IDENTIFICATION AND QUANTIFICATION OF SELECTED PESTICIDES IN SURFACE WATER IN SOUTHERN GAUTENG REGION

Malesole Nontutu Gadihele Bucibo

A dissertation submitted in fulfillment of the requirements for the degree

Magister Technologiae (Chemistry)

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Malesole Nontutu Gadihele Bucibo
B-Tech: Chemistry

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In the Department of Chemistry

Faculty of Applied and Computer Sciences
Vaal University of Technology

Supervisor	**:**	**Prof A.M. Sipamla, PhD (UPE)**
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DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree Magister Technologiae to the Department of Chemistry, Vaal University of Technology, Vanderbijlpark. It has not been submitted before for any degree or examination to any other university.

Signature: ……..

Date …….. JULY 2010

DEDICATION

This work is dedicated to the memory of my late brother Chwaro Donald Qwelane who passed away in 2006. He has been my source of inspiration from the beginning of the project until the end.

ACKNOWLEDGEMENTS

The author hereby wishes to express her sincere gratitude towards the following:

God almighty,

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Co-supervisor Dr L. L.Sibali

My Family,

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Mentors : Dr F Mtunzi and Mr Allen Haven (DWA)

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Brother : Nelson Qwelane

Chemistry department staff, for their support throughout the project

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ABSTRACT

The increased production and application of pesticides for agricultural and non-agricultural purposes has caused the pollution of air, soil, ground and surface water. This has a negative impact on the environment as well as human health due to direct exposure or through residues in food and drinking water.

The continuous monitoring of pesticides residues in environmental samples has great importance and demands high efficiency, unique selectivity and high sensitivity techniques. Gas chromatography and high performance liquid chromatography have been established for years as the techniques for the analysis of pesticides residues.

The dissertation deals with the qualitative and quantitative determination of selected pesticides in the Southern Gauteng region using Liquid- liquid extraction solid-phase extraction, high performance liquid chromatography, gas chromatography equipped with electron capture detector and gas chromatography mass spectrometry.

Liquid-liquid extraction and solid-phase extraction using dichloromethane, hexane and ethyl acetate as the extracting solvent were optimized and evaluated for the determination of pesticides in surface water in the Southern Gauteng region.

From the developed method the techniques were applied to water samples taken from different rivers selected namely: Zuikerbosch, Rand Water barrage and Kliprivier for sampling.

Dichloromethane was used as a solvent in this study since a recovery test was done between dichloromethane, Ethyl acetate and n-hexane. The percentage recovery test for 4,4-DDT, 4,4-DDE, 2,4-DDD and Endosulfan 1 & 2 ranged from

89.9% -97.3% for dichloromethane, 87.3%-96.8% for hexane 88.4%-97.1% for ethyl acetate.

The extracts obtained were subjected to column chromatography for clean up. Thereafter 1µl of the cleaned extracts were injected into the Gas chromatography equipped with an electron capture detector.

Organochlorines 4,4-DDT and its metabolites, Organophosphate Chlorypyriphos and carbamates were detected using Gas chromatography electron capture, Gas chromatography mass spectrometry and high performance liquid chromatography.

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DESCRIPTION OF ACRONYMS

“2,4-DDD”	**[2-(2-chlorophenyl)-2-(chlorophenyl)-1,1-dichloroethane];**
“4,4-DDD”	**[2,2-bis-(4-chorophenyl)-1,1-dichloroethane];**
“4,4-DDE”	**[2,2 bis-(4-chlorophenyl)-1,1-dichloroethane];**
“4,4-DDT”	**4,4-dichlorodiphenyltrichloroethane;**
“DWA”	**Department of Water Affairs ;**
“GC-ECD”	**Gas chromatography Electron Capture Detector;**
“GC-MS”	**Gas Chromatography-Mass Spectrometry;**
“HPLC”	**High performance liquid chromatography;**
“LLE”	**liquid liquid extraction;**
“LOD”	**Limits of Detection;**
“N/D”	**not detected;**
“ppm”	**parts per million;**
“% RSD”	**Percentage Relative Standard Deviation;**
“RT”	**Retention time;**
“SPE”	**solid phase extraction;**
“STD”	**Standard; and**
“WHO”	**World Health Organisation.**
“EPA”	**Environmental Protection Agency**

CHAPTER 1

INTRODUCTION

1.1 Introduction

Water is a very important constituent of the ecosystem on the Earth. The importance of water quality preservation and improvement constantly increases [1]. The term water quality is used to describe the physical, chemical, biological and aesthetic properties of water that determine its fitness for a variety of uses including consumption by humans and for the protection of aquatic ecosystems [2]. Many of these properties are controlled or influenced by constituents that are either dissolved or suspended in water [2].

Impaired water quality has a negative impact not only on potable use, but also on practically all water uses. It is further an unfortunate fact that most water uses are accompanied by deterioration in water quality.

Natural waters are contaminated with various pesticides or their transformation products. Herbicides and Nematicides are potential contaminants of natural waters because they are directly applied to the soil and are transported into ground water or leached to the surface water. Insecticides are transported into ground water in dust or rain water, which are washed out by precipitation and fall on the soil. The EEC Directive 80/778 concerning the quality of water designated for human consumption, establishes the maximum admissible concentration of each individual pesticide at 0.1ug. L-1 and the total amount of pesticides at 0.5 ug.L^{-1} [1].

During the last decade, the use of many chemical substances without any control and studies about their behavior has become a focus of interest for the European union **(EU)** [3]**.** Face to their capacity to be persistent and have a potential estrogenic activity, the aim of the registration evaluation and authorization of chemicals (**REACH**) system which came into force in June 2007 is to protect human health and the environment from impact of more than 32 million chemical substances registered to the Chemical Abstract Service [3]. Many high production volumes of chemicals, including some already identified as endocrine disrupting

compounds (EDCs), have domestic uses and are continually discharged in the urban wastewater system [3].

Rural communities depend on surface water from the rivers for potable uses such as washing of clothes, drinking and animals also drink from these rivers. Fishing is also one major use from the rivers and pesticides have also been indicated to be present in fish.

Environmental pollution is a major global concern. When sources of water pollution are enumerated, agriculture is, with increasing frequency, listed as a major contributor. Existing knowledge indicates that agricultural operations can contribute to water quality deterioration through the release of several materials into water, sediments, pesticides, animal manures and other sources of inorganic and organic matter. Many of these pollutants reach surface and groundwater resources through widespread runoff and percolation [4].

Groundwater supply, which is basically non-renewable, is diminishing and, in addition, anthropogenic groundwater pollution has become a fact of life. A majority of anthropogenic water pollutants are toxic, dangerous not only to humans but also to animals and plant [5].

Pesticides

The increasing production and application of pesticides for agricultural and non-agricultural purposes has caused the pollution of air, soil ground and surface water. This will have a negative impact on the environment as well as human health due to direct exposure or through residues in food and drinking water. In the world, alarming levels of pesticides have been reported in air, water, soil as well as foods and biological materials [6].

Pesticide residues reach the aquatic environment through direct run-off, leaching, careless disposal of empty containers, equipment washings [7].

Organochlorine such as dichlorodiphenyl-trichloroethane (DDT) pesticides are considered to be dangerous not only for the environment but for human beings as well [7]. They are very stable substances and it has been cited that the degradation of DDT in soil is 75-100% in 4-30 years [7]. Chlorinated pesticides are ubiquitous environmental contaminants [8]. Due to the long residence time of these substances in the environment, there is a great interest in examining the pollution they cause.

For decades, the extensive use of organophosphorus pesticides in predominantly agricultural areas has been favoured over the more persistent organochlorine pesticides mainly because of their quicker degradation rates [9].

Carbamates

The carbamates group represents a unique class of diverse compounds. The mode of action of these chemicals is similar to the organophosphates and inhibits the acetylcholonesterase. The symptoms of poisoning are cholinergic, salivation, miosis, convulsions and ultimately death. They are reversible inhibitors and are rapidly detoxified and eliminated from animal tissues. Consequently, carbamates do not accumulate in fats and are not excreted in milk. Carbaryl (Sevin) was first introduced in 1956 and is a contact insecticide [10].

It is only slightly soluble in water but highly soluble in organic solvents. The compound is mildly phototoxic but toxic to fish [10].

Aquatic ecosystems may be contaminated by pesticides used in crop protection. Pesticides are introduced into water systems from various sources such as industrial effluents, agricultural runoff and chemical spills. Even if these compounds are present only in very low concentrations in water, they are hazardous because some species of aquatic life are known to concentrate them 1000-fold or more [11]. These compounds may also be detrimental to fish by interfering with their metabolism and /or reduce egg hatchability. Also, entry of these pesticides into aquatic ecosystems will adversely affect many non target

organisms including fish and birds [12]. Effects of DDE include egg thinning, egg breakage and reduced clutch size, hatching success and subsequent productivity [13].

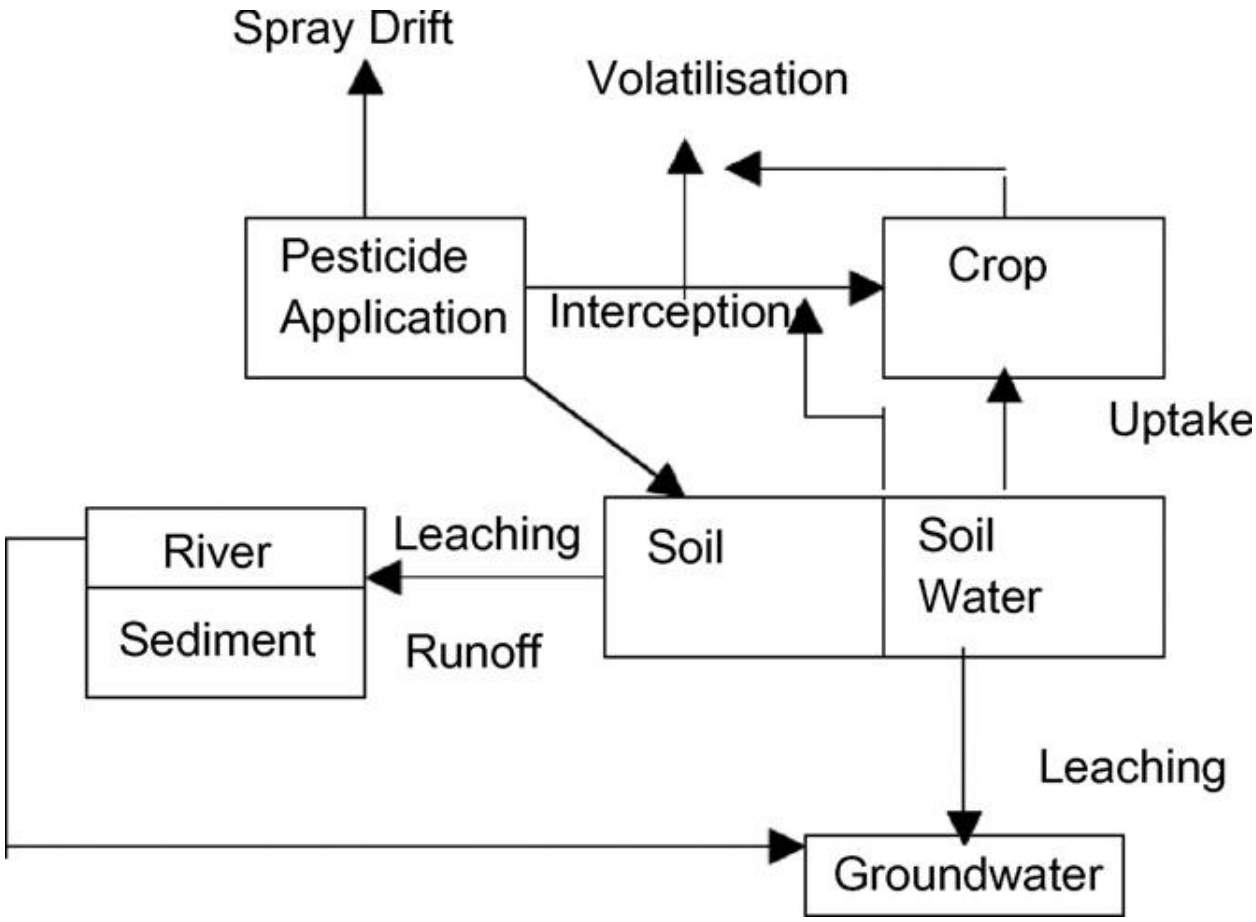


Figure 1Pathways of a pesticide applied to a crop [14].

1.2 Problem statement

Worldwide pesticides usage has increased dramatically during the last two decades, coinciding with changes in farming practices and the increasingly intensive agriculture. This widespread use of pesticides for agricultural and non-agricultural purposes such as in household for killing mosquitoes and bugs has resulted in the presence of their residues in various environmental matrices. Pesticides contamination of surface waters has been well documented and

constitutes a major issue that gives rise to concerns at local, regional, national and global scales [15].

1.3 Purpose of the study

Pesticides are a group of artificially synthesized substances used to fight pests and improve agricultural production [16].Pesticides are one class of compounds that, despite their benefits, may produce a wide range of toxic side effects that pose a potential hazard to the environment [7]. The purpose of this study is to determine the levels of contamination of organochlorines, organophosphorus and carbamates in surface water.

1.4 Objectives of this study

The primary objective of this project is assessing contamination levels of organophosphates, organochlorine and carbamates pesticides in surface water in selected areas in the Southern Gauteng Region.

1. Optimisation of the extraction methods.
2. Extraction and analysis of the selected pesticides in surface water using the extraction methods.
3. Qualitative and Quantitative analysis of the different pesticides using GC-ECD, GC-MS and HPLC.

1.5 Value of this study

The study will assist in giving an overall view of pesticides contamination levels and its effects on surface water quality.

CHAPTER 2

LITERATURE REVIEW

2.1 Background

Since before 2000BC, humans have utilized pesticides to protect their crops [17]. The first known pesticide was elemental sulfur used in summer about 4,500 years ago in ancient Mesopotamia. By the 15th century, toxic chemicals such as arsenic, mercury and lead were being applied to crops to kill pests. In the 17th century, nicotine sulfate was extracted from tobacco leaves for use as insecticide. The 19th century saw the introduction of two more natural pesticides, pyrethrum which is derived from chrysanthemums and rotenone which is derived from the roots of tropical vegetables [18].

Until the 1950s, arsenic-based pesticides were dominant [19]. Paul Muller discovered that DDT was a very effective insecticide. Organochlorines such as DDT were dominant, but they were replaced in the U.S. by the organophosphates and carbamates by 1975. Since then, pyrethrin compounds have become the dominant insecticides [19]. Herbicides became common in the 1960s, lead by triazine and other nitrogen-based compounds, carboxylic acids such as 2, 4-dichlorophenoxyacetic acid and glyphosate [19].

DDT is the best known pesticide, It was synthesized in 1874 in Germany but was recognized for its insecticidal properties in 1939 [20].

In the 1940s manufacturers began to produce large amounts of synthetic pesticides and their use became widespread some sources consider the 1940s and 1950s to have been the start of the pesticide era [21]. Pesticide use has increased 50-fold since 1950 and 2.3 million tonnes (2.5 million short tonnes) of industrial pesticides are now used each year [18]. Seventy-five percent of all pesticides in the world are used in developed countries, but use in developing countries is increasing [22].

2.2 General Description

The continued growth in human population has created a corresponding increase in the demand for the Earth's limited supply of freshwater. Thus, protecting the integrity of our water resources is one of the most essential environmental issues of the 21st century. Recent decades have brought increasing concerns for potential adverse human and ecological health effects resulting from the production, use and disposal of numerous chemicals that offer improvements in industry, agriculture and so on. Research has shown that many compounds can enter the environment, disperse and persist to greater extent than first anticipated [23]. Pesticides contamination of surface and ground waters from agricultural use have been well documented around the world. Pesticides are a group of artificially synthesized substances used to fight pests and to improve agricultural production [24].

Pesticides can be classified according to their mode or period of action, or their chemistry [25].

The widespread of pesticides for agricultural and non-agricultural purposes has resulted in the presence of their residues in various environmental matrices, such as soil, water and air [7]. More than 500 different pesticides formulations are being used in our environment, mostly in agriculture, although the control of biological public health hazards also continues to be an important field of application [26].
In the last 50 years, the use of pesticides have greatly increased the quantity and improved the quality of food for the growing world population [26]. However, with increasing amounts used, concern about their adverse effects on non target organisms, including human beings, has also grown [26]. Non target pesticide poisoning has been identified as the cause of fishes killing, reproductive failure in birds, and illness in humans.

In fact, it has been estimated that less than 0.1% of the pesticide applied to crops actually reaches the target pest; the rest enters the environment gratuitously, contaminating soil, water and air, where it can poison or otherwise adversely affect non target organisms [26].

Furthermore, many pesticides can persist for long periods in an ecosystem. Organochlorine insectides, for instance, were still detectable in surface waters 20 years after their use has been banned; and once a persistent pesticide has entered the food chain, it can undergo “biomagnifications”, i.e., accumulation in the body tissues of organisms, where it may reach concentrations many times higher than in the surrounding environment [26].

Pesticide-related deaths are a major public health problem worldwide. According to World Health Organization estimates published in 1990, there are around 3 million annual pesticides poisoning cases with 220.000 deaths [27].

A more recent study reported that the number of annual deaths from pesticide ingestion may actually be around 300,000 in China and South East Asia alone. Pesticide poisoning occupied the largest proportion of the deaths by all poisoning in 2001 in South Korea. Pesticides can be dangerous to consumers, workers and close bystanders during manufacture, transport, or during and after use [27].

Pesticides, the most cost-effective means of pest and weed control, allow the maintenance of current yields and so contribute to economic viability. Concern about the environmental impact of repeated pesticides use has prompted research into the environmental fate of these agents, which can emigrate from treated to air, other land and water bodies. How long the pesticide remains in the soil depends on how strongly it is bound by soil components and how readily it is degraded. It also depends on the environmental conditions at the time of application, e.g. soil water content. Pesticide use must ensure public safety and environmental protection with regards to both the chemical itself and their potentially harmful metabolites [16].

2.3 Classification

Pesticides can be classified by target organism, chemical structure and physical state. Pesticides can also be classed as inorganic, synthetic or biologicals (biopesticides) [27]. Prominent insecticide families include organochlorines, organophosphates and carbamates. Organochlorine hydrocarbons (e.g. DDT) could be separated into dichlorodiphenylethanes, cyclodiene compounds and other related compounds. They operate by disrupting the sodium/potassium balance of the nerve fiber, forcing the nerve to transmit continuously. Their toxicities vary greatly, but they have been phased out because of their persistence and potential to bioaccumulate [28]. Organochlorine pesticides are known to resist biodegradation and therefore they can be recycled through food chains and produce a significant magnification of the original concentration at the end of the chain.

Organophosphate and carbamates largely replaced organochlorines. Both operate through inhibiting the enzyme acetylcholinesterase, allowing acetylcholine to transfer nerve impulses indefinitely and causing a variety of symptoms such as weakness or paralysis [28].

Organophosphates are quite toxic to vertebrates and have in some cases been replaced by less toxic carbamates. Thiocarbamate and dithiocarbamates are subclasses of carbamates. Prominent families of herbicides include phenoxy and benzoic acid herbicides (e.g. 2, 4-D), triazines (e.g.) (e.g. atrazine), ureas (e.g. diuron) and Chloroacetanilides (e.g. alachlor), Phenoxy compounds tend to selectively kill broadleaved weeds rather than grasses. The phenoxy and benzoic acid herbicides function similar to plant growth hormones and grow cells without cell division, crushing the plants nutrient transport system. Triazines interfere with photosynthesis. Many commonly used pesticides are not included in these families, including glyphosate [28].

Name	For the control of
Algicides **or** algaecides	algae
Avicides	birds
Bactericides	bacteria
Fungicides	fungi and oomycetes
Herbicides **(e.g.** glyphosate**)**	weeds
Insecticides **(e.g.** organochlorines**,** organophosphates**,** carbamates**, and** pyrethroids**)**	insects - these can be ovicides (substances that kill eggs), larvicides (substances that kill larvae) or adulticides (substances that kill adults)
Miticides **or** acaricides	mites
Molluscicides	slugs and snails
Nematicides	nematodes
Rodenticides	rodents
Virucides	viruses

Table 1 List of Pesticides Classes[14].

2,4-D
Acetachlor (ESA, OXA)
Alachlor (+ESA)
Atrazine (+DEA, DIA, DACT, Hydroxy)
Carbaryl
Carbofuran
Chlorpyriphos
Glyphosate (+AMPA)
Propazine

Table 2 Pesticides of Water Quality Concern [14].

2.4 Organochlorine compounds (OCPs)

Synthetic organic pesticides have been widely used for more than 40 years and during their use have contributed greatly to the increase in worldwide food production and at the same improved human and animal health. However, these successes brought their side effects such as toxicities to non-target species [29].

Synthetic organochlorines such as DDTs polychlorinated biphenyls (PCBs) hexachlorocyclohexanes (HCHs), Chlordanes (CHLs), cyclodienes and hexachlorobenzene (HCB) also referred to as POP's (persistent organic pollutants) are highly resistant to degradation by biological, photochemical or chemical means. They are also liable to bioaccumulation and are toxic to humans. Most of these compounds are prone to long range transport [30, 31].

These compounds are also typically characterized as having low solubility in water and high lipid solubility. The organochlorines have been associated with significant environmental impact in a wide range of species and virtually all tropic levels.

Many organochlorines have been implicated in a broad range of adverse human health and environmental effects, including impaired reproduction, endocrine disruption, immunosuppression and cancer [31]. Exposure to organochlorines have been correlated with population decline in a number of marine mammals [30].

Prominent insecticide families include organochlorines, organophosphates and carbamates. Organochlorine hydrocarbons (e.g. DDT) could be separated into dichlorodiphenylethanes, cyclodiene compounds, and other related compounds [28].

They operate by disrupting the sodium/potassium balance of the nerve fiber, forcing the nerve to transmit continuously. Their toxicities vary greatly, but they have been phased out because of their persistence and potential to bioaccumulate [28].

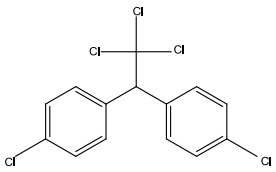
Dichlorodiphenyltrichloroethane (DDT) is the best known pesticide in this group. It was synthesized in 1874 in Germany but was recognized for its insecticidal properties in 1939. During this period DDT saved lives from malaria through pest control. However, due to its persistence in the environment, bioaccumulation in the adipose (fat) tissue of humans and wildlife and its biomagnification in the food chain, it is one of the most heralded pesticides and is banned in many countries and its use is severely restricted in others [32].

The major metabolite is via dehydrochorination to DDE or alternatively, dehalogenates to DDD and after a series of reductive chlorinations and oxidative steps from DDA (2, 2-bis (4-chlorophenyl) acetic acid). The acute toxicity of DDT is affected by the solvent vehicle e.g. if administered in oil to the rat, a typical median lethal dose is 250mg/kg of body weight. DDT is poorly absorbed through the skin [10].

The discovery of DDT and other organochlorine pesticides had important beneficial consequences in human health. However, many adverse effects after their application have also been detected. These pesticides are metabolized in

the liver and in some cases, such as heptachlor, its biotransformation metabolites are more toxic than the original product [33].Moreover, organochlorine pesticides are known to resist biodegradation, bioaccumulate due the capacity to bind to lipids and therefore can be redistributed through the food chain [33.]

Structural formula of 4, 4-DDT [14].



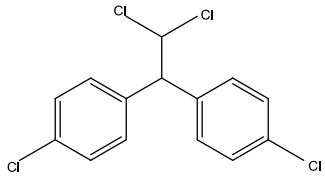
Potential mechanisms of action on humans are genetoxicity and endocrine disruption, DDT may be directly genotoxic, but may also induce enzymes to produce other genotoxic intermediates and DNA adducts. It is an endocrine disruptor [34].

DDT is classified as moderately toxic by the United States National Toxicology Program (NTP) [35.]DDT is classified as moderately toxic by the NTP and moderately hazardous by the World Health Organisation (WHO), based on the rat oral LD 50 of 113mg/kg [35].

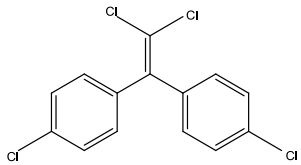
DDT metabolites

The DDT metabolite DDE acts as an antiandrogen (but not as an estrogen) [34]. DDE is found in the environment as a result of the breakdown of DDT, an insecticide. Human exposure to DDE appears to be primarily through food; in the United States in 1981, consumption of DDE in foods was estimated to be 0.001 parts per million per day (ppm/d). However, the levels of DDE in foods have been decreasing and are expected to continue. Levels of DDE in air and water samples are very low [36]. DDE has been listed as a pollutant of concern to EPA's Great Waters Program due to its persistence in the environment, potential to bioaccumulalte, and toxicity to humans and the environment [37.]

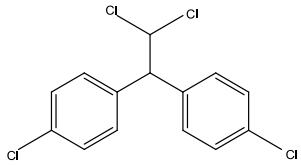
Structural formula of 4,4-DDE [14].



Structural formula of 2, 4-DDD [14].



Structural formula of 4, 4-DDD [14].



Endosulfan is an organochlorine compound that is used as an insecticide and acaricide. This colorless solid has emerged as a highly controversial agrichemical [38]. Due to its acute toxicity, potential for bioaccumulation, and role as an endocrine disruptor [38].

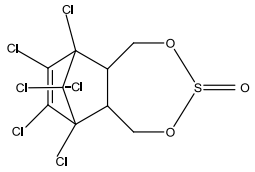
Endosulfan is one of the more toxic pesticides on the market today, responsible for many fatal pesticide poisoning incidents around the world [39]. Endosulfan is also a xenoestrogen, a synthetic substance that imitates or enhances the effect of estrogens and it can act as an endocrine disruptor, causing reproductive and developmental damage in both animals and humans. Whether Endosulfan can cause cancer is debated.

Endocrine disruption

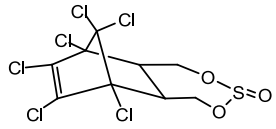
Theo Colborn, an expert on endocrine disruption, lists Endosulfan as a known endocrine disruptor [40], and both the EPA and the Agency for Toxic Substance

and Disease Registry consider Endosulfan to be a potential endocrine disruptor. Numerous *in vitro* studies have documented its potential to disrupt hormones and animal studies have demonstrated its reproductive and developmental toxicity, especially among males [41]. A number of studies have documented that it acts as an anti-androgen in animals [42]. Environmentally relevant doses of Endosulfan equal to the EPA's safe dose of 0.006 mg/kg/day have been found to affect gene expression in female rats similarly to the effects of estrogen [43]. It is not known whether Endosulfan is a human teratogen (an agent that causes birth defects); though it has significant teratogenic effects in laboratory rats [44] A 2009 assessment concluded that endocrine disruption occurs only at Endosulfan doses that cause neurotoxicity [45].

Structural formula of Endosulfan 1/Alpha [14].



Structural formula of Endosulfan 2/Beta [14].



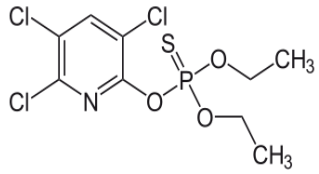
Due to the toxic effects of organochlorines in aquatic organisms, the use and/sale of most organochlorine pesticides has been banned or restricted in many developed countries such as United States of America and Sweden since the mid 1970s. Studies conducted on OCPs in aquatic environments in South Africa have shown a widespread occurrence of residues of these pesticides in environmental aquatic systems, despite the fact that they have been banned for decades. In developing countries such as South Africa, DDT is still used officially for malaria vector control in some parts of the country. It is believed that some group of OCPs may still be used clandestinely under unknown trade names in agriculture due to low cost and effectives for pest control [46].

2.5 Organophosphorus compounds

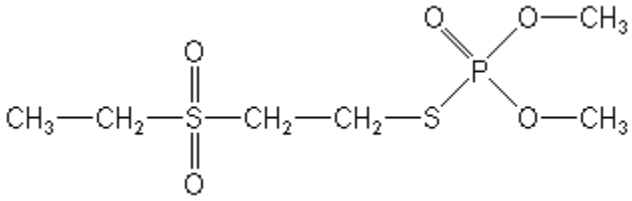
The first organophosphorus insecticide was tetraethyl pyrophosphate (TEPP). This group of pesticides was developed in Germany prior to World War II. Important organophosphorus pesticides includes, TEPP, disulfoton, azinphosmethyl, parathion, methyl parathion, chlorfenvinphos, dichlorvos, diazinon, dimethoate, trichlofon and malathion [10].

These chemicals act as irreversible inhibitors of the cholinesterase enzymes of the neuromuscular system. This immediate cause of death is asphyxia resulting from respiratory failure. Parthion was one of the earliest organophosphorus pesticides used in agriculture. It is slightly soluble in water, exerts a pseudo-systemic action in insects, is moderately persistent and is stable for a shorter time at higher temperature [10.]

Structural formula of Chlorpyriphos [14].



Structural formula of Demeton-S-methyl Sulfone [14].



2.6 Carbamates

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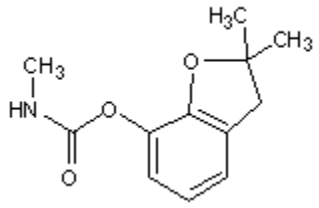
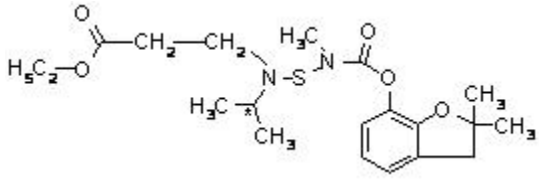
It is only slightly soluble in water but highly soluble in organic solvents. The compound is mildly phototoxic but toxic to fish [10.]

Structural formula of Carbofuran [14].



Structural formula of Benfuracarb [14]**.**



The increasing production and application of pesticides for agricultural and non-agricultural purposes such as use in households for killing of mosquitoes and bugs, has caused the pollution of air, soil, ground and surface water. This will have a negative impact on the environment as well as human health due to direct exposure or through residues in food and drinking water. In the world, alarming levels of pesticides have been reported in air, soil as well in foods and biological materials [6].

The continuous monitoring of pesticide residues in environmental samples has great importance and demands high efficiency, unique selectivity and high sensitivity techniques. Gas chromatography and high performance liquid chromatography have been established for years as the techniques for the analysis of pesticide residues [46].

Organohalogenated compounds (OHCs), particularly organochlorine pesticides (OCPs) are ubiquitous environmental contaminants. Of all the OCPs

dichlorodiphenyl-trichloroethane (DDT) has received most attention because of its early success for the eradication of malaria-carrying mosquitoes in many countries [47].

2.7 Environmental effects and toxicity of pesticides

A new study conducted by the Harvard School of public Health in Boston, has discovered a 70% increase in the risk of developing Parkinson's disease for people exposed to even low levels of pesticides [48].

Several studies reported the elevated concentrations of OCPs in fishes, mussel and birds collected from Asian countries including India, Vietnam and China. There is an indication of the presence of significant source of OCPs in these regions [49].

One study found that use of pesticides may be behind the findings that the rate of birth defects such as missing or very small eyes is twice as high in rural areas as in urban areas [50]. Another study found no connection between eye abnormalities and pesticides [50]. In the United States increase in birth defects is associated with conceiving in the same period of the year when agrichemicals are in elevated concentrations in surface water [51].

The World Health Organisation and the UN Environment Programme estimate that each year, 3 million workers in agriculture in the developing world experience severe poisoning from pesticides, about 18,000 of whom die [20,].According to one study, as many as 25 million workers in developing countries may suffer mild pesticide poisoning yearly [52].

According to researchers from the National Institutes of Health (NIH), licensed pesticide applicators who used chlorinated pesticides on more than 100 days in their lifetime were at greater risk of diabetes. One study found that associations between specific pesticides and incident diabetes ranged from a 20 percent to a

200 percent increase in risk. New cases of diabetes were reported by 3.4 percent of those in the lowest pesticide use category compared with 4.6 percent of those in the highest category. Risks were greater when users of specific pesticides were compared with applicators who never applied that chemical l[53].

It has been stated that DDT, lindane and atrazine were found to affect breakdown of estradiol, and thus increased the risk of breast cancer. It should, however, be noted that there is no conclusive evidence regarding this matter, and that more research has to be done to make a final conclusion [54].

The reproductive health of women is also affected by estrogen-mimicking substances, in the form of breast cancer, It has been concluded that breast cancer was four times common amongst women who had high blood levels of DDE, a breakdown product of DDT, than women who had low blood levels [55].

Children that were born to women who regularly consumed fish from Lake Michigan which was contaminated with PCBs and other organochlorines, were found to be smaller and had reduced postnatal growth. They had deficiencies in neural development and short term memory which lasted into childhood and breast milk [56].

The mechanism(s) of how these abnormalities are generated are unclear. Some researchers have concluded the following: during sexual differentiation there are a number of critical periods when the reproductive system is uniquely susceptible to chemically induced perturbations. At these times an inappropriate signal can result in infertility, whereas similarly exposed young adults are only transiently affected. Many of these abnormalities are not expressed during fetal and neonatal development and only become apparent after puberty [57].

Some scientists believe that estrogen-mimicking substances add to the adult estrogen exposure, and increase the risk of estrogen sensitive breast tumors in women while others say that exposure of the fetus in the womb leads to the most damage in both male and female [54].

It has been suggested that the apparent decline in male reproductive health might be caused by an excess of estrogenic compounds. These EDCs end up in the environment where animals and humans are exposed through various routes. In South Africa the vector control program for malaria uses both DDT and deltamethrin (DM) for indoor residual spraying. DDT exposure has negative reproductive health effects on humans and biota [58].
Sythetic pyrethroids such as DM were developed to limit the use of DDT [59], but pyrethroids are also EDCs [60]

2.8 Methods of detection and analysis of pesticides

In 1971 a study was designed to determine carbon adsorption-desorption efficiency and reproducibility in the recovery of organic pesticides, it was determined that carbon provided only 37% recovery rate for DDT compared to 70-85% for the other organochlorines. [61].

In 2000, water samples were analysed using GC-ECD from the Vaal River in the vicinity of Vereeniging and Vanderbijlpark for organochlorine pesticides. Pesticides of interest were, DDT total (all the forms of DDD, DDE and DDT), Methoxychlor, Endosulfan (Alpha and Beta isomers), Chlordane and Dicofol. Liquid-liquid extraction (LLE) was used in this study. Pesticides of interest were detected and found to be within the concentration range from 0.25 to 5ug/l [55].

In 2000, a study was conducted to investigate the quality status of surface and groundwater in the rural Western Cape for pesticides. Water samples were collected from Hex River suspected of contamination by pesticides. The results showed a wide degree of variability in the presence of pesticides detected. Pesticides detected were within the concentration range 00.2-3.86ug/l [62].

In another study in 2001, solid phase extraction and capillary GLC were used to provide the basis for selective determination of phthalate esters plasticizers in rivers and marine samples in the Eastern Cape Province. River and marine water

samples were found to be grossly polluted with several PAEs, such as dimethyl (DMP), diethyl (DEP) dibutyl (DBP) and diethylhexyl (DEHP) within the concentration range 0.03-2306+- 9.4µg/l [63].

In the Eastern Cape province of South Africa, a study was conducted in 2002 for the determination of OCPs from water and sediments. The levels of OCPs ranged from 5,5 Benzene, 1-Chloro-2- (2,2-dichloro-1 (4-chlorophenyl) ethyl (2,4-DDD) to 450+-0.10ng/l beta-Benzenehexachloride (beta-BHC0 in water samples and from 0.6 (aldrin and 2,4 DDD) to 184+-0.12ng/l (beta-BHC) in sediments. DDT, DDE, heptachlor, Endosulfan and chlordanes were also detected [64].

In 2005 a study was done for the confirmatory method for the determination of organochlorine pesticides and their metabolites in surface waters using SPE . Out of the seven samples, five were found to contain endosulfan sulfate, and only three of these could be qauntitated at levels of 0.31-0.50µL^{-1} [65].

In 2007 a study was conducted for the determination on semi-polar pesticides in Spain using on-line solid phase extraction-liquid chromatography-tandem-mass spectrometry Detection limits achieved for some compounds were below 5ng/L and 12ng/L for others [66].

It is well known that the use of large quantities of pesticides in agricultural activities is one of the main causes of pollution of surface and groundwaters. Consequently, very strict programs to control and monitor the levels of these contaminants in water sources have long been instituted in countries such as the United States and members of the European Community. These programs are based on the use of thoroughly validated analytical methods which allow determination of contaminants at trace levels [66].

There is a wide range of organic micropollutants with various chemical properties. When present in natural waters at the ng/L, they can generate toxic or organoleptic effects and some are known or suspected mutagens. Appropriate

water quality control is therefore necessary. The identification and quantification of organic micropollutants presents at trace levels together with thousands of other compounds is possible by using Gas Chromatograph/MS (GC-MS). However, extraction and concentration procedures are needed to enhance the sensitivity of GC-MS analysis [67].

The development and use of pesticides have played an important role in the increase of agricultural productivity. The majority of such substances are applied directly to soil or spayed over crop fields and hence released directly to the environment. For that, pesticides can enter as contaminants into natural waters either directly in applications or indirectly from drainage of agricultural lands. The amount and kind of pesticides in water of a given area depends largely on the intensity of production and kind of crops. However, the transport of pesticides out of their area of application results in the presence and accumulation of these compounds in many parts of the hydrosphere. For example, atmospheric precipitation is an important route of transport of pesticides, resulting in contamination of environmental waters far away from agricultural areas. Substantial amounts of pesticides have been found in ice and water of polar regions, lakes, seawater, rainwater or potable water [68].

To study the fate and transport of pesticides in natural waters, very low detection limits must be reached. The trace determination of pesticides requires both high performance analytical instruments and efficient sample preparation. Analysis of natural waters for pesticides is recognised as complex, since several hundred pesticides with different physical and chemical properties are widely used for agricultural purposes. Analysis is also considered difficult and prone to errors due to very low concentrations of the compounds [10].

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CHAPTER 3

EXPERIMENTAL

3.1 Analytical method development

Compound	Molecular Mass	Molecular Formular	%Purity
4,4' DDD	320.04 g/Mole	$C_{14}H_{10}C_{14}$	99.2%
4,4' DDE	318.03 g/Mole	$C_{14}H_8C_{l4}$	99.9%
4,4' DDT	354.49 g/Mole	$C_{14}H_9Cl_{15}$	99.1%
Endosulfan (Alpha + Beta)	406.93 g/Mole	$C_9H_6Cl_6O_3S$	99.9%
Chlorpyriphos	350.59 g/Mole	$C_9H_{11}Cl_3NO_3PS$	99.9%
Carbofuran	221.25 g/Mole	$C_{12}H_{15}NO_3$	99.9%
Benfuracarb	410.53 g/Mole	$C_{20}H_{30}N_2O_5S$	98.0%

Table 3 List of pesticides investigated in the study

3.1.1 Method Development

Validation and optimisation of solid- phase extraction (SPE) and liquid-liquid (LLE) methods of extraction.

Optimisation was performed in order to achieve maximum extraction.

Method development is essential as it confirms that an analytical method is effective in measuring the parameters it is intended to measure. The purpose of this chapter is to prove that the instrument method in the analysis of the selected pesticides is technically and statistically accurate to measure what is it intended to measure.

Successful validation of the method will confirm that the methods, procedures and protocols applied in the analysis produce reliable and accurate data and also ensure that valid conclusions are produced as a result of the validated method.

The optimisation was achieved by using different eluting solvents (SPE) and for LLE different extraction solvents.

Extracting solvents were chosen according to their different properties. Polarity as one of the properties that played a major role in choosing these solvents because compounds of interest have different polarities.

- N-Hexane
- Dichloromethane
- Ethyl Acetate
- Methanol

% Recovery of the spiked water samples Glassware used were thoroughly washed with liquid soap and rinsed with deionised water and acetone. 100ml of distilled water was measured into a 250ml separating flask and 1ml of 100ppm pesticides standard mixture was added. Spiked water samples were taken through the following extracting procedure.

Liquid -liquid extraction (LLE)

Procedure

Spiked samples were extracted with 3 x 15ml of extracting solvent/s (hexane; dichloromethane and ethyl acetate).

Silica gel column chromatography (clean up)

The chromatographic column (20cmx8mm I.D.) was slurry packed with 5.0 g of activated silica gel, which was made into slurry with about 1.5% (v/m) petroleum ether and then stirred well before use. About 1ml of anhydrous sodium sulphate was placed at the top of the column to absorb any water in the sample or the solvent. The column was pre-eluted with 15ml of petroleum ether. The extract was then eluted with 2x10ml portions of the extracting solvent. The eluate was collected, concentrated. Then 1ul was injected into the GC-ECD.

Solid phase extraction (SPE)

Procedure

Strata X 33um polymeric reversed phase 200mg/3ml was activated by treating the sorbent with 3ml methanol and the sorbent was prepared for optimized interaction with analyte with 3ml methanol. Thereafter 100ml of sample was passed through the cartridge at a flow rate of 4-8 ml / min. The cartridge was then rinsed with 3ml methanol to remove the impurities from the sample and then air dried. The retained analyte were then eluted with 3ml methanol. The extracts were collected evaporated and concentrated to a smaller volume 2ml. 1ul was injected into the GC-ECD.

The optimisation of GC-ECD instrument conditions

Method development was centered on the Optimisation of the following instrumental conditions using Perkin Elmer Clarus Gas Chromatograph with ECD detector and capillary column -Elite 5 (5% diphenyl-95% dimethylpolysiloxane, 30m x 0.25 mm i.d. and 0, 25ử m thickness) supplied by Perkin Elmer South Africa.

The parameters that were used before were before obtaining suitable conditions were as follows. The oven temperature was programmed as follows: 50^{O}C for 2 min ramp at 6 intermediate temperatures was 250^{O}C and the maximum was 300^{O}C. Injector and detector temperatures were 220^{O}C and 280^{O}C, respectively. Carrier gas used was Nitrogen (99.99%) at a flow rate 1.50ml/min and Air used as the make up gas. The split ratio was 20:1.

The oven temperature was programmed as follows: 60^{0}C for 1 min ramp at 5, intermediate temperature was 300^{0}C and the maximum was 320^{0}C. Injector and detector temperatures were 250^{O}C and 300^{O}C, respectively. Carrier gas used was Nitrogen (99.99%) at a flow rate of 1.20ml/min and Air used as the make up gas. The split ratio was 10:1. These conditions were found to be adequate for optimum results.

Procedure

Determination of instrument detection limits (IDLs) and retention times (tr)

The instrument detection limit for any instrument is the lowest detectable amount of each analyte that the instrument can detect and record. The IDL was computed using the method described by Miller and Miller (1998).

IDL = Yb + 3Sb

Where

Yb = Blank value

Sb = Standard error of the regression

The noise and thresholds were set during column background run so as to eliminate noise spikes being registered as peaks. Each standard was injected into the GC-ECD to determine its retention time.

Determination of instrumental limit of detection

Detection limit is the defined as the concentration that gives a readout that is double the electrical noise level inherent in the baseline. It is a qualitative parameter in the sense that it is the minimum concentration that can be detected, but not precisely determined, like a blip that is barely seen compared to the electrical noise on the baseline.

1000ppm of each pesticide standard was prepared by dissolving 10mg in 10ml volumetric flask and dissolved in toluene. Lower working concentrations of each pesticide standard were prepared from 1000ppm and 1.0ul of each was injected into the GC/ECD until the instrument could not detect and peaks.

Validation of the Method

The validation of SPE, LLE and gas chromatography was confirmed by carrying out the experiment to determine the following parameters.

Precision

Precision is the measure of the degree of repeatability on an analytical method under normal operation. For ease of reference, precision was categorized into Repeatability and reproducibility.

Repeatability

A 1 ppm standard was analyzed 11 times to determine the percentage relative standard deviation (%RSD)

3.2 Material and Methods

Chemicals and Reagents and glassware

All the reagents used dichloromethane, n-hexane, ethyl acetate, methanol, toluene and acetonitrile were HPLC grade and purchased from Merck and Sigma Aldrich. Acetone, petroleum and sodium sulphate were of analytical grade and were purchased from Merck. All the pesticides standards were purchased from Sigma Aldrich.

3.3 Study Area and Sampling

3.3.1 Description of the study area

The study areas for this research work Kliprivier river, Zuikerbosch and Rand water Barrage in Southern Gauteng South Africa. The rivers were chosen due to their proximity to farm areas, Klipriver is also close farm areas and an industrial area.

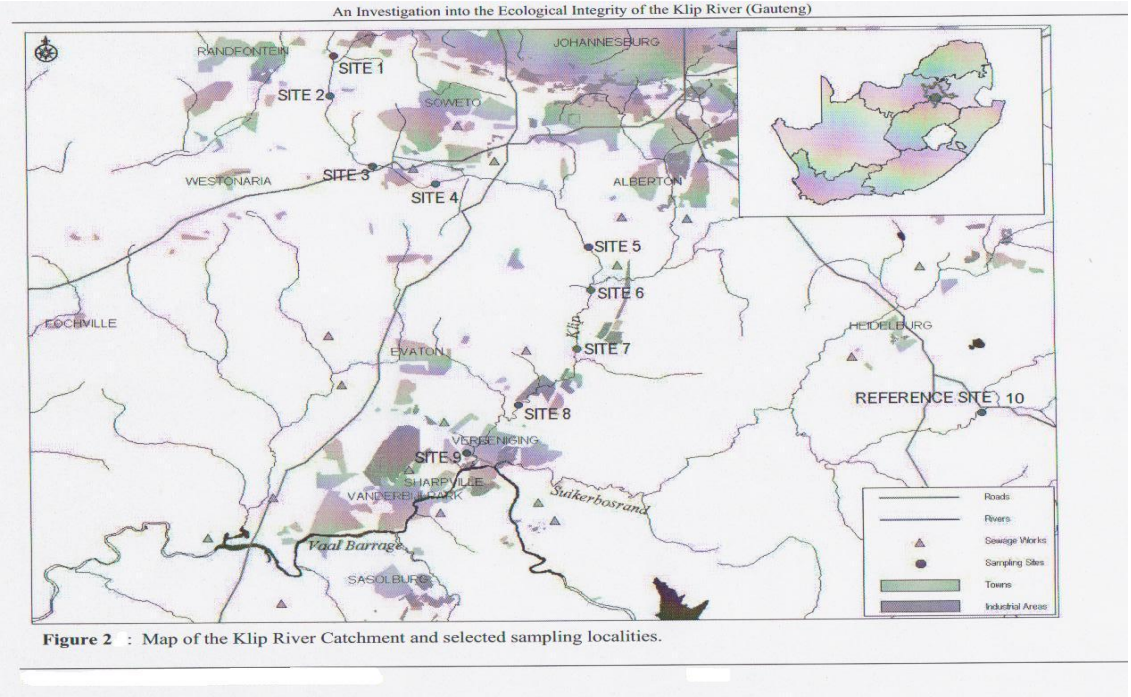


Figure 2 Map for the sampling areas

3.3.2 Sampling

The glassware that was used for sampling was washed thoroughly with soap, rinsed with distilled water and finally with acetone.

Water sampling

All water samples were collected in triplicates in 2.5 l pre-cleaned Winchester glass bottles from identified sites within the Kliprivier River, Zuikerboch and Barrage in Southern Gauteng region. Prior to use, the bottles were first rinsed with the water debris removed from the collection point and thereafter the bottles were immersed about 5 cm below the surface. 5 ml of concentrated sulphuric acid was added to the water samples for

preservation and tightly sealed. Samples were then transported in a cooler box with ice to the laboratory and stored in the fridge at 4 ^{o}C until they were extracted

Extraction of real water samples using liquid liquid extraction

In general, environmental water samples cannot be analyzed without some preliminary sample preparation; this step is frequently a major source of error. Thus, water samples are still widely processed by liquid-liquid extraction (LLE).

The extraction procedure was also repeated with hexane and dichloromethane. The choice of the solvents used was largely dictated by polarity and volatility of the solvents.

100ml of the acidified environmental water samples was measured in 250ml separating flask and extracted with 3 x 15ml of ethyl acetate. After drying with sodium sulphate, the sample was taken through a glass column packed with activated silica gel that was made into slurry with petroleum ether. The eluates were concentrated to about 5ml before GC analysis.

1 µl each of processed samples was injected into the Clarus 500GC (split mode)

Extraction of real water samples using solid phase extraction

Strata X 33µm polymeric reversed phase 200mg/3ml was activated by treating the sorbent with 3ml methanol and the sorbent was prepared for optimized interaction with analyte with 3ml methanol Thereafter 100ml of sample was passed through the cartridge at a flow rate of 4-8 ml / min. The cartridge was then rinsed with 3ml methanol to remove the impurities from the sample and then air dried. The retained analyte were then eluted with 3ml methanol. The extracts were collected evaporated and concentrated to a smaller volume 2ml.

GC-ECD-Conditions

1ul each of processed sample was injected into the Clarus 500 GC with electron capture detector (split mode) with a capillary column Elite 5 (5% diphenyl – 95% dimethyl polysiloxane, 30m x 0.25 mm i.d and 0.25 µm thickness. The oven temperature was programmed as follows for the injector and the detector temperatures were maintained at 250ºC and 300ºC respectively. The oven temperature was initially maintained at 60ºC and then programmed to increase to 300^{O}C. Nitrogen was used as a carrier gas (99.9%) at a flow rate of 1.20ml/min. Air and hydrogen were used as the make-up gas. The split ratio was 10:1. These

conditions were found to be adequate for optimum results. All analysis was carried out in triplicate and the injection volume was 1µL.

HPLC Conditions

20µl of processed sample was injected into SRI 2010D. The HPLC was fitted with a Synergi 4u Hydo-RP 80A column (250 x 4.6 mm ID). The mobile phase was 95% of (80% Acetonitrile) and (5% of 1% H_3PO_4). The detection was made with UV-Vis at 254nm. The gradient programmed was 95% to 100%.

GC-MS conditions

An Agilent Technologies 68920 Gas Chromatograph coupled with an Agilent technologies 5975 Mass spectrometer detector and capillary column DB5 (60m x 250um i.d) x 0.25 µm thickness). The oven temperature was programmed as follows: $70^{O}C$ was the initial temperature, the maximum was $320^{O}C$. The carrier gas used was Helium (99.99%) at a flow rate of 55.5ml/min. Air was used as the make up gas. The mode is splitless. These conditions were found to be adequate for optimum results. All the analyses were carried out in triplicate and the injection volume was 1µl.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

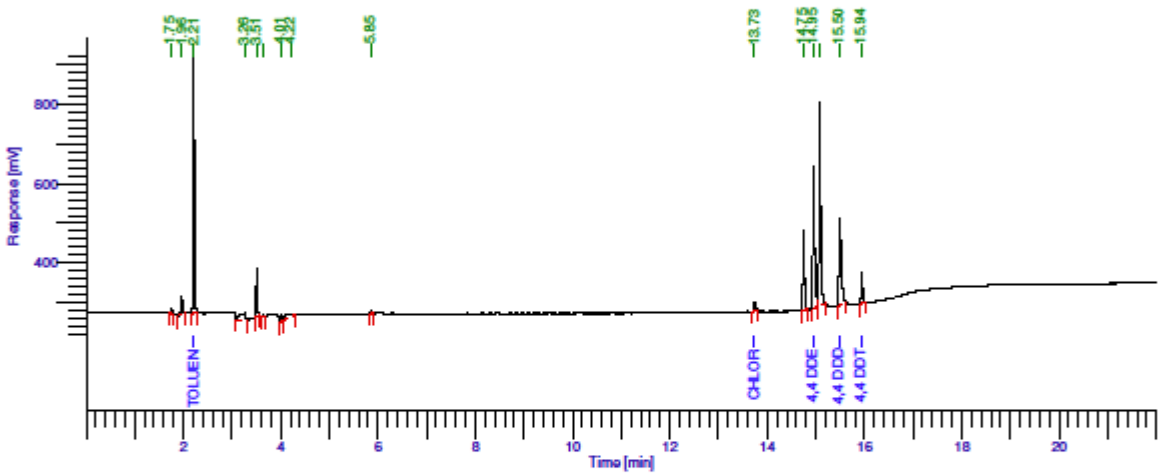
A method was developed as stated in chapter 3 for the determination of selected pesticides in surface water samples. Three solvents were chosen and tested for the best solvent that would recover the most pesticides and would be the one that would be applied as the solvent of extraction. The solvents were chosen due to their difference in polarity. The lowest detection limits for each pesticide were determined this was done to determine the concentration that gives a readout level that is double the electrical noise in the baseline. It is a qualitative parameter in the sense that it is the minimum concentration that can be detected. Different concentrations were prepared by dilution of the 1ppm standard solution.

Two methods were used for the extraction process.

The two extraction processes differ in terms of technique of extraction. The two extraction processes used were namely solid phase extraction and liquid- liquid extraction. The purposes for using two extraction processes were to determine the one which would extract more. The standards were identified on the GC-ECD to determine if there would be any matches to the environmental samples. GC-MS was also used to confirm the different compounds that were detected.

Software Version	: 6.3.1.0504	Date	: 2010/06/18 08:51:47 AM
Operator	: GC	Sample Name	:
Sample Number	: 001	Study	: 4.4 ddt
AutoSampler	: BUILT-IN	Rack/Vial	: 0/3
Instrument Name	: GC	Channel	: B
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 22.00 min
Sampling Rate	: 12.5000 pts/s		
Sample Volume	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 2010/03/16 09:23:03 AM	Cycle	: 1

Raw Data File : C:\Clarus 500\Data\datb001-20100316-092323.raw
Inst Method : C:\Clarus 500\Method\Chlorpyrifos channel B from C:\Clarus 500\Data\datb001-20100316-092323.raw
Proc Method : C:\Clarus 500\Method\Chlorpyrifos channel B.mth from
Calib Method : C:\Clarus 500\Method\Chlorpyrifos channel B.mth from
Report Format File: C:\Clarus 500\Method\ddd report.rpt
Sequence File : C:\Clarus 500\Sequence\repeatabilty test march.seq



DDD Report

Peak #	Component Name	Time [min]	Area [uV*sec]	ppm Amount	ppb amount
1		1.749	26351.14	0.026	26.3511
2		1.962	113153.24	0.113	113.1532
3	TOLUENE	2.208	1347221.56	1.347	1347.2216
4		3.261	156493.20	0.156	156.4932
5		3.506	197947.92	0.198	197.9479
6		3.638	15532.96	0.016	15.5330
7		4.012	35328.12	0.035	35.3281
8		4.222	120462.47	0.120	120.4625
9		5.855	13662.26	0.014	13.6623
10	CHLORPYRIPHOS	13.735	71241.46	0.071	71.2415
11		14.749	454485.81	0.454	454.4858
12	4,4 DDE	14.953	960482.66	0.960	960.4827
13		15.080	1262580.42	1.263	1262.5804
14	4,4 DDD	15.495	732865.71	0.733	732.8657
15	4,4 DDT	15.945	176917.75	0.177	176.9178
			5684726.67	5.685	5684.7267

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Figure 3 Chromatogram for mix at 1ppm

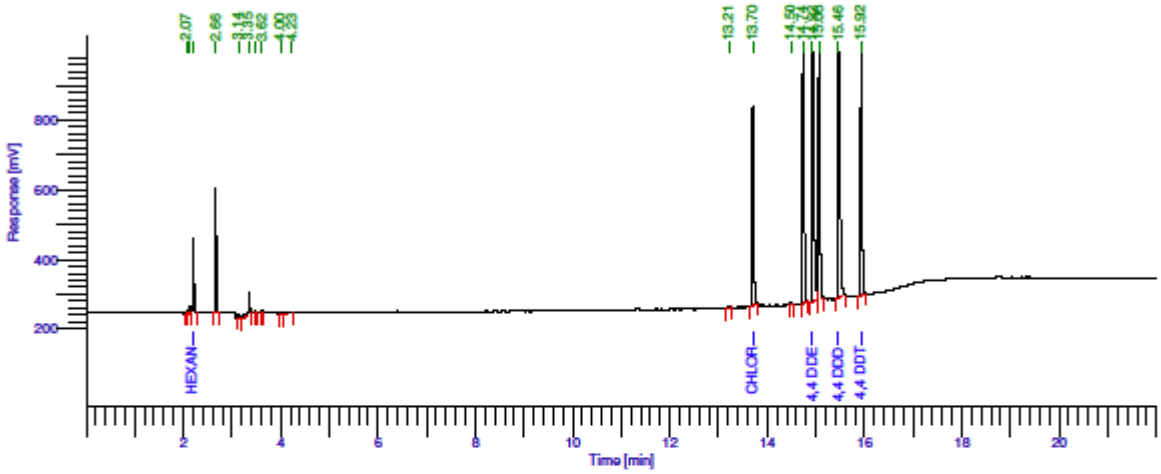
4.2 Recovery determinations

Recovery determinations were done using tree solvents namely hexane, dichloromethane and ethyl acetate and the recoveries are shown on the following figures for each solvent.

Software Version : 6.3.1.0504
Sample Name :
Instrument Name : GC
Rack/Vial : 0/11
Sample Amount : 1.000000
Cycle : 1

Date : 2010/06/24 12:50:01 PM
Data Acquisition Time : 2010/03/05 01:39:20 PM
Channel : B
Operator : GC
Dilution Factor : 1.000000

Result File :
Sequence File : C:\Clarus 500\Sequence\reproducibilitytest march05.seq



Report

Peak #	Component Name	Time [min]	Time [min]	Area [uV*sec]	Concentration ppm
1		2.072	2.072	8051.75	0.01
2		2.138	2.138	55311.40	0.06
3	HEXANE	2.203	2.203	374246.36	0.37
4		2.663	2.663	575531.53	0.58
5		3.144	3.144	45966.12	0.05
6		3.355	3.355	146979.74	0.15
7		3.479	3.479	5491.97	0.01
8		3.621	3.621	10192.23	0.01
9		4.002	4.002	15536.33	0.02
10		4.235	4.235	35841.48	0.04
11		13.215	13.215	19576.02	0.02
12	CHLORPYRIPHOS	13.700	13.700	1369727.96	1.37
13		14.498	14.498	19746.99	0.02
14		14.737	14.737	1941010.31	----------
15	4,4 DDE	14.924	14.924	2559945.15	----------
16		15.064	15.064	1901970.08	----------
17	4,4 DDD	15.464	15.464	2450552.22	----------
18	4,4 DDT	15.924	15.924	1780802.12	----------
				13316479.77	2.68

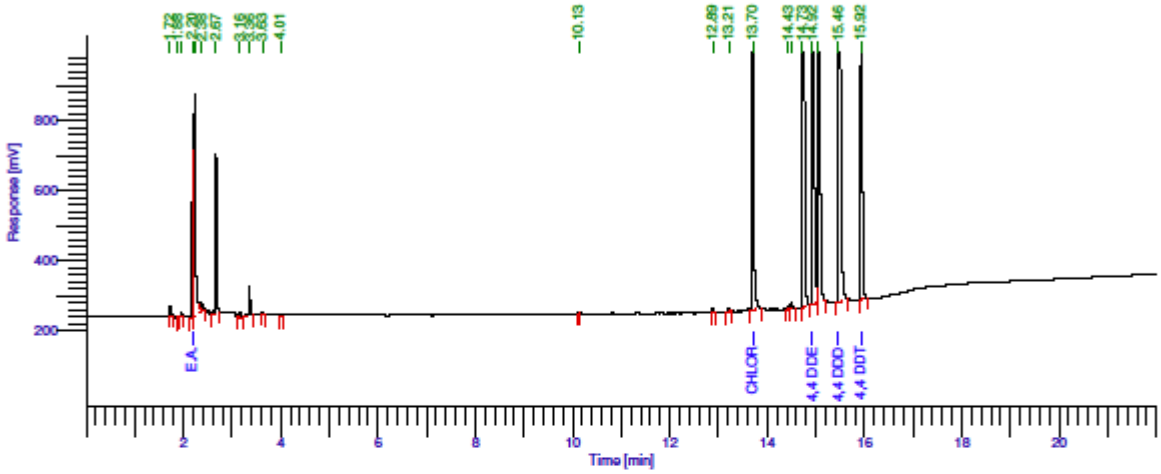
Warning -- Signal level out-of-range in peak

Figure 4 Chromatogram for Hexane recovery

Software Version : 6.3.1.0504
Sample Name :
Instrument Name : GC
Rack/Vial : 0/8
Sample Amount : 1.000000
Cycle : 1

Date : 2010/06/24 12:48:21 PM
Data Acquisition Time : 2010/03/05 12:05:16 PM
Channel : B
Operator : GC
Dilution Factor : 1.000000

Result File :
Sequence File : C:\Clarus 500\Sequence\reproducibilitytest march05.seq



Report

Peak #	Component Name	Time [min]	Time [min]	Area [uV*sec]	Concentration ppm
1		1.719	1.719	61620.76	0.06
2		1.880	1.880	8550.66	0.01
3		1.956	1.956	17443.77	0.02
4	E.A.	2.205	2.205	1173302.51	1.17
5		2.226	2.226	1410306.95	1.41
6		2.379	2.379	68144.34	0.07
7		2.672	2.672	789998.67	0.79
8		3.161	3.161	58887.82	0.06
9		3.362	3.362	159875.97	0.16
10		3.627	3.627	11776.83	0.01
11		4.006	4.006	14300.94	0.01
12		10.131	10.131	6097.31	0.01
13		12.885	12.885	23063.16	0.02
14		13.214	13.214	35255.55	0.04
15	CHLORPYRIPHOS	13.701	13.701	2055233.74	----------
16		14.426	14.426	11525.13	0.01
17		14.499	14.499	48619.80	0.05
18		14.728	14.728	2648476.93	----------
19	4,4 DDE	14.921	14.921	3047674.50	----------
20		15.055	15.055	2487681.49	----------
21	4,4 DDD	15.461	15.461	3440353.77	----------
22	4,4 DDT	15.921	15.921	2247369.92	----------
				19825560.50	3.90

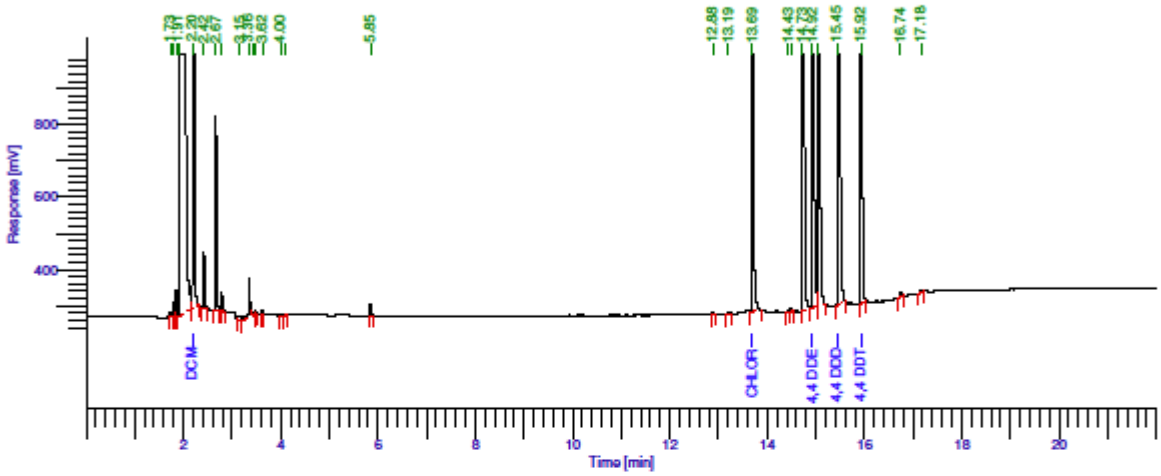
Warning -- Signal level out-of-range in peak

Figure 5 Chromatogram of Ethyl Acetate recovery

Software Version : 6.3.1.0504
Sample Name :
Instrument Name : GC
Rack/Vial : 0/3
Sample Amount : 1.000000
Cycle : 1

Date : 2010/06/24 12:46:01 PM
Data Acquisition Time : 2010/03/13 11:38:45 AM
Channel : B
Operator : GC
Dilution Factor : 1.000000

Result File :
Sequence File : C:\Clarus 500\Sequence\repeatabilty test march.seq



Report

Peak #	Component Name	Time [min]	Time [min]	Area [uV*sec]	Concentration ppm
1		1.733	1.733	24915.34	0.02
2		1.805	1.805	82481.95	0.08
3		1.853	1.853	106172.50	0.11
4		1.908	1.908	7421751.54	----------
5	DCM	2.201	2.201	2027272.80	----------
6		2.421	2.421	287253.73	0.29
7		2.668	2.668	1088264.22	1.09
8		2.788	2.788	104672.77	0.10
9		3.153	3.153	45471.95	0.05
10		3.357	3.357	245461.64	0.25
11		3.449	3.449	16265.19	0.02
12		3.480	3.480	21628.89	0.02
13		3.622	3.622	21462.13	0.02
14		3.998	3.998	23355.45	0.02
15		4.095	4.095	20066.10	0.02
16		5.846	5.846	66305.46	0.07
17		12.884	12.884	15633.21	0.02
18		13.195	13.195	27629.55	0.03
19	CHLORPYRIPHOS	13.688	13.688	2334134.51	----------
20		14.425	14.425	13054.78	0.01
21		14.496	14.496	24280.12	0.02
22		14.728	14.728	2599953.88	----------
23	4,4 DDE	14.921	14.921	2919064.36	----------
24		15.055	15.055	2426856.91	----------
25	4,4 DDD	15.455	15.455	2972457.22	----------
26	4,4 DDT	15.921	15.921	2257894.10	----------
27		16.740	16.740	39591.85	0.04
28		17.178	17.178	20077.94	0.02
				27253430.09	2.29

Figure 6 Gas chromatogram of Dichloromethane recovery

COMPOUND	DCM	HEXANE	ETHYL ACETATE
Chlorpyriphos	NR	NR	NR
4,4 DDE	97.3%	96.8%	97.1%
4,4 DDT	89.9%	87.3%	88.4%
2,4 DDD	98.5%	98.1%	98.3%
ENDOSULFAN 1/ALPHA	95.8%	94.3%	94.7%
ENDOSULFAN 2/BETA	96.9%	95.5%	96.6%
Demeton-s-methylsulfon	NR	NR	NR

Table 4 Recoveries for the different solvent

NR not recovered

Repeatability

A 1ppm standard was analyzed 10 times to determine the mean and the standard deviation

$$\%RSD = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

	Peak Area									
Endosulfan 1	**132109,29**	**127742,84**	**133109,25**	**132108,129**	**134170,09**	**136102,31**	**138809,50**	**133107,25**	**138695,20**	**13115.83**
Endosulfan 2	**52957,38**	**51496,22**	**52492,11**	**54452,72**	**52766,04**	**51710,75**	**55650,45**	**53981,17**	**51496,23**	**54456,76**
4,4 DDT	**95135,19**	**94149,84**	**90472,75**	**93533,97**	**92926,94**	**94050,08**	**94256,21**	**94050,40**	**95833,18**	**94257,21**
4,4 DDE	**88082,43**	**84839,31**	**75596,00**	**77378,21**	**75077,02**	**78217,20**	**83112,10**	**81258,45**	**78342,89**	**78341,79**
4,4 DDD	**94986,19**	**93408,35**	**92442,73**	**95280,07**	**89673,24**	**93730,85**	**95759,40**	**90848,18**	**94988,19**	**93409,35**
2,4 DDE	**59477,02**	**60632,35**	**62649,78**	**63333,79**	**66582,68**	**57681,08**	**56799,08**	**56798,09**	**65579.45**	**58950,84**
Endosulfan 1 & 2										
Chlopyriphos	**265830,09**	**265830,09**	**235881,85**	**226285,34**	**216892,14**	**217436,69**	**265831,09**	**223991,34**	**262910,31**	**224277,34**
Demeton-S-methyl Sulfone	**15889,87**	**15955,60**	**16139,75**	**15681,54**	**15302,17**	**15046,25**	**15286,48**	**15206,77**	**14633,25**	**14595,79**

Table 5 Peak Areas

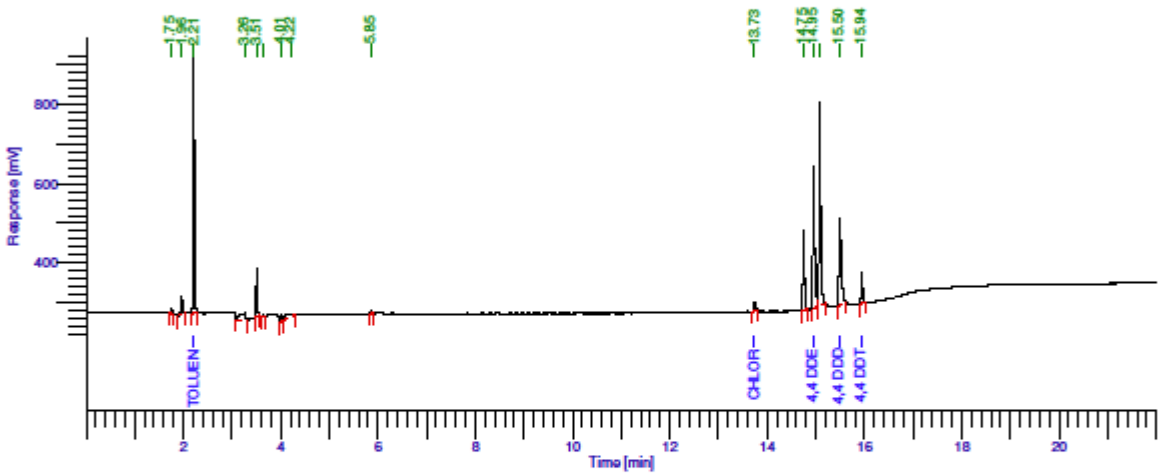
Compound	Mean	Standard Deviation	%RSD
4,4 DDT	93866,577	1431,418228	1.52%
4,4 DDE	80316,206	4177,06013	5,2%
4,4 DDD	93452,655	1987,14095	2,13%
2,4 DDE	60848,416	3544,149307	5,82%
ENDOSULFAN 1/ ALPHA	133906,228	3303,666986	2,48%
ENDOSULFAN 2/ BETA	53145,983	1433,967478	2,69%
CHLOPYRIPHOS	240516,728	21795,77594	9,06%
DEMETON-S-METHYL-SULFONE	15373,768	536,2409911	3,48%

Table 6 Indicating relative standard deviations for the different compounds

All compounds showed a % RSD of less than 10. These values of validation parameters indicated that the analysis using the instrument method is quite repeatable.

Software Version	: 6.3.1.0504	Date	: 2010/06/18 08:51:47 AM
Operator	: GC	Sample Name	:
Sample Number	: 001	Study	: 4.4 ddt
AutoSampler	: BUILT-IN	Rack/Vial	: 0/3
Instrument Name	: GC	Channel	: B
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 22.00 min
Sampling Rate	: 12.5000 pts/s		
Sample Volume	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 2010/03/16 09:23:03 AM	Cycle	: 1

Raw Data File : C:\Clarus 500\Data\datb001-20100316-092323.raw
Inst Method : C:\Clarus 500\Method\Chlorpyrifos channel B from C:\Clarus 500\Data\datb001-20100316-092323.raw
Proc Method : C:\Clarus 500\Method\Chlorpyrifos channel B.mth from
Calib Method : C:\Clarus 500\Method\Chlorpyrifos channel B.mth from
Report Format File: C:\Clarus 500\Method\ddd report.rpt
Sequence File : C:\Clarus 500\Sequence\repeatabilty test march.seq



DDD Report

Peak #	Component Name	Time [min]	Area [uV*sec]	ppm Amount	ppb amount
1		1.749	26351.14	0.026	26.3511
2		1.962	113153.24	0.113	113.1532
3	TOLUENE	2.208	1347221.56	1.347	1347.2216
4		3.261	156493.20	0.156	156.4932
5		3.506	197947.92	0.198	197.9479
6		3.638	15532.96	0.016	15.5330
7		4.012	35328.12	0.035	35.3281
8		4.222	120462.47	0.120	120.4625
9		5.855	13662.26	0.014	13.6623
10	CHLORPYRIPHOS	13.735	71241.46	0.071	71.2415
11		14.749	454485.81	0.454	454.4858
12	4,4 DDE	14.953	960482.66	0.960	960.4827
13		15.080	1262580.42	1.263	1262.5804
14	4,4 DDD	15.495	732865.71	0.733	732.8657
15	4,4 DDT	15.945	176917.75	0.177	176.9178
			5684726.67	5.685	5684.7267

Missing Component Report

Component	Expected Retention (Calibration File)

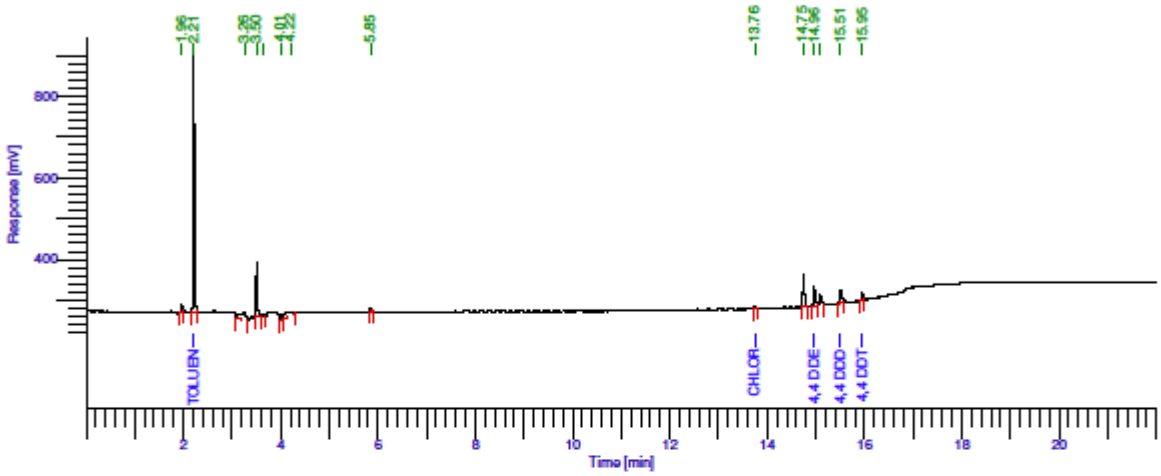
All components were found

Figure 7 Chromatogram for mixture at 1ppm

Software Version : 6.3.1.0504
Sample Name :
Instrument Name : GC
Rack/Vial : 0/4
Sample Amount : 1.000000
Cycle : 1

Date : 2010/06/18 09:07:36 AM
Data Acquisition Time : 2010/03/16 09:54:22 AM
Channel : B
Operator : GC
Dilution Factor : 1.000000

Result File :
Sequence File : C:\Clarus 500\Sequence\repeatabilty test march.seq



Report

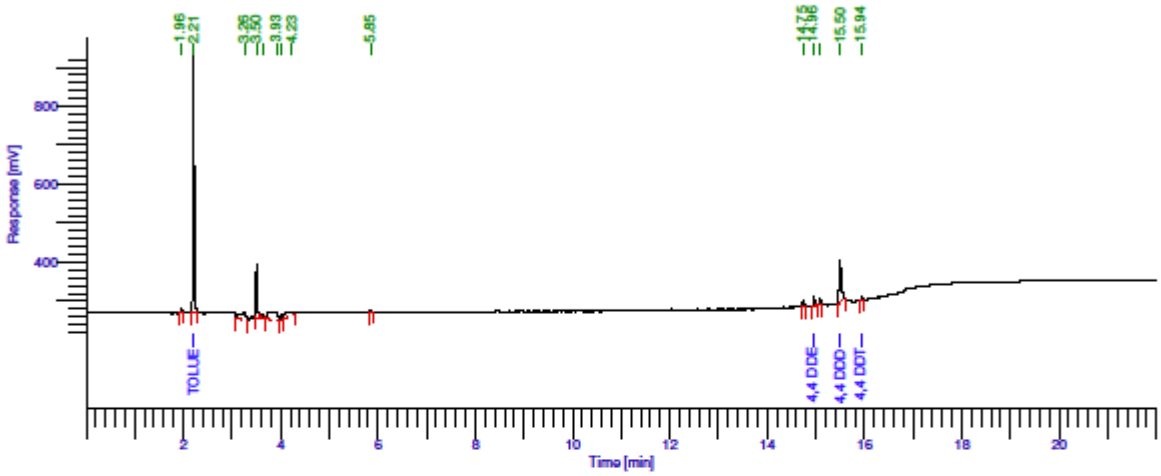
Peak #	Component Name	Time [min]	Time [min]	Area [uV*sec]	Concentration ppm
1		1.965	1.965	39892.88	0.04
2	TOLUENE	2.209	2.209	1329761.25	1.33
3		3.260	3.260	157396.30	0.16
4		3.505	3.505	228823.85	0.23
5		3.637	3.637	16581.90	0.02
6		4.010	4.010	35661.95	0.04
7		4.215	4.215	121663.79	0.12
8		5.850	5.850	16460.60	0.02
9	CHLORPYRIPHOS	13.756	13.756	10429.08	0.01
10		14.747	14.747	189281.70	0.19
11	4,4 DDE	14.964	14.964	151636.76	0.15
12		15.091	15.091	76769.46	0.08
13	4,4 DDD	15.508	15.508	109581.08	0.11
14	4,4 DDT	15.951	15.951	42939.57	0.04
				2526880.14	2.53

Figure 8 Chromatogram indicating the mixture at 0.5 ppm

Software Version : 6.3.1.0504
Sample Name :
Instrument Name : GC
Rack/Vial : 0/5
Sample Amount : 1.000000
Cycle : 1

Date : 2010/06/18 09:08:49 AM
Data Acquisition Time : 2010/03/16 10:25:41 AM
Channel : B
Operator : GC
Dilution Factor : 1.000000

Result File :
Sequence File : C:\Clarus 500\Sequence\repeatabilty test march.seq



Report

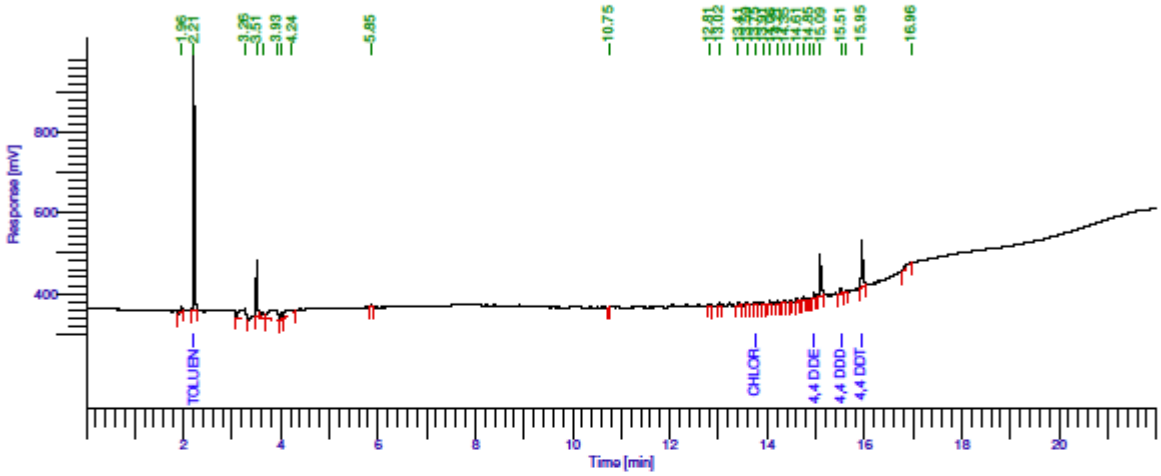
Peak #	Component Name	Time [min]	Time [min]	Area [uV*sec]	Concentration ppm
1		1.956	1.956	17458.75	0.02
2	TOLUENE	2.205	2.205	1336453.15	1.34
3		3.258	3.258	159788.77	0.16
4		3.503	3.503	245370.88	0.25
5		3.635	3.635	43725.74	0.04
6		3.929	3.929	275093.83	0.28
7		4.008	4.008	36501.18	0.04
8		4.227	4.227	124092.19	0.12
9		5.848	5.848	13575.72	0.01
10		14.745	14.745	45914.30	0.05
11	4,4 DDE	14.962	14.962	55401.56	0.06
12		15.087	15.087	34858.75	0.03
13	4,4 DDD	15.497	15.497	375116.51	0.38
14	4,4 DDT	15.943	15.943	21432.16	0.02
				2784783.50	2.78

Figure 9 Chromatogram indicating the mixture at 0.25ppm

Software Version : 6.3.1.0504
Sample Name :
Instrument Name : GC
Rack/Vial : 0/7
Sample Amount : 1.000000
Cycle : 1

Date : 2010/06/18 09:10:56 AM
Data Acquisition Time : 2010/03/16 11:28:39 AM
Channel : B
Operator : GC
Dilution Factor : 1.000000

Result File :
Sequence File : C:\Clarus 500\Sequence\repeatabilty test march.seq



Report

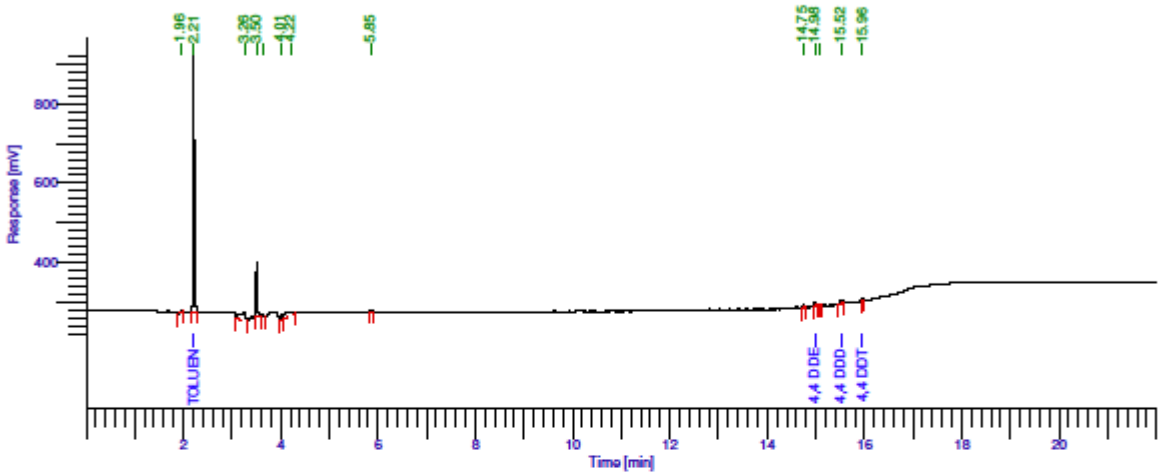
Peak #	Component Name	Time [min]	Time [min]	Area [uV*sec]	Concentration ppm
1		1.957	1.957	43009.61	0.04
2	TOLUENE	2.211	2.211	1403465.15	----------
3		3.262	3.262	235130.55	0.24
4		3.506	3.506	260667.72	0.26
5		3.636	3.636	50435.30	0.05
6		3.932	3.932	329555.73	0.33
7		4.010	4.010	45020.39	0.05
8		4.235	4.235	157193.23	0.16
9		5.852	5.852	11738.20	0.01
10		10.753	10.753	4097.30	0.00
11		12.807	12.807	14380.00	0.01
12		13.016	13.016	14792.10	0.01
13		13.411	13.411	15805.90	0.02
14		13.588	13.588	20598.97	0.02
15	CHLORPYRIPHOS	13.751	13.751	14124.51	0.01
16		13.910	13.910	14434.98	0.01
17		14.061	14.061	11297.63	0.01
18		14.207	14.207	12183.19	0.01
19		14.347	14.347	15785.43	0.02
20		14.480	14.480	13262.25	0.01
21		14.613	14.613	15839.07	0.02
22		14.745	14.745	24975.15	0.02
23		14.853	14.853	7575.52	0.01
24	4,4 DDE	14.970	14.970	32010.98	0.03
25		15.086	15.086	281030.27	0.28
26	4,4 DDD	15.515	15.515	30303.84	0.03
27		15.623	15.623	10470.04	0.01
28	4,4 DDT	15.945	15.945	313429.88	0.31
29		16.963	16.963	77420.38	0.08
				3480033.26	2.08

Figure 10 Chromatogram indicating the mixture 0.0625 ppm

Software Version : 6.3.1.0504
Sample Name :
Instrument Name : GC
Rack/Vial : 0/8
Sample Amount : 1.000000
Cycle : 1

Date : 2010/06/18 09:12:03 AM
Data Acquisition Time : 2010/03/16 12:00:04 PM
Channel : B
Operator : GC
Dilution Factor : 1.000000

Result File :
Sequence File : C:\Clarus 500\Sequence\repeatabilty test march.seq



Report

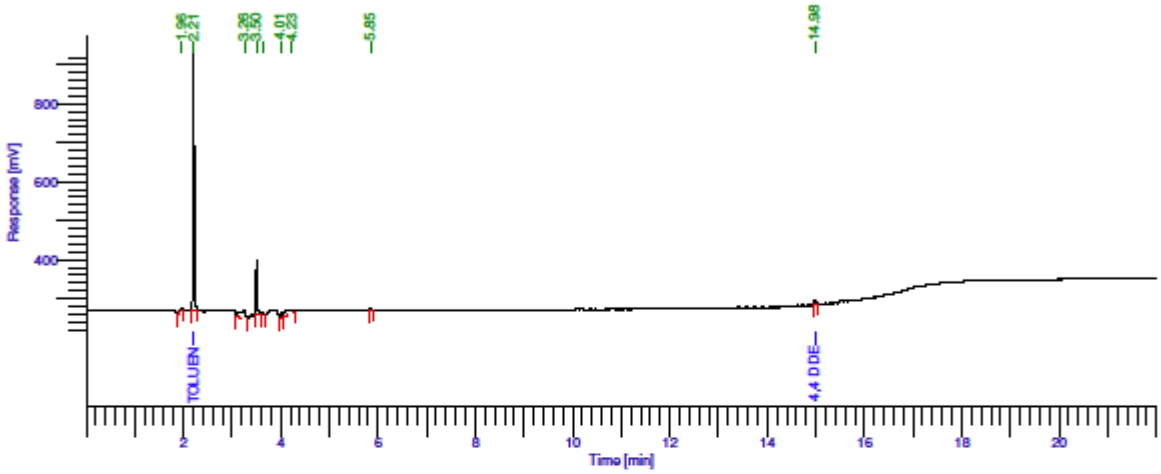
Peak #	Component Name	Time [min]	Time [min]	Area [uV*sec]	Concentration ppm
1		1.957	1.957	25499.13	0.03
2	TOLUENE	2.208	2.208	1356365.39	1.36
3		3.258	3.258	168237.73	0.17
4		3.503	3.503	236141.72	0.24
5		3.634	3.634	14958.85	0.01
6		4.008	4.008	35274.49	0.04
7		4.217	4.217	119425.75	0.12
8		5.849	5.849	10957.59	0.01
9		14.749	14.749	22145.56	0.02
10	4,4 DDE	14.979	14.979	20536.89	0.02
11		15.100	15.100	8122.32	0.01
12	4,4 DDD	15.522	15.522	37499.48	0.04
13	4,4 DDT	15.962	15.962	11447.71	0.01
				2066612.60	2.07

Figure 11 Chromatogram indicating the mixture at 0.03125 ppm

Software Version : 6.3.1.0504
Sample Name :
Instrument Name : GC
Rack/Vial : 0/9
Sample Amount : 1.000000
Cycle : 1

Date : 2010/06/18 09:13:17 AM
Data Acquisition Time : 2010/03/16 12:31:33 PM
Channel : B
Operator : GC
Dilution Factor : 1.000000

Result File :
Sequence File : C:\Clarus 500\Sequence\repeatabilty test march.seq



Report

Peak #	Component Name	Time [min]	Time [min]	Area [uV*sec]	Concentration ppm
1		1.958	1.958	34564.65	0.03
2	TOLUENE	2.207	2.207	1383081.07	1.38
3		3.259	3.259	164161.07	0.16
4		3.503	3.503	242400.56	0.24
5		3.635	3.635	15880.79	0.02
6		4.008	4.008	36043.19	0.04
7		4.226	4.226	122651.46	0.12
8		5.850	5.850	9447.53	0.01
9	4,4 DDE	14.983	14.983	36516.35	0.04
				2044746.66	2.04

Figure 12 Chromatogram indicating the mixture at 0.015625 ppm

Compound	Lowest detectable concentration
4,4- DDT	0.03125PPM
CHLORPYRIPHOS	0.5ppm
MIX	0.0156ppm
2,4- DDD	0.03125ppm
4,4- DDE	0,015625ppm
DEMETON-S-METHYL-SULFONE	1.0 ppm
ENDOSULFAN 1 & 2	1.0 ppm

Table 7. Indicating the lowest detection limits using GC-ECD

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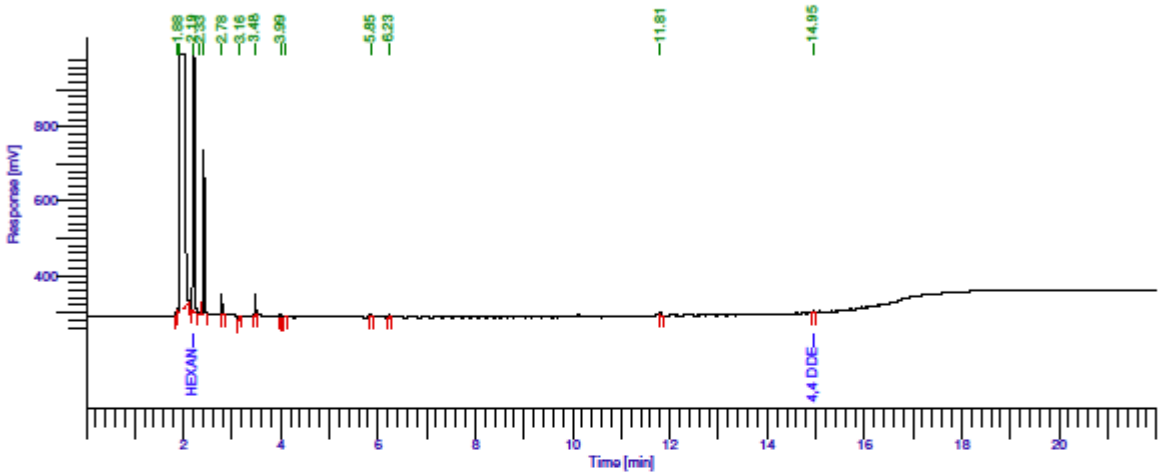
Extracts recovered

As indicated in chapter 3 samples were extracted from different rivers using dichloromethane as the solvent. The following chromatograms indicate examples of the extracts discovered from the three rivers using GC-ECD.

Software Version : 6.3.1.0504
Sample Name :
Instrument Name : GC
Rack/Vial : 0/6
Sample Amount : 1.000000
Cycle : 1

Date : 2010/06/25 12:14:10 PM
Data Acquisition Time : 2010/03/31 12:26:09 PM
Channel : B
Operator : GC
Dilution Factor : 1.000000

Result File :
Sequence File : C:\Clarus 500\Sequence\repeatabilty test march.seq



Report

Peak #	Component Name	Time [min]	Time [min]	Area [uV*sec]	Concentration ppm
1		1.879	1.879	34294.12	0.03
2		1.921	1.921	5854004.77	----------
3	HEXANE	2.195	2.195	1934450.99	----------
4		2.333	2.333	8100.83	0.01
5		2.420	2.420	704659.71	0.70
6		2.784	2.784	84371.61	0.08
7		3.162	3.162	21962.33	0.02
8		3.478	3.478	88418.05	0.09
9		3.993	3.993	28079.39	0.03
10		4.096	4.096	22544.02	0.02
11		5.847	5.847	10236.20	0.01
12		6.230	6.230	11444.09	0.01
13		11.807	11.807	12324.78	0.01
14	4,4 DDE	14.953	14.953	19605.34	0.02
				8834496.23	1.05

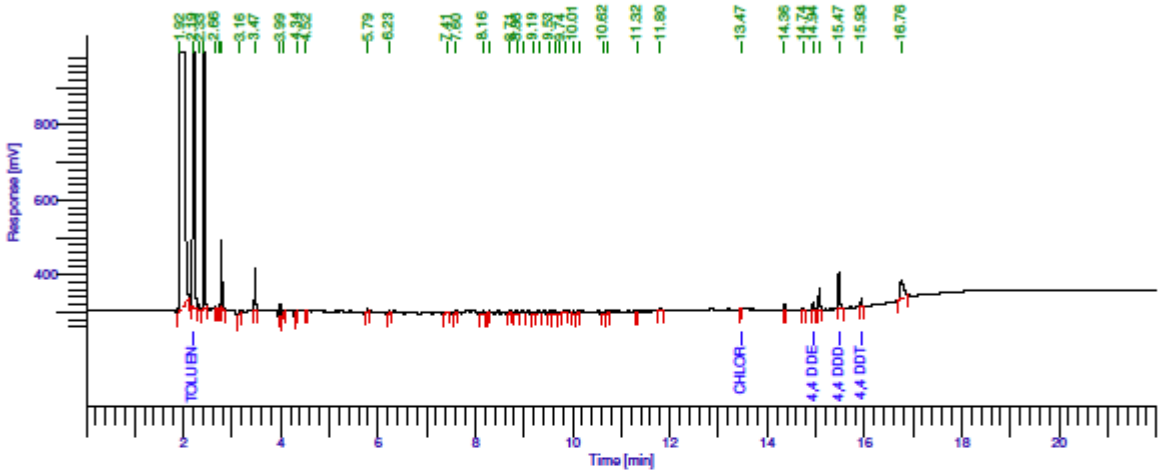
Warning -- Signal level out-of-range in peak

Figure 13 Chromatogram for the Kliprivier sample

Software Version : 6.3.1.0504
Sample Name :
Instrument Name : GC
Rack/Vial : 0/4
Sample Amount : 1.000000
Cycle : 1

Date : 2010/06/18 09:01:41 AM
Data Acquisition Time : 2010/04/23 09:38:19 AM
Channel : B
Operator : GC
Dilution Factor : 1.000000

Result File :
Sequence File : C:\Clarus 500\Sequence\repeatabilty test march.seq



Report

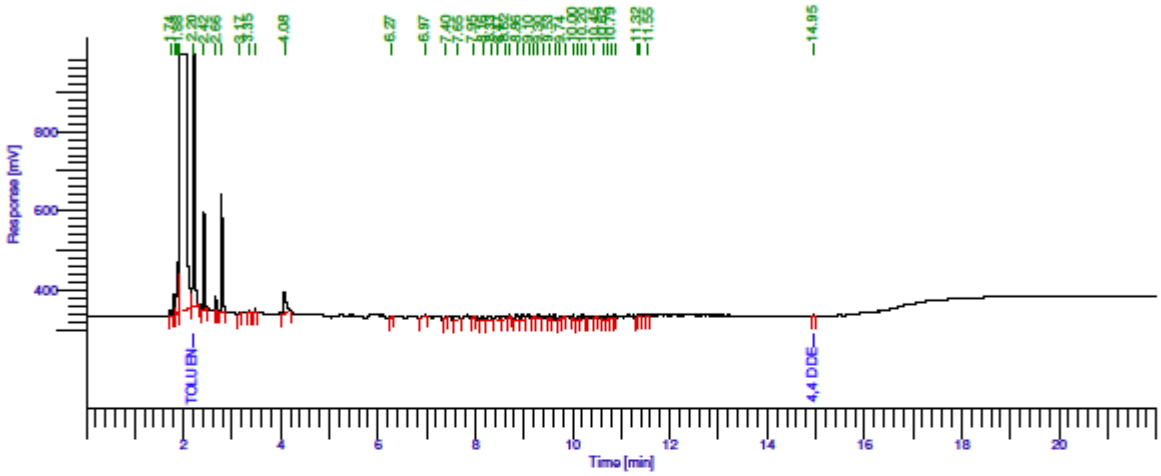
Peak #	Component Name	Time [min]	Time [min]	Area [uV*sec]	Concentration ppm
1		1.917	1.917	5901199.52	----------
2	TOLUENE	2.191	2.191	2316499.16	----------
3		2.330	2.330	20760.51	0.02
4		2.411	2.411	1458214.92	----------
5		2.655	2.655	5983.75	0.01
6		2.744	2.744	12827.91	0.01
7		2.782	2.782	302507.27	0.30
8		3.160	3.160	43807.82	0.04
9		3.472	3.472	190098.51	0.19
10		3.987	3.987	71054.46	0.07
11		4.067	4.067	25377.47	0.03
12		4.339	4.339	19914.15	0.02
13		4.516	4.516	4403.69	0.00
14		5.785	5.785	14633.25	0.01
15		6.225	6.225	12807.98	0.01
16		7.405	7.405	14914.40	0.01
17		7.596	7.596	14167.51	0.01
18		8.159	8.159	49553.43	0.05
19		8.277	8.277	9232.98	0.01
20		8.711	8.711	11970.08	0.01
21		8.855	8.855	38471.44	0.04
22		8.976	8.976	34068.89	0.03
23		9.187	9.187	22017.50	0.02
24		9.302	9.302	28663.94	0.03
25		9.528	9.528	30345.84	0.03
26		9.631	9.631	27387.14	0.03
27		9.743	9.743	18679.87	0.02
28		9.847	9.847	15384.37	0.02
29		10.007	10.007	20549.15	0.02
30		10.122	10.122	26210.73	0.03
31		10.619	10.619	17301.16	0.02
32		10.707	10.707	19889.56	0.02

Figure 14 Chromatogram for the Barrage sample

Software Version : 6.3.1.0504
Sample Name :
Instrument Name : GC
Rack/Vial : 0/9
Sample Amount : 1.000000
Cycle : 1

Date : 2010/06/18 09:05:38 AM
Data Acquisition Time : 2010/04/26 01:42:02 PM
Channel : B
Operator : GC
Dilution Factor : 1.000000

Result File :
Sequence File : C:\Clarus 500\Sequence\repeatabilty test march.seq



Report

Peak #	Component Name	Time [min]	Time [min]	Area [uV*sec]	Concentration ppm
1		1.738	1.738	38267.83	0.04
2		1.811	1.811	101152.14	0.10
3		1.884	1.884	292775.56	0.29
4		1.908	1.908	7004942.33	----------
5	TOLUENE	2.201	2.201	2011327.25	----------
6		2.423	2.423	444738.60	0.44
7		2.664	2.664	59880.42	0.06
8		2.789	2.789	545009.52	0.55
9		3.166	3.166	23092.88	0.02
10		3.351	3.351	7742.64	0.01
11		3.475	3.475	19146.77	0.02
12		4.077	4.077	234344.37	0.23
13		6.273	6.273	12059.01	0.01
14		6.969	6.969	23342.15	0.02
15		7.403	7.403	14807.93	0.01
16		7.645	7.645	81115.48	0.08
17		7.950	7.950	13604.16	0.01
18		8.157	8.157	63053.32	0.06
19		8.326	8.326	68658.99	0.07
20		8.472	8.472	56289.25	0.06
21		8.616	8.616	45865.49	0.05
22		8.708	8.708	13202.33	0.01
23		8.855	8.855	49159.14	0.05
24		8.977	8.977	50837.31	0.05
25		9.099	9.099	46289.55	0.05
26		9.183	9.183	7098.04	0.01
27		9.299	9.299	37172.67	0.04
28		9.419	9.419	37596.04	0.04
29		9.530	9.530	42955.37	0.04
30		9.631	9.631	38716.85	0.04
31		9.741	9.741	24094.41	0.02
32		9.845	9.845	11265.21	0.01

Figure 15 Chromatogram for the Zuikerbors sample

4.3 Results

As indicated in chapter 3, the extracts were also analyzed using HPLC for the determination of carbamates and results are indicated in the following chromatograms.

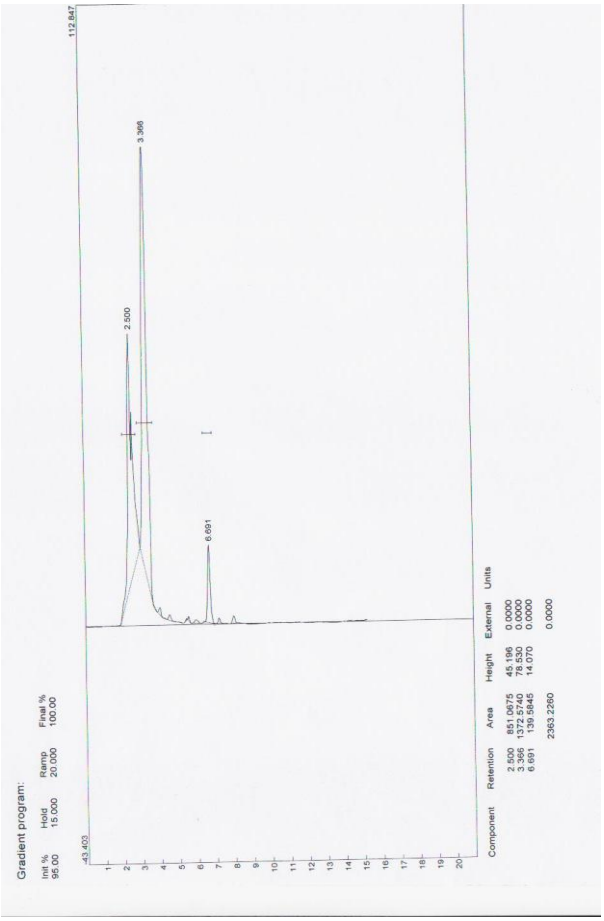


Figure 16 Zuikerbosch sample

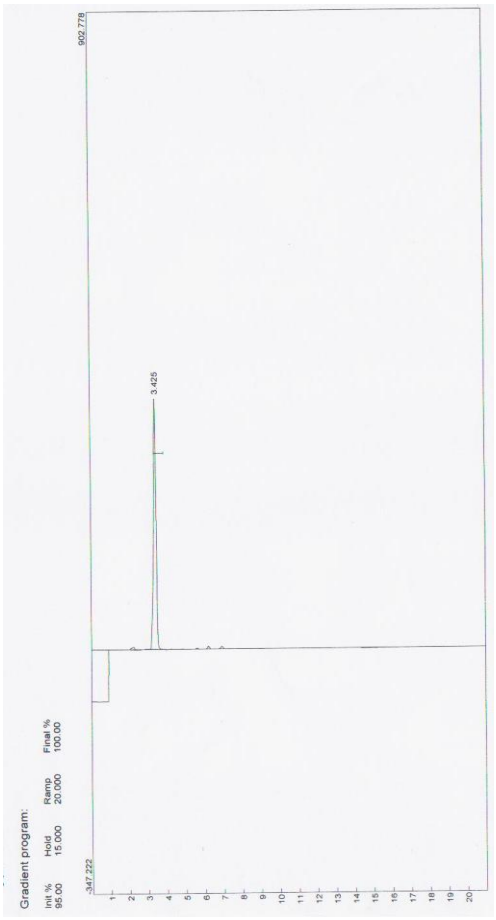


Figure 17Carbofuran standard

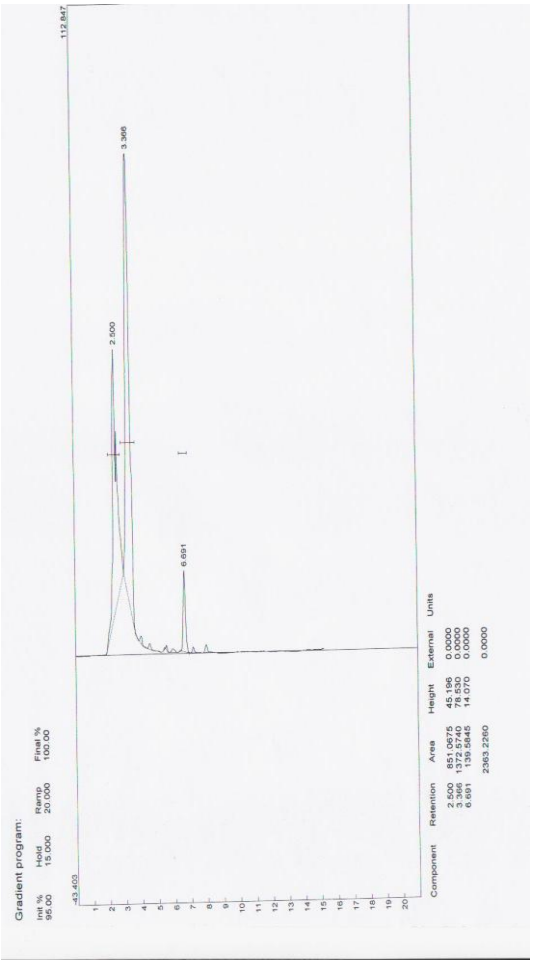


Figure 18 Kliprivier sample

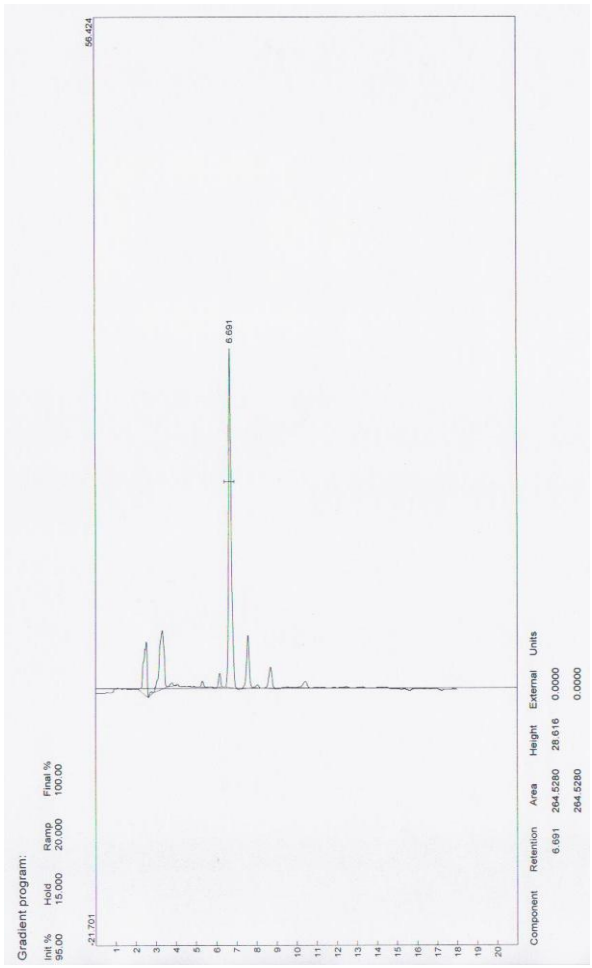


Figure 19 Benfuracarb standard

Standards	Retention Time(min)	
4,4- DDT	15.95	
4,4 -DDD	15.50	
4,4 -DDE	14.92	
2,4 -DDD	15.07	**GC-ECD**
Demeton-s-methylsulfon	13.35	
Chlorpyrifos	13.70	
Endosulfan 1&2	14.77 & 15.49	
Benfuracarb	6.691	
Carbofuran	3.425	**HPLC**

Table 8 Summary of the different retention times

4.4 Retention times

As indicated in chapter 3 the extracts were also analyzed using GC-MS to confirm the structures of the different pesticides. The following figures indicates the different retention times obtained using GC-MS.

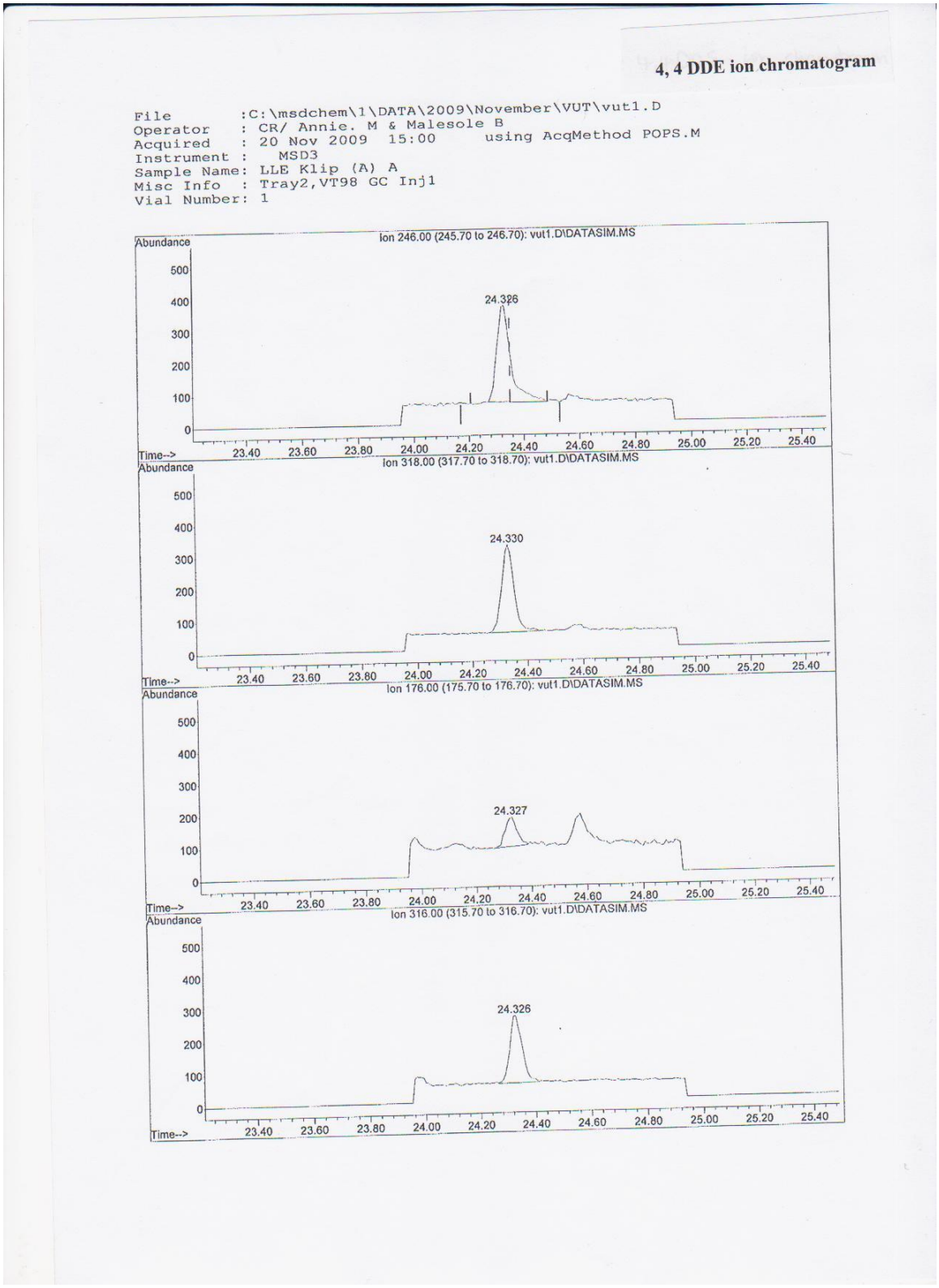


Figure 20 Chromatogram for the 4, 4 -DDE retention time

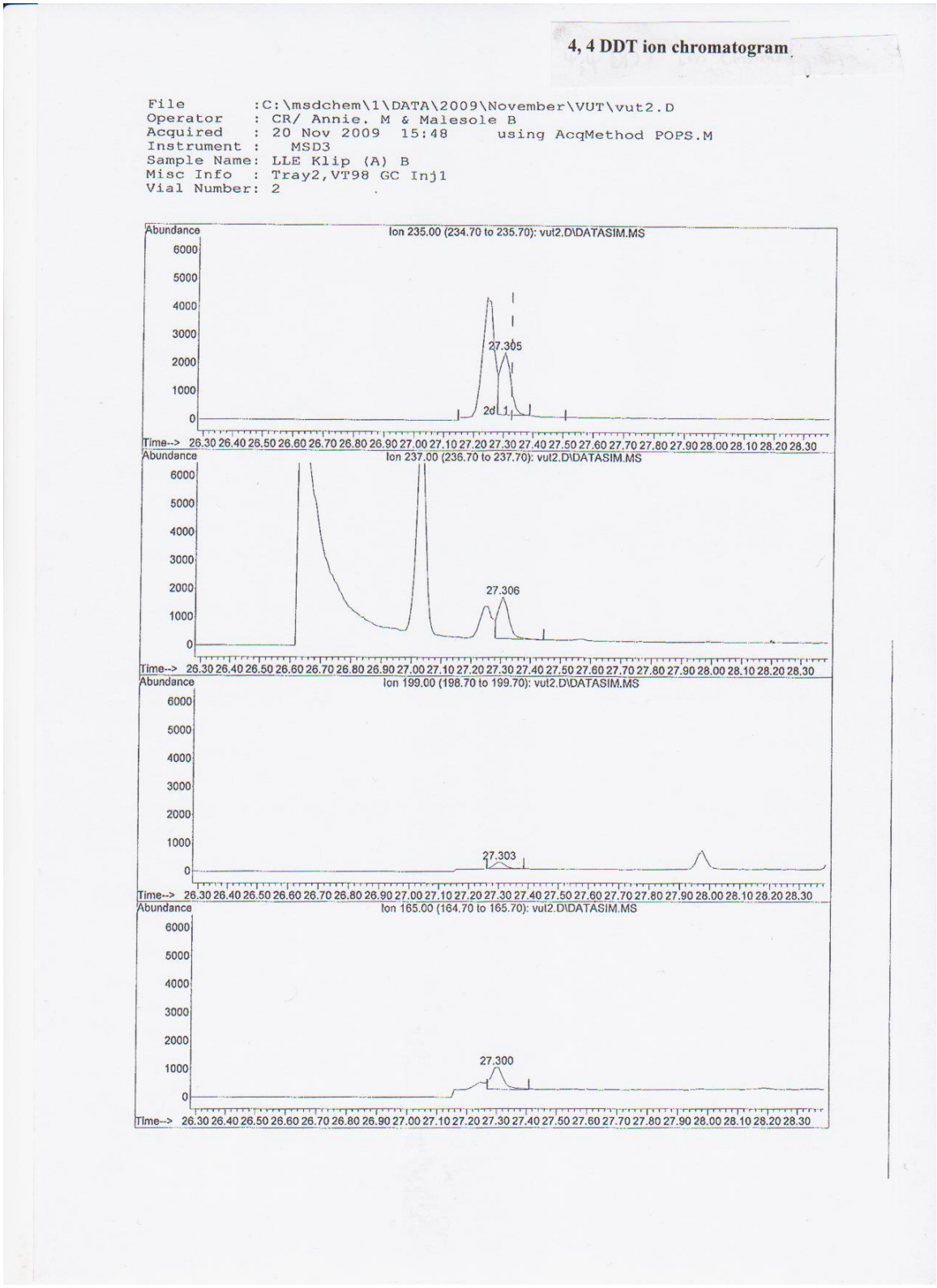


Figure 21 Chromatogram indicating 4, 4 -DDT retention time

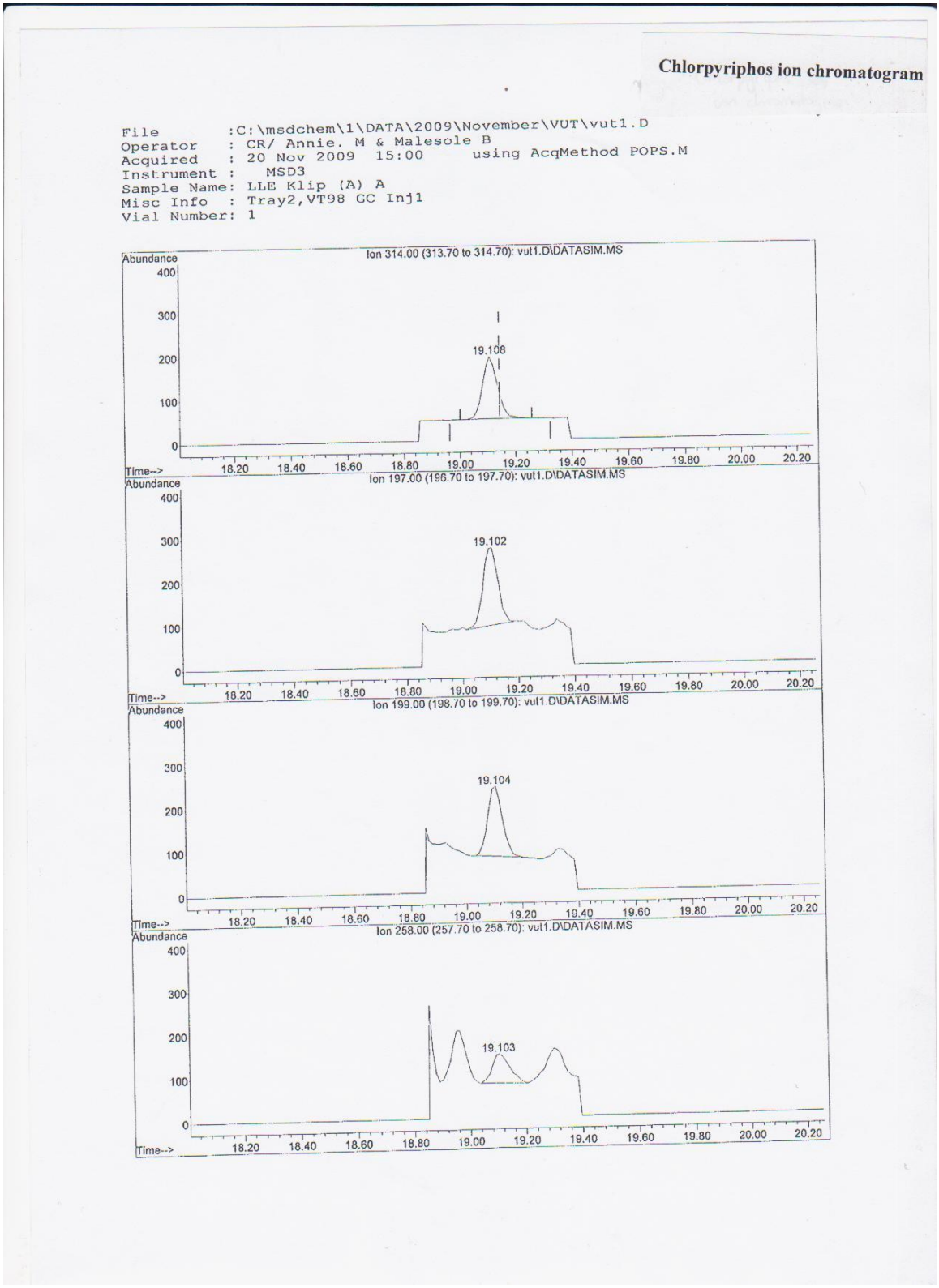


Figure 22 Chromatogram indicating chlorpyriphos retention time

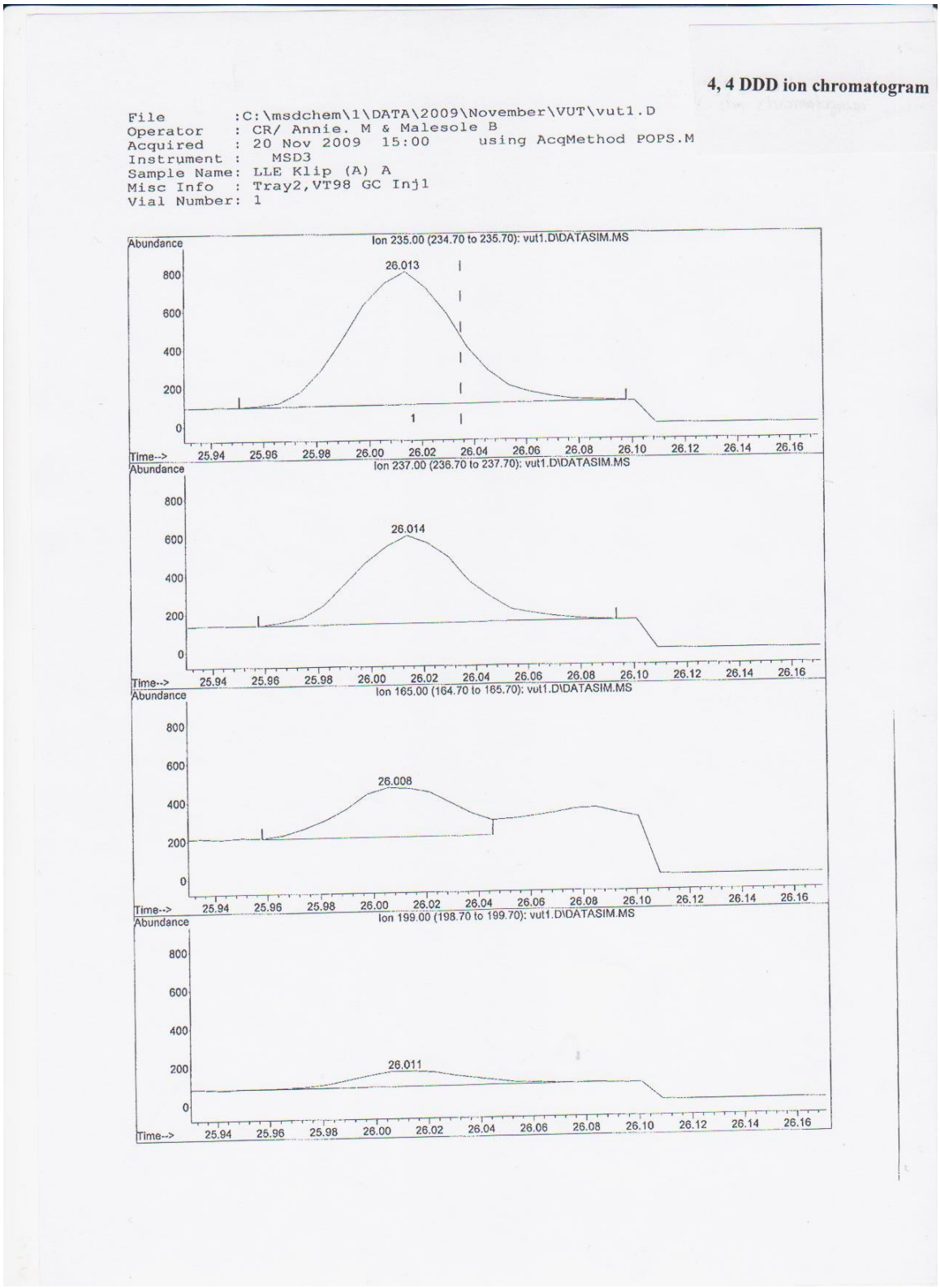


Figure 23 Chromatogram indicating 4, 4- DDD retention time

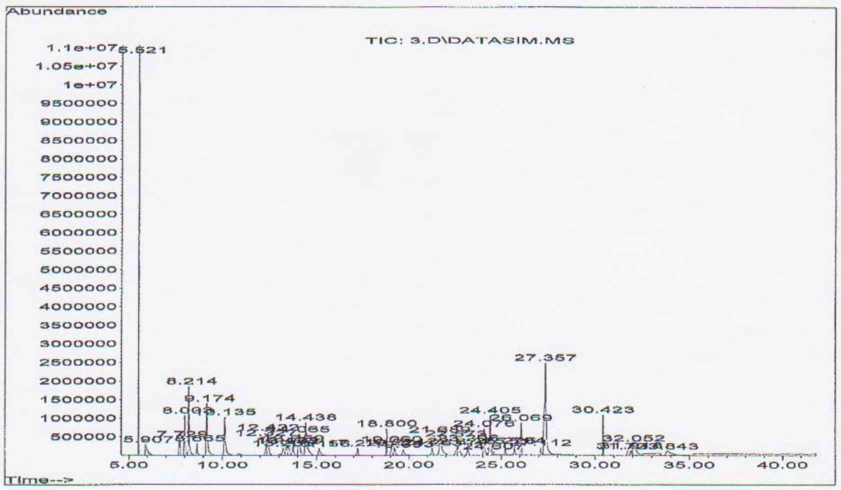
Standards	Retention Time (min)
4,4- DDT	27,3
4,4- DDE	24,3
4,4- DDD	26,0
Chlorpyriphos	19,1

Table 9 Summary of the retention times using GC-MS

GC-MS results for samples from the different rivers are shown on the following chromatograms

C:\msdchem\1\DATA\2010\April\20100325-39\VUT Samples\75.D

Sample Name	Klip AC LLE
Data File Name	75.D
Data File Path	C:\msdchem\1\DATA\2010\April\20100325-39\VUT Samples\
Vial Number	59
Date Acquired	11/10/2004 3:19
Acq. Method File	POPSCPSIL.M
Operator	CR\SM
Instrument Name	P&T Agilent MS 1



Name	Rt	Amt (ng/uL)	Target Resp	Man Int	Match	Q1	Expt d	Q2	Exp td	Q3	Expt d
1) Demeton-S-methyl	0.000	0.023	0	1	1	0	362	0	87	0	3
2) Chlorpyrifos ethyl	19.224	-0.263	909	0	93	95	112	110	109	55	55
3) Endosulfan (I) alpha	0.000	0.019	0	1	41	0	120	0	113	0	75
4) 4,4-DDE	24.016	0.005 ✓	1283	1	83	86	85	30	36	57	65
5) Endosulfan (II) beta	0.000	0.019	0	1	1	0	94	0	51	0	64
6) 4,4-DDD	25.670	0.009 ✓	4220	1	56	61	64	35	45	14	15
7) 4,4 DDT	26.989	0.016 ✓	2147	1	91	70	63	27	15	38	42
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0

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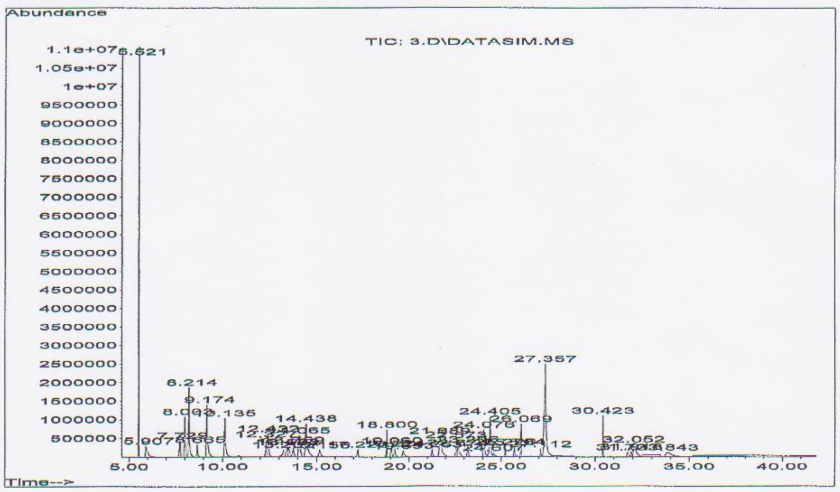
Figure 24 Chromatogram for Kliprivier sample with LLE

C:\msdchem\1\DATA\2010\April\20100325-39\VUT Samples\76.D

Sample Name	Klip BA LLE
Data File Name	76.D
Data File Path	C:\msdchem\1\DATA\2010\April\20100325-39\VUT Samples\
Vial Number	60
Date Acquired	11/10/2004 4:07
Acq. Method File	POPSCPSIL.M
Operator	CR\SM
Instrument Name	P&T Agilent MS 1



Name	Rt	Amt (ng/uL)	Target Resp	Man Int	Match	Q1	Expt d	Q2	Exp td	Q3	Expt d
1) Demeton-S-methyl	0.000	0.023	0	1	1	0	362	0	87	0	3
2) Chlorpyrifos ethyl	19.226	-0.264	452	0	94	107	112	99	109	57	55
3) Endosulfan (I) alpha	0.000	0.019	0	1	16	0	120	0	113	0	75
4) 4,4-DDE	24.016	0.005 ✓	1325	1	71	91	85	33	36	60	65
5) Endosulfan (II) beta	0.000	0.019	0	1	1	0	94	0	51	0	64
6) 4,4-DDD	25.670	0.007 ✓	2701	1	43	74	64	51	45	11	15
7) 4,4 DDT	26.988	0.014 ✓	1435	1	78	60	63	27	15	31	42
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0

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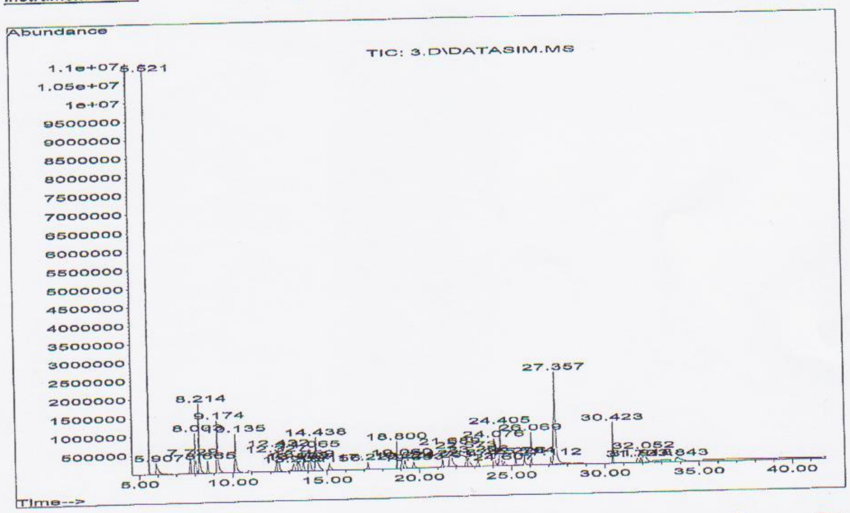
C:\msdchem\custrpt\GROBLRSDALTST.CRT

Figure 25 Chromatogram for Kliprivier sample with LLE

C:\msdchem\1\DATA\2010\April\20100325-39\VUT Samples\70.D

Sample Name	Klip DB SPE
Data File Name	70.D
Data File Path	C:\msdchem\1\DATA\2010\April\20100325-39\VUT Samples\
Vial Number	55
Date Acquired	10/10/2004 23:17
Acq. Method File	POPSCPSIL.M
Operator	CR\SM
Instrument Name	P&T Agilent MS 1



Name	Rt	Amt (ng/uL)	Target Resp	Man Int	Match	Q1	Expt d	Q2	Exp td	Q3	Expt d
1) Demeton-S-methyl	0.000	0.023	0	1	1	0	362	0	87	0	3
2) Chlorpyrifos ethyl	19.216	-0.264	363	0	86	105	112	124	109	36	55
3) Endosulfan (I) alpha	0.000	0.019	0	1	2	0	120	0	113	0	75
4) 4,4-DDE	24.016	0.004 ✓	383	1	1	111	85	44	36	108	65
5) Endosulfan (II) beta	0.000	0.000	0	0	0	0	94	0	51	0	64
6) 4,4-DDD	25.670	0.005 ✓	1068	1	29	86	64	52	45	18	15
7) 4,4 DDT	0.000	0.012 ✗	0	1	55	0	63	0	15	0	42
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0

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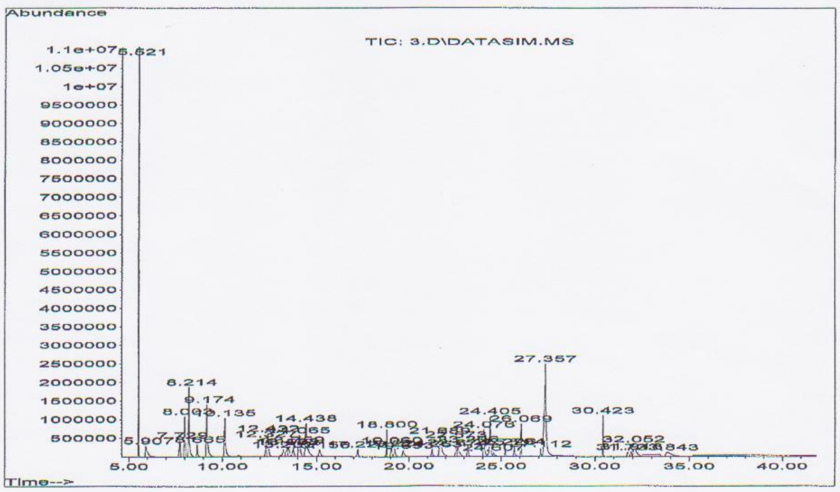
Figure 26 Chromatogram for Kliprivier sample using SPE

C:\msdchem\1\DATA\2010\April\20100325-39\VUT Samples\76.D

Sample Name	Klip BA LLE
Data File Name	76.D
Data File Path	C:\msdchem\1\DATA\2010\April\20100325-39\VUT Samples\
Vial Number	60
Date Acquired	11/10/2004 4:07
Acq. Method File	POPSCPSIL.M
Operator	CR\SM
Instrument Name	P&T Agilent MS 1



Name	Rt	Amt (ng/uL)	Target Resp	Man Int	Match	Q1	Expt d	Q2	Exp td	Q3	Expt d
1) Demeton-S-methyl	0.000	0.023	0	1	1	0	362	0	87	0	3
2) Chlorpyrifos ethyl	19.226	-0.264	452	0	94	107	112	99	109	57	55
3) Endosulfan (I) alpha	0.000	0.019	0	1	16	0	120	0	113	0	75
4) 4,4-DDE	24.016	0.005 ✓	1325	1	71	91	85	33	36	60	65
5) Endosulfan (II) beta	0.000	0.019	0	1	1	0	94	0	51	0	64
6) 4,4-DDD	25.670	0.007 ✓	2701	1	43	74	64	51	45	11	15
7) 4,4 DDT	26.988	0.014 ✓	1435	1	78	60	63	27	15	31	42
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0
) 0	0.000	0.000	0	0	0	0	0	0	0	0	0

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Figure 27 Chromatogram for Kliprivier sample using SPE

	KlipRivier		Zuikerbosch		Rand Water Barrage	
	GC	HPLC	GC	HPLC	GC	HPLC
4,4 DDT	P		P		N	
4,4 DDE	P		P		P	
4,4 DDD	P		P		N	
2,4 DDD	P		P		P	
Demeton-s-methylsulfon	N		N		N	
Chlorpyrifos	P		P		P	
Endosulfan 1&2	N		N		N	
Benfuracarb		P		N		N
Carbofuran		N		P		N

Table 10 Summary of the test samples N=Negative P=Positive

4.5 Quantitative analysis

The following concentrations from the water samples were observed for the following compounds in the different rivers.

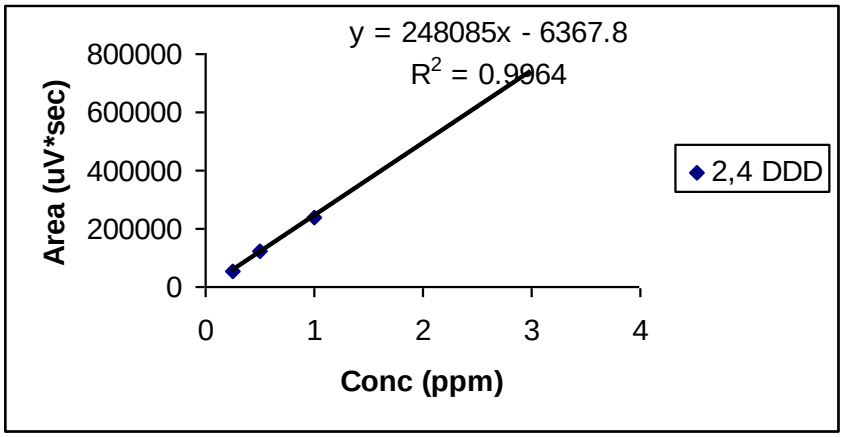

Figure 28 Calibration curve for 2, 4 DDD

Solid phase extraction (SPE)	
Kliprivier downstream	0.00736878 ppm RSD 4.7%
Kliprivier middle stream	0.131212286 ppm RSD 2.2%
Kliprivier upstream	N/D
Liquid liquid extraction (LLE)	
Kliprivier downstream	0.074436423 ppm RSD 7%
Kliprivier middle stream	0.161212286 ppm RSD 1.9%
Kliprivier upstream	N/D
Solid phase extraction (SPE)	
Barrage River downstream	0.045497148ppm RSD 4.5 %
Barrage River middle stream	N/D
Barrage River upstream	N/D
Liquid liquid extraction (LLE)	
Barrage River downstream	0.455211721ppm RSD 3.2%
Barrage River middle stream	N/D
Barrage River upstream	N/D
Solid phase extraction	
Zuikerbors River downstream	N/D
Zuikerbors River middle stream	N/D
Zuikerbors River upstream	N/D
Liquid liquid extraction	
Zuikerbosch River downstream	N/D
Zuikerbosch River middle stream	0.074995062 ppm RSD 4.2%
Zuikerbosch River upstream	N/D

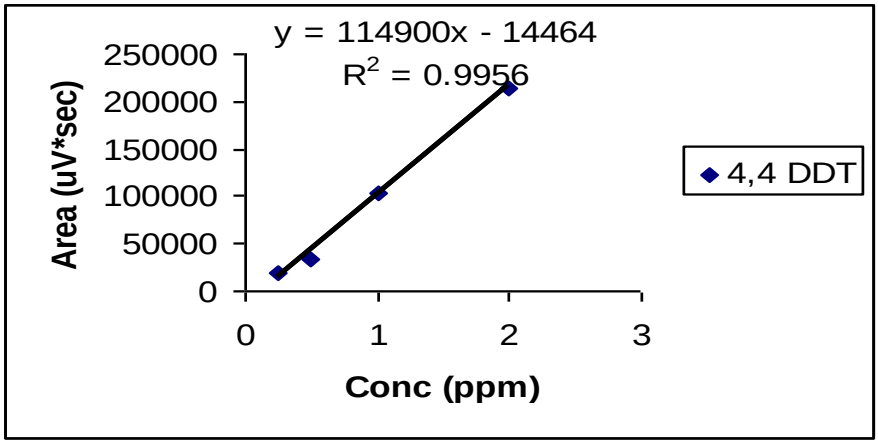

Figure 29 Calibration curve of 4, 4 DDT

SPE

Kliprivier downstream	N/D
Kliprivier middle stream	0.323125152 ppm RSD 4.2%
Kliprivier upstream	N/D

LLE

Kliprivier downstream	N/D
Kliprivier middle stream	0.367108006 ppm RSD 5.2%
Kliprivier upstream	N/D

SPE

Barrage River downstream	N/D
Barrage River middle stream	0.218659094 ppm RSD 2.3%
Barrage River upstream	N/D

LLE

Barrage River downstream	N/D
Barrage River middle stream	0.49381462 ppm RSD 4.2%
Barrage River upstream	N /D)

SPE

Zuikerbosch River downstream	N/D
Zuikerbosch River middle stream	N/D
Zuikerbosch River upstream	N/D

LLE

Zuikerbosch River downstream	0.208409153 ppm RSD 3.5%
Zuikerbosch River middle stream	1.455962228 ppm RSD 4.2%
Zuikerbosch River upstream	N/D

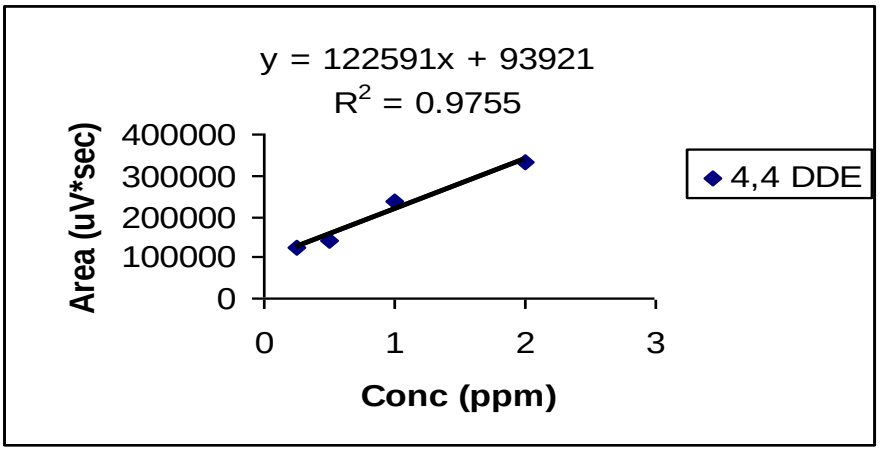

Figure 30 Calibration curve for 4,4 DDE

SPE

Kliprivier downstream	0.528865087 ppm RSD 6.2%
Kliprivier middle stream	0.211496276 ppm RSD 1.8%
Kliprivier upstream	N/D

LLE

Kliprivier downstream	0.618969663 ppm RSD 7.2%
Kliprivier middle stream	0.606208122 ppm RSD 4.3%
Kliprivier upstream	N/D

SPE

Barrage River downstream	0.560809847 ppm RSD 3.2%
Barrage River middle stream	0.423263045 ppm RSD 5.2%
Barrage River upstream	N/D

LLE

Barrage River downstream	0.410681289 ppm RSD 3.2%
Barrage River middlestream	N/D
Barrage River upstream	N/D

SPE

Zuikerbosch River middle stream	0.65032123 ppm RSD 4.2%
Zuikerbosch River upstream	0.637490761 ppm RSD 3.2%
Zuikerbosch River upstream	0.637490761 ppm RSD 4.2%

LLE

Zuikerbosch River downstream	N/D
Zuikerbosch River middle stream	0.543720746 ppm RSD 4.2%
Zuikerbosch River upstream	N/D

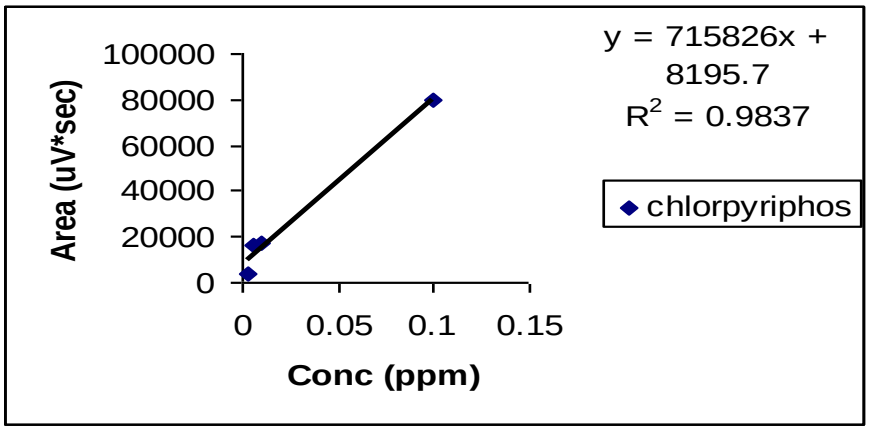

Figure 31 Calibration curve for Chlopyriphos

SPE

Kliprivier downstream	N/D
Kliprivier middle stream	0.002236325 ppm RSD 5.2%
Kliprivier upstream	N/D

LLE

Kliprivier downstream	N/D
Kliprivier middle stream	N/D
Kliprivier upstream	N/D

SPE

Barrage River downstream	N/D
Barrage River middle stream	N/D
Barrage River upstream	N/D

LLE

Barrage River downstream	N/D
Barrage River middle stream	N/D
Barrage River upstream	N/D

SPE

Zuikerbosch River downstream	N/D
Zuikerbosch River middle stream	N/D
Zuikerbosch River upstream	N/D

LLE

Zuikerbosch River downstream	N/D
Zuikerbosch River middle stream	N/D
Zuikerbosch River upstream	N/D

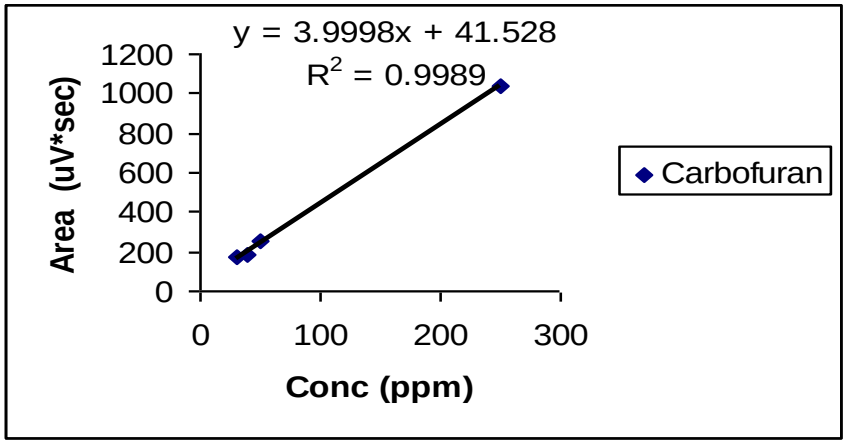

Figure 32 Calibration curve for Carbofuran

SPE

Zuikerbosch River downstream	0.00584252 ppm RSD 3.2%
Zuikerbosch River middle stream	0.00584252 ppm RSD 5.2%
Zuikerbosch River upstream	0.00534232 ppm RSD 4.5%

LLE

Zuikerbosch River downstream	0.006953164 ppm RSD 4.2%
Zuikerbosch River middle stream	0.006953164 ppm RSD 5.2%
Zuikerbosch River upstream	0.006953164 ppm RSD 5.2%

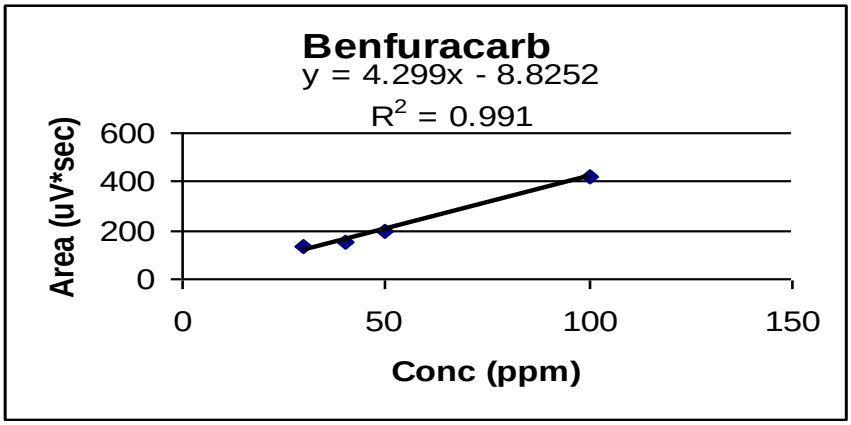

Figure 33 Calibration curve for Benfuracarb

SPE

Kliprivier downstream	0.018976651 ppm RSD 1.9%
Kliprivier middle stream	0.018976651 ppm RSD 3.3%
Kliprivier upstream	0.001655201 ppm RSD 4.2%

LLE

Kliprivier downstream	0.02896710929 ppm RSD 2.3%
Kliprivier middle stream	0.02896710929 ppm RSD 3.3%
Kliprivier upstream	0.02896710929 ppm RSD 4.2%

4.7 Discussion

Figure 3 shows the chromatogram of the mixture of the pesticides at 1ppm before extraction with LLE and SPE extraction processes. 4, 4 -DDE, 4,4- DDD 4, 4- DDT and chlorpyriphos are fairly well resolved at a reasonable time. Unidentified peaks can be linked to a series of contaminants. Since 4, 4- DDT and its metabolites can contain a series of contaminants. Figure 4 to 6 indicates the chromatograms for the different compounds after extraction with LLE. The peaks are clearly indicated that the compounds are fairly enriched after extraction which is the main point of extraction.

Table 4 shows the recovery percentages of the three solvents namely hexane, dichloromethane which dichloromethane indicated high recovery efficiency amongst the three. Table 5 shows the repeatability results with the peak areas. This test was done in order to test the repeatability of the GC-ECD in order to determine its suitability for the analysis of the extracts. Table 6 shows the mean, standard deviation and relative standard deviation of the different compounds. All compounds showed a % RSD of less than 10. These values of validation parameters indicated that the analysis using the GC-ECD method is quite repeatable.

Figure7 to fig12 show the different concentrations that were tested by dilution of the 1ppm mixture in order to determine the lowest detection limit of the mixture. This was done in order to determine the lowest concentration of the mixture of the compounds that could be detected by the GC-ECD. From the fig 12 it can be seen that the lowest compound to be detected was 4, 4 –DDE at a concentration of 0,015625 ppm.

Table 7 indicates the lowest detection limits for the different compounds obtained using GC-ECD.

Figure13 indicates a chromatogram for the Kliprivier sample, from the chromatogram it can be seen that the sample contained some 4, 4- DDE at a

retention time of 14, 95. It can clearly seen from the two chromatograms that Kliprivier contained some benfuracarb.

Figure 14 shows the Barrage sample that contains 4, 4 DDT at retention time of 15.93.

Figure 15 indicates that a Zuikerbosch sample contains a retention time of 14.95 which indicates that Zulkerbosch contains 4, 4-DDE.

Figure 16 indicates a Zuikerbosch sample and figure 17 indicates the carbofuran standard at a retention time of 3, 3 that shows that Zuikerbosch contains carbofuran.
Figure 18 indicates Kliprivier sample and figure 19 indicates Benfuracarb standards which clearly indicates that Kliprivier contains Benfuracarb at a retention time of 6, 6991

Figure 20 to fig 23 indicates the different chromatograms for the retention times obtained using the GC-MS instrument form DWA. Table 9 indicates a summary for the different retention times that were obtained using GC-MS.
Figure 24 and 25 indicates the Kliprivier samples using LLE and figure 26 and 27 indicates the same sample using SPE. It can be seen from these samples that LLE recovered more than SPE.

Table 10 indicates the summary for the tests done on the different rivers which clearly explains what compounds were detected in which river.

Fig 28 to fig 33 indicates he calibration curves for the following different compounds namely 2,4- DDD, 4,4-DDT, 4, 4- DDE and chlorpyriphos. These calibration curves were plotted in order to determine the different concentration from the rivers.

The GC-MS results were obtained at DWA to confirm the GC-ECD results that were obtained. There was a GC-ECD chromatogram that showed a retention time of 14.95 and the 4, 4- DDE standard was reading 14.92. The matrix effect must have been the reason why it is not exactly at 14.92 like the standard.

The results for the three rivers are listed in table and the concentrations are shown on table.

Most of the rivers did show the presence of DDT and its metabolites which might be would suggest a more prolonged contamination resulting in the breakdown of DDT into its metabolites.

From the chromatograms of two standards it can been seen that benfuracarb and carbofuran were detected in Kliprivier and Zuikerbosch waters.. Rand water barrage contains only 4,4 DDE and chlorpyrifos but no carbamates have been identified in this river. This is still is a concern for the enormous fishes in this river as well as the people who fish there. The water samples were taken close to living areas where these rivers are surely a source of direct drinking water for the people and the animals putting them in danger especially with pesticides like DDT and its metabolites.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The conditions of GC-ECD and HPLC were successfully optimized and the method developed was applied in the determination of selected pesticides studied. From the results carried out for the analysis in this study, it was revealed that DCM was the best solvent system for the determination of the selected pesticides in surface water. The analysis time was shortened and peak resolution was good in most cases.

SPE, LLE, GC and HPLC were used for the separation and quantification of organochlorine, organophosphates and carbamates pesticides in the southern Gauteng region. The methods applied were very effective since the presence of DDT, its metabolites, carbofuran and benfuracarb was clearly confirmed. This study has shown that LLE with DCM is an accurate and reliable and effective method for the determination of DDT and its metabolites, as well as benfuracarb and carbofuran at low concentrations in the environment. Further, it has been established that, although DDT has been banned, it is still present in the environment. The sources of these organochlorine compounds in the water systems might be from industrial effluents and or from diffuse sources such as run-off from agricultural lands. Both Kliprivier and Zuikerbosch rivers pass through agricultural areas in the Vaal region and thus the presence of DDT and some of its degradation residues in water systems can be attributed to their wide usage before their banning. Since they are persistent enough and degrade very slowly they accumulate in the soil and they are transported down to the water sources.

SPE and LLE were compared in terms of reproducibility and repeatability of the samples from the results it was deduced that LLE was the better extraction method between the two. It was also observed that analysis of carbamates did not produce satisfactory results using GC-ECD that it was why HPLC was chosen as the method of analysis for carbamates.

The presence of the pesticides confirmed in selected sampling sites is a great concern especially since some of the river namely Kliprivier is used as a source of drinking water. The possible sources might be sewage and waste dump sites around the river, or the fact that DDT and its metabolites are highly lipholic, bioaccumulative and are persistent in the environment in the case of Barrage River and Zuikerbosch.

5.2 Recommendations

Organochlorine and Organophosphate Pesticides

It is recommended that the organochlorine and organophosphates be analyzed using gas chromatography as a reliable and accurate method of analysis.

Carbamates

It is recommended that the carbamates should be analyzed using high performance liquid chromatography as the method of analysis. Carbamates pesticides should also be tested using HPLC-MS which might also confirm the presence of other carbamates pesticides since HPLC-MS will be capable of confirming structures of other pesticides present in our surface water.

Pesticides should be tested in soil and sediments which might give a more precise conclusion of these pesticides. Furthermore regular monitoring is needed to evolve a strategy to manage the environmental hazards due to these pesticides to avoid pollution of our water sources.

In the future, a new method has to be developed in order to investigate the occurrence and fate of target compounds in other environmental part like sludge sediments or soil samples.

Further study of the distribution of compounds capable of polluting the environment and the potential for human exposure is an urgent task.

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