INFLUENCE OF MATRIX EFFECT ON SELECTED ORGANOCHLORINE PESTICIDE RESIDUES IN WATER FROM THE JUKSKEI RIVER CATCHMENT: GAUTENG, SOUTH AFRICA

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This dissertation is presented in fulfilment of the requirements for the degree Magister Technologiae: Biotechnology at Vaal University of Technology.

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2011

DECLARATION BY CANDIDATE

I hereby declare that the dissertation submitted for M-Tech: Biotechnology, at Vaal University of Technology has never been submitted to any other institution of higher learning. I further declare that all cited sources are acknowledged by list of referencing.

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C. C Rimayi 22 November 2011

Vaal University of Technology 2011

ACKNOWLEDGEMENTS

I would like to thank the following;

- Firstly the almighty God for his never-ending grace and allowing me to undertake my MTech studies.

- My supervisors, Ms C. van Wyk, Dr F. Mtunzi and Dr A.O.D. Odusanya for their dedicated guidance throughout my research.

- Dr B. Naidoo and Professor M. Moloto for their technical input and guidance.

- I would like to thank my parents, Mr R.C. and Mrs B. Rimayi for their support and prayers.

- Dr N. Slabbert, Dr T. W. Duma and Joyce Lekekiso for granting me the permission to conduct my research at the Resource Quality Services.

- My colleagues from the Department of Water affairs, particularly Jennifer Tswai, Francinah Ngwandula, Nomthandazo Hans, Mihloti Taka, Seipati Motseng, Katlego Mashaba, Nosipho Molefe and Emmanuel Hlabane.

- My colleagues from the Vaal University of Technology, particularly Malesole Bucibo, Xoliswa Madlanga, Mrs Muleya and Ben Makoena.

- My colleagues Tichatonga Gonah, Shingirai Mutanga, Shepherd Tsoka, Garikai Gono, Rufaro Mwadiwa and Lloyd Mabonga for their encouragement and technological and academic assistance.

- My siblings Tendayi, Simbarashe, Tafadzwa and Kudakwashe for their encouragement and support.

- My nephew and niece Daniel and Charmaine.

- Dr A.I.C. Mwadiwa for encouraging me to undertake my MTech studies.

DEDICATION

This study is dedicated to my parents, Mr R. C. and Mrs B. Rimayi.

ABSTRACT

One of the major problems encountered in qualitative and quantitative determination of residual pesticides by gas chromatography is the matrix effects. Matrix components have a considerable effect on the way analysis is conducted and the quality of results obtained, introducing problems such as inaccurate quantification, low analyte detectability and reporting of false positive or even false negative results. It was aimed to develop and validate a suitable method for counteracting the matrix effects so as to improve the detection and quantification of selected organochlorine pesticide residues from real water samples. The real water samples used were sampled from three points along the Jukskei River catchment area in Gauteng, South Africa for a period of 7 months from January to July 2010 so as to create a representative sample.

An automated solid phase extraction (SPE) method coupled to Gas Chromatography-Mass Spectrometry (GC-MS) method for the analysis of 20 selected organochlorine pesticides was developed and validated for the purposes of studying the matrix effects. The analytical method showed a significant degree of validity when tested against parameters such as linearity, repeatability and sensitivity. Endosulphan beta, 4,4' Dichlorodiphenyldichloroethane, and Heptachlor-epoxide had the broadest linear calibration ranges of 1 ppm- 0.0156 ppm. Benzene hexachloride (BHC) delta and Lindane had the lowest statistical limits of detection of 0.018 ppm. Statistical hypothesis testing indicated that there was significant linearity in all selected organochlorine calibration curves.

Four different reversed sorbent phases, including LC18, SC18- E and Strata-X (styrene divinyl benzene) were tested for organochlorine retention efficiency. The LC-18 200 mg cartridge proved to be the most robust and effective sorbent phase as it produced better recoveries varying from 90-130% for most analytes. A breakthrough volume of 100 mL for the LC-18 200 mg cartridge was determined using an optimum matrix load curve. It was then concluded that the method developed was suitable for further research

towards the influence of the matrix on selective determination of the selected organochlorine pesticides.

Four different calibration methods, namely matrix-free external standard, matrix-matched external standard, matrix-free internal standard and matrix-matched internal standard were applied to test the efficiency of computing recoveries. All calibration curves for the 20 organochlorine pesticides showed significant linearity > 0.99 when plotted on both Chemstation and Excel. The calibration methods were tested on three different matrices composed of a high sample matrix (synthetic matrix), a low sample matrix (real sample matrix) and a no sample matrix (ultrapure water).

Statistical hypothesis testing led to the decision that there are significant differences between the mean recoveries of the three water sample matrices and also that the differences in the mean recoveries of the three sample matrices are independent of the both the two calibration techniques (internal standard and external standard) and calibration types (matrix-matched and matrix-free) applied. This led to the overall conclusion that the matrix effects have an overwhelming influence on the selective determination of the selected organochlorine pesticides.

List of Abbreviation and Symbols

Abbreviations

GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
MSD	Mass Spectrometric Detector
SIM	Selective Ion Monitoring
EI	Electron Impact Ionisation
m/z	Mass to charge ratio
amu	Atomic mass units
HRMS	High Resolution Mass Spectrometry
TOF	Time Of Flight
GCXGC	Two dimensional Gas Chromatography
FT	Fourier Transform
POP	Persistent Organic Pollutant
OC	Organochlorine pesticide
SPE	Solid Phase Extraction
LLE	Liquid-Liquid Extraction
BHC	Benzene Hexachloride
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
PCNB	Pentachloronitrobenzene
PAH	Poly Aromatic Hydrocarbon
IUPAC	International Union of Pure and Applied Chemistry
%	Percent

°C	Degrees Celsius
min	Minute
µL	Microlitre
mL	Millilitre
mg	Milligram
mg/L	Milligram per litre
ppm	Part per million
KPa	KiloPascals
USEPA	United States Environment Protection Agency
USFDA	United States Food and Drug Agency
QC	Quality Control
SPSS	Statistical Package for the Social Sciences
ANOVA	Analysis of Variance
MANOVA	Multivariate analysis of Variance
HSD	Tukey's Honestly Significant Difference
DF	Degrees of Freedom
Fcal	F-calculated value
Fcrit	F-critical value
Sig.	Level of significance
N	Number of variables
α	Error
STD	Standard
CI	Confidence interval
H_0	Null hypothesis
H_A	Alternative hypothesis
NIST	National Institute of Standards and Technology
RM	Reference Material
SRM	Standard Reference Material
CRM	Certified Reference Material
RF	Response factor
CF	Calibration Factor

IS	Internal Standard
MM	Matrix-Matched
MF	Matrix-Free
MFEXTSTD	Matrix-Free External Standard
MMEXTSTD	Matrix-Matched External Standard
MFIS	Matrix-Free internal Standard
MMIS	Matrix-Matched Internal Standard
LOD	Limit Of Detection
LOQ	Limit Of Quantification
RSD	Relative Standard Deviation
R^2	Coefficient of Regression
S/N	Signal to Noise ratio
ISO	International Standardisation Organisation
RSD	Relative Standard Deviation
DCM	Dichloromethane
Mg/L	Milligram per litre
≥	Greater than or equal to
>	Greater than
≤	Less than or equal to
<	Less than
±	Plus or minus
T	Target ion
Q	Qualifier ion
C	Calibration curve
μ_1	High sample matrix mean
μ_2	Low sample matrix mean
μ_3	No sample matrix mean
μ_{MM}	Matrix-matched standard mean
μ_{MF}	Matrix-free standard mean
μ_{IS}	Internal standard mean
μ_{EXTSTD}	External standard mean

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Generally, the sample matrix is believed to have some effects on the detection and quantification of target analytes. In analytical chemistry, matrix refers to components of a sample other than the analyte of interest and in Gas Chromatography (GC) analysis such as the analysis of residual organochlorine pesticide residues, matrix components may cause serious problems with respect to quantification and detection of analytes [1]. The aim of GC is to achieve the resolution of compounds of a given sample in the shortest possible time. GC is finding ever wider use in pesticide analysis [2]. The most used detectors in GC are the Electron Capture Detector (ECD), Nitrogen-Phosphorous Detector (NPD), Flame Photometric Detector (FPD) and most recently the Mass Spectrometer Detector (MSD) is fast becoming the detector of choice owing to its ability to effectively identify chromatographed compounds based to their mass to charge ratios (m/e) [3]. MSDs, particularly those with specific ion monitoring provide higher specificity due to their ability to give a detailed reflection on the molecular structure of a particular compound. Gas chromatography-Mass Spectrometry (GC-MS) is considered the most powerful analytical technique for quantitative analysis due to its inherent high sensitivity and selectivity. Common MSD's such as the quadrupole and the ion trap can however fail to provide unbiased confirmation about an analyte's identity [4].

The introduction of pesticides into the environment is considered a risk for human health due to the toxicity of the pesticides [1]. Semi-volatile persistent organic pollutants have been detected in natural waters from a variety of different regions. These include organochlorine pesticides such as Dichlorodiphenyltrichloroethane (DDT) and Benzene hexachlorides (BHC). Organochlorines have very low solubilities in water, are fat soluble and are resistant to metabolism [5]. Multiple determination of pesticides nowdays is applied to separate groups of semi-polar and thermally unstable contaminants with a wide range of physico-chemical properties within the same run in a relatively short period of time [6]. Multi-residue analysis has been identified as a cost effective and labour saving method for determination of a wide range of analytes within

a single run, but obtaining optimum recoveries for all analytes is practically impossible [7]. The analysis of pesticides in water samples involves isolation of the analytes from the sample matrix, removing the bulk co-extracts from the crude extract, identification and quantification of the pesticides.

Most analytical methods are based on liquid partitioning with organic solvents such as dichloromethane. Over the years new extraction techniques such as solid phase extraction have been developed to overcome the drawbacks caused by high amounts of glassware and toxic solvents in the classical liquid extraction methods [2]. Despite the increasing success of GC-MS, reports of matrix susceptibility have shown the limitations of this powerful analytical technique. Matrix components, which are unavoidably present in analysed samples, may be responsible for adverse effects impairing different stages of the GC-MS determinative step. The matrix effects therefore have to be investigated during the early development of any GC analytical method [8].

It is well known that matrix effects can seriously degrade the accuracy of GC-MS analysis results. The matrix effects can be defined as the effect of co-eluting residual matrix components on the ionisation of the target analytes [9]. They result in either signal suppression or enhancement. The matrix effect strongly depends on the nature of the analyte and on the properties of the co-eluting compounds, as some of the co-eluting compounds elute as chromatographic peaks and cause ionisation efficiency change only in a limited retention time range. The specific mechanism of the matrix effects is still not fully resolved [7].

Methods of taking the matrix effect into account have been studied but they do not necessarily reduce its influence [10]. Accounting for the matrix effects in principle can lead to corrected results, but for methods which undergo stronger ionisation suppression, its efficiency is limited [11]. Furthermore, since the nature and amount of these co-eluting compounds are usually variable between samples, the matrix effects can be highly variable and difficult to predict, making it difficult to compensate for them in practice [12]. Whilst different techniques can be applied to compensate for the matrix

effects and produce quantitatively accurate results, the loss in method sensitivity that is accompanied by signal suppression and the variability in method sensitivity that occurs between samples cannot be eliminated [7].

There are several commercially available robotic laboratory automation systems which serve to minimise and manage the matrix interferences by performing purification and extraction protocols. Matrix effects are however complex and system specific as each sample presents different management challenges and each analytical method is affected differently by the matrix components [13]. The most obvious way to reduce the matrix effects is to reduce the amount of matrix components entering the chromatographic system. An alternative strategy to reduce matrix effects is their compensation using appropriate calibration methods such as matrix-matched standards, standard addition method and the use of isotopically labelled calibration standards. Another alternative method is the use of analyte protectants which block the active sites in the injector [2].

For a solution, the concept of the matrix includes not only the compounds available naturally, but also those that may be present or added to the solution as part of the preparation because their nature and concentration may influence the intensity of the analyte signal. Two main types of matrices are a real sample matrix and a synthetic matrix [14].

A real sample matrix consists of a matrix and analytes that have combined with each other in nature and hence are naturally incorporated into the matrix of interest. They are also known as co-extracted matrix components. A real sample matrix is mainly made up of humic substances which are the most stable fraction of organic matter in soil and water and can persist for thousands of years [15]. Synthetic matrices are also called fortified matrices and consist of a matrix and analytes that have been combined together in a laboratory type production process rather than a natural setting [16]. Both types of matrices are useful and provide information that is critical to a successful

accreditation and certification program, although there is a strong debate about which type of matrix provides the best information about the analytical test [16].

1.2 Justification

In any analytical research, the matrix effect is always present in real samples and its reduction is one of the most challenging aspects of the method development process. The importance of this research is to investigate and understand the matrix effect phenomenon on GC-MS analysis of selected residual organochlorine pesticides with the aim of understanding and manipulating the mechanism of this phenomenon so as to improve quantification and detection of analytes.

1.3 Background of problem

In GC-MS analysis of residual pesticides, there is a propensity of the analytical system to produce inaccurate quantification, low analyte detectability and high background noise when all instrumental parameters indicate otherwise. Many researchers and laboratories also particularly noticed the trend of a systematic occurrence of reproducibly low recoveries even when all GC-MS parameters were optimally standardized, indicating that there is a factor within the sample that is adversely affecting the results (matrix factor) [16]. This research was mainly focused on organochlorine pesticides, testing different kinds of matrices and calibration techniques on their detection and quantification and also exploring th e use of matrix-matched standards to address these highlighted problems.

1.4 Aims and Objectives

The study aimed to produce data of sufficient quality to be able to meet the following research goals:

- To highlight and evaluate the role of the matrix effect in GC-MS pesticide residue analysis.
- To develop and establish a suitable method to handle the matrix effects in pesticide residue analysis with GC-MS.
- To determine the validity and significance of the matrix effects.
- To determine the significance of the matrix type.
- To investigate the use of matrix-matched standards versus matrix-free standards.
- To investigate the use of the internal standard and external standard calibration methods.

All research data collected underwent rigorous quality assurance review, which assessed, among other things, accuracy, precision, bias, representativeness, completeness, and comparability.

1.5 Precision, Accuracy, and Bias

Precision is the degree of agreement between replicate analyses of a sample under identical conditions and is a measure of the random error associated with the analysis, usually expressed as Relative Percent Difference (RPD) or Relative Standard Deviation (RSD). Accuracy is the measure of the difference between an analytical result and the true value, usually expressed as a percentage. The accuracy of a result is affected by both systematic errors (bias) and random errors (imprecision). Bias is the systematic or persistent distortion of a measurement process that causes errors in one direction. The precision, accuracy, and bias for the data collected were evaluated by one or more of the following quality control (QC) procedures:

- Analysis of various laboratory QC samples such as method blanks, matrix spikes, certified reference materials, duplicates/triplicates and positive and negative controls.

- Collection and analysis of field replicate samples. Field replicate results should exhibit a relative percent difference less than 150% in order for the evaluation of the spatial and aerial chemical concentrations to be meaningful.

1.6 Representativeness

Representativeness expresses the degree to which sample data accurately and precisely represents a characteristic of a population, parameter variations at the sampling point, or an environmental condition. Laboratory representativeness was achieved by proper preservation and storage of samples along with appropriate subsampling and preparation for analysis.

1.7 Comparability

Comparability is a qualitative parameter expressing the confidence with which one data set can be compared with another. This goal was achieved through using standard techniques to collect and analyze representative samples, along with standardized data validation and reporting procedures.

1.8 Scope

The scope of this research was limited to:

- 20 organochlorine pesticides which was sufficiently manageable by the researcher.
- GC-MS analysis so as to ensure more robust results on the matrix effects, by eliminating inter-instrument uncertainty.
- Water sample analysis so as to obtain a more uniform matrix and also to eliminate inter-sample uncertainty when computing and comparing analytical results.

1.9 Hypotheses

- There is no significant difference between real sample matrix and matrix-free analysis of residual organochlorine pesticides by GC-MS.
- There is no significant difference between real sample matrix and synthetic sample matrix analysis of residual organochlorine pesticides by GC-MS.
- There is no significant difference between the use of matrix-free standards and matrix-matched standards for calibration.
- There is no significant difference between external standard calibration and internal standard calibration.

1.10 Research Plan

This research is an experimental design and involved analytical method development, collection of water samples from three points along the Jukskei River catchment area in South Africa for laboratory analysis. The data obtained was statistically evaluated and validated by analysis of variance (ANOVA) using Statistical Package for the Social Science (SPSS).

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Gas chromatography-mass spectrometry (GC-MS) is one of the most powerful analytical techniques for the determination of multi-residue pesticides in a variety of matrices on a single run and is considered the most effective way to screen a large number of samples in a relatively short period of time [1,2]. Mass Spectrometry (MS) is the gold standard for performing qualitative analytical methods as its results are generally unquestioned. Each analysis however, requires careful deductive thinking on the part of the analyst to make a MS identification [3].

The analysis of pesticide residues typically involves the isolation, removal of analytes from the matrix followed by identification and quantification of the target analytes [4]. Due to the demand for lower detection limits, excessive sample cleanup is usually avoided as it leads to lower analyte recoveries [5]. Inadequate cleanup may lead to masking of residue peaks by co-eluted matrix components, resulting in inaccurate quantification of analytes. Attempts to analyse samples with heavy matrix burden with the aim of maximum target analyte retention will only lead to low production because of higher system maintenance due to the added contamination burden on the column [4]. Obtaining clean extracts where the matrix has been almost completely eliminated may not be possible in practice for multi-residue pesticide analysis, given the wide range of properties the pesticides tend to span [6].

Both the amount and type of matrix tend to affect the recovery. It is worth noting that the chemical structure as well as the concentration of the target analytes play an important role in producing good recoveries in matrix based extractions [7]. Individual pesticides may also show different increases or reductions in recoveries [8]. Common low resolution Mass spectrometric detectors (MSDs) such as the quadrupole may under some circumstances fail to provide unbiased confirmation of an analyte's identity due to the presence of interfering matrix ions [4]. When target analytes co-elute with matrix components yielding the same fragmentation ions as those generated by the target

analyte, the matrix ion's signal can suppress analyte signal, causing matrix signal suppression [9,10].

2.2 Recent developments in GC-MS

Before the 1980s MS instruments were less available and hence not very popular for use in routine monitoring laboratories [3]. Presently, the most common GC-MS in South Africa is the quadrupole GC-MS. High-resolution MS (HRMS) is becoming ever more popular in laboratories, particularly in the form of the Time-Of-Flight (TOF) MS. Other more powerful detectors in terms of resolution include the Fourier *trans*form (FT) MS and Orbitrap. All the above mentioned detectors have a full width half maximum resolving power of up to 10000. The TOF has a disadvantage in that it is less efficient in reducing matrix interferences. For this reason orthogonal, two dimensional (GCXGC) is usually coupled with the TOFMS [4].

Comprehensive GCXGC shows a remarkable enhanced separating power since it enables the chromatogram to spread into two independent dimensions in which the compounds in the mixture are separated by two independent mechanisms [4]. GCXGC in pesticide analysis has a theoretical gain in separation power of about 20 through having a second GC time window of 4 seconds with an average peak width of 0.2 seconds. In reality the GCXGC separation is significantly lower as three to four GCXGC cycles are used because of the need to generate broader peaks, thus analysis time is significantly longer. GCXGC however allows high quality identifications using only two ions in reconstructed SIM instead of three, at the same time providing increased sensitivity. Peak focusing in the second dimension also has the advantage of increasing the signal to noise ratio, thereby further increasing sensitivity [3].

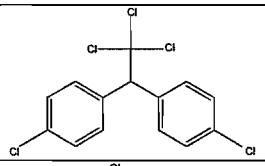
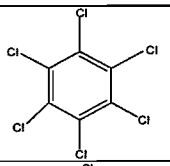
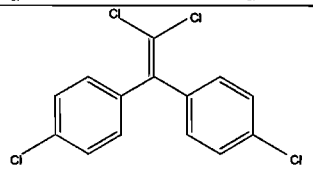
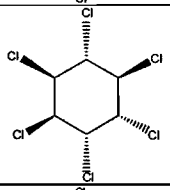
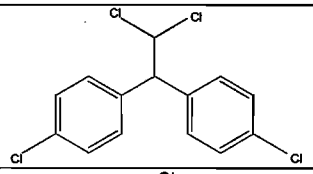
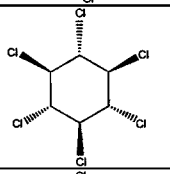
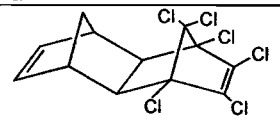
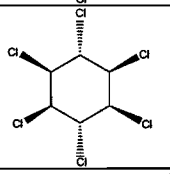
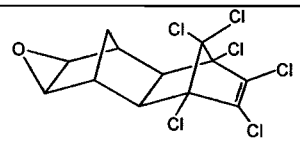
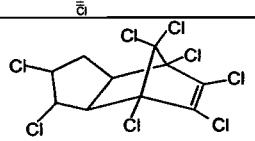
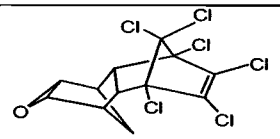
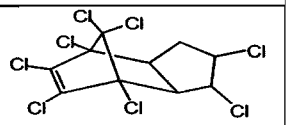
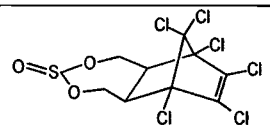
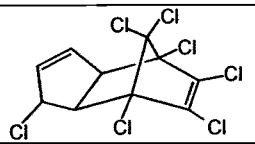
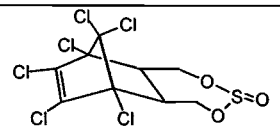
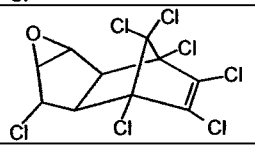
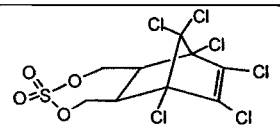
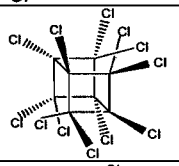
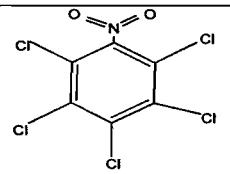
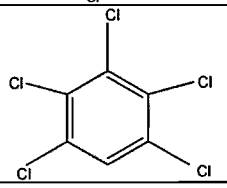
The use of even higher resolution orthogonal MS (HRMSXMS) truly excels in suppression of matrix interferences as it combines improved sensitivity and selectivity with resolutions of up to 0.0017 atomic mass units (amu) for a particular m/z [3,11]. Its main disadvantage is that it is not suitable for analysis of some organic compounds,

particularly where electron impact ionization (EI) is used due to ion instabilities, lack of enough product ions or low formation of high mass ions in MS to allow MSXMS. MSXMS is also disadvantageous in that it is target ion oriented and thus will miss any compound not in its target. The use of ions with a mass to charge ratio (m/z) of less than 91 and the use of ions with clusters of chlorine and bromine is discouraged as it may lead to false positives. In MSXMS determinations, chlorine and bromine have significant mass defects of -0.032 and -0.083 atomic mass units (amu) which can adversely affect accuracy [3].

2.3 Organochlorine pesticides

Organochlorine pesticides are chlorinated hydrocarbons composed primarily of carbon, hydrogen and chlorine [12]. Most of them are used as herbicides, insecticides and pesticides with forms varying from pellet application to sprays for seed and grain storage. Most organochlorine pesticides are classified as Persistent Organic Pollutants (POPs) because they break down very slowly and can remain in the environment long after application [13]. The organochlorine pesticides under study are Aldrin, Benzene hexachloride (BHC) alpha, Benzene hexachloride (BHC) beta, Benzene hexachloride (BHC) gamma, Pentachloronitrobenzene, Chlordane *cis* (alpha), Chlordane *trans* (gamma), 4,4' Dichlorodiphenyldichloroethane (DDD), 4,4' Dichlorodiphenyldichloroethylene (DDE), 4,4' Dichlorodiphenyltrichloroethane (DDT), Dieldrin, Endosulphan alpha, Endosulphan beta, Endosulphan sulphate, Endrin, Heptachlor, Heptachlor epoxide, Hexachlorobenzene and Mirex.

Table 2.1 : Typical chemical structures and names of the selected organochlorine compounds [10]

Pesticide	Chemical Structure	Pesticide	Chemical Structure
4,4'-DDT		Hexachlorobenzene	
4,4'-DDE		BHC alpha	
4,4'-DDD		BHC beta	
Aldrin		BHC gamma	
Endrin		Chlordane alpha	
Dieldrin		Chlordane *trans* gamma	
Endosulfan alpha		Heptachlor	
Endosulfan beta		Heptachlor epoxide	
Endosulfan sulphate		Mirex	
Pentachloro-nitrobenzene (PCNB		Pentachlorobenzene	

Organochlorine (OC) pesticides have been used worldwide commercially since the 1940s, but have since been banned and eliminated on their use and release into the environment in many countries due to their persistence in the environment and biological accumulation in the food chain [14]. POPs can be defined as hazardous and environmentally persistent substances which can be transported between countries by the earth's oceans and atmosphere. Organochlorine pesticides have been a major environmental issue, attracting much scientific concern because of their nature of toxicity, persistence, bioaccumulation, biomagnification and endocrine disrupting effects to non-target organisms and mutagenic and carcinogenic properties [15,16].

Organochlorines are readily detected even in remote regions of the earth with no historical usage and atmospheric transport is their major pathway for their global distribution [17]. Studies of the seasonal concentration changes of organochlorines in the European arctic indicate that long-range air transport from polluted areas might lead to significant concentration change even in the arctic air [18]. Persistent organochlorines have the following characteristics:

a) Long half-life in the environment, especially persistent biodegradation

b) Sufficient volatility which enables transport from source to remote areas and enrichment into water.

c) Bioaccumulation factors of at least 10^5 in the marine food chain [19].

Despite numerous countries having withdrawn the registered use of most persistent organochlorines, they still persist in considerable levels worldwide [18]. Organochlorines are lipophillic in nature and hence readily absorbed into the tissues of living organisms where they may have detrimental effects [20]. Modern agriculture depends on a variety of synthetic chemicals, including organochlorine pesticides, herbicides and insecticides. The release of the organochlorines daily has resulted in environmental pollution, leading to the detection of the pesticide residues in various environmental media such as riverines and estuarines.

2.4 Effects of the organochlorine pesticides on the environment

The effects of the organochlorine pesticides on the environment include:

- Toxicity to higher animals
- Potential to bioaccumulate
- Endocrine disruptors
- Mutagenicity
- Carcinogenicity
- Death of aquatic organisms
- Death of aquatic microorganisms

2.4.1 Methods of entry into aquatic environments

i) Drift from aerial ground application through soil erosion, including run-off, wash off and leaching from treated lands.

ii) Huge amounts added to water for effective pest control and enormous quantities discharged into water along with industrial and domestic sewer waters [21].

2.4.2 Regulation of the use of organochlorines and other POPs

The Stockholm convention on persistent organic pollutants governs the use of POPs including organochlorine pesticides in participating countries [22]. It is an international treaty signed on 23 May 2001 and effective from 17 may 2004, wh ose aim is to eliminate or restrict the production and use of persistent organic pollutants. Organochlorines approved for elimination include Aldrin, Chlordane, Dieldrin, Endrin, Heptachlor, Hexachlorobenzene, Mirex, BHC alpha, BHC beta, Lindane (BHC gamma) and Pentachlorobenzene. The use of DDT was restricted and the use of Hexachlorobenzene was also regulated [23].

2.4.3 4,4' Dichlorodiphenyltrichloroethane (4,4' DDT) and its derivatives

The compound 4,4' DDT is the most notorious organochlorine pesticide because it is one of the most persistent pesticides, its molecular formula is $C_{14}H_9Cl_{15}$ [24]. It was effectively used in the control of malaria in the 20th century as a contact pesticide against mosquitoes because of its low cost, ease of application availability and effectiveness [25]. Commercially available 4,4' DDT consists of 77% p'p'-DDT, 15% o'p'-DDT, 4% p'p'-DDE, and less than 1% o'p'-DDD, p'p'-DDD and o'p'-DDE [26]. Banned in most developed countries DDT's use is now restricted to certain developing countries under the supervision of the United Nations (UN) [27]. High levels of 4,4' DDT can affect the nervous system causing tremors, seizures and cancer.

4,4' Dichlorodiphenyldichloroethylene (DDE), is a 4,4' DDT derivative formed by the dehydrohalogenation (loss of hydrogen chloride) of 4,4' DDT. Its molecular formula is $C_{14}H_8Cl_4$ and its IUPAC name is 1,1-bis-(4-chlorophenyl)-2,2-dichloroethane. The organochlorine 4,4' Dichlorodiphenyldichloroethane (DDD) is another 4,4' DDT derivative formed by hydrogentation coupled with dehydrohalogenation of DDT. It is closely related to 4,4' DDT but is less toxic to animals. Its molecular formula is $C_{14}H_{10}Cl_4$ and its IUPAC name is 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene [26].

The compounds 4,4' DDT, 4,4' DDE and 4,4' DDD are rapidly broken down by sunlight but when they stick to soil, most of the 4,4' DDT is broken down to 4,4' DDE and 4,4' DDD by microorganisms. Half of the 4,4' DDT in soil is broken down in 2 to 15 years depending on the nature of the soil. The organochlorine 4,4' DDT and its derivatives are endocrine disruptors and have been proven to be reproductive toxicants to animals and some studies show that they are mutagenic as well as carcinogenic and also cause Alzheimer's and Parkinson's disease [24,28].

2.4.4 Aldrin

Aldrin has the molecular formula $C_6H_8Cl_6$ and IUPAC name (1R,4S,5S,8R)-1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene [29]. Its use is now banned and it was used previously at concentrations of 2.0-10.0 kg/Ha for pest control such as leatherjacket [16]. Aldrin is classified as a category B^2 carcinogen, based largely on liver tumours in mice fed with Aldrin [30].

2.4.5 Dieldrin

Dieldrin has the IUPAC name (1R4S,5S,8R)-1,2,3,4,10,10-hexachloro-,4,4a,5,6,7,7,8,8a-octa-hydro6,-7epoxy-1,4:5,8-dimethanonaphthalene and a molecular formula of $C_{12}H_8Cl_6O$ [30]. It is an extremely persistent organic pollutant and does not break down easily. It also tends to biomagnify as it is passed along the food chain. It has been linked to cancer and can adversely affect the reproductive and immune systems [29].

2.4.6 Endrin

Endrin, IUPAC name (1R,4S,5R,8S)-1,2,3,4,10,10-hexachloro-14,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene, is a stereoisomer of Dieldrin and was used primarily as a cotton insecticide. Unlike Dieldrin, it is rapidly metabolised by a wide range of organisms and is not recognised as a persistent compound [29].

2.4.7 Hexachlorobenzene

Hexachlorobenzene is both a pesticide and also an industrial by-product [31]. Sources of Hexachlorobenzene include the aerospace industry, agricultural chemicals, municipal waste incinerators, wood-preserving plants and industries involved in the manufacture of solvents. Its molecular formula is C_6Cl_6. Hexachlorobenzene is very toxic to aquatic animals and is a potential carcinogen. It was used mainly as a fungicide to protect

seeds and grain. It may be transferred to the foetus across the placenta during pregnancy and accumulate in foetal tissue [31].

2.4.8 Chlordane

Chlordanes are a mixture of organochlorine pesticides with a global production of 7 Kilotonnes between 1948 and 1988. It was mainly used on citrus crops and maize. The IUPAC name for Chlordane is octachloro-4,7-methanohydroindane [32]. The major components are *trans*-Chlordane and *cis*-Chlordane. Typical mixtures usually contain 24% *cis* and 19% *trans*. Chlordane *cis* and *trans* have the molecular formula $C_{10}H_6Cl_8$. It is highly toxic to fish and exposure to humans causes cancer, particularly testicular and prostate in men.

2.4.9 Hexachlorocyclohexanes (HCS)

Hexachlorocyclohexanes include three isomers, which are BHC alpha, BHC delta and Lindane (BHC gamma) [33]. Their molecular formula is $C_6H_6Cl_6$. BHC alpha has the IUPAC name α-1,2,3,4,5,6-hexachloricyclohexane and is the by-product of the production of Lindane. BHC beta is also a by-product of the manufacture of Lindane and has the IUPAC name β-1,2,3,4,5,6-hexachloricyclohexane [33]. It is stable to enzymatic degradation compared to the other BHC's and thus is more prominent in biota. Exposure to BHC beta causes damage to the brain and central nervous system and is also linked to Parkinson's and Alzheimer's disease. Lindane has the IUPAC name (1r.2R.3S,4r,5R,6S)-1,2,3,4,5,6-hexachlorocyclohexane and has been widely used both as a pesticide and as a pharmaceutical in the treatment of lice and scabies. Lindane is a neurotoxin which affects the nervous system and is also a carcinogen [34].

2.4.10 Mirex

Mirex, molecular formula $C_{10}Cl_{12}$, IUPAC name 1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1*H*-,3,4-(methanetriyl)cyclobuta[*cd*]pentalene, is a highly bioaccumulative and robust insecticide which is resistant to microbial degradation but is photo-labile as it is degraded to photomirex by sunlight. It was used mainly from 1962 to 1978 to control fire ants in the US. According to the USEPA, Mirex causes cancer, liver damage, damage to the central nervous system and miscarriages [35,36].

2.4.11 Endosulphan

Endosulphan sulphate, molecular formula $C_6H_6Cl_6O_4S$ is a highly toxic organochlorine pesticide. Its IUPAC name is 6,7,8,9,10-hexachloro-1,5,5a,6,9,9,9a-hexahydro-6,9-methano-2,3,4-benzodioxythiepin-3,3-dioxide [37].

Endosulphan alpha, molecular formula $C_9H_6Cl_6O_3S$ is also known as Endosulphan I. Its IUPAC name is 6,9-methano-2,4,3-benzidioxathiepin,6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-,3-oxide, (3.alpha.,5a.beta.,6.alpha.,9.alpha.,9a.beta) [38].
Endosulphan beta is also called Endosulphan II and has the molecular formula $C_9H_6Cl_6O_3S$. It is an isomer of Endosulphan I and its IUPAC name is 6,9-methano-2,4,3-benzidioxathiepin,6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-,3-oxide (3.alpha.,5a.alpha.,6.beta., 9.beta.,9a.alpha) [37].

2.4.12 Heptachlor

Heptachlor is an organochlorine insecticide used mainly for control of fire ants. It has a molecular formula $C_{10}H_5Cl_7$ and IUPAC name 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene. It is metabolised by soil microorganisms by epoxidation, hydrolysis or reduction to Heptachlor epoxide, another persistent organic pollutant or 1,2-dihydrooxydihydrochlordene. Its use has been limited by the USEPA due to its toxicity [25].

2.4.13 Heptachlor epoxide

Heptachlor epoxide is an insecticide used for killing insects in homes and on food crops until it was stopped in 1988. It has the molecular formula $C_{10}H_5Cl_70$ and its IUPAC name is 1,4,5,6,7,8,8-heptachloro-2,3,epoxy-3a,4,7,7,7a-tetrahydro-4,7-methanoindan. It is more likely to be found in the environment than its parent compound, Heptachlor. Heptachlor epoxide causes convulsions, seizures, coma and respiratory depression in humans exposed to it [39].

2.4.14 Pentachlorobenzene

Pentachlorobenzene is a persistent organochlorine which can be used to make another persistent organochlorine, Pentachloronitrobenzene. It was added to the list of chemical compounds covered by the Stockholm convention only in 2009. It has the molecular formula C_6HCl_5 and its IUPAC name is 1,2,3,4,5-pentachlorobenzene. It can be produced as a by-product of the manufacture of benzene or carbon tetrachloride. Exposure to Pentachlorobenzene causes damage to the liver, kidneys and central nervous system. Pentachlorobenzene can also be produced when organic compounds are burned in the presence of chlorine [40].

2.4.15 Pentachloronitrobenzene

Pentachloronitrobenzene is manufactured from Pentachlorobenzene and has the molecular formula $C_6Cl_5NO_2$. Its IUPAC name is Pentachloronitrobenzene. It is mainly used as a fungicide to protect seeds and grain and is also used in slime prevention in industrial waters. Exposure to Pentachloronitrobenzene may cause skin and eye irritation, itching and oedema. Its toxicity has not yet been established [31].

2.5 Recent developments in pesticide analysis

Modern instrumental extraction techniques such as Solid Phase Extraction (SPE) and Solid-Phase Microextraction (SPME) have been developed to replace classical protocol extraction methods such as Liquid-Liquid Extraction (LLE) [41]. SPE was introduced in the mid 1970s but only became popular in 1985. SPME has also gained widespread acceptance in recent years for a variety of matrices [41]. Although the instrumental conditions and configuration play an important role in the efficiency of the analytical method, it is the choice of the sample preparation technique that plays a key role in producing accurate results [4]. The use of chromatographic systems with high separating power such as orthogonal chromatography and higher resolutions will however go a long way to solve some of the problems encountered in GC analysis of complex samples, as even under the best experimental conditions, the probability of peak overlap in chromatographic separations can be quite severe [4,42]. It has been shown that the greater the complexity of the chromatographic separations, the bigger the burden to handle the enormous amounts of multivariate data and information generated [42].

2.6 Sources of errors in analytical chemistry

On numerous occasions the sample matrix causes interferences with the signal of the analyte of interest and if not accounted for will cause a systematic error which will affect the results and cause bias [11]. The main two types of systematic errors that may arise in GC-MS analysis are relative systematic errors and constant systematic errors [13]. Relative systematic errors are based on physical and chemical factors and do not lead to a response but affect the slope of the calibration curve. They can be caused by sample treatment steps such as sample extraction or by the presence of matrix components which modify the analyte signal [43]. Constant systematic errors are mainly caused by contaminant species which have a response of their own in addition to that of the analyte of interest, causing enhanced responses to be recorded in the samples [43].

This type of interference can be corrected by subtracting the blank sample analytical results from the real sample analytical results for each particular analyte of interest [44].

Another source of errors is random variability. Human errors are undoubtedly the greatest source of error with respect to qualitative identifications and confirmations, given the high quality and sophistication of technology and instrumentation in GC-MS. The number of random error mistakes depends on the diligence and intelligence of the analysts performing the analytical techniques [3].

2.7 Matrix effect phenomenon

The matrix effect is one of the main sources of errors in GC pesticide multi-residue analytical methods [44,45]. One of the ways in which the matrix effect occurs is in the column inlet and column of the GC when co-eluting molecules alter the signal of the component of interest [46]. The presence of several compounds from the matrix introduces bias during detection and quantification of residual pesticides in GC analysis [47].

The matrix effect phenomenon can either improve pesticide analysis or make it worse, as the main consequence of the matrix effect is an increasing analyte signal, a phenomenon known as matrix-induced response enhancement or decreasing the analyte signal, a phenomenon known as matrix-induced response diminishment [46]. The matrix effect is also related to the masking or formation of active sites (mainly silanol groups) within the chromatographic system [48].

2.7.1 Matrix-induced response enhancement

This phenomenon results when a sample matrix causes an enhancement in the chromatographic response of analytes in a matrix extract compared to the same concentration in a matrix-free solution [2,46].

When a real sample is injected into the GC, the matrix components are adsorbed and tend to block the active sites (silanol groups and metal ions) potentially present in even high quality GC inlet and column, thereby reducing loss of susceptible analytes caused by adsorption or degradation of these active sites [4]. This results in higher analyte signals in matrix containing solutions, leading to overestimations of the concentrations in the analysed samples [49].

The sample matrix may also reduce the thermal stress experienced by thermally labile analytes during vaporization in the hot injector, leading to an enhanced chromatographic response in the matrix sample [8]. Previous studies indicated that this type of matrix induced response enhancement can be manipulated to counteract low analyte signals. When the matrix increases the transfer of pesticides from the hot vaporizing injectors by reducing the thermal stress (degradation) for labile compounds, making them more stable at higher temperatures, it facilitates higher analyte signals on the GC [2,8].

Some of the organochlorine compounds susceptible to matrix induced response enhancements include BHC alpha, 4,4' DDT, Dieldrin, Endrin and Heptachlor-epoxide [8]. Many compounds are not affected by matrix induced enhancement either because they are thermally stable or have limited potential for adsorption interactions in hot vaporizing injectors or because the matrix is unable to provide a significant protecting effect [2].

2.7.2 Matrix- induced response diminishment

This phenomenon results when ion suppression and gradual accumulation of non-volatile matrix components occurs in the GC, resulting in formation of new active sites and gradual decrease in analyte responses [4]. The effect of ion suppression is of major concern in GC-MS analysis and as it has been shown to reduce the accuracy of an assay by as much as 26 % [10].

2.8 Sample preparation

The development of a complete analytical method includes a number of steps from sample collection to the final report of results. Sample preparation is the most time consuming step of any GC analytical method [50]. The main aim of sample preparation in GC analysis is to effectively remove or extract the analytes of interest from the sample containing potentially many interfering compounds and present the analytes in a suitable solvent for injection into the GC [51].

2.8.1 Solid Phase Extraction (SPE)

Solid phase extraction is a sample preparation technique used for extracting semi-volatile and non-volatile analytes from their matrices for subsequent chromatographic analysis. It is considered one of the most powerful techniques currently available for isolating trace amounts of organic compounds such as pesticides from water and other environmental samples [52,53]. It entails the use of SPE cartridges which are packed with silica bonded to a particular analyte adsorbing phase. SPE can be compared to other extraction techniques like liquid-liquid extraction although it is advantageous in that it provides better selectivity and extraction efficiency (recovery), eliminates problems associated with incomplete phase separation and yields quantitative extractions that are easy to perform [54].

SPE is particularly suited for the isolation of organic pesticides and has now become the method of choice in order to carry out simultaneous extraction and concentration of a wide array of pesticides [55]. The general approach to method development in solid-phase extraction is based on a trial and error approach rather than an empirical approach. Some computer-aided applications are however available to use instead of the trial and error approaches but require a high degree of theory [56].

It is important to note the fact that even the most sophisticated SPE sample cleanup protocols will not totally remove impurities that co-elute with the analytes of interest and cause matrix effects [5,10]. The principle behind SPE cleanup is to remove compounds that have different polarities and hydrophobicities to those of the analytes of interest. The compounds which co-elute with the analyte of interest actually do so because they have similar polarities and hydrophobicities to the analytes of interest [10].

2.8.2 SPE cartridges

A typical SPE cartridge consists of a short open syringe shaped barrel containing a sorbent phase packed between porous metal or plastic frits [56].

2.8.2.1 Strata-X (500 mg)

The Strata-X is a reversed phase bed cartridge suitable for the extraction of polar and non-polar analytes with hydrocarbon and aromatic groups which form a surface modified styrene divinyl benzene group. This gives it an advantage over other sorbents in that it is deconditioning-resistant and has better selectivity for polar and non-polar compounds [57]. The styrene divinyl structure also has the advantage of selective interaction with aromatic rings as those in DDT through formation of specific π-π interactions [58,59]. Reversed phase cartridges are frequently used in environmental chemistry to extract organic substances from aqueous samples such as water [60]. According to Ferrer and Barcello (1999), the cartridge is suitable for the analysis of Organochlorine compounds and the functional group is displayed below in figure 2 [53].

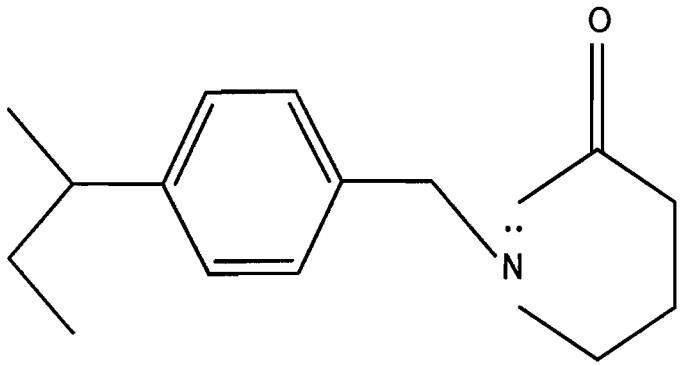


Figure 2.1: Functional group of Strata-X

2.8.2.2 LC-18 (Supelco) (200 mg)

The LC-18 (Supelco) cartridge consists of an octadecyl bonded end c apped silica reversed phase bed. It is suitable for non-polar to moderately polar compounds such as the organochlorine compounds under study. The hydrophilic silanol groups at the surface of the raw silica packing have been chemically modified with hydrophobic alkyl or aryl functional groups by reaction with the corresponding silates [61]. The functional group is displayed below in figure 2.2.

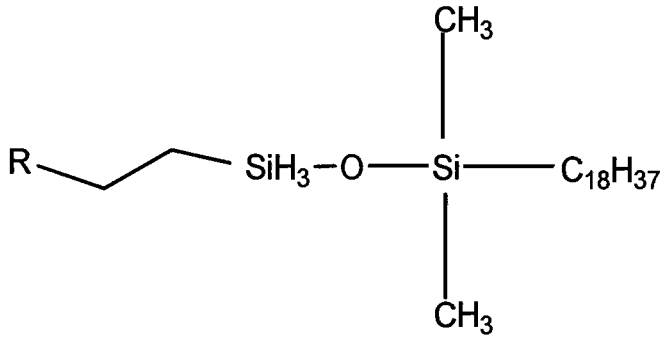
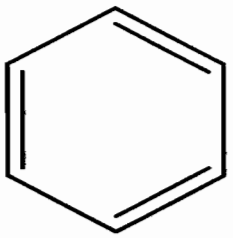


Figure 2.2: Functional group of LC-18 (Supelco)

2.8.2.3 Strata C18-E (200 mg) and Strata C18- E (500 mg)

The Strata C18-E (S C18-E) cartridge is a reversed phase absorbent with a hydrocarbon and aromatic functional group. Its retention mechanism is through hydrophobic interactions, hydrogen bonding and aromatic interactions [62,63,64]. A 200

mg and a 500 mg sorbent bed mass were used for the method development, particularly to test the effect of increasing the sorbent bed mass on analyte retention. The two functional groups of the Strata C18- E cartridge sorbent bed are shown below:



Benzene

octadecyl aryl group

Figure 2. 3: Functional groups of Strata C18- E

2.8.3 Automated liquid handling systems

Automation of SPE has been to date achieved by the use of robotics and dedicated liquid handling instruments which employ the use of flow processing and on-line analysers [52]. It is mainly used in areas of analytical chemistry where high sample throughput is required [50,65]. Automated liquid handling instruments are capable of performing the following cartridge treatment processes:

1) Conditioning cartridges

   This is done to activate the sorbent bed.

2) Loading cartridges

   This is done to introduce the analytes to the sorbent bed where they are adsorbed.

3) Drying cartridges

   This is done to remove any water remaining on the sorbent bed and prevent the introduction of water to the elute.

4) Eluting cartridges

   This is the process of desorbing the adsorbed analytes by adding a polar solvent that washes the analytes from the sorbent bed [56,66,67].

For effective mass transfer of analytes onto the sorbent a consistent flow rate applied at low pressure needs to be used [68]. Most automated instruments utilise positive pressure elution which makes it increasingly easy to control flow rates [66].

2.9 Recovery

The main purpose of calculating the recovery is to obtain a correction factor for computing analytical results and to provide validation data. Recovery is an estimation of the systematic error of the global extraction process [69]. The recovery data should be obtained from matrix Reference Materials (RMs), however RMs are seldom available so often analysts often have to resort to surrogate standards [70]. A surrogate standard in multiresidue pesticide analysis is a pure standard cocktail added to the test sample, whose chemical and physical properties are considered identical to the native test analytes. The degree to which the surrogate is transferred from the sample to the measurement phase is calculated as the recovery [43].

The surrogate cocktail is usually added to a blank matrix which has the same chemical and physical properties as the test samples to create a sample called a Fortified Matrix (FM). Since the FM contains different co-eluting compounds at different concentrations, recoveries for each particular analyte are usually different as the extent at which the sample matrix affects each analyte is different [10].

One of the most interesting practical considerations in recovery procedures is that recovery estimates based on spiking procedures do not reflect the real binding state of the compounds in the matrix since there is always a risk that the spike is more easily recovered than the naturally bound compound [43].

2.10 GC optimization- Selective Ion Monitoring (SIM)

Improvement and optimization of the chromatographic application can minimize co-elution of other compounds with the analyte of interest. GC-MS detector parameter settings like SIM are designed to detect only the specific analyte ions of interest so as not to detect the presence of other co-eluting compounds [15]. The assertion that coupled with retention time matching, the use of a target ion and three qualifier ions will give enough selectivity to positively identify most compounds is supported by many chemists, based on their experiences [3,43].

As a general guideline, at least three ions of the correct mass to charge ratio (m/z), a relative abundance ratio of ±10 % coupled with a ±2 % retention time error factor and a signal to noise ratio greater than three for the chromatographic peak of the least intense ion are required to make a definitive mass spectral match [3].

Implementing the general guidelines presents some major difficulties as even when the analyte has three or more intense ions, some of the target ions chosen or the analysis frequently have interference from the sample extracts at the retention time of interest so different ions must be chosen, depending on the matrix-analyte pair to increase selectivity and sensitivity [3]. A balance must be established between the number of analytes that can be included in the method versus retention time and the number of qualifier ions with careful consideration of the background matrix interferences. Another problem associated with using SIM is that compounds present in the sample but not configured into the SIM table could be considered false negatives since the GC-MS cannot detect them [45].

Previous studies by the National Institute of Standards and Technology (NIST) have shown that ions of higher mass to charge ratio (m/z) have less chance of potential interferences than those of lower m/z. In another study of GC-MS analysis of pesticides, the degree of matrix interference was actually shown to reduce exponentially by a factor of 20 fold per each 100-m/z increase [3]. The study also demonstrated how the

presence of a molecular ion greatly enhanced the ability to isolate the peak of interest in a complicated matrix. Ultimately the analyst must decisively make sound judgments when using SIM to make correct identifications [1].

2.11 Calibration standards

Calibration standards are defined as reference material used to define a unit of a quantity to serve as a reference standard. Calibration standards containing a single analyte in solution are called reference materials (RMs) [70]. RMs are defined as a substance or material whose properties are sufficiently homogenous and well established to be used for the calibration of an instrument or for assigning values to materials [43]. RMs are supplied accompanied by a certificate whose property values are certified by a procedure which establishes traceability and for which each certified value is accompanied by an uncertainty stated at a level of confidence.

The term Standard Reference Material (SRM) is sometimes used interchangeably with the term Certified Reference Material (CRM). CRMs are used principally as calibration verification materials where RMs are used, as measurement benchmarks in method development, and also as control materials when analysing a sequence of materials [70]. The CRM response is therefore used to plot quality control graphs. A correct result obtained with a CRM for matrix based analyses does not guarantee that correct results will be achieved when analysing unknown samples due to differences in matrix compositions [43].

The four main types of reference materials include

1) Pure standard reference material which consists of a chemically pure analyte in solution.
2) Standard solution reference material which consists of a mixture of pure substances prepared gravimetrically.

3) Matrix-matched reference material which consists of pure analyte prepared in a matrix.
4) Matrix-matched solution reference material which consists of a standard cocktail solution prepared gravimetrically into a matrix.

The matrix-matched standards contain the analytes of interest plus the chemical compounds characteristic of the matrix to be matched. In this study, the matrix-matched standards were prepared in real sample matrix but were tested against samples containing real sample matrices as well as samples containing synthetic matrices [43].

2.11.1 Neat standards

The use of neat standards entails one of the simplest and most widely used calibration techniques where matrix effects are considered to be negligible. Analysts who use neat standards usually prime the GC by injecting a real sample matrix before injection of the rest of the samples in the injection sequence [4].

2.12 Calibration techniques

Calibration can be defined as a set of operations that establish the relationship between responses obtained from an instrument and the corresponding values realized by standards [43]. The purpose of calibration is to produce a response curve through establishment of a quantitative relation between several known concentrations and their corresponding signals [42]. The interferences from a sample matrix can cause problems when using pure solvent standards for calibrating a GC-MS within the same injection sequence hence should be considered when choosing a calibration technique [44].

2.12.1 External standard calibration

External standard calibration technique is the most common calibration technique and usually involves the use of multiple point calibration points [43]. The main limitation of external calibration technique is the assumption that there is no difference between the solvent matrix which is the pure solvent, and the sample matrix. It is recommended to subject the calibration standards to the same process as the sample, thereby correcting any possible source of systematic error [42].

2.12.2 Internal standard calibration

The internal standard method is a robust calibration method which involves the use of response factors. It is mainly used to compensate for injection volume variability largely attributed to changes in solution viscosity due to temperature fluctuations or evaporation losses in the sample vial [71]. For this method a compound closely related to the analytes of interest and that is most unlikely to be found in the samples of interest being different enough so that it can be quantified separately, is chosen as the internal standard [73].

The internal standard must be added to the external calibration standards as well as the samples and must have a distinct retention time well separated from the test compounds so as to avoid any interference. In this respect Pentachlorobenzene was selected as the internal standard as it elutes before any of the test analytes. The response factor used for calibration is calculated using the ratio between the response of the internal standard and the response of the analyte standard. The analyte concentration is in turn computed from the response factor between the analyte and the internal standard [43,71].

The theory behind internal standard calibration is that even if the magnitudes of the individual responses for each analyte and the internal standard will differ in the presence or absence of the matrix effects, the ratio of the responses will be unaffected.

This means that the ratio of the analyte response/internal standard response versus the ratio of analyte concentration/internal standard concentration obtained will be the same whether the calibration standards are prepared in a matrix or pure solvent [10].

The internal standard calibration is practically carried out by spiking the same volume of a known concentration of an internal standard (C_{IS}) into both the calibration standard of known concentration (C_{STD}) and the sample [72]. The responses of the internal standard (R_{IS}) and the calibration standard (R_{STD}) are measured and used to calculate the response factor (RF) as follows:

$$RF = \frac{C_{STD}/ R_{STD}}{C_{IS}/ R_{IS}}$$

The actual analyte concentration (C_{ANAL}) is computed using the GC-MS response from the sample analyte analysis (R_{ANAL}) as follows [72]:

$$C_{ANAL} = RF \cdot \frac{C_{IS}}{R_{IS}} \cdot R_{ANAL}$$

For multi-point calibration, the same amount of internal standard was added to each of the calibration standards and the calibration curve obtained by plotting the ratio of the analyte standard signal and the internal standard signal on the y-axis against the analyte standard concentration on the x-axis [72,73].

The internal standard method is especially crucial for use in instances where the assay depends on very precise instrumental conditions that are difficult to control. Problems such as instrumental drift which may occur during a long sequence sample run, causing changes in instrument responses can be corrected using the internal standard method [43].

2.12.3 Matrix-matched calibration standards

Matrix-matched calibration standards are calibration standards made up by using extracts from blank samples (which is the actual sample containing the analytes) instead of a neat solvent [71]. This method is considered an effective way of avoiding errors arising from the matrix effects in the quantification and detection of analytes [10,44]. Results obtained using matrix-matched calibration standards are usually more reliable in interlaboratory studies compared to matrix-free standard determinations [8]. Higher recoveries recorded for matrix-matched standards compared to matrix-free standards were attributed to the protecting effects of the matrix in the matrix-matched standards which lead to a more complete transfer of analytes into the column. The response for the matrix-free calibration standards was less than it should have been for full transfer to the column because the solvent was unable to provide sufficient protection for the analytes [8]. It should be aimed to determine the maximum matrix burden that creates an optimum matrix-induced enhancement or diminishment effect to produce acceptable recoveries. This can be determined by estimating the breakthrough volume or optimum matrix load volume.

The use of matrix-matched standards is generally accepted internationally and used by several analytical laboratories in South Africa and many more worldwide [44]. It is also accepted by regulatory bodies in the European Union but is strictly forbidden by the United States Environmental Protection Agency (USEPA) and United States Food and Drug Administration (USFDA) for enforcement purposes [8,45]. The use and application of matrix-matched standards requires a sound knowledge of the experimental conditions and contribution of the interference to the measurement as the matrix calibration standards may contain one or more analytes not present in the sample. This could lead to matrix effects and consequently the negatives of the use of matrix-matched standards outweighing the benefits.

Another practical consideration is that in routine analysis there is no practical blank matrix that resembles all samples in a batch that can be spiked to prepare matrix-

matched calibration standards [10]. Matrix-matched standards however must be prepared freshly from the same matrix as for the samples therefore the most practical method would be to prepare a blank matrix from a mixture of all the samples in the batch to be analysed to create a representative blank matrix.

2.12.4 Matrix-matched standards versus matrix-free standards

The best way to provide a comparative analysis of the above mentioned calibration techniques is to perform indirect calibration using (i) chemical standards prepared from a pure analyte dissolved in a pure solvent and (ii) chemical standards prepared in the matrix co-extractives [44]. This approach was used by the researcher to study the differences in regression parameters of both calibration curves. Calibration is considered one of the most important parts of method validation even though systematic errors in calibration are rarely considered to be of relevant significance [69].

The use of matrix-free standards is advantageous economically as column contamination is minimal, hence the frequency of installing a new column is reduced. It also reduces the analysis time in terms of reducing the frequency of instrument and column maintenance. The use of matrix-matched standards may be disadvantageous in that it may increase the level of random errors, either as a constant, affecting the analytical blank or proportional, affecting the analytical sensitivity by causing problems related to loss of analytes at trace levels (near the LOD) and tailing of peaks which in turn leads to integration problems [4,69]. The concept of matrix matching is undoubtedly an important technique, however due to problems associated with sample to sample variation in matrix, coupled with the impossibility of exact matching of the matrix of the calibration standards with all samples, analysts are forced to use the standard addition method which is an effective calibration technique for producing more accurate data in GC analysis [10].

2.12.5 Standard addition calibration method

The standard addition method, as with the matrix-matched standards involves the addition of a standard analyte solution to the sample but with the purpose of effectively measuring the sample and analyte responses collectively, taking into consideration the analyte added is also the analyte of interest. This method is often used in instances where the sample matrix is so complex that the external and internal standard methods cannot be used with confidence due to significant matrix effects expected [10]. The main disadvantage of this method is that it is time consuming and laborious hence it is not an option for routine laboratories with a demand for high sample throughput.

Practically the standard addition method is carried out by first preparing a volume of sample (V_{sample}). A calibration factor is determined by spiking a second V_{sample} with a known analyte concentration (C_{std}) to create a V_{spiked} solution. The Calibration Factor (CF) is calculated as follows:

$$CF = \frac{(C_{std}).\ V_{spiked}}{V_{sample}}$$

the actual analyte concentration (C_{anal}) is calculated as

$$(C_{anal}) = CF \cdot \frac{R_{sample}}{R_{spiked} - R_{sample}}$$

Where R_{sample} and R_{spiked} are analytical responses from the sample solution and spiked solution respectively [43].

The multiple point standard addition calibration curve is calculated by:

a) Adding standard solutions with known amounts of analytes to portions of the sample
b) Analysing the above samples by GC-MS

c) Constructing a standard added linear calibration curve by extrapolating the value of R_{spiked} at concentration zero, assuming that the effect of adding a standard to the sample is insignificant and all matrices are identical thus the CF is taken to be a constant (K_{SA}).

The concentration of the analyte (C_{anal}) in the sample is calculated as follows;

$$C_{anal} = - K_{SA} . (R_{sample} - R_{matrix})$$

Where R_{matrix} is the matrix blank [43].

2.13 Standard distribution

In a sequence of injections, each prior injection has the potential to alter the activity of the next by depositing active matrix components into the liner. Where standards are made up in solvent (matrix-free) and are distributed over the working range, a gradual but significant increase in the area response for the standards over time is sometimes observed [8]. This leads to a negative concentration being attained at trace concentration levels.

2.14 Breakthrough volume

The breakthrough volume can be determined either experimentally by using a breakthrough curve or through the use of empirical models. Experimentally either an online method can be used or a more labour intensive offline method can be employed [52]. In theory, as the sample is loaded onto the solid phase, it adsorbs the analytes and the organic matrix up to the point of saturation, where the solid phase reaches its retention capacity. This point of saturation is equivalent to the optimum matrix load volume. Any further analytes introduced to the solid phase beyond this point will not be quantitatively retained by the solid phase. The breakthrough volume, by definition is

reached at the sample volume when the amount of analytes entering and leaving the solid phase becomes equal, due to saturation of the solid phase by analytes introduced [52].

2.15 Method validation

Method validation, according to ISO 9000 standard series, can be defined as a confirmation through the provision of objective evidence that the requirements for a specific method have been fulfilled [74]. Validation parameters include Limit of Detection (LOD), Limit of Quantification (LOQ), linearity, linearity verification, precision, trueness and statistical significance testing.

A typical method validation document must specify the intended use of the method, define the analytical performance requirements and most importantly provide reliable analytical data from validation experiments. In-house validation, as opposed to interlaboratory assays has an advantage in that it covers performance parameters such as linearity, matrix effects, selectivity and limits of detection [74].

2.15.1 Limits of Detection

The limit of detection can be calculated at 3 times the standard deviation of the blanks or low concentration samples or as 5 % of the error of detecting the analyte when it is not there [74]. The chromatographic LOD can also be determined as the response that gives a signal to noise (S/N) ratio of 3:1 [75]. The measurement of the LOD using S/N ratio is strongly recommended as it shows the skills of the analytical chemist to optimize the S/N ratio.

2.15.2 Limits of Quantification

The definition of LOQ should be based on values of precision, trueness or total error. The limit of quantification can be calculated determined as the response that gives a signal to noise (S/N) ratio of 10:1 or as a function of Relative Standard Deviation (RSD) [76]. Most researchers calculate LOQ as 10% RSD or simply as 10 times the standard deviation of the error associated with detection of the analyte in the blank sample [43].

2.15.3 Linearity

Linearity defines the ability of the method to obtain test results proportional to the concentration of the analyte [75]. It is recommended to establish calibration curves with five or more calibration points with the use of more than three replicates. The coefficient of regression (R^2) is used to assess the acceptability of a calibration curve [72]. One of the pitfalls of using R^2 is its bias towards the range of the data. Visual assessment is an acceptable criterion to define whether a calibration curve is linear or non-linear [43].

Validation of linearity should be done by means of a statistical test using the null hypothesis [74]. Analysis of Variance (ANOVA) should be applied to ensure that calibration curves obtained with each type of calibration is stable in repeatability conditions and hence data from at least three calibration curves should be compared using an F statistic at 95% confidence level [73].

2.15.4 Verification of linearity

The efficiency of computing the linearity of a calibration curve as calculated by relevant instrumental software often needs to be verified. Verification is the confirmation by examination and provision of objective evidence that specific requirements have been fulfilled [43].

2.15.5 Calibration Range

In the presence of matrix effects, the range of responses obtained from calibration can be wide hence ensuring the linearity over a wide range of concentrations above and below the added internal standard concentration is paramount for obtaining accurate data [42].

2.15.6 Precision

Precision is an important validation parameter and is specified as a requirement by most validation guidelines and is measured as a function of the true Relative Standard Deviation percentage (RSD%) [74]. For a defined number of replicates greater than three, a precision of <10% RSD is considered good. The precision should be expressed under the same operating conditions over a short interval of time [76]. It is recommended to calculate precision at three different concentrations.

2.15.7 Trueness

Trueness is defined as the difference between the average of an infinite number of replicate measured quantity values and a reference quantity value [71]. It is often erroneously confused with accuracy which is the difference between a measured value and the true quantity value of the measurand. In GC-MS analysis, trueness is best measured through the use of recoveries [74]. Assessing trueness implies estimating separately the proportional bias (in terms of recovery) and the constant bias of the analytical method [70].

2.15.8 Selectivity

Selectivity is the degree to which an extraction technique can separate the analyte from interferences in the original sample [53]. The choice of a sample preparation technique coupled with the analytical instrument of choice has a profound influence on the

selectivity of a particular analyte. For efficient selectivity the analytical method needs to be optimized for each particular analyte [43].

2.15.9 Measurement of uncertainty

Uncertainty is a parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand [77]. The uncertainty of a CRM should be taken into consideration even though it is generally negligible compared to the whole method uncertainties. Sources of uncertainties in GC methods analysis include;

1) Electronic balances
2) Volumetric glassware
3) GC injection volume on needle
4) Thermometers
5) Effect of temperature fluctuations
6) Purity of standards
7) Purity of reagents
8) Recovery results
9) Quality control data
10) Linearity of calibration curves
11) Bad separation or non-selective detection of the target analytes (from each other or from the matrix)
12) Re-isomerisation, decomposition and transformation of the target analytes
13) Low and largely differing detection sensitivity of some of the analytes
14) Integration error [48].

Matrix RMs usually have large uncertainties compared to RMs made up in pure solvents, hence proper diligence and attention should be taken with their use, furthermore it presents a challenge when applying them on samples as the same calibration should be used [43].

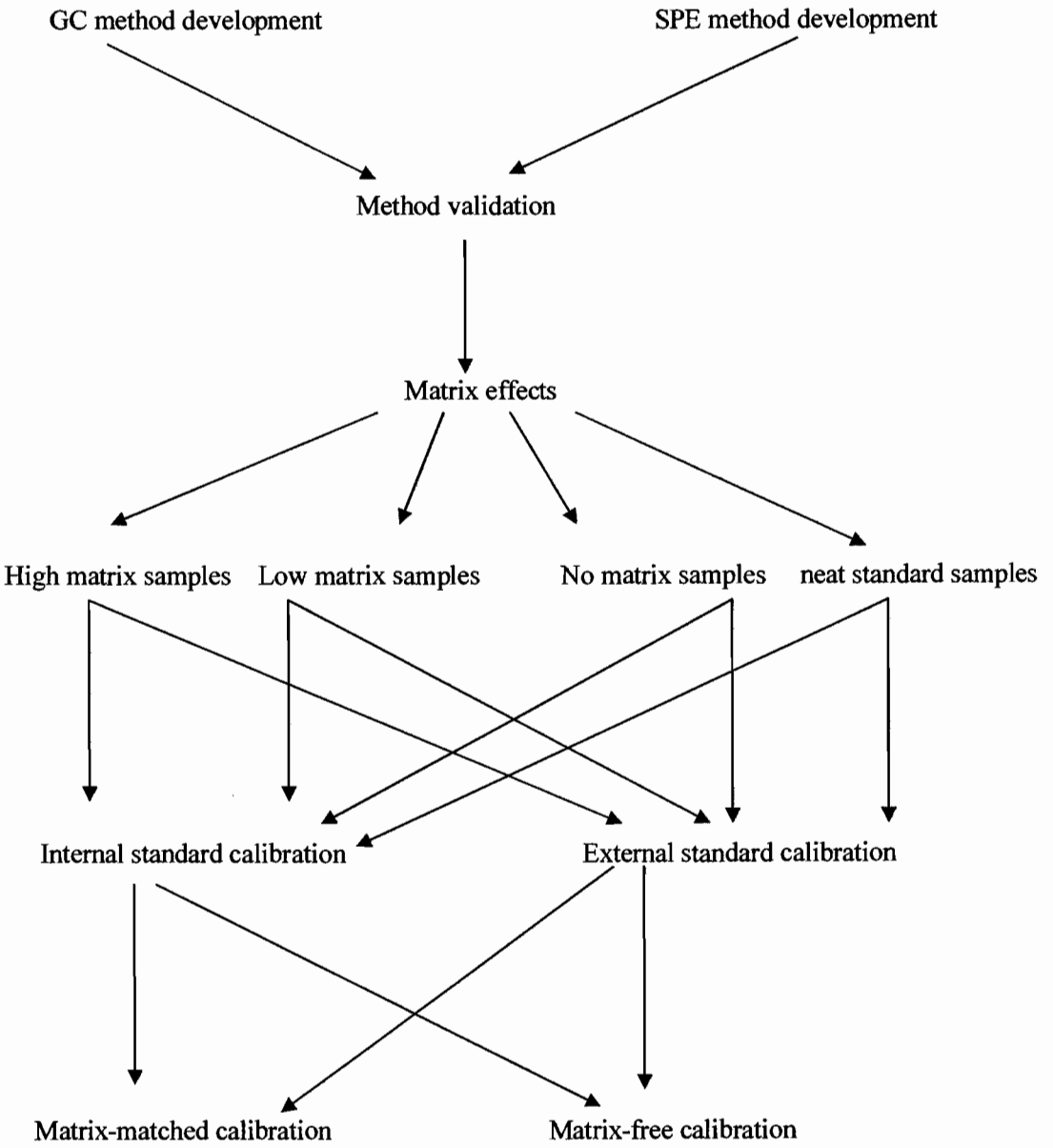


Figure 2. 4: Conceptual framework

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CHAPTER 3

EXPERIMENTAL METHODS

3.1 Introduction

The development of a robust sample collection and analytical methodology is important to ensure accurate measurements are subsequently attained. It was the goal of the sampling plan to preserve the hydrochemical properties of the water samples up to the point of sample analysis, particularly to safeguard any qualitative and quantitative properties potentially related to the integrity of the persistent organic pesticides potentially present within the samples collected. Emphasis was on the development and application of problem oriented sample handling techniques designed to maintain the time dependent characteristics of the water samples. The methodology was designed in line with the Stockholm convention on Persistent Organic Pollutants which includes the organochlorine pesticides under study and highlights the role of monitoring and controlling the discharge of toxic chemicals towards sustainable socio-economic development.

3.2 Research design

This research is a fully crossed design as the experimental units tested could take on all possible combinations across all factors tested (that is high-matrix, low-matrix and no-matrix) [1]. This allowed studying the effect of each factor on each variable (that is organochlorine pesticide, calibration type and calibration technique employed) as well as testing the interactions of different factors on each variable. The research involved the collection of real environmental samples from three main points on the Jukskei River identified as potential sources of persistent organic pollutants, particularly organochlorine pesticides. The analysis of the samples collected depended on successful completion of the following steps:

1) Planning

The planning stage was necessary to define the objective and purpose of the methodology applied and how the information obtained would be applied. Planning also helped to identify the limitations of the planned methodology and outline the implementation and interpretation of the findings [2].

2) Sample selection

The selection of the samples and sampling plan was guided by the purpose of the research. The sampling site selection was based on the presence of organic micro-pollutants within the water column.

3) Sample preparation and analysis

The main objective of sampling and sample analysis was to extract useful information about the properties of the sample, whose properties in turn determine the nature of the sample population, which is the Jukskei River catchment area. The information generated was used to create suitable sample matrices for use in support of the main specific objectives of this research.

4) Statistical analysis of the measurements

Statistics refer to the analysis and interpretation of data with a view towards objective evaluation of the reliability of the conclusions based on the data [1]. Both inferential and descriptive statistics were applied to this research. Statistical analysis for this research was done in line with method validation parameters such as the estimation of linearity, verification of linearity, accuracy, precision, Limit of Detection (LOD), Limit of Quantification (LOQ) and coefficient of regression (R^2) [3]. The descriptive statistics analysis tool generated a report of univariate statistics for the data and was used to summarise the data set obtained. Inferential statistics was used to draw conclusions on the population [1].

Univariate statistical analysis was done through the use of distributions and measures of dispersion such as standard deviation and calibration range. Inferential statistics was carried out through the use of a multi-way factorial Analysis of Variance (ANOVA) which assesses the effects of the variables (calibration techniques and different organochlorine pesticides) across three factors, namely high-matrix samples, low-matrix samples and no-matrix samples. A three factor analysis of variance was done using the statistical computer program Statistical Package for the Social Sciences (SPSS).

3.3 Sampling

The underlying principles of sampling are based upon the extrapolation of part of the sample population to obtain a representative sample. The population can be defined as the whole material whose properties are being investigated and a sample being a fraction of the population selected for analysis [4]. Since the water analytical procedure could not be done in situ, it was important to obtain representative volumes of water from the sampling points.

The main objective of the sampling was to obtain suitable environmental organic matrix water samples for the purpose of studying the influence of the sample matrix on the selective determination of selected organochlorine pesticides. The exact nature of the sample matrix at each day of sampling was not known therefore it was decided to obtain one representative sample from a mixture of all the samples collected at each sampling point.

3.3.1 Sampling plan

The sampling plan was designed taking into consideration the fact that the sample population is dynamic, that is, it changes over time therefore it was decided to sample over seven months in order ensure that a more representative sample matrix was attained.

3.3.2 Sampling site selection

The sampling sites were selected by using the stratified sampling method which is an important sampling technique as it allowed the use of logic and judgement to obtain samples with desired properties of interest [4]. This sampling technique was made possible by using previous pre-existing data gathered from water monitoring from these points which indicated that there were significant persistent organic pollutants to make up a significant organic matrix within the samples collected [5]. This enabled the population to be separated into three different strata or sub-groups whose properties formed a suitable organic matrix.

This sampling technique was very useful, particularly because the aim of the research was not to study the properties of the whole population but to obtain a representative real sample matrix required to fulfil the main specific objectives of this research. Since the variables by which the samples were stratified are strongly correlated with the variable of interest, that is the sample matrix, the stratified sampling method proved to be the best sampling technique to be employed for this research.

Identified sources of contamination observed near the sampling points include;
Site 1: agricultural land and a sewage treatment works (N14 along the Jukskei River).
The over-application of agrochemicals such as pesticides and herbicides in agricultural land and discharge of organic pollutants from the sewage treatment works were potential threats identified on site 1.
Site 2: an urban settlement and water treatment works (Midrand along the Jukskei River).
The proximity of sampling site 2 to an urban settlement exposed the water channel to a wide range of organic contaminants. Human activities have been identified as one of the contributors to organic pollution of freshwater bodies.
Site 3: an industrial area (Marlboro along the Jukskei River).

The industrial area situated upstream of site 3 is a source of synthetic organic micro-toxicants such as benzene hexachloride (BHC) isomers which are a by-product from industries such as paper and pulp factories and any other industrial processes which involve the use of chlorine bleaches.

The three sampling points were established downstream of the potentially polluting areas. The combination of socio-economic activities including urbanisation, industrial operations and agricultural production coupled with the naturally occurring toxicants within the same watershed created a complex sample matrix which is paramount for this research.

3.3.3 Sampling techniques

The samples were collected by a sub-surface grab method which is one of the simplest sampling techniques.

3.4 Sampling procedure

1) The four litre amber bottle was transported to the sampling site where the water appeared well mixed.
2) The bottle and cap of the bottle were rinsed twice with stream water.
3) The sample was taken 30 centimetres below the surface of the stream at a 45 degree angle to the direction of the flow.

3.4.1 Sampling frequency

Samples were collected every Monday at midday between 7 January and 26 July 2010 from the selected three points and stored at a temperature of ≤5 °C.

3.4.2 Sampling apparatus

A cooler box with ice, four litre glass amber bottles with caps and a water resistant waders were the standard sampling apparatus used.

3.5 Sample preparation

The sample preparation procedure consisted of the following steps:

- Sample receipt and storage
    - 102 samples were collected between January and July 2010.
- Sub-sampling
    - Often the samples collected are too large to be analysed using a laboratory procedure therefore sub-sampling may be essential. 196 mL of each of the 102 samples collected were sub-sampled and mixed into a 20 litre glass container to create a composite sample. The 20 litre glass container was stoppered and mixed by agitating and swirling the container.
- Sample filtration
    - The samples were filtered to remove any particulate matter that may potentially block the SPE sorbent bed.
- SPE analysis
    - The Gilson GX-274 ASPEC liquid handling instrument was used for sample preparation and the optimised methodology was used.

3.5.1 Developed methodology

Samples and cartridges were arranged in corresponding wells on the Gilson liquid handling instrument deck.

Figure 3.1: Automated Gilson GX-274 ASPEC liquid handling instrument

The sample analysis sequence was set up on the Gilson liquid handling instrument and simulated using the following parameters;

- Conditioning the cartridge
- Loading sample onto the cartridge
- Drying the cartridge using nitrogen gas
- Eluting the cartridge.

After a successful simulation the sample sequence was run, the extracts were removed from the well and 5 µL dodecane (keeper) reagent was added to each sample before blowing down the sample to near dryness using nitrogen gas. 1 mL toluene was added to the test tube and mixed using a vortex mixer before being transferred to a 2 mL sample vial. The sample vial was then transferred to the GC autosampler for subsequent GC-MS analysis after setting up the injection sequence.

3.6 Apparatus and reagents

The apparatus and reagents used include; sample containers, sample waste containers, Pasteur pipettes and rubber bulbs, calibrated glass pipettes, calibrated micro-syringes, measuring cylinders, SPE cartridges, toluene, dichloromethane, acetone, dodecane, nitrogen gas, a vortex mixer, weighing boats, calibrated volumetric flasks and organochlorine standards .

3.7 Preparation of stock solutions

Stock standard solutions of 100 mg/L were prepared by weighing 10 mg of pure standard material into a weighing boat before transferring to a 100 mL volumetric flask and topping up to the mark with toluene and carefully dissolving by using the vortex mixer.

The 1 mg/L cocktail solution was prepared by adding 1 mL of each of the 20 pure organochlorine pesticide solutions into a 100 mL volumetric flask and topping up to the mark with toluene and mixing well by shaking the volumetric flask. The calibration standards were made up by serial dilution from the 1 mg/L cocktail solution to yield 8 calibration level standards [6].

3.7.1 External standard calibration method

For the external standard calibration method the calibration standards and samples were analysed on the GC-MS without any modifications.

3.7.2 Internal standard calibration method

For the internal standard calibration technique, the calibration standards and samples were spiked with 10 µL of a 100 mg/L solution of the selected internal standard, Pentachlorobenzene and analysed on the GC-MS.

3.7.3 Recovery (spike) solution

The quality control and spike cocktail solution with a concentration of 0.4 mg/L was made up by pipetting 40 mL of the 1 mg/L cocktail solution into a 100 mL volumetric flask and topping to the mark with acetone.

3.7.4 Surrogate solution

1 µL of the 100 mg/L surrogate solution, 4,4' dichlorobiphenyl was added to each sample prior to analysis to test the efficiency of the sample extraction analytical procedure. The surrogate standard was monitored during sample analysis.

3.8 Bias and sampling errors

Bias was an important consideration in this research as it was aimed to obtain samples with a particular characteristic. The stratified sampling method was selected because it provided enough bias to obtain a representative sample with the properties of interest. Bias is also termed systematic error and is calculated as the standard error. It is however difficult to calculate the sampling standard error in this research, considering that the sample population is dynamic. The degree of heterogeneity can however be estimated from the sample data using standard deviation. Samples collected using stratified sampling methods tend to have both significantly high sampling errors and bias.

3.9 Sample analytical procedure

The aim of the sample analytical procedure was to configure a methodology that would promote the highest sample analyte extraction. Different sample and apparatus treatment processes were applied to determine the optimum extraction efficiency processes.

3.10 Quality control

All volumetric flasks and pipettes were calibrated before use. Analytical balances were calibrated annually and verified using reference masses daily. Grade A volumetric glassware and analytical (pesticide) grade reagents were also used for the entire analysis with a purity >99%. All cartridge testing for SPE method development was done in at least duplicate analyses. Deionised ultrapure water was sourced from a Millipore Milli-Q system. The water was passed through an organic compound scavenger resin bed before passing to the Milli-Q system. The certified pesticide neat standards had a purity of at least 98.5% (obtained from Dr Ehrenstorfer and Chemservice) and 100 mg/L stock solution and subsequent cocktails were prepared in toluene and stored at ≤-18°C. Spiking solutions were prepared in acetone. Temperatures for the laboratory atmosphere and freezers were monitored daily.

3.11 GC-MS instrument configuration

An Agilent Technologies 6890 GC coupled to an Agilent Technologies 5975 Quadrupole Mass Selective Detector was used for analysis using a 30m x 0.25mm x 0.25µm DB-5MS column with stationary phase 5% phenyl and 95% dimethylpolysiloxane. The mobile phase of choice used was 99.999% helium gas supplied by Airliquide South Africa.

Total runtime for the analysis was 31.87 minutes with initial temperature of 70 °C and hold time of 2 minutes. Ramp 1 was 25 °C/ min to 150 °C, with no hold time. Ramp 2 was 3 °C/min to 200°C, with no hold time and ramp 3 was 8 °C/min to 280 °C with no hold time. A constant pressure of 129.9 KPa was maintained with an average velocity of 50 cm/second. Data was analysed using Chemstation software from Agilent Technologies. A 1 µL volume of sample was injected using Gerstel autosampler [7].

Figure 3.2: The Gas Chromatography-Mass Spectrometer (GC-MS)

3.12 Peak identification and data assessment

To identify the peaks of interest, 10 ppm neat standards were injected to determine the retention time for each analyte. To increase the specificity of the analytical method, particularly in the presence of a matrix, Selective ion monitoring (SIM) mode was configured into the GC-MS. An average of 4 major ion fragments from each analyte were selected for use in identification of the compounds, using criteria of a balance between the highest mass and abundance, since each compound has a specific ion spectrum, with reference to ions from previously published work [7].

Table 3.1: Target and qualifier ions used for SIM analysis

Peak No.	Peak name	(min)	T	Q1	Q2	Q3
1	Pentachlorobenzene (IS)	8.96	250	108	213	252
2	BHC-alpha	12.08	181	183	217	219
3	Hexachlorobenzene	12.431	284	249	142	214
4	BHC-beta	13.194	181	109	219	217
5	Lindane (BHC gamma)	13.444	181	217	109	219
6	PCNB	13.705	237	295	249	239
7	BHC-delta	14.505	183	219	217	109
8	Heptachlor	16.824	272	237	337	135
9	Aldrin	18.578	263	293	66	186
10	Heptachlor-epoxide	20.765	353	237	263	253
11	Chlordane, *trans* (gamma)	22.076	375	272	237	263
12	Endosulfan alpha	22.663	170	241	195	265
13	Chlordane *cis* (alpha)	22.868	373	272	263	339
14	Dieldrin	23.899	263	277	265	108
15	DDE 4,4'	24.059	246	318	176	316
16	Endrin	24.753	263	245	81	317
17	Endosulfan beta	25.178	195	237	265	159
18	DDD 4,4'	25.738	235	237	165	199
19	Endosulfan SO4	26.783	272	229	387	237
20	DDT 4,4'	27.009	235	237	199	165
21	Mirex	29.887	272	274	237	332

T= Target ion; Q= Qualifier ion; IS= Internal standard

3.13 Investigation of the matrix effects

The following parameters were investigated for the efficiency of quantification and detection of the organochlorine pesticides under study;

- Effect of the synthetic matrix
- Effect of the real sample matrix
- Effect of matrix-matched standards
- Effects of using internal standard and external standard calibration

3.14 Test matrices used

The test matrices used to test the matrix effects were:

- Blank matrix neat sample which consisted of the OC cocktail in toluene solvent
- Ultrapure spiked water (no-matrix) sample which consisted of deionised water spiked with 1 mL of the 0.4 ppm OC cocktail
- Low-matrix water sample which consisted of the representative real sample water spiked with 1 mL of the 0.4 ppm OC cocktail.
- High-matrix water sample which consisted of the low-matrix water sample spiked with 5 mL of a 1 mg/µL solution which consisted of 40 persistent organic pollutants excluding any organochlorine pollutants but including organophosphates, phthalates, triazines, Poly Aromatic Hydrocarbons (PAHs) and pyrethroids.
- Blank neat individual standards which consisted of individual organochlorine standards in toluene solvent.

3.15 Matrix-matched standards

Matrix-matched standards were also treated and analysed as samples. Seven calibration levels were selected which included the 1 ppm, 0.5 ppm, 0.25 ppm, 0.125 ppm, 0.0625 ppm, 0.0313 ppm and 0.0156 ppm standards. 100 mL of the real sample matrix were spiked with 1 mL of each calibration level and analysed as for the other samples to obtain a 1 mL extract which was used for calibration.

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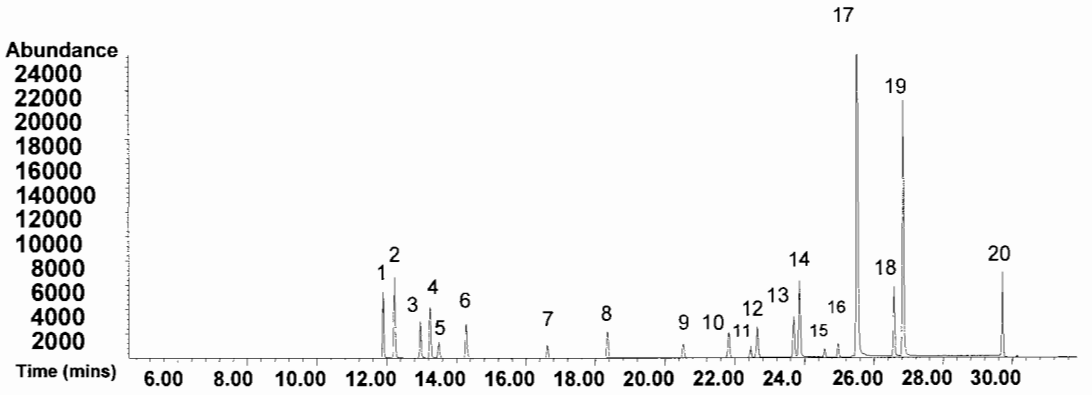
CHAPTER 4

PRESENTATION OF RESULTS

4.1 Method development for GC-MS

The recent technological developments in the synthesis of commercial pesticides has led to the production of more polar and thermally labile compounds, rendering their analysis with GC more complicated. The GC-MS method development was aimed at developing and validating a highly selective and sensitive multiresidue analytical method for the analysis of the selected organochlorine pesticides.



1. BHC alpha 2. Hexachlorobenzene 3. BHC beta 4. BHC gamma 5. Pentachloronitrobenzene 6. BHC delta 7. Heptachlor 8. Aldrin 9. Heptachlor epoxide 10. Chlordane trans (gamma) 11. Endosulphan alpha 12. Chlordane cis 13. Dieldrin 14. 4,4' DDE 15. Endrin 16. Endosulphan beta 17. 4,4' DDD 18. Endosulphan sulphate 19. 4.4'DDT 20. Mirex

Figure 4.1: SIM chromatogram of organochlorine cocktail.

Figure 4.1 above shows the SIM Chromatogram of a 1 mg/L organochlorine cocktail analysed using the developed GC-MS methodology.

4.1.1 GC-MS instrument method validation

Method validation is essential as it confirms that an analytical method is effective in measuring the parameters it is intended to measure. Successful validation of this instrument method validation confirms that the methods, procedures and protocols applied in the analysis produce reliable and accurate data and also ensure that valid conclusions are postulated as a result of the validated method.

4.1.2 Validation parameters

For the purposes of method validation the parameters tested were linearity, linearity verification by Excel, working range, repeatability, reproducibility, limits of detection, limits of quantification and analysis of variance (ANOVA).

4.1.3 Linearity

Eleven independent calibration curves were prepared for the purposes of validating the linearity of each analyte. The results are displayed below.

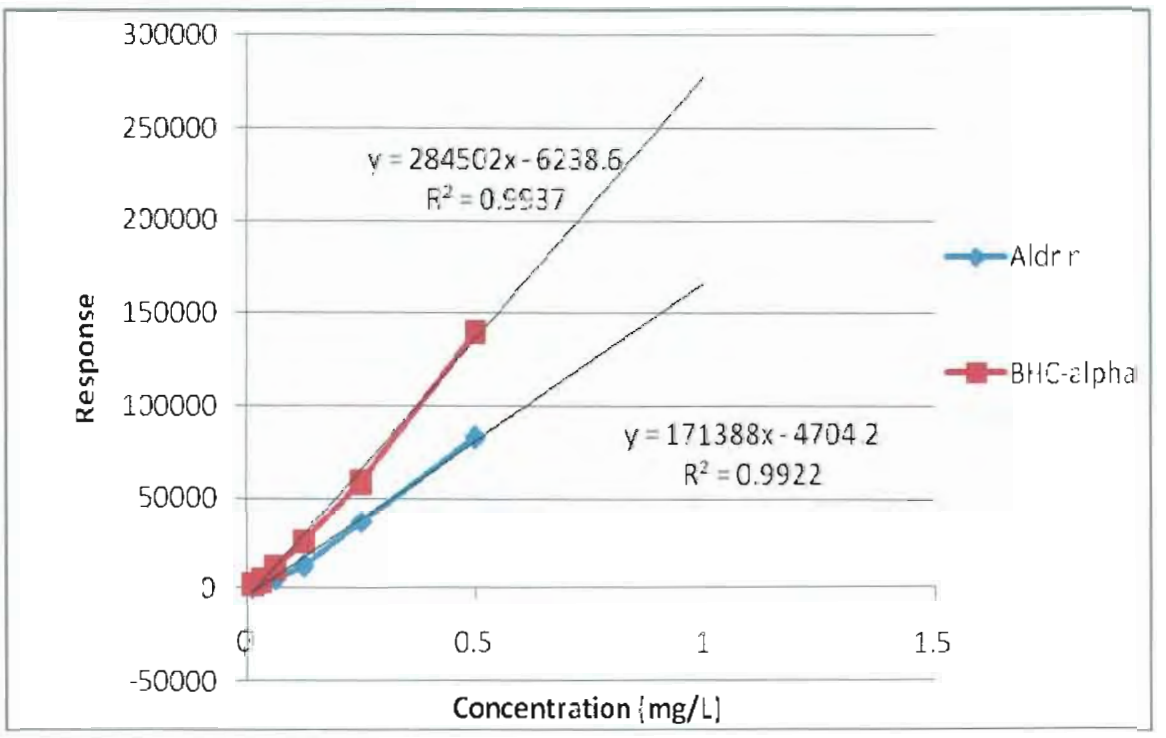


Figure 4.2: Calibration curves for Aldrin and BHC alpha

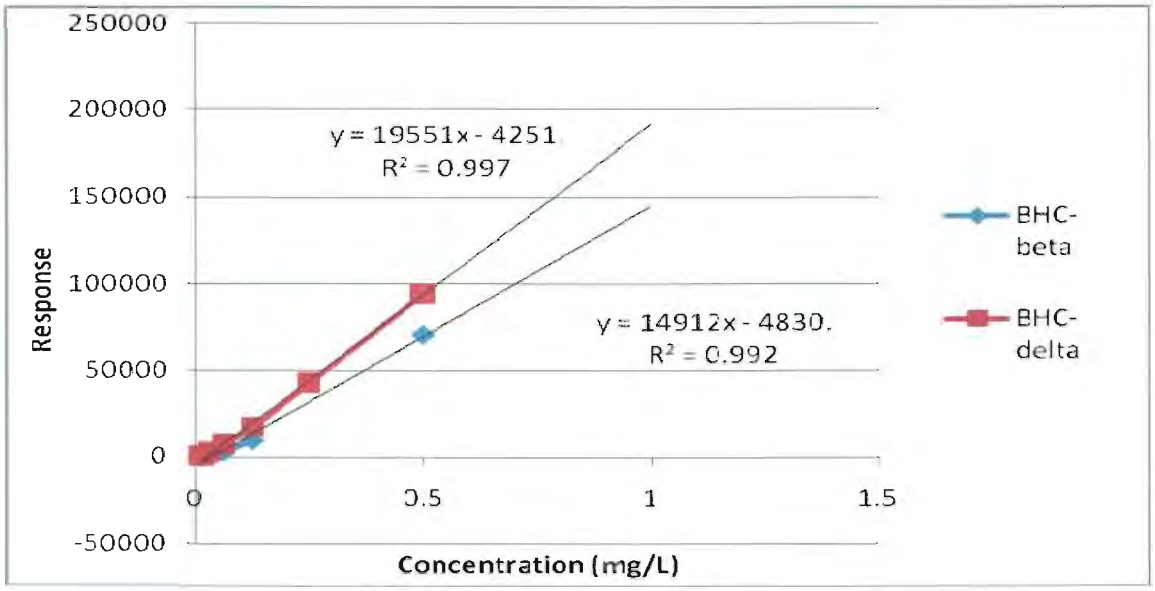


Figure 4.3: Calibration curves for BHC beta and BHC delta

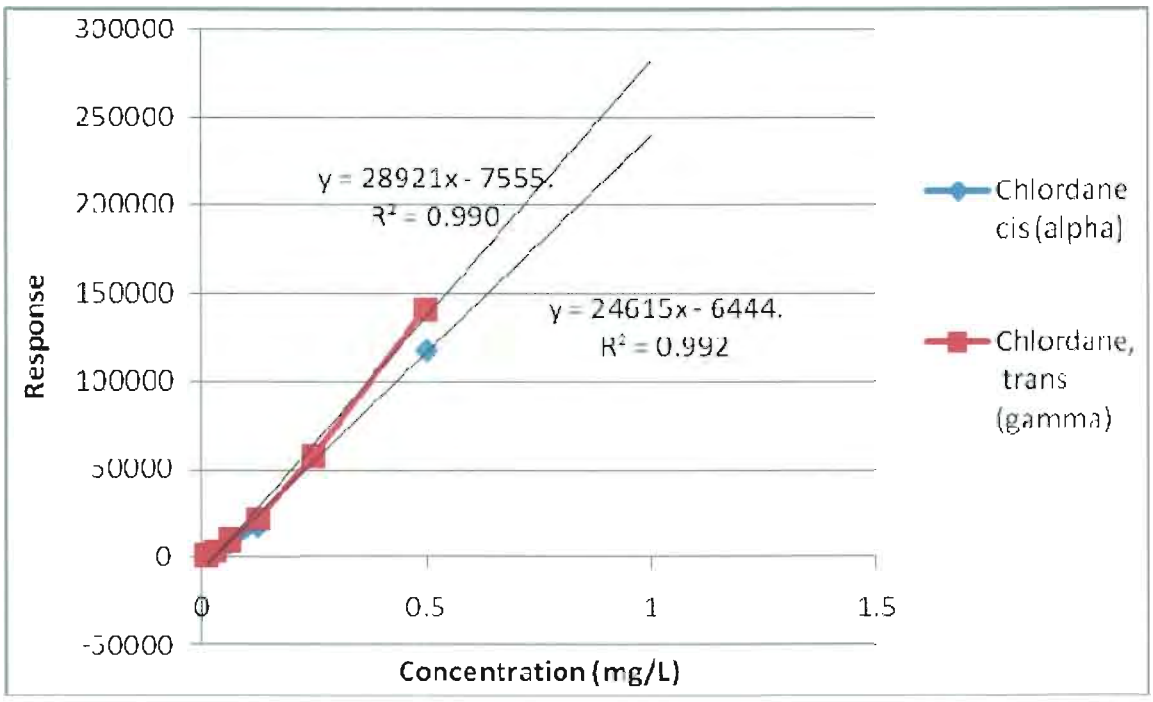


Figure 4.4: Calibration curves for Chlordane *cis* (alpha) and Chlordane *trans* (gamma)

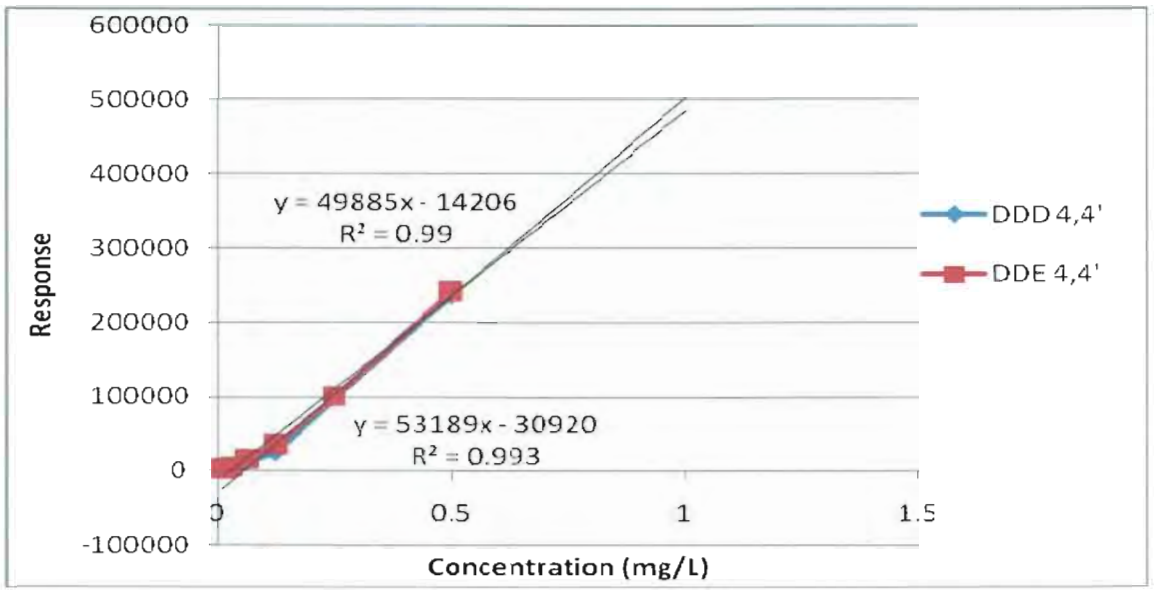


Figure 4.5: Calibration curves for 4,4' DDD and 4,4'DDE

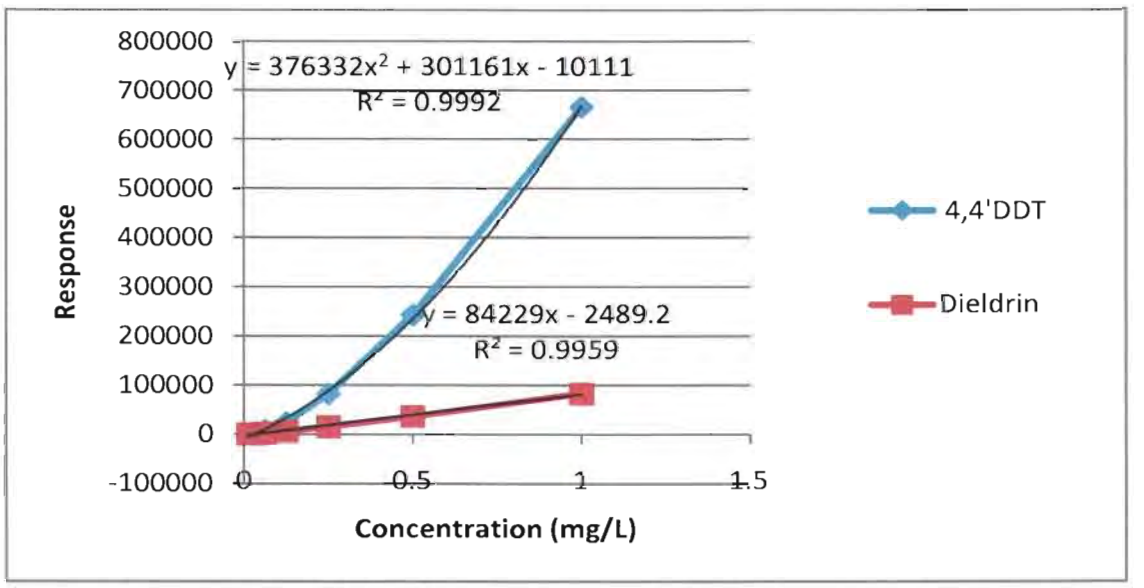


Figure 4.6: Calibration curves for 4,4'DDT and Dieldrin

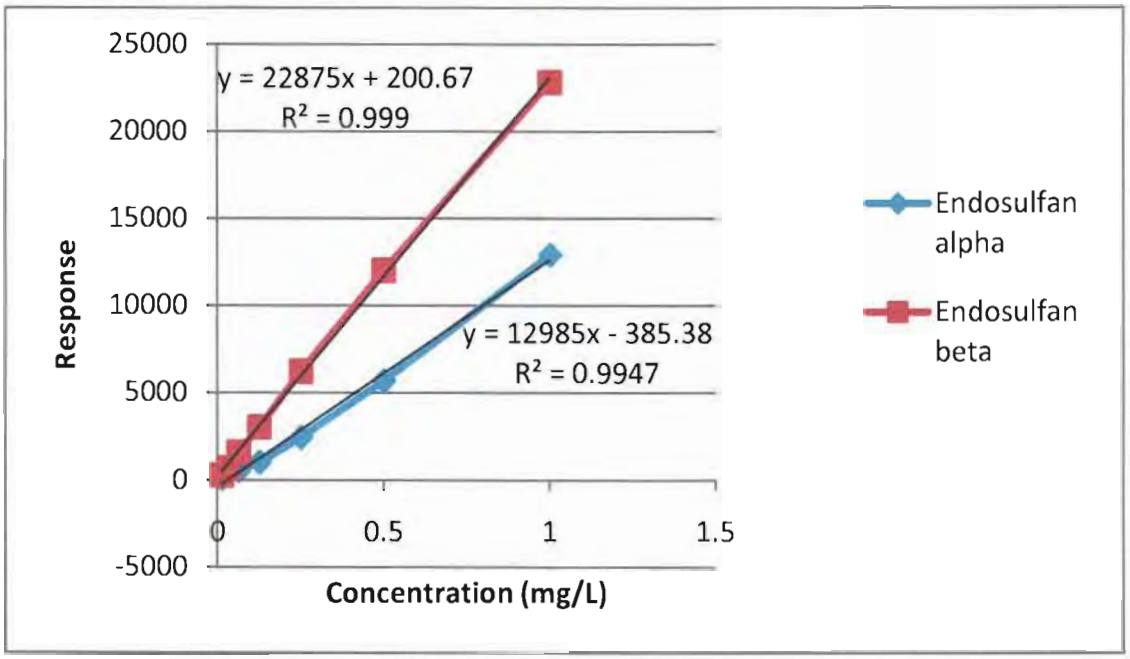


Figure 4.7: Calibration curves for Endosulphan alpha and Endosulphan beta

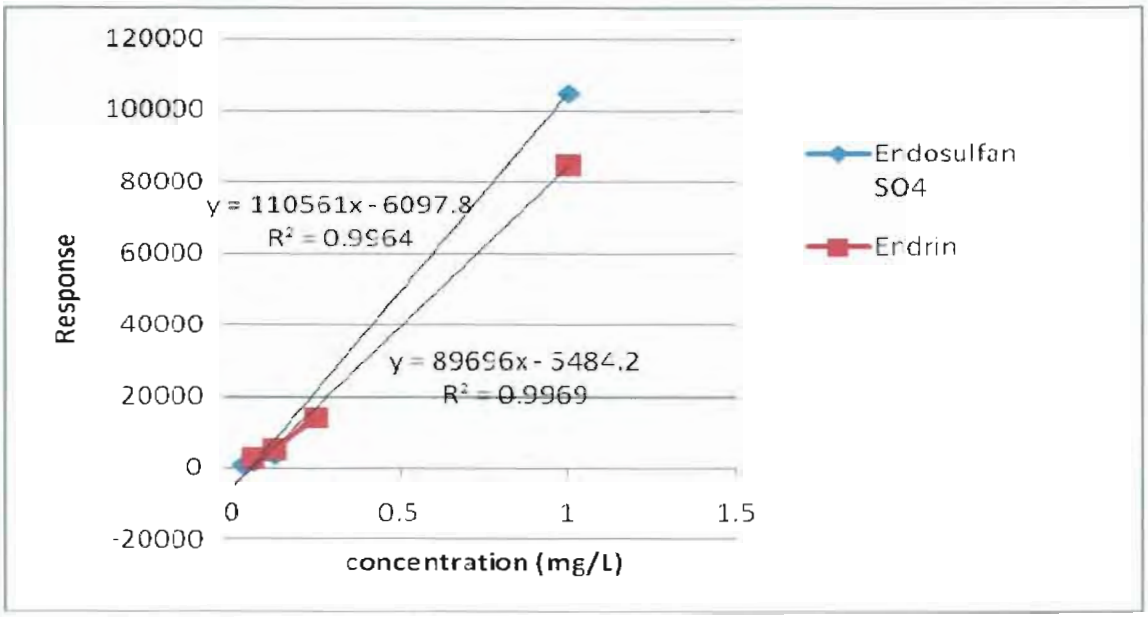


Figure 4.8: Calibration curves for Endosulphan Sulphate and Endrin

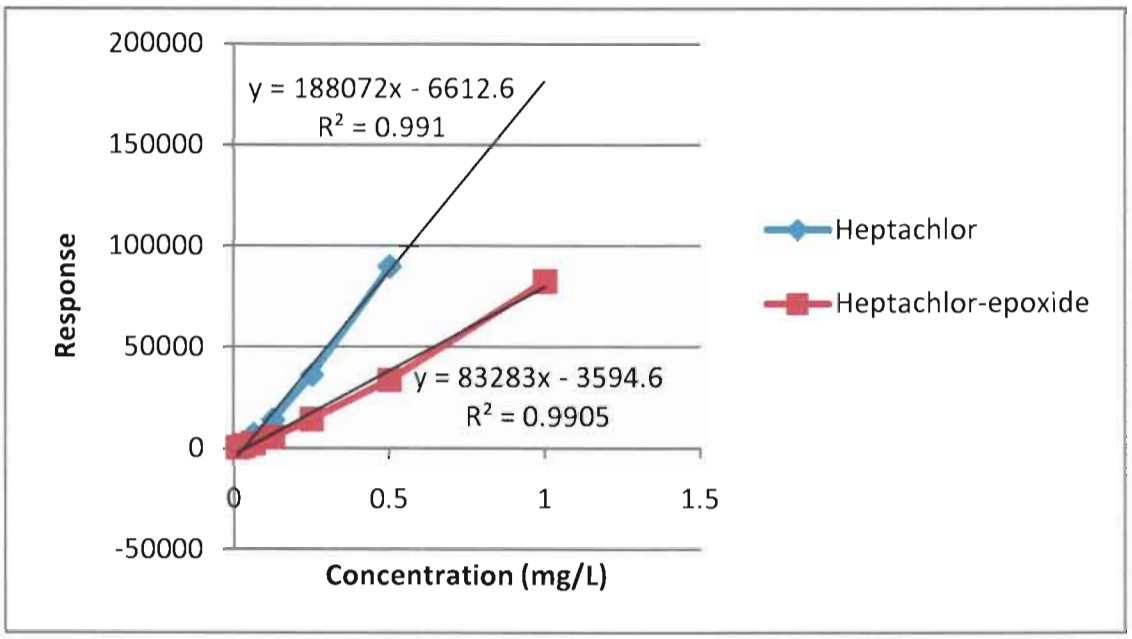


Figure 4.9 Calibration curves for Heptachlor and Heptachlor-epoxide

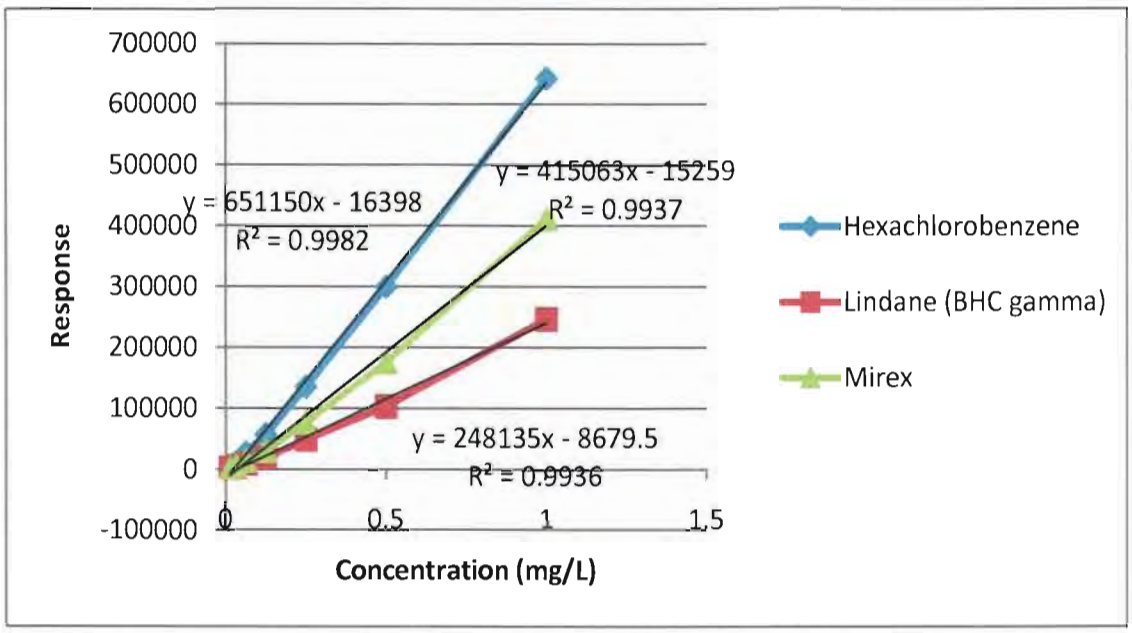


Figure 4.10 Calibration curves for Hexachlorobenzene, Lindane and Mirex

Table 4.1: Linear and non linear regression

Organochlorine Compound	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
Aldrin	0.9988	0.9994	0.9989	0.9990	0.9986	0.9985	0.9997	0.9986	0.9985	0.9986	0.9996
BHC-alpha	0.9996	0.9997	0.9986	0.9995	0.9986	0.9982	0.9987	0.9996	0.9993	0.9995	0.9994
BHC-beta	0.9980	0.9992	0.9981	0.9983	0.9984	0.9986	0.9987	0.9991	0.9989	0.9994	0.9991
BHC-delta	0.9988	1.0000	0.9990	0.9995	0.9988	0.9985	0.9984	0.9989	0.9999	0.9993	0.9985
Chlordane *cis* (alpha)	0.9992	0.9989	0.9983	0.9991	0.9987	0.9990	0.9985	0.9980	0.9982	0.9989	0.9988
Chlordane, *trans* (gamma)	0.9988	0.9998	0.9987	0.9988	0.9991	0.9982	0.9990	1.0000	0.9983	0.9990	0.9987
DDD 4,4'	0.9994	0.9986	0.9982	**1.0000**	0.9990	0.9989	0.9995	0.9996	0.9991	0.9988	**1.0000**
DDE 4,4'	0.9994	0.9997	0.9987	0.9991	0.9983	0.9990	0.9987	0.9983	0.9980	0.9990	0.9984
DDT 4,4'	**0.9999**	0.9987	**0.9998**	0.9998	**0.9998**	**1.0000**	**0.9999**	0.9995	**1.0000**	**1.0000**	**1.0000**
Dieldrin	0.9998	0.9994	0.9987	0.9984	0.9983	0.9985	0.9981	0.9988	0.9991	0.9996	0.9986
Endosulfan alpha	0.9988	0.9986	0.9981	0.9984	0.9990	0.9999	0.9990	0.9984	0.9986	0.9983	0.9988
Endosulfan beta	0.9999	0.9997	0.9992	0.9998	0.9997	0.9997	0.9995	0.9991	1.0000	0.9983	0.9995
Endosulfan SO4	0.9996	0.9998	0.9986	0.9998	0.9992	0.9986	**1.0000**	0.9995	0.9988	**1.0000**	0.9989
Endrin	0.9982	0.9991	0.9991	0.9989	0.9985	0.9987	0.9991	0.9995	**1.0000**	0.9999	0.9982
Heptachlor	0.9981	**0.9999**	0.9995	0.9980	0.9983	0.9993	0.9989	0.9992	0.9989	0.9987	**1.0000**
Heptachlor-epoxide	0.9982	0.9998	0.9998	0.9992	0.9981	0.9991	0.9995	0.9992	0.9983	0.9991	**1.0000**
Hexachlorobenzene	0.9989	0.9998	0.9988	0.9994	0.9988	0.9990	0.9987	0.9988	1.0000	0.9987	0.9991
Lindane (BHC gamma)	0.9981	0.9985	0.9991	0.9986	0.9981	0.9995	0.9983	0.9987	0.9990	0.9988	0.9997
Mirex	0.9986	0.9989	0.9997	0.9981	0.9988	0.9988	0.9982	0.9987	0.9992	0.9985	0.9993

Key

Non-Bold figures= linear fit; Bold figures= non-linear fit; C= calibration curve regression

For most instruments, a linear response is expected from calibration standards made by serial dilution from a stock solution. Not all compounds analysed by GC-MS displayed a linear fit on calibration though, this can largely be attributed to either re-isomerisation, decomposition or transformation of the target analytes, either within the chromatographic system or before introduction into the chromatographic system [1]. This leads to greater uncertainties in GC measurements. The MS detector responds to changes in the sample concentration then displays a non linear fit. Previous studies have shown that this loss of linearity in most compounds may largely be attributed to breakdown of the compounds due to high GC oven temperature [2]. Other researchers indicate that the breakdown of the compounds increases as the GC oven temperature increases therefore it is better to start off with lower GC oven temperatures on analysis. Contaminants from sample processing or analyte extraction from physiological matrices can be ionized together with the compound of interest, causing a phenomenon called

matrix signal suppression effects [3]. This effect can lead to loss of linearity especially if the samples are sandwiched evenly in between the standards during a sequence run.

For the purposes of this study, a linear curve graph displaying a regression of ≥0.998 with at least four calibration levels was considered to be significantly linear. A total of seven calibration levels were used to test the linearity of the fit and also to determine the calibration range. The linear curve does not always pass through the origin as this characteristic depends on the detection limit. The calibration curves show that the lower the detection limit, the closer the curve is to the graph origin (0,0).

The regression data displayed in bold shows calibration curves with a non-linear fit and those in non-bold show a linear regression. It can be deduced from the above data that Aldrin, BHC-alpha, BHC- beta, BHC delta, BHC gamma, Chlordane *cis* (alpha), Chlordane *trans* (gamma), 4,4' DDE, Dieldrin, Endosulphan alpha, Endosulphan beta, Hexachlorobenzene and Mirex all display distinct linear fit. Although Endrin, 4,4' DDD, Endosulphan sulphate, Heptachlor epoxide, and Heptachlor displayed between two and one non-linear calibration curves, it can also be deduced that they also show a linear fit.

Experience shows that problems associated with obtaining a non-linear calibration fit lie mainly within the chromatographic system [1]. Previous studies by Scientific Services Inc. have proved that the selection of the liner type, liner packing type, liner packing position, solvent volume used, injection volume used, injection technique, and oven temperatures used all have a profound effect on the linearity of a calibration fit of a specific compound.

4.1.4 Verification of linearity

The validity of the Chemstation software for computing the regression was determined by calculating the regression using Microsoft Excel. The results indicate that the Chemstation software was indeed effective in regression calculation. Minor differences in the resultant regression values between Chemstation and Excel were expected as

Chemstation overtly has advantages in manipulating plots such as forcing the curve through the origin so as to improve the quantification of analytes just near the detection limits, an action which is usually detrimental to achieving better coefficients of regression.

Table 4.2: Verification of linearity

Target Compound Name	0.0156 ppm Peak area	0.0313 ppm Peak area	0.0625 ppm Peak area	0.125 ppm Peak area	0.25 ppm Peak area	0.5 ppm Peak area	1 ppm Peak area	Excel Coeff-icient.	Chem-station Coeff-icient
Aldrin	828	2081	5509	12391	36234	82915	187754	0.9984	0.9989
BHC-alpha	2011	4265	11268	25493	58965	139748	328678	0.9990	0.9986
BHC-beta	216	618	3398	9971	27865	70698	175970	0.9985	0.9981
BHC-delta	526	2951	7466	17316	43235	94858	229780	0.9980	0.9990
Chlordane *cis* (alpha)	1243	2991	7788	17514	47731	118258	275377	0.9985	0.9983
Chlordane, *trans* (gamma)	1763	4005	10755	22208	58097	141647	330427	0.9993	0.9987
DDD 4,4'	872	2347	8267	25468	99298	287653	770668	0.9982	0.9982
DDE 4,4'	2901	5927	15950	36004	100849	242661	597541	0.9993	0.9987
DDT 4,4'	1144	2755	8645	23663	82610	243007	665803	**0.9984**	**0.9998**
Dieldrin	816	1365	3336	6833	16388	37147	83573	0.9999	0.9987
Endosulfan alpha	89	326	540	1021	2445	5690	12919	0.9986	0.9981
Endosulfan beta	295	702	1609	3049	6234	12020	22818	0.9990	0.9992
Endosulfan SO4	208	575	1445	3488	11482	39640	104852	0.9988	0.9986
Endrin	499	1043	2641	5365	14214	33876	84781	0.9994	0.9991
Heptachlor	1193	2587	6612	13874	36155	89913	225088	0.9980	0.9995
Heptachlor-epoxide	366	1092	2387	5416	14242	33721	82622	0.9991	0.9998
Hexachlorobenzene	5239	9797	26419	56425	134799	299664	642994	0.9983	0.9988
Lindane (BHC gamma)	1591	3340	8295	18814	48772	103280	246781	0.9982	0.9991
Mirex	2481	5564	14209	30241	76221	175292	411540	0.9995	0.9997

It should however be noted that the coefficient displayed for DDT 4,4' is the regression for the quadratic fit.

4.1.5 Hypothesis testing

ANOVA was used to test the hypothesis H_0 = there is no significant linearity for the selected organochlorine pesticides. Besides the fact that 4,4' DDT mostly showed no

significant linearity within the calibration range tested, it was also tested for linearity using the eight non-linear regression coefficients and three linear regression coefficients from the calibration curves

Table 4.3: F-calculated (F-cal) and F-critical (F-crit)values for ANOVA F-test

Target Compound Name	DF	F-Cal	F-Crit	Result
Aldrin	10	1228.5	6.613	Reject H_0
BHC-alpha	6	634.2	19.25	Reject H_0
BHC-beta	10	451.9	6.613	Reject H_0
BHC-delta	6	1907.4	19.25	Reject H_0
Chlordane *cis* (alpha)	10	725.4	6.613	Reject H_0
Chlordane, *trans* (gamma)	6	994.8	19.25	Reject H_0
DDD 4,4'	6	297.2	19.25	Reject H_0
DDE 4,4'	6	487.5	19.25	Reject H_0
*DDT 4,4'	12	224.4	4.818	Reject H_0
Dieldrin	8	858.7	9.013	Reject H_0
Endosulfan alpha	10	820.3	6.613	Reject H_0
Endosulfan beta	12	4856.5	4.818	Reject H_0
Endosulfan SO4	4	810.6	215.7	Reject H_0
Endrin	4	1544.9	215.7	Reject H_0
Heptachlor	4	504.9	215.7	Reject H_0
Heptachlor-epoxide	10	521.0	6.613	Reject H_0
Hexachlorobenzene	6	1179.8	19.25	Reject H_0
Lindane (BHC gamma)	8	1636.0	9.013	Reject H_0
Mirex	6	767.1	19.25	Reject H_0

*Calibration curves showed no significant linearity within the calibration range tested.

Applying the decision rule: If F-cal > F-crit = reject H_0, the F-test indicated that for all selected organochlorine compounds the decision was to reject the null hypothesis

therefore there was indeed significant linearity within the selected organochlorine compounds tested for linearity.

Table 4.4: Calibration ranges, coefficient of regression, repeatability and sensitivity for selected organochlorine compounds

Compound Name	Linear Range	R^2	Repeatability (RSD%)	LOD	LOQ
Aldrin	1-0.0313	0.9990	6.45	0.038	0.125
BHC-alpha	1-0.125	0.9984	9.63	0.062	0.205
BHC-beta	1-0.0313	0.9985	3.92	0.060	0.201
BHC-delta	0.5-0.0313	0.9980	4.59	0.018	0.060
Chlordane *cis* (alpha)	1-0.0313	0.9995	3.22	0.048	0.161
Chlordane, *trans* (gamma)	1-0.125	0.9993	3.23	0.049	0.162
DDD 4,4'	1-0.125	0.9982	5.93	0.083	0.277
DDE 4,4'	1-0.0156	0.9993	4.31	0.068	0.228
DDT 4,4'	Non linear	Non linear	5.25	0.078	0.261
Dieldrin	0.5-0.0313	0.9999	3.75	0.025	0.082
Endosulfan alpha	1-0.0313	0.9986	4.23	0.047	0.155
Endosulfan beta	1-0.0156	0.9990	2.65	0.019	0.063
Endosulfan SO4	1-0.25	0.9988	7.17	0.027	0.091
Endrin	0.5-0.125	0.9994	5.81	0.021	0.071
Heptachlor	0.5-0.125	0.9980	5.17	0.037	0.123
Heptachlor-epoxide	0.5-0.0156	0.9991	4.39	0.029	0.095
Hexachlorobenzene	0.5-0.0625	0.9983	2.75	0.023	0.077
Lindane (BHC gamma)	0.5-0.0313	0.9982	4.20	0.018	0.059
Mirex	1-0.125	0.9995	4.41	0.056	0.187

The above Table 4.4 shows the calibration range in which acceptable accuracy, linearity and precision can be obtained.

4.1.6 Calibration range

The calibration ranges for the selected organochlorine compounds were tested using 1 ppm, 0.5 ppm, 0.25 ppm, 0.125 ppm, 0.0625 ppm, 0.0313 ppm, 0.0156 ppm and 0.0078 ppm calibration standards. The 0.0078 ppm standard was then rejected as it was extremely difficult to distinguish between background (noise) peaks and the analyte

4.2 SPE method Validation

The first parameter tested on the three cartridges, namely Strata-C18 E 200 mg, (Supelco) LC-18 200 mg and Strata-X 500 mg was the effect of conditioning versus non-conditioning of the cartridges. Table 4.5 below shows the responses and concentration obtained when 2 mL of a 1 ppm OC cocktail solution was loaded onto the unconditioned cartridges and the elute obtained after loading was analysed on the GC-MS. The results indicate that no analytes were retained at all by the cartridges when the cartridges were not conditioned.

Table 4.5: Results of analyses of elute from non-conditioned cartridges

	Strata C-18-E 200 mg (1)		Strata C-18-E 200 mg (2)		LC-18 200 mg		Strata-X 500 mg	
Organochlorine Name	Conc (ng/uL)	Resp	Conc (ng/uL)	Resp	Conc (ng/uL)	Resp	Conc (ng/uL)	Resp
Pentachlorobenzene	1.113	1462403	1.167	1533079	1.214	1595141	1.18	1550510
BHC-alpha	1.184	595261	1.147	576705	1.272	639636	1.131	568568
Hexachlorobenzene	1.024	1287321	1.006	1263891	1.091	1370946	0.98	1231640
BHC-beta	1.24	344372	1.236	343198	1.689	468851	0.906	251495
Lindane (BHC gamma)	1.114	463596	1.138	473308	1.35	561478	1.132	471060
Pentachloronitrobenzene	1.117	335068	1.211	363207	1.333	399689	1.21	362933
BHC-delta	1.149	403163	1.088	381712	1.295	454221	0.663	232569
Heptachlor	1.186	420281	1.193	422814	1.307	463271	1.226	434529
Aldrin	1.156	353107	1.146	350098	1.227	374607	1.151	351379
Heptachlor- epoxide	1.043	432743	1.084	449961	1.175	487594	1.065	441810
Chlordane *trans* (gamma)	1.052	591642	1.094	615146	1.197	673305	1.049	590035
Endosulfan (I) alpha	1.117	118907	1.169	124414	1.249	132999	1.147	122121
Chlordane *cis* (alpha)	1.108	612582	1.124	621501	1.218	673773	1.078	596319
Dieldrin	1.058	175624	1.092	181178	1.124	186598	1.114	184923
4,4'-DDE	1.127	1048065	1.205	1120256	1.334	1240591	1.144	1063264
Endrin	1.292	82119	1.346	85556	2.131	135409	2.101	133500
Endosulfan (II) beta	1.134	147288	1.242	161394	1.4	181970	1.247	161968
4,4'-DDD	1.082	6764500	1.133	7082600	1.402	8769624	1.119	6996213
Endosulfan sulphate	1.154	262244	1.23	279459	1.449	329309	1.197	272079
4,4' DDT	1.478	3581441	1.506	3649397	2.049	4963794	1.664	4030629
Mirex	1.083	1003676	1.091	1011243	1.108	1027480	1.068	989883

The data above shows that the elute showed a higher response and concentration than the original 1 ppm concentration initially loaded onto the cartridge. This shows the

phenomenon called matrix-induced enhanced chromatographic effects and it also explains the rationale for poor accuracy for some data generated by routine GC methods employing traditional calibration strategies for quantification of analytes [4,5]. External calibration methods of injecting neat calibration standards were performed for quantification and the results indicate that the synthetic matrix indeed had an effect on the quantification of the analytes after elution even in the absence of a real sample matrix.

4.2.1 Results of conditioned cartridges

2 mL of a 1 ppm cocktail solution of the organochlorine compounds was loaded onto a cartridge previously conditioned using 2 mL methanol. The elute obtained after loading was collected and analysed by GC-MS. The results of GC-MS analysis of the elute indicate that there was significant analyte retention by all cartridges, particularly the LC-18 and Strata X cartridges whose results indicate that significant quantities of the analytes were adsorbed by the solid phase as shown by low analyte concentrations detected within the elute as shown in figure 4.11 below. Most notably is the absence of BHC-delta in the elute extracted using the LC-18 cartridge indicating that there was up to 100% retention. It can therefore be deduced that it is essential to condition the cartridges before use, with the degree of conditioning depending upon the nature of the sorbent bed and the sorbent bed mass. These results are in sync with findings by Poole *et al.* (2000) who postulated that the high surface tension of water often causes slow and uneven flow rates through solid phases when cartridges are not conditioned first before loading the sample, resulting in low analyte recovery [6].

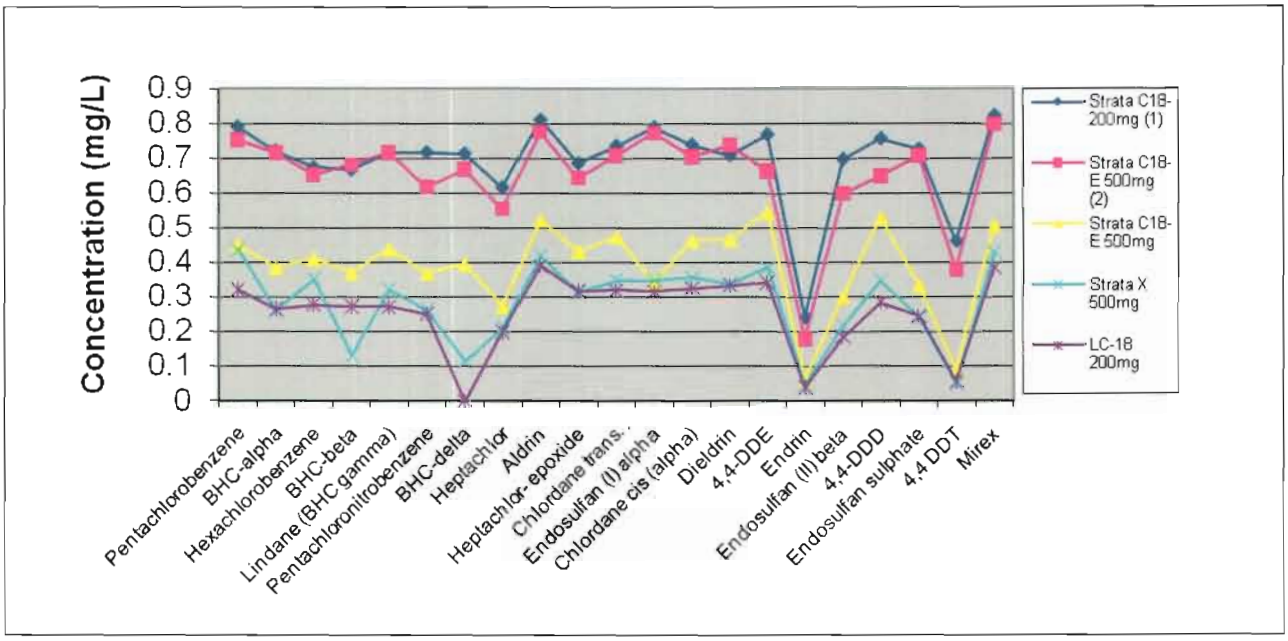


Figure 4.11: Results of conditioned cartridges

By comparing the Strata C18-E 200 mg with the Strata C18-E 500 mg it can be deduced that to some extent increasing the sorbent bed mass increases the degree of analyte retention significantly. From the results in Figure 4.11 above, it can be deduced that the LC-18 cartridge is more efficient in retaining the organochlorine compounds as the elute overall showed the lowest analyte concentration.

4.2.2 Recovery of test cartridges

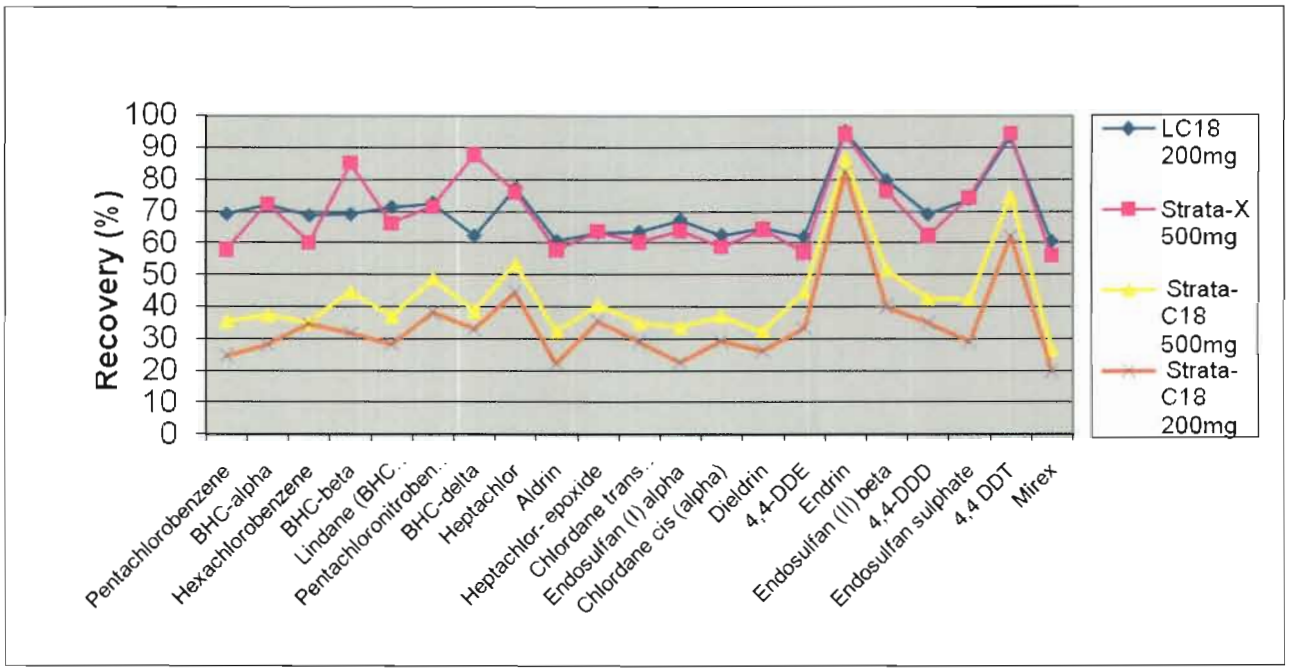


Figure 4.12: Results of analyte recovery after conditioning in percentage

Most researchers decline to indicate their acceptable recoveries especially for matrix based determinations as it is difficult to maintain strict recovery targets particularly when the nature of the matrix under study is unknown. Poole (2007) however indicated that recoveries above 90% were acceptable. For this research, recoveries of 100 ± 30% for determinations were considered to be acceptable [7]. The above graph indicates that the LC18 and Strata X cartridges showed the best recoveries, although some of the method development parameters were yet to be optimised in order to meet the target of 100 ± 30%.

4.2.3 Effect of conditioning volume on analyte retention

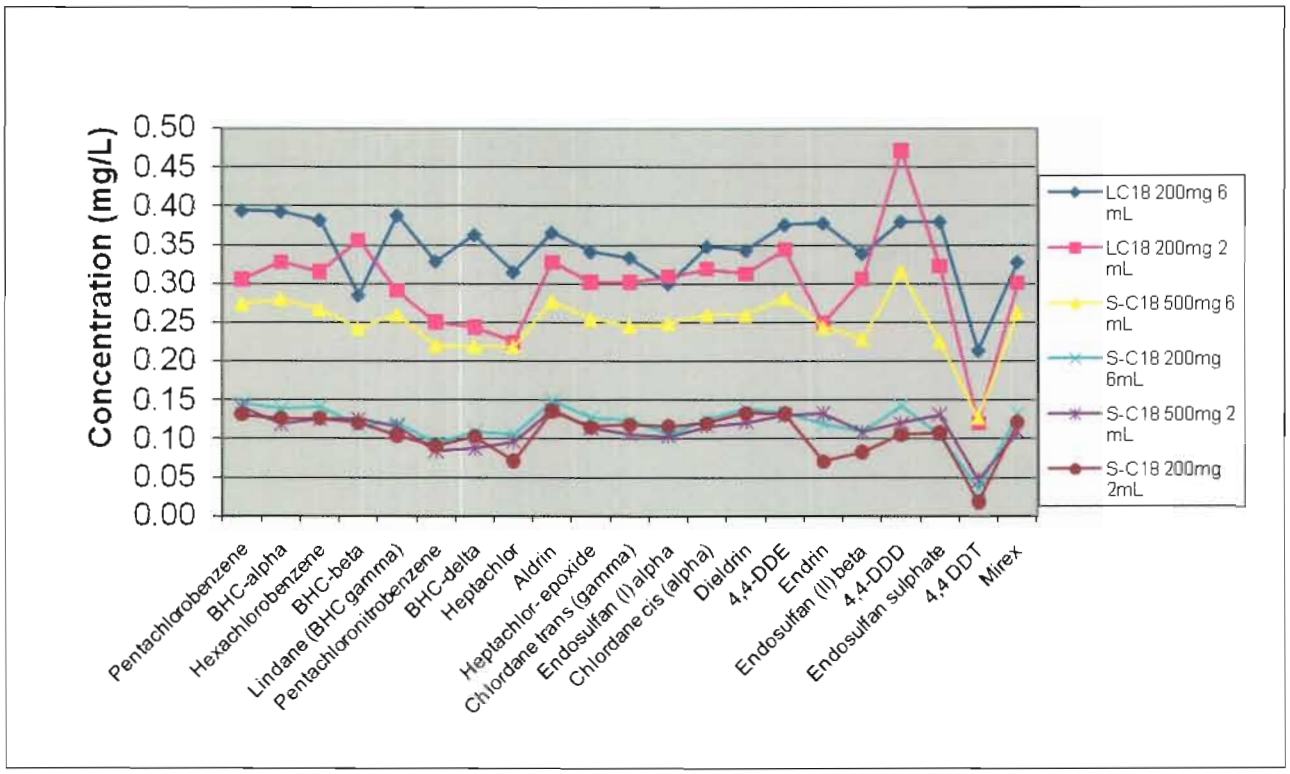


Figure 4.13: Effect of conditioning volume on analyte retention

Figure 4.13 above shows the results obtained after conditioning the cartridges with both 2 mL and 6 mL methanol, followed by loading 2 mL of 0.4 ppm cocktail solution before collecting the elute into a 2 mL final volume. The elute collected was analysed by GC-MS and the results show that the volume of conditioning solvent used has a significant effect on the efficiency of analyte retention of the cartridges. The conditioning volume had a greater effect on the SC-18 E 500 mg cartridges as it shows a greater difference in analyte retention when the conditioning volume is increased from 2 mL to 6 mL. Once again the LC-18 displays the greatest efficiency and robustness as the increase from 2 mL to 6 mL does not have such a significant effect on the efficiency of analyte retention as when compared to other cartridges. Conditioning of the LC-18 cartridge with 6 mL methanol proved to provide the optimum cartridge performance and considerably increased the recovery of most analytes to the target range of 100 ± 30%.

4.2.4 Effect of elution volume on cartridge efficiency

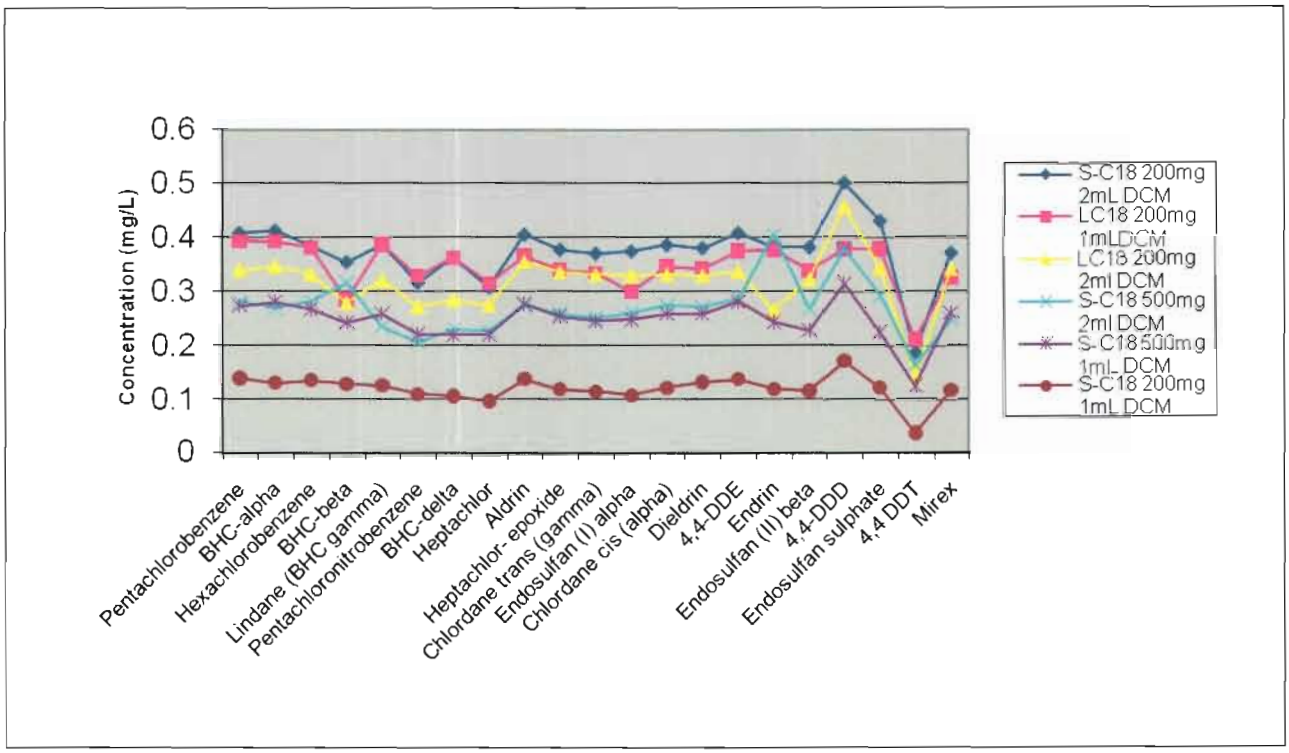


Figure 4.14: Effect of elution volume on analyte retention

Figure 4.14 above shows the effect of the elution volume tested on the LC-18 200 mg, SC-18 E 200 mg and SC-18 E 500 mg cartridges. The cartridges were firstly conditioned with 6 mL methanol before loading with 1 mL of a 0.4 ppm organochlorine cocktail. Increasing the elution volume twofold from 1 mL to 2 mL produced considerable changes in the amount of analytes desorbed from the sorbent bed, particularly for the SC-18 E 200 mg and LC-18 200 mg cartridges. This indicates that 1 mL eluent was insufficient to desorb all analytes from the sorbent bed. A volume of 2 mL DCM was found to be optimally capable of desorbing most analytes from the LC-18 200 mg and SC-18 E 200 mg cartridges. Subsequent analysis of further 2 mL aliquots on the same cartridges proved that 6 mL DCM was the most efficient volume required to desorb any remaining analyte traces from the solid phase. The above data show that in some cases, increasing the sorbent mass does not necessarily lead to greater cartridge efficiency as the SC-18 E 200 mg cartridge proved to be more efficient than the SC-18

E 500 mg cartridge. Furthermore, increasing the elution volume for the SC-18 E 500 mg cartridge from 1 mL to 2 mL produced no significant difference.

4.5 Developed sample preparation technique

The matrix is a burden on pesticide residue analysis [7]. Unfortunately it is presently impossible to completely eliminate the matrix from a real sample matrix in order to isolate the analyte of interest. Dedicated SPE application techniques have been developed to deal with extracts with comparatively low-matrix burden but several problems still arise in the GC analysis of the pesticide residues.[7] The following sample preparation conditions were developed for optimum analyte extraction and recovery:

- Condition with 6 mL methanol with flow rate 6 mL/min
- Load 10 mL sample with flow rate 1.5 mL/min
- Dry using nitrogen gas for 2 minutes with flow rate 6 mL/min
- Elute with 6 mL DCM with rate 1.5 mL/min.

4.6 Results of real sample and blank analysis

Each of the four test cartridges were individually loaded with both 10 mL real sample water (s) spiked with a 1 ppm organochlorine cocktail solution and another four with 10 mL blank deionised water (b) spiked with a 1 ppm organochlorine cocktail solution. The cartridges were analysed through the developed SPE method using the above mentioned optimised conditions.

The results in Figure 4.15 below indicate that the real sample recoveries on the LC-18 cartridge were the most acceptable as most analytes were in the 100 ± 30% range. The sample seemed to exhibit matrix induced enhanced chromatographic effect on the LC-18 cartridge as all but one of the analytes produced recoveries greater than 100%. Other cartridges produced recoveries of less than 100% for both the real sample and

blank determinations. This indicates that there was either inefficient extraction or a matrix induced diminished chromatographic response. It is not unusual to obtain recoveries as high as >200% in pesticide residue analysis in the presence of a real sample matrix as many laboratories worldwide have documented such cases [8].

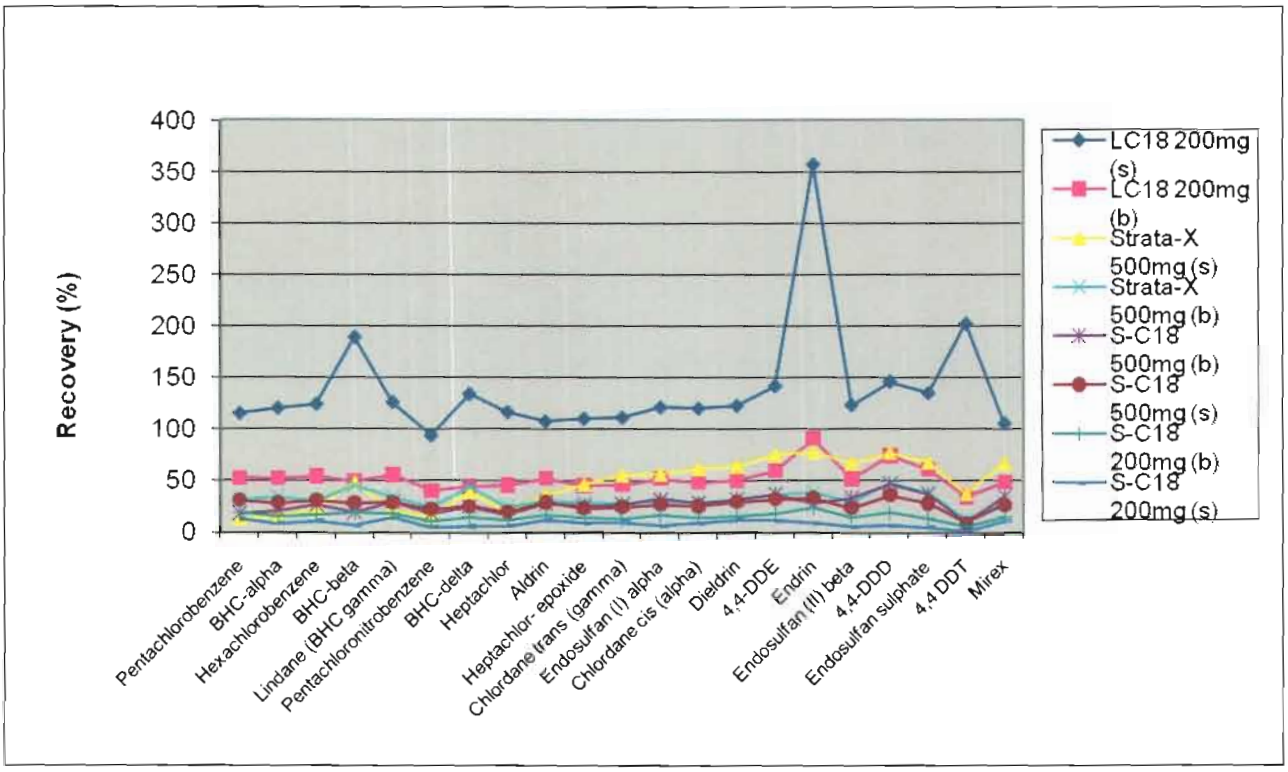


Figure 4.15: Recovery of spiked sample and blank water

4.7 Repeatability of the Gilson GX-271 liquid handling instrument

The Table 4.6 below displays duplicate analysis done using the SC-18 200 mg cartridge on the real sample and blank sample. The results indicate that the SPE method developed showed a significant degree of reproducibility and repeatability. As expected, matrix-free extractions generally display a greater degree of repeatability compared to matrix-based extractions.

Table 4.6: Repeatability of the Gilson GX-271 liquid handling instrument.

	S-C18 E sample 1	S-C18 E sample 2	S-C18E blank 1	S-C18 E blank 2	RSD % Sample	RSD % blank
Pentachlorobenzene	0.053	0.036	0.079	0.08	27.0	0.89
BHC-alpha	0.036	0.030	0.067	0.066	12.9	1.06
Hexachlorobenzene	0.047	0.038	0.072	0.071	15.0	0.99
BHC-beta	0.028	0.031	0.082	0.073	7.2	8.21
Lindane (BHC gamma)	0.059	0.036	0.077	0.081	34.2	3.58
Pentachloronitrobenzene	0.021	0.017	0.044	0.053	14.9	13.12
BHC-delta	0.025	0.022	0.062	0.051	9.0	13.77
Heptachlor	0.026	0.024	0.049	0.055	5.7	8.16
Aldrin	0.05	0.038	0.069	0.074	19.3	4.94
Heptachlor- epoxide	0.038	0.033	0.058	0.059	10.0	1.21
Chlordane *trans* (gamma)	0.042	0.035	0.055	0.062	12.9	8.46
Endosulfan (I) alpha	0.028	0.026	0.071	0.057	5.2	15.47
Chlordane *cis* (alpha)	0.038	0.033	0.062	0.064	10.0	2.24
Dieldrin	0.052	0.041	0.067	0.071	16.7	4.10
4,4'-DDE	0.048	0.042	0.077	0.078	9.4	0.91
Endrin	0.038	0.041	0.099	0.097	5.4	1.44
Endosulfan (II) beta	0.024	0.017	0.062	0.059	24.1	3.51
4,4'-DDD	0.028	0.029	0.078	0.084	2.5	5.24
Endosulfan sulphate	0.021	0.026	0.056	0.07	15.0	15.71
4,4' DDT	0.006	0.007	0.025	0.023	10.9	5.89
Mirex	0.046	0.037	0.061	0.065	15.3	4.49

For most organochlorine compounds, the presence of the matrix caused deterioration in the quality of repeatability data for real samples, compared to blanks. Pentachlorobenzene blank shows a repeatability as low as RSD% of 0.89% and deteriorated to 27.0% in the presence of a matrix. The compound 4,4' DDE also shows a repeatability of 0.91% which deteriorated to 9.4% due to the presence of the matrix.

Table 4.7: Optimum matrix load volume test results

Organochlorine compound	10 mL Sample % recovery	50 mL Sample % recovery	100 mL Sample % recovery	500 mL Sample % recovery	750 mL Sample % recovery	1000 mL Sample % recovery
BHC-alpha	18.32	81.37	101.24	150.31	177.95	174.53
Hexachlorobenzene	23.02	66.87	62.48	57.00	79.29	58.83
BHC-beta	49.16	97.39	132.46	275.89	366.39	421.19
Lindane (BHC gamma)	28.63	85.59	102.29	134.19	147.32	139.56
Pentachloronitrobenzene	16.94	82.64	104.55	141.32	188.84	162.40
BHC-delta	62.47	118.58	135.79	180.67	200.50	190.77
Heptachlor	24.01	73.89	80.17	80.91	125.99	118.23
Aldrin	22.40	65.42	63.36	45.97	62.48	59.82
Heptachlor-epoxide	56.57	109.43	127.51	154.87	185.94	163.68
Chlordane *trans* (gamma)	54.91	98.66	93.07	80.97	98.35	83.45
Endosulphan alpha	55.87	73.91	75.65	55.87	74.20	48.59
Chlordane *cis* (alpha)	60.13	95.82	96.76	85.49	102.71	83.61
Dieldrin	70.92	107.42	98.03	93.86	99.77	79.61
4,4' DDE	70.11	115.89	115.58	91.58	100.74	84.95
Endrin	130.49	259.81	261.55	287.77	342.52	300.58
Endosulphan beta	82.71	168.57	155.96	164.02	176.64	143.34
4,4' DDD	108.99	206.68	195.38	178.25	179.71	78.01
Endosulphan sulphate	117.70	208.85	193.39	223.51	241.35	210.04
4,4' DDT	176.82	374.09	380.91	347.73	358.64	289.09
Mirex	59.47	100.59	101.78	81.66	78.40	66.86

Table 4.7 above indicates that the 100 mL real-sample volume proved to be more robust as it produced the most recoveries within the 100 ± 30% range for the organochlorine analytes, compared to other sample volumes. The optimum matrix volume load, unlike the breakthrough volume was determined using an offline detection method and was determined for each specific analyte. In theory, as the sample is loaded onto the solid phase, it adsorbs the analytes and the organic matrix up to the point of saturation, where the solid phase reaches its retention capacity. This point of saturation is equivalent to the optimum matrix load volume. Any further analytes introduced to the solid phase beyond this point will not be quantitatively retained by the solid phase. The breakthrough volume, by definition is reached at the sample volume when the amount of analytes entering and leaving the solid phase becomes equal, due to saturation of the solid phase by analytes introduced [6]. The optimum matrix volume

load curves for Lindane, Pentachloronitrobenzene (PCNB) and BHC alpha form the data extracted from table 7 are shown in figure 4.16 below.

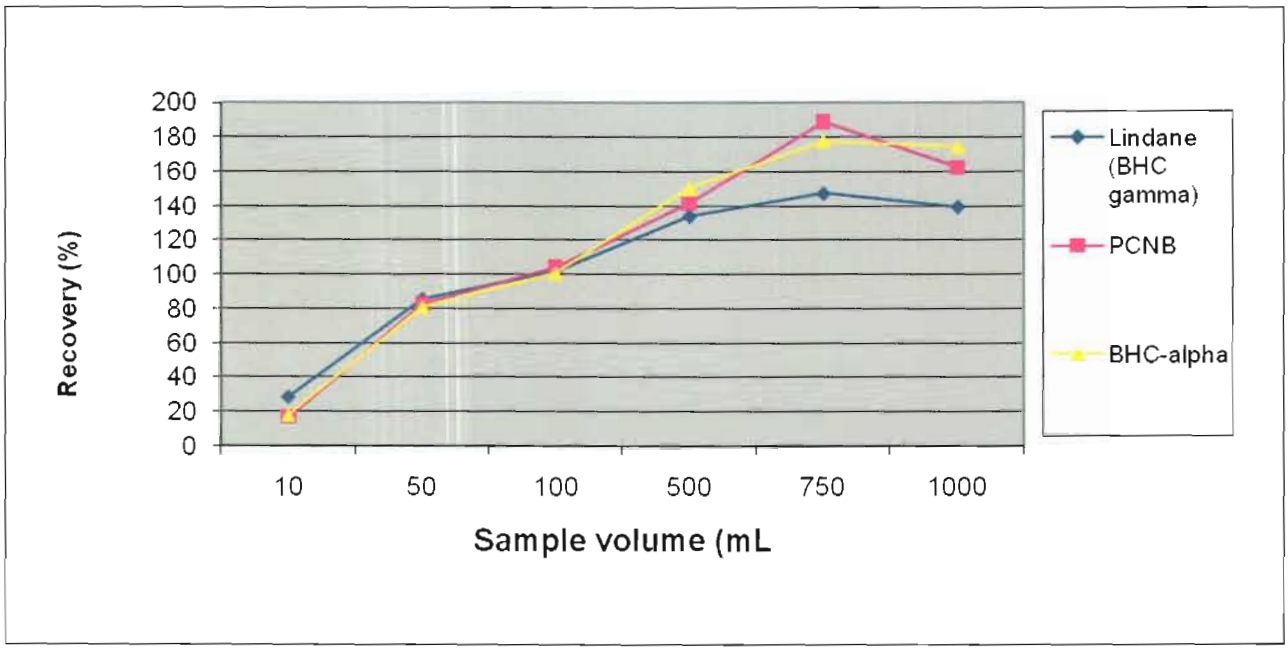


Figure 4.16: Optimum matrix load curve

Both the breakthrough volume and optimum matrix volume load curve theoretically take the form of a sigmoid curve. The optimum matrix load volume for a particular analyte is the volume which produces a 100% recovery. One of the pitfalls of using optimum matrix volume load curves for multi-residue analysis is that the optimum volume varies for each particular analyte. For this research, 100 mL was selected as the optimum matrix volume load curve and will be used for all further analyses.

4.8 Effects of the sample matrix on chromatography

Figure 4.17 below shows chromatograms of a 1 ppm matrix-free and a 1 ppm matrix based cocktail injected successively into the GC. The effect of the matrix can be clearly seen in the SIM chromatograms below as it caused a high background noise, low analyte delectability and also reporting of false positive and even false negative results

[9]. The matrix tends to reduce the quality of data generated as some co-eluted matrix components are potentially capable of masking the analyte peak of interest [8]. It has been widely noted that most polar pesticides cannot be determined because of their co-elution with matrix peaks which are mainly concentrated at the beginning of the chromatogram [10].

The adverse effects of the matrix on quantification and detection of analytes can to some extent be addressed by using selective ion monitoring. In SIM, only data from the ion representing the ion signal of interest is generated, excluding information about the occurrence of other compounds. This gives the illusion that other compounds that co-elute with the analyte of interest do not interfere with the results [11].

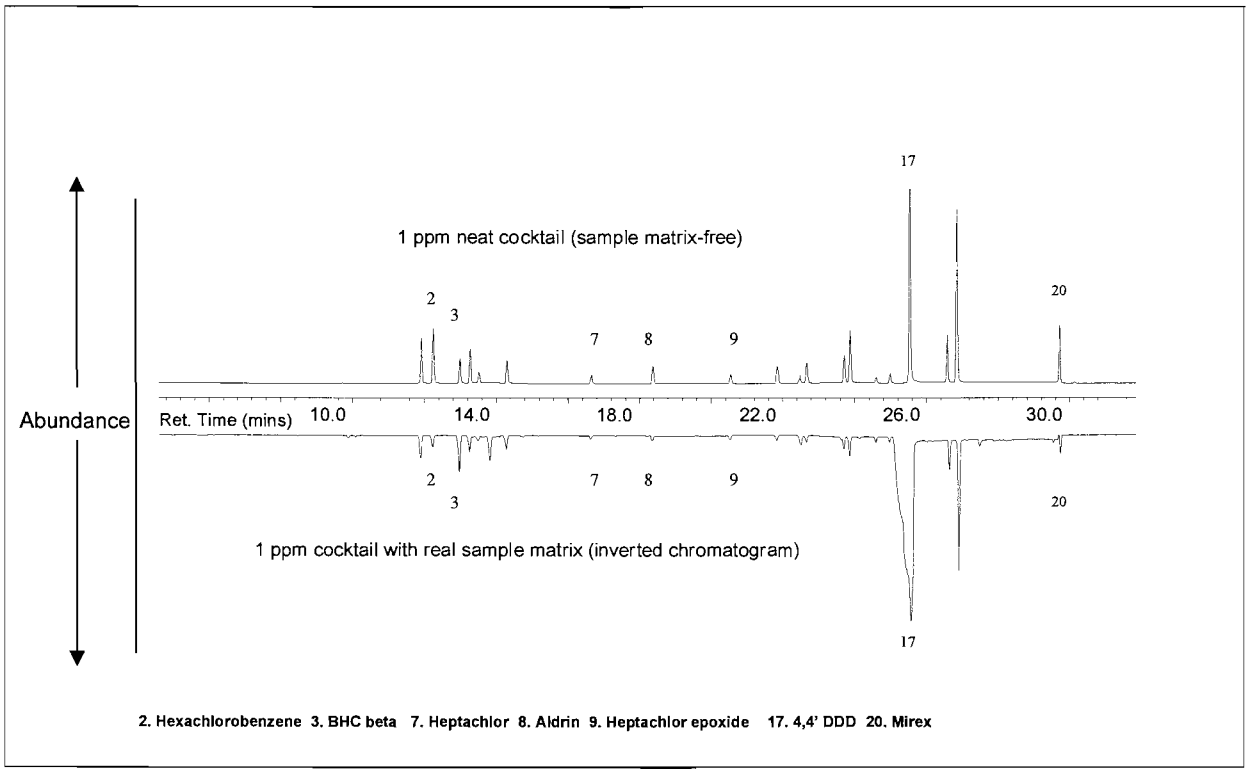


Figure 4.17: SIM chromatogram of spiked blank and real sample matrix.

Often when the sample matrix is ionised together with the analytes, it presents problems associated with matrix signal suppression. Matrix signal suppression can be clearly

observed, most notably on Hexachlorobenzene and Mirex, where the presence of the matrix leads to a significant reduction in the peak heights [3]. This phenomenon is also called matrix-induced response diminishment effects as the matrix induced a lower chromatographic response compared to the matrix-free extract. BHC beta on the other hand exhibited matrix induced response enhancement effects as the matrix caused an enhanced chromatographic response compared to the matrix-free extract [4].

Some of the clearly visible problems caused by the matrix in SIM analysis are:
Bad separation
Loss of efficiency (sharp and narrow peaks)
Lower plate numbers
Loss of selectivity
Loss of resolution
Lower baseline separation
Broader peaks
Lower detection sensitivity
Higher background noise
Suppressed peak heights
Enhanced peak heights
Peak fronting and tailing

Other problems that result during data processing include
Integration errors
Reduced ruggedness (long term reproducibility)
Inaccurate quantification
Reporting false positive results
Reporting false negative results
Recording recoveries of up to 1000% [1].

4.9 Conclusions

The method developed for the analysis of the selected organochlorine pesticides showed a significant degree of validity in terms of trueness. Most validation criteria such as repeatability, linearity, sensitivity and ANOVA were met. The LC-18 cartridge proved to be the most robust for the analysis of the selected organochlorine pesticides as it overall produced better recoveries even when significant changes were made to the sample preparation procedure. The method development has clearly shown that the matrix does have a significant effect on the quantification and detection of analytes. Automation of SPE has proved to be an important aspect of SPE analysis and is equally as beneficial as the use of a GC automatic sample injector in terms of drastically increasing precision and accuracy and also substantially reducing the chances of human error. However it should be noted that the issue of defining acceptable recoveries remains a controversial and subjective issue where matrix-based extractions are involved.

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CHAPTER 5

PRESENTATION OF FINDINGS

5.1 Calibration curves for matrix-matched and matrix-free calibration types coupled to internal standard and external standard calibration techniques

Figures 5.1 to 5.40 below display the four different calibration techniques applied to the sample matrices; high-matrix samples, low-matrix samples and no-matrix samples.

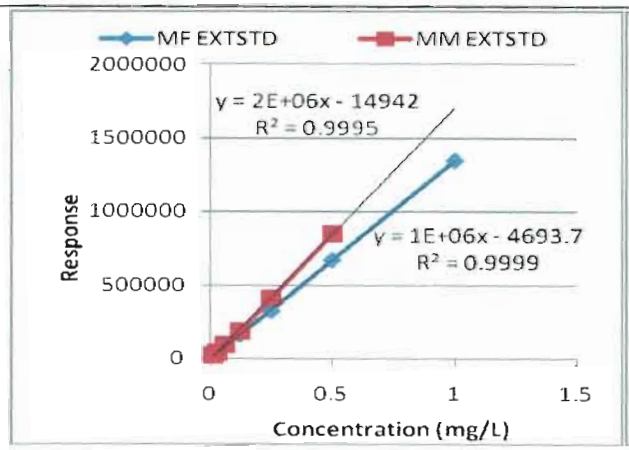

Figure 5.1: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

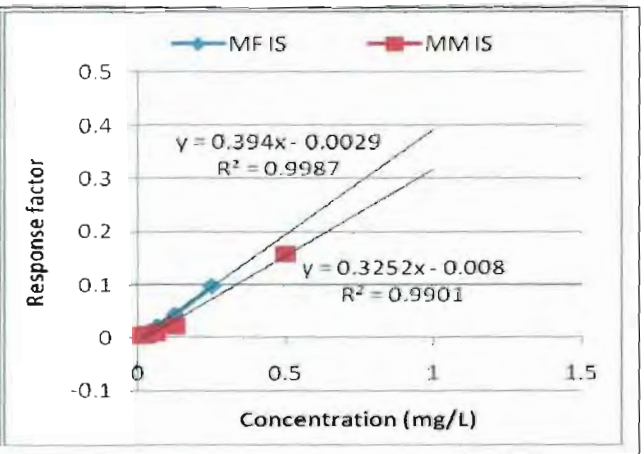

Figure 5.2: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

BHC alpha

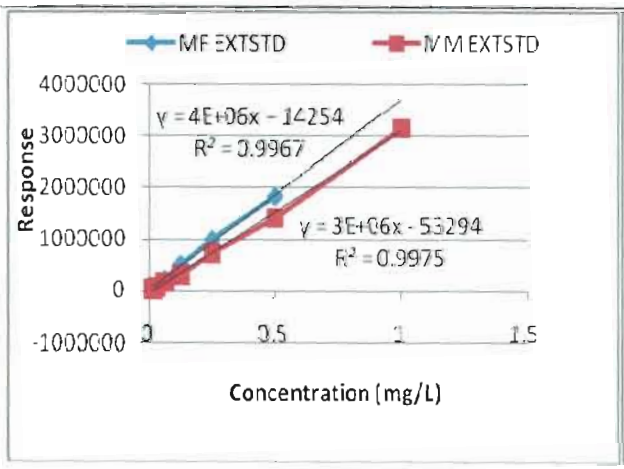

Figure 5.3: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

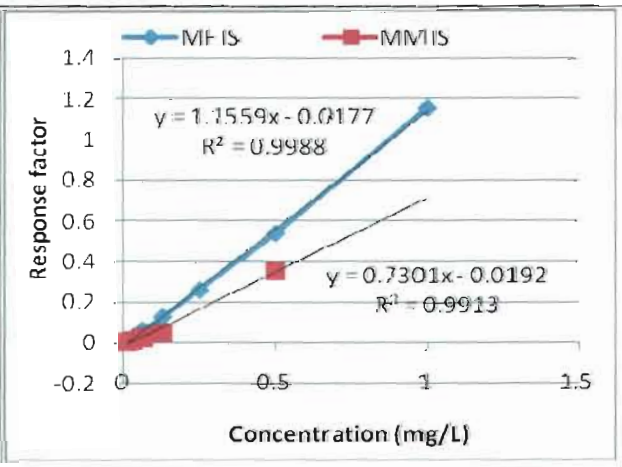

Figure 5.4: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Hexachlorobenzene

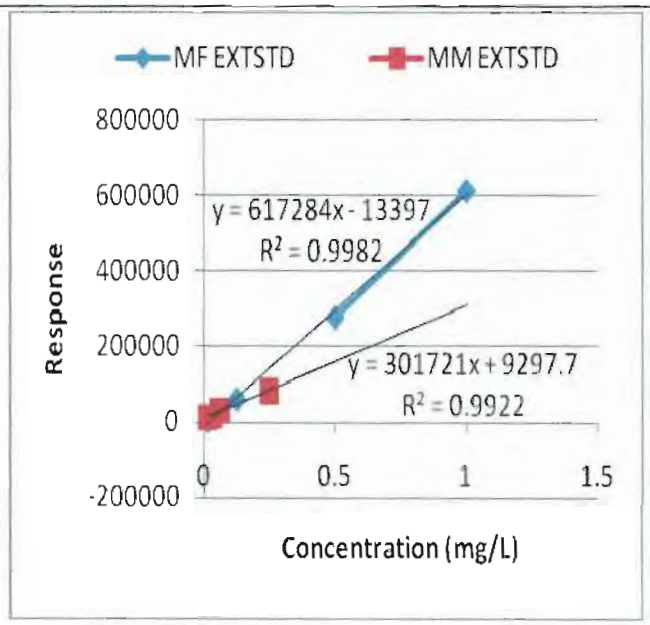

Figure 5.5: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

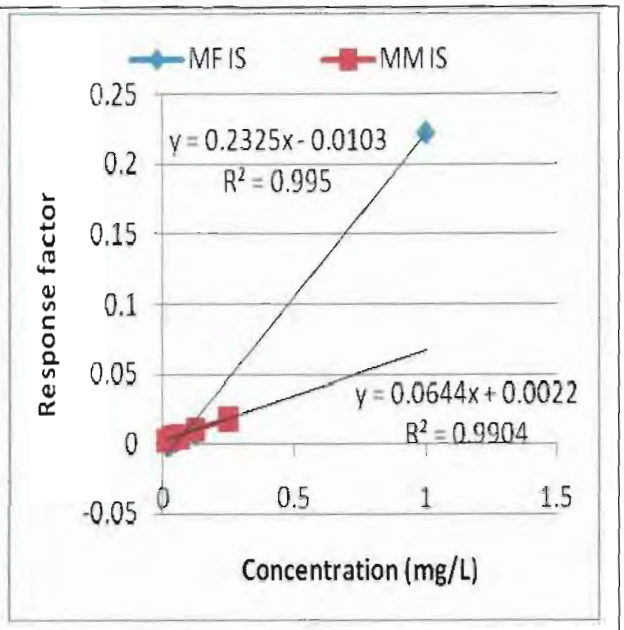

Figure 5.6: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

BHC beta

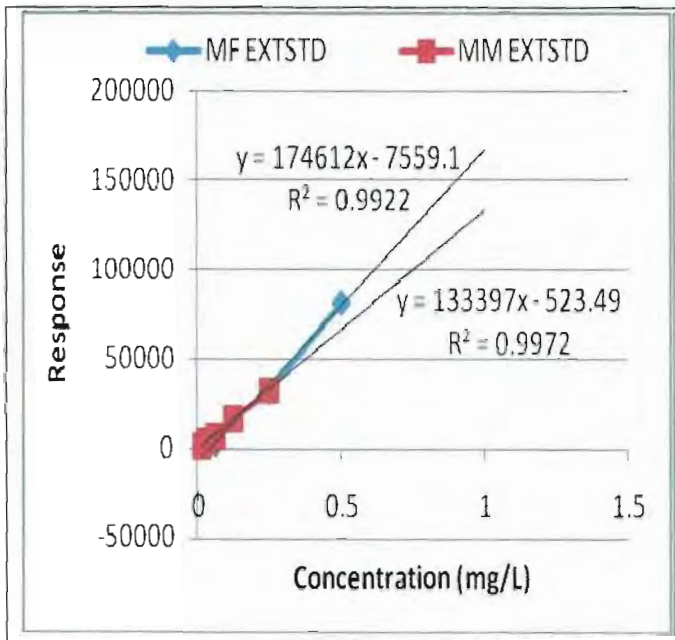

Figure 5.7: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

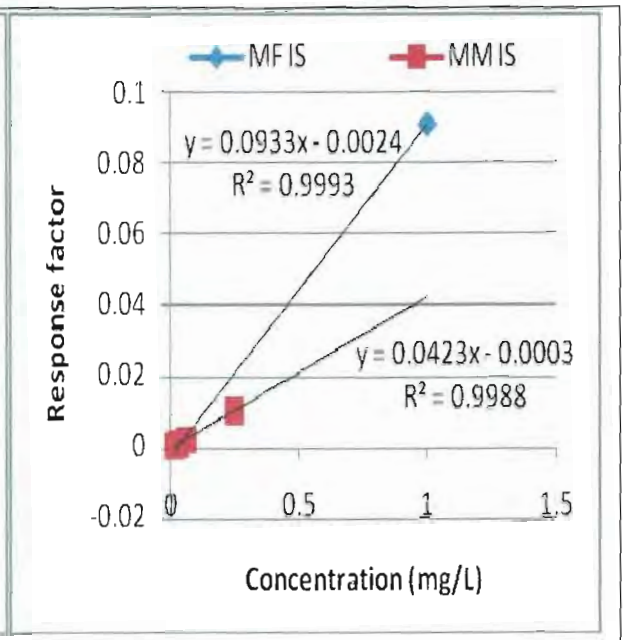

Figure 5.8: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Pentachloronitrobenzene

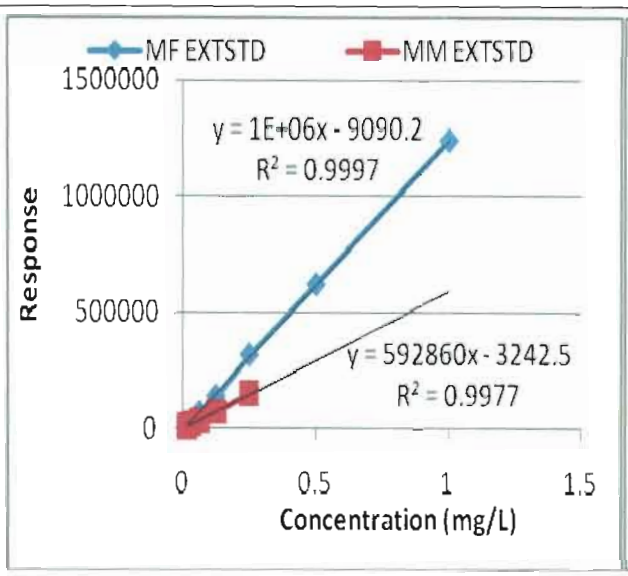

Figure 5.9: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

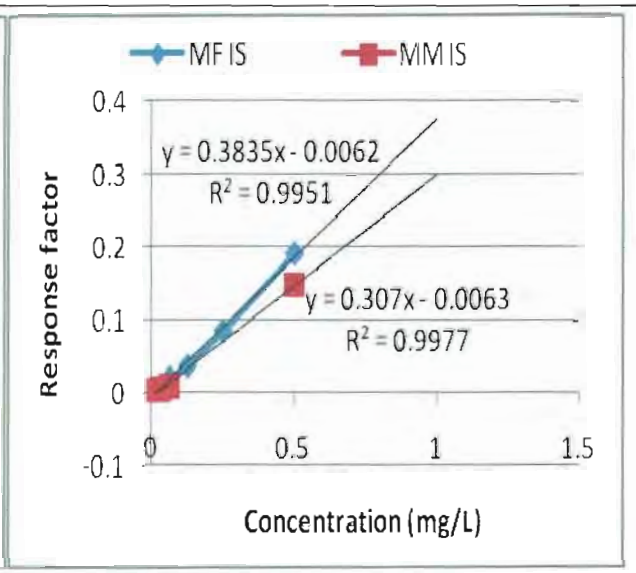

Figure 5.10: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Lindane (BHC gamma)

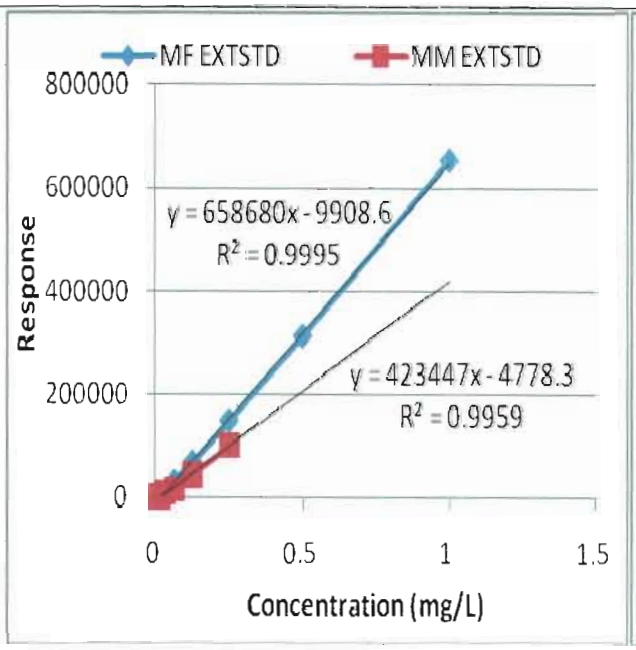

Figure 5.11: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

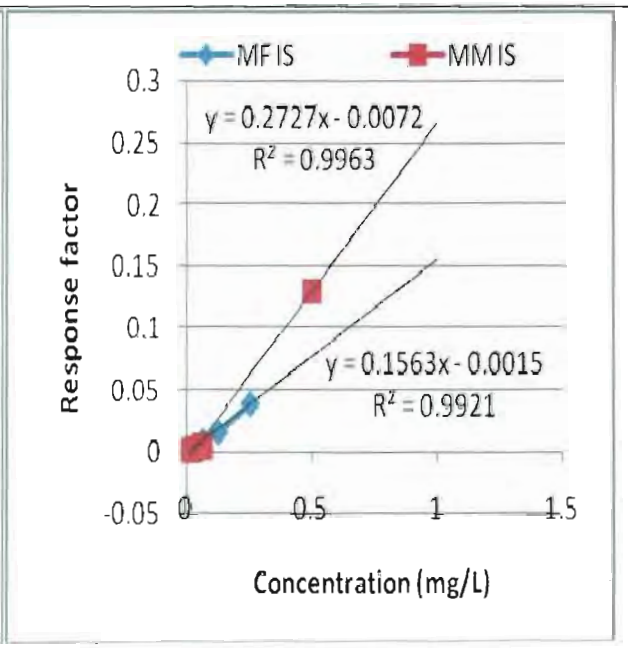

Figure 5.12: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

BHC delta

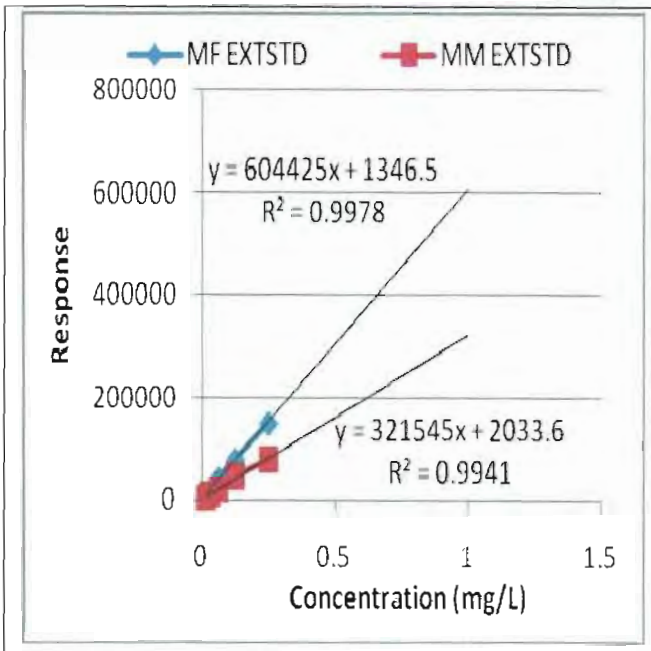

Figure 5.13: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

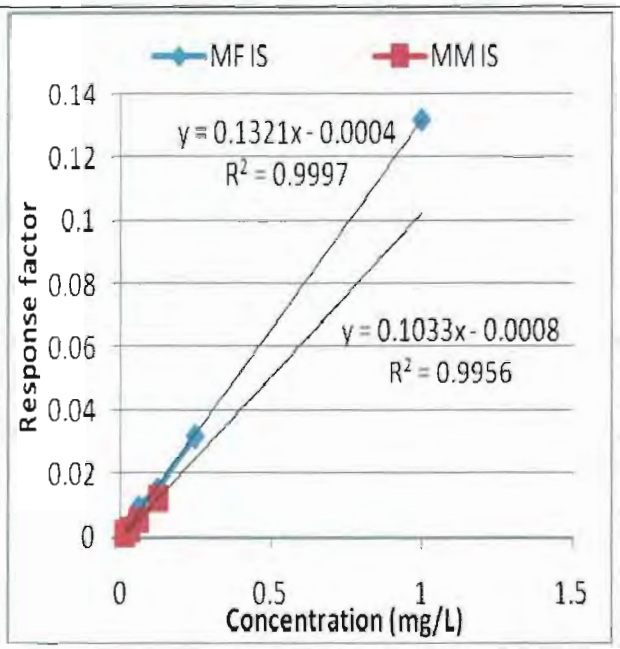

Figure 5.14: Matrix-Free Internal Standard and Matrix- Matched Internal Standard calibrations

Heptachlor

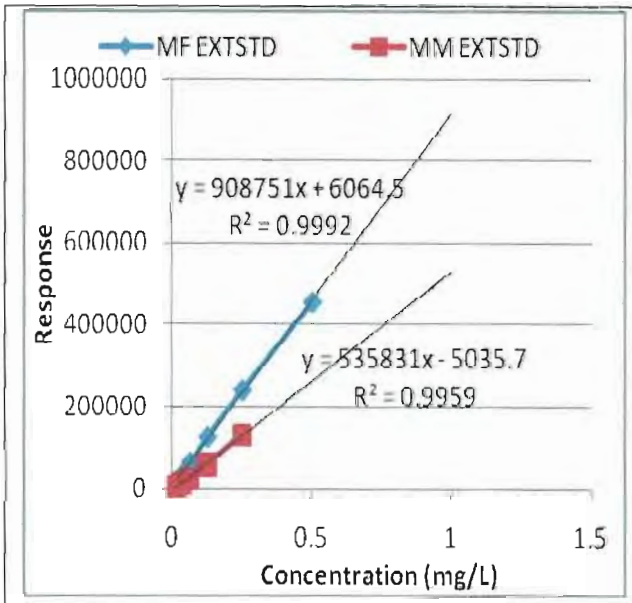

Figure 5.15: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

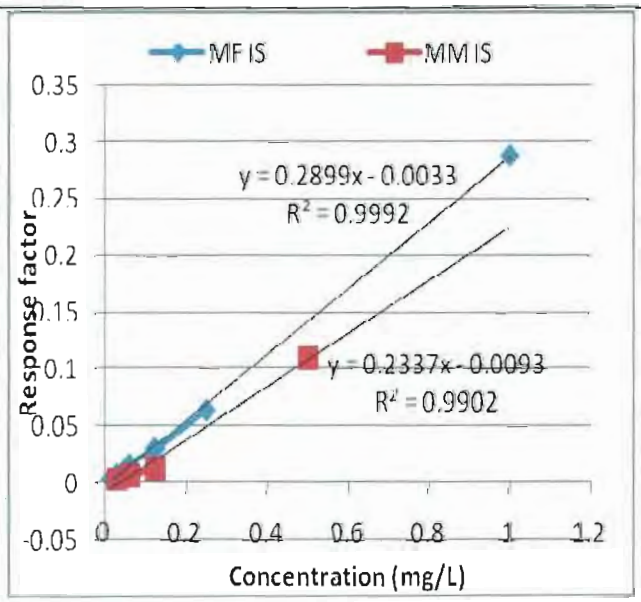

Figure 5.16: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Aldrin

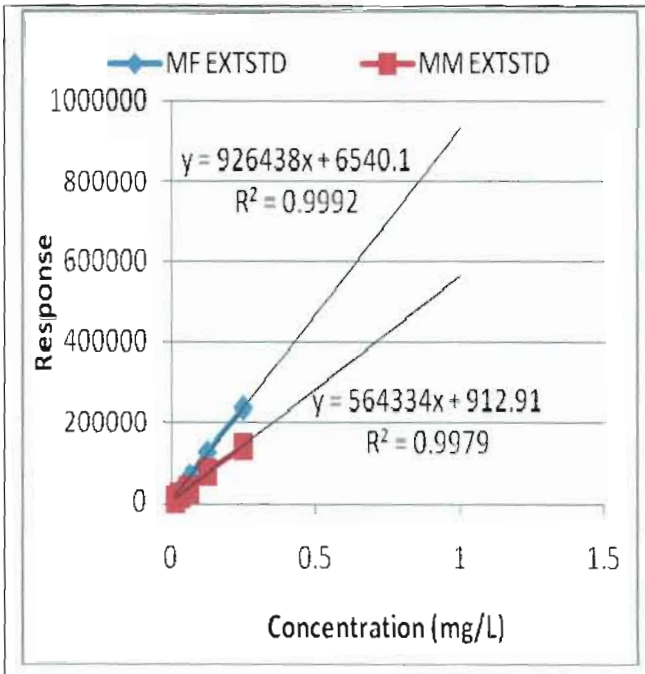

Figure 5.17: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

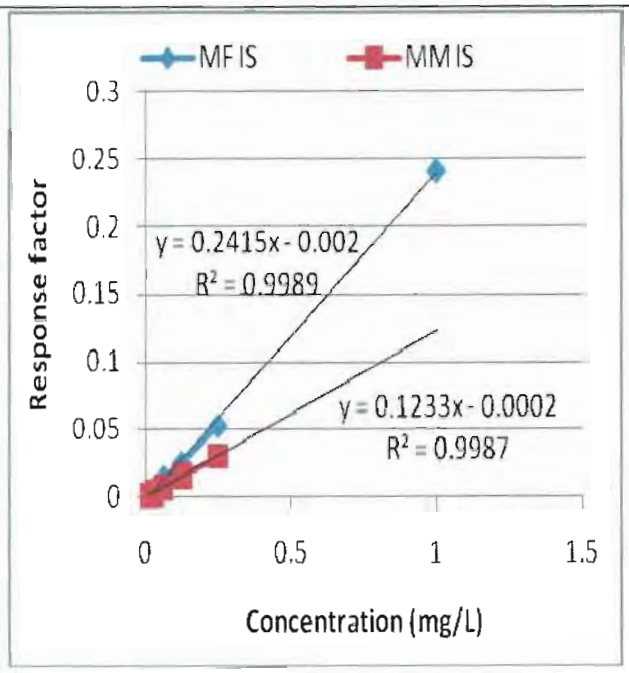

Figure 5.18: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Heptachlor epoxide

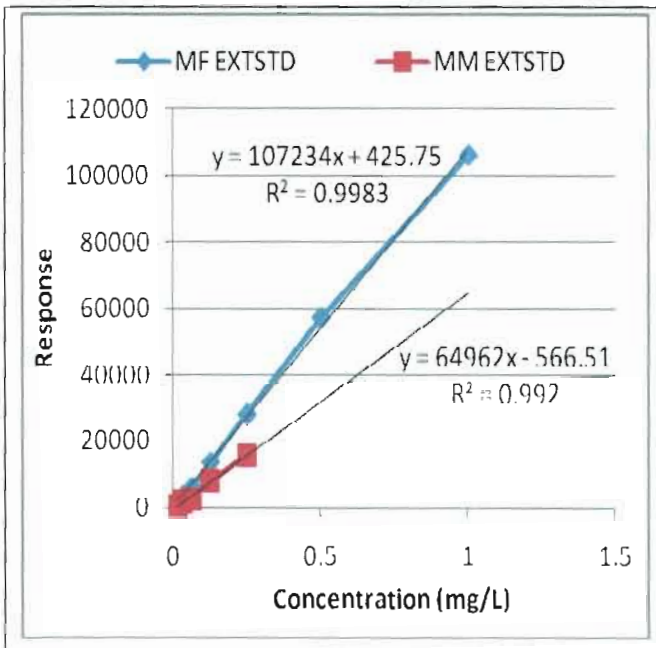

Figure 5.19: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

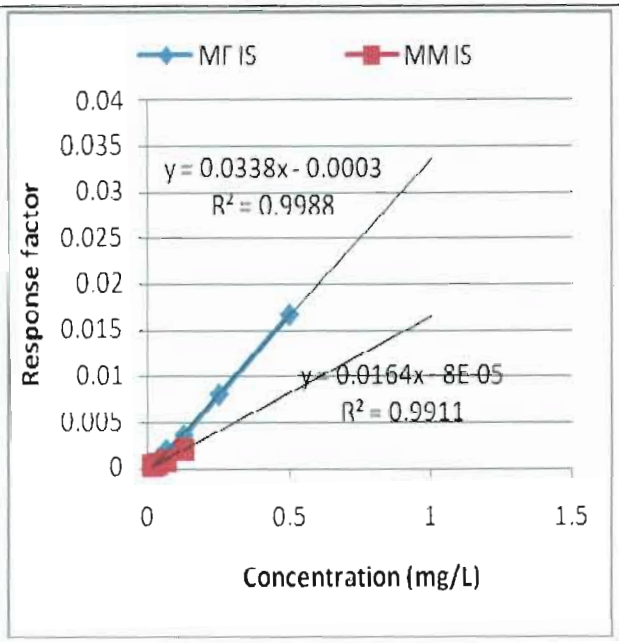

Figure 5.20: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Endosulphan alpha

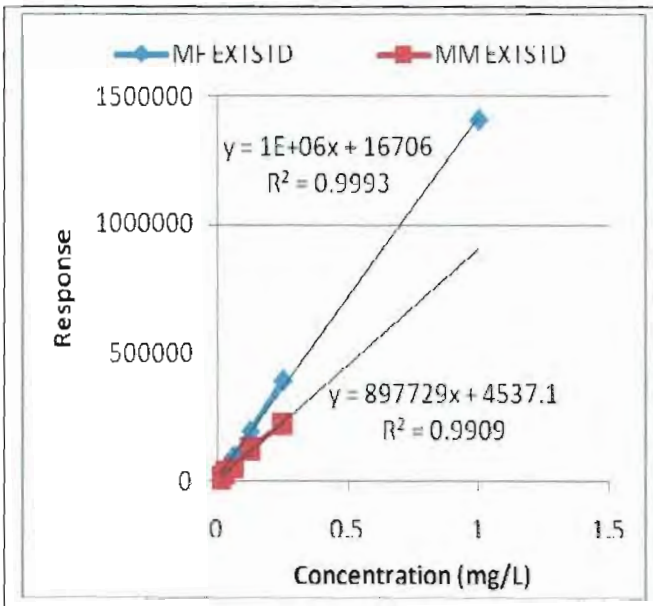

Figure 5.21 Matrix-Free External Standard and Matrix-Matched External Standard calibrations

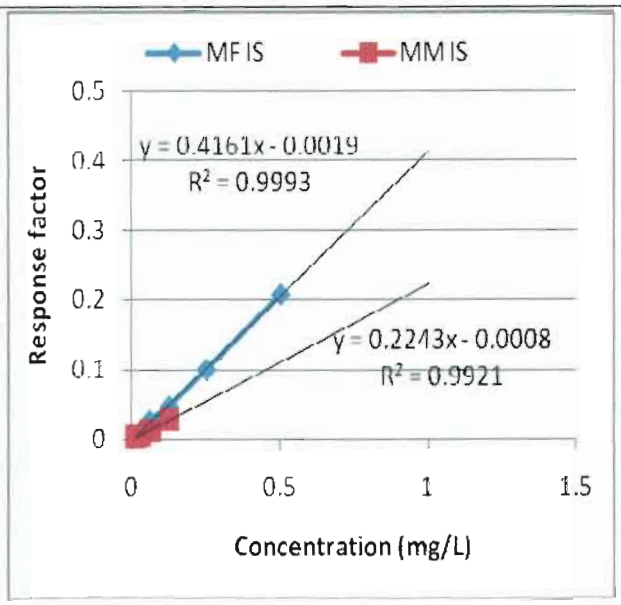

Figure 5.22: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Chlordane *trans* (gamma)

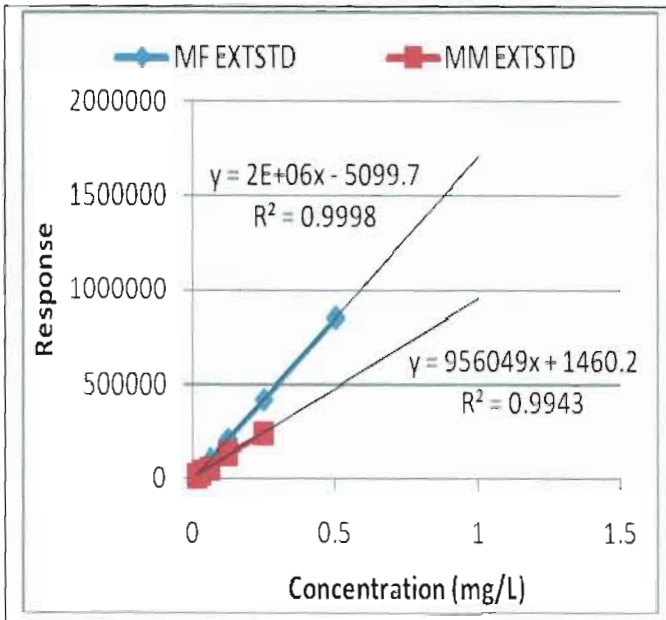

Figure 5.23: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

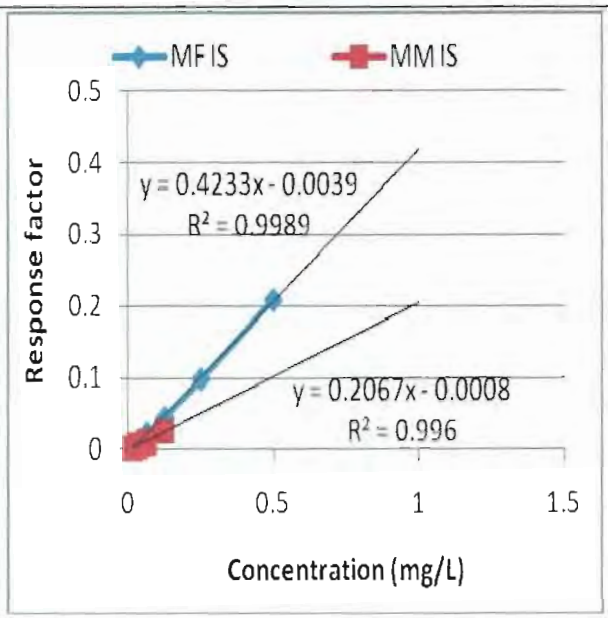

Figure 5.24: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Chlordane *cis* (alpha)

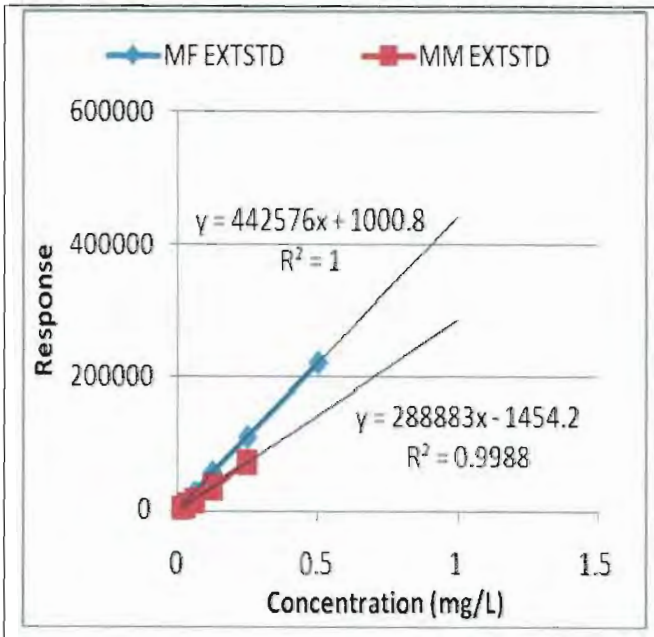

Figure 5.25: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

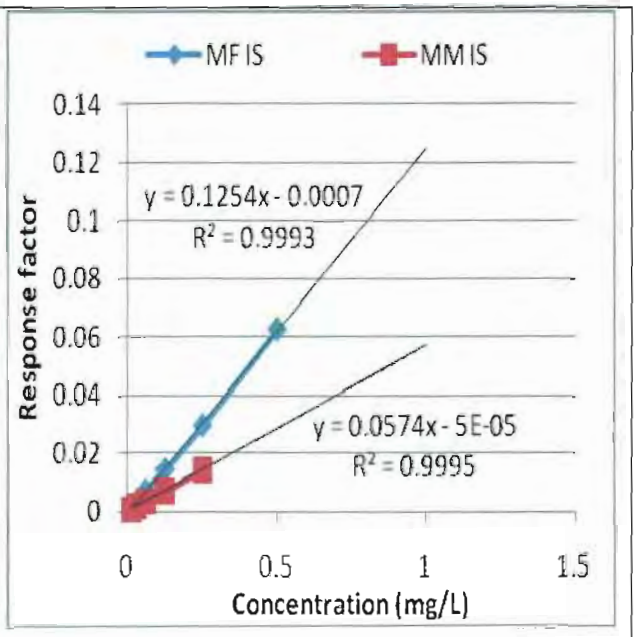

Figure 5.26: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Dieldrin

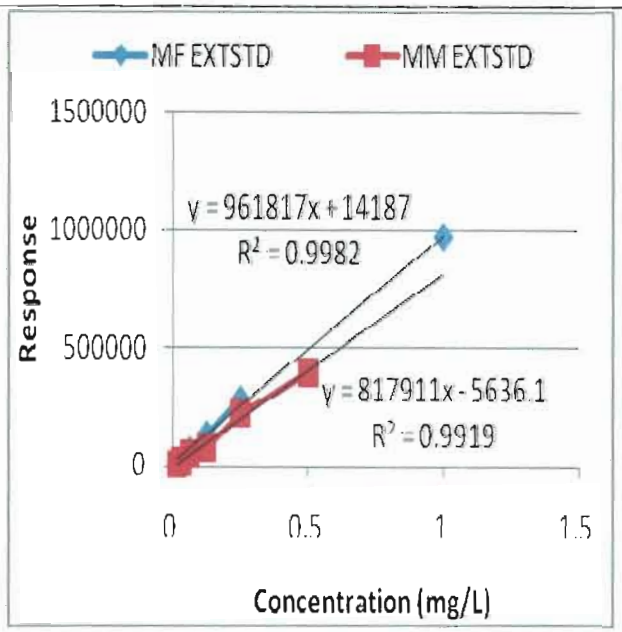

Figure 5.27: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

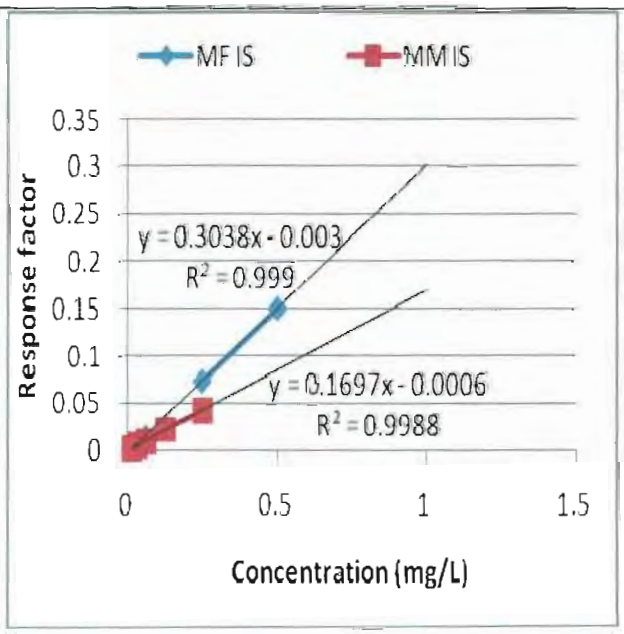

Figure 5.28: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

4,4'-dichlorodiphenyldichloroethylene (DDE)

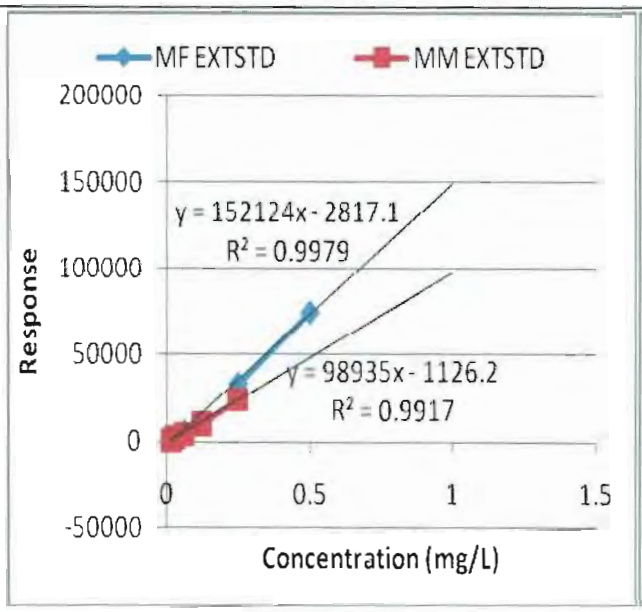

Figure 5.29: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

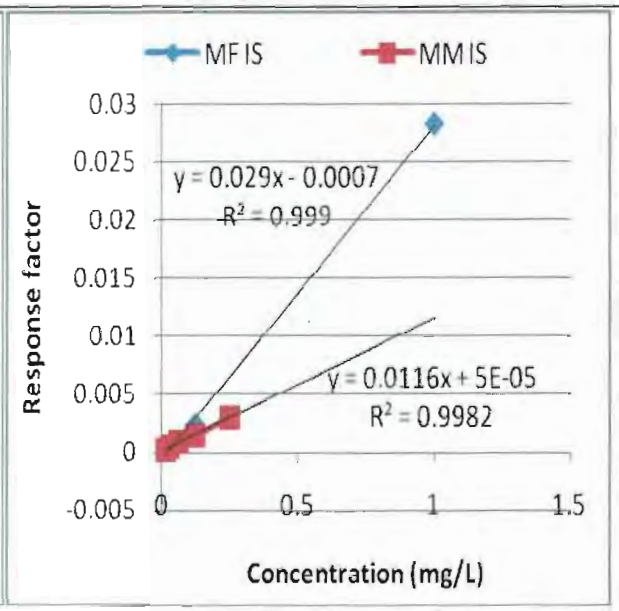

Figure 5.30: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Endosulphan beta

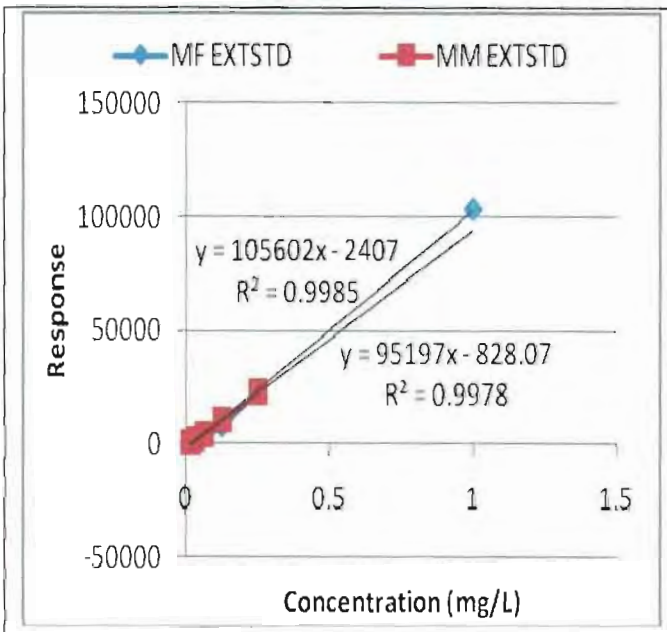

Figure 5.31: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

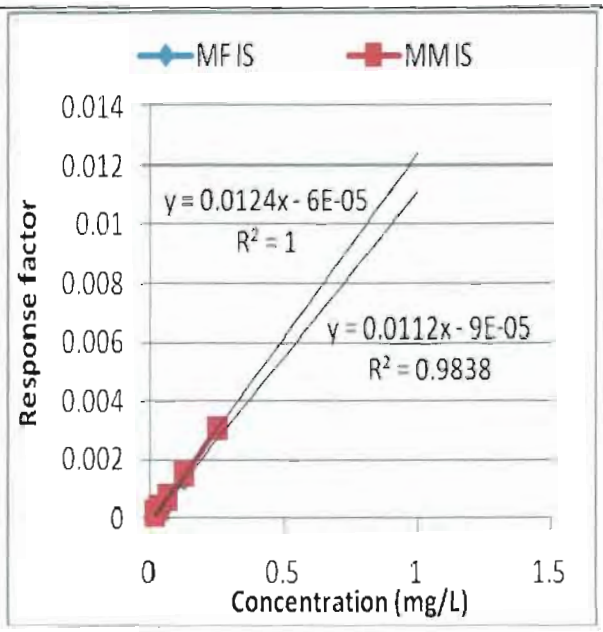

Figure 5.32: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Endrin

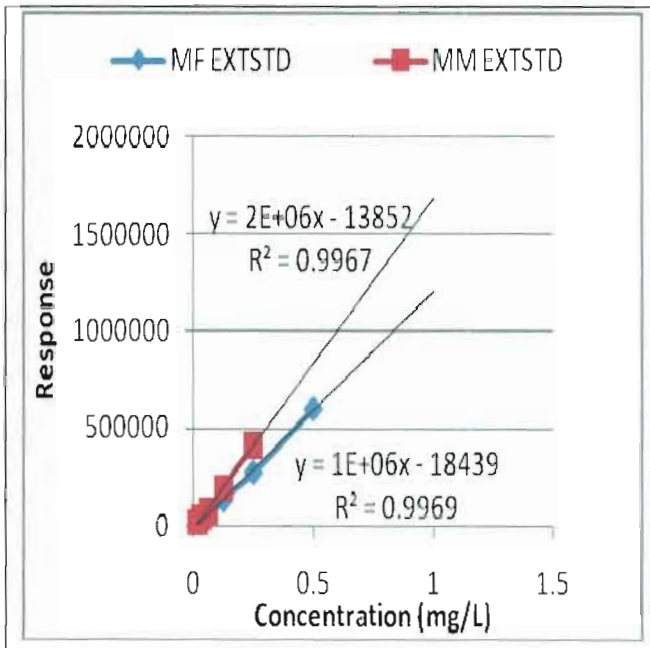

Figure 5.33: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

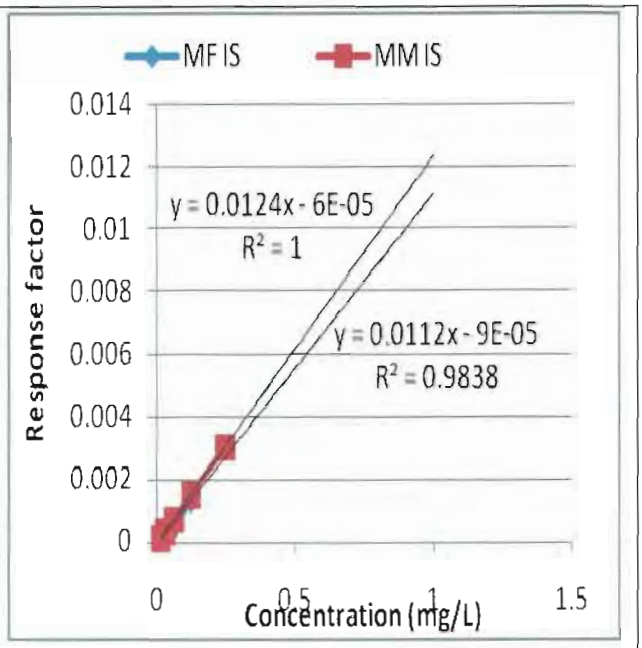

Figure 5.34: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

4,4'- dichlorodiphenyldichloroethane (DDD)

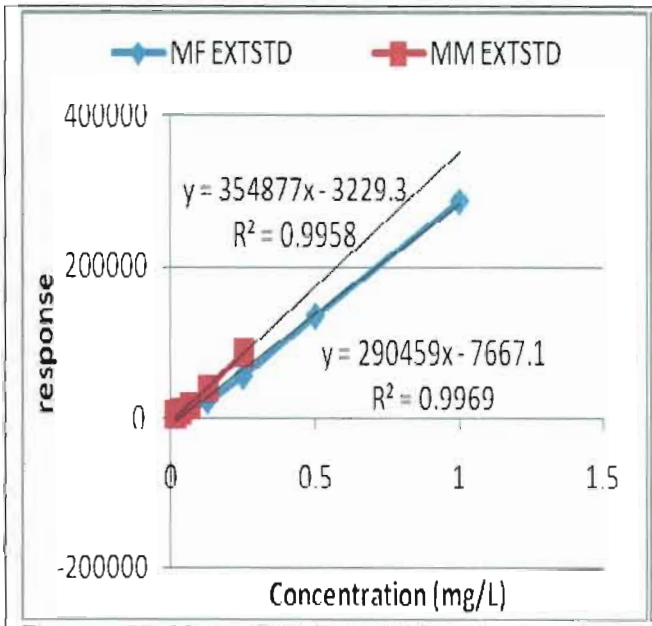

Figure 5.35: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

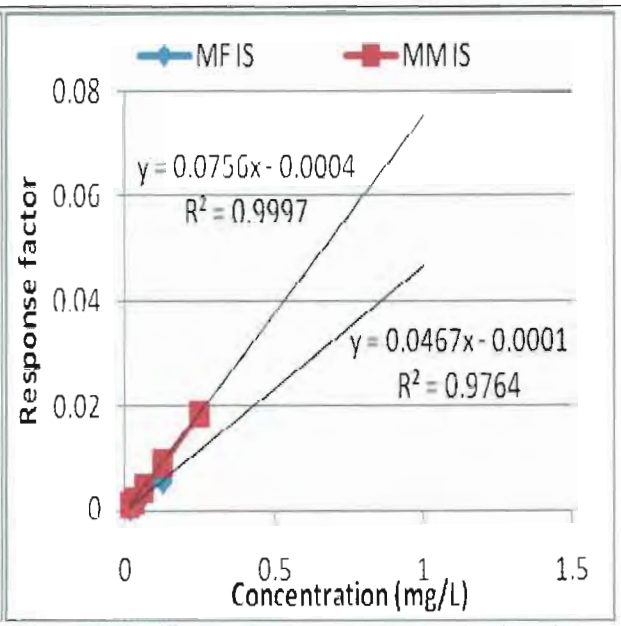

Figure 5.36: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Endosulphan sulphate

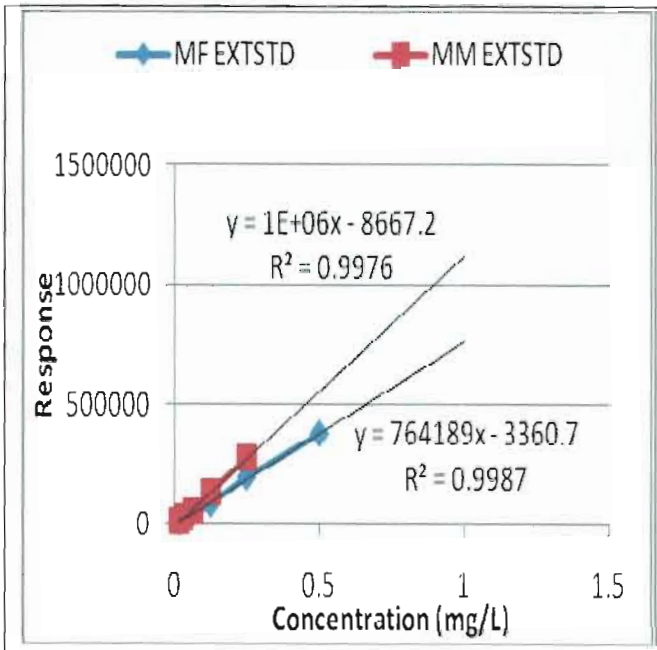

Figure 5.37: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

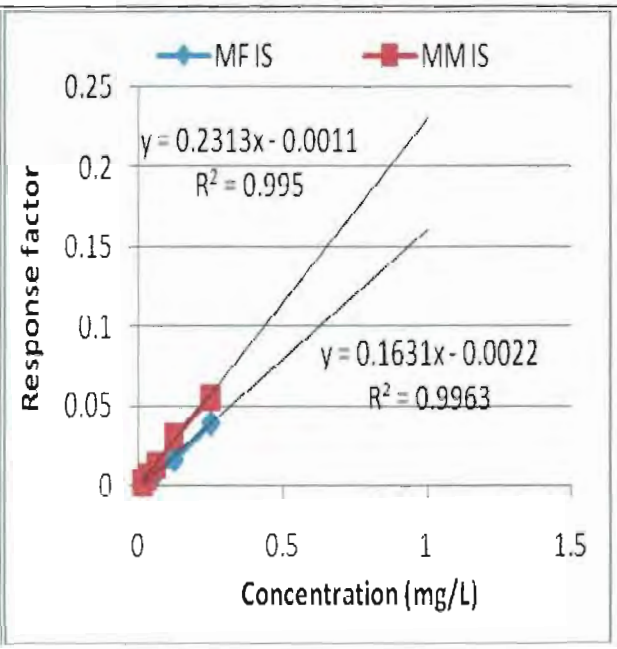

Figure 5.38: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

4,4'-dichlorodiphenyltrichloroethane (DDT)

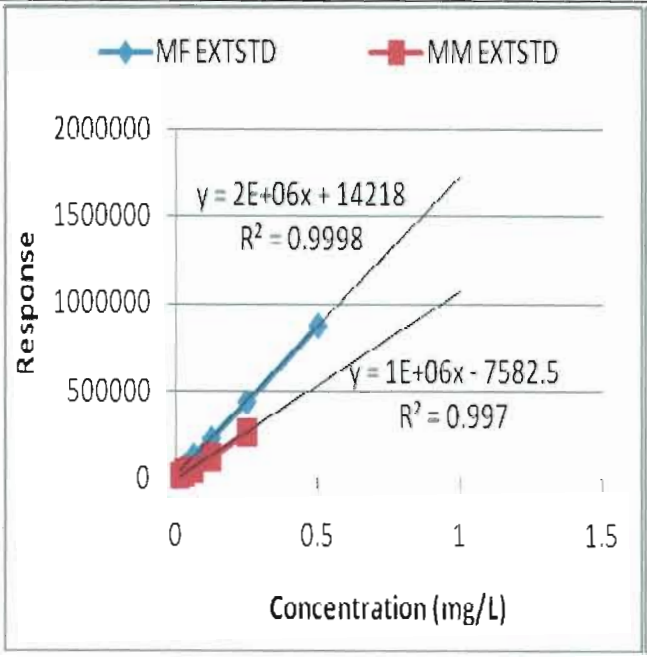

Figure 5.39: Matrix-Free External Standard and Matrix-Matched External Standard calibrations

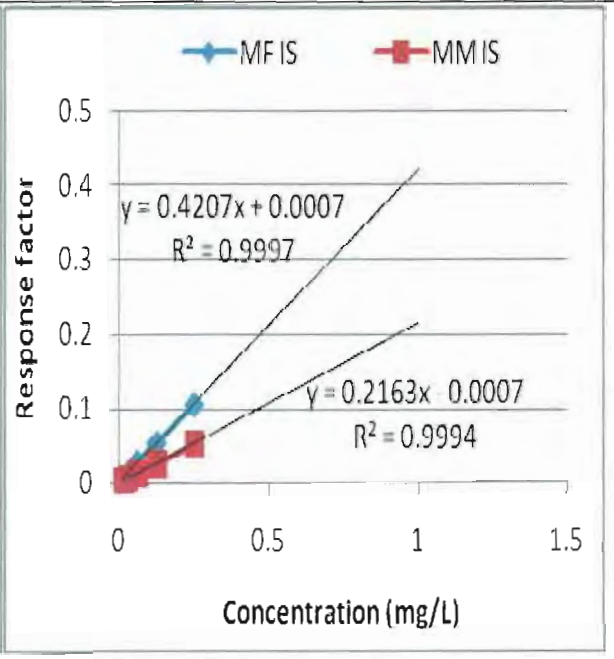

Figure 5.40: Matrix-Free Internal Standard and Matrix-Matched Internal Standard calibrations

Mirex

Calibration curves for external standard calibration displayed a response on the Y-axis and calibration curves for internal standard displayed a response factor in the Y-axis. All calibration curves showed significant linearity as they all displayed a coefficient of regression of >0.99. The calibration curves show that matrix-free standards generally have higher responses than matrix-matched standards as shown by the occurance of a higher gradient in the matrix-free calibration curves. This indicates the occurance of matrix-induced response diminishment due to the presence of the sample matrix. A general matrix-induced response enhancement was shown only for MMIS calibration curves for BHC delta (figure 5.11), MFEXTSTD and MMIS Endosulphan sulphate (figures 5.35 and 5.36), and MFEXTSTD and MMIS DDT (figures 5.37 and 5.38). This indicated that matrix induced response enhancement effects were generally not as prominent as matrix-induced response diminishment effects.

From the calibration curves above, the majority of the curves exhibited high detection limits as the Y-intercept from the equation of the curves was negative. Graphs for BHC alpha, PCNB, Lindane, BHC delta, Endosulphan sulphate and DDT indicate that the calibration curve crosses the Y-intercept on a negative value for all four calibration methods, MMFEXTSTD, MMEXTSTD, MFIS and MMIS. No compound had all calibration curves crossing the Y-intercept at a positive value for all four calibration methods.

Table 5.1: Coefficients of regression from Chemstation calibration curves

Organochlorine	MMEXTSTD	MMIS	MFEXTSTD	MFIS
BHC-alpha	0.9995	0.9989	0.9999	0.9996
Hexachlorobenzene	0.9975	1.0000	0.9983	0.9989
BHC-beta	0.9983	0.9946	0.9982	0.9974
Pentachloronitrobenzene	0.9968	0.9988	0.9998	0.9977
Lindane (BHC gamma)	0.9996	0.9998	0.9997	0.9986
BHC-delta	0.9982	0.997	0.9995	0.9995
Heptachlor	0.9979	0.9998	0.9978	0.9997
Aldrin	0.9995	0.9992	0.9992	0.9998
Heptachlor- epoxide	0.9994	0.9987	0.9992	0.9989
Endosulfan (I) alpha	0.9991	0.9996	0.9983	0.9981
Chlordane *trans* (gamma)	0.999	0.9999	0.9987	0.9984
Chlordane *cis* (alpha)	0.9987	0.9999	0.9998	0.9989
Dieldrin	0.9997	0.9995	1.0000	0.9978
4,4'-DDE	0.9976	0.9988	0.9987	0.999
Endosulfan (II) beta	0.9983	0.9982	0.9997	0.9981
Endrin	0.9984	1.0000	0.9985	0.9976
4,4'-DDD	1.0000	0.9996	0.9986	0.9991
Endosulfan sulphate	0.9996	0.9997	0.9984	0.9996
4,4' DDT	0.9987	1.0000	0.9987	0.9992
Mirex	0.9995	0.9994	0.9998	0.9997

The above data was analysed using SPSS to give the descriptive statistics in Table 5.2 below.

Table 5.2: Descriptive Statistics for Chemstation coefficients of regression

Dependent variable: Recovery (%)

	N	Minimum	Maximum	Mean	Std. Deviation
MMEXTSTD	20	0.9968	1.0000	0.998765	0.0008719
MMIS	20	0.9946	1.0000	0.999070	0.0012905
MFEXTSTD	20	0.9978	1.0000	0.999040	0.0006924
MFIS	20	0.9974	0.9998	0.998780	0.0007777

The descriptive statistics for the Chemstation calibration coefficients of regression indicate that there was a high degree of linearity in all the calibration methods. The minimum coefficient of regression recorded was 0.9946 for the MMIS. The highest were recorded for MMEXTSTD, MMIS and MFIS with a coefficient of regression of 1, which shows perfect linearity. MFIS displayed the lowest standard deviation of 0.0007777 for coefficients of regression of the 20 calibration curves constructed. This shows that the

MFIS calibration curve coefficients of regression had the highest repeatability whilst MMIS had the lowest with a standard deviation of 0.0012905.

5.2 Three factor analysis of variance

Dependant Variable = Recovery (%)

Factor A = sample matrix: (3)
High-matrix, low-matrix and no-matrix

Factor B = matrix calibration type:(2)
Matrix-matched and matrix-free

Factor C = calibration technique: (2)
Internal standard and external standard

5.3 Hypotheses

H_0 : There are no significant differences between all the three sample matrices ($\mu_1 = \mu_2 = \mu_3$)
H_A : There are significant differences between all the three sample matrices ($\mu_1 \neq \mu_2 \neq \mu_3$)

[μ_1= high sample matrix mean; μ_2 = low sample matrix mean; μ_3 = no sample matrix mean]

H_0 : There is no significant difference between the matrix-matched calibration and matrix-free calibration types ($\mu_{MM} = \mu_{MF}$).

H_A : There is a significant difference between the matrix-matched calibration and matrix-free calibration types ($\mu_{MM} \neq \mu_{MF}$).

[μ_{MM}= matrix-matched standard mean; μ_{MF} = matrix-free standard mean]

H_0 : There are no significant differences in the mean recoveries for both internal standard and external standard calibration technique ($\mu_{IS} = \mu_{EXTSTD}$).

H_A : There are significant differences in the mean recoveries for both internal standard and external standard calibration technique ($\mu_{IS} \neq \mu_{EXTSTD}$).

[μ_{IS}= matrix internal standard mean; μ_{EXTSTD} = external standard mean]

H_0 : Differences in mean recovery among the three sample matrices are independent of the two matrix calibration types (Testing A X B)
H_A : Differences in mean recovery among the three sample matrices are not independent of the two matrix calibration types

H_0 : Differences in mean recovery among the three sample matrices are independent of the two calibration techniques (Testing A X C)
H_A : Differences in mean recovery among the three sample matrices are not independent of the two calibration techniques

H_0 : Differences in mean recovery between the two matrix calibration types are independent of the two calibration techniques (Testing B X C)
H_A : Differences in mean recovery between the two matrix calibration types are not independent of the two calibration techniques

H_0 : Differences in mean recovery among the three sample matrices are independent of the two other factors (Testing A X B X C interaction)
H_A : Differences in mean recovery among the three sample matrices are not independent of the two other factors

Table 5.3: High matrix sample recoveries

	Matrix-matched calibration		Matrix-free calibration	
Organochlorine pesticide	External Standard	Internal Standard	External Standard	Internal Standard
BHC-alpha	80.38	67.63	46.25	31.00
Hexachlorobenzene	27.00	25.00	13.50	11.33
BHC-beta	116.50	99.00	44.92	88.88
Pentachloronitrobenzene	33.83	27.88	45.25	26.88
Lindane (BHC gamma)	87.63	79.00	35.75	37.63
BHC-delta	106.08	91.63	83.25	63.00
Heptachlor	36.38	22.00	19.38	13.50
Aldrin	26.00	23.88	14.25	9.25
Heptachlor epoxide	102.38	76.88	60.88	38.38
Endosulfan alpha	88.25	87.38	51.13	36.13
Chlordane *trans*(gamma)	98.13	88.38	53.50	40.88
Chlordane *cis* (alpha)	110.25	93.25	62.00	41.00
Dieldrin	91.75	92.63	58.00	42.00
4,4'DDE	100.75	100.13	79.00	56.63
Endosulfan (II) beta	65.25	88.50	57.13	44.13
Endrin	82.75	78.50	77.50	77.25
4,4' DDD	114.00	105.08	189.25	113.50
Endosulfan sulphate	95.33	105.63	120.83	104.92
4,4' DDT	111.00	108.88	161.58	102.75
Mirex	95.33	111.00	66.75	55.63

Table 5.4: Low-matrix samples recoveries

	Matrix-matched calibration		Matrix-free calibration	
Organochlorine pesticide	External Standard	Internal Standard	External Standard	Internal Standard
BHC-alpha	109.25	94.33	64.58	47.13
Hexachlorobenzene	86.13	80.75	43.58	30.50
BHC-beta	122.83	97.67	80.00	89.50
Pentachloronitrobenzene	104.00	77.13	85.50	72.25
Lindane (BHC gamma)	115.50	97.38	60.92	50.00
BHC-delta	124.13	99.25	88.92	70.13
Heptachlor	106.83	67.38	56.92	41.38
Aldrin	99.08	92.33	55.08	39.75
Heptachlor epoxide	108.50	82.63	65.33	42.13
Endosulfan alpha	98.92	99.50	57.33	42.25
Chlordane *trans*(gamma)	107.33	96.58	59.08	45.42
Chlordane *cis* (alpha)	114.38	100.83	66.83	47.63
Dieldrin	92.58	94.58	58.75	45.38
4,4'DDE	125.00	108.75	89.33	63.58
Endosulfan (II) beta	71.25	108.50	68.00	58.25
Endrin	90.38	100.63	77.50	94.17
4,4' DDD	143.00	125.25	251.42	138.88
Endosulfan sulphate	111.00	103.17	143.25	119.38
4,4' DDT	151.38	119.88	224.92	166.63
Mirex	136.88	136.38	86.25	71.33

Table 5.5: No-matrix sample recoveries

	Matrix-matched calibration		Matrix-free calibration	
Organochlorine pesticide	External Standard	Internal Standard	External Standard	Internal Standard
BHC-alpha	63.50	51.25	35.00	23.88
Hexachlorobenzene	62.63	56.50	30.42	23.13
BHC-beta	77.13	41.00	44.67	41.25
Pentachloronitrobenzene	36.00	30.33	44.75	31.00
Lindane (BHC gamma)	72.92	60.83	36.08	33.42
BHC-delta	84.50	56.13	56.25	38.50
Heptachlor	62.17	34.38	32.67	22.50
Aldrin	62.25	57.08	34.25	24.25
Heptachlor epoxide	76.08	52.50	45.17	29.25
Endosulfan alpha	65.42	62.83	37.25	27.67
Chlordane *trans*(gamma)	67.25	59.75	37.25	30.88
Chlordane *cis* (alpha)	74.58	62.50	42.17	30.25
Dieldrin	73.33	78.88	46.58	37.88
4,4'DDE	63.42	48.33	42.33	31.75
Endosulfan (II) beta	69.50	76.50	48.83	45.75
Endrin	54.88	51.13	47.33	55.63
4,4' DDD	78.63	50.67	88.88	43.75
Endosulfan sulphate	69.25	50.42	78.08	62.75
4,4' DDT	57.13	33.83	71.75	49.25
Mirex	64.13	49.92	34.17	27.50

5.4 Statistical analysis hypothesis testing

5.4.1 Descriptive and inferential statistics

The data in Tables 5.3, 5.4 and 5.5 was used to test the 7 Hypotheses.

5.4.1.1 Hypothesis 1

H_0 : There are no significant differences between all the three sample matrices ($\mu_1 = \mu_2 = \mu_3$).

Descriptive statistics were applied in testing hypothesis 1 to describe the main characteristics of the data collected.

Table 5.6: Descriptive tests for Hypothesis 1

Dependent variable :Recovery (%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Low matrix	80	91.3105	38.10322	4.26007	82.8310	99.7900	30.50	251.42
No Matrix	80	50.5446	16.83411	1.88211	46.7984	54.2909	22.50	88.88
High Matrix	80	70.2925	35.92127	4.01612	62.2986	78.2864	9.25	189.25
Total	240	70.7159	35.75349	2.30788	d66.1695	75.2623	9.25	251.42

The data in Table 5.6 above indicate that the analytical method, coupled with the overall different calibration techniques applied was best suited for the low-matrix samples as indicated by the highest mean recovery of 91.3105%. The high-matrix samples had a mean recovery of 70.2925% and the no-matrix samples had the lowest mean recovery of 50.5446%. Low-matrix samples had the most precise upper bound mean at 95% Confidence Interval (CI) of 99.7900% and also the highest lower bound mean of 82.8310%. No-matrix samples had both the lowest upper bound and lower bound means of 54.2909% and 46.7984% respectively. The no-matrix samples displayed the

lowest standard deviation at 95% confidence level of 1.88211% and the low-matrix displayed the highest standard deviation of 4.26007%.

Inferential statistics applied on hypothesis 1 was tested using a one way Multivariate Analysis of Variance (MANOVA). Firstly, to test the homogenety of the variances of the high, low and no-matrices, assuming equal variances, the Levene's F- test for equality of variance was used to verify the assumption that the variances were equal across all groups, that is high-matrix samples, low-matrix samples and no-matrix samples.

Table 5.7: Test of Homogeneity of variances for hypothesis 1

Dependent variable: Recovery (%)

Levene's Statistic	DF1	DF2	Sig. (P-value)
15.716	2	237	0.000

Table 5.7 above indicates that the Levene's F statistic has a significance level of 0.000 at 2 degrees of freedom. Applying the decision rule;
If p-value ≤ α (0.05)= assume unequal variances
If p-value > α (0.05)= assume equal variances;

It can be deduced that the assumption of homogenety is not met. This indicates that the high, low and no-matrix samples do not have similar variances therefore the ANOVA F-test is not applicable and the robust test of equality of means is applicable.

Table 5.8: Robust Tests of Equality of Means for Hypothesis 1

Dependent variable: Recovery (%)

	Statistic[a]	DF1	DF2	sig. (p-value)
Welch's test	42.588	2	136.557	0.000

a. Asymptotically F distributed.

The Welch's test was applied for sample groups as the Levene's F- test indicated that there were unequal variances. The Welch's test was used to determine whether there were any significant differences between the high, low and no-matrix samples.
The decision rule applied was:
p-value ≤ 5 = the sample groups have statistically significant differences
p-value > 5 = the sample groups have statistically insignificant differences.

The above table shows that the p-value for the Welch 's test of 0.000 at 2 degrees of freedom, is less than 0.05 therefore leading to the decision that there are statistically significant differences between the groups.

5.4.1.1.1 Post –hoc analysis

To test which which groups (high, low and no-matrices) differ from each other, having proven that there are significant differences between the three groups as a whole, multicomparisons between the groups were applied. The Tukey's honestly significant difference (HSD) test was applied for the comparisons.

Table 5.9: Multiple Comparisons for hypothesis 1

Dependent variable: Recovery (%)

	(I) Sample matrix	(J) Sample matrix	Mean Difference (I-J)	Std. Error	Sig. (p-value)	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Low-matrix	No-Matrix	40.76588*	5.02127	0.000	28.9230	52.6087
		High-Matrix	21.01800*	5.02127	0.000	9.1752	32.8608
	No-Matrix	Low-matrix	-40.76588*	5.02127	0.000	-52.6087	-28.9230
		High-Matrix	-19.74788*	5.02127	0.000	-31.5907	-7.9050
	High-Matrix	Low-matrix	-21.01800*	5.02127	0.000	-32.8608	-9.1752
		No-Matrix	19.74788*	5.02127	0.000	7.9050	31.5907

Applying the decision rule:
p-value ≤0.05 = the sample matrices have statistically significant differences

p-value >0.05 = the sample matrices have statistically insignificant differences;
it can be deduced that there are statistically significant differences in recoveries of all combinations of groups as all the p-values are 0.000, a value less than 0.05.

5.4.1.2 Hypothesis 2

H_0 : There is no significant difference between the matrix-matched calibration and matrix-free calibration types ($\mu_{MM} = \mu_{MF}$).

Descriptive statistics for grouped recoveries for matrix-matched standard calibration and matrix-free standard calibration types are shown below. A total of 120 variables were grouped and analysed for both matrix-matched and matrix-free calibration. The descriptive statistics (group statistics for 120 variables) are shown in Table 5.10 below.

Table 5.10: Descriptive tests for Hypothesis 2

Dependent variable: Recovery (%)

	Calibration Type	N	Mean	Std. Deviation	Std. Error Mean
Recovery	Matrix-Matched	120	81.5481	28.14724	2.56948
	Matrix-Free	120	59.8837	39.22313	3.58057

Tabe 5.10 above indicates that the mean for matrix-matched calibration was more precise than the mean for matrix-free calibration which were 81.5481% and 59.8837% respectively. This indicates that the matrix-matched calibration produced more precise recoveries than than matrix-free calibration. Matrix-matched calibration also had a lower standard deviation at 95% confidence and a lower standard error of 28.14724% and 2.56948% respectively. This shows that matrix-matched calibration type is generally a more robust calibration technique than matrix-free calibration.

Inferential statistics for Hypothesis 2 was analysed using a T-test for independent samples. The T-test assumes that the variability of each calibration type (matrix-matched and matrix-free) is approximately equal and therefore the Levene's test for

equality of variances at 95% confidence level was applied as it was assumed that the variances were not equal, using the decision rule;

If p-value ≤ α (0.05)= reject null hypothesis

If p-value > α (0.05)= accept null hypothesis.

Table 5.11: Independent Samples Test

Dependent variable: Recovery (%)

		Levene's Test for Equality of Variances		T-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig. (p-value)	T	DF	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Recov	Equal variances assumed	1.135	0.288	4.916	238	0.000	21.66442	4.40712	12.98248	30.34635
	Equal variances not assumed			4.916	215.873	0.000	21.66442	4.40712	12.97793	30.35090

The above Table 5.11 shows that the p-value for the Levene's test was 0.288, therefore the null hypothesis was accepted, implying that the variances are equal. From Table 5.11 above, the T-value, (labelled under column "T") assuming equal variances, is 4.916 and has 238 degrees of freedom. The decision rule applied for the 2-tailed test is;

If p-value ≤ α (0.05)= reject null hypothesis

If p-value > α (0.05)= accept null hypothesis.

The p-value for the Levene's test for equality of variances at 95% confidence level was 0.000, leading to the decision to reject the null hypothesis. The results point to the deduction that the mean recoveries are not the same for both matrix-matched and matrix-free calibration types.

5.4.1.3 Hypothesis 3

H_0 : There are no significant differences in the mean recoveries for both internal standard and external standard calibration technique ($\mu_{IS} = \mu_{EXTSTD}$).

Group descriptive statistics on 120 variables on internal standard and external standard calibration techniques show that the internal standard calibration technique produced an overally more precise mean recovery of 77.0885% than external standard calibration technique which had an overall mean of 64.3433%.

Table 5.12: Descriptive statistics (group statistics for 120 variables)

Dependent variable: Recovery (%)

	Calibration Technique	N	Mean	Std. Deviation	Std. Error Mean
Recovery	Internal Standard	120	77.0885	38.37882	3.50349
	External Standard	120	64.3433	31.82026	2.90478

The internal standard calibration technique had both a higher standard deviation and standard error at 95% confidence level of 38.3788 and 3.50350 than external standard calibration techniques of 31.8203 and 2.9048 respectively. This indicates that the internal standard calibration is a more robust calibration technique than the external standard calibration technique for the selected organochlorine compounds.

Hypothesis 3 was tested using the independent sample T-test, assuming equal variances. The Levene's test for equality of variances at 95% confidence level was applied using the decision rule;

If p-value ≤ α (0.05)= reject null hypothesis

If p-value > α (0.05)= accept null hypothesis.

Table 5.13: Independent Samples Test for Hypothesis 3

Dependent variable: Recovery (%)

		Levene's Test for Equality of Variances		T-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	DF	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Recovery	Equal variances assumed	0.162	0.687	2.800	238	0.006	12.74525	4.55106	3.77974	21.71076
	Equal variances not assumed			2.800	230.104	0.006	12.74525	4.55106	3.77816	21.71234

The above table 5.13 shows that the p-value for the Levene's test was 0.678, therefore the null hypothesis was accepted, implying that the variances are equal. Assuming equal variances, the T-value was 2.800 at 238 degrees of freedom. The decision rule applied for the 2-tailed test was;

If p-value ≤ α (0.05)= reject null hypothesis

If p-value > α (0.05)= accept null hypothesis.

The p-value for the Levene's test for equality of variances at 95% confidence level was 0.006, hence the decision to reject the null hypothesis. It can therefore be deduced that the mean recoveries are not the same for both internal standard and external standard calibration techniques.

5.4.1.4 Hypothesis 4

H_0 : Differences in mean recovery among the three sample matrices are independent of the two matrix calibration types (Testing A X B).

Comparing sample matrix and matrix calibration type, the mean recovery descriptive statistics overally indicate that matrix-matched calibration type produces more precice recoveries than matrix-free calibration type. The most precise mean recovery of 103.6420% was shown for low-matrix samples coupled with matrix-matched calibration type. Matrix-free calibration type coupled with no-matrix calibration type showed the lowest precision with a mean recovery of 41.1027%.

Table 5.14: Descriptive Statistics for Hypothesis 4

Dependent Variable: Recovery (%)

Sample Matrix	Calibration Type	Mean	Std. Deviation	N
Low-matrix	Matrix-Matched	103.6420	19.80257	40
	Matrix-Free	78.9790	47.29551	40
	Total	91.3105	38.10322	80
No-Matrix	Matrix-Matched	59.9865	13.27476	40
	Matrix-Free	41.1027	14.66125	40
	Total	50.5446	16.83411	80
High-Matrix	Matrix-Matched	81.0158	29.51776	40
	Matrix-Free	59.5693	38.81480	40
	Total	70.2925	35.92127	80
Total	Matrix-Matched	81.5481	28.14724	120
	Matrix-Free	59.8837	39.22313	120
	Total	70.7159	35.75349	240

The above standard deviation data also indicate that overtly, the matrix-matched calibration type produced more precice recoveries than matrix-free standards for all matrix types, that is high, low and no-matrix. The standard deviation for all matrix-matched standards is lower than matrix-free standards for all three matrix types.

Levene's Test of Equality of Error Variances was performed to test whether the error variance of the two matrix calibration types were equal across all the three sample matrix types.

Table 5.15: Levene's Test of Equality of Error Variances for Hypothesis 4

Dependent Variable: Recovery (%)

F	DF1	DF2	Sig.
7.637	5	234	0.000

The decision rule applied was;

If p-value ≤ α (0.05)= assume unequal variances

If p-value > α (0.05)= assume equal variances.

The p-value for the Levene's test for equality of variances at 95% confidence level was 0.000, hence the decision to assume unequal variances.

The two-way ANOVA shown below was used to test Hypothesis 4;

Table 5.16: Tests of Between-Subjects Effects for Hypothesis 4

Dependent Variable: Recovery (%)

Source	Type III Sum of Squares	DF	Mean Square	F	Sig.
Corrected Model	94992.011[a]	5	18998.402	21.117	0.000
Intercept	1200176.394	1	1200176.394	1334.007	0.000
Sample matrix	66495.772	2	33247.886	36.955	0.000
Calibration Type	28160.817	1	28160.817	31.301	0.000
Sample matrix * Calibration Type	335.422	2	167.711	0.186	0.830
Error	210524.564	234	899.678		
Total	1505692.969	240			
Corrected Total	305516.575	239			

a. R Squared = 0.311 (Adjusted R Squared = 0.296)

Table 5.16 above shows the test of in-between subject effects between the sample matrices and calibration types. The results show that the p-value is 0.830. Using the decision rule:

If p-value ≤ α (0.05)= reject null hypothesis

If p-value > α (0.05)= accept null hypothesis;

the results show that the differences in mean recovery among the three sample matrices are indeed independent of the two matrix calibration type. It can therefore be deduced that there are significant differences in the mean recoveries between sample matrix (p= 0.000) and calibration type (p= 0.000).

5.4.1.5 Hypothesis 5

H_0 : Differences in mean recovery among the three sample matrices are independent of the two calibration techniques (Testing A X C).

The descriptive statistics show that the internal standard calibration technique coupled with low-matrix sample produced the most precise mean recovery of 99.3243%. The lowest mean recovery was reported for the external standard calibration technique coupled with no-matrix samples.

Table 5.17: Descriptive Statistics for Hypothesis 5

Dependent Variable: Recovery (%)

Sample matrix	Calibration Technique	Mean	Std. Deviation	N
Low-matrix	Internal Standard	99.3243	42.41942	40
	External Standard	83.2967	31.77729	40
	Total	91.3105	38.10322	80
No-Matrix	Internal Standard	56.7145	16.66501	40
	External Standard	44.3748	14.77266	40
	Total	50.5446	16.83411	80
High-Matrix	Internal Standard	75.2268	38.48120	40
	External Standard	65.3583	32.90920	40
	Total	70.2925	35.92127	80
Total	Internal Standard	77.0885	38.37882	120
	External Standard	64.3433	31.82026	120
	Total	70.7159	35.75349	240

The internal standard calibration techniques showed a higher standard deviation than the external standard calibration techniques for all sample matrices.

Levene's Test of Equality of Error Variances was performed to determine whether the error variance of the two matrix calibration techniques were equal across all the three matrix types.

Table 5.18: Levene's Test of Equality of Error Variances for Hypothesis 5

Dependent Variable: Recovery (%)

F	DF1	DF2	Sig.
7.969	5	234	0.000

Using the decision rule;

If p-value ≤ α (0.05)= assume unequal variances

If p-value > α (0.05)= assume equal variances;

unequal variances can be assumed since the p-value for the Levene's test for equality of variances at 95% confidence level was 0.00.

To test hypothesis 5, the two-way ANOVA shown below was used.

Table 5.19: Tests of Between-Subjects Effects for Hypothesis 5

Dependent Variable: Recovery (%)

Source	Type III Sum of Squares	DF	Mean Square	F	Sig.
Corrected Model	76626.522[a]	5	15325.304	15.667	0.000
Intercept	1200176.394	1	1200176.394	1226.970	0.000
Sample matrix	66495.772	2	33247.886	33.990	0.000
Calibration Technique	9746.484	1	9746.484	9.964	0.002
Sample matrix * Calibration Technique	384.266	2	192.133	0.196	0.822
Error	228890.053	234	978.163		
Total	1505692.969	240			
Corrected Total	305516.575	239			

a. R Squared = 0.251 (Adjusted R Squared = 0.235)

The test of in-between subject effects between the sample matrices and calibration types indicate that the p-value is 0.822. Using the decision rule:
If p-value ≤ α (0.05)= reject null hypothesis
If p-value > α (0.05)= accept null hypothesis;

the null hypothesis is accepted, therefore it can be deduced that there are significant differences in the mean recoveries between sample matrix (p= 0.000) and calibration technique (p= 0.000).

5.4.1.6 Hypothesis 6

H_0 : Differences in mean recovery between two the matrix calibration types are independent of the two calibration techniques (Testing A X C)

Hypothesis 6 compares the effects of the calibration types with the effects of the calibration techniques used.

Table 5.20: Descriptive Statistics for hypothesis 6

Dependent Variable: Recovery (%)

Calibration Technique	Calibration Type	Mean	Std. Deviation	N
Internal Standard	Matrix-Matched	86.5525	27.77292	60
	Matrix-Free	67.6245	44.91467	60
	Total	77.0885	38.37882	120
External Standard	Matrix-Matched	76.5437	27.85121	60
	Matrix-Free	52.1428	31.04486	60
	Total	64.3433	31.82026	120
Total	Matrix-Matched	81.5481	28.14724	120
	Matrix-Free	59.8837	39.22313	120
	Total	70.7159	35.75349	240

The descriptive statistics in Table 5.20 above show that matrix-matched calibration type coupled with the internal standard calibration technique produced the most precise

mean recovery of 86.5525% and the matrix-free calibration type coupled with the external standard calibration technique produced the least precise mean recovery of 52.1428%. The matrix-matched calibration coupled with internal standard calibration type calibration technique also showed the lowest standard deviation of 27.77292.

Levene's Test of Equality of Error Variances was performed to determine whether the error variance of the two matrix calibration techniques were equal across all the three matrix types.

Table 5.21: Levene's Test of Equality of Error Variances for Hypothesis 6

Dependent Variable: Recovery (%)

F	DF1	DF2	Sig.
0.988	3	236	0.399

The decision rule:

If p-value ≤ α (0.05)= assume unequal variances

If p-value > α (0.05)= assume equal variances;

led to the decision to assume equal variances since the p-value = 0.399.

Hypothesis 6 was tested using the two-way ANOVA shown below.

Table 5.22: Tests of Between-Subjects Effects for Hypothesis 6

Dependent Variable: Recovery (%)

Source	Type III Sum of Squares	DF	Mean Square	F	Sig.
Corrected Model	38356.579[a]	3	12785.526	11.294	0.000
Intercept	1200176.394	1	1200176.394	1060.195	0.000
Calibration Technique	9746.484	1	9746.484	8.610	0.004
Calibration Type	28160.817	1	28160.817	24.876	0.000
Calibration Technique * Calibration Type	449.279	1	449.279	0.397	0.529
Error	267159.995	236	1132.034		
Total	1505692.969	240			
Corrected Total	305516.575	239			

a. R Squared = 0.126 (Adjusted R Squared = 0.114)

Using the decision rule;

If p-value ≤ α (0.05)= reject null hypothesis

If p-value > α (0.05)= accept null hypothesis;

given that the p-value = 0.529 at 1 degree of freedom, the null hypothesis is accepted therefore the mean recovery between two the matrix calibration types are independent of the two calibration techniques.

5.4.1.7 Hypothesis 7:

H_0 : Differences in mean recovery among the three sample matrices are independent of the two other factors (Testing A X B X C interaction).

Table 5.23: Descriptive statistics for hypothesis 7

Dependent Variable: Recovery (%)

Calibration Technique	Calibration Type	Sample matrix	Mean	Std. Deviation	N
Internal Standard	Matrix-Matched	Low-matrix	109.4740	20.63789	20
		No-Matrix	66.7350	10.45092	20
		High-Matrix	83.4485	29.72307	20
		Total	86.5525	27.77292	60
	Matrix-Free	Low-matrix	89.1745	55.23331	20
		No-Matrix	46.6940	15.79399	20
		High-Matrix	67.0050	44.87506	20
		Total	67.6245	44.91467	60
	Total	Low-matrix	99.3243	42.41942	40
		No-Matrix	56.7145	16.66501	40
		High-Matrix	75.2268	38.48120	40
		Total	77.0885	38.37882	120
External Standard	Matrix-Matched	Low-matrix	97.8100	17.53269	20
		No-Matrix	53.2380	12.51451	20
		High-Matrix	78.5830	29.87531	20
		Total	76.5437	27.85121	60
	Matrix-Free	Low-matrix	68.7835	36.35798	20
		No-Matrix	35.5115	11.22286	20
		High-Matrix	52.1335	31.02096	20
		Total	52.1428	31.04486	60
	Total	Low-matrix	83.2967	31.77729	40
		No-Matrix	44.3748	14.77266	40
		High Matrix	65.3583	32.90920	40
		Total	64.3433	31.82026	120
Total	Matrix-Matched	Low matrix	103.6420	19.80257	40
		No-Matrix	59.9865	13.27476	40
		High-Matrix	81.0158	29.51776	40
		Total	81.5481	28.14724	120
	Matrix-Free	Low matrix	78.9790	47.29551	40
		No Matrix	41.1027	14.66125	40
		High Matrix	59.5693	38.81480	40
		Total	59.8837	39.22313	120
	Total	Low-matrix	91.3105	38.10322	80
		No-Matrix	50.5446	16.83411	80
		High-Matrix	70.2925	35.92127	80
		Total	70.7159	35.75349	240

Using the Levene's Test of Equality of Error Variances it was determined that the variance between the calibration type, calibration technique and sample matrix type were unequal since p= 0.000.

Table 5.24: Levene's Test of Equality of Error Variances for Hypothesis 7

Dependent Variable: Recovery (%)

F	DF1	DF2	Sig.
4.391	11	228	0.000

The decision rule used was;

If p-value ≤ α (0.05)= assume unequal variances

If p-value > α (0.05)= assume equal variances;

Hypothesis 7 was tested using the two-way ANOVA shown below.

Table 5.25: Tests of Between-Subjects Effects for Hypothesis 7

Dependent Variable: Recovery (%)

Source	Type III Sum of Squares	DF	Mean Square	F	Sig.
Corrected Model	106030.948[a]	11	9639.177	11.017	0.000
Intercept	1200176.394	1	1200176.394	1371.729	0.000
Calibration Technique	9746.484	1	9746.484	11.140	0.001
Calibration Type	28160.817	1	28160.817	32.186	0.000
Sample matrix	66495.772	2	33247.886	38.000	0.000
Calibration Technique* Calibration Type	449.279	1	449.279	0.513	0.474
Calibration Technique * Sample matrix	384.266	2	192.133	0.220	0.803
Calibration Type * Sample matrix	335.422	2	167.711	0.192	0.826
Calibration Technique * Calibration Type * Sample matrix	458.909	2	229.454	0.262	0.770
Error	199485.627	228	874.937		
Total	1505692.969	240			
Corrected Total	305516.575	239			

a. R Squared = 0.347 (Adjusted R Squared = 0.316)

Using the decision rule;

If p-value ≤ α (0.05)= reject null hypothesis

If p-value > α (0.05)= accept null hypothesis;

The null hypothesis was accepted as the p-value = 0.770. This indicates that the differences in mean recovery among the three sample matrices are independent of the calibration type and/or calibration technique.

Table 5.26 below shows the results of analysis of a real sample matrix using the external standard calibration method on matrix-free and matrix-matched standards. It can be clearly observed that for most analytes the use of matrix-matched standards adversely increased the detection limit for the detected organochlorine residues.

5.5 Analytical results of real samples

Table 5.26: Matrix-matched and matrix-free calibration of real samples

	MFEXTSTD calibration (mg/L)			MMEXTSTD calibration (mg/L)		
Organochlorine pesticide	Real sample 1	Real sample 2	Real sample 3	Real sample 1	Real sample 2	Real sample 3
BHC-alpha	0.01	0.013	0.012	0.011	0.015	0.016
Hexachlorobenzene	0	0	0	0	0	0
BHC-beta	0.04	0.055	0.046	0.011	0.041	0.028
Pentachloronitrobenzene	0	0	0	0	0	0
Lindane (BHC gamma)	0	0	0	0	0	0
BHC-delta	0	0	0	0	0	0
Heptachlor	0	0	0	0	0	0
Aldrin	0	0	0	0	0	0
Heptachlor epoxide	0	0	0	0	0	0
Endosulfan alpha	0	0	0	0	0	0
Chlordane *trans*(gamma)	0.004	0.004	0.004	0.001	0.001	0.001
Chlordane *cis* (alpha)	0.003	0.003	0.003	0.001	BQ	BQ
Dieldrin	0	0	0	0	0	0
4,4'DDE	0	0	0	0	0	0
Endosulfan (II) beta	0	0	0	0	0	0
Endrin	0	0	0	0	0	0
4,4' DDD	0	0	0	0	0	0
Endosulfan sulphate	0.023	0	0	0.009	0	0
4,4' DDT	0	0	0	0	0	0
Mirex	0	0	0	0	0	0

BQ= Below Quantification

The results of table 5.2.6 indicate that matrix-free calibration method applied on the determination of Chlordane *cis* in real sample matrix produced a concentration of 0.03 ng/µL whilst the matrix-matched calibration method could not quantify it above the detection limit. Matrix-matched calibration computed lower concentrations for Endosulphan sulphate, Chlordane *trans*, and BHC beta than the matrix-free calibration. The use of matrix-matched standards therefore had a suppressing effect on the analyte response, save for only BHC alpha where matrix-matched standards computed higher concentrations than matrix-free standards calibration.

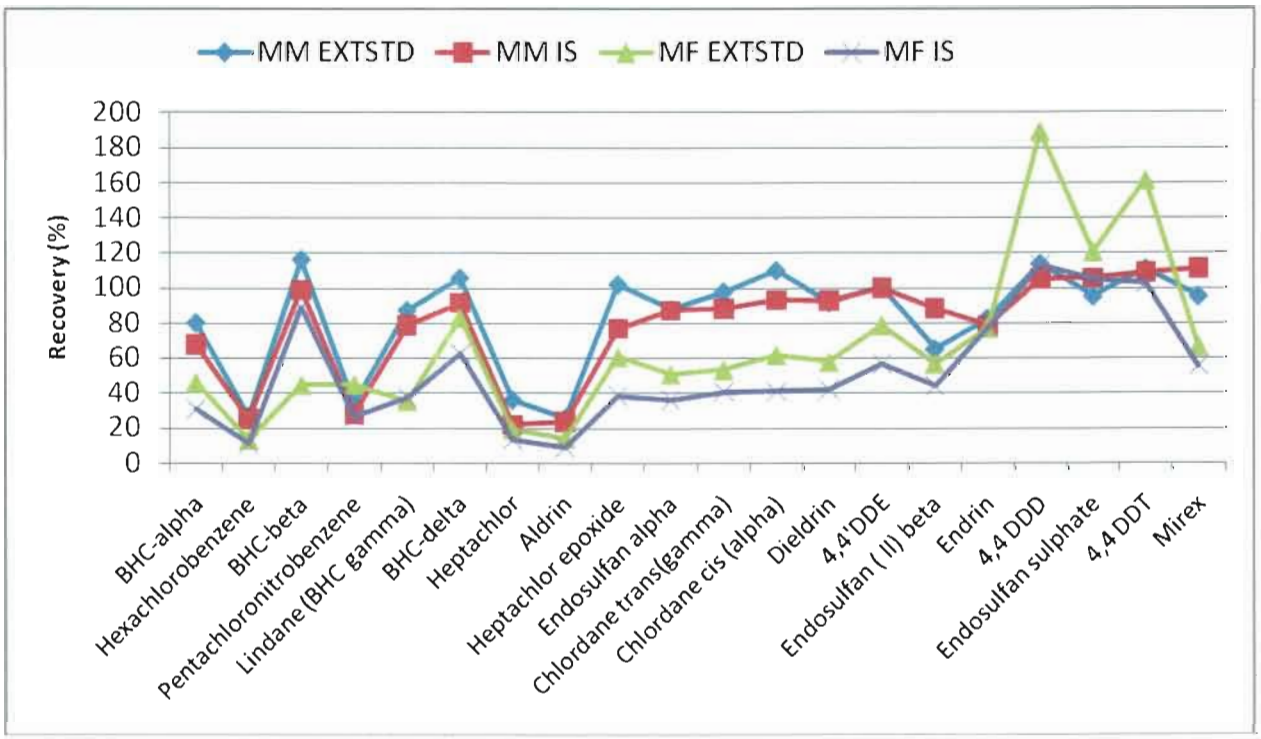

Figure 5.41: High-matrix samples

From figure 5.41 above, it can be deduced that the GC-MS-SPE method was not very efficient in quantifying some of the organochlorine pesticides in the high-matrix samples as shown by low recoveries for Hexachlorobenzene, Pentachloronitrobenzene, Heptachlor and Aldrin. The matrix-matched external standard and internal standard methods were however found to be efficient in quantifying more than 50% of the

selected organochlorine compounds. Most notably is the matrix-matched external standard calibration for which most of the selected organochlorine compounds produced recoveries within the 100±30% range. Matrix-matched external standard calibration method however showed the highest degree of precision and matrix-free internal standard showed the least precise recoveries.

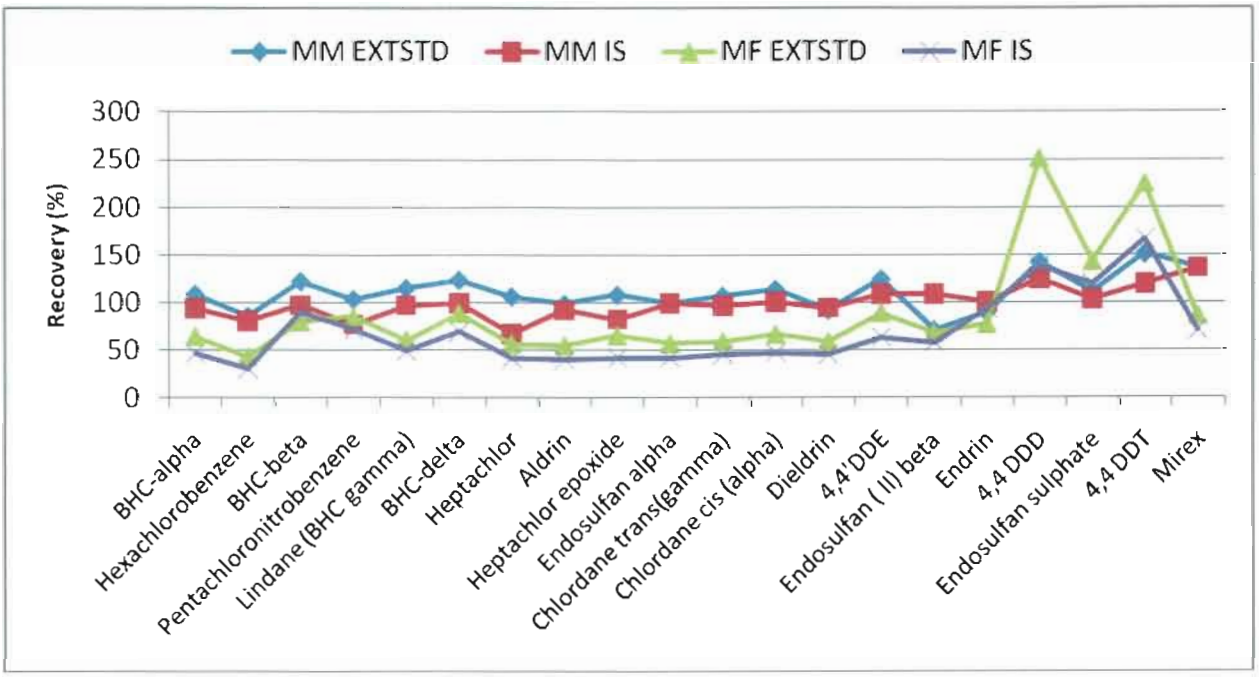

Figure 5.42: Low-matrix samples

Figure 5.42 above indicates that the GC-MS-SPE method was efficient in quantifying the selected organochlorine compounds in the low-matrix samples; most notably the matrix-matched external standard and matrix-matched internal standard methods which produced precise recoveries with almost all the selected organochlorine compounds being in the 100±30% range. Matrix-free external standard and matrix-free internal standard calibration methods showed a low degree of precision in computing recoveries with half of the organochlorine compounds analysed showing a recovery of <50%. This indicated that matrix-matched standard calibration, particularly coupled with external standard calibration is the best calibration technique to use for the analyses of the selected organochlorine pesticides as it produced more precise quantification results.

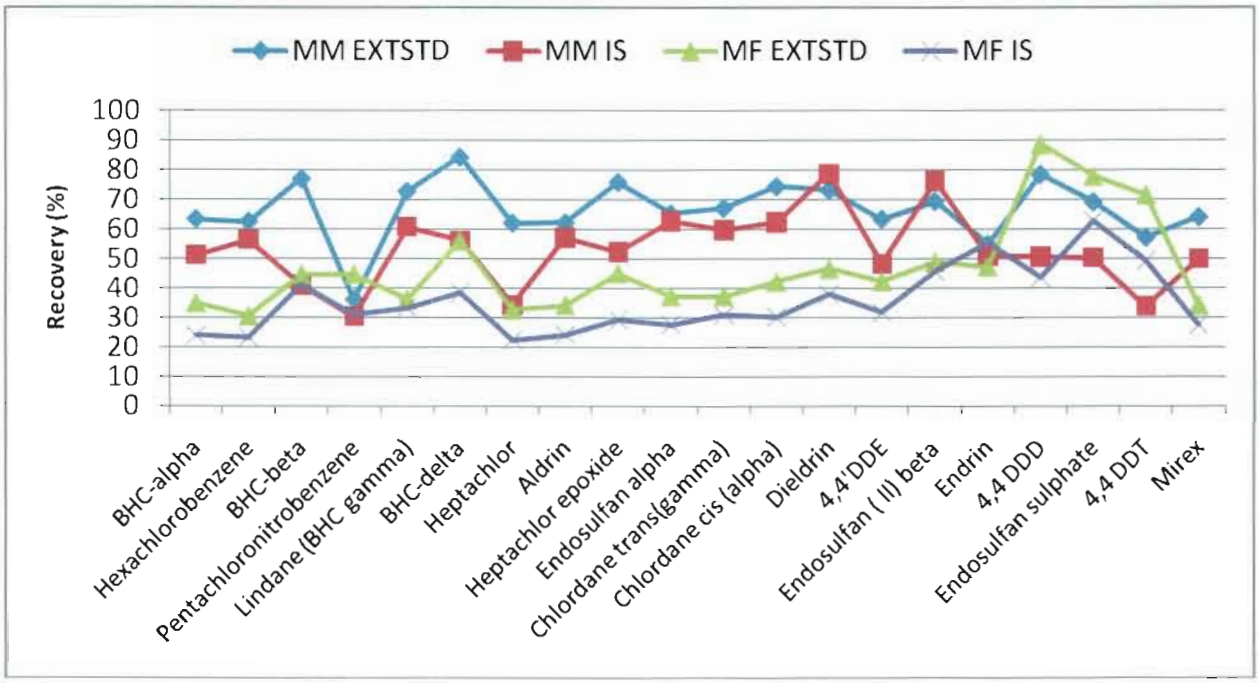

Figure 5.43: No-matrix samples

All calibration techniques used generally showed low precision in computing the recoveries for the selected organochlorine compounds on the no-matrix samples. This indicates that the GC-MS-SPE method was less efficient for analysing matrix-free extracts than matrix-based extracts. The matrix-matched external standard technique however showed the highest precision in computing recoveries with most organochlorine compounds displaying recoveries of >60%. Matrix-free internal standard showed the least precision with most compounds producing <50% recoveries.

5.6 Application of the matrix-matched external standard calibration method on spiked real samples

The matrix-matched external calibration method was applied to real-sample matrix samples spiked with a 0.4 mg/L cocktail of the selected organochlorine compounds. The results show a high degree of precision and accuracy in the three replicates and recoveries. The relative standard deviation for all the replicates were all <10%, with 4,4'

DDT showing a RSD% of 0.102%, indicating that the method had a high degree of repeatability.

Table 5.27: Recoveries of real spiked samples analysed by Matrix-matched external standard calibration method

Organochlorine compound	Real sample 1 (mg/L)	Real sample 2 (mg/L)	Real sample 3 (mg/L)	Sample Mean (mg/L)	RSD%	Mean recovery (%)
BHC-alpha	0.388	0.437	0.426	0.417	6.165	104.25
Hexachlorobenzene	0.380	0.371	0.388	0.380	2.240	94.88
BHC-beta	0.416	0.433	0.488	0.446	8.444	111.42
Pentachloronitrobenzene	0.411	0.399	0.423	0.411	2.920	102.75
Lindane (BHC gamma)	0.449	0.475	0.462	0.462	2.814	115.50
BHC-delta	0.459	0.414	0.437	0.437	5.155	109.13
Heptachlor	0.385	0.410	0.397	0.397	3.147	99.33
Aldrin	0.409	0.379	0.401	0.396	3.920	99.08
Heptachlor epoxide	0.418	0.402	0.432	0.417	3.597	104.33
Endosulfan alpha	0.393	0.370	0.424	0.396	6.849	98.92
Chlordane *trans*(gamma)	0.413	0.411	0.464	0.429	6.997	107.33
Chlordane *cis* (alpha)	0.426	0.439	0.433	0.433	1.503	108.13
Dieldrin	0.380	0.367	0.394	0.380	3.550	95.08
4,4'DDE	0.462	0.458	0.460	0.460	0.435	115.00
Endosulfan (II) beta	0.349	0.361	0.365	0.358	2.324	89.58
Endrin	0.353	0.377	0.400	0.377	6.242	94.13
4,4' DDD	0.459	0.475	0.467	0.467	1.713	116.75
Endosulfan sulphate	0.475	0.413	0.444	0.444	6.982	111.00
4,4' DDT	0.491	0.490	0.491	0.491	0.102	122.63
Mirex	0.443	0.454	0.431	0.443	2.599	110.63

The recoveries for all the selected organochlorine compounds were well within the 100±30% quality control range, with most of them averaging just below or above 100%. Heptachlor and Pentachloronitrobenzene showed an average recovery of 99.33% and 102.75% respectively. It can therefore be deduced that the method developed was effective in counteracting the matrix effects so as to produce accurate and precise recoveries.

5.7 Conclusion

The findings of this research point out the fact that the matrix-matched external standard calibration method overally proved to be the best calibration method as it

produced more accurate and precise recoveries. Statistical analysis also indicated that the sample matrix type has a significant effect on the quality of both qualitative and quantitative analytical data produced. It can therefore be concluded that the GC-MS-SPE method developed was effective in counteracting the matrix effects so as to produce accurate and precise recoveries.

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CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

Validation of the GC-MS method developed for the analysis of the selected organochlorine pesticides indicated that the GC-MS method was robust in measuring what it is intended to measure. Validation criteria such as linearity ($R^2 \geq 0.998$), regression, repeatability, sensitivity (in terms of LOD and LOQ) and statistical hypothesis testing through ANOVA F-test led to the conclusion that there was significant linearity in all the organochlorine compounds with the exception of DDT whose calibration curve could not produce a significant linearity, R^2 of ≥0.998 for the calibration range of 1 ppm - 0.0156 ppm tested.

The results of SPE method development indicated that conditioning of the cartridges used was essential as poor recoveries were produced when cartridges were not conditioned compared to when the cartridges were conditioned. It was also evident that both the conditioning and elution parameters employed were critical in obtaining optimum extraction of the organochlorine pesticides from the samples. The SPE method development proved that the LC-18 cartridge was most robust for the analysis of the selected organochlorine pesticides as it overally produced more precise recoveries even when significant changes were made to the sample preparation procedure. Recovery calculations used to test the validity of the automated SPE method procedure on real sample matrices using the LC-18 cartridge indicated that the SPE method was overwhelmingly precise in extracting most of the organichlorine pesticides tested. A percentage of 80% of the organochlorine pesticides showed a recovery within the target range of 100±30% indicating that the SPE method developed was valid.

When the optimum breakthrough volume of 100 mL for the LC-18 cartridge was tested against a spiked real sample matrix, the following matrix effects were clearly identified when the chromatogram was compared to a spiked matrix-free chromatogram; matrix-induced response enhancement chromatographic effects, matrix-induced response

diminishment chromatographic effects, loss of efficiency (sharp and narrow peaks), loss of resolution, higher background noise, peak fronting and peak broadening.

The matrix-matched external standard calibration method proved to be the best for the organochlorine compounds tested as it produced more precice recoveries when tested against high, low and no-matrix samples. The matrix-free external standard calibration method also had the widest calibration range.

It was clearly evident that the GC-MS-SPE method was more efficient in quantitating low-matrix samples than the high-matrix and no-matrix samples as shown by the more precise recoveries produced by the low-matrix samples.

Statistical hypothesis testing proved the following;

1) The mean recoveries between the three sample matrices, that is high-matrix, low matrix and no-matrix are not the same.
2) The mean recoveries between both the matrix-matched and matrix-free standard calibration types are not the same.
3) The mean recoveries are not the same for both internal standard and external standard calibration techniques.
4) The differences in mean recoveries among the three sample matrices (high, low and no-matrix) are independent of the two matrix calibration types (matrix-matched and matrix-free).
5) The differences in mean recoveries among the three sample matrices (high, low and no-matrix) are independent of the two matrix calibration techniques (internal standard and external standard).
6) The differences in mean recoveries between two the matrix calibration types are independent of the two calibration techniques.
7) The differences in mean recoveries among the three sample matrices are independent of both the calibration technique and calibration type used.

It can therefore be concluded from the study that the matrix effects do have a considerable effect with respect to the detection and quantification of the selected organochlorine pesticides. Furthermore it can be concluded that the use of matrix-matched standards, coupled to the use of the external standard calibration technique will help to produce more accurate and precise quantification in matrix-based extracts from water samples.

6.2 Recommendations

The matrix effects are a very delicate and controversial issue, therefore it is recommended to study and understand them before developing any GC analytical method. It is also recommended to validate each developed method prior to implementation. To improve quantification and detection of organichlorine compouds, It is also recommended;

To test different cartridges to determine which sorbent phase works best for a particular water sample matrix.

To test the breakthrough volume for a partucular selected cartridge to determine its optimum maximum matrix load.

To test each water sample matrix by applying matrix-matched calibration, matrix-free calibration, internal standard calibration and external standard calibration to determine which calibration method is best for the particular type of sample.

To employ automated systems in performing sample preparation as is is faster, more accurate, precise and also drastically reduces the chances of errors.

6.3 Recommendations for further studies

The study of a wider variety of matrices such as sediment is recommended as there are more serious matrix effects which need to be investigated.

The study of problematic pesticides which have high detection limits such as organophosphates should be conducted to improve their detection and quantification.

This study can also be extended to Liquid chromatography (LC) for determination and study of the matrix effects in thermally labile pesticides.

The use of high resolution and orthogonal separating systems can be employed in more in-depth to the study of the matrix effects.