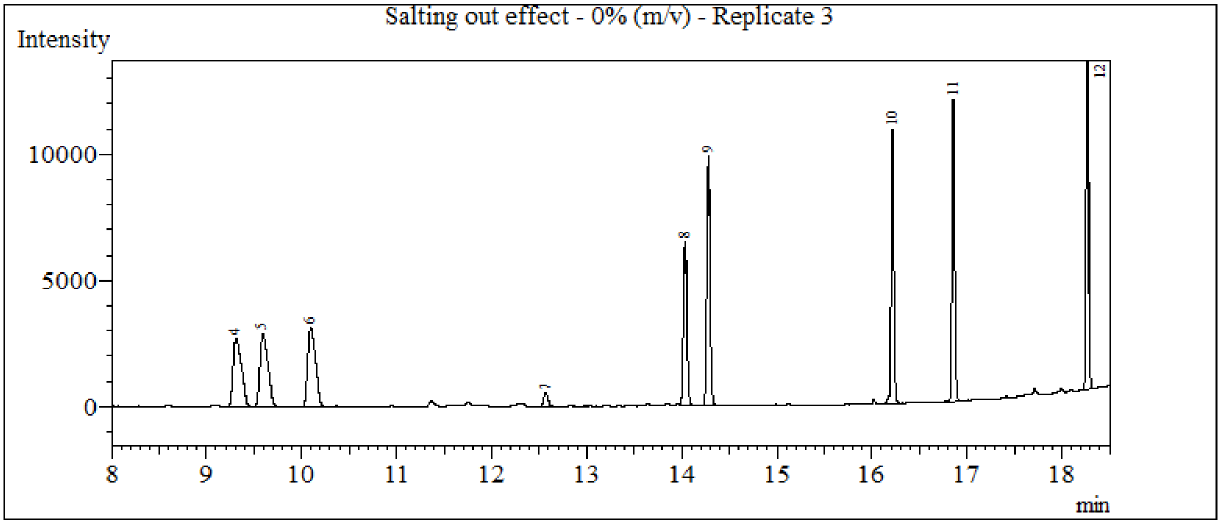
Peak #	Ret time	Retention time	Area	Height
5	1,3-DCB	9.306	12866.3	1926.6
6	1,4-DCB	9.590	15112.3	2311.5
7	1,2-DCB	10.094	12998.9	1698.4
13	1,2,4-TCB	14.280	19045.6	7683.7
14	1,2,4,5-TeCB	16.216	20640.1	10238.8
15	1,2,3,4-TeCB	16.857	23401.1	12384.5
18	PCB	18.265	26380.8	15335.5


Salting out effect - 0% (m/v) - Replicate 1

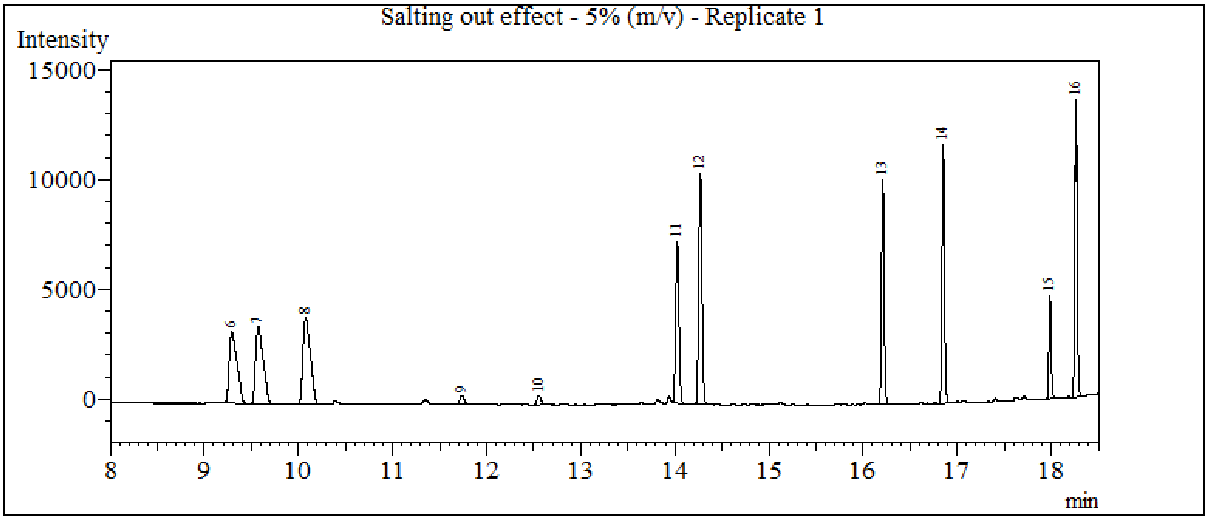
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.308	18425.9	2912.8
5	1,4-DCB	9.590	18961.4	3151.5
6	1,2-DCB	10.093	19729.9	3363.2
9	1,2,4-TCB	14.280	27945.5	11226.6
10	1,2,4,5-TeCB	16.216	24621.4	12397.8
11	1,2,3,4-TeCB	16.857	25273.9	13407.9
12	PCB	18.265	27764.4	16046.8



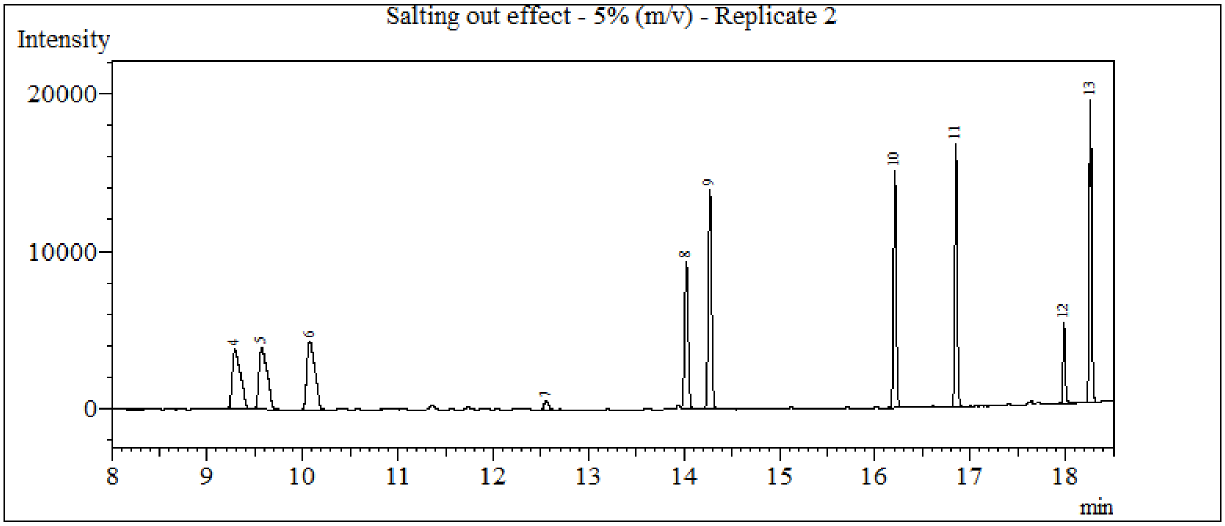
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.312	16323.6	2720.6
5	1,4-DCB	9.594	16820.2	2872.1
6	1,2-DCB	10.098	17853.8	3125.0
9	1,2,4-TCB	14.281	24104.3	9755.1
10	1,2,4,5-TeCB	16.217	21045.2	10716.3
11	1,2,3,4-TeCB	16.857	21896.9	11649.2
12	PCB	18.265	25491.1	14673.2



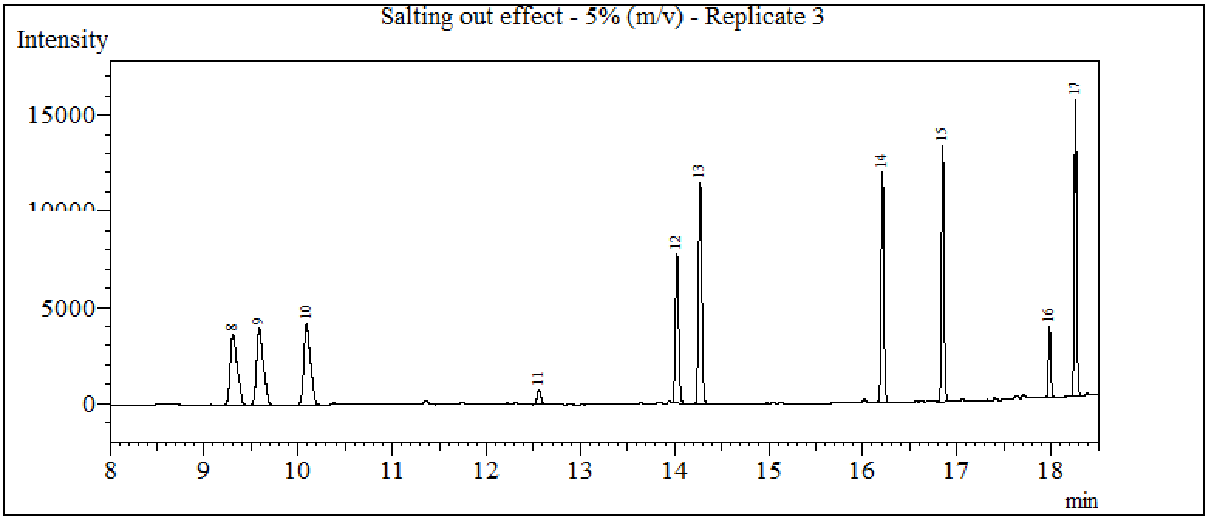
Peak #	Ret time	Retention time	Area	Height
2	1,3-DCB	9.308	19715.6	3102.7
3	1,4-DCB	9.589	19745	3227.0
4	1,2-DCB	10.095	20775.4	3529.3
6	1,2,4-TCB	14.281	33328.2	13519.1
7	1,2,4,5-TeCB	16.217	33723.5	17150.5
8	1,2,3,4-TeCB	16.858	34070.3	18244.6
9	PCB	18.267	43596.3	24943.6



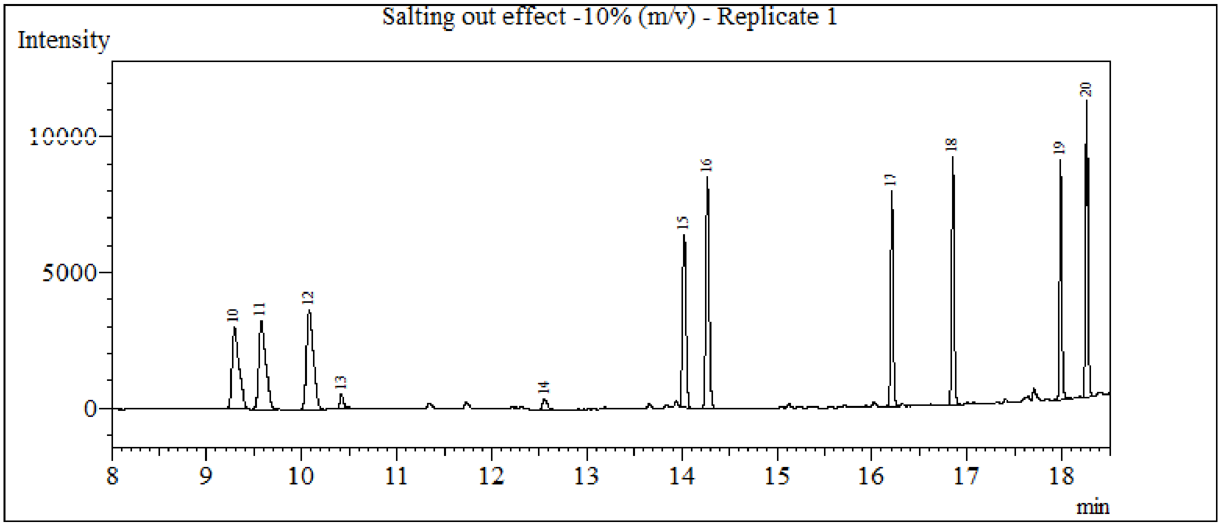
Peak #	Ret time	Retention time	Area	Height
6	1,3-DCB	9.294	18794.3	3255.8
7	1,4-DCB	9.576	19629.3	3534.3
8	1,2-DCB	10.079	21359	3922.3
12	1,2,4-TCB	14.272	25529.2	10448.4
13	1,2,4,5-TeCB	16.210	19720.7	10183.5
14	1,2,3,4-TeCB	16.851	21360.8	11606.1
16	PCB	18.267	23165.5	13314.1



Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.293	22892.8	3781.7
5	1,4-DCB	9.576	23466.8	3949.5
6	1,2-DCB	10.079	25168	4360.2
9	1,2,4-TCB	14.271	34401.8	13796.6
10	1,2,4,5-TeCB	16.210	28860.4	14964.0
11	1,2,3,4-TeCB	16.851	30321.2	16409.4
13	PCB	18.261	32850.9	18836.3

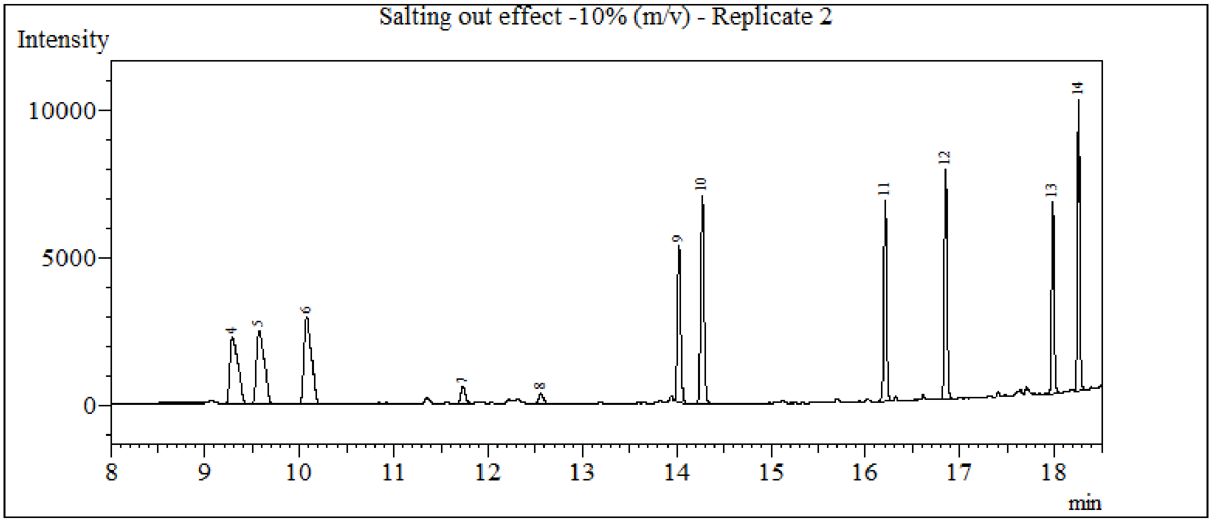


Peak #	Ret time	Retention time	Area	Height
8	1,3-DCB	9.308	18827.9	3693.1
9	1,4-DCB	9.588	19844.2	3988.8
10	1,2-DCB	10.090	20758.4	4212.1
13	1,2,4-TCB	14.273	27558	11458.7
14	1,2,4,5-TeCB	16.210	22686.6	11897.1
15	1,2,3,4-TeCB	16.851	24229.2	13100.6
17	PCB	18.260	26267.9	15273.7

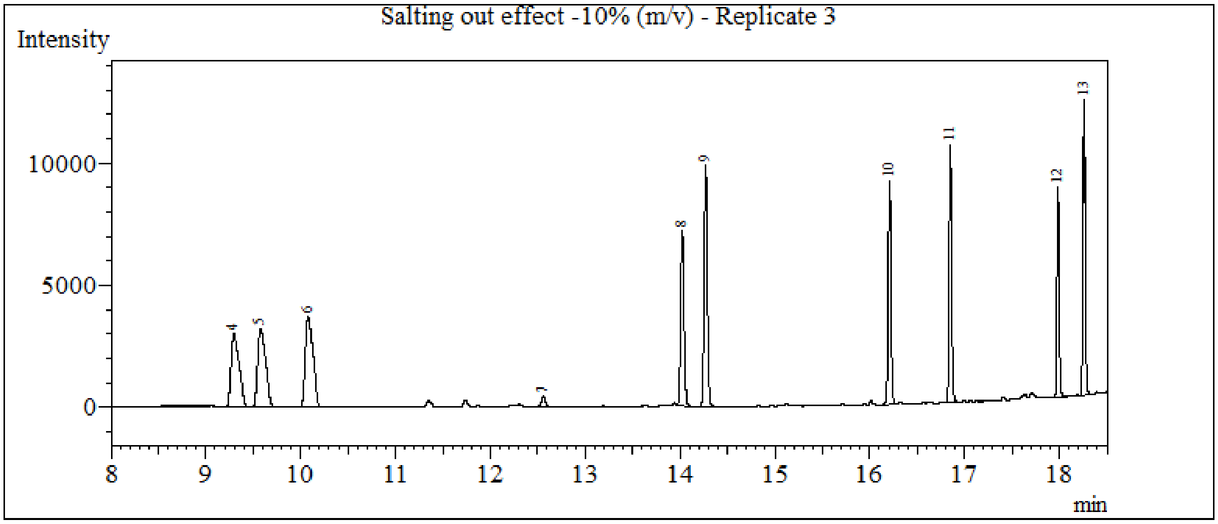


Salting out effect -10% (m/v) - Replicate 1

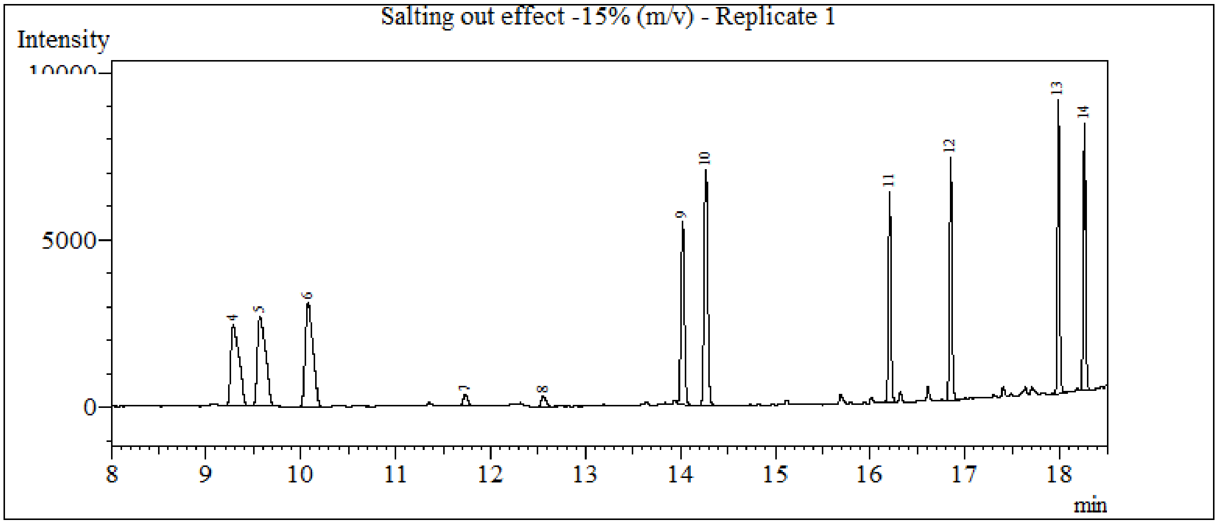
Peak #	Ret time	Retention time	Area	Height
10	1,3-DCB	9.294	15413.6	3010.0
11	1,4-DCB	9.576	16707.6	3252.4
12	1,2-DCB	10.080	18524.6	3679.6
16	1,2,4-TCB	14.270	20510.4	8472.0
17	1,2,4,5-TeCB	16.209	15117.3	7865.0
18	1,2,3,4-TeCB	16.850	16903.5	9109.6
20	PCB	18.259	18640.6	10850.0



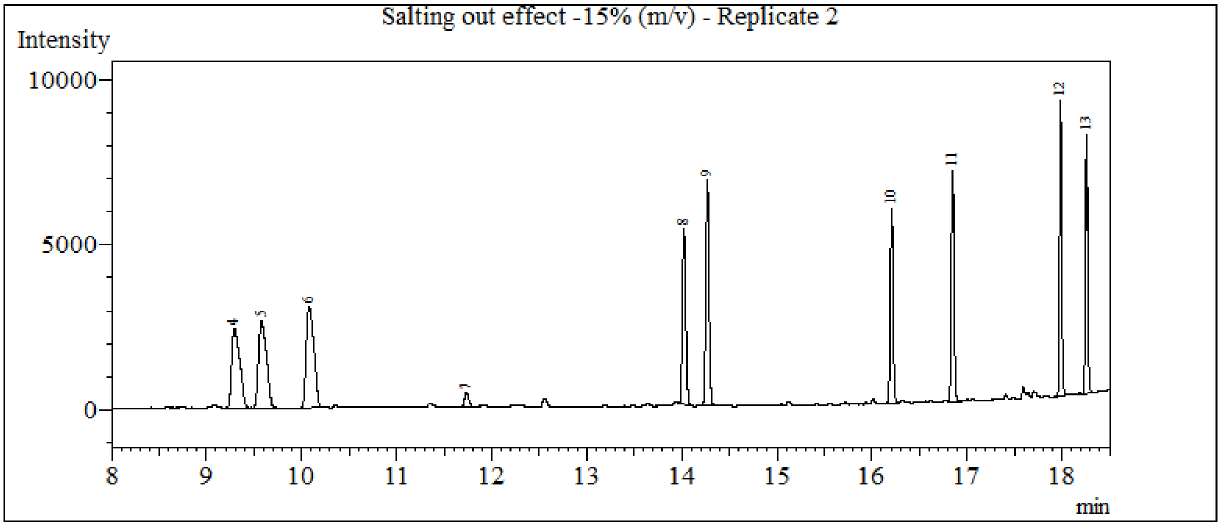
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.292	13431.3	2258.2
5	1,4-DCB	9.575	14675.3	2470.9
6	1,2-DCB	10.079	16447	2978.9
10	1,2,4-TCB	14.270	17256	6975.4
11	1,2,4,5-TeCB	16.208	13055	6650.0
12	1,2,3,4-TeCB	16.850	14299.7	7742.4
14	PCB	18.259	16857.7	9752.6



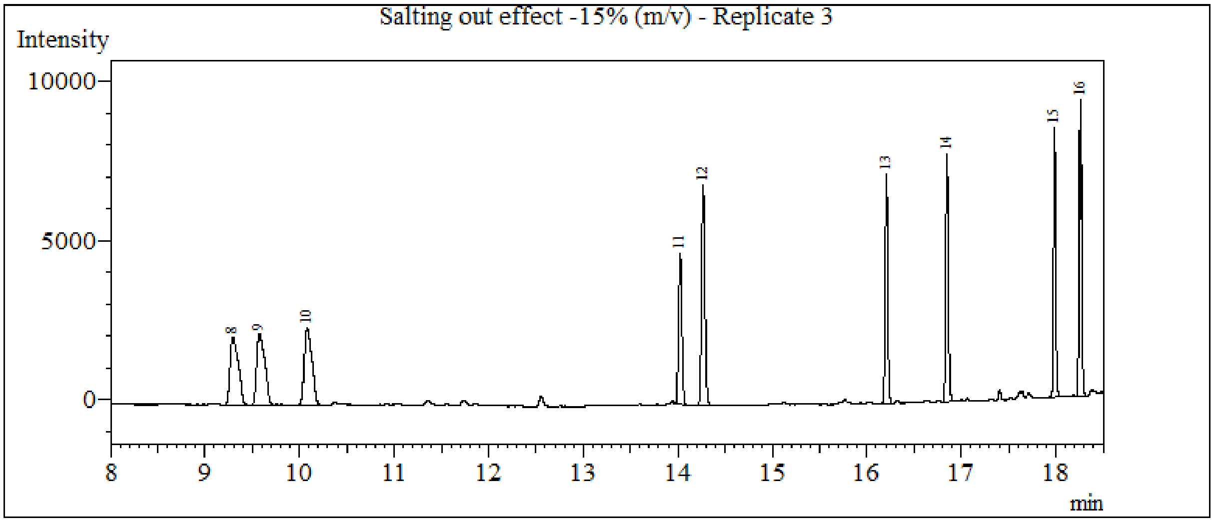
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.297	17768.1	2993.8
5	1,4-DCB	9.577	18978.4	3219.3
6	1,2-DCB	10.081	20991.3	3705.4
9	1,2,4-TCB	14.270	23718.0	9799.7
10	1,2,4,5-TeCB	16.208	17705.5	8980.5
11	1,2,3,4-TeCB	16.850	19429.3	10518.0
13	PCB	18.259	20560.0	11995.0



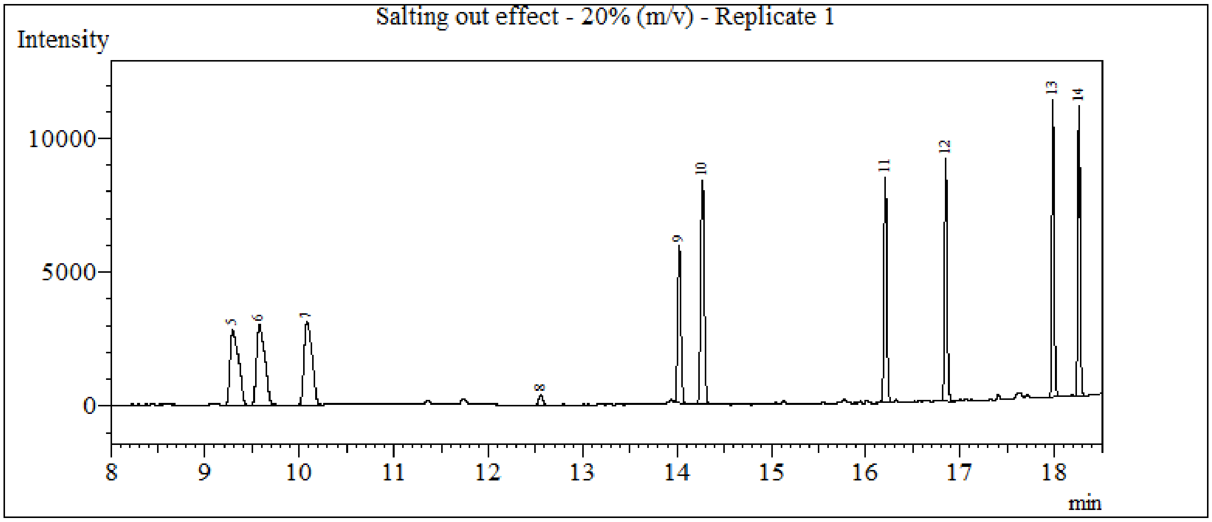
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.290	14695.0	2423.1
5	1,4-DCB	9.572	16087.3	2687.7
6	1,2-DCB	10.076	17689.0	3088.9
10	1,2,4-TCB	14.270	17439.3	7018.6
11	1,2,4,5-TeCB	16.208	12108.3	6178.4
12	1,2,3,4-TeCB	16.849	13388.3	7206.3
14	PCB	18.258	13705.3	7885.2



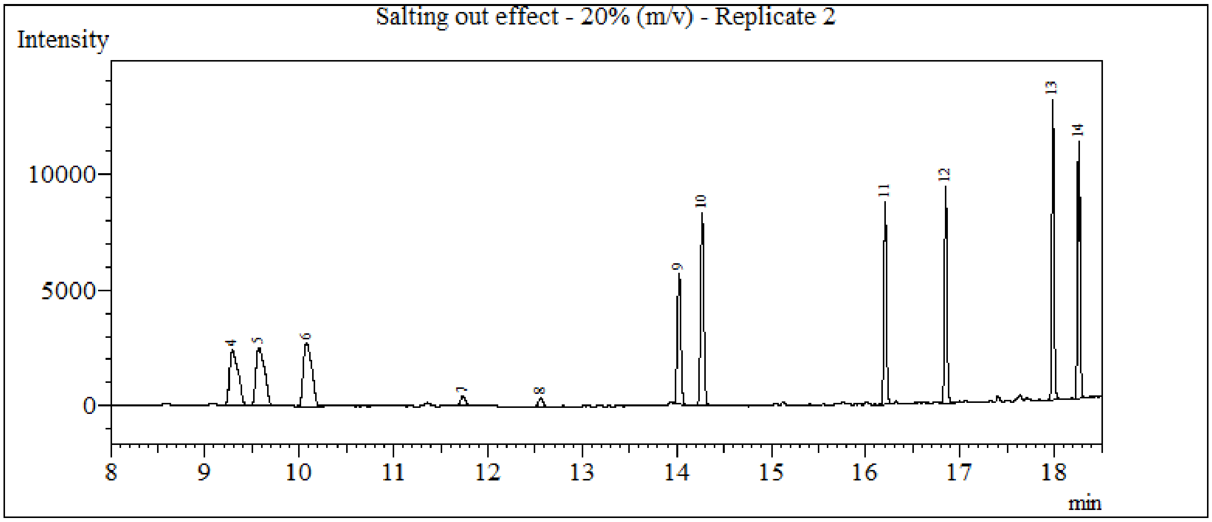
Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.296	13950.4	2387.5
5	1,4-DCB	9.580	15296.0	2618.8
6	1,2-DCB	10.082	17141.3	3044.2
9	1,2,4-TCB	14.270	16553.3	6791.6
10	1,2,4,5-TeCB	16.208	11428.6	5842.8
11	1,2,3,4-TeCB	16.849	12837.0	6932.2
13	PCB	18.258	13466.0	7769.4


Salting out effect -15% (m/v) - Replicate 3

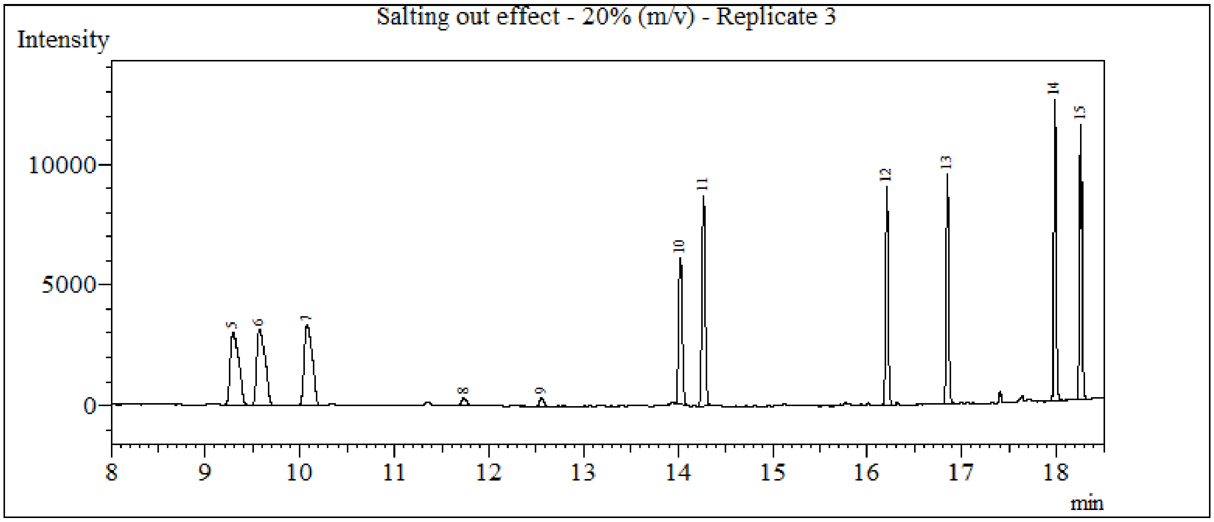
Peak #	Ret time	Retention time	Area	Height
8	1,3-DCB	9.296	12768.8	2110.1
9	1,4-DCB	9.576	13200.6	2234.4
10	1,2-DCB	10.081	13720.3	2431.1
12	1,2,4-TCB	14.270	16922.1	6871.7
13	1,2,4,5-TeCB	16.208	13786.4	7050.4
14	1,2,3,4-TeCB	16.849	14441.4	7731.9
16	PCB	18.258	15823.6	9192.3



Peak #	Ret time	Retention time	Area	Height
5	1,3-DCB	9.295	17688.5	2799.0
6	1,4-DCB	9.576	18530.9	2973.3
7	1,2-DCB	10.082	18773.9	3115.8
10	1,2,4-TCB	14.270	20489.0	8286.3
11	1,2,4,5-TeCB	16.208	16228.3	8283.1
12	1,2,3,4-TeCB	16.849	16704.3	9023.9
14	PCB	18.258	18442.6	10735.5



Peak #	Ret time	Retention time	Area	Height
4	1,3-DCB	9.292	15279.2	2390.1
5	1,4-DCB	9.573	15872.0	2527.6
6	1,2-DCB	10.078	16727.1	2729.0
10	1,2,4-TCB	14.270	20469.6	8260.1
11	1,2,4,5-TeCB	16.207	16740.5	8569.5
12	1,2,3,4-TeCB	16.849	17188.1	9316.9
14	PCB	18.258	19070.3	10985.5



Salting out effect - 20% (m/v) - Replicate 3

Peak #	Ret time	Retention time	Area	Height
5	1,3-DCB	9.296	18615.7	2982.4
6	1,4-DCB	9.575	19251.3	3169.8
7	1,2-DCB	10.077	19631.4	3341.8
11	1,2,4-TCB	14.270	21606.6	8703.8
12	1,2,4,5-TeCB	16.208	17238.6	8914.3
13	1,2,3,4-TeCB	16.849	17609.6	9485.6
15	PCB	18.258	19579.9	11322.4

ADDENDUM III

PUBLICATION

A rapid method for determining chlorobenzenes in dam water systems

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ABSTRACT

A method using direct immersion solid phase microextraction (DI-SPME) coupled to gas chromatography equipped with a flame ionisation detector (GC-FID) was developed for the analysis of 7 chlorinated benzenes in dam water. The main parameters affecting the DI-SPME process were optimised. The optimised method comprises the use of a 100 µm polydimethylsiloxane (PDMS) fibre coating; 5 mℓ sample size; 700 r/min rate of agitation and an extraction time of 30 min. The calibration curve was linear with correlation coefficients ranging from 0.9957–0.9995 for a concentration range of 1–100 ng/mℓ. The limits of detection and quantification ranged from 0.020–0.265 ng/mℓ and 0.204–2.65 ng/mℓ, respectively. Recoveries ranged from 83.6–107.2% with relative standard deviation of less than 9.2%, indicating that the method has good precision. The method is reliable and is free of matrix interferences. Water samples collected from Grootdraai Dam were analysed using the optimised conditions to assess the potential of the method for trace level screening and quantification of chlorobenzenes. The method proved to be efficient, as 1,3 dichlorobenzene, 1,4-dichlorobenzene and pentachlorobenzene were detected at concentrations of 0.429 ng/mℓ, 1.685 ng/mℓ and 1.433 ng/mℓ, respectively.

Keywords: chlorobenzenes, Grootdraai Dam, water, dam, SPME, DI-SPME, GC-FID

INTRODUCTION

Among persistent organic pollutants (POPs), chlorobenzenes are some of the most frequently encountered compounds in aqueous systems. These compounds can enter the environment via natural and anthropogenic sources, and are ubiquitous due to their extensive use over the past several decades (Soonthorntantikul et al., 2009). Several chlorobenzene compounds, once in the environment, can be biologically accumulated, and are reputed to be carcinogens and extremely hazardous to health. Chlorobenzene compounds are listed as priority pollutants by the United States Environmental Protection Agency (USEPA) (He et al., 2000). Excessive exposure to these compounds affects the central nervous system, irritates skin and upper respiratory tract, hardens skin and leads to haematological disorders including anaemia (Kozani et al., 2007; Khajeh et al., 2006). In spite of these harmful effects, chlorobenzenes are still used widely as process solvents and raw materials in the manufacture of pesticides, chlorinated phenols, lubricants, disinfectants, pigments and dyes (Grosjean, 1991; Vidal et al., 2007). In the light of the above, it is imperative to continuously monitor the levels of chlorinated benzenes in all types of surface waters, using a low-cost but sensitive method.

Analysis of chlorobenzenes at trace and ultra-trace levels requires pre-concentration as well as sample matrix clean-up to remove any interfering components (Chisvert et al., 2009). Various pre-concentration techniques have been used for the isolation of chlorobenzenes from water. These include traditional liquid-liquid extraction (Ormad et al., 1996); solid phase extraction (Wang and Lee, 1998; Liu et al., 2004); single drop microextraction (Tor, 2006; Khajeh et al., 2006; Vidal et al., 2005); stir bar sorptive extraction (Montero et al., 2004) and solid phase micro-extraction (SPME) (He et al., 2000; Wang et al., 2003; Huang et al., 2007). These pre-concentration steps precede analysis using gas chromatography coupled to an appropriate detector for identification. Whereas mass spectroscopy (MS) and electron capture detectors (ECD) are excellent detectors for the analysis of these compounds, due to superior sensitivity, accuracy and resolution, they incur relatively high capital, operational and maintenance costs which results in decreased throughput. Although relatively low detection limits can be attained with MS and ECD as detectors, the actual limits set by environmental protection agencies are much higher and can be attained with less expensive systems. This report describes a system comprising a pre-concentration device, a standard chromatograph and a flame ionisation detector (FID). Pre-concentration was effected through direct immersion solid phase micro-extraction (DI-SPME).

Experimental parameters affecting the extraction efficiency of the selected chlorobenzenes, such as fibre type, sample size, rate of agitation, salting-out effect and extraction time, were optimised and applied to the Grootdraai Dam water samples for the quantification of chlorobenzenes.

EXPERIMENTAL

Reagents and materials

The chlorobenzenes selected for this study were: 1,2-dichlorobenzene (1,2-DCB); 1,3-dichlorobenzene (1,3-DCB); 1,4-dichlorobenzene (1,4-DCB); 1,2,4-trichlorobenzene (1,2,4-TCB); 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB); 1,2,4,5-tetrachlorobenzene (1,2,4,5-TeCB) and pentachlorobenzene (PCB). These standards were high purity (> 98%) and were purchased together with HPLC grade methanol (99.9%) from Sigma Aldrich (Milwaukee, WI, USA). American reagent grade sodium chloride (> 99%) was purchased from Merck Darmstadt, Germany. 100 µm polydimethylsiloxane (PDMS) and 85 µm polyacrylate (PA) SPME fibres were purchased from Supelco (Bellefonte, PA, USA). 1 000 mg stock solutions of individual standards were prepared. A 1 000 mg/ℓ multi-compound solution was prepared, from which a 100 µg/ℓ working standard was prepared daily by a series of dilutions. All solutions were prepared in methanol and stored under refrigeration at 4°C. Purified water (18 MΩ·cm resistivity) for this study was taken from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Sampling

Grab samples were taken from the Grootdraai Dam in 1 ℓ Schott bottles, which were subsequently stored under refrigeration at 4°C. The samples were taken at the dam wall and from a depth of approximately 0.5 m. No samples were taken from the point of discharge. The Grootdraai water samples were filtered through a 0.45 µm membrane filter, prior to analysis, to eliminate particulate matter. The Grootdraai Dam is an impoundment on the Vaal River which is situated northeast of Standerton in the province of Mpumalanga, South Africa (locality: 26°54’33.6”S 29°23’42.5”E) (Van Ginkel, 2001). The dam was built in 1981, has a gross storage capacity of 364 million m^3, surface area of 39 km^2 and an average depth of 27 m. The

Grootdraai Dam is upstream of the Vaal Dam, forming part of the Usuthu – Vaal Scheme (Strauss, 2006; Jeleni and Mare, 2007).

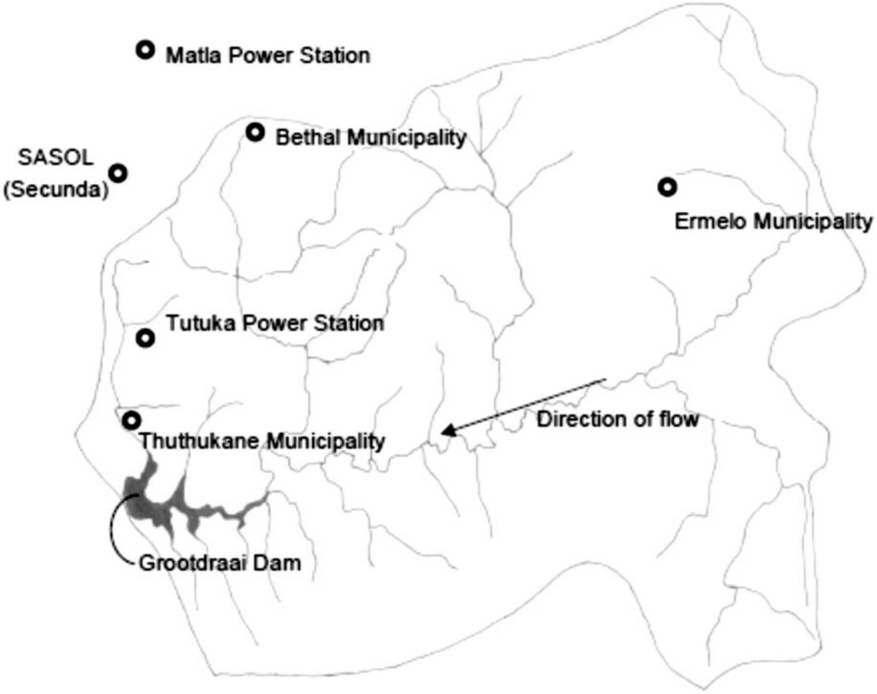

Figure 20: Grootdraai Dam catchment (Adapted from Strauss, 2006)

With the exception of a small local community, there is limited domestic use of water directly from the dam. The Grootdraai Dam catchment has a surface area of 7 928 km^2 and the major water users are industries and municipalities (Fig. 1) (Strauss, 2006; Van Ginkel, 2001).

SPME procedure

The optimisation of the main parameters influencing an SPME extraction was performed using Millipore water fortified with the seven chlorobenzene isomers. All SPME extractions were performed in 22 mℓ amber glass vials. For a typical experiment, an aliquot of 5 mℓ of Millipore water was fortified with a mass of 10 ng of each analyte. In the case of the experiments for salting-out effect, 5 mℓ sodium chloride solutions of 5%, 10%, 15% and 20% (m/v) instead of pure Millipore water were used. To enhance the transfer of analytes to the fibre, agitation was performed using a 2 cm Teflon stir bar, coupled to a magnetic stirrer. The vials were then sealed with screw-type plastic caps containing a PTFE/silicon septum. The SPME manual holder needle was then used to penetrate the septum, and the fibre was then directly immersed into the sample solution for a pre-determined time. To enhance exposure of the fibre to the analytes, precaution was taken to ensure that the fibre was exposed to the outside flow of the vortex rather than directly within the vortex. After extraction, the fibre was retracted back into the protective needle and removed from the sample vial. Lint free tissue paper was then used to remove any water molecules present at the entrance of the protective needle. The protective needle was then used to penetrate the septum of the GC, and the fibre was then exposed to thermal conditions of the injector port for desorption. All samples were freshly prepared minutes before the extraction to reduce/eliminate any losses due to volatilisation or decomposition due to prolonged storage. SPME fibres were conditioned for 30 min every morning and blank fibre injections were performed to assess the presence of any contamination within the fibre.

GC parameters

Analysis was performed with a Shimadzu (Tokyo, Japan) QP-5000 GC–FID system equipped with a SBPX-5 capillary column (30 m x 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as the carrier gas which was passed through an oxygen, moisture and hydrocarbon trap prior to entering the GC. All GC injections were done in the splitless mode and the injector port was equipped with a glass insert for splitless injection. The vent valve of the split outlet was closed for 30 s and then opened fully. The GC conditions were as follows: injector port and FID temperatures were 250°C and 300°C, respectively; constant column flow rate was 1 mℓ/min; oven temperature programme was 60°C held for 2 minutes, ramped at 10°C/min to 70°C and held for 4 min, ramped at 5°C/min to 85°C, ramped at 15°C/min to 205°C, ramped at 25°C/min 280°C and held for 5 min. The total analysis time was 26 min. All chromatographic data were processed using the GC solution post-run software (Shimadzu, version 2.30).

RESULTS AND DISCUSSION

Optimisation of parameters in the DI-SPME of chlorobenzenes

Several analytical parameters can be optimised to enhance the extraction efficiency of chlorobenzenes from water using the DI-SPME method. These parameters included SPME fibre coating, sample size, rate of agitation, salting-out effect and extraction time.

SPME fibre coating

Fibres coated with polyacrylate (PA) and polydimethlylsiloxane (PDMS) were tested for extraction efficiency. The results, depicted in Fig. 1, show that the PDMS-coated fibre gives better extraction for all seven analytes tested. This is also consistent with the fact that PDMS is more non-polar than PA and that the analytes have relatively low polarity. The PDMS fibre was used for the rest of the study.

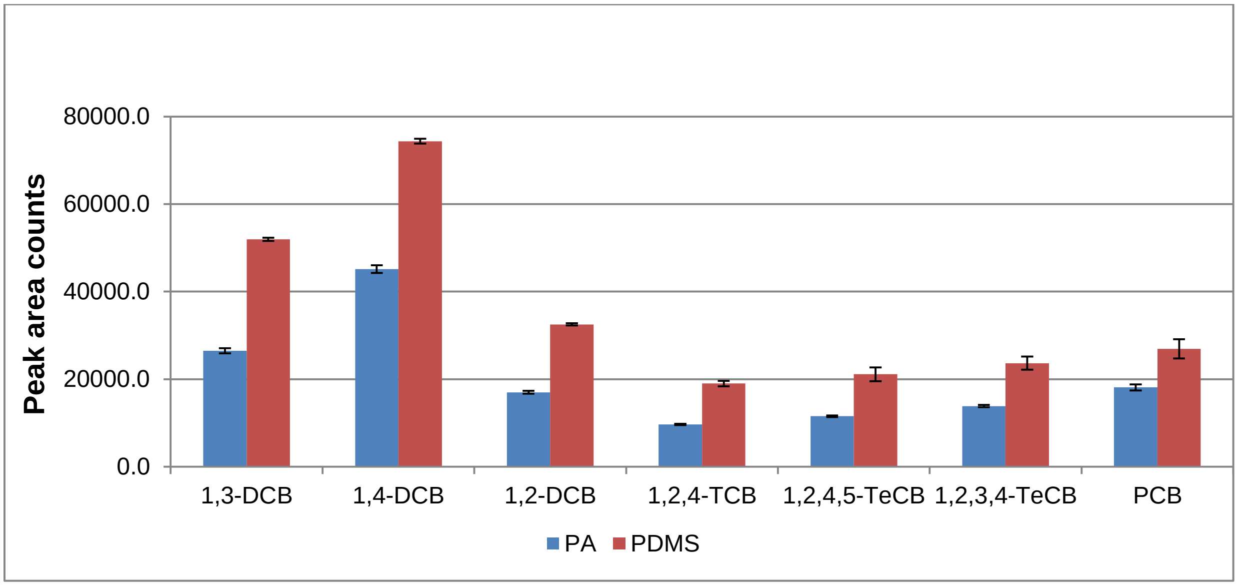

Figure 21: Extraction efficiencies of the PA and PDMS coating; error bars indicate standard error of experiment (*n* = 3)

Sample size

In DI-SPME studies performed on water matrices, such as those of Filho et al. (2010), Lopez-Darias et al. (2010a), Lopez-Darias et al. (2010b), Meng and Anderson, (2010), Li et al. (2009), Luan et al. (2007) and Junior and Re-Poppi (2007), a constant sample size was used while optimising all other parameters. Since this study aimed to take advantage of any enhancement in extraction efficiency, 3 sample volumes (5 mℓ, 10 mℓ and 15 mℓ) were investigated.

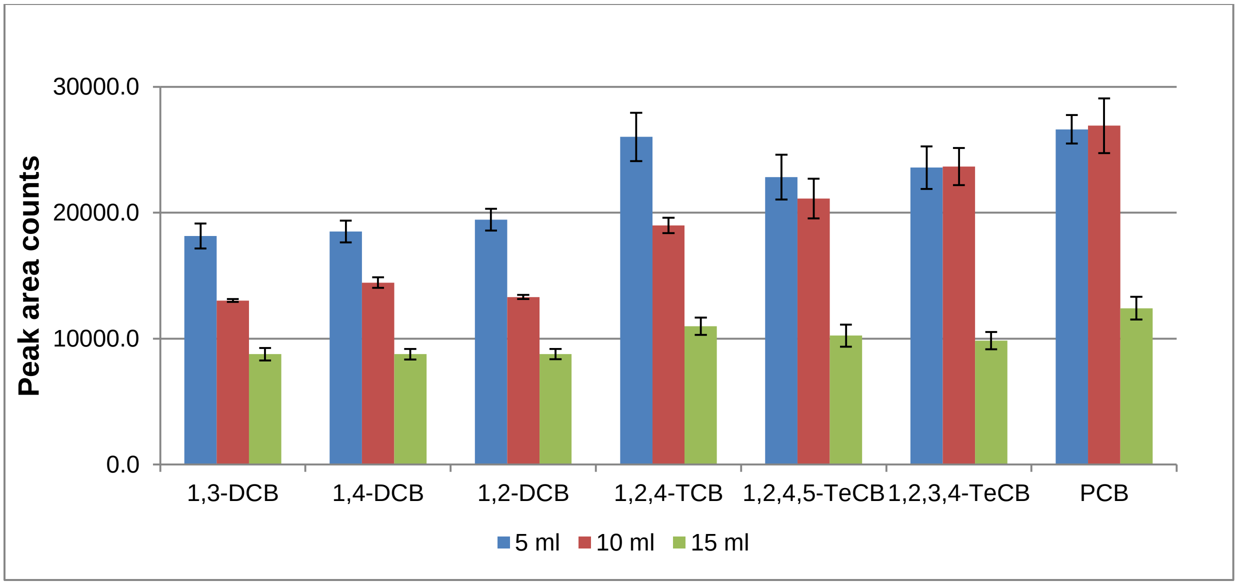

Figure 22: Influence of sample size on extraction efficiency, error bars indicates standard error of experiment (*n* = 3)

Figure 3 shows that superior extraction efficiency was obtained using a sample volume of 5 mℓ. Further increases in sample volume resulted in decreased extraction efficiency. The enhanced extraction efficiency at lower sample volume can be attributed to a larger phase ratio between volume of the fibre coating and sample. In light of the above, a sample size of 5 mℓ was used for the rest of the study.

Effect of rate of agitation

In view of the observation (Pawliszyn, 1997) that equilibration times are dependent on the rate at which the solution is stirred, 4 stir speeds were tested while other parameters were kept constant. The results are shown in Fig. 4. It is evident that a stir speed of 700 r/min resulted in the highest extraction efficiency; while further increase to 1 100 r/min resulted in a decrease in extraction efficiency. Kin (2008) experienced a similar phenomenon, which is due to the vibration of the stir bar and the irregular agitation of the sample at high stir speeds. Furthermore, a stir rate of 1 100 r/min resulted in splashing of sample onto the walls of the glass vial. This could have resulted in the loss of analytes and decreased extraction efficiency. In addition, higher random errors were obtained at higher stir rates, indicating that experiments at this stir speed were a lot more difficult to reproduce, thereby decreasing method precision.

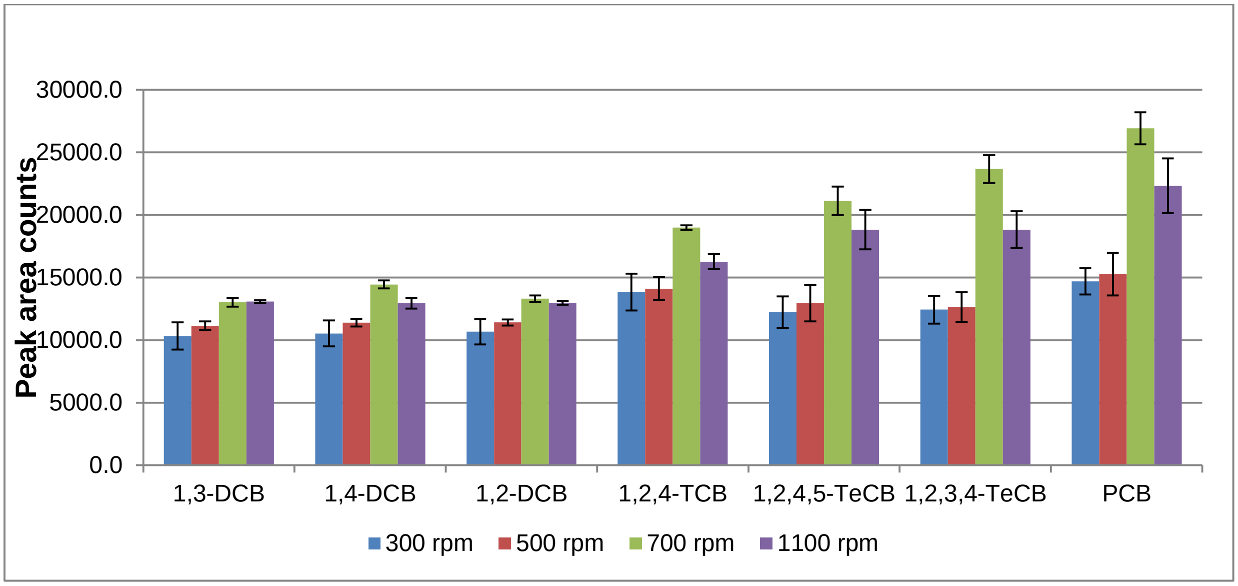

Figure 23: Influence of rate of agitation on extraction efficiency; error bars indicate standard error of experiment (*n* = 3)

From Fig. 4, it is apparent that the rate of agitation had a minimum effect on the lower chlorinated benzenes (1,3-DCB, 1,2-DCB and 1,4-DCB, 1,2,4-TCB), while higher stir rates promoted an enhanced extraction efficiency for the higher chlorinated benzenes (1,2,4,5- and 1,2,3,4-TeCB and PCB). This suggests that the rate of mass transfer from the bulk of the sample to the fibre is sufficient for low molecular weight chlorobenzenes at low rates of agitation. The converse is true of high molecular weight chlorobenzenes; therefore a stir speed of 700 r/min was deemed optimum and was used for the rest of the study.

Salting-out effect

Salt is added, in SPME, with the aim of effecting a modification to the sample matrix. An increase in ionic strength reduces the solubility of some analytes. A concomitant increase in extraction efficiency is expected on the basis that more of the analyte is available for adsorption onto the fibre (Kin, 2008). As such, sodium chloride was added to aqueous samples to attain concentrations (m/v) ranging from 0– 20%.

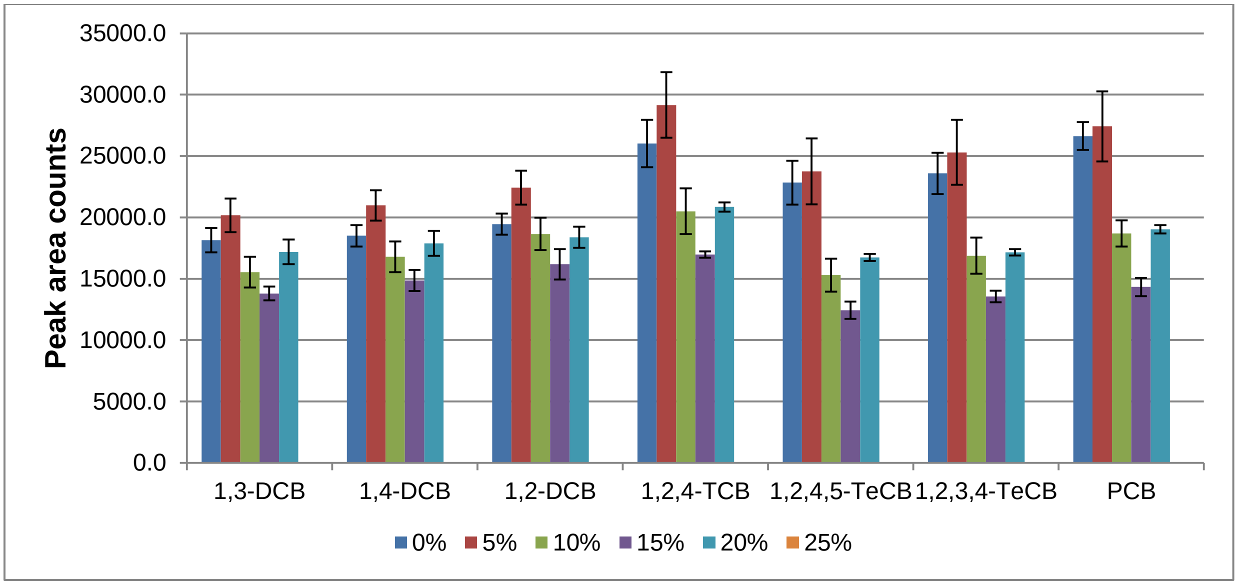

Figure 24: Impact of salting-out effect on extraction efficiency; error bars indicate standard error of experiment (*n* = 3)

Figure 5 indicates that 5% (m/v) salt concentration had a slight improvement on the extraction efficiency while a further increase in salt concentration resulted in a drastic decrease in extraction efficiency. Ionic strength has a greater effect on analytes which have high water solubilities (Santos et al., 1996). Therefore, it is evident that the compounds with higher water solubilities (1,2-DCB, 1,3-DCB, 1,4-DCB and 1,2,4-TCB) were extracted to a greater extent (9–13%). By contrast, ionic strength had a minimal influence on compounds with lower water solubilities (1,2,3,4-TeCB; 1,2,4,5-TeCB and PCB) resulting in a 2–6% enhancement in extraction efficiency. These observations are attributed to the effect of hydration spheres around the salt molecules. These hydration spheres reduce the amount of water molecules available to dissolve the analytes, which results in an increased mass transfer of certain analytes into the extraction phase (He et al., 2000; Psillakis, 2002). He et al. (2000) reported an increase in extraction efficiency with ionic strength, in the extraction of chlorobenzenes from river water using HS-SPME. These authors reported an optimum ionic strength of 20% (m/v). In contrast, the optimum ionic strength obtained in this study was 5% (m/v). This discrepancy can be explained in terms of the mode of extraction used. He et al. (2000) used the headspace technique and thus the SPME fibre was not exposed to the ionic species and other interferences in the sample matrix. Since this study used the direct immersion mode the fibre was directly exposed to the ionic species and interferences in the sample matrix. Initially, an increase in extraction efficiency will be experienced (salting-out effect) at lower salt concentrations. However, further increases in salt concentration will result in a decrease in extraction efficiency. This is due to an increase in the interactions between the analytes and the ionic species, which reduces the analyte's ability to move into the extracting phase.

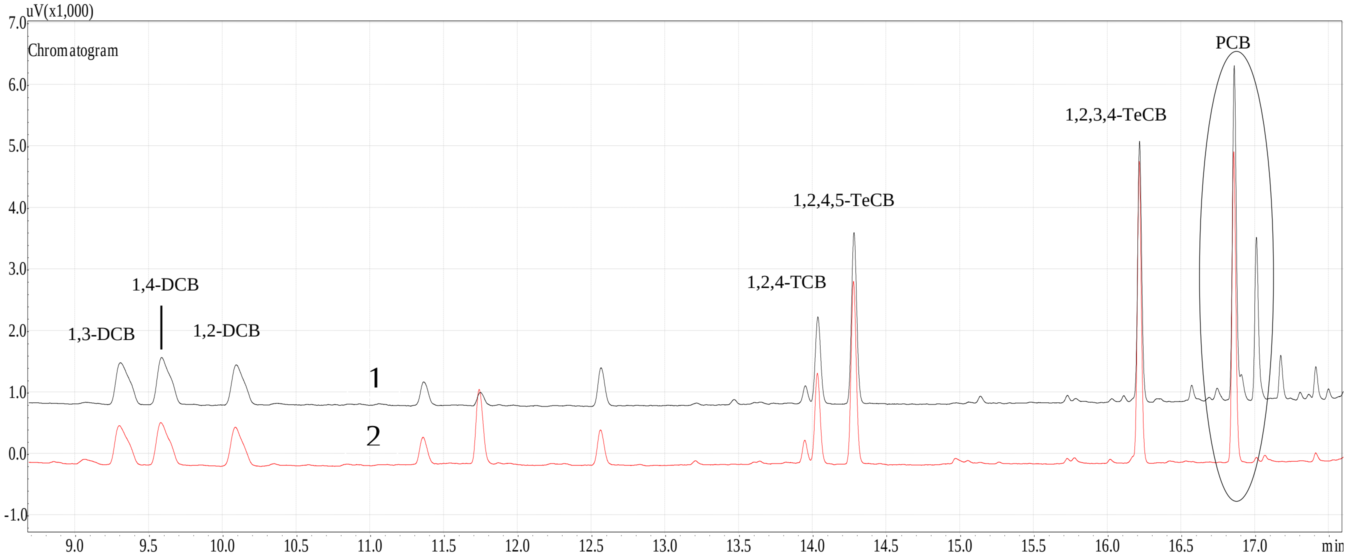

Figure 25: Interfering compound due to the addition of salt; (1) extraction with the addition of 5% (m/v) salt; (2) extraction with 0% (m/v) salt

However, an addition of salt resulted in the introduction of several interferences in the sample matrix as can be seen in the chromatogram given as Fig. 6. Although there was no co-elution, these interferences would have resulted in a decrease in both the limits of detection and quantification. Since the salting-out effect had a slight effect on the extraction efficiency, it was decided to carry out further experiments without the addition of any salt.

Effect of duration of extraction

SPME is an equilibrium process; therefore it is imperative to determine the time required for each analyte to attain equilibrium with the fibre coating. While maintaining all other extraction parameters at optimum levels, the duration of extraction was varied from 5 min to 120 min. The results are depicted in Fig. 7.

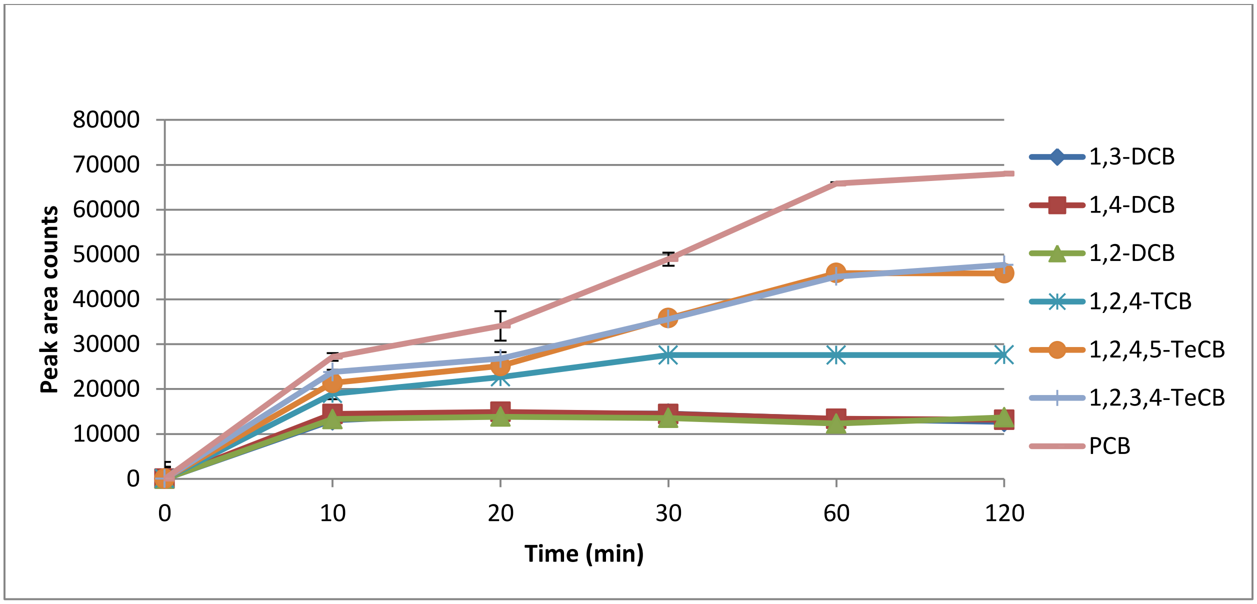

Figure 26: The effect of extraction time on extraction efficiency; error bars indicate standard error of experiment (*n* = 3)

The equilibration time is defined as the time after which the amount of analyte extracted remains constant (Pawliszyn, 1997). Figure 7 indicates that the DCB isomers attained equilibrium rapidly. 1,2,4-TCB required 30 min to attain equilibrium while 1,2,3,4-TeCB, 1,2,4,5-TeCB and PCB required 60 min. The results indicate that equilibrium times are compound dependent, having a trend of increasing times with increasing degree of chlorination and molecular weight. The chlorobenzene compounds with higher vapour pressures and octanol-water partition coefficients and lower molecular mass attained equilibrium rapidly. To enhance throughput, a 30-min extraction was selected for the rest of this study. It should be noted that by terminating the extraction process prior to equilibration of PCB, 1,2,3,4- and 1,2,4,5-TeCB being attained, reduced method reproducibility can be anticipated. This occurs during 'pre-equilibrium' of analytes. Small changes in time result in large change in the amounts of analytes adsorbed (Vas and Vekey, 2004).

Validation of the optimised DI-SPME method

A newly-developed analytical method needs to be validated to determine its suitability for the intended application. The figures of merit of the validation experiments are shown in Table 1.

Linearity

The linearity of the analytical method was determined within an analyte mass range of 1–100 ng/mℓ. The R^2-values of the analytes ranged from 0.9957–0.9995 (Table 1). This indicates good linearity of the optimised method as there is a linear relationship between analyte mass and detector response within the range of trace concentration level. He et al. (2000) reported correlation coefficients of greater than 0.9910 for each analyte, at a linearity range of 0.2–20 ng/mℓ using HS-SPME coupled to GC-MS. This study produced slightly improved correlation coefficients, of greater than 0.9957, over a wider concentration range, indicating that there is a more accurate mathematical relationship between detector response and analyte concentration.

Limits of detection and quantification

As generally accepted, the LOD was taken to be the concentration of analyte which yielded a signal to noise ratio of 3, and the LOQ as the concentration of analyte which yielded a signal to noise ratio of 10. Table 1 shows the LOD and LOQ obtained for each analyte. Furthermore, the LOQs obtained in this study are well below the regulatory limits of the World Health Organisation (WHO) and USEPA drinking water guidelines. This indicates that the LOQ obtained in this study is well within the levels required for trace concentration analysis and is suitable for the routine analysis of chlorobenzenes in drinking water.

Table 34: Figures of merit obtained during method validation

Compound	Linearity[a] (R^2–value)	LOD (ng/mℓ)	LOQ (ng/mℓ)	Recovery[b] (%)	% RSD[b]	WHO Guidelines[c] (ng/mℓ)	USEPA Drinking Water Guidelines[d] (ng/mℓ)	LOD[e] (ng/mℓ)
1,3-DCB	0.9987	0.0204	0.2048	105.6	2.2	-	-	-
1,4-DCB	0.9957	0.0429	0.4293	102.4	1.9	300	75	0.1
1,2-DCB	0.9995	0.0219	0.2193	101.4	5.6	1 000	600	0.1
1,2,4-TCB	0.9984	0.2650	2.650	93.4	0.6	-	70	0.5
1,2,4,5-TeCB	0.9966	0.0910	0.9105	83.6	9.2	-	-	-
1,2,3,4-TeCB	0.9975	0.0730	0.7305	102.8	0.5	-	-	-
PCB	0.9996	0.0720	0.7203	107.2	7.2	-	-	-

a – Five-point linear range (1, 10, 25, 50, 100 ng/mℓ)

b – *n* = 3; spiking level 5 ng/mℓ

c – WHO, 2011

d – USEPA, 2003

e – Limit of detection obtained from Zhao et al. (2009)

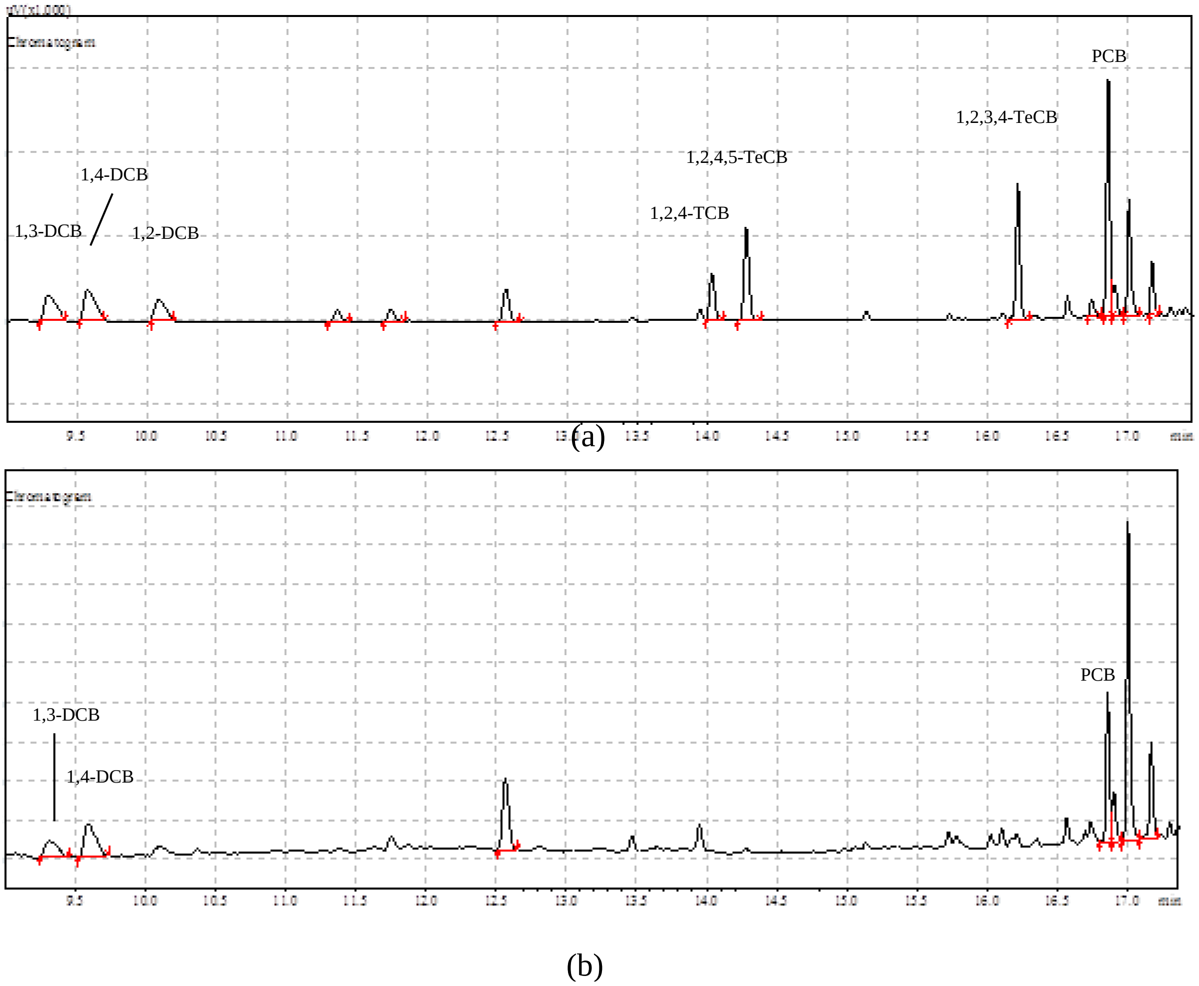

Figure 27: (a) Dam sample fortified with 5 ng/mℓ of each analyte; (1) 1,3-DCB; (2) 1,4-DCB; (3) 1,2-DCB; (4) 1,2,4-TCB, (5) 1,2,4,5-TeCB; (6) 1,2,3,4-TeCB; (7) PCB; (b) Dam sample blank

Recovery experiments

Recovery experiments were performed to determine the efficiency of the method for the extraction of chlorobenzene from the Grootdraai Dam water samples, as well to investigate the presence of any matrix interferences. The optimised method was initially applied to 5 mℓ of water collected from the Grootdraai Dam. The chromatogram in Fig. 8a indicates the presence of 1,3-DCB, 1,4-DCB and PCB. The optimised method was then applied to 5 mℓ of Grootdraai Dam water which was fortified with 10 ng/mℓ of each analyte (Fig. 8b). The average recoveries obtained in this study were within acceptable limits ranging between 83.6% and 107.32% with RSD values of less than 10%. This indicates that the optimised method is quantitative, free of any matrix interferences and has good repeatability. However, raw water samples, having interfering compounds such as humic and fulvic acids, may require additional clean-up procedures to increase the efficiency of the pre-concentration technique.

Comparisons with literature reports

The data from 5 reports published in journals have been tabulated in Table 3 to indicate the relative rank and value of the method reported herein compared with reputedly more sophisticated and sensitive methods. Although the LODs for the GC-FID method are much higher, relative to the studies listed Table 3 (Vidal et al., 2005; Vidal et al., 2007; Li et al., 2010; Bagheri and Aghakhani, 2011), it is worth noting that these LODs are adequate for monitoring samples to assess compliance with regulatory limits specified in international standards and guidelines (WHO in this case). In addition, the GC-FID is easy to use and is relatively inexpensive. However, upon comparison with a study using a similar detection system coupled to HS-SDME (Zhao et al., 2009), the LOD obtained in this study for 1,4-DCB, 1,2-DCB and 1,2,4-DCB is 1.9–4.6-fold lower. Additionally, the method presented in this study offers a higher degree of precision. This indicates that the DI-SPME method developed in this study is superior to the HS-SDME method of pre-concentration.

Table 35: Comparisons with other studies

Reference	Sample preparation and detection method	Extraction time (min)	Number of analytes, Linear range (ng/mℓ), Range of recoveries, % Relative standard deviation Limit of detection (ng/mℓ)
Vidal et al. (2005)	Single drop micro-extraction (SDME); GC-MS	5	10, 0.2–50, 97% mean, 2.1–13.2%, 0.003–0.031
Vidal et al. (2007)	Micro-wave assisted headspace (HS)-SDME; HPLC	10-20	8, not specified, 99% mean, 2.3–8.3%, 0.016–0.039
Li et al. (2010)	Novel SPME coating; GC-ECD	15	7, not specified , 91% mean, 2.1–4.9%, 0.00032–0.00225
Bagheri and Aghakhani (2011)	Novel nanofibre coating – HS-SPME ; GC-MS	Not specified	not specified, 0.050–1, 94–102%, 3–8%, 0.010
Zhao et al. (2009)	SDME, FC-FID	20	5, 1–1 000, 88.9–110.9%, 7.73–12.39%, 0.1–0.5
This study (2012)	Direct immersion SPME GC-FID	30	7, 1–100 , 83.6–107.2%, 0.5–9.2 %, 0.020–0.265

Occurrence of chlorobenzenes in the Grootdraai Dam

There were 3 chlorobenzene isomers detected in the Grootdraai Dam. These included 1,3-DCB; 1,4-DCB and PCB, at concentrations of 0.429 ng/mℓ, 1.685 ng/mℓ and 1.433 ng/mℓ, respectively. The major water users of the Grootdraai Dam are industries and municipalities (Table 3).

Table 36: Water use trends for the Grootdraai Dam (Adapted from Strauss, 2006)

User	**Demand** m^3/a	**Return** m^3/a
Irrigation	321 500	-
Power Station A	47 420 000	-
Power Station B	53 838 000	-
Industry A	91 250 000	4 015 000
Municipality A	3 600 000	1 982 124
Municipality B	5 420 250	3 011 250
Thuthukane Township	1 427 556	642 400

The contamination by chlorobenzene isomers in the Grootdraai Dam could be due to agricultural chemical run-off, irresponsible discharge of effluent from chemical industries, air emissions during chemical manufacturing, return flow from wastewater treatment plants or disposal of materials containing these compounds within the catchment of this impoundment. 1,3-DCB and 1,4-DCB are commonly used in the manufacture of pesticides, indicating that agricultural activities within the catchment which use these pesticides are a possible source (McPherson et al., 2002). Various environmental influences, particularly stormwater run-off, can mobilise the chlorobenzenes which will ultimately enter the waterways emptying into the Grootdraai Dam through surface runoff.

The occurrence of PCB in the Grootdraai Dam is quite noteworthy, as there is currently no large-scale use and commercial production of the chemical. However, since PCB is produced in small quantities during the chlorination of benzenes, one can anticipate the presence of PCB when DCB is detected. Historically, PCB has been used in the manufacture of fungicides, and, more recently, as a flame retardant. This could indicate past PCB contamination, which has been re-mobilised through environmental processes, and eventually entered and accumulated in the Grootdraai Dam.

Domestic and industrial discharge could also be a source of chlorobenzenes. These compounds can be introduced into the Grootdraai Dam via return flows from wastewater treatment plants within the catchment. 1,4-DCB is used in common household products such as air fresheners, moth balls and urinal deodorisers. These chlorobenzene compounds are also common components of industrial degreasing solvents (McPherson et al., 2002). Negligent and poor waste disposal protocols at both domestic and industrial level can lead to contamination. The major industries in the Grootdraai catchment are Power Station A, Power Station B and Industry A. Table 2 indicates that both power stations have a zero-discharge policy; as a result, contamination is negligible. Industry A, as well as the Thuthukane Township, Municipalities A and B are the main contributors to return flow into the Grootdraai Dam. As a result, one cannot rule out these as possible sources of chlorobenzene contamination.

Comparison to drinking water guidelines

The South African Drinking Water Standard (SANS) 241:2005 for drinking water does not regulate any of the detected chlorobenzenes. The SANS 241:2005 is derived from the World Health Organisation (WHO) Guidelines for drinking water quality. The SANS 241:2005 standard indicates that, for the organic determinants which are not listed, one should refer to the WHO Guidelines for drinking water for comparative purposes (DWAF, 2005). The WHO has set a health-based guideline value of 300 ng/mℓ for 1,4-DCB (WHO, 2011). This indicates that the level of 1,4-DCB within the Grootdraai Dam (1.685 ng/mℓ) is well within the WHO guideline and does not pose a significant health risk. Since WHO has not derived a health-based guideline for the other two isomers detected in this study; a health risk assessment cannot be derived.

CONCLUSIONS

The results show that the technique of immersing the SPME fibre coupled with analysis by GC-FID is viable for routine monitoring of dam water samples for the presence of chlorinated benzenes. Furthermore, it is adequate to meet the limits set for two of the three chlorobenzenes three chlorobenzenes regulated by USEPA and for one of the two listed by WHO. Furthermore, the optimised DI-SPME method produced lower LODs than the HS-SDME. An added advantage of this method is that it is relatively cheap and easy to use. It should therefore be of service to organisations which monitor their drinking water, as well as source water, for levels of chlorobenzenes, in order to ensure public health protection and safety of its customers.

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