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DEVELOPMENT OF SAMPLE PREPARATION AND CHROMATOGRAPHIC MASS SPECTROMETRIC TECHNIQUES FOR DETERMINATION OF SELECTED ORGANIC POLLUTANTS IN WASTEWATER

By

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of the

UNIVERSITY OF JOHANNESBURG

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DECLARATION

I hereby declare that this dissertation, which I herewith submit for the research qualification for

<u>DOCTOR OF PHILOSOPHY (PhD) DEGREE IN CHEMISTRY</u>

to the University of Johannesburg, Department of Chemistry, is, apart from the recognised assistance of my supervisors, my own work and has not previously been submitted by me to another institution to obtain a research diploma or degree.

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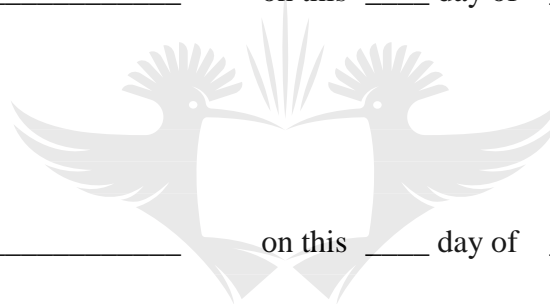
DEDICATION

I dedicate this work to the Almighty God for this far He has brought me and the far He is taking me in my career as woman in science.

ACKNOWLEDGEMENTS

ABSTRACT

In the recent past, there has been a great concern on the ever-increasing emergence of organic contaminants in the various environmental compartments, that pose great health concerns to humans and aquatic life. These organic pollutants have been ubiquitous in the environment for decades, however, they were not identifiable until the emergence of new and advanced analytical technologies. Therefore, the main objective of this study was to develop robust and efficient analytical and modelling techniques, for the extraction and analysis of selected multi-class organic contaminants from wastewater samples. This is because their analytical determination is very challenging due to their occurrence in trace levels (ng L^{-1} to µg L^{-1}) in the environment. The analytical techniques comprise of optimization of both the sample preparation procedures and instrumental analysis for detection and quantification. Solid phase extraction (SPE), dispersive liquid-liquid microextraction (DLLME) and ultrasonic-assisted magnetic solid phase dispersive extraction (UA-MSPDE) were the selected sample preparation techniques used for the extraction and preconcentration of methylparaben, ethylparaben, propylparaben, ethoprofos, parathion methyl, azinphos methyl and chlorpyrifos in water samples. This was followed by instrumental analysis for their detection and quantification using liquid chromatography tandem mass spectrometry (LC-MS/MS).

The developed analytical techniques were applied in real environmental samples obtained from different water treatment stages of a local wastewater treatment plant in Gauteng province, South Africa. Experimental factors that had an influence on the analytical response in terms on highest percentage recoveries were optimized using both univariate (one factor a time) and multivariate approach for all the experiments in this study. Multivariate optimization was accomplished using Statistica and Minitab software. The performance characteristics of the LC-MS/MS facilitated the determination of these organic contaminants at trace levels. Multiple reaction monitoring mode (MRM) was used for specific and sensitive targeted analysis, where the quadrupole analyzers were set at multiple ion frequencies for the specific analytes under investigation together with their product fragment ions. MRM is ideally suitable for trace level analysis of complex mixtures.

Oasis HLB cartridges were found to be suitable for extraction of parabens giving satisfactory results. Vortex assisted dispersive liquid-liquid microextraction (VA-DLLME) was used for the extraction and enrichment of organophosphorus pesticides in wastewater samples. Selection of the appropriate organic solvent (extractant and disperser solvents) used for this method was of utmost importance and was performed using univariate optimization.

The results revealed chloroform to be the most suitable extractant solvent while acetone was the optimum disperser solvent. This was followed by the chemometric optimization of the independent variables that significantly affect the outcome of the analytical response. The organophosphorus compounds that were extracted in wastewater samples using this technique with satisfactory results were ethoprofos, parathion methyl and azinphos methyl.

Also, a novel method was developed for the extraction and preconcentration of multi-class organic compounds (parabens and organophosphorus pesticides) using synthesized pristine carbon nanodots (CNDs) applied as SPE adsorbent. A comparison between the synthesized CNDs and commercial based SPE sorbent was analyzed. Two-level factorial design and response surface methodology based on central composite design were used for multivariate optimization of the experimental variables. Furthermore, the CNDs were also functionalized with magnetite. The magnetic CNDs were applied for the development of magnetic solid phase dispersive extraction method with ultrasonic dispersion for the simultaneous extraction of chlorpyrifos and triclosan in environmental water samples. This method offered a very rapid and simple extraction and preconcentration of these organic contaminants with satisfactory results.

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LIST OF ABBREVIATIONS

AA	Acetic acid
ACN	Acetonitrile
ANOVA	Analysis of Variance
API	Atmospheric pressure ionization
CCD	Central composite design
CE	Collision energy
CID	Collision Induced Dissociation
CND	Carbon nanodots
CNT	Carbon nanotubes
CNF	Carbon nanofibers
CPF	Chlorpyrifos
C18	Octadecylsilane
DLLME	Dispersive liquid-liquid microextraction
DSPME	Dispersive solid phase microextraction
DV	Disperser volume
ESI	Electrospray ionization
ESI-MS	Electrospray ionization-mass spectrometry
EthPB	Ethylparaben
ET	Extraction time
EV	Elution Volume
EU	European Union
FA	Formic acid
FTIR	Fourier transform infra-red
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometry
HLB	Hydrophilic Lipophilic Balance
HPLC	High performance liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LDR	Linear dynamic range
LOD	Limit of detection
LOQ	Limit of quantification
MA	Mass of adsorbent

ME	Matrix effect
MeOH	Methanol
MePB	Methylparaben
Min	minute
mL	millilitre
MNP	Magnetic nanoparticles
MRM	Multiple reaction mode
MS/MS	Tandem mass spectrometry
MWCNT	Multiwalled carbon nanotubes
m/z	mass-to-charge ratio
OPPs	Organ phosphorous pesticides
PCPs	Personal care products
ProPB	Propylparaben
QqQ	Tripe quadrupole
R	Correlation coefficient
RSD	Relative standard deviation
RSM	Response surface methodology
SEM	Scanning electron microscope
SPE	Solid phase extraction
SPME	Solid phase microextraction
SV	Sample volume
TEM	Transmission electron microscope
TCS	Triclosan
TIC	Total ion chromatogram
UHP	Ultra high pure water
UV	Ultraviolet
$\mu g\ L^{-1}$	microgram per litre
UA-MSPDE	Ultrasonic assisted magnetic solid phase dispersive extraction
UHPLC	Ultra high perfomance liquid chromatography mass spectrometry
VA-DLLME	Vortex asssited dispersive liquid-liquid microextraction
VSM	Vibrating sample magnetometer
WWTP	Wastewater treatment plant
XRD	X-ray diffraction

CONFERENCE AND SYMPOSIUM PRESENTATIONS

V. A. Muckoya, P. N. Nomngongo, J. C. Ngila. Preconcentration of organophosphorus pesticides in wastewater using dispersive liquid-liquid microextraction prior to their determination using liquid chromatograph with mass spectrometric detection. **Africa Food Safety Workshop 2018, Pretoria South Africa, 4-8th June 2018. Poster presentation**

V. A. Muckoya, P. N. Nomngongo, J. C. Ngila. Optimization of solid phase extraction method for determination of parabens in wastewater using factorial design and ultra-high-performance liquid chromatography triple quadrupole mass spectrometry, **5th International Conference on Pure and Applied Chemistry (ICPAC), 2-6th July 2018, Mauritius. Poster presentation and conference proceedings**

V. A. Muckoya, P. N. Nomngongo, J. C. Ngila. Factorial design optimization of solid phase extraction for preconcentration of personal care products in wastewater using UHPLC-MS/MS, **Analitika Conference 2018, 22-26 July 2018, Mookgophong, Limpopo. Oral presentation**

V. A. Muckoya, P. N. Nomngongo, J. C. Ngila Analysis of Multi-class Organic pollutants in wastewater using LC-MS/MS. **AOAC INTERNATIONAL Sub-Sahara Africa Inaugural meeting, Improving the Quality of Testing to assure Food Safety, Public Health and Trade, Pretoria South Africa, 5-7th November 2018. Young scientist oral presentation**

V. A. Muckoya, P. N. Nomngongo, J. C. Ngila. Ultrasonic Assisted Magnetic Solid Phase Dispersive Extraction for Determination of Chlorpyrifos and Triclosan in Wastewater Samples Prior to Liquid Chromatography-Tandem Mass Spectrometric. **50th General Assembly & 47th IUPAC World Chemistry Congress 5 – 12th July 2019 - Paris, France. Poster presentation.**

LIST OF PUBLICATIONS

- ❖ **Vallerie A. Muckoya,** Philiswa N. Nomngongo and J. Catherine Ngila, Factorial Design Optimisation of Solid Phase Extraction for Preconcentration of Parabens in Wastewater Using Ultra-High-Performance Liquid Chromatography Triple Quadrupole Mass Spectrometry, Current Analytical Chemistry (2018) 14: 1-10.

- ❖ **Vallerie A. Muckoya**, Philiswa N. Nomngongo and J. Catherine Ngila. Preconcentration of Organophosphorus Pesticides in Wastewater Samples using Vortex Assisted Dispersive Liquid-Liquid Microextraction with Liquid Chromatography-Mass Spectrometry, International Journal of Science and Technology. Accepted.

- ❖ **Vallerie A. Muckoya**, Azeez. O. Idris, Philiswa N. Nomngongo, J. Catherine Ngila 2019. Synthesized Carbon Nanodots for Simultaneous Extraction of Personal Care Products and Organophosphorus Pesticides in Wastewater Samples using UHPLC-MS/MS. Analytical Bioanalytical Chemistry, Analytical and Bioanalytical Chemistry 411 (**23**):6173-6187.

- ❖ **Vallerie A. Muckoya,** Philiswa N. Nomngongo and J. Catherine Ngila Ultrasonic Assisted Magnetic Solid Phase Dispersive Extraction for Preconcentration of Chlorpyrifos and Triclosan in Wastewater Samples Prior to Liquid Chromatography Tandem Mass Spectrometric Detection. Chromatographia (Under review).

CHAPTER 1:
INTRODUCTION

1.1 BACKGROUND

The occurrence of emerging or newly identified contaminants in our water bodies is still of great concern for the health and safety of the consuming public, ecosystems, and economies. The increase of contamination with thousands of organic pollutants in fresh waters systems worldwide is among the key environmental challenges facing the world [1]. A report by the UN-Water has indicated that nearly two-thirds of freshwater species are considered endangered [2]. Polluted water most often than not has led to the waterborne disease outbreaks with long term acute and long-term health effects [3, 4]. This is because the chemicals and toxins affect humans directly or bioaccumulate in food either from the sea or agricultural land that is consumed by humans causing developmental and or neurological damage [5, 6].

A wide array of organic pollutants are released into the environment as a result of human activities, with a small percentage due to natural activities such as volcanic eruptions, but the primary source of anthropogenic water pollution is mostly from poorly treated or untreated municipal sewage, wastewater treatment plants, discharge from individual septic systems, wastes from livestock agriculture, industrial wastes, drainage from mines, spilt petrochemical wastes among other sources [7-9]. These wastes contain complex mixtures of pollutants which can be categorized as inorganic, organic and biological in nature. The inorganic pollutants comprise mainly of heavy metals and ionic pollutants such as sulphates, nitrates, phosphates, fluorides chlorides and oxalates [10]. The organic components include pesticides, aromatic hydrocarbons, phenols, polychlorinated biphenyls, detergents, oils, and grease and others [11]. Biological pollutants include harmful microbial contaminants like bacteria, fungi, algae, plankton, amoeba, viruses etc. All these pollutants co-exist either as a colloid, suspension or in solvated form [12]. The route of entry of organic pollutants into the water bodies is also of great concern. This is because once the pollutants have been introduced into the receiving water bodies such as lake and rivers or groundwater via effluent discharge, or on land surfaces via disposal of either treated or untreated sludge deposits, they are transported back in the water cycle [12]. The main challenge of the frequent occurrence of these recalcitrant organic compounds is their gradual accumulation in the different environmental compartments, that can potentially result in detrimental negative effect to human health, aquatic ecosystems as well as wildlife [13, 14].

1.2 CLASSES OF ORGANIC CONTAMINANTS

Organic contaminants are quite diverse, numerous, ubiquitous and are thus classified that define their purpose, application, occurrence amongst other characteristics. As aforementioned, they are substances that are already known to cause adverse effects to human life and aquatic ecosystems, when exposed to the different environmental compartment to certain levels and concentration over stipulated time periods [15, 16]. Organic contaminants that were previously unknown and consequently unregulated have recently been reported. This is attributed to the increase in advanced analytical technologies with the capabilities of detecting known and unknown compounds [11]. Examples of these organic contaminants include pharmaceuticals, personal care products, pesticides, surfactants, disinfection-by-products, polyaromatic hydrocarbons and detergents [4, 11]. Due to the myriad organic contaminants present in the environment, of great interest in this review are the personal care products, pesticides and polyaromatic hydrocarbons.

1.2.1 Personal care products

The number of organic chemicals that comprise personal care products (PCPs) are in thousands. These products are used daily and in large quantities by large communities of the world including South Africa where the current study was done. PCPs are composed of heterogeneous group of compounds included in items such as shampoo, soaps, lotions fragrances and cosmetic products, dental care products among others [17]. Unlike pharmaceuticals which are intended for intended for internal use, PCPs are dermally applied and only enter the wastewater mostly through wash off the human body, improper disposal in toilets, sinks or trash as they go down the drain. They may also be absorbed into the body and released through urine or in other cases excreta [18]. Due to external application, PCPs are not subjected to metabolic alterations, hence they are released into the environment unaltered via municipal WWTPs. Despite these compounds being environmentally persistent, with a potential risk of bioaccumulation, they have not received much attention, unlike pharmaceuticals which have been studied extensively [19, 20]. The major classes of PCPs include UV filters, preservatives, disinfectants, antimicrobials fragrances, insect repellants, plasticizers (e.g. phthalates) among others [21]. Among the PCPs classes mentioned, the focus in this review will be on parabens as a type of preservatives and triclosan, an antimicrobial that is widely applied in PCPs.

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1.2.1.1 Parabens

The most widely used family of preservatives in PCPs are the p-hydroxybenzoic acid esters (parabens) [4]. They include the parent compounds; methylparaben, ethylparaben, propylparaben and butylparaben, and the derivatives; isobutyl, isopropyl, phenyl- benzyl and pentylparabens. Parabens have got numerous advantages that have rendered them the most preferred preservatives in PCPs. They include; chemical stability, broad-spectrum activity, inertness, adequate water solubility, low systemic toxicity, low production cost, among others [4, 22, 23]. Despite the advantages that accrue with the use of parabens in PCPCs, there are diverse shortcomings to their usage. For instance, the benzylparaben is reportedly the most acutely toxic derivative, as shown in a study conducted on aquatic organisms, of the chronic effects of parabens [24, 25]. These in-vivo studies also demonstrated potential effects due to continued exposure to low levels of parabens. This is as attributed by the endocrine-disrupting with oestrogenic and androgenic-like properties that these compounds exhibit [26, 27]. The oestrogenic activity increases with an increase in alkyl chain length (methyl to n-butylparaben) [28, 29] or with alkyl chain branching (n-butylparaben to isobutyl paraben) [30]. The widespread usage and production of these preservatives have increased water pollution, as they enter the environment mainly through incomplete removal from WWTPs as well as run-off from non-point sources [4, 31]. Moreover, recent studies have reported parabens in the air and dust [32] as well as biota [33] which have additionally led to increased exposures to these endocrine disrupting compounds. The structures of the parabens investigated in this study are shown in **Figure 1.1.**
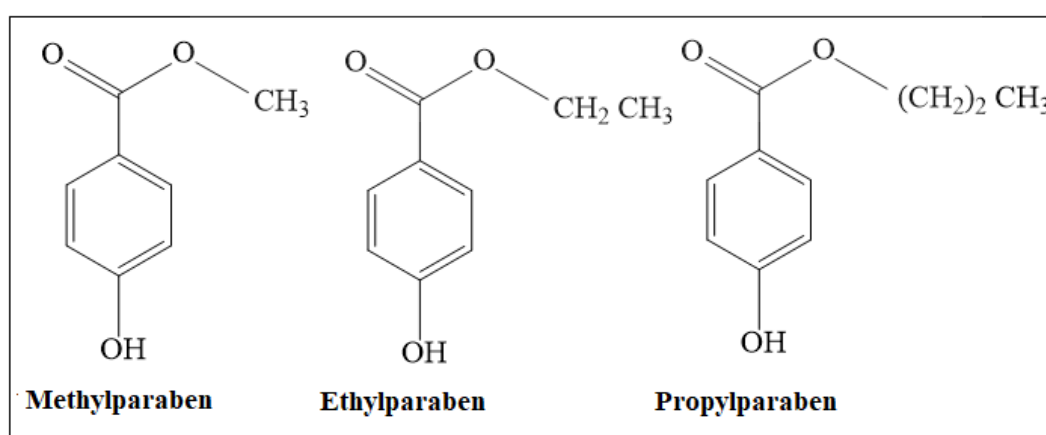
Figure 1.1: Chemical structures of parabens

1.2.1.2 Triclosan

Triclosan is an antimicrobial agent used in a wide variety of consumer products such as detergents, soaps, toothpaste and lotions, among others with a concentrations ranging from 0.1-0.3% (w/w) product weight [34, 35]. Due to its halogenated biphenyl ether structure, concerns have been raised over triclosan potentially as an endocrine disruptor, specifically disrupting the thyroid hormone homeostasis [36]. The widespread application of triclosan has led its release into the terrestrial and aquatic environments via WWPTs, and other water sources affecting the ecosystems and human health [37]. Chlorination or methylation of triclosan can result in the formation of persistent and toxic compound such as methyl-triclosan, biphenyl ethers and chlorinated phenols [38]. For instance, 2,4,6- trichlorophenol (2,4,6-TCP), a known endocrine disruptor is reported to cause birth defects, cancer and development disorders in offspring. Other reports have also revealed that methyl-triclosan exhibits more lipophilic properties than its parent compound and can potentially bioaccumulate in wildlife and humans [39].In view of the above, it is therefore critical to develop sensitive and robust methods for effective identification and quantitation of triclosan in water systems. The chemical structure of triclosan is given in **Figure 1.2.**
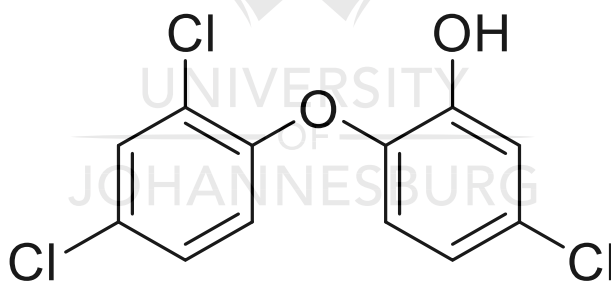
Figure 1.2: Chemical structure of triclosan

1.2.2 Pesticides

Pesticides comprise a collective group of organic chemical compounds used for various purposes such as fungicides, herbicides, insecticides, rodenticides among other uses. They are extensively used worldwide in agriculture for enhanced food production, by preventing the infestation of pests on crops, the growth of harmful insects, invasive plants, thereby averting hazardous diseases in crops and animals [40, 41]. The use of pesticides for non-agricultural

purposes is also on the increase. This can be observed by the application of pesticides in domestic purposes for pest control, industrial usage, maintenance of recreational facilities e.g. (golf courses, parks, sports grounds etc.) and care of pets [40, 42]. With the increase in both agricultural and nonagricultural applications, it is evident that there will be the continuous release of pesticides residues into the environment. Despite their several benefits, pesticides are among the most notorious environmental organic pollutants globally due to their mobility, toxicity, environmental persistence, bioaccumulation and long-term effect on humans and aquatic life [43, 44]. Some of the effects to human health are enhancements of cancer development, genetic mutations, diseases that affect the liver or central nervous system, among other effects [45]. In general, pesticides can undergo several chemical or biological transformation and be transported to other compartments in the environment, exerting toxic effects outside the area applied, on non-targeted species [46]. Surface run-off from agriculturally related use has been the most predominant source of entry of pesticide contamination into the environment, while WWTP represents one of the main sources of pesticide contamination in urban areas mostly attributed to non-agricultural uses [47]. Evidently, there is an uncontrolled discharge of these pesticides residues into in the environment, at both high and trace levels (ng L^{-1} to µg L^{-1}), which results in subsequent accumulation in different environmental compartments with potentially detrimental effects on human health and aquatic life [48, 49]. The ubiquitous presence of these pesticide residues in the environment compromises natural water resources meant for human consumption, for instance, groundwater as well as water used for aquaculture activities [46]. Pesticides are quite broad and can be categorized into four major groups namely, organochlorines, organophosphorus, carbamates and pyrethroids [50]. For our study purpose, we focused on organophosphorus pesticides.

1.2.2.1 Organophosphorus pesticides

Organophosphorus pesticides (OPPs) are esters, amides or thiol derivatives of phosphonic, phosphoric acid, phosphinic or thiophosphoric acid. They were introduced to replace organochlorine pesticides which were highly persistent and bioaccumulate in ecosystems and subsequently banned or restricted [51]. They are applied extensively in agriculture and veterinary medicine as insecticides, parasites respectively [52] and in the industry as flame retardants, solvent and plasticizers [53]. Even though OPPs have relatively low persistent in the environment, they are readily soluble in water. In addition, their extensive usage has

resulted in their accumulation and subsequent pollution in the environment, as evidenced by the detection of OPPs residues in different water matrices [54]. This possesses great health concern to humans. Chronic exposure to OPPs can be via inhalation, ingestion and/or skin inhalation. This can consequently result in adverse effects such as cancer, neurodegenerative disorders including Parkinson, Alzheimer, autism among other [55]. Furthermore, OPPS are also known to be strong inhibitors of cholinesterase that function as neurotransmitters. This inhibition can result in the accumulation of acetylcholine at the neuron/muscle synapses, leading to dysfunction of autonomic and behavioural systems which can result in respiratory paralysis and/or fatalities [56]. Therefore, there is a dire need for the continuous monitoring of these compounds in the environment to mitigate the risk of exposure of these compounds to humans as well as aquatic life.

OPPs can be classified according to their structure, comprising of central phosphorus atom that is either doubly bonded to an oxygen atom P=O (phosphoric bond), or sulphur atom P=S (thiophosphoric bond) also referred to as oxon and thion group respectively [57]. Under certain environmental conditions (oxygen and light), the OPPs can undergo oxidation reactions where the sulphur atom in the thion group is replaced by oxygen, which exponentially increases the toxicity of these compounds. This is primarily because the OPPs with oxon group are strong inhibitors of the enzyme acetylcholinesterase (AcHE) resulting in major neurotoxic effects [58]. The OPPs selected for the purpose of this study were azinphos-methyl, ethoprofos, parathion-methyl and chlorpyrifos as shown in **Figure 1.3**.
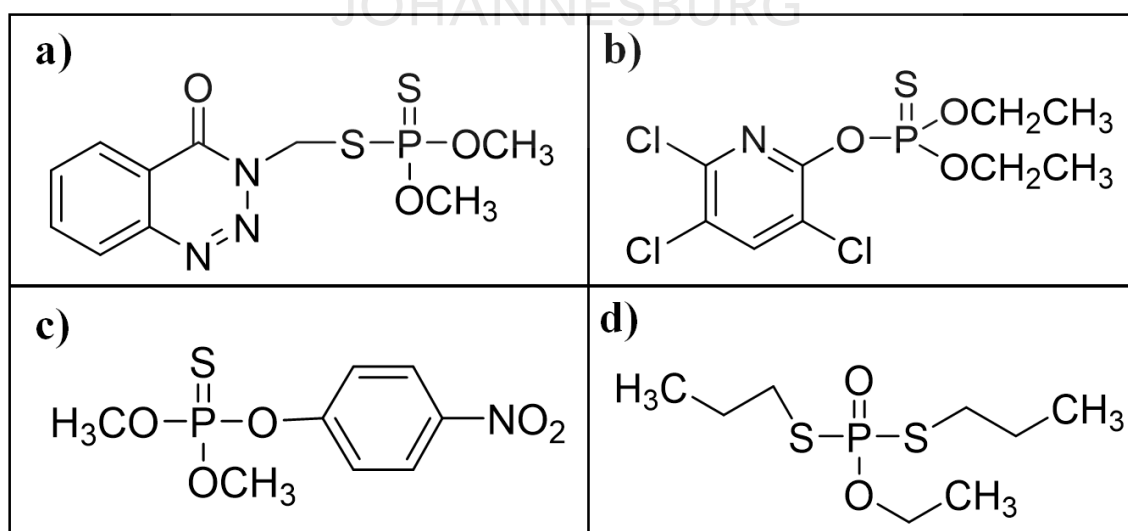
Figure 1.3: Chemical structures organophosphorus pesticides; a) azinphos-methyl, b) Chlorpyrifos, c) parathion-methyl, d) ethoprofos

1.3 PROBLEM STATEMENT

Gauteng province operates 56 wastewater treatment plants (WWTPs) which are either small, medium, large or macro-sized [59]. These plants receive water that is heavily polluted with organic contaminants from different sources and of various kinds such as PAHs, polychlorinated biphenyls (PCBs), pesticides, pharmaceuticals, PCPs, DBPs, persistent organic pollutants (POPs) and many others [7, 60]. The removal efficiency of organic pollutants in WWTPs largely depends on the technology implemented in the WWTPs. Despite the advanced technologies put in place, the WWTPs still experience challenges in meeting the standards for the disposal of the quality of effluent discharged into the receiving water bodies. This is because the secondary conventional processes (trickling filters and activated sludge) that constitute the most intensely used processes, were not precisely designed to remove the numerous emerging organic contaminants. It, therefore, leads to the partial removal of pollutants from the WWTPs, and subsequent introduction into the receiving water bodies (lakes, rivers and coastal water) [61]. This then becomes a source of the deteriorating water quality, as the waters are released to the environment poorly treated [62]. Furthermore, precautionary and monitoring actions are were not well established, in some cases.

The processes involved in treating wastewater in most of the WWTPs, include sedimentation, coagulation, filtration, disinfection and advanced oxidation process [63]. The disinfection process which mostly employs chlorination, in the tertiary treatment stage, has been reported to result in the formation of secondary contamination [64, 65]. The organic contaminants that accrue due to this process include disinfection-by-products (DBPs), such as the chlorinated methanes and acetic acids. Advanced oxidation processes such as ultraviolet combined with hydrogen peroxide treatment (UV/H_2O_2) have also been reported to enhance the formation of DBPs after post chlorination [66, 67]. The underperformance of the WWTPs to completely remove these organic contaminants is expected to rise if urgent measures are not put in place to provide advanced monitoring, identification, and rapid quantification procedures. It is therefore imperative to study and investigate the occurrence and concentrations of these selected organic contaminants in local WWTPs.

1.4 JUSTIFICATION

Numerous studies have been reported on the exponential increase of emerging organic pollutants in the aquatic environment [68]. These compound possess diverse physicochemical properties such as polarity, volatility, thermal stability, molecular mass, chemical structure

among others, that renders them very difficult to analyze and detect using conventional methods [69]. These organic compounds also do not occur singly, but rather as complex mixtures, resulting in synergistic effects. Most of these organic contaminants can exist in water at trace levels, thereby increasing the complexity associated in detecting them in low concentrations [70]. In addition, wastewater is a very complex matrix composed of high content of natural organic matter (NOM). It, therefore, requires sensitive and selective sample extraction methods to accurately isolate the target compounds prior to their determination. Analytical methodologies that can analyze more than one class of compounds are necessary for the simultaneous determination of these organic contaminants in water. Some of the advanced analytical methodologies include chromatographic methods hyphenated to mass spectrometry techniques such as liquid chromatography-tandem mass spectrometry (LC-MS and gas chromatography-mass spectrometry (GC-MS) techniques. GC-MS is adopted mainly for analysis for thermally labile, volatile organic compounds whereas LC-MS is employed for the more polar, less volatile, high molecular weight organic compounds [71]. The complimentary use of these instrumentation techniques provides a holistic and comprehensive overview of the presence of the organic contaminants in wastewater [72]. In addition, advanced multivariate optimization of sample preparation methodologies provides for faster and more efficient sample extraction procedures.

1.5 HYPOTHESIS

There exist significant levels of organic pollutants in WWTPs effluents that have the capacity to highly pollute the ecosystem and other drinking water sources. The extraction and preconcentration of these organic contaminants can be accomplished using solid phase extraction comprising of different adsorbents and dispersive liquid-liquid extraction. In addition, the use of simulated wastewater helps to mimic real environmental conditions in the method development prior to real sample application. Furthermore, the use of chemometric based approaches for experimental optimization could provide for a more enhanced and efficient extraction and analysis, in monitoring the levels of these organic contaminants in WWTPs. Due to the existence of these contaminants in trace levels, the use of LC-MS/MS offers the most appropriate technique due to its excellent characteristics in robustness, high selectivity and sensitivity. Consequently, the need to ensure accurate identification and quantification of the analytes in trace levels in study is crucial in order to avoid false positive

or false negatives. Therefore, the application of a strict criteria is vital for quantification and confirmation of the compounds in complex matrix samples.

1.6 AIMS AND OBJECTIVES

1.6.1 Aim of the study

The main aim of the study was to develop robust and efficient sample preparation techniques for the extraction of selected classes of organic contaminants from wastewater samples collected at different treatment stages (Primary, secondary and tertiary treatment stages) of a WWTP prior to chromatographic-mass spectrometry detection.

1.6.2 Specific Objectives

1. Develop solid phase extraction protocols for separation and preconcentration of parabens in wastewater samples using UHPLC-MS/MS.

- Evaluation of different SPE cartridges for simultaneous preconcentration of organic compounds in water matrices.
- Multivariate optimization and validation of the analytical parameters, such as pH, sample volume and elution volume.
- Application of developed method in spiked and real wastewater samples, both influent and effluent

2. Develop a Vortex assisted, dispersive liquid-liquid microextraction (VA-DLLME) for the determination of organophosphorus pesticides in wastewater.

- Univariate selection of extraction and dispersion solvents.
- Application of screening and response surface design for optimization of VA-DLLME parameters (sample pH, extractant volume, disperser volume, ionic strength).
- Validation using spiked raw influent and treated effluent water samples.
- Application of VA-DLLME in preconcentration of organophosphorus in real wastewater samples prior to UHPLC-MS/MS.

3. Preparation of carbon nanodots for preconcentration and extraction of multi-class organic contaminants in wastewater.

- Green synthesis of carbon nanodots (CNDs) for extraction on parabens and organophosphorus pesticides in water samples.
- Characterization of the prepared CNDs.

- Optimize for optimum extraction parameters (sample volume, pH, sorbent mass, elution concentration) using multivariate techniques.
- Use UHPLC-MS/MS for the determination of parabens and organophosphorus pesticides co-extracted from wastewater using the CNDs.

4. Application of magnetic CND (m-CNDs) for extraction of triclosan and chlorpyrifos in water samples and determination using ultra high-performance liquid chromatography-tandem mass spectrometry

- Preparation of magnetic carbon nanodots.
- Multivariate optimization of experimental variables for the preconcentration of triclosan and chlorpyrifos in spiked water samples.
- Application of m-CNDs, in the extraction on triclosan and chlorpyrifos in real environmental water samples.

1.7 THESIS OUTLINE

A brief description of the contents of this thesis is highlighted below.

Chapter 1: This introductory chapter gives a general background of organic contamination in the environment, via the introduction of partially treated wastewater effluent from WWTP into the environment. This is followed by a problem statement, justification, hypothesis as well as the aim and objectives of this research.

Chapter 2: A detailed literature review is presented on the different classes of organic contaminants, with major focus on the selected class of compounds in this study. This was followed by the sample preparation techniques applied in extraction and separation of organic contaminants as well as the analytical techniques used for detection and quantification. Emphasis was made on the techniques used in this research. The use of design of experiments (DOE) for the optimization of sample preparation procedures was also reviewed.

Chapter 3: This chapter presents general experimental methodology on the sample preparation techniques as well as the instrumentation techniques used in this study. Preparation and characterization of the synthesized carbon nanodots were also enumerated.

Chapter 4, paper 1 (Current Analytical Chemistry, 2018,*14,*1-10): This Chapter describes the Factorial Design Optimization of Solid Phase Extraction for Preconcentration of Parabens in Wastewater Using Ultra-High-Performance Liquid Chromatography Triple Quadrupole Mass Spectrometry.

Chapter 5: This chapter discusses the preconcentration of organophosphorus pesticides from wastewater samples, using dispersive liquid-liquid microextraction technique coupled with detection using UHPLC-MS/MS.

Chapter 6: This Chapter discusses the synthesis, preparation and application of carbon nanodots (CNDs) as adsorbents for solid phase extraction of multi-class organic compound in water samples, using UHPLC-MS/MS. Organic contaminants occur as mixtures in the environment, hence a method suitable for multi-class determination of organic compounds is crucial. This chapter also entails the comparison of the synthesized sorbent material with the commercially available SPE sorbents in the separation and preconcentration of the parabens and organophosphorus compounds in water.

Chapter 7: This chapter describes the application of magnetic carbon nanodots (m-CNDs) as adsorbent for ultrasonic assisted magnetic solid phase dispersive extraction and preconcentration of triclosan and chlorpyrifos in wastewater samples.

Chapter 8: This chapter gives general conclusions of the developed techniques and their application in wastewater analysis well as the future recommendations.

1.8 REFERENCES

[1] R. Loos, R. Carvalho, D.C. Antonio, S. Comero, G. Locoro, S. Tavazzi, B. Paracchini, M. Ghiani, T. Lettieri, L. Blaha, EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents, Water research 47 (2013) 6475-6487.

[2] B.L.D.U. Francesc H.S. (University of Valencia), Javier, M.Q.U.-I. M.S. (IWMI), Economiv valuation of waste water UNEP, UNEP, 2015, pp. 72.

[3] I. Xagoraraki, D. Kuo, Water pollution: Emerging contaminants associated with drinking water, International Encyclopedia of Public Health 6 (2008) 539-550.

[4] D. Błędzka, J. Gromadzińska, W. Wąsowicz, Parabens. From environmental studies to human health, Environment international 67 (2014) 27-42.

[5] S.D. Richardson, M.l.J. Plewa, E.D. Wagner, R. Schoeny, D.M. DeMarini, Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research, Mutation Research/Reviews in Mutation Research 636 (2007) 178-242.

[6] P.D. Darbre, P.W. Harvey, Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks, Journal of Applied Toxicology 28 (2008) 561-578.

[7] A. Pal, K.Y.-H. Gin, A.Y.-C. Lin, M. Reinhard, Impacts of emerging organic contaminants on freshwater resources: Review of recent occurrences, sources, fate and effects, Science of the Total Environment 408 (2010) 6062-6069.

[8] C.G. Daughton, Pharmaceuticals in the environment: sources and their management, Analysis, Removal, Effects and Risk of Pharmaceuticals in the Water Cycle—Occurrence and Transformation in the Environment. Elsevier, New York, NY, USA (2013) 37-69.

[9] M.L. Hladik, M.J. Focazio, M. Engle, Discharges of produced waters from oil and gas extraction via wastewater treatment plants are sources of disinfection by-products to receiving streams, Science of the Total Environment 466 (2014) 1085-1093.

[10] G. Bharath, E. Alhseinat, N. Ponpandian, M.A. Khan, M.R. Siddiqui, F. Ahmed, E.H. Alsharaeh, Development of adsorption and electrosorption techniques for removal of organic and inorganic pollutants from wastewater using novel magnetite/porous graphene-based nanocomposites, Separation and Purification Technology 188 (2017) 206-218.

[11] S.E. Madhumitha R., Katharine, B.W. M., Contaminants of Emerging Concern in Water, (2013).

[12] V.K. Gupta, I. Ali, T.A. Saleh, A. Nayak, S. Agarwal, Chemical treatment technologies for waste-water recycling—an overview, Rsc Advances 2 (2012) 6380-6388.

[13] C.G. Daughton, T.A. Ternes, Pharmaceuticals and personal care products in the environment: agents of subtle change?, Environmental health perspectives 107 (1999) 907.

[14] P.K. Jjemba, Excretion and ecotoxicity of pharmaceutical and personal care products in the environment, Ecotoxicology and environmental safety 63 (2006) 113-130.

[15] M. Farre, L. Kantiani, M. Petrovic, S. Perez, D. Barcelo, Achievements and future trends in the analysis of emerging organic contaminants in environmental samples by mass spectrometry and bioanalytical techniques, J Chromatogr A 1259 (2012) 86-99.

[16] G. Vandermeersch, H.M. Lourenço, D. Alvarez-Muñoz, S. Cunha, J. Diogène, G. Cano-Sancho, J.J. Sloth, C. Kwadijk, D. Barcelo, W. Allegaert, Environmental contaminants of emerging concern in seafood–European database on contaminant levels, Environmental research 143 (2015) 29-45.

[17] B. Lubliner, M.B. Redding, D. Ragsdale, Pharmaceuticals and personal care products in municipal wastewater and their removal by nutrient treatment technologies, Washington State Department of Ecology2010.

[18] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, Analytical methods for personal-care products in environmental waters, TrAC Trends in Analytical Chemistry 30 (2011) 749-760.

[19] R. Celano, A.L. Piccinelli, L. Campone, L. Rastrelli, Ultra-preconcentration and determination of selected pharmaceutical and personal care products in different water matrices by solid-phase extraction combined with dispersive liquid–liquid microextraction prior to ultra high pressure liquid chromatography tandem mass spectrometry analysis, Journal of Chromatography A 1355 (2014) 26-35.

[20] T.A. Ternes, A. Joss, H. Siegrist, Peer reviewed: scrutinizing pharmaceuticals and personal care products in wastewater treatment, ACS Publications, 2004.

[21] J.M. Brausch, G.M. Rand, A review of personal care products in the aquatic environment: environmental concentrations and toxicity, Chemosphere 82 (2011) 1518-1532.

[22] P. Guadarrama, S. Fomine, R. Salcedo, A. Martínez, Construction of simplified models to simulate estrogenic disruptions by esters of 4-hydroxy benzoic acid (parabens), Biophysical chemistry 137 (2008) 1-6.

[23] M. Terasaki, M. Makino, Determination of chlorinated by-products of parabens in swimming pool water, International Journal of Environmental and Analytical Chemistry 88 (2008) 911-922.

[24] M. Terasaki, M. Makino, N. Tatarazako, Acute toxicity of parabens and their chlorinated by-products with Daphnia magna and Vibrio fischeri bioassays, Journal of Applied Toxicology 29 (2009) 242-247.

[25] I. Bazin, A. Gadal, E. Touraud, B. Roig, Hydroxy benzoate preservatives (parabens) in the environment: data for environmental toxicity assessment, Xenobiotics in the urban water cycle, Springer2010, pp. 245-257.

[26] S. Terasaka, A. Inoue, M. Tanji, R. Kiyama, Expression profiling of estrogen-responsive genes in breast cancer cells treated with alkylphenols, chlorinated phenols, parabens, or bis-and benzoylphenols for evaluation of estrogenic activity, Toxicology letters 163 (2006) 130-141.

[27] J.J. Prusakiewicz, H.M. Harville, Y. Zhang, C. Ackermann, R.L. Voorman, Parabens inhibit human skin estrogen sulfotransferase activity: possible link to paraben estrogenic effects, Toxicology 232 (2007) 248-256.

[28] E.J. Routledge, J. Parker, J. Odum, J. Ashby, J.P. Sumpter, Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic, Toxicology and applied pharmacology 153 (1998) 12-19.

[29] J. Byford, L. Shaw, M. Drew, G. Pope, M. Sauer, P. Darbre, Oestrogenic activity of parabens in MCF7 human breast cancer cells, The Journal of steroid biochemistry and molecular biology 80 (2002) 49-60.

[30] P. Darbre, J. Byford, L. Shaw, R. Horton, G. Pope, M. Sauer, Oestrogenic activity of isobutylparaben in vitro and in vivo, Journal of Applied Toxicology: An International Journal 22 (2002) 219-226.

[31] Y. Yu, Q. Huang, Z. Wang, K. Zhang, C. Tang, J. Cui, J. Feng, X. Peng, Occurrence and behavior of pharmaceuticals, steroid hormones, and endocrine-disrupting personal care products in wastewater and the recipient river water of the Pearl River Delta, South China, Journal of Environmental Monitoring 13 (2011) 871-878.

[32] R.A. Rudel, D.E. Camann, J.D. Spengler, L.R. Korn, J.G. Brody, Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust, Environmental science & technology 37 (2003) 4543-4553.

[33] J.-W. Kim, B.R. Ramaswamy, K.-H. Chang, T. Isobe, S. Tanabe, Multiresidue analytical method for the determination of antimicrobials, preservatives, benzotriazole UV stabilizers, flame retardants and plasticizers in fish using ultra high performance liquid chromatography coupled with tandem mass spectrometry, Journal of Chromatography A 1218 (2011) 3511-3520.

[34] J.V. Rodricks, J.A. Swenberg, J.F. Borzelleca, R.R. Maronpot, A.M. Shipp, Triclosan: a critical review of the experimental data and development of margins of safety for consumer products, Critical reviews in toxicology 40 (2010) 422-484.

[35] H. Rothe, R. Fautz, E. Gerber, L. Neumann, K. Rettinger, W. Schuh, C. Gronewold, Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment, Toxicology Letters 205 (2011) 97-104.

[36] N. Veldhoen, R.C. Skirrow, H. Osachoff, H. Wigmore, D.J. Clapson, M.P. Gunderson, G. Van Aggelen, C.C. Helbing, The bactericidal agent triclosan modulates thyroid hormone-associated gene expression and disrupts postembryonic anuran development, Aquatic toxicology 80 (2006) 217-227.

[37] J.-L. Zhao, Q.-Q. Zhang, F. Chen, L. Wang, G.-G. Ying, Y.-S. Liu, B. Yang, L.-J. Zhou, S. Liu, H.-C. Su, Evaluation of triclosan and triclocarban at river basin scale using

monitoring and modeling tools: implications for controlling of urban domestic sewage discharge, Water research 47 (2013) 395-405.

[38] E.M. Fiss, K.L. Rule, P.J. Vikesland, Formation of chloroform and other chlorinated byproducts by chlorination of triclosan-containing antibacterial products, Environmental science & technology 41 (2007) 2387-2394.

[39] G.S. Dhillon, S. Kaur, R. Pulicharla, S.K. Brar, M. Cledón, M. Verma, R.Y. Surampalli, Triclosan: current status, occurrence, environmental risks and bioaccumulation potential, International journal of environmental research and public health 12 (2015) 5657-5684.

[40] A. Samsidar, S. Siddiquee, S.M. Shaarani, A review of extraction, analytical and advanced methods for determination of pesticides in environment and foodstuffs, Trends in Food Science & Technology (2017).

[41] N.I. Rousis, R. Bade, L. Bijlsma, E. Zuccato, J.V. Sancho, F. Hernandez, S. Castiglioni, Monitoring a large number of pesticides and transformation products in water samples from Spain and Italy, Environmental research 156 (2017) 31-38.

[42] F.P. Garcia, S.Y.C. Ascencio, J.C.G. Oyarzun, A.C. Hernandez, P.V. Alavarado, Pesticides: classification, uses and toxicity. Measures of exposure and genotoxic risks, Journal of Research in Environmental Science and Toxicology 1 (2012) 279-293.

[43] O. Ogbeide, I. Tongo, L. Ezemonye, Risk assessment of agricultural pesticides in water, sediment, and fish from Owan River, Edo State, Nigeria, Environmental monitoring and assessment 187 (2015) 654.

[44] A. Chopra, M.K. Sharma, S. Chamoli, Bioaccumulation of organochlorine pesticides in aquatic system—an overview, Environmental monitoring and assessment 173 (2011) 905-916.

[45] J.M. Dabrowski, J.M. Shadung, V. Wepener, Prioritizing agricultural pesticides used in South Africa based on their environmental mobility and potential human health effects, Environment international 62 (2014) 31-40.

[46] F.P. Carvalho, Pesticides, environment, and food safety, Food and Energy Security 6 (2017) 48-60.

[47] M. Köck-Schulmeyer, M. Villagrasa, M.L. de Alda, R. Céspedes-Sánchez, F. Ventura, D. Barceló, Occurrence and behavior of pesticides in wastewater treatment plants and their environmental impact, Science of the Total Environment 458 (2013) 466-476.

[48] T. Radović, S. Grujić, A. Petković, M. Dimkić, M. Laušević, Determination of pharmaceuticals and pesticides in river sediments and corresponding surface and

ground water in the Danube River and tributaries in Serbia, Environmental monitoring and assessment 187 (2015) 4092.

[49] C. Ribeiro, A.R. Ribeiro, M.E. Tiritan, Occurrence of persistent organic pollutants in sediments and biota from Portugal versus European incidence: a critical overview, Journal of Environmental Science and Health, Part B 51 (2016) 143-153.

[50] A.S. Chau, Analysis of Pesticides in Water: Volume I: Significance, Principles, Techniques, and Chemistry of Pesticides, CRC press2018.

[51] T. Galloway, R. Handy, Immunotoxicity of organophosphorous pesticides, Ecotoxicology 12 (2003) 345-363.

[52] V.M.R.d. Santos, C.L. Donnici, J.B.N.d. Costa, J.M.R. Caixeiro, Compostos organofosforados pentavalentes: histórico, métodos sintéticos de preparação e aplicações como inseticidas e agentes antitumorais, (2007).

[53] M.B. Abou-Donia, Organophosphorus ester-induced chronic neurotoxicity, Archives of Environmental Health: An International Journal 58 (2003) 484-497.

[54] Q. Zhou, H. Bai, G. Xie, J. Xiao, Trace determination of organophosphorus pesticides in environmental samples by temperature-controlled ionic liquid dispersive liquid-phase microextraction, Journal of Chromatography A 1188 (2008) 148-153.

[55] S. Mostafalou, M. Abdollahi, The link of organophosphorus pesticides with neurodegenerative and neurodevelopmental diseases based on evidence and mechanisms, Toxicology 409 (2018) 44-52.

[56] M. Jokanović, Medical treatment of acute poisoning with organophosphorus and carbamate pesticides, Toxicology Letters 190 (2009) 107-115.

[57] D. Sud, P. Kaur, Heterogeneous photocatalytic degradation of selected organophosphate pesticides: a review, Critical reviews in environmental science and technology 42 (2012) 2365-2407.

[58] T. Elersek, M. Filipic, Organophosphorous pesticides-mechanisms of their toxicity, Pesticides-the impacts of pesticides exposure, InTech2011.

[59] DWA, <WWTPs Chapter 5-Gauteng province-DWA.pdf>, 1-38.

[60] Bolong, A.F. Ismail, M.R. Salim, T. Matsuura, A review of the effects of emerging contaminants in wastewater and options for their removal, Desalination 239 (2009) 229-246.

[61] B. Petrie, R. Barden, B. Kasprzyk-Hordern, A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring, Water research 72 (2015) 3-27.

[62] <WRC policy brief on improving the quality if waste water treatment services>.

[63] C. Chen, X. Zhang, W. He, W. Lu, H. Han, Comparison of seven kinds of drinking water treatment processes to enhance organic material removal: A pilot test, Science of the Total Environment 382 (2007) 93-102.

[64] S.D. Richardson, Disinfection by-products and other emerging contaminants in drinking water, TrAC Trends in Analytical Chemistry 22 (2003) 666-684.

[65] S.K. Golfinopoulos, A.D. Nikolaou, Survey of disinfection by-products in drinking water in Athens, Greece, Desalination 176 (2005) 13-24.

[66] A.D. Dotson, V.S. Keen, D. Metz, K.G. Linden, UV/H2O2 treatment of drinking water increases post-chlorination DBP formation, Water research 44 (2010) 3703-3713.

[67] R. Chinn, T. Lee, S. Krasner, M. Dale, S.D. Richardson, J. Pressman, T. Speth, R. Miltner, J.E. Simmons, Solid-phase extraction of 35 DBPs with analysis by GC/ECD and GC/MS, Proceedings of the 2007 Water Quality Conference, 2007, pp. 1-20.

[68] D. Fatta-Kassinos, S. Meric, A. Nikolaou, Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research, Analytical and bioanalytical chemistry 399 (2011) 251-275.

[69] S. Huntscha, H.P. Singer, C.S. McArdell, C.E. Frank, J. Hollender, Multiresidue analysis of 88 polar organic micropollutants in ground, surface and wastewater using online mixed-bed multilayer solid-phase extraction coupled to high performance liquid chromatography–tandem mass spectrometry, Journal of Chromatography A 1268 (2012) 74-83.

[70] Y. Luo, W. Guo, H.H. Ngo, L.D. Nghiem, F.I. Hai, J. Zhang, S. Liang, X.C. Wang, A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment, Science of the Total Environment 473 (2014) 619-641.

[71] E. Pitarch, T. Portoles, J.M. Marin, M. Ibanez, F. Albarran, F. Hernandez, Analytical strategy based on the use of liquid chromatography and gas chromatography with triple-quadrupole and time-of-flight MS analyzers for investigating organic contaminants in wastewater, Analytical and Bioanalytical Chemistry 397 (2010) 2763-2776.

[72] M. Petrović, S. Gonzalez, D. Barceló, Analysis and removal of emerging contaminants in wastewater and drinking water, TrAC Trends in Analytical Chemistry 22 (2003) 685-696.

CHAPTER 2:

LITERATURE REVIEW ON SAMPLE PREPARATION METHODOLOGIES AND CHROMATOGRAPHIC MASS SPECTROMETRIC TECHNIQUES FOR DETERMINATION OF ORGANIC CONTAMINANTS IN WASTEWATER

PREAMBLE

This chapter outlines the review of the analytical procedures used in sample preparation and techniques used for the detection of these organic contaminants in wastewater. Emphasis was made on the extraction and detection techniques employed in this study. A synopsis of liquid chromatography and gas chromatography hyphenated with mass spectrometry is enumerated along with their principles of operation and application in environmental analysis. Finally, the application of chemometric techniques in the optimization of sample extraction procedures is also highlighted at the close of this chapter.

2.1 SAMPLE PREPARATION TECHNIQUES

Sample preparation in analytical chemistry remains an absolutely important procedure, despite being the most laborious and time-consuming step (~80% analysis time) in the entire analytical process [1]. This is because of the interrelated tasks that accompany the sample preparation procedures. They include pretreatment and preservation of sample, extraction of the analyte, extract "clean-up", derivatization and extract storage, among others [2].

Analysis of organic contaminants is often challenging due to the complexity, diversity and other interfering compounds that the sample matrices exhibit. These interfering compounds include humic and fulvic acids, natural organic matter, protein and lipids, among other compounds [3]. In addition, the analytes exist in trace amounts, making them difficult to analyze, if extraction, preconcentration and clean-up steps are not performed [4]. The organic contaminants as already mentioned, exist as complex mixtures from various classes of compounds exhibiting different physicochemical properties [3, 5]. To alleviate these setbacks, researchers need to employ adequate sample preparation methodologies or develop new ones, for efficient and reproducible isolation and separation of these organic contaminants from interfering compounds in sample matrices. In several occasions, these pollutants have been ubiquitous in the environment for decades, however, they were not identifiable until the emergence of new and advanced analytical technologies [3]. Presently, sample preparation techniques are geared toward environmental friendliness, simplicity,

miniaturization as well as low cost [6, 7]. In addition, certain aspects should be considered in determining the applicability of a chosen extraction method such as the capacity to remove interferences, recovery of analytes and ease of operation. Furthermore, sample preparation procedures are very crucial in improving instrument sensitivity, since co-extracted matrix components may compromise instrument performance [8].

Sample preparation methodologies include conventional and newly developed techniques such as liquid-liquid extraction, solid phase extraction, magnetic solid phase extraction, dispersive solid phase extraction, solid phase microextraction, dispersive liquid-liquid microextraction among others. The factors that advised the selection of sample preparation method in these research work, was selected based on the simplicity of operation, inexpensive, robustness, novelty and capability for multivariate optimization. Therefore, the choice of sample preparation methods in this research were solid phase extraction, magnetic solid phase extraction and dispersive liquid-liquid microextraction. The chosen methods have been discussed in detail whilst the other methods have been mentioned briefly.
[9, 10], rain and stormwater[11] , surface water [12] as well as soil matrices [13].

2.1.1 Dispersive Liquid-Liquid Microextraction (DLLME)

Myriad of analytical chemists have explored the dispersive liquid-liquid microextraction (DLLME) technique as observed by the numerous publications, since its introduction in 2006 by Assadi and co-workers [14]. This is because of the simplicity of the method's operation, which is based on the injection of a premixed solution of few microliters (μl) of an organic extractant solvent and few μl to millilitres (mL) of a disperse solvent rapidly into the aqueous sample forming a cloudy solution. The presence of the aqueous sample layer and organic layer leads to the establishment of a two-phase system, resulting in the dispersion of the extractant onto the aqueous sample-disperser mixed phase forming small droplets, resulting in analyte extraction. A state of equilibrium is achieved quickly between the extractant and the aqueous sample due to the large surface area between them, thereby rendering the extraction time very short [15]. Besides the ease of operation of this method, other advantages include low-cost, high enrichment factors, speed and low sample volumes. It is also referred to as a green technique due to the reduced consumption of hazardous chlorinated solvents. Furthermore, it is quite versatile as it can be coupled with a wide range of spectrophotometric and chromatographic instruments either directly or after solvent exchange [16].

The extractants and disperser solvent types and volumes are the most critical factors that affect DLLME efficiency. Types of extractant solvent used in classical DLLME include chlorinated solvents which were first employed in the development of this technique. They include chloroform, chlorobenzene, carbon tetrachloride, methylene chloride and tetrachloroethylene [17, 18]. Some of the characteristics of the choice of extractant solvent are that they are water immiscible, possess high extractability of the compounds of interest with good chromatographic compatibility [19]. The disperser solvents that are mostly employed in DLLME include methanol, acetone, acetonitrile and ethanol. They are selected based on their miscibility with the extractant solvent and the aqueous matric sample. The volumes of the extractant and disperser volume are among the key parameters that must be optimized during method development of DLLME. The preconcentration factor (PF) is affected by the extractant volume. The higher the extractant volume the lower the PF and vice-versa. The disperser volume is equally very critical for the extraction efficiency. Different disperser solvents affect the final volume of the sedimented phase of the extractant solvent. Higher disperser volumes result in poor extraction efficiency due to dilution effects [20]. At low disperser volumes, the cloudy solution is not properly formed resulting in low extraction efficiency [21].

Due to the toxicity of the extractant solvents and their limitations in extracting a wide array of analytes with various polarities several modifications were made to allow the use of less toxic and less polluting solvents [22]. This led to the application of less dense solvents such as the long-chained alcohols (1-undecanol, 1-dodecanol, 1-decanol, 1-hexanol, 1-octanol) [23-25]. These solvents are lighter than water and hence float at the top of the centrifuge tube after extraction. Other solvents that are employed in modified DLLME to replace the toxic chlorinated solvents include ionic liquids (IL) [22, 26] and supramolecular solvents (SUPRAS) [27, 28].

Besides the solvent types and volumes used in DLLME, other factors such as sample pH, salt concentration (ionic strength) and extraction time, have an influence on the extraction recovery of the organic compounds from the sample matrices.

The existence of the analytes in different forms is determined by the pH of the sample. This affects the extraction efficiency as the molecules will either be in nonionized or ionic form. If the analytes are in ionized form, they will have less affinity for the extractant solvent, leading to low extraction recoveries, hence pH must be adjusted to make the analytes nonionic. Ionizable compounds exist in nonionized form when the sample pH is less than pKa of the compound [29].

In DLLME, extraction time is referred to as the time between the injection of the premixed extractant and disperser solvents and the centrifugation step. As previously mentioned, the high surface area between the aqueous and the extraction solvent accelerates the extraction process (the mass transfer of the analytes to the extractant phase) and the equilibrium is reached within a short time [30].

The addition of salt is performed to enhance the ionic strength of the sample solution. This helps in the mass transfer of analytes from the aqueous phase to the extractant phase. Generally, an increase of ionic strength results in a decrease in analyte solubility in the sample solution, leading to an improved extraction efficiency due to the salting out effects. However, in some cases, the use of salt addition does not affect extraction efficiency [21, 31]. DLLME has been reported by many researchers in sample pretreatment in various fields of applications. More importantly in the environmental aspects, it has been successfully applied in the determination of the emerging organic contaminants in various environmental compartments. It has been applied often in analysis of pesticides in water samples. For instance, organophosphorus pesticides [32], triazine herbicides [18], carbamates [33], have been analyzed using DLLME. In addition, it has been applied in the determination of personal care products such as parabens [34], UV filters [35], as well as PAHs [36].

2.1.2 Solid Phase Extraction (SPE)

Since most hydrophilic analytes do not typically partition from the aqueous phase into the organic solvent in (liquid-liquid extraction) LLE, this results in poor extraction capabilities, and hence solid phase extraction (SPE) provides an alternative to the time and solvent consuming LLE [2]. SPE methodologies employ a packing of an appropriate bonded phase material (florisil, silica gel, alumina, C-18) in a cartridge, where analytes on an aqueous sample are adsorbed onto the sorbent material (stationary phase) by retention mechanisms. Thereafter, they are desorbed with an appropriately selected elution solvent that will interrupt the binding mechanism [37]. The eluate can thereafter be evaporated for solvent exchange with an appropriate solvent or to obtain higher preconcentration factors prior to instrumental analysis [38].

Over the past decades, SPE has evolved with numerous improvements making it a valuable tool in sample preparation procedures. This is attributed to its notable advantages such as various sorbents types with capabilities of extracting a wide array of analytes,

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flexibility, fast and less labour intensive, relatively low cost and high sample throughput with automation possibilities [38].

Due to the challenges that accrue with the wide array of organic compounds of different physicochemical properties, careful consideration of the choice of adsorbent is vital. This is because different packings have different modes of operation. The sorbent type is critical as it controls the capacity, selectivity and affinity of how the mechanism of extraction of the compounds take place. The basic mechanisms of retention are based on hydrophobic interactions, hydrophilic interactions and pi-pi interactions [39]. For instance, retention of polar phases is a result of dipolar interactions, hydrogen bonding and dispersion forces [40]. Compounds with acidic, basic or amphoteric character can be separated on ion-exchange adsorbents. Polar compounds are easily lost due to low adsorption affinity or there may be inefficient desorption of non-polar analytes as a result of high retention on hydrophobic adsorbents [41]. Hydrophilic adsorbents retain polar compounds, allowing non-polar components to pass through unretained.

SPE has been applied widely in the extraction of the myriad of compounds different fields including environmental analysis [42-45], industrial applications [46, 47], pharmaceutical and biological applications [48, 49], as well as food applications [50, 51]. The different types of commercially available adsorbents applied in the different fields of application are described below.

2.1.2.1 Commercial SPE sorbents

Many commercially prepacked reversed-phase solid phase extraction sorbents (RP-SPEs) are available for a wide range of analytes and applications. As aforementioned, the materials commonly used in classical RP-SPEs include alkyl-bonded silica (C2, C8, or C18), and co-polymer sorbents based on styrene-divinylbenzene such as cross-linked polystyrene divinylbenzene, hydrophilic-lipophilic balance (HLB) polymers, N-vinyl pyrrolidone (strata-X) among others [52]. Other bonded SPEs with varying polarities include quaternary amine bonded silica with chloride ion (Cl^-), sulfonic, and carboxylic acid bonded with sodiated counter ion (Na^+) [53]. The commercial SPEs which have been mostly preferred in the environmental application are the alkyl bonded C18 and the Oasis HLB sorbents due to their availability, stability in wide pH ranges, good extraction efficiency and relatively high recovery [40]. **Table 2.1** gives a summary of available SPEs as reported in literature used in the preconcentration and extraction of organic contaminants from water.

Despite the advantages of the SPEs mentioned above, there is an ever-increasing number of organic contaminants that render the limited applicability of the conventional SPEs. Therefore, the development of novel carbon-based nanomaterials with much higher selectivity and specificity towards target analytes and applications is of great importance as described below.

Table 2.1: Application of solid phase extraction (SPE) technique for extraction of organic contaminants in environmental matrices [93, 96-102]

Matrix	SPE adsorbent	Organic contaminants	Level found% recoveries	Detection	References
Wastewater	Strata-X Phenomenex	Pharmaceuticals (inflammatory and analgesic)	0.46 to 600.5 ng/L	UHPLC-MS/MS	[49]
River and lake water	Polymeric	Parabens	466-44 ng L^{-1}	LC-MS/MS	[54]
Wastewater	Oasis HLB	Parabens, triclosan, triclocarban	>85 %	LC-ESI-MS	[55]
Surface water and wastewater	Oasis HLB	Multi-class pesticides	50-130 %	LC-TOF	[56]
Surface and wastewater	Anion exchange	Antimicrobials	84.5-105.6 %	UHPLC-ESI-MS/MS	[57]
River water	Bond Elut Plexa	Personal care products	69-101%	UHPC-MS/MS	[58]
Surface ground, wastewater	C-18	OPPs, OCs, PBE, BDEs, PAHS phenols,	25-82 ng L^{-1}	GC-MS/MS	[59]
Ground water	C-18 and OASIS HLB	pesticides	> 65-68 %	GC-MS	[60]

MEPS=microextraction by packed sorbent

2.1.2.2 Carbon-based nano-adsorbents

With the advancement in technologies, there are newly developed sorbent materials such as immunosorbents [61], molecularly imprinted polymers [62], and carbon-based nanomaterials [63] of various types, that have been introduced to enhance the extraction efficiency as well as to expand the scope of the ever-increasing number of organic contaminants in the environment. Novel carbonaceous nanomaterials are made of unique structures that render them very valuable in SPE techniques due to their particle size at the nanoscale as well as their miniaturization in application [64]. The mode of interaction with organic compounds can occur via hydrophobic interactions, electrostatic forces and hydrogen bonding. Examples of these materials include fullerenes, graphene, carbon nanotubes, carbon nanofibers, carbon nanodiamonds, carbon nanocones and horns as well as their functionalized forms [65]. Graphene and carbon nanotubes are easily synthesized and can be functionalized to meet specific needs. Carbon nanodiamonds, nanohorns and nanocones are not easily available like other materials, hence there is limited research on these materials [66]. Sample preparation techniques employing nanomaterials exhibit more advantages than the conventional SPEs. These include high surface-to-volume ratio, chemically active surface areas, enrichment capability and high selectivity, stability over extreme basic of acidic conditions as well as re-usability of the columns with satisfactory results [64, 65, 67].

A summary of the carbon-based adsorbents applied for extraction and precontraction of organic pollutants is shown in **Table 2.2**. In this work, however, the emphasis has been made on carbon nanodots (CND) a novel sorbent of interest in this study as it has been sparsely used as an adsorbent in SPE.

Table 2.2 Application of carbon-based SPE adsorbents for the extraction of organic contaminants in environmental matrices [67-73]

Analyte	Matrix	Sorbent type	Detection Technique	Recovery %	References
Pesticides	Surface water	MWCNT	GC-MS	82.0–103.7	[68]
PAHs	Water	SWCNT	GC-TOF-MS	21-96	[69]
PAHs	Tap, well, river, wastewater	m-G/CNF	GC-FID	95.5–99.9	[70]
Organophosphorus	Runoff, mineral and tap water	MWCNT	GC-NPD	67-107	[71]
Triclosan	River and lake water	MIP-MWCNT	HPLC-UV	91-95	[72]
6 PCB	Tap and river water	Fe3O4/MWCNTs-COOH	GC-MS	71-99	[73]

PCBs=polychlorinated biphenyls, MWCNT=multi-walled carbon nanotubes, WWCNT=single-walled carbon nanotubes,
m-G/CNF=magnetic-graphene-carbon nanofiber, MIP=molecularly imprinted polymers

2.1.2.2.1 Carbon nanodots

Carbon nanodots (CNDs) are a relatively new class of nanoparticles with a quasi-spherical dimension with a typical size of less than 10 mm [74, 75]. They were first discovered during a purification process of single-walled carbon nanotubes via preparative electrophoresis [76]. CNDs have gained wide popularity due to their benign, simplicity in preparation with a wide range of raw materials, low-cost in nature, low toxicity, eco-friendly and water solubility [77, 78]. In addition, a key feature of CNDs that has generated a lot of interest in the recent past is that they can be prepared in large scale from biomass derived waste, using a one-step pathway such as candle burning, laser ablation methods and in-situ dehydration reactions [75]. CNDs possess diverse characteristics which makes them attractive for a myriad of applications such as analytical methodologies [79], electrochemical applications [80], bioimaging [81], biosensing [82] and drug delivery [83]. Several methods have been reported for the synthesis of CNDs such as pyrolysis, acidic oxidation, laser ablation, ultrasonic passivation and hydrothermal treatments. However, there are novel methods that are based on green synthesis, using natural raw materials such as honey [84], coffee ground [85] and soy milk [86] as a source of carbon for the CNDs synthesis. This study employed the use of oats as the raw materials for the synthesis of CNDs. To the best of our knowledge, there is sparse or no application of CND as an adsorbent in SPE for preconcentration of organic contaminants in water samples.

2.1.3 Dispersive solid phase extraction (DSPE)

Dispersive solid phase extraction (DSPE) technique is a simplified microextraction technique, which continues to be extensively applied in sample preparation procedures since its inception in 2004 by Anastassiades and coworkers [87]. Unlike the SPE technique, DSPE employs the dispersion of the solid sorbent in the sample matrix for the extraction process. The solid sorbent, based on either silica, polymer-based, or synthesized nanomaterials, is introduced directly into the sample and immediately dispersed [88-90]. Thereafter, the sorbent is retrieved via centrifugation or filtration process. The analytes are then desorbed from the sorbent with an appropriate elution solvent prior to instrumental analysis. The dispersion of the sorbent allows for maximum contact area and interaction between the analyte and the sorbent. This facilitates selective extraction or pre-concentration of analytes from the sample matrix [91].

The type of interaction, as with most methods that employ solid adsorbents, and depending on the physicochemical properties of the compounds are principally hydrogen bonding, hydrophobic and hydrophilic interactions, van-der-Waals forces and electrostatic interactions [92, 93]. This technique has attracted much interest over the years due to its unique advantages such as being rapid, high selectivity, high sample throughput, robustness and is also inexpensive due to low consumption of solvent consumption [94-96]. Several authors have reported the applications of DSPE for the preconcentration of organic contaminants in various environmental samples [97-99] biological samples [100, 101] as well as food matrices [102, 103].

2.1.4 Magnetic Solid Phase Extraction (MSPE)

Magnetic solid phase extraction (MSPE), is a relatively new procedure for the preconcentration of analytes targeted mostly from aqueous sample matrices by using magnetic or magnetizable adsorbents, as was developed by Safarikova and Safarik [104]. Amongst the setbacks of using the conventional SPE procedure is; relatively low recovery of the analytes, poor isolation and preconcentration of target compounds and time-consuming extraction steps [105]. Magnetic nanoparticles (MNPs) provide a much better alternative, by alleviating the limitations that are inherent to SPE. In MSPE, the synthesized sorbent material is dispersed into the sample solution by vortex or sonication. After a certain period, the analytes are adsorbed onto the surface of the MNPs. Separation of the magnetic sorbent with adsorbed analytes is achieved by application of an external magnetic field (magnet), on the exterior of the extraction vessel. This eliminates the need for centrifugation and or filtration of the sample, thereby quickening the overall extraction process. The analytes are desorbed from the sorbent using an appropriate organic solvent for further analysis [106]. The advantages of MSPE include 1) high extraction efficiency due to high surface area of the adsorbent, 2) dispersibility in aqueous solution, 3) rapid analyte separation by magnetic force, 4) time effective with reduced laborious approach, 5) high enrichment factor and durability, 6) reusability with washing and desorption cycles [107, 108].

Numerous methods have so far been developed for analysis of organic contaminants using MSPE based on different nanomaterials combined with magnetic nanoparticles as sorbents [108-110]. These magnetized nanomaterials include carbon nanotubes (m-CNT) [111], magnetic carbon nanofibers (m-CNF) [112] magnetic graphene-based sorbents [113], magnetic graphitic carbon nitride (g-C_3N_4) [114].

Among the most commonly used sources of magnetic material used in the preparation of the magnetic adsorbents are the iron oxide particles, (Fe_3O_4) and (Fe_2O_3). This is mainly because of their ease of preparation, surface modification capabilities, very good dispersibility in aqueous solution and reusability [108]. The iron oxide particles can then be functionalized with other materials as highlighted above, achieving different properties of the magnetic material such as large surface area, more adsorption sites as well as pH flexibility and stability [115]. The common procedure for synthesizing the magnetic carbon materials is by the introduction of the magnetic particles into the carbon material or addition of the carbon material into the magnetic source. This can be achieved via different physical or chemical methods such as hydrothermal synthesis [116], adsorption processes with the aid of magnetic stirring [117], or chemical co-precipitation of the magnetic source (Fe^{2+} and Fe^{3+}) in alkaline solution in the presence of carbon material [118]. The magnetic characteristic of the synthesized magnetic carbon materials are evaluated using a magnetometer by magnetization curves [119]. Due to the benefits that accrue with the use of magnetic nanoparticles for magnetic solid phase extraction, many researchers have reported their application for the determination of organic pollutants in water systems. Li et al, reported the use of MSPE for determination of triclosan in wastewater samples [120]. Furthermore, other researchers reported application of MSPE in analysis of various organic contaminants such as organophosphorus pesticides and other personal care products as detailed in the literature [121].

2.1.5 Solid Phase Microextraction (SPME)

Solid phase microextraction (SPME) is another method widely used in the extraction of organic compounds prior to instrumental analysis. Fibre–SPME entails the partitioning of analytes between the coated fibre which is the stationary phase and the sample until equilibrium is reached, thereby accomplishing sample extraction and pre-concentration in single step unlike in SPE procedures [122]. The fibre coating can be solid, liquid polymer or combination of both. Examples of fibre coatings used in SPME include polyacrylate (PA), polydimethylsiloxane (PDMS), divinylbenzene (DVB) or a combination of carboxen/PDMS/DVB [123]. Extraction takes place by immersing the fibre into sample matrix (non-volatiles extraction), or via headspace sampling by exposing the coating to the gaseous phase of the sample (volatiles and semi-volatiles extraction) for a predetermined time [124]. Thereafter the analytes are desorbed for instrumental analysis. SPME is characterized

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by the many advantages including small sample volume, simplicity in operation, short extraction time, automation for online extraction and versatility [125, 126]. Various studies have been reported using this sample extraction technique for the determination of organic contaminants in the environment such as wastewater [9, 10], rain and stormwater [11], surface water [12] as well as soil matrices [13].

2.2 ANALYTICAL TECHNIQUES - CHROMATOGRAPHIC SEPARATION AND DETECTION

Analytical separation is a vital component of the analysis of complex matrices. After successful extraction of target analytes, instrumental separation of these compounds is important to combat background noise, avoid matrix ionization effects reduce the risk of obtaining false negative and false positives and obtain precise and reliable results [127]. For successful determination of the myriad of organic compounds in a sample mixture, separation techniques are employed. The methods that are typically used include gas chromatography (GC) and liquid chromatography (LC) methods. For the identified analytes to be quantitated, these techniques are coupled and/hyphenated with different detectors each with unique functionalities. They include ultraviolet-visible detectors (UV-VIS) which is coupled to LC, electron capture detector (ECD) and flame ionization detector (FID) which are connected to GC, as well as mass spectrometric detectors (MS). The focus of this review, however, was based on reversed phase LC and GC techniques hyphenated to mass spectrometers as has been discussed below in detail.

2.2.1 Gas chromatography (GC)

Gas chromatography is a separation technique employed for separation of volatile and semi-volatile organic compounds or compounds that can be transformed into volatile derivatives. A microliter sample is injected into the heated GC injection port and the sample is volatilized to gaseous phase and transported with the heated carrier gas into the GC column (stationary phase) for subsequent separation. The injection port temperature is set high enough (200-300 °C) to ensure immediate and complete vaporization of the sample components before rapid transfer into the column. The separation of the sample constituents in the column is determined by the volatility difference and degree of interaction with the stationary phase [128].

Depending on the type of analysis, two injection systems are used, split or spitless injection modes. This is primarily to prevent overloading onto the GC capillary column due to its small internal volume capacity. In split injection mode, part of the injected sample volume is diverted to waste and only a small portion is injected into the column. In spitless injection mode, all the sample is injected into the column for separation. Spitless mode is applied for trace analysis. Split injection mode is utilized for the highly concentrated sample to avoid, instrument damage such as filament failure [129].

The principle of separation in GC is based on temperature difference. As has been mentioned, volatility of analytes plays a key role in GC separation and is temperature dependent. The column is placed in a column oven where the temperatures are controlled by computer software for fast and efficient separation. At low temperatures, high volatile organic compounds are separated while at high temperatures, low volatile organic compounds (high boilers) are separated. The distribution of the sample components between the carrier gas and stationary phase determines the rate of retention in the column. Increase in temperature increases the volatility hence reduces the retention, and vice-versa. The control of the oven temperature can be operated in two modes for sample acquisition: isothermal analysis and temperature programming. In the isothermal analysis, the temperature is kept constant, while in temperature programming, there is a gradual increase in temperature with time at certain rate intervals during the separation. Samples of similar volatility can be analyzed using isothermal mode. Temperature programming is most often used since most methods employ analysis of complex mixtures of analytes of different physicochemical properties [128, 130]. The stationary phase in GC is a material packed or coated in a column while the mobile phase is the carrier gas, that transports the analytes through the column for separation. There are different types of carrier gases used in GC like, nitrogen, helium and hydrogen. Nitrogen is a relatively large molecule, compared to hydrogen, and will consequently interfere with the mass transfer of analytes resulting in broadened peaks. Helium is the most preferred carrier gas, as it is safer to use than hydrogen. In addition, 40% of the analysis time is reduced by using helium gas [128].

In general, GC analysis mainly preferred for more volatile compounds. In addition, analyte polarity and thermal stability limit the application of GC. Due to elevated temperatures required for GC separation, thermally unstable compounds cannot withstand the high temperatures. Furthermore, polar compounds are more prone to peak tailing during GC analysis [130]. Therefore, more polar, thermally unstable high molecular weight compounds are more amenable to LC analysis as has been discussed in the preceding section below.

2.2.2 Liquid chromatography (LC)

In the conventional high-performance liquid chromatography (HPLC), a liquid mobile phase is pumped through a column, where sample components are separated on a column, based on the physical or chemical affinity of the analytes to the stationary phase of the column. Most LC based separation methods are based on reversed phase principle, where the mobile phase is an aqueous solution and the stationary phase is hydrophobic [131].

The mobile phase is the carrier of the sample component through the column for separation into individual components. It comprises a mixture of ultra-pure water and an organic solvent that is miscible with waters usually methanol or acetonitrile. In most cases, methanol is preferred over acetonitrile due to its low toxicity and low cost. However, methanol as a modifier forms viscous solvent mixtures with water leading to increased back pressure in the LC instrument.

There are two modes in which the mobile phase is delivered in the LC chromatograph for separation: isocratic and gradient elution [132]. In isocratic elution, the composition of the mobile phase is constant whereas, in gradient elution, the solvent composition is varied during the analysis, commencing with the weaker elution mobile phase composition. This results in an increased elution strength, leading to a faster and efficient elution of later eluting substances with greater sensitivity. As a result, there is improved detection limits, reduction in analysis time, and improved peak shapes of the analytes, especially the later eluting ones. In addition, gradient elution is also very useful as it cleans our the strongly retained analytes on the column [133]. Overall, the best chromatographic conditions are obtained using gradient elution.

The heart of the liquid chromatography is the column, where the separation takes place as the analytes partition themselves between the stationary phase and the mobile phase. The most used column packing is based on octadecylsilylsilica (ODS) material that has been functionalized to obtain different stationary phases for different functionalities such as reversed phase separation, ion exchange, chiral separation and hydrophilic interactions [134]. Over the years, the columns in LC have been characterized by very small particle size packings (3-5μm), which ultimately result in good column efficiency (high number of theoretical plates). Reduction in particle size leads to faster analysis using shorter columns as well as the low limit of detection and limit of quantification. Using smaller particle size, allows for increased mobile phase flow rates for rapid separations with minimal loss in resolution, thus allowing for the fast development of LC methods.

The column packing technologies have continued to improve tremendously over the years, with column particle sizes of 1.5-2 µm. This miniature particle size requires instruments that can withstand much higher back pressure. This led to the development of ultra-high-performance liquid chromatography instruments.

UHPLC is marked by substantial performance enhancement as compared to conventional HPLC systems and hence attractive for rapid and robust method development needs. UHPLC instruments have pumps and autosampler that can withstand pressures up to 1000-1500 bars. The utilization of columns packed with particle sizes of sub-2µm in 2 mm or 3 mm internal diameters formats, allows for better separation with much narrower peaks, better chromatographic sensitivity and efficiency and resolution [135]. As compared to HPLC, the extra efficiency in UHPLC is due to higher flow rates achieved by shorter run times. The high flow rates result in high back pressure and /or column clogging which can be avoided by keeping the column temperatures at 30-40 ^{o}C to reduce mobile phase viscosity or maintaining the flow rates below 0.5 mL min $^{-1}$. In addition, shorter column lengths can also be employed. Using shorter columns with smaller internal diameters results in reduced consumption of the mobile phase and analysis time (faster equilibration) consequently saving on cost [136]. Other advantages of using UHPLC, include transfer of existing HPLC methods directly to UHPLC, high analysis throughput, the speed of analysis is increased 3-10-fold with high resolution, versatility in development of wide variety of methods for complex matrices, higher sensitivity and performance as well as automation of method development for faster UHPLC [137].

2.2.3 Overview of Detection techniques used with chromatography

Different compounds require different detectors to be ''seen''. In GC analysis, the standard detectors used include electron capture detector (ECD), flame ionization detector (FID) and nitrogen phosphorous detector (NPD). The most common detector used in HPLC is ultraviolet (UV). These detectors are popular due to their ease of use, low cost and relatively low detection limits. The ECD detector, for instance, is used in the determination of compounds with high affinity for electrons such as halogens, phosphorous and nitro groups. The degree of electron capture is proportional to the concentration of the analyte in the sample [138]. GC-ECD is a very sensitive technique, however, it is limited to the electronegative compounds only and has also been widely adopted in the analysis of various organic compounds in the environment [139, 140]. FID, on the other hand, is a universal detector with wide applicability

to most non-polar organic compounds. It has also been used in many applications such as analysis of organophosphorus pesticides [141] and PAHs [142] in different matrices. NPD, also known as thermionic detector (TID) is a highly sensitive and selective for the analysis of analytes containing, nitrogen or phosphorus. The selectivity and sensitivity of NPD make it appropriate for the analysis of some organic compounds such as pharmaceuticals [143] pesticides [144] and drugs of abuse [145]. For HPLC instruments, the ultraviolet (UV) is included as a standard detector. HPLC-UV is fairly easy to operate with high operational stability. In addition, it is also preferred where sensitivity is not much needed. For instance, Farajzadeh et.al., [146] developed a method for the determination of diazinon, ethion and fenitrothion, in water samples using HPLC-UV. These detectors, however, are still limited in performance, in terms of robustness and trace level determination of multi-class organic compounds. Mass spectrometry detectors have become extensively applied in numerous fields as they are hyphenated to both GC and LC chromatographs as discussed in below.

2.2.3.1 Gas chromatography-Mass spectrometry (GC-MS)

GC-MS is the synergistic combination of the separation features of gas chromatography hyphenated to mass spectrometry (mass analyzer) detector for the separation and analysis of volatile and semi-volatile sample components according to their mass-to-charge values. As previously mentioned, GC is limited to compounds that can easily volatilize and are thermally stable. However, compounds which are not suited for GC analysis in their natural state, but have the capability of forming stable volatile derivatives, can be analyzed using GC-MS [147]. The capillary column separates the mixture of sample components with excellent efficiency over time, with a very high number of theoretical plates, as the MS collects data (mass spectrum) that gives structural identification of the individual sample components [148]. These sample components exit the capillary column in a purified gas state and enter the mass spectrometer via an ionization source, where the ionization takes place as discussed in the sections below. Some of the advantages of this hyphenation technique include (1) identification and confirmation capabilities of compounds in complex sample mixtures, (2) quantification of analytes and (3) low detection and quantification limits due to the application of special data acquisition modes [147].

The gas chromatography is linked to the mass spectrometer via an interface where ionization takes place. Efficient production of ions is of utmost importance as fundamentally the MS measures ions. The two primary ionization techniques used in GCMS are electron

ionization and chemical ionization [149]. CI is a soft ionization technique and is less sensitive than EI. In general, CI is seldom used in most GC-MS applications.

Electron ionization (EI) is the most commonly adopted in GCMS due to its extensive fragmentation patterns. The ionization is accomplished by passing a beam of electrons generated from a tungsten filament to the components coming off the GC column. Radicals are formed via the electron removal when an electron collides with a neutral analyte molecule, forming positively charged radical ions. The formed radical cation then reacts with another electron to form another radical cation. This cascade of ionisation depicts the fragmentation rich nature of EI [148]. EI generally employs an ionization potential of 70 eV. This voltage is regarded as the maximum energy required for the ionization of all volatile molecules amenable for GC-EI-MS. EI is advantageous due to its high efficiency in ionisation, reproducible fragmentation patterns, enabling the use of spectral library searching and versatility in ionising compounds. These attributes have rendered (EI) the most adopted ionization GCMS [128]. However, one of the drawbacks of EI is that in some instances, the extensive fragmentation of components with similar structures can result in identical sample spectra, making it difficult to distinguish the different components in the sample [150].

2.2.3.2 Liquid chromatography-Mass spectrometry techniques (LC-MS)

Liquid chromatography-Mass spectrometry (LC-MS) is a hyphenated analytical technique that combines the powerful separation capabilities of HPLC and/or UHPLC (LC) with the mass analysis technologies of mass spectrometry (MS) [147]. The separated sample components from the LC enter the mass spectrometer ion source in gaseous form, for identification, quantification or structure elucidation by observing the fragmentation ions. To achieve optimum analytical results using LC-MS, the choice of the mobile phase is very important. Unlike HPLC, the mobile phases applicable for LC-MS are restricted. Eluents coming from the LC are in liquid form (mobile phase), which are converted into gaseous form at the atmospheric pressure interfaces (API). Therefore, it is imperative that the mobile phase comprises of volatile components and of high purity, preferably LC-MS grade for effective ionization [147]. The most common mobile phase additives used in LC-MS include formic acid, acetic acid, ammonium formate and ammonium acetate. These additives also enhance the ionization of analytes in the interface, thus gaining better sensitivity [151]. The types of APIs used in most modern LC-MS instruments are briefly discussed below.

The coupling of the LC with the MS is achieved using an API where the analytes from LC eluent (liquid form) are ionized and transferred into gas phase. The mobile phase solvent is desolvated under normal atmospheric conditions (without vacuum) during the ionization process. Positive or negative ions can be formed by the addition of removal of a proton in a molecule [152]. The ions formed are then separated in the mass analyzer based on their mass-to-charge ratios *(m/z)*, where *m* is the analyte mass and *z* is the analyte charge [133]. There are several interfaces used in the generation of ions in LC-MS analysis such as electrospray ionization (ESI) [153], atmospheric pressure chemical ionization (APCI) [154] and atmospheric pressure photo-ionization (APPI) [155] each with different applications. APCI is not suitable for large biomolecules, but more amenable to small and relatively nonpolar compounds [133, 153]. APPI is also used for small relatively non-polar compounds, that are not properly ionized in ESI or APCI [156]. ESI is the most commonly used ionization technique in LC-MS applications and is the method of choice in this study.

In ESI, the eluent from the column enters a high capillary voltage (3-5kV) where it is nebulized leading to the formation of a fine spray of charged droplets. The droplet formation is facilitated by the presence of the nebulizing gas (N_2) that mixes with the liquid flow. A drying gas (desolvation gas) is also introduced flowing in the opposite direction to facilitate the evaporation of the solvent. Positive and negative charged ions can be formed and detected, by switching between the two ionization modes [157]. The ions formed enter the MS by applying a positive or negative potential gradient as they pass through the ion optics of the mass analyzer [153]. A schematic representation of the essential features ESI is as shown below.
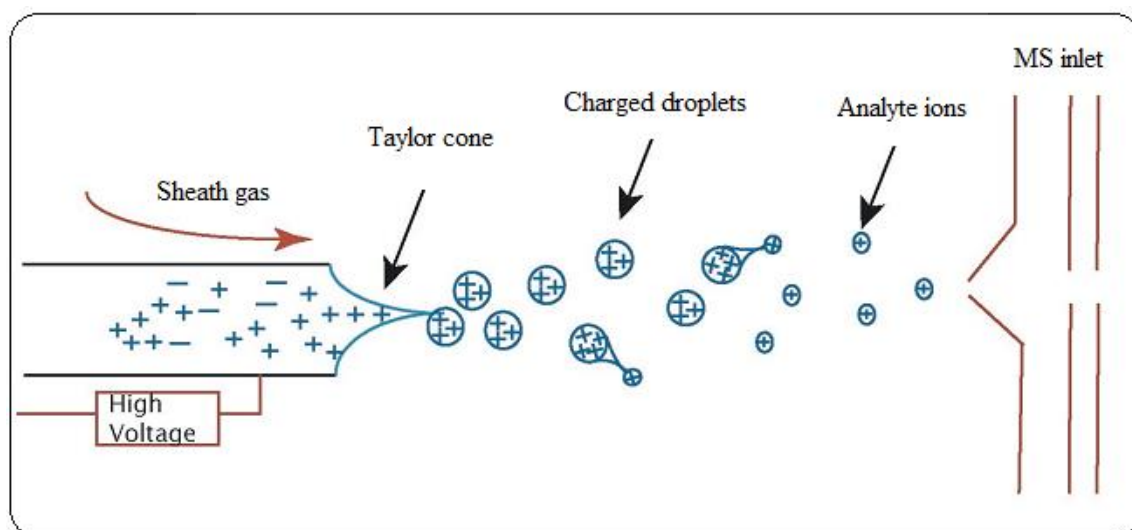
Figure 2.1: Electrospray ionization interface [153]

In LC-ESI-MS/MS, a compromise must always be achieved between chromatographic separation and ESI sensitivity regarding the mobile phase. Most reversed phase analysis comprises an increase of organic solvent during the analysis to achieve baseline separation. This is advantageous in that better sensitivity is achieved when compounds are eluted with higher organic solvent composition [158]. In most applications, methanol and acetonitrile are the most commonly used organic solvents. However, methanol is preferred over acetonitrile for several reasons, 1) it offers a slightly better efficiency in ionization acetonitrile, 2) better peak shapes are obtained for basic compounds, 3) it has a lower elution strength allowing elution of compounds at higher organic composition [153]. The prerequisite in ESI is that analytes must be already ionized in the liquid phase, to enhance ionization in the ESI. This is normally facilitated by the addition of low concentration (~5-10 mM) mobile phase additives such as formic acid, acetic acid, ammonium formate or ammonium acetate [159].

2.2.3.3 Mass analyzers detectors

Mass analyzer measures gas phase ions based on their m/z values. Mass measurement on facilitated by the charge addition, allowing the molecule to be affected by the electrical fields [152]. The performance of mass analyzers is characterized by the following: by the mass resolution, mass accuracy, scan speed, mass range and tandem analysis capabilities. Mass resolution (R), also known as resolving power, is the ability of the MS to separate m/z ratios effectively from each other. A higher R-value connotes better separation of closely related m/z values [133]. Mass accuracy (E) is the difference between the measured and the theoretical m/z values. A correctly measured mass value is signified by a low E value. Scan speed is the rate at which a particular mass range is scanned by the mass analyzer [133]. Mass range is the m/z range of the mass analyzer. Below are examples of the most commonly used mass analyzer detectors.

2.2.3.3.1 Triple-Quadrupole (QqQ)

A triple quadrupole (QqQ) MS is a tandem mass spectrometer which consists of two quadruples (Q1 and Q3) and a collision cell (Q2) which is placed in the middle of the two quadrupoles [160]. The geometry of the collision cell is a quadruple but can take a different geometry such as hexagonal or octagonal depending on the specification of different manufacturers. Tandem analysis is the ability of the mass analyzer to separate varying

molecular ions, generate fragment ions from the molecular ion and measure the mass of the fragmented ions [147].

A quadrupole uses oscillating electric fields for separation of ions according to their m/z values. It is comprised of four cylindrical rods in a parallel radical arrangement, connected electrically. A direct current (DC) voltage or and radio-frequency (RF) alternating current is applied on two opposite rods causing the creation of an oscillating electric field. The ions are introduced into the oscillating electric field via low accelerating potential. The flight path towards the detector is not a straight line due to the oscillating electric field, causing the ions to move in the z- y- and x-directions [152]. The ions of specific m/z values, with stable trajectories, traverse through the filter towards the detector when a given combination of DC and RF voltages are applied on the quadrupoles. Ions of other m/z with unstable trajectories will collide on quadrupoles and eventually get lost in the vacuum system and will not be detected. A whole spectrum is therefore obtained by varying DC and RF voltages in a controlled manner [161].

The collision cell (Q2) which is operated in the RF mode only, is the heart of the QqQ, where selected ions are bombarded with neutral gas molecules (argon or nitrogen gas) resulting in collision-induced dissociation (CID) [160]. This mechanism gives rise to fragment ions. As a result, QqQ can be operated in four different acquisition modes; precursor ion scan, product ion scan, neutral loss scan and multiple reaction monitoring. In precursor-ion-scan, a specific ion selected from the ions generated in the ion source is channelled to the collision cell for fragmentation. The fragment ions are then transferred to the Q3 for mass analysis [162]. In product-ion-scan, the fragment ion produced in Q2 is transmitted to Q3, where they are scanned to give information of the fragment ions obtained. The product ions spectrum obtained acts as a 'fingerprint' used to confirm with certainty the identity of a compound since the fragmentation pattern is unique for each compound [157]. In neutral loss scan, both Q1 and Q3 are scanned simultaneously and Q2 is offset by the neutral loss being investigated (mass of the neutral loss). Compounds in the same class can be identified if they have a characteristic neutral loss [133].

Multiple reaction monitoring is the most commonly used analysis mode for quantitative analysis for a wide array of analytes in a single run. The product ion signals from the multiple precursor ions are measured and the signal of the most intense product ion (quantifier) is used for quantification of multiple analytes, while the one with the least intense product ion signal (qualifier) is used for confirmation [163]. The above-mentioned analysis modes render the triple quad MS highly selective with excellent identification, confirmation

and quantitation capabilities with very low limits of detection and quantification. However, one of the setbacks of triple quadruple is the limitation in structure elucidation of non-target compounds, which is also attributed to the lack of libraries in LC-MS/MS spectra for identification of unknown compounds. As a result, other mass analyzers capable of this function such as time-of-flight were introduced [164].

2.2.3.3.2 Time-of-Flight (TOF)

A time-of-flight mass analyzer (TOF) consist of a field-free tube where m/z values of an ion are determined by the time the ions take to traverse from the ion source to the detector. An electric field is used to accelerate the ions through the tube with the same potential and the time taken to reach the detector is measured [152]. Particles having similar charges, will have identical kinetic energies, however, their velocities are dependent on their masses hence the lighter ions will possess a shorter flight time than heavier ions, and the separation will be according to their m/z values. TOF mass spectrometers are generally characterized by mass accuracy, good sensitivity in wide mass range scanning and high resolution. An advanced version of the TOF called high resolution-time-of-flight (HR-TOF-MS), exhibits higher resolution and is characterized by a longer flight path than a standard TOF mass analyzer allowing ions to traverse the lengthy flight path without losing sensitivity and attaining high resolution [165]. HR-TOF-MS are also characterized by low mass accuracy (<5ppm) making it excellent for qualitative purposes such as screening for several compounds in one run [166]. Moreover, the mass accuracy allows the attainment of extracted ion chromatograms with narrow mass windows, allowing the removal of large chemical background and isobaric interferences, thereby tremendously improving the signal-to-noise ratios.

Due to the advantages that accrue with TOF-MS, they are used more specifically in identification and structure elucidation of unknown compounds [167]. In addition, they are advantageous in the detection a myriad of organic contaminants that may be present in the samples other than the target analytes, thereby giving more information useful for various applications [168].

2.2.3.3.3 Quadrupole Ion trap (QIT)

The quadrupole ion trap (QIT) mass analyzers are different from the above-mentioned mass analyzers in that they run at a relatively higher pressure 10^{-1} Pa as compared to 10^{-4} Pa for the quadrupoles and 10^{-7} Pa for the TOF mass analyzers [147]. The QIT comprises of three

electrodes; two end cap electrodes and the third one is a circular ring electrode which is positioned symmetrically between the end-cap electrodes. The geometries of the electrodes are defined so as to produce an ideal three-dimensional quadrupole field which in turn produces a parabolic potential well for the confinement of ions [169]. Varieties of potentials can be applied to the end-cap electrodes to allow for trapping of all ions within or above specified m/z ratios, trapping of ions only at a selected m/z ratio, or ejection of ions of specified m/z ratios [170]. The trapped ions are then scanned out for detection using mass selective instability scan mode of operation. In this mode, all ions having m/z ratios above a given value can be stored initially but eventually ejected to sequential m/z ratios by ramping the (r.f) voltage applied to the ring electrode, where upon ions are destabilized in order of increasing m/z ratios. As the ions exit the QIT in the axial direction, they are detected by the electron multiplier.

The QIT operates in a pulsed mode, so that it can accumulate ion masses selectively over time, hence ''tandem-in-time mass spectrometry'' [170]. Three stages involved in the tandem experiment. First is the isolation of ion species designated as the precursor ion during and after ionization. The second step is collision induced dissociation (CID) to determine the *m/z* ratios of the product ions and the final step is mass analysis of the products ions produced [171]. Precursor ion isolation is achieved by a r.f. ramping in conjunction with axial modulation to resonantly eject ions of lower *m/z* ratios than the precursor ion. A second broadband isolation waveform is then applied to eject ions of higher *m/z* ratios than the precursor ion. Ion isolation takes place as a result of destabilization of unwanted ions leaving only the precursor ion of interest followed by CID, where the precursor ions collide with the buffer e.g. helium to form product ions [169]. Similar to the QqQ, high sensitivity and selectivity of QITs can also be achieved in selected reaction monitoring (SRM) and selected ion monitoring (SIM) experiments.

2.2.3.4 Matrix effect in liquid chromatography mass spectrometry

UHPLC-MS/MS is a powerful tool applied for the determination of organic compounds. However, it is still faced with limitations for being prone to matrix effects [172]. Matrix effect (ME) is signal enhancement or suppression of an analyte due to coeluting matrix components which interfere with the ionization process. It can be easily detected by comparing the analyte signal of a standard solution with that of a post-extraction spike sample (matrix-matched standard) [173]. The difference in the two signals indicated suppression or enhancement of

the signal. ME occurs as a result of the competition between the analytes of interest and the non-volatile components in the matrix for the access to the surface of the droplet for transfer to the gaseous phase [174]. The non-volatile components, which can arise from either sample components or mobile phase additive, may precipitate during the desolvation process and prevent the formed analyte ions to be converted from droplet to gaseous phase. In addition, a decrease in the rate of formation of charged droplets caused by the high boiling point of the solution leading to inefficient solvent evaporation which can result in an increased ionization suppression [174].

Moreover, mobile phase additives are also known to cause signal enhancement or suppression, since analyte ionization is greatly influenced by the composition of the mobile phase. In a study by Benijts et al., 2004 reported an increase in signal suppression when comparing 0.01% of formic acid and 0.1% of acetic acid concentration (v/v) as well as 1 and 5 mM concentrations of ammonium acetate and formate [175]. There are two types of ME; absolute and relative ME. Absolute matrix effect indicates the variations between the responses of the standard solution and the post extracted spike matrix, whereas relative matrix effects indicate the differences in various lots of post extracted spiked samples. Absolute ME affects the accuracy of the method while relative ME affects the precision of the method [176]. In view of the above, it is important to develop analytical methods for reducing or compensating ME when using mass spectrometric methods for quantitation.

There are different approaches that have been employed for removing constituents responsible for matrix effect such as improved sample clean-up by using a more selective extraction technique as discussed in the previous section of this chapter. This reduces the matrix components introduced into the instrument prior to sample analysis [172]. Alternatively, the matrix interferences on the accuracy and/or precision of the method can be eliminated or compensated for. This can be achieved by standard addition, matrix-matched calibrations and the use of internal standards such as ^{13}C labelled standards or an analogue internal standard, to compensate for signal alteration. However, the application of using isotopically labelled standards is limited due to the cost involved in obtaining these standards, and also the availability for only a limited number of target analytes, especially for multiclass analysis [176]. Matrix-matched calibration is another way of compensating for ME. However, this method is faced with the challenge of selecting suitable blanks for preparation of the matrix-matched standards, for several matrices that need to be investigated. This renders this method laborious and time-consuming. If matrix blanks are not adequately available, standard

addition approach provides an alternative in obtaining precise and accurate results in LC-MS analysis [159].

2.2.4 Application of LC-MS and GC-MS for determination of organic pollutants in Environmental samples

The complementary application of both GC and LC coupled with tandem mass spectrometric detection is necessary for obtaining wholistic overview of the organic contamints present in the water samples. The advantage of coupling tandem mass spectrometric detectors to GC or LC chromatographs unlike other conventional detection techniques such as UV, is the ability to identify and quantify a myriad of organic compounds with diverse physicochemical properties in one analytical run using either (MRM), TOF or QIT mass analyzers [163]. The superiority offered by these hyphenated systems include greater sensitivity and selectivity without derivatization, good reproducibility, mass accuracy, improved sensitivity, and reduced interference effects [177]. This excellent performance is made possible by the measurement of the molecular mass of the parent compounds and that of the fragment ions. Furthermore, excellent performance can be obtained for other analytes that may be present in the sample matrix that can be included in the list of target analytes [178].

The application of GC-MS had previously been the predominant method of choice for monitoring of contaminants in environmental samples. Albeit being a powerful technique, the handling and its maintenance is demanding and time consuming; sample preparation procedures can be long and tedious with the need of derivatization [179]. However, in the recent past, tremendous technological advancement has enabled the use of LC-MS and LC-MS/MS instruments that allows for the detection of wide variety of polar and non-volatile organic contaminants that are not acquiescent with GC-MS [180] .

The release of emerging contaminants and disposal of new and existing chemicals into the environment has led to the growing efforts geared toward the development of GC-MS and LC-MS based techniques, in various environmental matrices (water, soil, sediments etc.). For instance, a study by Gracia-Lor *et. al.* [181], developed a target UHPLC-MS/MS method with a QqQ to determine 17 selected PPCP in surface and wastewater. Three MRM transitions were selected for most of the compounds for reliable quantification, while two MRM transitions were chosen for compounds with poor fragmentation. Vulliet and co-workers described a sensitive and selective method for the investigation of organic contaminants in

ground water [182]. This was accomplished using both GC-TOFMS and LC-MS following SPE. Among the compounds originally targeted 36 could be determined.

A summary of recent analytical methods developed for the determination of organic contaminants in different water matrices using LC-MS and GC-MS have been tabled below.

Table 2.3: Application of LC-MS and GC-MS for the determination of organic pollutants in water samples [12,42,90,136, 181-187]

Matrix	Sample treatment	Technique	Compounds	References
Surface water, effluent wastewater	SPE	UHPLC-MS/MS	PPCPs	[181]
Ground water	SPE	GC-TOFMS &LC-MS/MS	Multiclass organic compounds	[182]
Drinking water surface water	SPE	LC-ESI-MS	Carbamates and triazines	[183]
Drinking, sea, river and wastewater	DLLME	UHPLC-MS/MS	PPCPs	[184]
Tap, river sea water	dSPE	GC-MS	UV-filters	[90]
Drinking water	SPE	HPCL-APCI-MS	pesticides	[185]
Underground water	SPE	GC-MS	Organophosphorus pesticides	[186]
Influent wastewater	SPE	LC-MS	15 pharmaceuticals	[136]
Ground, surface wastewater	Online SPE	LC-MS	Multiclass polar compounds	[42]
Surface water	SPME	GC-HRMS	Pesticides, PAHS, BDEs, PCBs	[12]
Rainwater	SPME	GC-MS	16 PAH	[187]

MEPS=microextraction by packed sorbent, PPCP= personal care products, PCBs=polychlorinated biphenyls BDE=brominated diphenyl ethers

2.2.5 The choice of hyphenated chromatographic technique used for the current study

In this study, the analysis of the selected organic contaminants in wastewater samples was investigated using UHPLC-MS/MS. This was attributed to the sensitivity, selectivity and amenability of the selected compounds in this study towards this technique as well as low detection levels of the triple quadruple mass spectrometer detector. Based on the type of compounds in this study, GC-MS was however not found to be a suitable technique for quantitative analysis. This was also because the GC, hyphenated to a TOF-MS is applied mostly for screening purposes and therefore limited in achieving low detection limits required for trace level analysis of the organic contaminants in this research. The parabens and triclosan are not amenable to GC due to their polarity and would require derivatization step for analysis. The application of UHPLC-MS/MS in this study is as discussed in chapters 4 to 7

2.3 METHODS FOR EXPERIMENTAL DESIGN OPTIMIZATION OF ANALYTICAL TECHNIQUES

The optimization of analytical methodologies using experimental design (ED) has become very important in obtaining optimum, valid and reliable results, with minimum effort, time and resources. Two or more experimental variables are predetermined simultaneously as having an influence on the experimental responses [188]. The classical way of optimization involves one-variable-at-a-time optimization approach, while other parameters are kept constant. However, this method does not take into consideration the interactive effects between factors, requires numerous experiments with an increase in the number of factors and the optimum conditions might rely on the initial conditions [189].

Multivariate optimization approaches, on the other hand, vary numerous parameters simultaneously. The goal of multivariate optimization approaches is to establish effective factors, estimate the impact of these factors on responses, determine the main and interactive effects between factors, as well as optimization and modelling to establish a mathematical relationship between the factors and their respective responses, with minimal number of experiments. This, in turn, helps in saving time and cost when good experimental conditions are obtained [190]. All this is performed because, in analytical chemistry, pretreatment of environmental samples is a vital prerequisite in the chromatographic determination of organic compounds from complex sample matrices. The sample extraction techniques inherently

exhibit numerous steps with a large number of factors that influence the extraction efficiency such as solvent type, temperature, sample pH, sample volume, extraction time and ionic strength, depending on the extraction method used [188]. Therefore, these extraction parameters must be optimized with the aid of multivariate approaches to obtain the best experimental conditions as well as the best model for the relationship between variables.

There are two broad categories in which ED can be classified, depending on the objective of the experiment: screening designs also referred to as first order models and response surface designs also known as second-order designs [191].

2.3.1 Screening designs

In performing a design of laboratory experiments, screening design is usually the initial step in determining the experimental variables to be investigated, that have the most critical influence on the outcome of the analytical results [192]. In screening design, fewer experiments can be conducted with a high number of variables. The variables are examined at two extreme levels [192]. The factors that are most important are investigated further in the optimization phase for determination of the best conditions. From previous studies, it is evidenced that full factorial design, fractional factorial design and Placket-Burman design are the most popular screening designs [193, 194]. This study focused on factorial design.

2.3.2 Response surface design

In cases where the screening design does not represent the experimental data sufficiently, response surface designs can be used in obtaining the actual optimal values. Additional experiments are conducted and the results used in obtaining a quadratic response surface with a curvature that can be used to envisage factor levels that produce high or low response values, as described in **equation 2.1** below [195].

$$y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 \hspace{2cm} \textbf{(2.1)}$$

where y represents the measured response, x_1 and x_2 are the factors selected, b_0 is the intercept, b_1 and b_2 are first order parameters, b_{12} is an interaction parameter, b_{11} and b_{22} are second order parameters. A 3-D response surface gives a better understanding of the behaviour of the system by indicating the contribution of the independent variables [188]. Analysis of variance (ANOVA) is performed to verify model quality fit to the data after calculation of the model

coefficients and their standards errors. Random execution of experiments is essential, to obtain an accurate estimation of experimental error [196]. There different types of response designs include Central Composite design (CCD), Box Behnken (BBD), three-level full factorial and Doehlert designs. In this study central composite design was applied in the optimization of optimal sample extraction conditions of the extraction techniques selected.

2.3.1 Central Composite

Central composite design (CCD) is the most common and prevalently used response design (second-order models) [191]. It consists of three components 1) two-level, factorial design, 2) axial points (star points) and 3) a centre points at the centre region of the experiment where all factors consist of central coding. These extra points facilitate additional properties such as the rotatability or orthogonality to fit the quadratic polynomial [191]. The advantages that make CCD more popular is that it is more rotatable and gives detailed information at once than other designs [190] and utilizes fewer number of experiments than three-level full factorial designs [191].

REFERENCES

[1] J. Pawliszyn, Unified theory of extraction, Comprehensive Analytical Chemistry, Elsevier2002, pp. 253-278.

[2] A.P. Daso, O.J. Okonkwo, Conventional Extraction Techniques: Soxhlet and Liquid–Liquid Extractions and Evaporation, Analytical Separation Science (2015) 1437-1468.

[3] M. Lorenzo, J. Campo, Y. Picó, Analytical challenges to determine emerging persistent organic pollutants in aquatic ecosystems, TrAC Trends in Analytical Chemistry (2018).

[4] M. Farré, L. Kantiani, M. Petrovic, S. Pérez, D. Barceló, Achievements and future trends in the analysis of emerging organic contaminants in environmental samples by mass spectrometry and bioanalytical techniques, Journal of chromatography A 1259 (2012) 86-99.

[5] A.T. Lawal, Polycyclic aromatic hydrocarbons. A review, Cogent Environmental Science 3 (2017) 1339841.

[6] J. Płotka-Wasylka, N. Szczepańska, M. de la Guardia, J. Namieśnik, Miniaturized solid-phase extraction techniques, TrAC Trends in Analytical Chemistry 73 (2015) 19-38.

[7] R.M. Smith, Before the injection—modern methods of sample preparation for separation techniques, Journal of chromatography A 1000 (2003) 3-27.

[8] V. Matamoros, D. Calderón-Preciado, C. Domínguez, J.M. Bayona, Analytical procedures for the determination of emerging organic contaminants in plant material: a review, Analytica chimica acta 722 (2012) 8-20.

[9] I. Dominguez, F.J. Arrebola, R. Gavara, J.L.M. Vidal, A.G. Frenich, Automated and simultaneous determination of priority substances and polychlorinated biphenyls in

wastewater using headspace solid phase microextraction and high resolution mass spectrometry, Analytica chimica acta 1002 (2018) 39-49.

[10] A. Godayol, E. Besalú, E. Anticó, J.M. Sanchez, Monitoring of sixteen fragrance allergens and two polycyclic musks in wastewater treatment plants by solid phase microextraction coupled to gas chromatography, Chemosphere 119 (2015) 363-370.

[11] E. Rianawati, R. Balasubramanian, Optimization and validation of solid phase micro-extraction (SPME) method for analysis of polycyclic aromatic hydrocarbons in rainwater and stormwater, Physics and Chemistry of the Earth, Parts A/B/C 34 (2009) 857-865.

[12] I. Domínguez, F.J. Arrebola, R. Romero-González, A. Nieto-García, J.L. Martínez Vidal, A. Garrido Frenich, Solid phase microextraction and gas chromatography coupled to magnetic sector high resolution mass spectrometry for the ultra-trace determination of contaminants in surface water, Journal of Chromatography A 1518 (2017) 15-24.

[13] F.S. Higashikawa, M.L. Cayuela, A. Roig, C.A. Silva, M.A. Sánchez-Monedero, Matrix effect on the performance of headspace solid phase microextraction method for the analysis of target volatile organic compounds (VOCs) in environmental samples, Chemosphere 93 (2013) 2311-2318.

[14] M. Rezaee, Y. Assadi, M.-R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, Determination of organic compounds in water using dispersive liquid–liquid microextraction, Journal of Chromatography A 1116 (2006) 1-9.

[15] M. Rezaee, Y. Yamini, M. Faraji, Evolution of dispersive liquid–liquid microextraction method, Journal of Chromatography A 1217 (2010) 2342-2357.

[16] Z. Xiao-Huan, W. Qiu-Hua, M.-Y. ZHANG, X. Guo-Hong, W. Zhi, Developments of dispersive liquid-liquid microextraction technique, Chinese Journal of Analytical Chemistry 37 (2009) 161-168.

[17] S. Berijani, Y. Assadi, M. Anbia, M.-R.M. Hosseini, E. Aghaee, Dispersive liquid–liquid microextraction combined with gas chromatography-flame photometric detection: very simple, rapid and sensitive method for the determination of organophosphorus pesticides in water, Journal of Chromatography A 1123 (2006) 1-9.

[18] D. Nagaraju, S.-D. Huang, Determination of triazine herbicides in aqueous samples by dispersive liquid–liquid microextraction with gas chromatography–ion trap mass spectrometry, Journal of Chromatography A 1161 (2007) 89-97.

[19] J. Xia, B. Xiang, W. Zhang, Determination of metacrate in water samples using dispersive liquid–liquid microextraction and HPLC with the aid of response surface methodology and experimental design, Analytica chimica acta 625 (2008) 28-34.

[20] E.G. Primel, S.S. Caldas, L.C. Marube, A.L.V. Escarrone, An overview of advances in dispersive liquid–liquid microextraction for the extraction of pesticides and emerging contaminants from environmental samples, Trends in Environmental Analytical Chemistry 14 (2017) 1-18.

[21] H. Xu, Z. Ding, L. Lv, D. Song, Y.Q. Feng, A novel dispersive liquid–liquid microextraction based on solidification of floating organic droplet method for determination of polycyclic aromatic hydrocarbons in aqueous samples, Analytica Chimica Acta 636 (2009) 28-33.

[22] H. Yan, H. Wang, Recent development and applications of dispersive liquid–liquid microextraction, Journal of Chromatography A 1295 (2013) 1-15.

[23] K. Seebunrueng, Y. Santaladchaiyakit, S. Srijaranai, Vortex-assisted low density solvent based demulsified dispersive liquid–liquid microextraction and high-performance liquid chromatography for the determination of organophosphorus pesticides in water samples, Chemosphere 103 (2014) 51-58.

[24] J. López-Darias, M. Germán-Hernández, V. Pino, A.M. Afonso, Dispersive liquid–liquid microextraction versus single-drop microextraction for the determination of several endocrine-disrupting phenols from seawaters, Talanta 80 (2010) 1611-1618.

[25] C. Wu, H. Liu, W. Liu, Q. Wu, C. Wang, Z. Wang, Determination of organophosphorus pesticides in environmental water samples by dispersive liquid–liquid microextraction with solidification of floating organic droplet followed by high-performance liquid chromatography, Analytical and Bioanalytical Chemistry 397 (2010) 2543-2549.

[26] N.R. Biata, L. Nyaba, J. Ramontja, N. Mketo, P.N. Nomngongo, Determination of antimony and tin in beverages using inductively coupled plasma-optical emission spectrometry after ultrasound-assisted ionic liquid dispersive liquid-liquid phase microextraction, Food chemistry 237 (2017) 904-911.

[27] A. Mpupa, G.P. Mashile, P.N. Nomngongo, Vortex assisted-supramolecular solvent based microextraction coupled with spectrophotometric determination of triclosan in environmental water samples, Open Chemistry 15 (2017) 255-262.

[28] S. Jafarvand, F. Shemirani, Supramolecular-based dispersive liquid–liquid microextraction: A novel sample preparation technique utilizes coacervates and reverse micelles, Journal of separation science 34 (2011) 455-461.

[29] Y. Assadi, M.A. Farajzadeh, A. Bidari, 2.10 - Dispersive Liquid–Liquid Microextraction, in: J. Pawliszyn (Ed.), Comprehensive Sampling and Sample Preparation, Academic Press, Oxford, 2012, pp. 181-212.

[30] M.I. Leong, S.-D. Huang, Dispersive liquid–liquid microextraction method based on solidification of floating organic drop for extraction of organochlorine pesticides in water samples, Journal of Chromatography A 1216 (2009) 7645-7650.

[31] M. El-Shahawi, H. Al-Saidi, Dispersive liquid-liquid microextraction for chemical speciation and determination of ultra-trace concentrations of metal ions, TrAC Trends in Analytical Chemistry 44 (2013) 12-24.

[32] Q. Zhou, H. Bai, G. Xie, J. Xiao, Trace determination of organophosphorus pesticides in environmental samples by temperature-controlled ionic liquid dispersive liquid-phase microextraction, Journal of Chromatography A 1188 (2008) 148-153.

[33] S. Zhang, C. Li, S. Song, T. Feng, C. Wang, Z. Wang, Application of dispersive liquid–liquid microextraction combined with sweeping micellar electrokinetic chromatography for trace analysis of six carbamate pesticides in apples, Analytical Methods 2 (2010) 54-62.

[34] Q. Yin, Y. Zhu, Y. Yang, Dispersive Liquid–Liquid Microextraction Followed by Magnetic Solid-Phase Extraction for Determination of Four Parabens in Beverage Samples by Ultra-performance Liquid Chromatography Tandem Mass Spectrometry, Food Analytical Methods 11 (2018) 797-807.

[35] P.-P. Zhang, Z.-G. Shi, Q.-W. Yu, Y.-Q. Feng, A new device for magnetic stirring-assisted dispersive liquid–liquid microextraction of UV filters in environmental water samples, Talanta 83 (2011) 1711-1715.

[36] M. Jowkarderis, F. Raofie, Optimization of supercritical fluid extraction combined with dispersive liquid–liquid microextraction as an efficient sample preparation method for determination of 4-nitrotoluene and 3-nitrotoluene in a complex matrix, Talanta 88 (2012) 50-53.

[37] Y. Guo, K. Kannan, Analytical methods for the measurement of legacy and emerging persistent organic pollutants in complex sample matrices, Comprehensive Analytical Chemistry, Elsevier2015, pp. 1-56.

[38] V. Davankov, M. Tsyurupa, Solid-Phase Extraction of Organic Contaminants With Hypercrosslinked Sorbents, Comprehensive Analytical Chemistry, Elsevier2011, pp. 523-565.

[39] D.M. Pavlović, S. Babić, A.J.M. Horvat, M. Kaštelan-Macan, Sample preparation in analysis of pharmaceuticals, TrAC Trends in Analytical Chemistry 26 (2007) 1062-1075.

[40] S. Moldoveanu, V. David, Chapter 7 - Solid-Phase Extraction, in: S. Moldoveanu, V. David (Eds.), Modern Sample Preparation for Chromatography, Elsevier, Amsterdam, 2015, pp. 191-286.

[41] V. Pichon, Solid-phase extraction for multiresidue analysis of organic contaminants in water, Journal of Chromatography A 885 (2000) 195-215.

[42] S. Huntscha, H.P. Singer, C.S. McArdell, C.E. Frank, J. Hollender, Multiresidue analysis of 88 polar organic micropollutants in ground, surface and wastewater using online mixed-bed multilayer solid-phase extraction coupled to high performance liquid chromatography–tandem mass spectrometry, Journal of Chromatography A 1268 (2012) 74-83.

[43] I. Racamonde, J. Quintana, B, E. Villaverde-de-Sáa, R. Cela, Determination of benzodiazepines, related pharmaceuticals and metabolites in water by solid-phase extraction and liquid-chromatography–tandem mass spectrometry, Journal of Chromatography A 1352 (2014) 69-79.

[44] G.O. Bosire, J.C. Ngila, H. Parshotam, Comparison of Three Solid Phase Materials for the Extraction of Carboxylic Acids from River Water Followed by 2D GC×GC-TOFMS Determination, International journal of analytical chemistry 2016 (2016).

[45] L.M. Madikizela, S.F. Muthwa, L. Chimuka, Determination of triclosan and ketoprofen in river water and wastewater by solid phase extraction and high performance liquid chromatography, South African Journal of Chemistry 67 (2014).

[46] M. Tian, J. Feng, Selective enrichment of phenols from coal liquefaction oil by solid phase extraction method, Energy Sources, Part A 31 (2009) 1646-1653.

[47] P.N. Nomngongo, J.C. Ngila, Multivariate optimization of dual-bed solid phase extraction for preconcentration of Ag, Al, As and Cr in gasoline prior to inductively coupled plasma optical emission spectrometric determination, Fuel 139 (2015) 285-291.

[48] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography–electrospray tandem mass spectrometry, Analytical and Bioanalytical Chemistry 391 (2008) 1293-1308.

[49] P. Paíga, A. Lolić, F. Hellebuyck, L.H. Santos, M. Correia, C. Delerue-Matos, Development of a SPE–UHPLC–MS/MS methodology for the determination of non-

steroidal anti-inflammatory and analgesic pharmaceuticals in seawater, Journal of pharmaceutical and biomedical analysis 106 (2015) 61-70.

[50] A. Azzouz, E. Ballesteros, Multiresidue method for the determination of pharmacologically active substances in egg and honey using a continuous solid-phase extraction system and gas chromatography–mass spectrometry, Food chemistry 178 (2015) 63-69.

[51] Y. Nolvachai, C. Kulsing, R.I. Boysen, M.T. Matyska, J.J. Pesek, P.J. Marriott, M.T. Hearn, Comparison of the performance of different silica hydride particles for the solid-phase extraction of non-volatile analytes from dark chocolate with analysis by gas chromatography–quadrupole mass spectrometry, Food chemistry 174 (2015) 434-439.

[52] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase extraction of organic compounds: a critical review (Part I), TrAC Trends in Analytical Chemistry 80 (2016) 641-654.

[53] H. Kataoka, Pharmaceutical Analysis | Sample Preparation☆, Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, Elsevier 2018.

[54] A. Zgoła-Grześkowiak, M. Jeszka-Skowron, B. Czarczyńska-Goślińska, T. Grześkowiak, Determination of parabens in polish river and lake water as a function of season, Analytical Letters 49 (2016) 1734-1747.

[55] I. González-Mariño, J.B. Quintana, I. Rodríguez, R. Cela, Simultaneous determination of parabens, triclosan and triclocarban in water by liquid chromatography/electrospray ionisation tandem mass spectrometry, Rapid Communications in Mass Spectrometry 23 (2009) 1756-1766.

[56] J. Robles-Molina, F.J. Lara-Ortega, B. Gilbert-López, J.F. García-Reyes, A. Molina-Díaz, Multi-residue method for the determination of over 400 priority and emerging pollutants in water and wastewater by solid-phase extraction and liquid chromatography-time-of-flight mass spectrometry, Journal of Chromatography A 1350 (2014) 30-43.

[57] N.H. Tran, H. Chen, T.V. Do, M. Reinhard, H.H. Ngo, Y. He, K.Y.-H. Gin, Simultaneous analysis of multiple classes of antimicrobials in environmental water samples using SPE coupled with UHPLC-ESI-MS/MS and isotope dilution, Talanta 159 (2016) 163-173.

[58] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, Ultra-high-performance liquid chromatography–tandem mass spectrometry for determining the presence of eleven personal care products in surface and wastewaters, Journal of Chromatography A 1216 (2009) 6994-7000.

[59] E. Pitarch, C. Medina, T. Portolés, F.J. López, F. Hernández, Determination of priority organic micro-pollutants in water by gas chromatography coupled to triple quadrupole mass spectrometry, Analytica chimica acta 583 (2007) 246-258.

[60] A. Kouzayha, A.R. Rabaa, M. Al Iskandarani, D. Beh, H. Budzinski, F. Jaber, Multiresidue method for determination of 67 pesticides in water samples using solid-phase extraction with centrifugation and gas chromatography-mass spectrometry, American Journal of Analytical Chemistry 3 (2012) 257.

[61] C. Baggiani, P. Baravalle, G. Giraudi, C. Tozzi, Molecularly imprinted solid-phase extraction method for the high-performance liquid chromatographic analysis of fungicide pyrimethanil in wine, Journal of Chromatography A 1141 (2007) 158-164.

[62] E. Turiel, A. Martín-Esteban, Molecularly imprinted polymers for sample preparation: a review, Analytica Chimica Acta 668 (2010) 87-99.

[63] S.C. Smith, D.F. Rodrigues, Carbon-based nanomaterials for removal of chemical and biological contaminants from water: a review of mechanisms and applications, Carbon 91 (2015) 122-143.

[64] J. Płotka-Wasylka, N. Szczepańska, M. de la Guardia, J. Namieśnik, Modern trends in solid phase extraction: new sorbent media, TrAC Trends in Analytical Chemistry 77 (2016) 23-43.

[65] Y. Wen, L. Chen, J. Li, D. Liu, L. Chen, Recent advances in solid-phase sorbents for sample preparation prior to chromatographic analysis, TRAC Trends in Analytical Chemistry 59 (2014) 26-41.

[66] B.-T. Zhang, X. Zheng, H.-F. Li, J.-M. Lin, Application of carbon-based nanomaterials in sample preparation: a review, Analytica chimica acta 784 (2013) 1-17.

[67] K.M. Dimpe, A. Mpupa, P.N. Nomngongo, Microwave assisted solid phase extraction for separation preconcentration sulfamethoxazole in wastewater using tyre based activated carbon as solid phase material prior to spectrophotometric determination, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 188 (2018) 341-348.

[68] S. Wang, P. Zhao, G. Min, G. Fang, Multi-residue determination of pesticides in water using multi-walled carbon nanotubes solid-phase extraction and gas chromatography–mass spectrometry, Journal of Chromatography A 1165 (2007) 166-171.

[69] J.M. Jiménez-Soto, S. Cárdenas, M. Valcárcel, Evaluation of single-walled carbon nanohorns as sorbent in dispersive micro solid-phase extraction, Analytica chimica acta 714 (2012) 76-81.

[70] M. Rezvani-Eivari, A. Amiri, M. Baghayeri, F. Ghaemi, Magnetized graphene layers synthesized on the carbon nanofibers as novel adsorbent for the extraction of polycyclic aromatic hydrocarbons from environmental water samples, Journal of Chromatography A 1465 (2016) 1-8.

[71] M.Á. González-Curbelo, A.V. Herrera-Herrera, J. Hernández-Borges, M.Á. Rodríguez-Delgado, Analysis of pesticides residues in environmental water samples using multiwalled carbon nanotubes dispersive solid-phase extraction, Journal of separation science 36 (2013) 556-563.

[72] R. Gao, X. Kong, F. Su, X. He, L. Chen, Y. Zhang, Synthesis and evaluation of molecularly imprinted core–shell carbon nanotubes for the determination of triclosan in environmental water samples, Journal of Chromatography A 1217 (2010) 8095-8102.

[73] S. Zeng, Y. Cao, W. Sang, T. Li, N. Gan, L. Zheng, Enrichment of polychlorinated biphenyls from aqueous solutions using Fe3O4 grafted multiwalled carbon nanotubes with poly dimethyl diallyl ammonium chloride, International journal of molecular sciences 13 (2012) 6382-6398.

[74] Z. Peng, X. Han, S. Li, A.O. Al-Youbi, A.S. Bashammakh, M.S. El-Shahawi, R.M. Leblanc, Carbon dots: biomacromolecule interaction, bioimaging and nanomedicine, Coordination Chemistry Reviews 343 (2017) 256-277.

[75] S.N. Baker, G.A. Baker, Luminescent carbon nanodots: emergent nanolights, Angewandte Chemie International Edition 49 (2010) 6726-6744.

[76] X. Xu, R. Ray, Y. Gu, H.J. Ploehn, L. Gearheart, K. Raker, W.A. Scrivens, Electrophoretic analysis and purification of fluorescent single-walled carbon nanotube fragments, Journal of the American Chemical Society 126 (2004) 12736-12737.

[77] Y. Hu, J. Yang, J. Tian, L. Jia, J.-S. Yu, Waste frying oil as a precursor for one-step synthesis of sulfur-doped carbon dots with pH-sensitive photoluminescence, Carbon 77 (2014) 775-782.

[78] I.Y. Goryacheva, A.V. Sapelkin, G.B. Sukhorukov, Carbon nanodots: mechanisms of photoluminescence and principles of application, TrAC Trends in Analytical Chemistry 90 (2017) 27-37.

[79] X. Qin, W. Lu, A.M. Asiri, A.O. Al-Youbi, X. Sun, Microwave-assisted rapid green synthesis of photoluminescent carbon nanodots from flour and their applications for sensitive and selective detection of mercury (II) ions, Sensors and Actuators B: Chemical 184 (2013) 156-162.

[80] S.S. Gwebu, P.N. Nomngongo, N.W. Maxakato, Pt-Sn Nanoparticles Supported on Carbon Nanodots as Anode Catalysts for Alcohol Electro-oxidation in Acidic Conditions, Electroanalysis 30 (2018) 1125-1132.

[81] S. Hu, Z. Wei, Q. Chang, A. Trinchi, J. Yang, A facile and green method towards coal-based fluorescent carbon dots with photocatalytic activity, Applied Surface Science 378 (2016) 402-407.

[82] Q. Wang, Y.-J. Zhong, J.-P. Yuan, L.-H. Shao, J. Zhang, L. Tang, S.-P. Liu, Y.-P. Hong, R.A. Firestone, Y. Li, Targeting therapy of hepatocellular carcinoma with doxorubicin prodrug PDOX increases anti-metastatic effect and reduces toxicity: a preclinical study, Journal of translational medicine 11 (2013) 192.

[83] C. Liu, P. Zhang, X. Zhai, F. Tian, W. Li, J. Yang, Y. Liu, H. Wang, W. Wang, W. Liu, Nano-carrier for gene delivery and bioimaging based on carbon dots with PEI-passivation enhanced fluorescence, Biomaterials 33 (2012) 3604-3613.

[84] X. Yang, Y. Zhuo, S. Zhu, Y. Luo, Y. Feng, Y. Dou, Novel and green synthesis of high-fluorescent carbon dots originated from honey for sensing and imaging, Biosensors and Bioelectronics 60 (2014) 292-298.

[85] P.-C. Hsu, Z.-Y. Shih, C.-H. Lee, H.-T. Chang, Synthesis and analytical applications of photoluminescent carbon nanodots, Green Chemistry 14 (2012) 917-920.

[86] X.J. Zhao, W.L. Zhang, Z.Q. Zhou, Sodium hydroxide-mediated hydrogel of citrus pectin for preparation of fluorescent carbon dots for bioimaging, Colloids and Surfaces B: Biointerfaces 123 (2014) 493-497.

[87] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, Journal of AOAC international 86 (2003) 412-431.

[88] W.-H. Tsai, T.-C. Huang, J.-J. Huang, Y.-H. Hsue, H.-Y. Chuang, Dispersive solid-phase microextraction method for sample extraction in the analysis of four tetracyclines in water and milk samples by high-performance liquid chromatography with diode-array detection, Journal of Chromatography A 1216 (2009) 2263-2269.

[89] T. Khezeli, A. Daneshfar, Development of dispersive micro-solid phase extraction based on micro and nano sorbents, TrAC Trends in Analytical Chemistry 89 (2017) 99-118.

[90] I.P. Román, A. Chisvert, A. Canals, Dispersive solid-phase extraction based on oleic acid-coated magnetic nanoparticles followed by gas chromatography–mass spectrometry for UV-filter determination in water samples, Journal of Chromatography A 1218 (2011) 2467-2475.

[91] Z. Li, B. Yu, H. Cong, H. Yuan, Q. Peng, Recent development and application of solid phase extraction materials, Rev. Adv. Mater. Sci 48 (2017) 87-111.

[92] M.R. Jamali, A. Firouzjah, R. Rahnama, Solvent-assisted dispersive solid phase extraction, Talanta 116 (2013) 454-459.

[93] L. Gao, Y. Wei, Fabrication of a novel hydrophobic/ion-exchange mixed-mode adsorbent for the dispersive solid-phase extraction of chlorophenols from environmental water samples, Journal of separation science 39 (2016) 3186-3194.

[94] B. Socas-Rodríguez, A.V. Herrera-Herrera, M. Asensio-Ramos, J. Hernández-Borges, Dispersive Solid-Phase Extraction, Analytical Separation Science (2015) 1525-1570.

[95] G. Islas, I.S. Ibarra, P. Hernandez, J.M. Miranda, A. Cepeda, Dispersive Solid Phase Extraction for the Analysis of Veterinary Drugs Applied to Food Samples: A Review, International Journal of Analytical Chemistry 2017 (2017).

[96] T. Boontongto, K. Siriwong, R. Burakham, Amine-Functionalized Metal–Organic Framework as a New Sorbent for Vortex-Assisted Dispersive Micro-Solid Phase Extraction of Phenol Residues in Water Samples Prior to HPLC Analysis: Experimental and Computational Studies, Chromatographia 81 (2018) 735-747.

[97] A. Alsharaa, M. Sajid, C. Basheer, K. Alhooshani, H.K. Lee, Determination of haloacetic acids in water using layered double hydroxides as a sorbent in dispersive solid-phase extraction followed by liquid chromatography with tandem mass spectrometry, Journal of separation science 39 (2016) 3610-3615.

[98] Y. Yu, Y. Tao, F. Dong, J. Xu, X. Liu, X. Wu, M. Ji, Y. Zheng, Performance comparison of dispersive solid-phase extraction and multiplug filtration cleanup methods for the determination of tefuryltrione in plant and environmental samples using UHPLC–MS/MS, Journal of separation science 40 (2017) 4420-4430.

[99] J. Guan, D. Li, Y. Guo, S. Shi, F. Yan, Simultaneous determination of seven pesticide residues in soil samples using ultrasound-assisted dispersive solid-phase extraction combined with UHPLC–MS/MS, Separation Science Plus 1 (2018) 296-305.

[100] Z. Aladaghlo, A. Fakhari, M. Behbahani, Efficient sample preparation method based on solvent-assisted dispersive solid-phase extraction for the trace detection of butachlor in urine and waste water samples, Journal of separation science 39 (2016) 3798-3805.

[101] F. Khalilian, S. Ahmadian, Molecularly imprinted polymer on a SiO2-coated graphene oxide surface for the fast and selective dispersive solid-phase extraction of Carbamazepine from biological samples, Journal of separation science 39 (2016) 1500-1508.

[102] N. Razavi, A. Sarafraz Yazdi, New application of chitosan-grafted polyaniline in dispersive solid-phase extraction for the separation and determination of phthalate esters in milk using high-performance liquid chromatography, Journal of separation science 40 (2017) 1739-1746.

[103] L. Li, H. Zhang, C. Pan, Z. Zhou, S. Jiang, F. Liu, Multiresidue analytical method of pesticides in peanut oil using low-temperature cleanup and dispersive solid phase extraction by GC-MS, Journal of separation science 30 (2007) 2097-2104.

[104] M. Šafaříková, I. Šafařík, Magnetic solid-phase extraction, Journal of Magnetism and Magnetic Materials 194 (1999) 108-112.

[105] M. Wierucka, M. Biziuk, Application of magnetic nanoparticles for magnetic solid-phase extraction in preparing biological, environmental and food samples, TrAC Trends in Analytical Chemistry 59 (2014) 50-58.

[106] M. Safarikova, I. Kibrikova, L. Ptackova, T. Hubka, K. Komarek, I. Safarik, Magnetic solid phase extraction of non-ionic surfactants from water, Journal of magnetism and magnetic materials 293 (2005) 377-381.

[107] M. Zare Jeddi, R. Ahmadkhaniha, M. Yunesian, N. Rastkari, Magnetic solid-phase extraction based on modified magnetic nanoparticles for the determination of phthalate diesters in water samples, Journal of chromatographic science 53 (2014) 385-391.

[108] X.-S. Li, G.-T. Zhu, Y.-B. Luo, B.-F. Yuan, Y.-Q. Feng, Synthesis and applications of functionalized magnetic materials in sample preparation, TrAC Trends in Analytical Chemistry 45 (2013) 233-247.

[109] H.R. Nodeh, W.A.W. Ibrahim, M.A. Kamboh, M.M. Sanagi, New magnetic graphene-based inorganic–organic sol-gel hybrid nanocomposite for simultaneous analysis of polar and non-polar organophosphorus pesticides from water samples using solid-phase extraction, Chemosphere 166 (2017) 21-30.

[110] R. Hamedi, A. BG Aghaie, M.R. Hadjmohammadi, Magnetic core micelles as a nanosorbent for the efficient removal and recovery of three organophosphorus pesticides from fruit juice and environmental water samples, Journal of separation science 41 (2018) 2037-2045.

[111] C. Herrero-Latorre, J. Barciela-García, S. García-Martín, R.M. Peña-Crecente, J. Otárola-Jiménez, Magnetic solid-phase extraction using carbon nanotubes as sorbents: A review, Analytica Chimica Acta 892 (2015) 10-26.

[112] A. Sarafraz-Yazdi, T. Rokhian, A. Amiri, F. Ghaemi, Carbon nanofibers decorated with magnetic nanoparticles as a new sorbent for the magnetic solid phase extraction of selected polycyclic aromatic hydrocarbons from water samples, New Journal of Chemistry 39 (2015) 5621-5627.

[113] J. Sun, Q. Liang, Q. Han, X. Zhang, M. Ding, One-step synthesis of magnetic graphene oxide nanocomposite and its application in magnetic solid phase extraction of heavy metal ions from biological samples, Talanta 132 (2015) 557-563.

[114] M. Wang, X. Yang, W. Bi, Application of magnetic graphitic carbon nitride nanocomposites for the solid-phase extraction of phthalate esters in water samples, Journal of separation science 38 (2015) 445-452.

[115] X.-S. Li, J.-H. Wu, Y. Zhao, W.-P. Zhang, Q. Gao, L. Guo, B.-F. Yuan, Y.-Q. Feng, Preparation of magnetic polymer material with phosphate group and its application to the enrichment of phosphopeptides, Journal of Chromatography A 1218 (2011) 3845-3853.

[116] Z. Zhang, X. Yang, X. Chen, M. Zhang, L. Luo, M. Peng, S. Yao, Novel magnetic bovine serum albumin imprinted polymers with a matrix of carbon nanotubes, and their

application to protein separation, Analytical and bioanalytical chemistry 401 (2011) 2855.

[117] Y.-B. Luo, Z.-G. Shi, Q. Gao, Y.-Q. Feng, Magnetic retrieval of graphene: extraction of sulfonamide antibiotics from environmental water samples, Journal of Chromatography A 1218 (2011) 1353-1358.

[118] Q. Wu, M. Liu, X. Ma, W. Wang, C. Wang, X. Zang, Z. Wang, Extraction of phthalate esters from water and beverages using a graphene-based magnetic nanocomposite prior to their determination by HPLC, Microchimica acta 177 (2012) 23-30.

[119] V. Wanjeri, C. Sheppard, A. Prinsloo, J. Ngila, P. Ndungu, Isotherm and kinetic investigations on the adsorption of organophosphorus pesticides on graphene oxide based silica coated magnetic nanoparticles functionalized with 2-phenylethylamine, Journal of environmental chemical engineering 6 (2018) 1333-1346.

[120] G. Li, R. Deng, G. Peng, C. Yang, Q. He, Y. Lu, H. Shi, Magnetic solid-phase extraction for the analysis of bisphenol A, naproxen and triclosan in wastewater samples, Water Science and Technology (2018) wst2018137.

[121] H. Heidari, H. Razmi, Multi-response optimization of magnetic solid phase extraction based on carbon coated Fe3O4 nanoparticles using desirability function approach for the determination of the organophosphorus pesticides in aquatic samples by HPLC–UV, Talanta 99 (2012) 13-21.

[122] P.C. Lindholm, J.S. Knuutinen, H.S.J. Ahkola, S.H. Herve, Analysis of trace pharmaceuticals and related compounds in municipal wastewaters by preconcentration, chromatography, derivatization, and separation methods, BioResources 9 (2014) 3688-3732.

[123] S. Allard, J.W. Charrois, C.A. Joll, A. Heitz, Simultaneous analysis of 10 trihalomethanes at nanogram per liter levels in water using solid-phase microextraction and gas chromatography mass-spectrometry, Journal of Chromatography A 1238 (2012) 15-21.

[124] E. Boyacı, A. Rodriguez-Lafuente, K. Gorynski, F. Mirnaghi, E.A. Souza-Silva, D. Hein, J. Pawliszyn, Sample preparation with solid phase microextraction and exhaustive extraction approaches: Comparison for challenging cases, Analytica chimica acta 873 (2015) 14-30.

[125] A. Spietelun, Ł. Marcinkowski, M. de la Guardia, J. Namieśnik, Recent developments and future trends in solid phase microextraction techniques towards green analytical chemistry, Journal of Chromatography A 1321 (2013) 1-13.

[126] É.A. Souza-Silva, N. Reyes-Garcés, G.A. Gómez-Ríos, E. Boyacı, B. Bojko, J. Pawliszyn, A critical review of the state of the art of solid-phase microextraction of complex matrices III. Bioanalytical and clinical applications, TrAC Trends in Analytical Chemistry 71 (2015) 249-264.

[127] M. Kuster, M.L. de Alda, D. Barceló, Liquid chromatography–tandem mass spectrometric analysis and regulatory issues of polar pesticides in natural and treated waters, Journal of Chromatography A 1216 (2009) 520-529.

[128] S. Pedersen-Bjergaard, Gas Chromatography (GC), in: S.H.H.a.S. Pedersen-Bjergaard (Ed.), Bioanalysis of Pharmaceuticals2015.

[129] O.D. Sparkman, Z. Penton, F.G. Kitson, Gas chromatography and mass spectrometry: a practical guide, Academic Press2011.

[130] J.A. Masucci, G.W. Caldwell, Techniques for gas chromatography/mass spectrometry, Modern practice of gas chromatography (2004) 339-401.

[131] S.H. Hansen, General chromatographic theory and principles, Bioanalysis of Pharmaceuticals: Sample Preparation, Separation Techniques, and Mass Spectrometry (2015) 31-60.

[132] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC method development, John Wiley & Sons2012.

[133] S.H. Hansen, L. Reubsaet, High-Performance Liquid Chromatography (HPLC) and High-Performance Liquid Chromatography–Mass Spectrometry (LC-MS), Bioanalysis of Pharmaceuticals: Sample Preparation, Separation Techniques, and Mass Spectrometry (2015) 123-172.

[134] O. Núñez, K. Nakanishi, N. Tanaka, Preparation of monolithic silica columns for high-performance liquid chromatography, Journal of Chromatography A 1191 (2008) 231-252.

[135] V. Pérez-Fernández, L.M. Rocca, P. Tomai, S. Fanali, A. Gentili, Recent advancements and future trends in environmental analysis: Sample preparation, liquid chromatography and mass spectrometry, Analytica chimica acta 983 (2017) 9-41.

[136] I. Tarcomnicu, A.L. van Nuijs, W. Simons, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, Simultaneous determination of 15 top-prescribed pharmaceuticals and their metabolites in influent wastewater by reversed-phase liquid chromatography coupled to tandem mass spectrometry, Talanta 83 (2011) 795-803.

[137] M.W. Dong, K. Zhang, Ultra-high-pressure liquid chromatography (UHPLC) in method development, TrAC Trends in Analytical Chemistry 63 (2014) 21-30.

[138] C. Cordero, E. Liberto, B. Sgorbini, P. Rubiolo, C. Bicchi, Chapter 11 - Gas Chromatography, in: Y. Picó (Ed.), Chemical Analysis of Food: Techniques and Applications, Academic Press, Boston, 2012, pp. 311-373.

[139] A. Asati, G.N.V. Satyanarayana, V.T. Srivastava, D.K. Patel, Determination of organochlorine compounds in fish liver by ultrasound-assisted dispersive liquid–liquid microextraction based on solidification of organic droplet coupled with gas chromatography-electron capture detection, Journal of Chromatography A 1561 (2018) 20-27.

[140] D. Xia, L. Gao, S. Zhu, M. Zheng, Separation and screening of short-chain chlorinated paraffins in environmental samples using comprehensive two-dimensional gas chromatography with micro electron capture detection, Analytical and bioanalytical chemistry 406 (2014) 7561-7570.

[141] M.A. Farajzadeh, M.R.A. Mogaddam, S.R. Aghdam, N. Nouri, M. Bamorrowat, Application of elevated temperature-dispersive liquid-liquid microextraction for determination of organophosphorus pesticides residues in aqueous samples followed by gas chromatography-flame ionization detection, Food chemistry 212 (2016) 198-204.

[142] O.S. Olatunji, O.S. Fatoki, B.O. Opeolu, B.J. Ximba, Determination of polycyclic aromatic hydrocarbons [PAHs] in processed meat products using gas chromatography–Flame ionization detector, Food chemistry 156 (2014) 296-300.

[143] J.A. Adamovics, Chromatographic analysis of pharmaceuticals, Routledge2017.

[144] M. Słowik-Borowiec, E. Szpyrka, S. Walorczyk, Gas chromatographic determination of pesticide residues in white mustard, Food chemistry 173 (2015) 997-1005.

[145] S. Dubey, C.P. Shinde, T. Kaur, S.P. Singh, M. Chakraboroty, A. Beotra, S. Jain, Development and validation of a comprehensive method for detection of eighty stimulants, narcotics & other drugs by gas chromatography nitrogen phosphorous detector/mass spectrometry in sports doping analysis, (2016).

[146] M.A. Farajzadeh, M. Bahram, M.R. Vardast, M. Bamorowat, Dispersive liquid-liquid microextraction for the analysis of three organophosphorus pesticides in real samples by high performance liquid chromatography-ultraviolet detection and its optimization by experimental design, Microchimica Acta 172 (2011) 465-470.

[147] J.T. Watson, O.D. Sparkman, Introduction to mass spectrometry: instrumentation, applications, and strategies for data interpretation, John Wiley & Sons2007.

[148] J.N. Gushue, Principles and Applications of Gas Chromatography Quadrupole Time-of-Flight Mass Spectrometry, Comprehensive Analytical Chemistry, Elsevier2013, pp. 255-270.

[149] F.K. Tadjimukhamedov, P.B.C. Forbes, Chapter 10 - Mass Spectrometry and Ion Mobility Spectrometry, in: P.B.C. Forbes (Ed.), Comprehensive Analytical Chemistry, Elsevier2015, pp. 311-329.

[150] D.J. Harvey, Mass Spectrometry—Ionization Methods Overview☆, Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, Elsevier2018.

[151] P. Meena, H.G. Peter, A. Lucy, J.C. Michael, C.S. Juliet, y.S. Courtne, Clearing the Waters A focus on the water quality solutions, (2010) 1-91.

[152] J.-S. Kang, Principles and applications of LC-MS/MS for the quantitative bioanalysis of analytes in various biological samples, Tandem Mass Spectrometry-Applications and Principles, InTech2012.

[153] R. Kostiainen, T.J. Kauppila, Effect of eluent on the ionization process in liquid chromatography–mass spectrometry, Journal of Chromatography A 1216 (2009) 685-699.

[154] F.J. Andrade, J.T. Shelley, W.C. Wetzel, M.R. Webb, G. Gamez, S.J. Ray, G.M. Hieftje, Atmospheric pressure chemical ionization source. 1. Ionization of compounds in the gas phase, Anal. Chem. 80 (2008) 2646-2653.

[155] A. Raffaelli, A. Saba, Atmospheric pressure photoionization mass spectrometry, Mass Spectrometry Reviews 22 (2003) 318-331.

[156] S.J. Bos, S.M. van Leeuwen, U. Karst, From fundamentals to applications: recent developments in atmospheric pressure photoionization mass spectrometry, Analytical and bioanalytical chemistry 384 (2006) 85.

[157] J.J. Pitt, Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry, The Clinical Biochemist Reviews 30 (2009) 19.

[158] R. Dams, T. Benijts, W. Günther, W. Lambert, A.D. Leenheer, Influence of the eluent composition on the ionization efficiency for morphine of pneumatically assisted electrospray, atmospheric-pressure chemical ionization and sonic spray, Rapid communications in mass spectrometry 16 (2002) 1072-1077.

[159] W.M. Niessen, Liquid chromatography-mass spectrometry, Crc Press2006.

[160] J.A. Rodrigues, A.M. Taylor, D.P. Sumpton, J.C. Reynolds, R. Pickford, J. Thomas-Oates, Mass spectrometry of carbohydrates: newer aspects, Advances in carbohydrate chemistry and biochemistry 61 (2007) 59-141.

[161] U. Karst, Wilfried MA Niessen: Liquid chromatography–mass spectrometry, Analytical and Bioanalytical Chemistry 389 (2007) 1303-1304.

[162] F. Hernández, J.V. Sancho, O.J. Pozo, Critical review of the application of liquid chromatography/mass spectrometry to the determination of pesticide residues in biological samples, Analytical and Bioanalytical Chemistry 382 (2005) 934-946.

[163] A. Kruve, R. Rebane, K. Kipper, M.-L. Oldekop, H. Evard, K. Herodes, P. Ravio, I. Leito, Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part I, Analytica chimica acta 870 (2015) 29-44.

[164] Y. Picó, C. Blasco, G. Font, Environmental and food applications of LC–tandem mass spectrometry in pesticide-residue analysis: An overview, Mass spectrometry reviews 23 (2004) 45-85.

[165] F. Hernández, T. Portolés, E. Pitarch, F.J. López, Gas chromatography coupled to high-resolution time-of-flight mass spectrometry to analyze trace-level organic compounds in the environment, food safety and toxicology, TrAC Trends in Analytical Chemistry 30 (2011) 388-400.

[166] R. Peters, A. Stolker, J. Mol, A. Lommen, E. Lyris, Y. Angelis, A. Vonaparti, M. Stamou, C. Georgakopoulos, M. Nielen, Screening in veterinary drug analysis and sports doping control based on full-scan, accurate-mass spectrometry, TrAC Trends in Analytical Chemistry 29 (2010) 1250-1268.

[167] S. Lacorte, A.R. Fernandez-Alba, Time of flight mass spectrometry applied to the liquid chromatographic analysis of pesticides in water and food, Mass spectrometry reviews 25 (2006) 866-880.

[168] T. Čajka, J. Hajšlová, Gas chromatography–high-resolution time-of-flight mass spectrometry in pesticide residue analysis: advantages and limitations, Journal of Chromatography A 1058 (2004) 251-261.

[169] R.E. March, An introduction to quadrupole ion trap mass spectrometry, Journal of mass spectrometry 32 (1997) 351-369.

[170] Y. Song, G. Wu, Q. Song, R.G. Cooks, Z. Ouyang, W.R. Plass, Novel linear ion trap mass analyzer composed of four planar electrodes, Journal of The American Society for Mass Spectrometry 17 (2006) 631-639.

[171] K.R. Jonscher, J.R. Yates III, The quadrupole ion trap mass spectrometer—a small solution to a big challenge, Analytical biochemistry 244 (1997) 1-15.

[172] T. Reemtsma, Liquid chromatography–mass spectrometry and strategies for trace-level analysis of polar organic pollutants, Journal of Chromatography A 1000 (2003) 477-501.

[173] W. Niessen, P. Manini, R. Andreoli, Matrix effects in quantitative pesticide analysis using liquid chromatography–mass spectrometry, Mass Spectrometry Reviews 25 (2006) 881-899.

[174] R. King, R. Bonfiglio, C. Fernandez-Metzler, C. Miller-Stein, T. Olah, Mechanistic investigation of ionization suppression in electrospray ionization, Journal of the American Society for Mass Spectrometry 11 (2000) 942-950.

[175] T. Benijts, R. Dams, W.E. Lambert, A. De Leenheer, Countering matrix effects in environmental liquid chromatography–electrospray ionization tandem mass spectrometry water analysis for endocrine disrupting chemicals, Journal of Chromatography A 1029 (2004) 153-159.

[176] B. Matuszewski, M. Constanzer, C. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC− MS/MS, Anal. Chem. 75 (2003) 3019-3030.

[177] A. Samsidar, S. Siddiquee, S.M. Shaarani, A review of extraction, analytical and advanced methods for determination of pesticides in environment and foodstuffs, Trends in Food Science & Technology (2017).

[178] E. Pitarch, T. Portoles, J.M. Marin, M. Ibanez, F. Albarran, F. Hernandez, Analytical strategy based on the use of liquid chromatography and gas chromatography with triple-quadrupole and time-of-flight MS analyzers for investigating organic contaminants in wastewater, Analytical and Bioanalytical Chemistry 397 (2010) 2763-2776.

[179] M. Vogeser, F. Kirchhoff, Progress in automation of LC-MS in laboratory medicine, Clinical biochemistry 44 (2011) 4-13.

[180] R. Rosen, Mass spectrometry for monitoring micropollutants in water, Current Opinion in Biotechnology 18 (2007) 246-251.

[181] E. Gracia-Lor, M. Martínez, J.V. Sancho, G. Peñuela, F. Hernández, Multi-class determination of personal care products and pharmaceuticals in environmental and wastewater samples by ultra-high performance liquid-chromatography-tandem mass spectrometry, Talanta 99 (2012) 1011-1023.

[182] E. Vulliet, M. Tournier, A. Vauchez, L. Wiest, R. Baudot, F. Lafay, A. Kiss, C. Cren-Olivé, Survey regarding the occurrence of selected organic micropollutants in the groundwaters of overseas departments, Environmental Science and Pollution Research 21 (2014) 7512-7521.

[183] J. Nogueira, T. Sandra, P. Sandra, Multiresidue screening of neutral pesticides in water samples by high performance liquid chromatography–electrospray mass spectrometry, Analytica chimica acta 505 (2004) 209-215.

[184] R. Celano, A.L. Piccinelli, L. Campone, L. Rastrelli, Ultra-preconcentration and determination of selected pharmaceutical and personal care products in different water matrices by solid-phase extraction combined with dispersive liquid–liquid microextraction prior to ultra high pressure liquid chromatography tandem mass spectrometry analysis, Journal of Chromatography A 1355 (2014) 26-35.

[185] T.L. Potter, M.A. Mohamed, H. Ali, Solid-phase extraction combined with high-performance liquid chromatography− atmospheric pressure chemical ionization− mass spectrometry analysis of pesticides in water: Method performance and application in a reconnaissance survey of residues in drinking water in Greater Cairo, Egypt, Journal of agricultural and food chemistry 55 (2007) 204-210.

[186] J. Ma, R. Xiao, J. Li, X. Zhao, B. Shi, S.g. Li, Determination of organophosphorus pesticides in underground water by SPE-GC-MS, Journal of chromatographic science 47 (2009) 110-115.

[187] E. Rianawati, R. Balasubramanian, Optimization and validation of solid phase micro-extraction (SPME) method for analysis of polycyclic aromatic hydrocarbons in rainwater and stormwater, Physics and Chemistry of the Earth, Parts A/B/C 34 (2009) 857-865.

[188] P.K. Sahu, N.R. Ramisetti, T. Cecchi, S. Swain, C.S. Patro, J. Panda, An overview of experimental designs in HPLC method development and validation, Journal of pharmaceutical and biomedical analysis (2017).

[189] B. Dejaegher, Y. Vander Heyden, Ruggedness and robustness testing, Journal of chromatography A 1158 (2007) 138-157.

[190] L. Mousavi, Z. Tamiji, M.R. Khoshayand, Applications and opportunities of experimental design for the dispersive liquid–liquid microextraction method-A review, Talanta (2018).

[191] C.R.T. Tarley, G. Silveira, W.N.L. dos Santos, G.D. Matos, E.G.P. da Silva, M.A. Bezerra, M. Miró, S.L.C. Ferreira, Chemometric tools in electroanalytical chemistry: methods for optimization based on factorial design and response surface methodology, Microchemical journal 92 (2009) 58-67.

[192] B. Dejaegher, Y. Vander Heyden, Experimental designs and their recent advances in set-up, data interpretation, and analytical applications, Journal of pharmaceutical and biomedical analysis 56 (2011) 141-158.

[193] A. Mpupa, G.P. Mashile, P.N. Nomngongo, Ultrasound-assisted dispersive solid phase nanoextraction of selected personal care products in wastewater followed by their determination using high performance liquid chromatography-diode array detector, Journal of Hazardous Materials (2018).

[194] B. Dejaegher, Y. Vander Heyden, The use of experimental design in separation science, Acta Chromatographica 21 (2009) 161-201.

[195] N.A. Armstrong, Pharmaceutical experimental design and interpretation, CRC Press2006.

[196] S.L.C. Ferreira, R.E. Bruns, E.G.P. da Silva, W.N.L. Dos Santos, C.M. Quintella, J.M. David, J.B. de Andrade, M.C. Breitkreitz, I.C.S.F. Jardim, B.B. Neto, Statistical designs and response surface techniques for the optimization of chromatographic systems, Journal of chromatography A 1158 (2007) 2-14.

CHAPTER 3:
GENERAL EXPERIMENTAL METHODS

This chapter describes the experimental work including sample preparation and instrumentation techniques applied in achieving the objectives of this study. The application of materials used for sample extraction and preconcentration are also highlighted. However, the details of the sample preparation procedures are enumerated in the subsequent chapters (4-7).

3.1 REAGENTS AND MATERIALS

The analytical standards methylparaben, ethylparaben, propylparaben, triclosan, azinphos-methyl, ethoprofos, chlorpyrifos and parathion-methyl and were all purchased from Merck (Darmstadt, Germany). Methanol, acetonitrile and formic acid were of HPLC grade and ultra-high pure water (18mΩ) was used throughout the experimental runs. All other reagents and chemicals were of analytical grade. Working standard solutions were prepared by appropriate dilution with deionized water. Hydrochloric acid and sodium hydroxide solutions were used in adjusting the pH of the samples. Filtration of environmental samples was done using 0.45 µm PVDF syringe filters. Commercially based SPE sorbents (Oasis HLB) were obtained from Merck (Darmstadt, Germany). Environmental samples were collected from a local wastewater treatment plant in Gauteng province in South Africa. Samples were obtained from primary, secondary and tertiary treatment stages of the WWTP.

3.2 ENVIRONMENTAL WASTEWATER SAMPLES

Waste water sampled were obtained from a local wastewater treatment plant in Gauteng province in south Africa. Pre-cleaned sampling bottles were used for sampling in triplicate. After collection of the samples, they were placed in cooler boxes containing ice, transported to the laboratory and stored at 4 °C prior to filtration, extraction and instrumental analysis. The samples comprised of influent raw wastewater, primary influent, secondary influent and final effluent. The samples were analyzed for the selected organic contaminants and used for validation of the developed methods in the study for suitability in real life applications. All the samples were analyzed alongside the reagent blanks and un-spiked ultrapure water, to ensure no contamination in the equipment and the analytical procedures. Positive identification of target analytes in the samples was based on the LC retention time and ion ratio abundance of

the MRM transitions of the quantifier and qualifier ions. The retention time of the positive samples was compared to the analytical standards with acceptable deviation of ± 0.10 min

3.3 SAMPLE PREPARATION TECHNIQUES

3.3.1 Solid phase extraction

Solid phase extraction was carried out using Oasis HLB cartridges and synthesized carbon nanodots (CNDs), for separation of parabens and organophosphorus pesticides in water samples. The CNDs were dry packed in empty 3 ml SPE cartridges prior to extraction. The experimental factors such as sample pH, sample volume, elution solvent, elution volume and mass of adsorbent were optimized by either univariate or multivariate approach. Factorial design was used for screening of variables while central composite design was used in optimizing the experimental conditions. SPE vacuum manifold was used in automatically loading the samples through the preconditioned cartridges as seen in **Figure 3.1**.
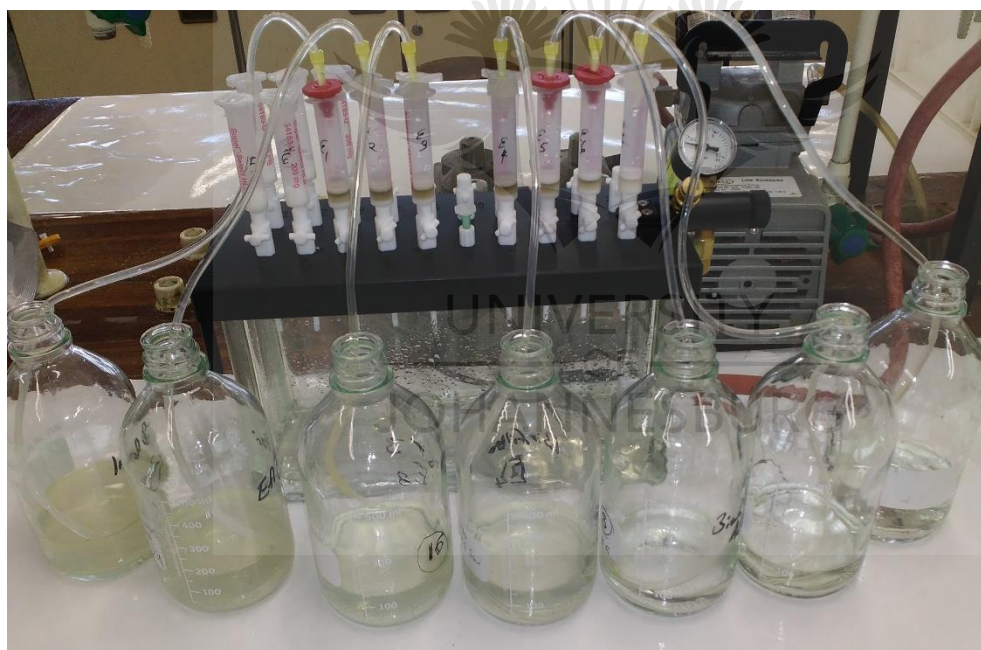
Figure 3.1: SPE set up for extraction of parabens in wastewater samples using Oasis HLB cartridges

3.3.2 Vortex assisted- dispersive liquid-liquid extraction

This technique was used for extraction of ethoprofos, azinphos-methyl and parathion methyl in wastewater samples. Chloroform and acetone were employed as optimum extractant and disperser solvents. The sample solution and the organic solvents were vortex mixed and the analytes were extracted into chloroform droplets. Centrifugation was used to separate the two

immiscible liquids formed. The sedimented liquid (chloroform) was obtained and transferred to a vial where it was evaporated to dryness. The residue was re-dissolved in mobile phase prior to instrumental analysis. Chemometric techniques were used in the optimization of final experimental conditions. The variables optimized were, sample pH, disperser volume (mL), extractant volume (mL).

3.3.3 Ultrasonic-assisted magnetic solid phase dispersive extraction

In this procedure, magnetized CNDs were used for the extraction of triclosan and chlorpyrifos in environmental water samples under ultrasonic dispersion of the nanomaterial in the sample solution. The experimental variables optimized using central composite design include sample pH, mass of the adsorbent (mg) and extraction time (minutes). The elution solvent was however optimized univariately. The magnetic properties of the magnetic material were confirmed by the placing an external magnet on the wall of the sample bottle for rapid separation of the nanocomposite material from aqueous solution after extraction. A vibrating sample magnetometer (VSM) was also used to ascertain the magnetic properties of the magnetic material.

3.4 SYNTHESIS OF NANOMATERIALS

3.4.1 Green synthesis of carbon nanodots

In this study, carbon nanodots were synthesized following a previously published method in literature with slight modification [1]. In a nutshell, 10 g of oats cereal were weighed, crushed to fine powder, placed in a crucible and thereafter transferred to the muffle furnace and pyrolyzed at 400 °C for 2 hrs. The color of the obtained product was black and was allowed to cool at ambient temperature before being further crushed to fine powder. The material was then dispersed in ultra-pure water and thereafter centrifuged at 7800 rpm to remove the larger particles. The carbon nanodots aqueous suspension was filtered and the CNDs residue dried in an oven for 24 hrs. at 80 ˚C. A schematic representation of this synthesis is shown in **Figure 3.2**.
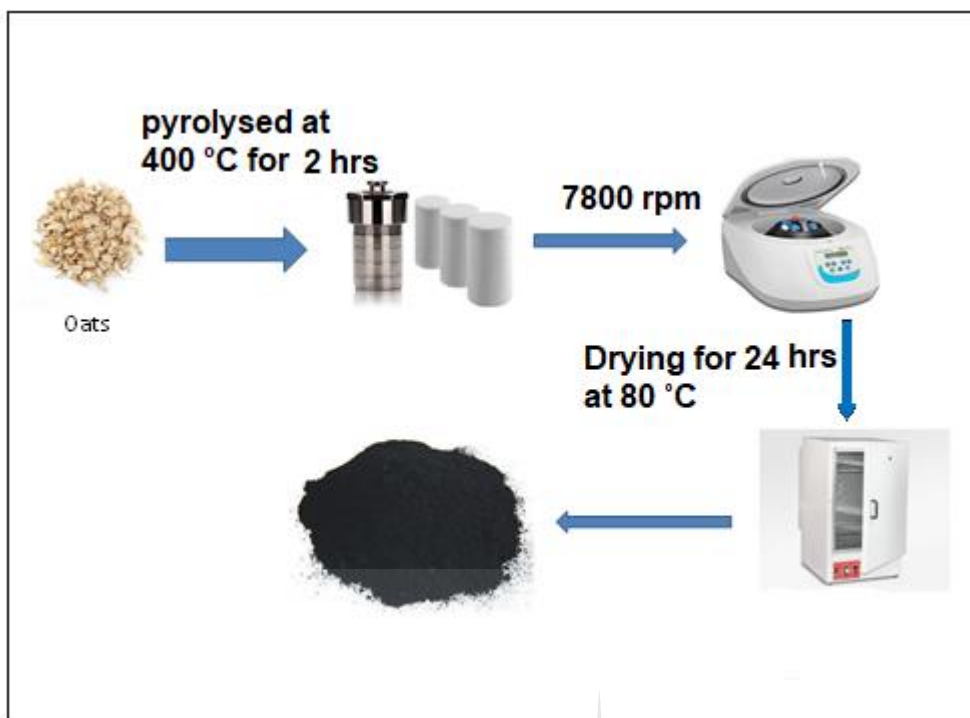
Figure 3.2: Synthesis of carbon nanodots from oats

3.4.2 Synthesis of Fe₃O₄ and Fe₃O₄@CNDs

Magnetite (Fe_3O_4) nanoparticles were synthesized using a chemical co-precipitation procedure from a method reported in literature with minor modifications [2, 3]. 16 g of $FeCl_3 \cdot 6H_2O$ and 7 g of $FeCl_2 \cdot 4H_2O$ were dissolved in 150 mL deionized water under nitrogen atmosphere with vigorous magnetic stirring under a heated oil bath at 90 ^{o}C. Afterward, 50 mL of ammonia solution (25 % v/v) was added rapidly into the above solution. The mixture was stirred for another 30 minutes under the same conditions. After the reaction finalized, the solution was cooled to room temperature. The resulting black Fe_3O_4 nanoparticles were collected by magnetic decantation and washed severally with de-ionized water and ethanol with centrifugation (7800 rpm). The synthesized nanoparticles were then dried at 60 ^{o}C for 6 hrs. in an oven, then further ground to finer particles. The obtained magnetic Fe_3O_4 nanoparticles were used in functionalizing the carbon nanodots for magnetic solid phase extraction. This was accomplished using a method from literature with slight alterations [4, 5]. Briefly, a simple co-mixing method with magnetic stirring was employed. 250 mg of the prepared pristine CNDs was dissolved in 50 mL ethanol under ultrasonication. Then 1 g of Fe_3O_4 nanoparticles was dispersed into the prepared CNDs solution and the mixture was subjected to overnight stirring at room temperature. After this process, the obtained nanocomposite was separated by an

external magnet and washed with ultrapure water severally and then dried at 60 ºC for 6 hrs. for further use.

3.5 CHARACTERIZATION TECHNIQUES

The characterization of the synthesized nanomaterials was assessed using various spectroscopic techniques. Firstly, scanning electron microscope (SEM) was employed in getting images of the CNDs, that detailed the morphology of the surface of the CNDs. The images were taken using Vegas TC3 software. An accelerating voltage of 10KV was used in operating the instrument. A high-resolution transmission electron microscope (HR-TEM) was used in further characterizing the CNDs and the magnetic-CNDs. Prior to the analysis the samples were first dispersed in ethanol under ultrasonication for 10 minutes, then a drop of the sample solution was placed on a copper grid ready for analysis. Fourier transform infrared (FTIR) spectrophotometer was used to establish the functional groups on the surface of the nanomaterials. The CNDs or magnetic-CNDs were mixed with potassium bromide (KBr) at a ratio of 1:100 and, then compressed with a hydraulic press to from 1 mm discs, prior to the analysis. X-ray diffraction (XRD) was carried out to investigate the crystalline nature of the nanomaterials. X-ray diffraction (XRD) was measured using a PANalytical X'Pert PRO X-ray diffractometer in a ranging at 4–90° of 2θ at room temperature.

3.6 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

The UHPLC was equipped with two LC-30AD pumps, DGU-20A5R degasser unit, a SIL-30AC nexera autosampler, and a CTO-30A column oven, was used for the analysis. The UHPLC was coupled to an LC-MS 8040 triple quadrupole mass spectrometer, installed with orthogonal electrospray ionization (ESI) source. A raptor ARC-18 column (100 mm x 2.1 mm, 3 μm) coupled with a C18 guard column (2.1 × 5 mm, 1.8 μm, RESTEK, USA) was used for the chromatographic separations. Peak detection, instrument control, data analysis, method optimization was carried out using LabSolutions (Tokyo, Japan). The mass spectrometry detection was acquired in multiple reaction monitoring (MRM) mode. The total ion chromatogram (TIC) obtained for the compounds in this study together with the mass spectra are highlighted in the appendices (**Figure A1-A8**). The matrix-matched calibration curves used for quantification are also shown in the appendices (**Figure A9-A10**).

3.7 REFERENCES

[1] L. Shi, X. Li, Y. Li, X. Wen, J. Li, M.M. Choi, C. Dong, S. Shuang, Naked oats-derived dual-emission carbon nanodots for ratiometric sensing and cellular imaging, Sensors and Actuators B: Chemical 210 (2015) 533-541.

[2] Z. Liu, Y. Liu, K. Yao, Z. Ding, J. Tao, X. Wang, Synthesis and magnetic properties of Fe 3 O 4 nanoparticles, Journal of materials synthesis and processing 10 (2002) 83-87.

[3] A.H. Lu, E.e.L. Salabas, F. Schüth, Magnetic nanoparticles: synthesis, protection, functionalization, and application, Angewandte Chemie International Edition 46 (2007) 1222-1244.

[4] S. Gwebu, P. Nomngongo, P. Mashazi, T. Nyokong, N. Maxakato, Platinum Nanoparticles Supported on Carbon Nanodots as Anode Catalysts for Direct Alcohol Fuel Cells, Int. J. Electrochem. Sci 12 (2017) 6365-6378.

[5] Y. Guo, J. Qian, A. Iqbal, L. Zhang, W. Liu, W. Qin, Pd nanoparticles immobilized on magnetic carbon dots@Fe3O4 nanocubes as a synergistic catalyst for hydrogen generation, International Journal of Hydrogen Energy 42 (2017) 15167-15177.

CHAPTER 4:

FACTORIAL DESIGN OPTIMISATION OF SOLID PHASE EXTRACTION FOR PRECONCENTRATION OF PARABENS IN WASTEWATER USING ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TRIPLE QUADRUPOLE MASS SPECTROMETRY

ABSTRACT

The solid phase extraction (SPE) method for preconcetration of three parabens in wastewater and subsequent determination using liquid chromatography-mass spectrometry (UHPLC-MS/MS) was successfully optimized and developed. A two-level (2^k) full factorial design was used for investigation of experimental variables that have the most significant effect on the analytical response. According to the ANOVA results sample pH and eluent volume were the statistically most significant parameters. The method developed was validated for accuracy, precision, limits of detection (LOD), quantification (LOQ) and linearity. The LODs and LOQs established under those optimized conditions varied between 0.08-0.12 μgL^{-1} and 0.14-0.40 μgL^{-1} respectively. The linearity ranged between 5-100 $\mu g\ L^{-1}$ with good determination coefficient ($r^2 > 0.995$). The use of matrix-matched external calibration provided extraction recoveries between 70-120 % with relative standard deviations at 2-11% for two spike levels (10 and 100 μgL^{-1}) in three different water matrices (simulated wastewater, influent and effluent water). Finally, the method was applied to the analyses of parabens in wastewater samples at different sampling points of a wastewater treatment plant, revealing the presence of parabens at concentrations up to 3 μgL^{-1}.

Keywords: Factorial design, Methylparaben, Ethylparaben, Propylparaben, Solid phase extraction, LC-MS/MS, Wastewater

4.1 INTRODUCTION

Parabens belong to a group of synthetic esters of p-hydroxybenzoic acid. They include methylparaben (MePB) ethylparaben (EthPB), propylparaben (PrpPB), butylparaben (BuPB), isobutylparaben (IBPB), isopropylparaben (IPPB) and benzylparaben (BePB) [1]. These compounds have a widespread application in personal care products such as cosmetics, toiletries, pharmaceuticals and food, as preservatives and bactericides [1].

The extensive use of the products containing these compounds has brought about a great concern to the potential health effects. This is because it poses to humans over a prolonged period of exposure through inhalation, ingestion or dermal contact. These compounds have been reported to exhibit endocrine disruptive properties, that can lead to adverse effects such as the development of breast cancer. They are also reported to affect male reproductive functions as a result of the combination of oestrogenic and anti-androgenic properties [1-3]. The prevalent use of parabens has resulted in their abundant concentrations in the environment. Despite these compounds being biodegradable under aerobic conditions, they remain ubiquitous in the environment due to constant consumption and nonstop entry into the environment. One of the main sources of the introduction of the parabens into the aquatic environment is urban wastewater [4]. Because of inadequate removal of these compounds during the treatment processes, they are potentially released into the environment through effluent discharge and subsequently entering drinking water sources [5]. Therefore, it is of utmost importance to monitor the levels these compounds in wastewater.

Various extraction techniques either conventional or newly developed, have been employed for the determination of parabens in wastewater. They include dispersive liquid-liquid microextraction (DLLME) [6], solid phase microextraction (SPME) [7], dispersive ionic liquid (IL)-DLLME [8], magnetic solid phase extraction (MSPE) [9], rotating disk sorptive extraction (RDSE) [10], among many others. However, the most common and robust extraction and pre-concentration method, for extraction of parabens is solid phase extraction (SPE) [2, 7]. This is largely due to its versatility in retaining these compounds and the availability of a wide array of adsorbents, chemistries and sizes of the SPE cartridges, making it robust and selective extraction technique [11]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the most frequently used method for determination of parabens due to its sensitivity, selectivity and very low detection levels (μg L^{-1} to ng L^{-1}) [12]. In addition, no derivatization is required as it is the case with gas chromatography (GC) analysis [10, 13]. Ultra-high-performance liquid chromatography technique (UHPLC) uses of sub-2-μm particle size columns which makes it

more favorable over HPLC as it tremendously improves resolution with increased peak capacity and shortened analysis times [14].

This study is aimed at developing and validating a robust novel analytical technique to preconcentrate and determine three parabens namely, methylparaben (MePB), ethylparaben (EthPB) and propylparaben (ProPB), in wastewater using SPE and UHPLC-MS/MS. Experimental factors (sample pH, sample volume and eluent volume) were optimized using a two-level (2k) full factorial design in conjunction with response surface methodology (RSM). The chemometric approach is advantageous in that it decreases the number of experimental runs resulting in reduced analysis times reagents, sample volumes as well as the cost of analysis [10].

In this study, Oasis HLB SPE cartridges as the adsorbent for preconcentration of these parabens in synthetic and wastewater samples. The Oasis HLB cartridge was selected for the analysis because it has been reported to have high rates of recovery for most compounds including parabens, from water samples [1, 11, 15]. In addition, it is characterized by a hydrophilic-lipophilic balance that facilitates the wetting properties of the hydrophilic N-vinylpyrrolidine monomer. This makes it most suitable for the extraction of acidic analytes, without acidifying the sample, as well as extraction of neutral analytes, over a wide range of polarity [16]. A simulated wastewater matrix was employed throughout the method development and validation stages, in contrast to using spiked deionized water, as is commonly reported [10, 17]. This approach was adopted to mimic the real environmental sample from the onset of the method development procedures. To the best of our knowledge, this approach has not been employed for the extraction of parabens in wastewater samples.

4.2 EXPERIMENTAL

4.2.1 Chemical and reagents

Methylparaben (MePB), ethylparaben (EthPB) and propylparaben (ProPB) were all purchased from Merck (Darmstadt, Germany). Individual stock solutions (1000 mgL^{-1}) were prepared in methanol, as well as the mixed solution of the three analytes and stored at -18 ^{o}C until use. 10 mgL^{-1} standard working solution was prepared in methanol. Calibration standards were prepared daily in matrix solution (simulated wastewater) from the 10 mgL^{-1} stock solution. HPLC grade methanol and formic acid (98 % purity) were supplied by Merck (Darmstadt, Germany). Ultrapure water from Millipore filtration system with a specific resistance of 18.2 MΩcm was used for preparing the matrix solution. The simulated wastewater was prepared

using, urea, meat extract, peptone, sodium chloride (NaCl), Dipotassium hydrogen phosphate (K_2HPO_4), Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) and Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H2O$) were purchased from Merck (Darmstadt, Germany).

4.2.2 Sample collection

Water samples were obtained from Pretoria (Daspoort wastewater treatment plant), which was the representative of urban and domestic activities. The WWTP is divided into two plants, east and west. There were 7 sampling sites on the east plant and 6 on the west plant.

Sampling was carried out in pre-cleaned sampling bottles. Prior to the collection, the bottles were rinsed thrice with the sample. A tracer (fluorescein sodium salt) was dosed before and after each process unit to validate the design of the WWTP regarding the hydraulic retention time (HRT). The samples were collected before and after each process unit (two samples per sampling site), while observing the retention times calculated by the use of the tracer [1]. The samples were packed in cooler boxes containing ice, transported to the laboratory and were refrigerated at 4 °C.

4.2.3 Solid phase extraction procedure

Extraction of parabens from the wastewater samples was performed using Oasis HLB cartridges (6 mL, 200 mg). Prior to the extraction, the samples were filtered on a Millipore filtration unit using 0.45 µm filter paper to remove any suspended matter that may otherwise interfere with the SPE extraction due to clogging. 0.1 M sodium hydroxide (NaOH) was used in adjustment of the sample pH. Before commencement of SPE extraction, 5 mL methanol, followed by 5 mL ultra-pure water (UPW) was used in conditioning the cartridges. Thereafter, the filtered water samples were percolated into the pre-conditioned cartridge with the aid of an SPE vacuum manifold. After sample loading, de-ionized water (5 mL) was passed to clean the cartridge before vacuum drying for 15 minutes. 6 mL of methanol was then used in the elution of the retained analytes.

4.2.4 Design of experiment

A multivariate experimental design was employed for optimization of SPE experimental conditions. The conventional way of varying one variable at a time does not guarantee that the results obtained are optimum. This is because the interaction between variables is not taken into account [2]. The simultaneous interaction of various parameters influences the overall

analytical response. In this study, the design of experiment (DOE) based optimization approach was used to investigate the effect of three dependent variables that influence the analytical response in the extraction of parabens from wastewater. The variables studied were sample volume, elution volume and sample pH. A two-level (2^3) (where 2 is the number of levels and 3 is the number of factors) full factorial design was initially employed in the optimization as shown in **Table 4.1,** resulting in a total of 11 experiments. The levels for each variable are assigned either as high, low or central values, as indicated in **Table 4.1.** This DOE was accomplished using Statistica version 8 (StatSoft, USA)**.**

Table 4.1: Experimental variables and levels used in 2^3 factorial design for SPE of parabens in wastewater.

Variable	Low level (-1)	Central Point (0)	High Level (+1)
pH	3.5	6.5	9.5
Sample Volume (mL)	50	125	200
Elution Volume (mL)	3	4.5	6

4.2.5 Liquid chromatography-tandem mass spectrometry conditions

Chromatographic experimental runs were conducted using a Shimadzu Nexera Ultra High-Performance Liquid Chromatography (Tokyo, Japan). Separation of the analytes was obtained using a pinnacle DB biphenyl column of 100 x 2.1 mm and 3µm particle size (RESTEK, USA). The column compartment was maintained at 40 ^{0}C whereas the autosampler was kept at 4 ^{0}C. The mobile phase used for the gradient elution comprised of 0.1 % formic acid in de-ionized water (mobile phase A) and 0.1 % formic acid in methanol (mobile phase B). The initial starting conditions of the mobile phase gradient started with 50 % of B held for 0.5 minutes, followed by a linear ramp to 95 % of B in 3 minutes. This was held for a further 2 minutes, with a post-run time of 7 minutes for re-equilibration back to original conditions of the mobile phase. The flow rate used was 0.2 mL min $^{-1}$ and the injection volume of 30 µl was used for all the analyses. An LCMS 8030 (Shimadzu, Japan) triple quadrupole mass spectrometer with an electrospray ionization (ESI) manifold, was used in acquiring data in the multiple reaction monitoring modes (MRM). Nitrogen gas was used as desolvation gas and argon as the collision gas. The optimum conditions for MS analyses were: nebulizing gas flow 3 Lmin^{-1}drying gas flow 15 Lmin^{-1}; DL temperature 250 ^{0}C; heat block 400 ^{0}C; probe voltage 4.5Kv. Peak detection,

instrument control, data analysis and method optimization were carried out using LabSolutions software (Tokyo, Japan).

4.2.6 Method validation

The method development and validation were carried out using simulated wastewater which was prepared according to the guidelines stipulated in OECD 303A. The assay preparation is shown in **Table 4.2**. This solution contains 25 mg L^{-1} of DOC (dissolved organic carbon) [3]. To reduce the effect of matrix on the experimental run, matrix-matched standards were employed throughout the method development and validation. Recovery studies on the extraction efficiency were determined by spiking 3 different water matrices at two concentration levels, 10 µg L^{-1} and 100 µg L^{-1}, before and after SPE. The precision of the method was established using repeatability and reproducibility runs with repeated injections (n=6). Linearity, LOD and LOQ were determined using matrix- matched solutions

Table 4.2: Constituents of simulated wastewater

Constituents	Concentration (mg L^{-1})
Peptone	160
Meat Extract	110
Urea	30
Anhydrous dipotassium hydrogen phosphate	28
Sodium chloride	7
Calcium chloride dihydrate	4
Magnesium sulphate heptahydrate	2

4.3 RESULTS AND DISCUSSION

4.3.1 Factorial Design

A two-level full factorial experimental design was applied for the optimisation of solid phase extraction of methylparaben, (MePB) ethylparaben (EthPB), and propylparaben (ProPB) in aqueous samples. The factors affecting the solid phase extraction that were assessed in this study were sample pH, sample volume (SV) and elution volume (EV). The pH of the solution is very important as it determines the state in which the analytes exist and thereby the interactions between the analytes and the adsorbent [4]. The sorbent type selected for SPE extraction was the polymeric reversed-phase Oasis hydrophilic-lipophilic (HLB) sorbent and it has been proven to be efficient in obtaining better recoveries in the various literature [5, 6].

The factors that have the potential to affect the analytical response of parabens were simultaneously investigated using a two-level (2^3) full factorial design with triplicates of the central point [7]. The mean % recovery was used as the analytical response. The factorial design matrix and the analytical response are presented in **Table 4.3**. Analysis of variance (ANOVA) was conducted on the analytical results in order to establish the reliability of the model [8]. As observed in **Figure 4.1,** there is a good relationship between the predicted and observed experiment data for methylparaben.

Table 4.3: Experimental design using two-level full factorial design with their corresponding and analytical responses.

				MePB	EthPB	ProPB
Experimental Runs	pH	SV	EV		% Recovery	
1	3.5	50.0	3.0	101	89	75
2	9.5	50.0	3.0	114	104	89
3	3.5	200.0	3.0	104	94	77
4	9.5	200.0	3.0	114	101	84
5	3.5	50.0	6.0	107	100	90
6	9.5	50.0	6.0	120	112	104
7	3.5	200.0	6.0	102	96	82
8	9.5	200.0	6.0	107	102	87
9	6.5	125.0	4.5	110	95	88
10	6.5	125.0	4.5	107	100	90
11	6.5	125.0	4.5	110	101	87
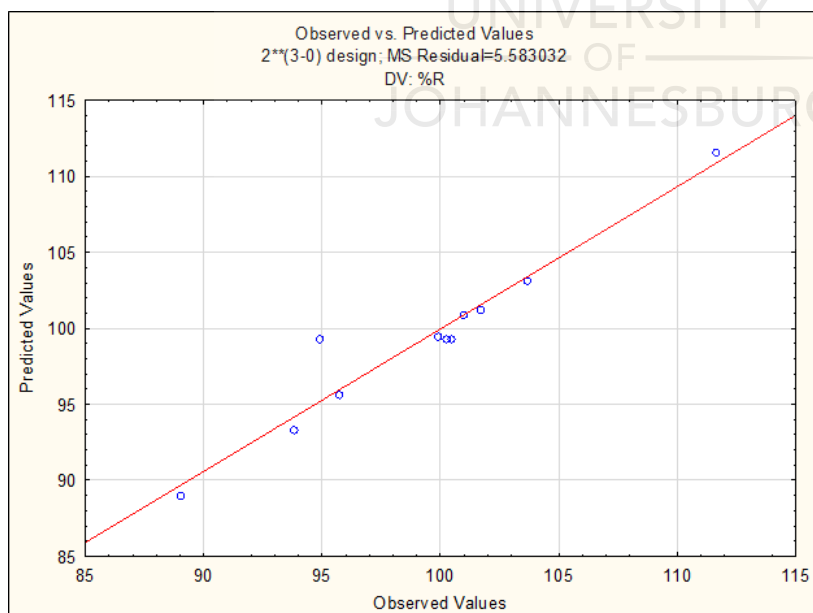
Figure 4.1: The plot of predicted versus experimental values on methylparaben extraction yield

The analytical results in **Table 4.3** were also evaluated using ANOVA as represented in the Pareto charts in **Figure 4.2.** The charts aid in visualization of the main effects and their interactions. The red line indicated on the Pareto chart determines whether the main parameter or an interactive effect is statistically significant at 95 % confidence level ($p \leq 0.05$) [7, 8]. It can be observed in the Pareto chat that sample pH was the most significant parameter on the analytical response for the three parabens. This is also confirmed by the coefficient values for each of the 3 compounds, which is largest for pH. For MePB, three factors had the most significant effect on the analytical response at 95% confidence level. They are pH, the interactive effect of SV & EV, and lastly the individual effect of SV. For EthPB, only pH and EV were significant at 95% confidence level. ProPB had the highest number of factors that were significant at 95% confidence level. They included the main parameters i.e pH, SV, EV, interactive effects of pH with SV, and pH with EV. Also, it can be observed overall that elution volume (EV) and pH were the only parameters that positively affected the analytical response across all the three compounds. This is as shown with the algebraic sign on the coefficient (+ or -) on each parameter in the Pareto chart **(Figure 4.2).** A positive sign implies that as the factor increases, the analytical response also increases whereas a negative sign on the coefficient connotes that as the factors increase the analytical response is decreased. The strength of the relationship is also depicted by the absolute value of the coefficient [9].
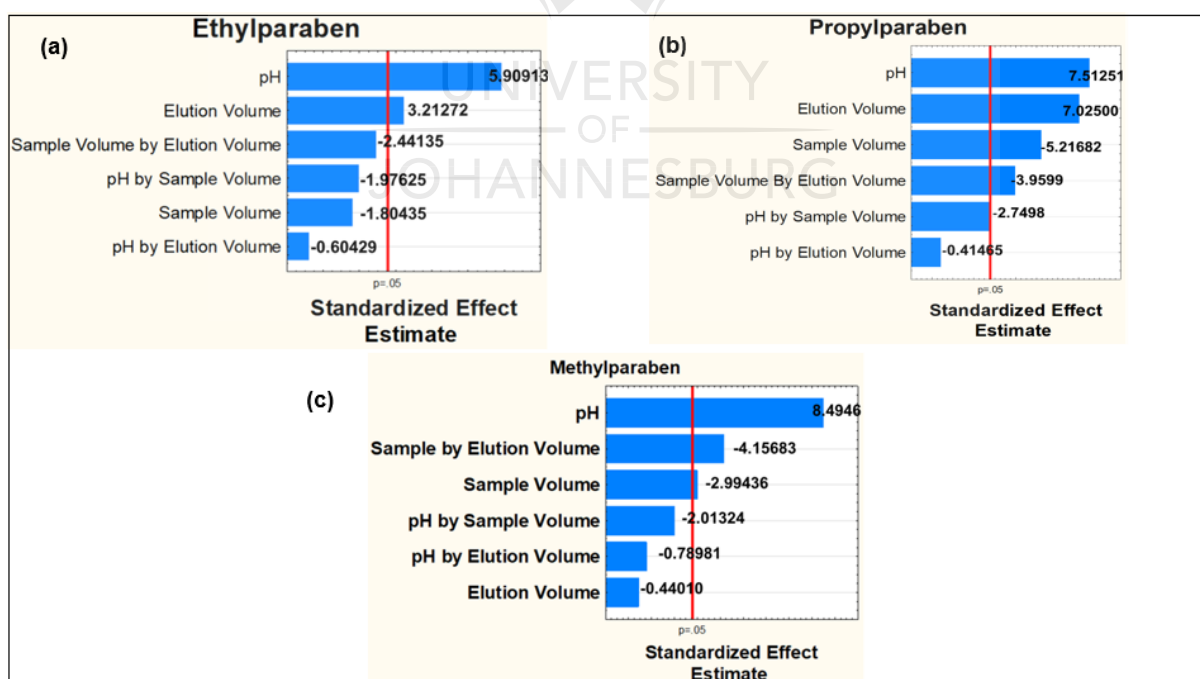
Figure 4.2: Pareto chart of standardised effects for variables in the solid phase extraction of MePB, EthPB, ProPB

4.3.2 Response surface plots

A graphical representation in the form of a three-dimensional (3D) response surface plots was drawn to show the relationship between the three independent variables (pH, SV and EV) and the analytical response [10, 11]. The 3D plot also shows the kind of interaction between the two test parameters. The responses were plotted against two experimental parameters while keeping the third parameter constant at its central value. **Figure 4.3, 4.4** & **4.5** shows the response surface plots for the three compounds. **Figure 4.3** (a & c), **Figure 4.4** (a & c) and **Figure 4.5** (a & c) show that as the pH is increased, the percentage recovery on extraction yield is increased. The interactive effect of the sample pH and SV for the three compounds is such that at high pH and low sample volume, higher extraction yield is obtained. This is because, at basic media, there is ion-exchange and also because the pK_a of parabens is at 8.4, the hydroxyl group dissociation is higher at (pH>8.4) rendering the analytes to be anionic [6, 12, 13]. **Figure 4.3 (b)**, **Figure 4.4 (b)** & **Figure 4.5** (b) shows interactive effect between sample volume and elution volume for MePB, EthPB & ProPB, respectively. When EV is increased and SV is decreased, it results in high extraction yield of the analyte. When both EV and SV are high, there is a decrease in extraction yield. This is because at high SV, the cartridge is depleted of the active sites, and therefore the analytes can no longer be adsorbed due to saturation. In view of the above experimental data, the target of obtaining high extraction yield as seen in the % recovery was achieved. The optimum conditions that resulted in high % recovery as shown in bold in **Table 4.3** were determined as follows; pH 9.5, sample volume 50 mL, and elution volume of 6 mL.

It can be observed also in **Figure 4.5** that the analytical response of ProPB was slightly lower as compared to the MePB and EthPB. This is because of the difference in polarity due to the longer alkyl chain length of ProPB.
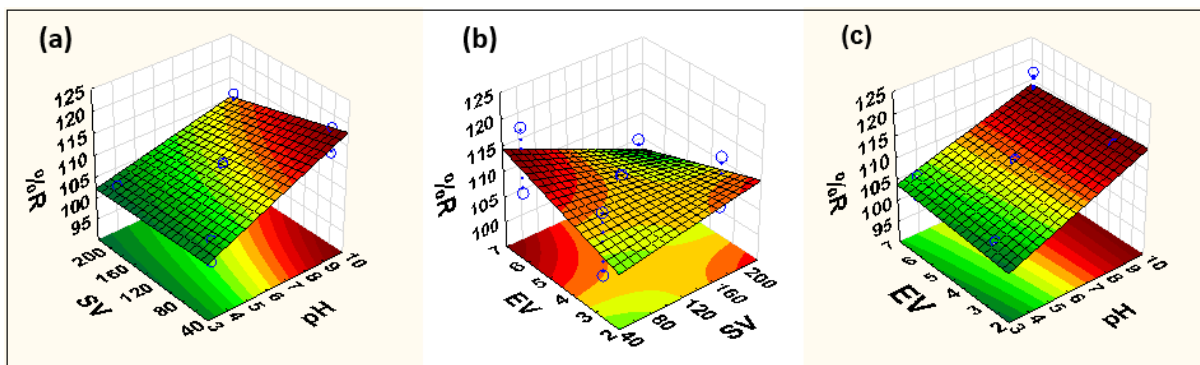
Figure 4.3: Response surface plot for interactive effects between SV and pH for (a) EV and SV in (b) and interaction between EV and pH in (c) for MePB
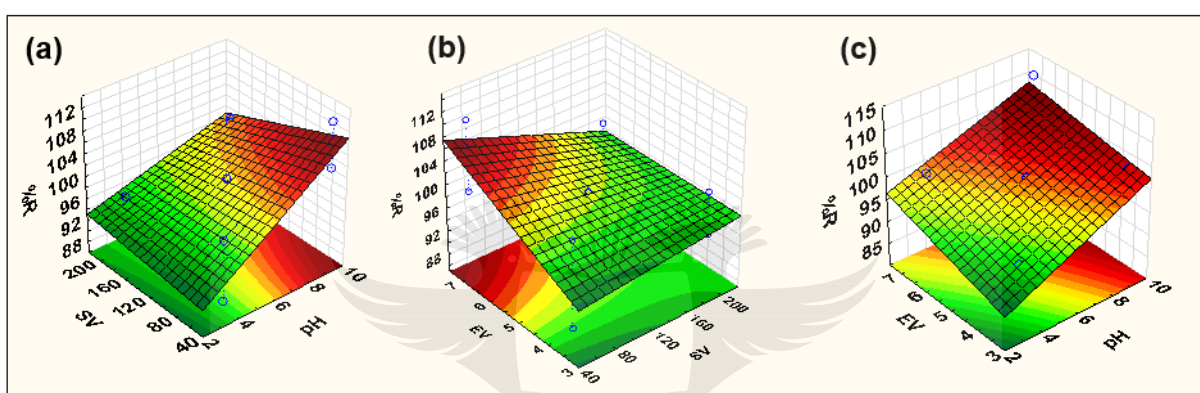
Figure 4.4: Response surface plot for interactive effects between SV and pH for (a) EV and SV in (b) and interaction between EV and pH in (c) for EthPB
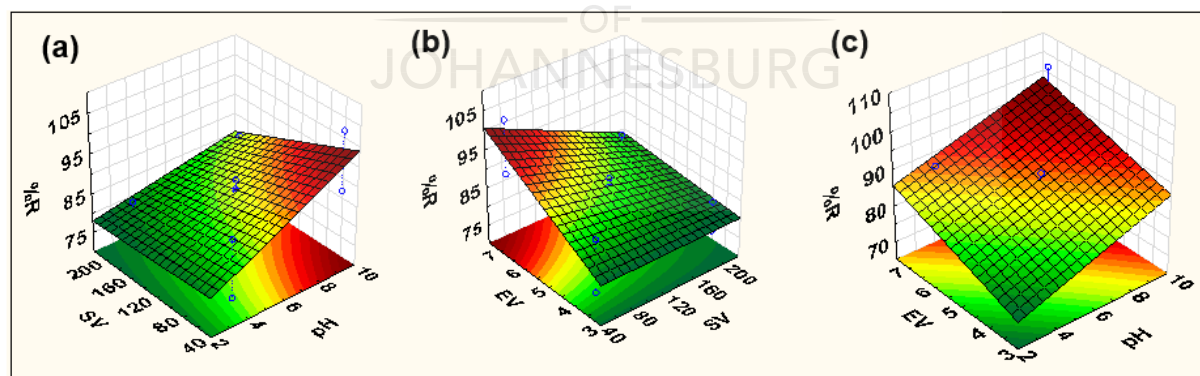
Figure 4.5: Response surface plot for interactive effects between SV and pH for (a) EV and SV in (b) and interaction between EV and pH in (c) for ProPB

4.3.3 Liquid chromatography-tandem Mass spectrometry analysis

Mass spectrometry parameters were optimized by direct infusion of the 1µg mL^{-1} standard solution to select the optimum conditions for the precursor ions, the product ion and the collision energies of each compound. As shown in **Table 4.4,** the precursor ions in this study corresponded to deprotonated molecules [M-H]$^{-}$ ionized in the negative mode, that showed the best detection sensitivity similar to what has been reported in the literature in the analysis of parabens [6, 14]. The most intense product ion was selected for quantification, while the least intense was used for qualification. For all the parabens m/z 92 was used as the quantifier ion, which was formed due to loss of CO_2. The secondary product ion of m/z 136 was as a result of loss of either methyl, ethyl or propyl group, as indicated in **Table 4.4** for each analyte. LCMS based organic solvents (methanol and acetonitrile) were studied as mobile phase eluents for chromatographic separation, with the addition of formic acid (FA). Milli-Q water was used as the aqueous mobile phase. The optimum responses were obtained with methanol spiked with FA (0.1%) and Milli-Q water consisting of FA (0.1%) that resulted in excellent gaussian peak shapes and greater analyte signal sensitivity, as also observed in other literature [15]. The order of elution as seen in **Table 4.4**, increased with the increase in the molecular weight of the compounds.

Table 4.4: Multiple reaction monitoring (MRM) conditions, retention time and proposed product ions for determination of parabens.

Analyte	Empirical formula	MRM transition (m/z)	Product ion	Retention time (min)	Collision energy (eV)
MePB	$C_8H_8O_3$	151>92	[M-H-CH$_3$-CO$_2$]$^{-}$	2.5	23.0
		151>136	[M-H-CH$_3$]$^{-}$		14.0
EthPB	$C_9H_{10}O_3$	165>92	[M-H-CH$_2$CH$_3$-CO$_2$]$^{-}$	3.5	23.0
		165>136	[M-H-CH$_2$CH$_3$]$^{-}$		20.0
ProPB	$C_{10}H_{12}O_3$	179>92	[M-H-CH$_2$(CH$_3$)$_2$-CO$_2$]$^{-}$	4.8	25.0
		179>136	[M-H-CH$_2$(CH$_3$)$_2$]$^{-}$		18.0

4.3.4 Method Accuracy and Recovery

To evaluate the suitability of the method developed, different sample matrices (simulated wastewater, influent wastewater and effluent wastewater) were spiked at 2 concentration levels (10 µg L^{-1} and 100 µg L^{-1}), for matrix spike recovery. Six replicate samples were spiked for each water matrix simultaneously. **Table 4.5** shows the summary of the recoveries obtained

for each analyte in the different sample matrices. The recoveries obtained for the three analytes spiked in simulated wastewater were higher (90-128 %) as compared those obtained in the spiked real wastewater samples which ranged between 75-115 % for influent wastewater and 70-114 % for treated effluent. This can be attributed to higher matrix effect exhibited in the real water samples as has been previously reported by other researchers [16]. The method reproducibility was also remarkable with a relative standard deviation (% RSD) below 10 % for all the compounds as shown in **Table 4.5.** This is a good reflection of the precision of the SPE procedure similar to the previously reported literature [14, 17].

Table 4.5: Compound matrix recoveries with RSD in three different water matrices (n=6)

	Simulated Wastewater		Influent Water		Effluent Water	
Analyte	%Recovery (%RSD)		%Recovery (%RSD)		%Recovery (%RSD)	
	10 µgL^{-1}	100 µg L^{-1}	10 µg L^{-1}	100 µg L^{-1}	10 µg L^{-1}	100 µg L^{-1}
MePB	121 (1.7)	128 (2.1)	117 (5.7)	105 (10)	114 (2.9)	106 (5.3)
EthPB	101 (2.0)	122 (2.6)	87 (5.9)	81 (10)	79 (0.7)	77 (5.1)
PropPB	90 (10)	127 (2.8)	75 (7.6)	75 (9.2)	78 (2.3)	71 (6.4)

4.3.5 Method precision, sensitivity and linearity

The method precision was determined as the relative standard deviation (RSD) of six replicate measurements. Intra-day variability was carried out by replicate injections over the same operating conditions, in 3-hour intervals. Interday precision was established by six replicate measurements in three different days. The measurements were carried out using 10 and 50 µg L^{-1} matrix matched standards. The method precision for intra-day and interday variability was lower than 10 % for MePB and lower than 15 % for EthPB and ProPB, **Table 4.6**. Linearity range using matrix-matched calibration standards was from 5 to 100 µg L^{-1}. The determination coefficient (r^2) obtained ranged from 0.995-0.997 for the three analytes, **Table 4.6**. Limit of detection (LOD) calculated as the lowest concentration giving a signal to noise ratio (S/N) of 3:1, ranged from 0.04 and 0.12 µg L^{-1}. The limit of quantification (LOQ) was calculated in a similar way, corresponding to a S/N ratio of 10:1, and the results ranged between 0.14 and 0.27 µg L^{-1} for the three compounds as observed in **Table 4.6.** The above validation results were compared with previously reported studies that determined parabens **Table 4.7**. As we can observe, the recoveries and the % RSD were similar. In addition, this method also proves to exhibit better performance in respect of LOD and LOQ values except one [18].

Table 4.6: Linearity, LOD, LOQ and Precision obtained for MePB, EthPB, ProPB using SPE

Analyte	R^2	LOD (μg L^{-1})	LOQ (μg L^{-1})	% RSD (Intra-day n=6)		% RSD (Interday n=6)		% ME
				10 μgL^{-1}	50 μgL^{-1}	10 μgL^{-1}	50 μgL^{-1}	
MePB	0.995	0.08	0.27	4.5	8.5	5.1	9.0	39.48
EthPB	0.997	0.12	0.40	12	5.2	4.6	7.7	50.36
ProPB	0.997	0.04	0.14	11	4.8	9.0	12	64.60

Table 4.7: Method performance comparison of different extraction and detection techniques for parabens determination.

Analytical Method	Instrument	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	%RSD	% Recovery	Matrix	Reference
IL-DLLME	CE-UV	0.45–0.72	1.50–2.40	9.5	72-119	mouthwash	[19]
RDSE	GC-MS	0.02-0.05	0.06–0.15	9.7	79-91	water	[18]
DF-µLPME	HPLC-UV	1.6-3.5	5-12	10	84–100	water	[20]
DLLME-MSPE	UHPLC-MS/MS	0.5–1.53	1.60 -4.78	8.3	58-89	beverage	[21]
SPE	LC-MS/MS	0.04-0.12	0.14-0.40	10.9	75-128	water	**This work**

DLLME: Dispersive Liquid-Liquid Microextraction, SPME: Solid Phase Microextraction, IL: Ionic Liquid, MSPE: Magnetic Solid Phase Extraction, RDSE: Rotating Disk Sorptive Extraction, DF-µLPME: Double Flow Microfluidic Liquid Phase Microextraction

4.3.6 Matrix effect

Matrix effect (ME), is one of the drawbacks that accrue with the usage of LC-MS coupled with an ESI source. This phenomenon leads to signal enhancement or suppression due to inherent matrix compounds which get co-extracted with the analytes of interest [6]. The ionization efficiency of the analyte is compromised by the matrix compounds that compete with the analytes during the ionization process [22]. In this study, matrix effect was therefore evaluated by comparing the slope obtained from the calibration plots of standards in matrix, with the slope obtained from the calibration plots of standards in Milli-Q water (5-100 µg L^{-1}). The calculation was performed using **equation 4.1** [23].

$$\%ME = \frac{Slope(matrix-matched)}{Slope\ (solvent)} x100 \qquad (4.1)$$

A value of 100 % means no matrix effect, indicating similar responses in both the Milli-Q water and in the matrix. A value <100 % indicates signal suppression and a value of >100 % indicates a signal enhancement [16, 24]. Co-eluting matrix component can result in signal suppression or enhancement. As observed in **Table 4.6,** MePB exhibited the significant signal suppression (ME = 39.48 %). EthPB and ProPB had an ME of 50 % and 64 % respectively. This scenario of signal suppression has been observed in other previously reported literature [6, 25]. Despite having signal suppression, high % recovery was still obtained as seen from the results in **Table 4.5**. This was as a result of incorporating matrix-matched calibration to compensate for signal suppression [4].

4.3.7 Environmental water sample analysis

The developed analytical technique was used in the extraction and quantification of the three parabens in real water samples, drawn from a domestic municipal wastewater treatment plant in Pretoria, South Africa. The WWTP is divided into two plants i.e. east and west. The east plant is the trickling unit and west is the biological nutrients removal (BNR) unit. The various sampling points are as shown in **Table 4.8** sampling code one being influent as it progresses to effluent with sampling code 7. The concentrations obtained for the three parabens in this study are also shown in **Table 4.8**. **Figure 4.6** displays the UHPLC-MS/MS total ion chromatogram (TIC) of an un-spiked influent water sample. The highest concentration was found in the samples corresponded to MePB, and ProPB. This is in line with what is expected as MePB and

ProPB are the mostly used parabens in products such as toothpaste, body creams, shampoos etc. [26, 27]. Also because of their synergistic effects, they are formulated together and hence the observed high concentrations as compared to ethylparaben [26, 28]. It was also observed that the concentrations decreased from E1-E7. This is expected since the influent samples are more complex matrices with higher organic matter than effluent samples. In general, however, the levels obtained were very low as observed with the highest concentration found to be 3.3 $\mu g\ L^{-1}$. Comparison of the results obtained for the two plants (east and west) does not show much difference in the parabens concentration and is indicative of adequate removal of these parabens. These findings are comparable with other studies reporting the determination of parabens from WWTP [27].

Table 4.8: Application of SPE in extraction of MePB, EthPB and ProPB in wastewater samples (n=6)

Sampling code	Sampling Point	Methylparaben		Ethylparaben		Propylparaben	
		Conc (μgL^{-1})	RSD %	Conc (μgL^{-1})	RSD %	Conc (μgL^{-1})	RSD %
E1	Division box	3.33	1.63	0.40	0.17	1.82	0.96
E2	Grit	2.86	6.39	0.54	4.88	1.48	2.42
E3	Primary settling Tank	1.98	3.83	ND		0.82	3.36
E4	Siphoning tank	1.85	0.12	ND		0.47	2.54
E5	Trickling Filters	ND		ND		ND	
E6	Humas Tank	ND		ND		ND	
E7	CCT Chlorine contact dam	ND		ND		ND	
W1	Division box	2.97	2.95	<LOQ		2.17	2.62
W2	Grit	2.30	1.86	ND		1.58	0.95
W3	Primary Settler	2.56	2.25	ND		1.54	0.28
W4	BNR Activated sludge reactor	ND		ND		ND	
W5	Humas Tank	ND		ND		ND	
W6	CCT Chlorine contact dam	ND		ND		ND	

ND: not detected, Conc: concentration, E: East, W: West, BNR: biological nutrients removal
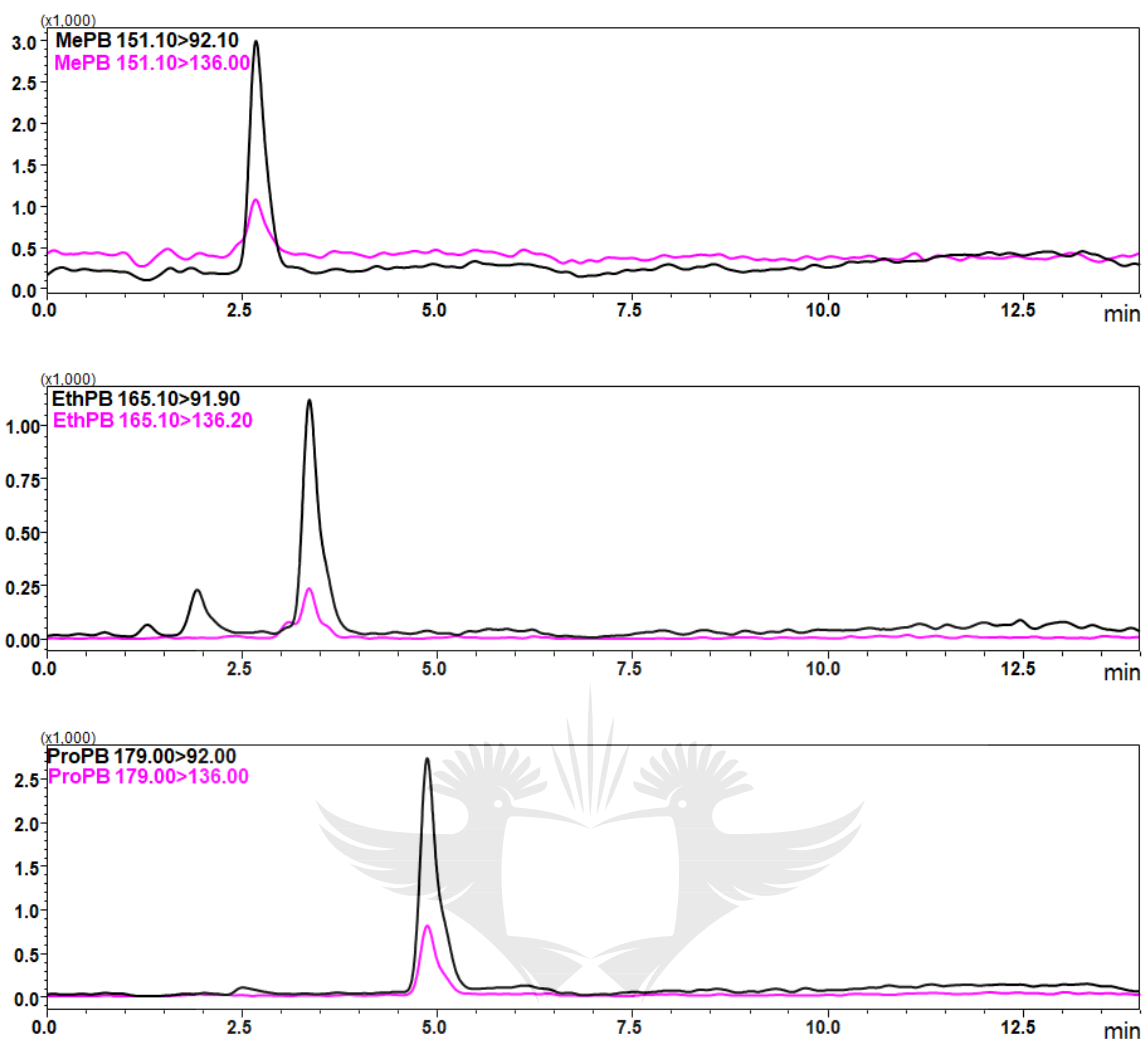
Figure 4.6: Extracted ion chromatogram (EIC) of an unspiked influent wastewater sample after SPE.

4.4 CONCLUSIONS

In this study we report a novel, fast and reliable method, employing simulated water matrix that mimics the real environmental sample throughout method development stages, for the extraction of parabens in wastewater. The method is indeed fast as it employs an automated sample delivery setup into SPE cartridges with minimal sample volume (50 mL). UHPLC-MS/MS was successfully employed in carrying out all sample analysis. The SPE extraction procedures were optimized using two-level factorial design to obtain the optimum conditions of the extraction parameters which resulted in high extraction yield. This multivariate optimization approach revealed that sample pH and sample volume had the most significant effect on the analytical response (recovery) of the analytes (the three parabens). The results obtained provided high recoveries (78-120 %) with minimal sample extraction volume (50

mL). The LOD and LOQ obtained were 0.04-0.12 µg L^{-1} and 0.14-0.40 µg L^{-1} respectively. The method was properly validated with real wastewater samples obtained from the local WWTP with concentrations ranging between 0.40-3.36 µg L^{-1} for the three analytes. The results obtained here-in demonstrate the suitability and applicability of the method in the determination of three parabens namely (MePB, EthPB and ProPB), in wastewater samples.

4.5 REFERENCES

[1] U. Kunkel, M. Radke, Reactive tracer test to evaluate the fate of pharmaceuticals in rivers, Environmental science & technology 45 (2011) 6296-6302.

[2] R. Leardi, Experimental design in chemistry: A tutorial, Analytica chimica acta 652 (2009) 161-172.

[3] S. Gartiser, F. Flach, C. Nickel, M. Stintz, S. Damme, A. Schaeffer, L. Erdinger, T.A. Kuhlbusch, Behavior of nanoscale titanium dioxide in laboratory wastewater treatment plants according to OECD 303 A, Chemosphere 104 (2014) 197-204.

[4] M.J. Gómez, M.M. Gómez-Ramos, O. Malato, M. Mezcua, A.R. Férnandez-Alba, Rapid automated screening, identification and quantification of organic micro-contaminants and their main transformation products in wastewater and river waters using liquid chromatography–quadrupole-time-of-flight mass spectrometry with an accurate-mass database, Journal of Chromatography A 1217 (2010) 7038-7054.

[5] E. Blanco, M.d.C. Casais, M.d.C. Mejuto, R. Cela, Combination of off-line solid-phase extraction and on-column sample stacking for sensitive determination of parabens and p-hydroxybenzoic acid in waters by non-aqueous capillary electrophoresis, Analytica chimica acta 647 (2009) 104-111.

[6] I. González-Mariño, J.B. Quintana, I. Rodríguez, R. Cela, Simultaneous determination of parabens, triclosan and triclocarban in water by liquid chromatography/electrospray ionisation tandem mass spectrometry, Rapid Communications in Mass Spectrometry 23 (2009) 1756-1766.

[7] P.N. Nomngongo, J.C. Ngila, Multivariate optimization of dual-bed solid phase extraction for preconcentration of Ag, Al, As and Cr in gasoline prior to inductively coupled plasma optical emission spectrometric determination, Fuel 139 (2015) 285-291.

[8] R. Abdelmajid, M.R. L., E.S. Salah, E.H. Mohammadine, Use of response factorial design for process optimization of basic dye adsorption onto activated carbon derived from Persea species, Microchemical Journal (2017) 129-136.

[9] P.N. Nomngongo, J.C. Ngila, T.A.M. Msagati, B. Moodley, Chemometric optimization of hollow fiber-liquid phase microextraction for preconcentration of trace elements in diesel and gasoline prior to their ICP-OES determination, Microchemical Journal 114 (2014) 141-147.

[10] S. Gbashi, P. Njobeh, I.P. Steenkamp, H. Tutu, N. Madala, The effect of temperature and methanol–water mixture on pressurized hot water extraction (PHWE) of anti-HIV analogoues from Bidens pilosa, Chemistry Central Journal 10 (2016).

[11] D.B. Hibbert, Experimental design in chromatography A tutorial review, Journal of Chromatography B 910 (2012) 2-13.

[12] T.A.M. Msagati, T. Barri, J.A. Jonsoon, Analysis and quantification of parabens in cosmeticproducts by utilizing hollow fibre-supported liquidmembrane and high performance liquidchromatography with ultraviolet detection, International Journal of Cosmetic Science 30 (2008) 297-307.

[13] F. Han, Y.Z. He, C.Z. Yu, On-line pretreatment and determination of parabens in cosmetic products by combination of flow injection analysis, solid-phase extraction and micellar electrokinetic chromatography, Talanta 74 (2008) 1371-1377.

[14] M. Pedrouzo, F. Borrull, R.M. Marcé, Pocurull, Ultra-high-performance liquid chromatography–tandem mass spectrometry for determining the presence of eleven personal care products in surface and wastewaters, Journal of Chromatography A 1216 (2009) 6994-7000.

[15] N.H. Tran, H. Chen, T.V. Do, M. Reinhard, H.H. Ngo, Y.Z. He, K.Y. Gin, Simultaneous analysis of multiple classes of antimicrobials in environmental water samples using SPE coupled with UHPLC-ESI-MS/MS and isotope dilution, Talanta 159 (2016) 163-173.

[16] T. Benijts, R. Dams, W.E. Lambert, A. De Leenheer, Countering matrix effects in environmental liquid chromatography–electrospray ionization tandem mass spectrometry water analysis for endocrine disrupting chemicals, Journal of Chromatography A 1029 (2004) 153-159.

[17] C. Moreta, M.-T. Tena, K. Kannan, Analytical method for the determination and a survey of parabens and their derivatives in pharmaceuticals, Environmental research 142 (2015) 452-460.

[18] M. Becerra-Herrera, V. Miranda, D. Arismendi, P. Richter, Chemometric optimization of the extraction and derivatization of parabens for their determination in water samples by rotating-disk sorptive extraction and gas chromatography mass spectrometry, Talanta 176 (2018) 551-557.

[19] T. Ma, Z. Li, Q. Jia, W. Zhou, Ultrasound-assisted temperature-controlled ionic liquid emulsification microextraction coupled with capillary electrophoresis for the determination of parabens in personal care products, Electrophoresis 37 (2016) 1624-1631.

[20] M. Ramos-Payan, S. Maspoch, A. Llobera, A simple and fast Double-Flow microfluidic device based liquid-phase microextraction (DF-µLPME) for the determination of parabens in water samples, Talanta 165 (2017) 496-501.

[21] Q. Yin, Y. Zhu, Y. Yang, Dispersive Liquid–Liquid Microextraction Followed by Magnetic Solid-Phase Extraction for Determination of Four Parabens in Beverage Samples by Ultra-performance Liquid Chromatography Tandem Mass Spectrometry, Food Analytical Methods 11 (2018) 797-807.

[22] A. Jaume, l.R. Danie, Z. Bozo, P. Sandra, B. Damià, Liquid Chromatography–Mass Spectrometry quantification and confirmation aspects, (2015).

[23] S.J. Lehotay, K.A. Son, H. Kwon, U. Koesukwiwat, W. Fu, K. Mastovska, E. Hoh, N. Leepipatpiboon, Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables, Journal of Chromatography A 1217 (2010) 2548-2560.

[24] J.C. Van De Steene, K.A. Mortier, W.E. Lambert, Tackling matrix effects during development of a liquid chromatographic–electrospray ionisation tandem mass spectrometric analysis of nine basic pharmaceuticals in aqueous environmental samples, Journal of Chromatography A 1123 (2006) 71-81.

[25] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography–electrospray tandem mass spectrometry, Analytical and Bioanalytical Chemistry 391 (2008) 1293-1308.

[26] L. Núñez, J.L. Tadeo, A.I. García-Valcárcel, E. Turiel, Determination of parabens in environmental solid samples by ultrasonic-assisted extraction and liquid chromatography with triple quadrupole mass spectrometry, Journal of Chromatography A 1214 (2008) 178-182.

[27] P. Canosa, I. Rodríguez, E. Rubí, M.H. Bollaín, R. Cela, Optimisation of a solid-phase microextraction method for the determination of parabens in water samples at the low ng per litre level, Journal of Chromatography A 1124 (2006) 3-10.

[28] B. Albero, R.A. Pérez, C.o. Sánchez-Brunete, J.L. Tadeo, Occurrence and analysis of parabens in municipal sewage sludge from wastewater treatment plants in Madrid (Spain), Journal of hazardous materials 239-240 (2012) 48-55.

CHAPTER 5:

DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES IN WASTEWATER SAMPLES USING VORTEX ASSISTED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION WITH LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

ABSTRACT

A simple and rapid method for determination of azinphos-methyl, parathion-methyl and ethoprofos, group of organophosphorus pesticides (OPPs) in wastewater matrices is presented. A chemometric approach for the optimisation of vortex assisted dispersive liquid-liquid microextraction (VA-DLLME) experimental conditions prior to liquid chromatography tandem mass spectrometry (LC-MS/MS) detection was applied. In this method, a high-density organic solvent (chloroform) was used as the extractant, with acetone as the disperser solvent. Vortex mixing of the sample and the organic solvents was applied, while centrifugation was used for phase separation of the organic phase (sedimented layer of extractant) and the aqueous layer. A two-level full factorial design (2^4) was employed initially for the screening process, and final optimisation of the significant parameters was performed using response surface methodology (RSM) based on central composite design (CCD). The method performance characteristics investigated included linear dynamic range (LDR, 5-100 µg L^{-1}) with a good determination coefficient (r^2>0.999). The method precision expressed as intra-day and inter-day relative standard deviation (%RSD) were in the range of 7.8-8.2 % and 8.1-9.4 % respectively. The influence of matrix was found to be negligible with recoveries ranging from 99.9-106.7%. The proposed method was then applied in real wastewater samples. Extraction recoveries performed at two spiking levels (25 and 100 µg L^{-1}) in untreated (influent) and treated (effluent) wastewater matrices ranged between 95-120 %.

Keywords: Design-of-experiment; Azinphos-methyl; Parathion-methyl; Ethoprofos; Extraction; Wastewater

5.1 INTRODUCTION

Organophosphorus pesticides are among the group of organophosphorus compounds that are used worldwide in the environment mainly for agricultural purposes to protect crops and animal production from pests [1]. They are among the most extensively used insecticides until the 21st century. As such they are constantly being introduced to the aquatic environment in greater concentrations. The quality of the surface and groundwater which constitutes the largest source of drinking water in most places is thereby compromised. Some of the major ways in which they are introduced in the environment is from farmlands and from various effluent point sources [2]. Continuous release of these pesticides in the aquatic environment results in various physical and chemical effects such as bioaccumulation which produces adverse effects on humans and aquatic life [2]. These effects include but not limited to carcinogenicity, mutagenicity and endocrine disruptive effects [1]. As such, it is of utmost importance to develop low-cost high throughput methods that will aid in continuously monitoring their levels in different sources of water.

Various analytical methods have been developed in the monitoring and evaluation of the concentrations of these compounds in different water samples. They include, gas chromatographic methods (GC) [3, 4], gas chromatographic-mass spectrometry (GC-MS) [5, 6], high-performance liquid chromatography (HPLC) [7-9] as well as liquid chromatography-mass spectrometry (LC-MS) methods [9, 10]. However, prior to the instrumental analysis, the samples must be extracted and preconcentrated first. This is an extremely important step in the development of the analytical procedure, to obtain accurate and sensitive results, remove potential matrix interferences inherently present in the sample, as well as to protect the instruments [7, 9]. Several pre-treatment methods for the extraction and preconcentration of organophosphorus in water samples have been developed and reported. They include liquid-liquid extraction (LLE) [11, 12], solid phase extraction (SPE) [10, 13], solid phase microextraction (SPME) [1, 14] and liquid phase microextraction (LPME) [7, 15]. Methods based on microextraction techniques have in the recent past gained wide popularity in the extraction of organic compounds in wastewater, to overcome the setbacks that characterize the conventional extraction techniques such as lengthy extraction times and large amounts of organic solvents required [16, 17].

Dispersive liquid-liquid microextraction (DLLME) [18] is one of such methods being used in sample preparation. DLLME is a miniaturized, highly efficient, rapid extraction technique, with low cost and simplicity of operation employing high enrichment factors. It uses

very low volumes of organic solvents as well as sample volumes. It employs the use of water-immiscible extraction solvent and water-miscible dispersive solvent mixtures. A mixture of the two solvents in microliter volumes is introduced swiftly into the sample using a microsyringe. Dispersion of fine droplets of the extraction solvent takes place in the aqueous phase, forming a cloudy solution. The analytes get extracted into the fine droplets of extractant and the two phases, organic and aqueous are centrifuged to further separate them [6, 18, 19]. However, to the best of our knowledge, in reported literature, there are few or no reports on the simultaneous determination of the three OPPs compounds in wastewater samples using VA-DDLME as the extraction and preconcentration technique, coupled to LC-MS/MS with chemometric method optimization.

Therefore, the aim of this study was to accurately develop a sensitive VA-DLLME method for the determination of three organophosphorus pesticides (azinphos-methyl, ethoprofos and methyl parathion) in wastewater samples using LC-MS/MS. Design of experiment was used to investigate and obtain the optimum conditions for the experimental factors that have the highest influence on the analytical response (% recovery). The chemometric approach was selected as most analytical methods do not consider the effect of interaction between factors. This can result in failure to obtain accurate and precise results when conventional optimization strategies such as the one-variable-at-a-time (OVAT) are used. The experimental parameters multivariately investigated in this study include sample pH, extractant and disperser volumes, and ionic strength (salting out effects).

5.2 EXPERIMENTAL

5.2.1 Chemical and reagents

Mixed organophosphorus pesticide standards were purchased from Merck (Darmstadt, Germany). The purity of all standards was 98-99 %. Stock solutions of the mixed standards were prepared at 10 mg L^{-1} in acetonitrile and stored at 4 °C. Working standards were prepared daily from the 10 mg L^{-1} stock solutions in Milli-Q water with a purity of 18.2 MΩcm (Millipore USA). Methanol, acetonitrile and formic acid (98 % purity) were of HPLC grade, supplied by Merck (Darmstadt, Germany). All other chemicals, dichloroethane, 1,1,2,2-tetrachloroethane, chloroform, acetone, ethanol, and ammonium sulphate were of analytical reagent grade purchased from Merck (Darmstadt, Germany).

5.2.2 Sample collection

Environmental samples were collected from a local wastewater treatment plant in Pretoria, South Africa. This included untreated raw wastewater (influent), and treated wastewater (final effluent), sampled in triplicate. Glass amber bottles, precleaned before collection with the real samples, were used to collect the samples and placed in a cooler box with ice. They were then transported to the laboratory and stored at 4 °C prior to filtration, extraction and instrumental analysis.

5.2.3 Liquid chromatography-tandem mass spectrometry (LC-MS/MS) conditions

Chromatographic analysis was carried out using Shimadzu Nexera 8030 UHPLC (Tokyo, Japan). Baseline separation was performed on a raptor ARC-18 column (100 mm x 2.1 mm, 3 µm) (RESTEK, USA) using a binary mixture of solvents comprising of 0.1% (v/v) formic acid in de-ionized water (eluent A) and methanol as (eluent B). The flow rate used was kept at 0.2 mL min $^{-1}$ and the injection volume of 30 µl was used for all the analyses. Column compartment was maintained at 40 °C whereas the autosampler was kept at 4 °C. The optimized gradient elution programme was as follows: initial starting condition was 50 % B held for 0.5 minutes, followed by a linear ramp to 75 % of B in 3 minutes, followed by another ramp to 100 % B in 3 minutes. This was kept isocratic for 5 minutes, before re-establishing the initial conditions in 1 minute maintained for 7 minutes. For mass spectrometry (MS) analysis, an LCMS 8030 (Tokyo, Japan) triple quadrupole mass spectrometer with electrospray ionization (ESI) manifold, was used in acquiring data in the multiple reaction monitoring modes (MRM), positive ionization, with a dwell time of 50 ms. The first (Q1) and third (Q3) quadrupoles, (mass analyzers) were operated in unit mass resolution. Nitrogen gas was used as desolvation gas at the electrospray ionization source (ESI). Argon with a purity of 99.999% was used as the collision-induced dissociation (CID) gas at the second quadrupole (Q2) to produce the product ions at Q3 for each of the analytes. The optimum conditions for MS analyses were: nebulizing gas flow rate of 3 L min $^{-1}$; drying gas flow rate of 15 L min $^{-1}$; DL temperature 250 °C; heat block 400 °C; probe voltage 4.5KV. Peak detection, instrument control, data analysis, method optimization was carried out using LabSolutions software (Tokyo, Japan).

5.2.4 Vortex assisted dispersive liquid-liquid microextraction analytical procedure

An aliquot of 5 mL sample was placed in 15 mL centrifuge tubes and a mixture of extraction solvent (0.29 mL chloroform in 0.28 mL acetone) was introduced rapidly into the sample. The

extractant was dispersed into the sample solution via vortex mixing for 0.5 minutes resulting in the formation of a cloudy solution (water/acetone/chloroform). The analytes were extracted into the fine droplets of chloroform [19]. Centrifugation was applied to separate the two immiscible layers at 4400 rpm for 3 minutes. The sedimented organic layer (chloroform) was thereafter quantitatively transferred into a 2 mL vial and evaporated to dryness at 60 °C. Thereafter, the residue was reconstituted in 1 mL mobile phase and vortexed prior to injection into the LC-MS/MS.

5.2.5 Design of experiment

A multivariate approach was employed for the optimisation of VA-DLLME experimental conditions that influence the analytical response in the extraction of organophosphorus pesticides in water. The selection of the disperser solvent was however done univariately. The experimental factors investigated in this study were sample pH, extractant volume and disperser volume. A two-level (2^4) full factorial design was initially employed for screening of the most influential experimental factors. Further optimization was carried out using response surface methodology based on central composite design to obtain the optimum experimental conditions. The levels for each variable was assigned a maximum, minimum and a central value. Statistica version 13 (StatSoft, USA) was used to carry out the statistical analysis. The levels are as shown in **Table 5.1**

Table 5.1: Variables and levels selected for the two-level (2^4) full factorial design

Variable	Low level (-1)	High Level (+1)
Sample pH	3.0	9.0
Extractant Volume (mL)	100	250
Disperser Volume (mL)	0.5	1.5
Ionic strength %	5	25

5.2.6 Method validation parameters

The method performance characteristics were evaluated based on accuracy (% recovery), limits of detection (LOD), limit of quantification (LOQ), method precision (repeatability and reproducibility) linearity and matrix effect. The method precision was performed by analyzing fortified samples (n=10) at 50 µg L^{-1}. Different environmental samples matrices were fortified at two concentration levels (25 µg L^{-1} and 100 µg L^{-1}) to establish the accuracy of the method

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from the obtained mean recoveries. Matrix effect assessment was evaluated using two sets of samples at 25 µg L^{-1}. The first set of samples (n=5) comprised of analytes present in the mobile phase solvent as reagent blank, while in the second set (n=5), influent wastewater samples were first extracted using the developed method and the analytes spiked into the sample extracts (post extraction spike). The peak area ratios of the analytes in solvent solution with that of the analytes in matrix solution were compared to ascertain the presence or absence of matrix effect.

5.3 RESULTS AND DISCUSSION

5.3.1 LC-MS/MS Analysis

In the LC-MS/MS method development, methanol and acetonitrile were tested separately, as the organic mobile phase components, while the aqueous mobile phase was kept at 0.1 % formic acid in deionized water. It was observed that when the analytes were eluted while using methanol as the mobile phase, higher peak areas with good peak resolution were obtained, as compared to when acetonitrile was used. In addition, co-elution between parathion-methyl and ethoprofos when acetonitrile was used as the mobile phase was also observed. This scenario can be attributed to unfavorable elution characteristics of acetonitrile, as compared to methanol which gave good peak resolution [20]. Methanol was therefore chosen as the optimum organic eluant/mobile phase. The pump flow rate was optimized at 0.2 mL min^{-1} and the column oven temperature kept at 40 °C. The mass spectrometry analysis was performed on multiple reaction monitoring modes (MRM) on positive ionization. The mass spectrometric conditions (precursor-ion, product-ion, collision energies) were automatically optimized in MRM mode for each of the compounds. The precursor ions were characterized as [M+H]$^{+}$. Two transition levels (product ions) were selected as quantifier and qualifier ions. To increase the sensitivity of the analysis, time range windows for acquisition were automatically preset for each analyte at time ranges of 3.77-7.77; 3.93-7.93 and 4.78-8.78 minutes for azinphos-methyl, methyl-parathion and ethoprofos, respectively.

5.3.2 Univariate selection and optimization of dispersive and extraction solvents

Prior to the multivariate optimization of the method, the extractant and disperser solvents were optimized univariately. From previous studies, the mostly used disperser solvents in DLLME experiments include acetone, acetonitrile, methanol [21]. These solvents possess miscibility with aqueous solutions, as well as the extractant solvents, which is the main criteria in the selection of disperser solvent for DLLME. The method reported by [19] was modified and

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adopted in extracting spiked Milli-Q water ($50\,\mu g\,L^{-1}$) to determine the most suitable extractant and disperser solvents. Chloroform, 1,2-dichloroethane and tetrachloroethane were tested as extractant solvents. The sample solutions were initially extracted using 1 mL of methanol as the disperser followed by extraction with $250\,\mu L$ with each of the extractant solvents one at a time. **Figure 5.1a** illustrates that chloroform as the extraction solvent yielded higher recoveries (90-110 %) in extracting the three organophosphorus pesticides in water. In addition, chloroform, having the lowest boiling point (61.2 °C), was more favorable for the evaporation step as compared to 1,2-dichloroethane (83.47 °C) and tetrachloroethane (146.7 °C), making the overall extraction procedure much quicker (~10 minutes). Another set of spiked water samples were extracted using chloroform as the extractant, with 1ml of each disperser solvents (acetone, acetonitrile, methanol). In the results shown in **Figure 5.1b**, there was not much difference on the % recoveries, however, acetone was selected as it showed slightly better recoveries. Also, the selection was based on its low cost and low toxicity [3]. Therefore, in this study, chloroform and acetone were selected for further optimization.
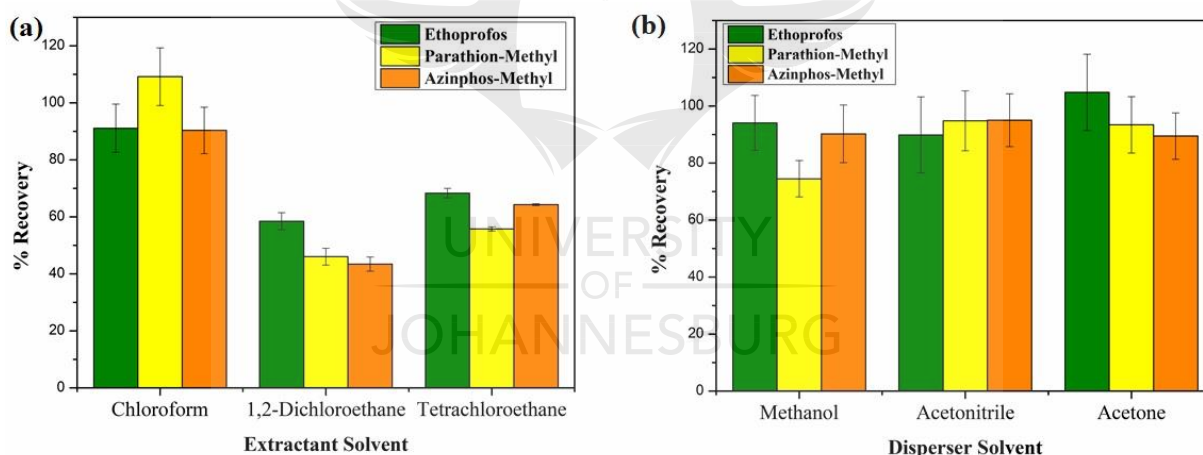
Figure 5.1: Optimization of extractant and disperser solvent for DLLME: for each solvent regime. The error bars correspond to the RSD of the mean recovery (n = 3)

5.3.3 Two-level (2^4) full factorial design screening

The factors affecting the method performance of VA-DLLME of methyl parathion, ethoprofos and azinphos-methyl in water were investigated. They included sample pH, extractant volume (EV) disperser volume (DV) and ionic strength (% IS). The sample pH was adjusted using 0.1 mol L^{-1} NaOH. Full factorial design (2^4) was used initially for screening. As shown in **Table 5.2**, the % recovery of each compound was used as the analytical response. **Figure 5.2** portrays the analysis of variance (ANOVA) results displayed in the form of a Pareto chart of the

standardized main effects and their interactive effects on the investigated parameters for the three OPPs. The length of the bar signifies proportionality to the absolute effect whereas the vertical line indicates a 95 % confidence level [22]. The positive or negative sign connotes signal enhancement or reduction of that particular variable or the effect of two variables [23]. It can be observed in **Figure 5.2** that among the main independent variables, EV and sample pH exhibited a positive sign on the coefficient. EV showed a relatively stronger effect that impacted the extraction efficiency. Although they were not statistically significant at the 95% confidence level, the analytical response in terms of % recoveries (% R) was still low, since higher recoveries were expected, **Table 5.2**. Therefore, increasing the values of these variables were expected to increase the extraction efficiency. The ionic strength (IS), as evaluated by ammonium sulphate (NH_4SO_4) concentration, had a negative sign and did not impact significantly on the efficiency of the extraction. This would have probably been attributed to low salting-out effect. It was therefore excluded from further optimization. The variables in the screening study that were subjected to further optimization were sample pH, EV and DV. The sample volume was kept constant at 5 mL.
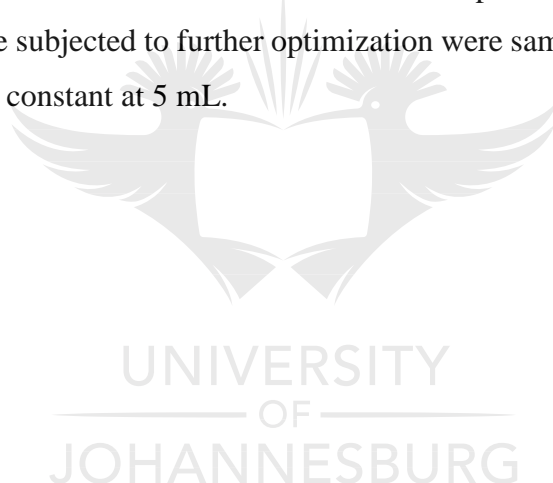
Table 5.2: Response corresponding to full factorial design (2^4) matrix design optimization

Standard Run	EV (uL)	DV (mL)	pH	IS (%)	Ethoprofos	Parathion-methyl	Azinphos-Methyl
						% Recovery	
1	100	0.50	3.0	5.00	65	75	75
2	250	0.50	3.0	5.00	41	34	42
3	100	1.50	3.0	5.00	41	41	41
4	250	1.50	3.0	5.00	52	53	50
5	100	0.50	9.0	5.00	56	52	47
6	250	0.50	9.0	5.00	70	66	70
7	100	1.50	9.0	5.00	57	56	52
8	250	1.50	9.0	5.00	44	42	41
9	100	0.50	3.0	25.00	29	27	39
10	250	0.50	3.0	25.00	80	75	79
11	100	1.50	3.0	25.00	25	35	44
12	250	1.50	3.0	25.00	38	33	48
13	100	0.50	9.0	25.00	48	38	44
14	250	0.50	9.0	25.00	80	74	89
15	100	1.50	9.0	25.00	34	33	46
16	250	1.50	9.0	25.00	22	17	22
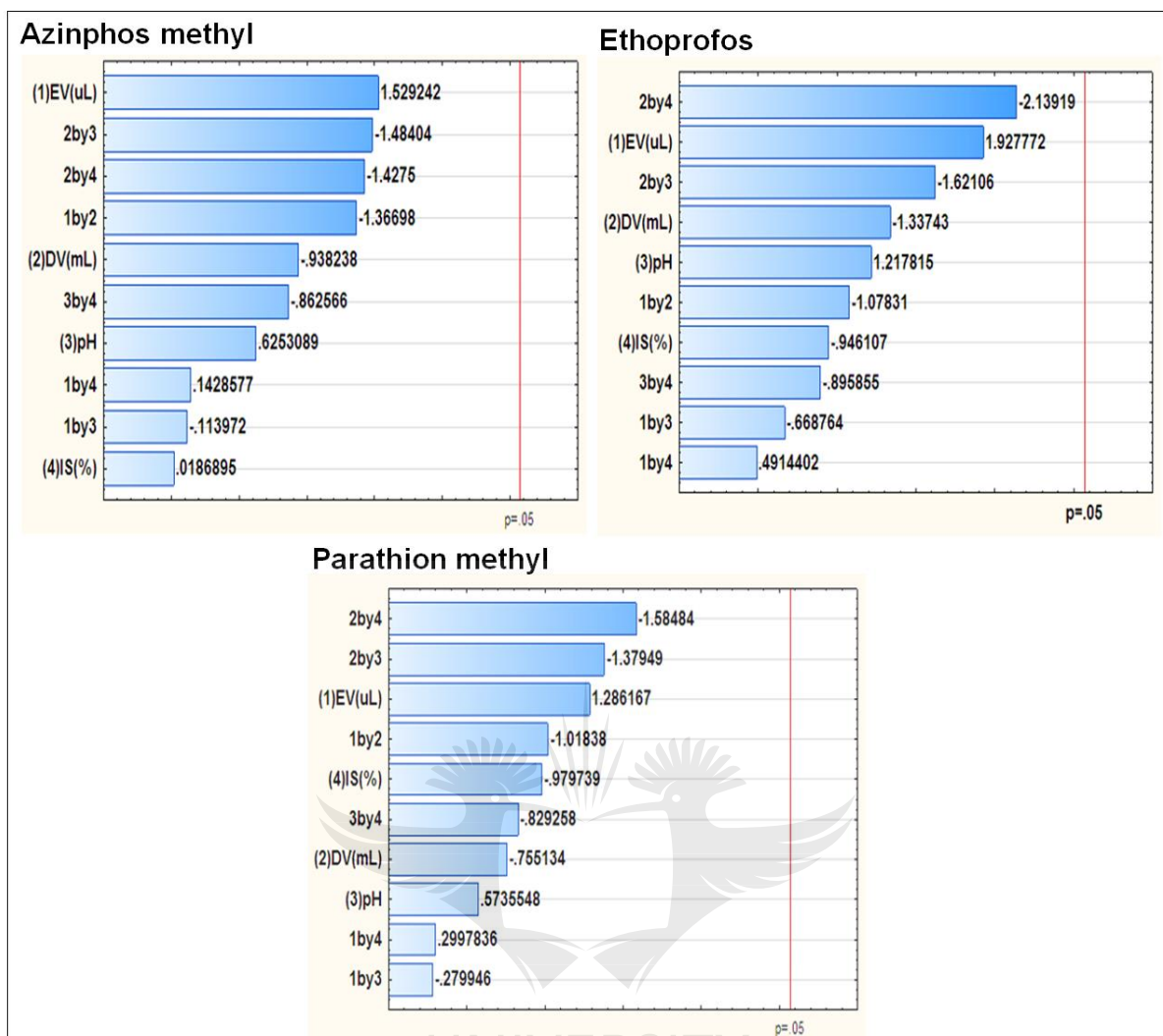
Figure 5.2: Pareto chart of standardised effects for variables in the VA-DLLME of azinphos-methyl, ethoprofos and parathion-methyl

5.3.4 Central composite design optimization

Further optimization of the three variables (EV, pH and DV) was conducted by using a central composite design (CCD) matrix composed of 23 experimental runs including nine central points, **Table 5.3**. The advantage of using CCD is that it allows the determination of parameters with various levels to be conducted simultaneously, with the evolution of the interrelation between parameters [24]. The combination of two-level factorial with additional points such as star points and centre points were employed to obtain rotatability, to fit of the quadratic polynomials. The replicate (n=9) centre points, were performed to ensure a good experimental error estimate [25]. A nonlinear quadratic model was obtained to demonstrate a semi-empirical display of the dependence of % recovery with respect to the variables under investigation, at the optimized conditions, **equation 5.1**.

%R = 94.3815+3.46091 pH-0.739755 pH×pH - 48.3977 DV (mL) -9.16051 DV(mL)×DV(mL) -0.130635 EV(uL) -0.000205056 EV(uL)×EV(uL) + 4.92839 pH×DV (mL) + 0.0189835 pH×EV(uL) + 0.136446 DV(mL)×EV(uL) **(5.1)**

The coefficient in the above quadratic equation connotes the magnitude of the intensity where the positive or negative sign defines the nature of influence. A positive sign connotes an increase in response when the variable is increased while a negative sign decreases the response when the variable is increased [23].

5.3.5 Response surface methodology

Response surface methodology (RSM) plots for Ethoprofos in **Figure 5.3,** were developed to portray the interaction between a pair of independent variables on the analytical response (% R) while keeping the third variable constant at the centre point [8]. Response surface plots for azinphos-methyl and parathion-methyl are shown in supplementary data (Fig S3 & S4). As observed in the surface plot in **Figure 5.3a**, the combined effects of EV and DV on the analytical response was investigated and the pH was fixed at a central point. The quadratic effect of DV on the %R is very strongly negative. From the curvature, it can be observed that the optimum values fall in-between the minimum and maximum values for EV and DV. Also, at constant pH, when EV and DV are increased, there is a slight increase in the %R, then a minor decrease when approaching the maximum values, due to the quadratic effect. **Figure 5.3b** depicts the response surface plots for EV versus pH whilst keeping the DV at 1 mL. There is a very strong quadratic effect of pH on the % R. At constant DV, when the pH is low, there is low % R and as the pH gradually increases toward neutral (pH 7-8), there is an observed optimal recovery. Between pH 7-8, the OPPs exist in their molecular form, having a high affinity for extractability into chloroform. This effect may be attributed to partial hydrolysis of these compounds whose pKa values range between 5-7.15 [26] The effect of EV on % R is however negligible. Lastly, **Figure 5.3c** shows the response surface plots obtained as a function of pH versus DV, with a constant EV of 175 µL. We can observe that at constant EV when DV is increased, the % R drops significantly. High DV, results in the low extraction efficiency of the analytes into the extractant solvent. This is because of the dilution effects of the OPPs in water, thereby decreasing the distribution coefficient. Also, the formation of the cloudy solution is dependent on the disperser solvent volume [25, 27]. The effect of pH on the % R is also observed to be optimal towards a neutral as highlighted above.

Based on the overall RSM and the polynomial quadratic equations, the optimum conditions for the three variables that result in high extraction efficiency and preconcentration factors of the 3 OPPs were: sample pH=7.9, EV=291µL, DV=0.276 µL. These optimized experimental conditions were used for evaluation of the method performance and application to real environmental samples.
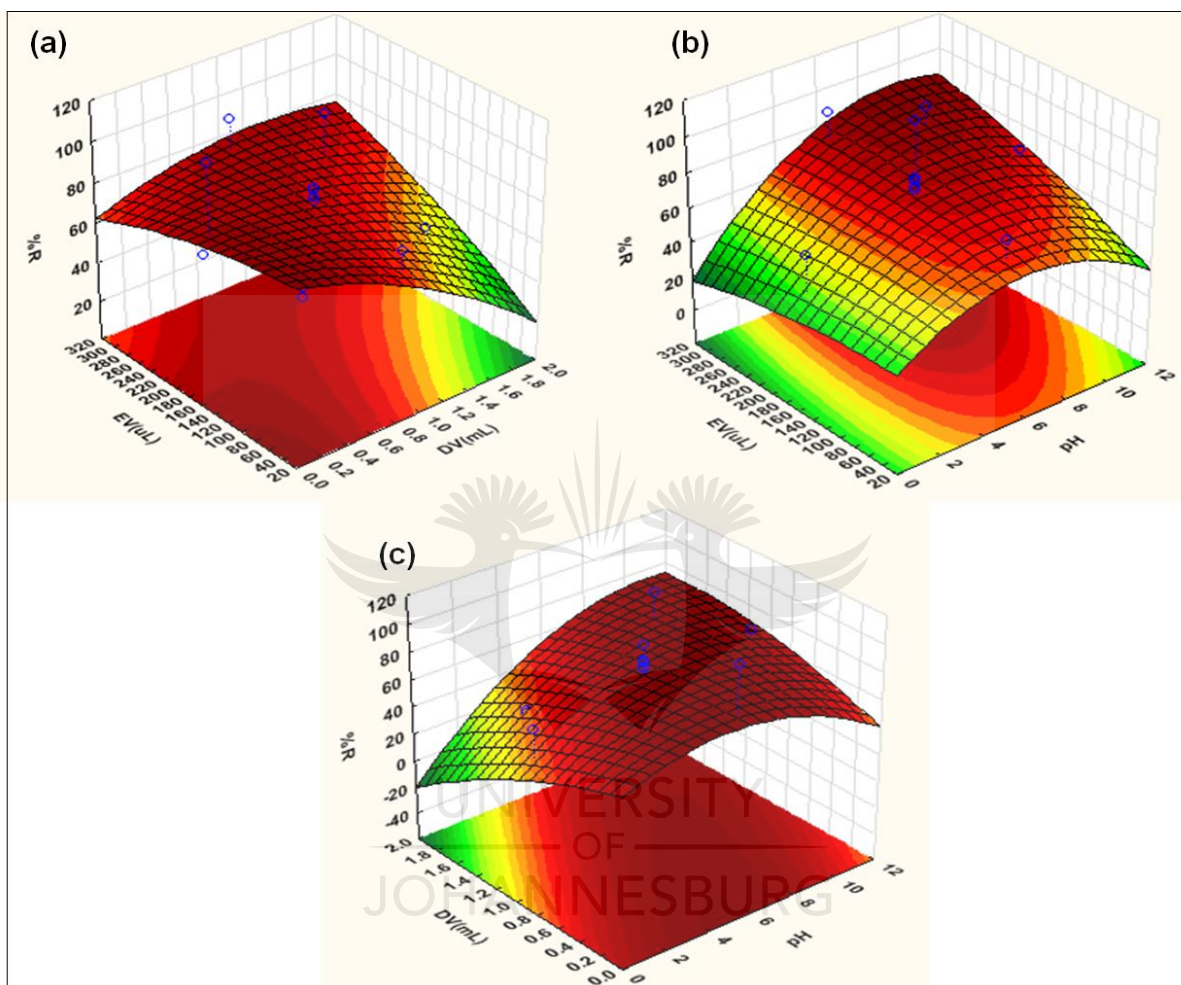
Figure 5.3: Response surface plot for; (a) interactive effects between EV and DV, (b) EV and pH, (c) DV and pH, for Ethoprofos.

Table 5.3: Central composite design experimental factors and levels during optimization of the three variables (EV, pH and DV)

				Ethoprofos	Parathion Methyl	Azinphos-Methyl
Standard Run	pH	DV (mL)	EV (uL)		% Recovery	
1	3.0	0.5	100	41	54	36
2	3.0	0.5	250	36	48	33
3	3.0	1.5	100	27	39	25
4	3.0	1.5	250	24	40	23
5	9.0	0.5	100	43	56	39
6	9.0	0.5	250	51	67	48
7	9.0	1.5	100	48	73	41
8	9.0	1.5	250	82	127	65
9	1.0	1.0	175	67	67	56
10	11	1.0	175	75	74	107
11	6.0	0.2	175	100	125	95
12	6.0	1.8	175	40	47	35
13	6.0	1.0	50	164	239	157
14	6.0	1.0	301	62	90	57
15	6.0	1.0	175	60	76	53
16	6.0	1.0	175	60	82	52
17	6.0	1.0	175	63	79	55
18	6.0	1.0	175	61	78	52
19	6.0	1.0	175	60	77	51
20	6.0	1.0	175	63	76	55
21	6.0	1.0	175	59	67	51
22	6.0	1.0	175	64	80	61
23	6.0	1.0	175	59	84	59

5.3.6 Characteristic features of the VA-DLLME method

Under optimum conditions, the analytical performance of the proposed method for the determination of OPPs in wastewater was evaluated using several parameters as summarized in **Table 5.4**. Method precision was evaluated by injecting 50 μg L^{-1} spiked-standard solutions, analyzed in a day, over a period of three days. The % RSD ranged between 7.70-8.2 % and 8.1-9.4 % for intraday (n=10) and inter-day (n=10 x 3 days), respectively, showing a good overall method precision of <10 %. The linear dynamic range (LDR) of the method was optimum between 5-100 μg L^{-1} with a good determination coefficient (R^2) higher than 0.999, indicative of very good linearity and applicability of the quantitative measurements. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using 3 SD/b and 10 SD/b respectively, where SD is the residual standard deviation of the linear regression and b is the

slope. The optimized method provided LODs and LOQ in the range of 0.67-0.83 µg L^{-1} and 2.2-2.8 µg L^{-1} respectively, for the three OPPs compounds studied.

5.3.7 Matrix effect

Quantitative LC-MS/MS analysis is associated with matrix effect (ME) caused by co-eluting residual matrix components. This can result in impendence of the ionization efficiency leading to inaccurate quantification of the compounds. Therefore, it was imperative to investigate matrix effects during method development and validation [28]. In this study, effluent wastewater samples were used to investigate the impact of the matrix on the VA-DLLME method. The % ME was determined by comparing the analyte signal (peak area) of the post extracted sample matrices with the analyte signal of the standards solution of the analyte prepared in the mobile phase, **equation 5.2** [28].

$$\%ME = \frac{\text{Analyte signal (post extraction spiked matrix)}}{\text{Analyte signal(solvent)}} \times 100 \qquad \textbf{(5.2)}$$

Table 5.4 shows the summary of the % ME for the 3 OPPs, evaluated at 25 µg L^{-1} in the spiked wastewater matrices. From the results, the % ME ranged from 99-106 % for three OPPs compounds which signify negligible matrix interference from coeluting matrix components. This is also attributed to efficient removal of matrix interferences by the developed sample pre-treatment method.

Table 5.4: Analytical features of method performance characteristics (n=10)

Analyte	LOD (µg L^{-1})	LOQ (µg L^{-1})	LDR (µg L^{-1})	R^2	Intra-day % RSD	Inter-day %RSD	% Matrix effect
Azinphos-methyl	0.83	2.8	5.0-100	0.9993	7.89	8.12	99.7
Parathion-methyl	0.67	2.2	5.0-100	0.9995	7.69	9.38	107
Ethoprofos	0.82	2.7	5.0-100	0.9993	8.22	8.76	106

5.3.8 Application to real environmental samples

The accuracy, validity and applicability of the developed method were tested in real environmental samples. Due to unavailability of a secondary reference material (SRM), fortification experiments using OPPs standards, were conducted in different water matrices

(influent and effluent water) at two concentration levels (25 and 100 µg L^{-1}). For each spike level, seven replicate measurements (n=7) were performed using the developed VA-DLLME procedure. The results obtained indicated that no OPPs compounds were found at the quantification level of the method. A summary of the spike recovery results obtained for each analyte in the two different sample matrices is indicated in **Table 5.5**. High recoveries ranging between 94-119 % were obtained for the OPPs spiked in both influent and effluent wastewater matrices. The RSDs obtained ranged between 5.1 - 9.8 %. These results illustrate good reproducibility and suitability of the developed VA-DLLME procedure in determining these OPPs. Furthermore, the results obtained for the non-spiked (blank) influent and effluent wastewater samples were non-detectable at the LOD of the method for each analyte. This also reveals that the method is free of interferences that could inhibit the correct identification and quantification of these compounds. **Figure 5.4** shows the extracted ion chromatogram (EIC) of the influent wastewater sample spiked at 25 µg L^{-1}.
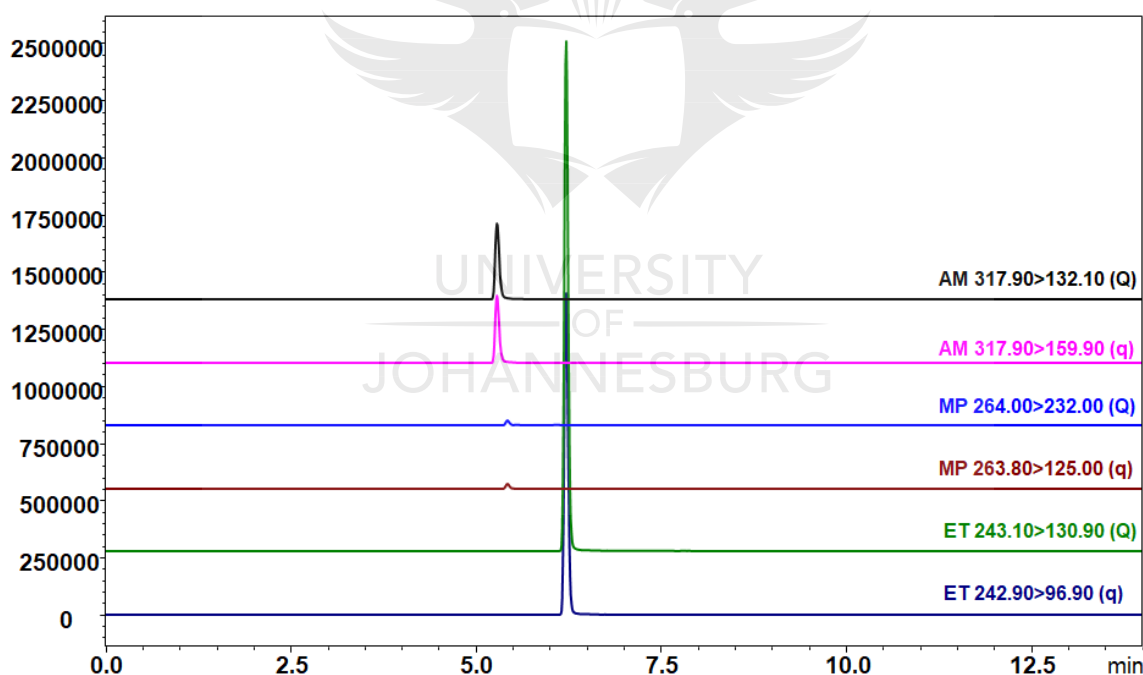
Figure 5.4: Extracted ion chromatogram (EIC) of influent wastewater sample spiked at 25 µg L^{-1} with: Azinphos-methyl, Parathion-methyl and Ethoprofos (ET) at the quantifier (Q) and qualifier (q) *m/z* transitions.

5.3.9 Comparison of VA-DLLME with other sample preparation techniques

Table 5.6 illustrates the comparison of the developed method characteristic performance with previous studies reported on determination of OPPS. The methods compared with were liquid-phase microextraction LPME [29], cloud point extraction CPE [17], single drop microextraction SDME [30], alkanol-based supramolecular solvent microextraction Al-SSME [31], ultrasound assisted dispersive magnetic solid phase extraction UADM-SPE [32], magnetic solid phase extraction (MSPE) [33] and Hollow fibre liquid phase microextraction HF-LPME [34]. From the Table, we observed that VA-DLLME has comparable, linearity, RSDs and % recoveries with the previous methods. In addition, the developed method is superior in terms of small sample size required, minimal solvent consumption, high sample throughput minimal extraction time (<10 min) compared to methods such as SPE. Also the sensitivity and shape of the chromatographic peaks obtained in this study (**Figure 5.4)** are better compared to those reported in other methods.

Table 5.5: Compound matrix recoveries of three organophosphorus pesticides in wastewater matrices (n=7)

Analyte	Spike level ($\mu g\ L^{-1}$)	Influent water			Effluent Water		
		Mean Conc ($\mu g\ L^{-1}$)	R %	RSD %	Mean Conc ($\mu g\ L^{-1}$)	R %	RSD %
Azinphos-methyl	0	ND			ND		
	25	27.31	119	9.8	26.86	110	7.4
	100	89.23	101	5.7	100.64	120	5.2
Parathion-methyl	0	ND			ND		
	25	24.95	111	8.8	24.77	98	5.3
	100	94.22	115	7.0	110.44	115	5.1
	0	ND			ND		
Ethoprofos	25	30.00	110	9.2	27.51	105	7.8
	100	91.20	95	6.5	98.06	95	6.6

ND -Non-detectable

Table 5.6: Comparison of VA-DLLME -UHPLC-MS/MS with other analytical techniques in analyzing OPPs [17,29,30-35]

Method	Detection	Linearity (µg L^{-1})	LOD (µg L^{-1})	%RSD	% Recovery	Reference
CPE	HPLC-UV	50-5000	1-30	0.9-2.2	82.7-107	[17]
LPME	HPLC-UV	0.5-400	0.1-0.35	2.0-5.7	92.2-107	[29]
SDME	GC-NPD	0.05-50	0.012-0.02	<6	70.6-107	[30]
Al-SSME	HPLC	1.3-500	0.5-1.3	<7	>94	[31]
UADM-SPE	HPLC-UV	0.2-800	0.08-0.13	<6	84-97	[32]
MSPE	GC-MS	50-3000	5.0	<10.7	-	[33]
HF-LPME	GC-MS	0.14-200	0.04-0.44		85.17-114.73	[34]
SPE	LC-MS	0.1-200	0.005-0.1	3.2-9.4	71.7-78.5	[35]
VA-DLLME	LC-MS/MS	5.0-100	0.74-0.91	5.1-9.8	95.0-119	**This work**

LPME: liquid-liquid microextraction, CPE: cloud point extraction, SDME: single drop microextraction, Al-SSME: alkanol-based supramolecular solvent microextraction, UADM-SPE: ultrasound assisted dispersive magnetic solid phase extraction, MSPE: magnetic solid phase extraction, HF-LPME: Hollow fibre liquid phase microextraction

5.4 CONCLUSIONS

In the present study, a method for the determination of three organophosphorus pesticides (azinphos-methyl, parathion-methyl and ethoprofos) in wastewater samples using VA-DLLME/ LC-MS/MS was successfully developed for the first time. The LC-MS/MS technique provided a robust and sensitive analysis. In comparison to other methods in the literature, the developed method is relatively fast and simple in the analysis of OPPs in water, with very minimal organic solvent consumption, ease of use with negligible matrix interference (99-106 %). The concentrations of the wastewater samples analyzed were below the LOD. Excellent method performance was obtained following the optimized experimental conditions using RSM based on CCD. Overall, the main advantages of the proposed analytical technique are high extraction recoveries (94.95-119.47 %) with minimal sample volume (5 mL), low intra-day and inter-day % RSDs (<9.5 %) with minor matrix interference.

5.5 REFERENCES

[1] H. John, F. Worek, H. Thiermann, LC-MS-based procedures for monitoring of toxic organophosphorus compounds and verification of pesticide and nerve agent poisoning, Analytical and Bioanalytical Chemistry 391 (2008) 97-116.

[2] M. Tankiewicz, J. Fenik, M. Biziuk, Determination of organophosphorus and organonitrogen pesticides in water samples, TrAC Trends in Analytical Chemistry 29 (2010) 1050-1063.

[3] S. Berijani, Y. Assadi, M. Anbia, M.-R.M. Hosseini, E. Aghaee, Dispersive liquid–liquid microextraction combined with gas chromatography-flame photometric detection: very simple, rapid and sensitive method for the determination of organophosphorus pesticides in water, Journal of Chromatography A 1123 (2006) 1-9.

[4] M.R. Khalili-Zanjani, Y. Yamini, N. Yazdanfar, S. Shariati, Extraction and determination of organophosphorus pesticides in water samples by a new liquid phase microextraction–gas chromatography–flame photometric detection, Analytica chimica acta 606 (2008) 202-208.

[5] Z. Cai, D. Wang, W.T. Ma, Gas chromatography/ion trap mass spectrometry applied for the analysis of triazine herbicides in environmental waters by an isotope dilution technique, Analytica Chimica Acta 503 (2004) 263-270.

[6] D. Nagaraju, S.-D. Huang, Determination of triazine herbicides in aqueous samples by dispersive liquid–liquid microextraction with gas chromatography–ion trap mass spectrometry, Journal of Chromatography A 1161 (2007) 89-97.

[7] Q. Zhou, H. Bai, G. Xie, J. Xiao, Trace determination of organophosphorus pesticides in environmental samples by temperature-controlled ionic liquid dispersive liquid-phase microextraction, Journal of Chromatography A 1188 (2008) 148-153.

[8] J. Xia, B. Xiang, W. Zhang, Determination of metacrate in water samples using dispersive liquid–liquid microextraction and HPLC with the aid of response surface methodology and experimental design, Analytica chimica acta 625 (2008) 28-34.

[9] C. Wu, H. Liu, W. Liu, Q. Wu, C. Wang, Z. Wang, Determination of organophosphorus pesticides in environmental water samples by dispersive liquid–liquid microextraction with solidification of floating organic droplet followed by high-performance liquid chromatography, Analytical and Bioanalytical Chemistry 397 (2010) 2543-2549.

[10] G. Gervais, S. Brosillon, A. Laplanche, C. Helen, Ultra-pressure liquid chromatography–electrospray tandem mass spectrometry for multiresidue determination of pesticides in water, Journal of Chromatography A 1202 (2008) 163-172.

[11] N.M. Brito, S. Navickiene, L. Polese, E.F.G. Jardim, R.B. Abakerli, M.L. Ribeiro, Determination of pesticide residues in coconut water by liquid–liquid extraction and gas chromatography with electron-capture plus thermionic specific detection and solid-phase extraction and high-performance liquid chromatography with ultraviolet detection, Journal of Chromatography A 957 (2002) 201-209.

[12] J.J. Jiménez, J.L. Bernal, M.J.d. Nozal, C. Alonso, Liquid–liquid extraction followed by solid-phase extraction for the determination of lipophilic pesticides in beeswax by gas chromatography–electron-capture detection and matrix-matched calibration, Journal of Chromatography A 1048 (2004) 89-97.

[13] J.-B. Baugros, B. Giroud, G. Dessalces, M.-F. Grenier-Loustalot, C. Cren-Olivé, Multiresidue analytical methods for the ultra-trace quantification of 33 priority substances present in the list of REACH in real water samples, Analytica Chimica Acta 607 (2008) 191-203.

[14] C. Cháfer-Pericás, R. Herráez-Hernández, P. Campíns-Falcó, In-tube solid-phase microextraction-capillary liquid chromatography as a solution for the screening analysis of organophosphorus pesticides in untreated environmental water samples, Journal of Chromatography A 1141 (2007) 10-21.

[15] P. Liang, L. Guo, Y. Liu, S. Liu, T.-z. Zhang, Application of liquid-phase microextraction for the determination of phoxim in water samples by high performance liquid chromatography with diode array detector, Microchemical Journal 80 (2005) 19-23.

[16] P. Soisungnoen, R. Burakham, S. Srijaranai, Determination of organophosphorus pesticides using dispersive liquid–liquid microextraction combined with reversed electrode polarity stacking mode—micellar electrokinetic chromatography, Talanta 98 (2012) 62-68.

[17] K. Seebunrueng, Y. Santaladchaiyakit, P. Soisungnoen, S. Srijaranai, Catanionic surfactant ambient cloud point extraction and high-performance liquid chromatography for simultaneous analysis of organophosphorus pesticide residues in water and fruit juice samples, Analytical and bioanalytical chemistry 401 (2011) 1703.

[18] M. Rezaee, Y. Assadi, M.-R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, Determination of organic compounds in water using dispersive liquid–liquid microextraction, Journal of Chromatography A 1116 (2006) 1-9.

[19] M.A. Farajzadeh, M. Bahram, M.R. Vardast, M. Bamorowat, Dispersive liquid-liquid microextraction for the analysis of three organophosphorus pesticides in real samples by high performance liquid chromatography-ultraviolet detection and its optimization by experimental design, Microchimica Acta 172 (2011) 465-470.

[20] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC method development, John Wiley & Sons2012.

[21] Q. Yin, Y. Zhu, Y. Yang, Dispersive Liquid–Liquid Microextraction Followed by Magnetic Solid-Phase Extraction for Determination of Four Parabens in Beverage Samples by Ultra-performance Liquid Chromatography Tandem Mass Spectrometry, Food Analytical Methods 11 (2018) 797-807.

[22] K.M. Dimpe, A. Mpupa, P.N. Nomngongo, Microwave assisted solid phase extraction for separation preconcentration sulfamethoxazole in wastewater using tyre based activated carbon as solid phase material prior to spectrophotometric determination, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 188 (2018) 341-348.

[23] C. Stalikas, Y. Fiamegos, V. Sakkas, T. Albanis, Developments on chemometric approaches to optimize and evaluate microextraction, Journal of Chromatography A 1216 (2009) 175-189.

[24] T. Rakić, I. Kasagić-Vujanović, M. Jovanović, B. Jančić-Stojanović, D. Ivanović, Comparison of full factorial design, central composite design, and box-behnken design

in chromatographic method development for the determination of fluconazole and its impurities, Analytical Letters 47 (2014) 1334-1347.

[25] H. Sereshti, M. Karimi, S. Samadi, Application of response surface method for optimization of dispersive liquid–liquid microextraction of water-soluble components of Rosa damascena Mill. essential oil, Journal of Chromatography A 1216 (2009) 198-204.

[26] Y. Liu, C. Zhang, X. Liao, Y. Luo, S. Wu, J. Wang, Hydrolysis mechanism of methyl parathion evidenced by Q-Exactive mass spectrometry, Environmental Science and Pollution Research 22 (2015) 19747-19755.

[27] M. Rezaee, Y. Yamini, M. Faraji, Evolution of dispersive liquid–liquid microextraction method, Journal of Chromatography A 1217 (2010) 2342-2357.

[28] B. Matuszewski, M. Constanzer, C. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC− MS/MS, Anal. Chem. 75 (2003) 3019-3030.

[29] P. Zohrabi, M. Shamsipur, M. Hashemi, B. Hashemi, Liquid-phase microextraction of organophosphorus pesticides using supramolecular solvent as a carrier for ferrofluid, Talanta 160 (2016) 340-346.

[30] F. Tian, W. Liu, H. Fang, M. An, S. Duan, Determination of six organophosphorus pesticides in water by single-drop microextraction coupled with GC-NPD, Chromatographia 77 (2014) 487-492.

[31] M. Peyrovi, M. Hadjmohammadi, Alkanol-based supramolecular solvent microextraction of organophosphorus pesticides and their determination using high-performance liquid chromatography, Journal of the Iranian Chemical Society 14 (2017) 995-1004.

[32] R. Hamedi, A. BG Aghaie, M.R. Hadjmohammadi, Magnetic core micelles as a nanosorbent for the efficient removal and recovery of three organophosphorus pesticides from fruit juice and environmental water samples, Journal of separation science 41 (2018) 2037-2045.

[33] J. Xie, T. Liu, G. Song, Y. Hu, C. Deng, Simultaneous Analysis of Organophosphorus Pesticides in Water by Magnetic Solid-Phase Extraction Coupled with GC–MS, Chromatographia 76 (2013) 535-540.

[34] H.C. Menezes, B.P. Paulo, M.J.N. Paiva, Z.L. Cardeal, A simple and quick method for the determination of pesticides in environmental water by HF-LPME-GC/MS, Journal of analytical methods in chemistry 2016 (2016).

[35] H. Zhang, D. West, H. Shi, Y. Ma, C. Adams, T. Eichholz, Simultaneous Determination of Selected Trace Contaminants in Drinking Water Using Solid-Phase Extraction-High

Performance Liquid Chromatography-Tandem Mass Spectrometry, Water, Air, & Soil Pollution 230 (2019) 28.

CHAPTER 6:

SYNTHESIZED CARBON NANODOTS FOR SIMULTANEOUS EXTRACTION OF PERSONAL CARE PRODUCTS AND ORGANOPHOSPHORUS PESTICIDES IN WASTEWATER SAMPLES PRIOR TO LC-MS/MS DETERMINATION

ABSTRACT

A simple, rapid and efficient solid phase extraction method based on synthesized carbon nanodots was developed for the preconcentration and extraction of personal care products and organophosphorus pesticides in environmental matrices. Factorial (screening) and central composite designs were employed for the optimization of experimental conditions that could potentially influence the percentage recoveries of the target analytes. The experimental variables including sample pH, mass of adsorbent, eluent volume and sample volume, were examined. Under the optimized conditions, the developed method was validated, and acceptable analytical results obtained showed good performance. The method accuracy carried out at two spiking levels (10 and 100 μg L^{-1}) in different sample matrices ranged between 63-120%. The method precision based on relative standard deviation (% RSD) was <10%. The linear range studied had a coefficient of determination of (R^2>0.995). The limits of detection (LOD) and limit of quantification (LOQ) established varied between 0.015-0.125 μg L^{-1} and 0.05-0.415 μg L^{-1} respectively. The ensuing method was applied successfully in analysis of real wastewater samples with concentrations ranging between 0.13-3.51 μg L^{-1}. The influent and effluent wastewater samples were obtained from a municipal WWTP located in Pretoria, South Africa.

6.1 INTRODUCTION

The occurrence, fate and behaviour of organic contaminants in the environment are subjects that have sparked major interest in recent research globally [1]. These organic contaminants encompass a diverse group of compounds such as organophosphorus pesticides (OPPs) and personal care products (PCPs) [2, 3]. Due to the increasing demand in their application in various sectors, these organic contaminants are ubiquitous in the environment depending on their pattern of use and the application mode. Parabens belonging to the class of personal care products that are applied externally with no metabolic changes in their structure. This causes them to be released easily in the aquatic environment via industrial and domestic effluent discharge [4]. OPPs are extensively applied in agricultural activities and are considered among the most acutely toxic group of pesticides according to the environmental protection agency (EPA) classification [5]. These organic contaminants enter the aquatic environment primarily through discharge from poorly treated effluents from wastewater treatment plants (WWTPs), secondary terrestrial run-offs and municipal landfill leachates [6, 7]. Long-term exposure of these organic contaminants at trace levels to humans and aquatic life has raised great health concerns due to the carcinogenic, mutagenic and endocrine disruptive effects exhibited by these compounds [8]. For instance, since 2011, the addition of propylparaben and butylparaben in children cosmetics had been banned in Denmark [9]. However, these compounds are still extensively used in PCPs in other countries like South Africa as reported in previous studies [10]. Moreover, water contamination caused by these organic contaminants in the aquatic environment significantly affects the possibility of the reuse of water from treated industrial water and municipals effluents [11]. Furthermore, due to their presence in complex mixtures, there is a potential risk of increased toxicological activity due to antagonism or synergism phenomena [12].

Consequently, there is a need to develop multi-class methods for extraction and determination of these compounds at trace levels, with the utmost sensitivity, selectivity and reliability. This is, in fact, a prerequisite for definitive risk assessment and evaluation of the quality of the waste, surface and drinking water [13]. Due to matrix interference and the existence of the compounds in trace concentrations, a clean-up and preconcentration step is indispensable, to obtain low detection levels. Additionally, the development of multi-class methods for monitoring these compounds is important since these compounds exist as complex mixtures in the environment [14]. Also, increasing the number of analytes that can be determined simultaneously in a single run, is a key factor in high-throughput analyses [15].

For quantitative determination of these compounds, chromatographic methods coupled with mass spectrometric detection techniques such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been employed [15, 16]. LC-MS/MS is a powerful detection technique with the advantage of being able to separate, identify and quantify these multi-class organic contaminants in a single run at trace levels [17]. However, it is essential to develop adequate sample enrichment methods to achieve low limits of detection (LODs) and quantification (LOQs) of organic contaminants in complex environmental matrices.

Several sample enrichment procedures such as solid phase extraction (SPE) [8, 18], solid phase microextraction (SPME) [19] and disperse solid phase microextraction (DSPME) [20], have been used extensively in the past decades. SPE is a suitable extraction technique that provides high enrichment of analytes, extract clean up, single step preconcentration and can be easily incorporated into automated analytical procedures [21]. The type of sorbent in use and its interaction with the analyte play an important role in obtaining high enrichment efficiency of analytes [22]. Sorbent materials commonly used in SPE cartridges include C18, Oasis HLB (divinylbenzene/*N*-vinylpyrrolidone copolymer) and bonded silica [20, 23]. In the recent past, carbonaceous nanomaterials have also been employed and integrated with SPE techniques, which provide additional trapping sites than previous sorbents [24]. Carbon nanodots (CNDs) with a typical size of less than 10 nm, have emerged as novel nanomaterials with increasing application in the areas of biosensing, bioimaging and photocatalysis [25-27]. This is attributed to the following advantages; inexpensive, readily scalable, high aqueous solubility, low toxicity, excellent chemical stability and inertness, easy preparation and functionalisation and colloidal stability [28]. Furthermore, they can be prepared using a variety of methods, including green synthesis procedures that employ readily available and inexpensive resources such as corn, papaya or sweet pepper as the initial starting material [29-31] without the use of any chemicals for preparation as was reported by [32]. To this end, despite the excellent features of the CNDs, they are yet to be fully exploited in the analytical applications employing extraction of organic contaminants in water samples.

This study demonstrates the use of grain oats as carbon source for a simple, economical and cost-effective green approach for the direct synthesis of CNDs without any further modification. The application of the CNDs as SPE nanosorbents for extraction and preconcentration of three parabens and two OPPs compounds in wastewater has been demonstrated. Liquid chromatography-tandem mass spectrometry method was also developed for their sensitive and reproducible quantitative analysis of these organic compounds. A comparison between the commonly used commercial-based adsorbents (Oasis HLB) and

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CNDs was performed. To the best of our knowledge, the application of pristine CNDs as SPE sorbent for preconcentration of multi-class analytes (pesticides and parabens) in wastewater samples, has not been reported in the literature. The effect of operational variables influencing the extraction efficiency of the proposed method was optimized using a multivariate approach. The ensuing method was evaluated for analytical performance and thereafter applied to real samples analysis.

6.2 EXPERIMENTAL

6.2.1 Chemicals and Reagents

Methylparaben (MePB), ethylparaben (EthPB) and propylparaben (ProPB) azinphos-methyl and parathion-methyl, were purchased from Merck (Darmstadt, Germany). Individual stock solutions of each of the parabens (1000 mg L^{-1}) were prepared in methanol, as well as the mixed solution of the three parabens and stored at -18 °C until use. A mixed standard working solution of 10 mg L^{-1} was prepared in acetonitrile comprising all the analytes and stored at -18 °C. Calibration standard mixtures of all the analytes were prepared prior to the analytical run by appropriate dilution of 10 mg L^{-1} stock standard solution in water/acetonitrile (80:20 v/v). HPLC grade methanol (MeOH), acetonitrile (ACN) and acetic acid (96 %) and formic acid (FA) (98%) were supplied by Merck (Darmstadt, Germany). Hydrochloric acid (HCl) (37%) and sodium hydroxide (NaOH) also supplied by Merck (Darmstadt, Germany) were used to adjust the pH of the model solutions and real water samples. Ultra-pure water was obtained from a Millipore filtration system with a specific resistance of 0.55µs. Oasis HLB cartridges purchased from Sigma-Aldrich (St. Louis, MO, USA) were used for comparison with the synthesised CNDs-SPE cartridges. Amber glass vials (2 ml), 0.45 µm PVDF syringe filers and 10 ml syringes were obtained from RESTEK (RESTEK, USA). Grain oats were obtained from local stores.

6.2.2 Sample collection

Environmental samples were collected from a local wastewater treatment plant in Pretoria, South Africa. This included untreated raw wastewater (influent), and treated wastewater (final effluent) systems, sampled in triplicates. Glass amber bottles, precleaned before collection with the real samples, were used to collect the samples and placed in a cooler box with ice. They were then transported to the laboratory and stored at 4 °C. Samples were filtered using 0.45 µm PVDF syringe filters prior to extraction and chromatographic mass-spectrometric analysis.

6.2.3 LC-MS/MS operating conditions

The instrumental analysis was carried out using a Shimadzu UHPLC-MS/MS system. The UHPLC was equipped with two LC-30AD pumps, DGU-20A5R degasser unit, a SIL-30AC nexera autosampler, a CTO-30A column oven, and a CBM-20A communication module. The UHPLC was coupled to an LC-MS 8040 triple quadrupole mass spectrometer, installed with orthogonal electrospray ionisation (ESI) source. The HPLC separation was done using a raptor ARC-18 column (100 mm x 2.1 mm, 3 µm) (RESTEK, USA). A C18 guard column (2.1×5 mm, 1.8 µm, RESTEK, USA) was used to protect and extend the chromatographic column useful life.

Mobile phase A comprised of 0.1 % (*v/v*) FA in de-ionised water and mobile phase B was methanol. The column oven temperature was 40 °C and autosampler was kept at 4 °C. Sample injection volume was 30 µL. The flow rate was maintained at 0.2 mL min^{-1} and the column was equilibrated with 50 % B prior to injection. The optimised gradient elution programme started at 50% B and was increased to 75 % B in 4 min, increased to 100 % B in 1 min, maintained at 100 % B for 4 min, and re-equilibrated at 50% B for 5 min.

Multiple reaction monitoring (MRM) mode was used for the identification and quantification of the compounds in positive mode for OPPs and negative mode for parabens [33, 34]. The optimum conditions for MS analyses were: the nebulizing gas flow rate of 3 L min^{-1}; drying gas flow rate of 15 L min^{-1}; desolvation line (DL) temperature 250 °C; heat block 400 °C; ion source spray voltage 4.5kV. Argon with a purity of 99.999% was used as the collision-induced dissociation (CID) gas. Peak detection, instrument control, data analysis, method optimisation was carried out using LabSolutions (Tokyo, Japan).

6.2.4 Green synthesis of CNDs

Carbon nanodots were synthesized according to previous literature with slight modification [35]. Briefly, 10 g of oats grains were weighed, crushed, placed in a crucible and transferred into a muffle furnace and pyrolyzed at 400 °C for 2 hrs. The black product obtained was cooled at ambient temperature before being finally mechanically crushed to a fine powder. The product obtained was dispersed in ultrapure water and centrifuged at 7800 rpm several times to remove larger particles. The carbon nanodots aqueous suspension was filtered and the CNDs residue dried in an oven for 24 hrs at 80 °C.

6.2.5 Characterization of CNDs

The synthesised CNDs were evaluated using various characterisation techniques. Fourier transform Infrared (FTIR) spectroscopy was used to determine the functional groups present in the CNDs. Scanning electron microscope (SEM) (TESCAN Model Vega 3LMH) was used to determine the surface morphology and particle sizes. High-resolution transmission electron microscope (HRTEM) was used in obtaining micrographs showing the shape of the CNDs. An acceleration voltage of 200 Kv was used. Samples for TEM analysis were prepared by dispersing the CNDs in ethanol with ultrasonication for 10 min. A drop of the dispersion was placed onto a copper grid (200 mesh size Cu-grid). X-ray diffractometer (Phillips X'Pert-PRO PANalytical) was used to examine the crystallographic patterns of the nanomaterial. A VacMaster-24 sample SPE station (Waters Corporation Milford, USA) was used for the SPE experiments.

6.2.6 CNDs-SPE procedure

Extraction of MePB, EthPB, ProPB, azinphos-methyl and parathion-methyl from the wastewater samples was performed using pre-packed SPE cartridges with the CNDs. Supelco polyethene columns (Supelco, PA, USA, 1.35 cm in diameter and 3.5 cm in length) with frits were employed for SPE in a VacMaster-24 sample station. The powdered CNDs (170 mg) was dry packed into empty SPE cartridges. Porous frits were placed at the bottom and at the top of the column for the proper settling of the sorbent material. Prior to the extraction, the samples were filtered using 0.45 µm PVDF syringe filters to eliminate any suspended solids that may otherwise interfere with the extraction due to clogging. Sample pH adjustment was done using 0.1 M HCl. Sorbent conditioning with 3 mL ACN, followed by 3 mL de-ionised water was carried out prior to sample loading. Spiked and blank (un-spiked) water samples (50 mL), were loaded into the pre-conditioned cartridge with the aid of an SPE vacuum manifold. Teflon tubes were connected between the sample bottles and the SPE cartridges, for automatic sample loading. De-ionized water (5 mL) was passed to clean the cartridge before vacuum drying for 20 minutes. Elution of retained analytes was done using 6 mL of 10 % (v/v) acetic acid in acetonitrile. This sample eluate was filtered using 0.22 µm PVDF syringe filters and an aliquot of 200 µL was diluted 5x with the mobile phase ready for instrumental analysis. For the initial optimization experiments, model solutions spiked with the working mixed standards at 100 µg L^{-1} were prepared in de-ionised water.

6.2.7 Design of experiment

A two-fold multivariate optimization strategy was performed for the optimisation of the SPE extraction procedure. Firstly, two-level (2^4) (where 2 is the number of levels and 4 is the number of factors), full factorial design was used in the screening of the independent variables that has an influence in the extraction recovery. They included sample pH, the mass of adsorbent (MA), sample volume (SV) and elution volume (EV). The maximum and minimum values assigned to these variables are indicated in **Table 6.1.** The second phase of optimization was the application of response surface methodology (RSM) based on central composite design (CCD) to optimise the optimum experimental conditions, in terms of % recovery as the analytical response. Pareto charts and RSM plots were obtained using Statistica version 13 (StatSoft, USA). The final optimum experimental conditions were obtained using Minitab 17 statistical software (Minitab Lt. Conventry, UK). The selection of the best elution solvent was however done univariately where the following solvents were investigated; 100 % methanol, 100 % acetonitrile, and 10 % (v/v) acetic acid in acetonitrile and 10 % (v/v) acetic acid in methanol.

Table 6.1: Variables and levels selected for the two-level (2^4) full factorial design

Variable	Low level (-1)	High level (+1)
Sample pH	3	9
Mass of adsorbent (mg)	90	150
Sample volume (mL)	50	150
Elution Volume (mL)	3	6

6.2.8 Method validation

The method analytical figures of merit were evaluated based on accuracy (% recovery), limits of detection (LOD), limit of quantification (LOQ), method precision (repeatability and reproducibility) linearity and matrix effect. The method precision was performed by analysing fortified samples (n=8) at 50 µg L^{-1}. Influent and effluent wastewater samples matrices were fortified at two concentration levels (10 µg L^{-1} and 100 µg L^{-1}) to establish the accuracy of the method from the obtained mean recoveries. Matrix effect assessment was performed by spiking the analytes in two different sets of samples; mobile phase solvent and in real water samples at 25 µg L^{-1} (n=5). To ascertain the presence or absence of matrix effect, the peak area ratios of the analytes in solvent solution with that of the analytes in matrix solution were compared.

6.2.9 Regeneration studies

The CNDs-SPE cartridge that had been previously employed for the extraction of spiked de-ionised water sample containing 25 ug L^{-1} of the target analytes, was cleaned thoroughly and repeatedly, averagely 10 times using acetonitrile. The cartridge was then dried under vacuum, conditioned and utilized for the subsequent round of extractions. This procedure was evaluated 5 times, to ascertain the re-usability of the adsorbent. Analyte recoveries for each extraction were determined.

6.3 RESULTS AND DISCUSSION

6.3.1 Characterisation

The TEM image of the CNDs revealed that they are spherical in shape, **Figure 6.1a**. The CNDs appear as black spots and are well monodispersed from each other as observed in the TEM image. A corresponding particle size distribution of the CNDs as shown in the insert image was obtained by statistical analysis of approximately 100 particles. The diameter of the CNDs ranged between 1-7 nm with an average diameter of 3.45 ± 0.92 nm (Figure 1a). Also, a spherical nanostructure was observed for the SEM image of the synthesised CNDs, **Figure 6.1b.**

The functional groups present in the synthesized CNDs were investigated using FTIR as shown in **Figure 6.1c.** The strong absorption band at 3430 cm^{-1} is ascribed to the stretching vibration of the –OH and 2923 cm^{-1} corresponds to C-H [36]. The peak at 1620 cm^{-1} and 1391 cm^{-1} relate to the asymmetric and symmetric stretching of the carboxylate anions respectively, while the peak at 1038 cm^{-1} similarly corresponds to the -OH vibration of water [31]. The typical XRD pattern of the CNDs is presented in **Figure 6.1d**. Two diffraction peaks at 2θ values of 24.63° and 42.64° are observed. The former represented (111) lattice plane and the later to a diamond phase in the carbon nanodots. The predominant broad diffraction peak centred at 24.63° suggest that the synthesised CNDs consist mainly of amorphous carbon [37]. The presence of the mentioned functional groups enables good extractability and long-term stability.
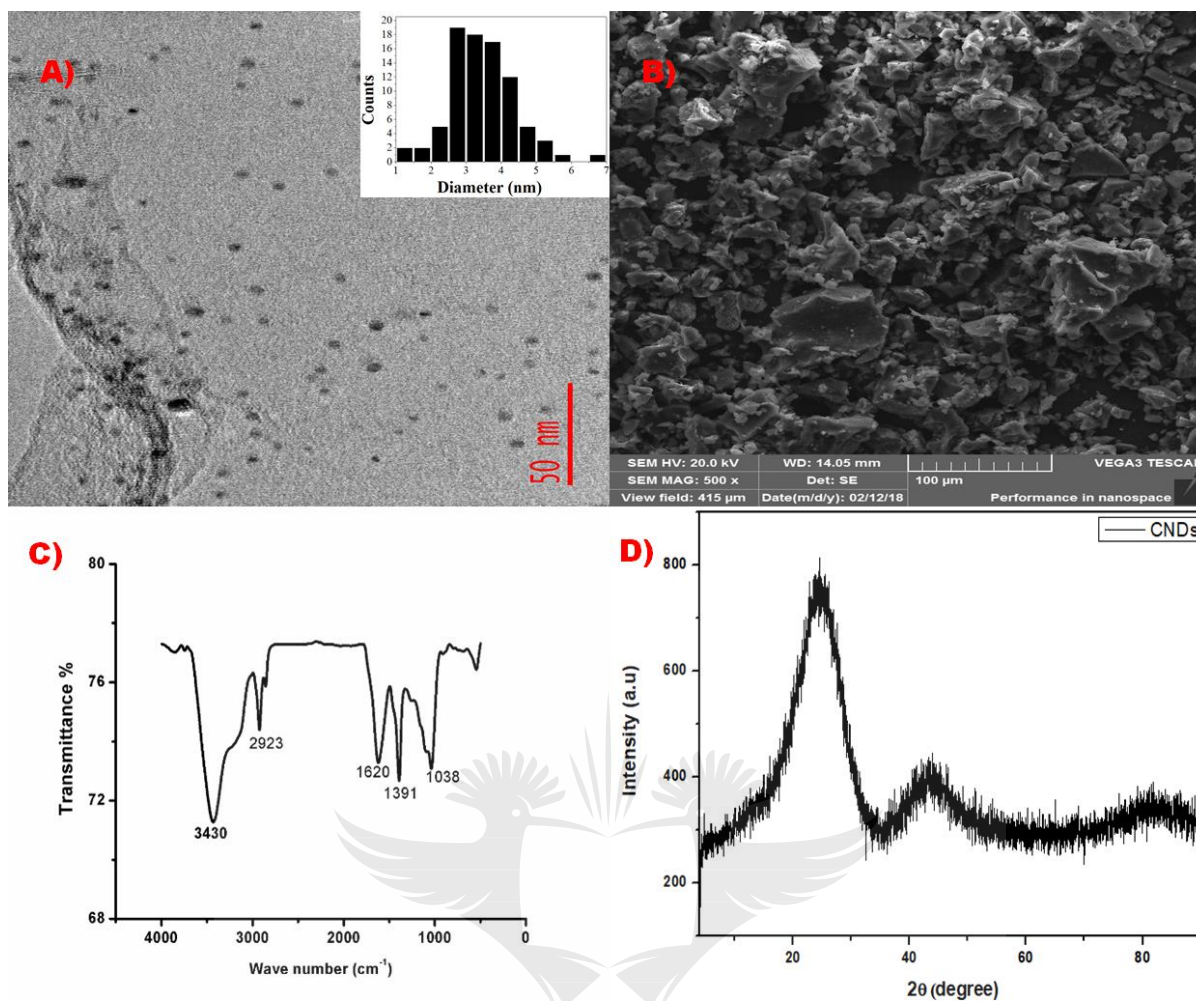
Figure 6.1: Characterization results of CNDs for A) TEM-The insert shows the average diameter of the CNDs, B) SEM, C) FTIR, D) XRD

6.3.2 LC-MS/MS optimization

The maximum selectivity and sensitivity of the mass spectrometry conditions were achieved using multiple reaction monitoring (MRM) mode, as detailed in **Table 6.2**. Scheduled MRM and polarity switching permitted the simultaneous acquisition of the 10 transitions in the same chromatographic run. The parabens achieved maximum sensitivity in the negative mode, characterized by the deprotonated molecular ion at $[M-H]^-$ as the precursor ion. The OPPs, on the other hand, were more sensitive in the positive mode giving precursor ions characterized as $[M+H]^+$. Product ions were obtained by the collision-induced dissociation of the precursor ions. Two product ions were monitored for each analyte. The most intense product ion in terms of peak area was selected as the target mass-to-charge ratio (m/z) for quantitative analysis in all experimental runs. The least intense product ion was used for confirmatory purposes. The chromatographic separation using gradient elution programmed is as detailed in section 2.3.

During the preliminary experiments of the mobile phase, gradient conditions were starting at lower % organic (20%). Late elution for all the analytes were observed, with the first analytes eluting at >5 min and longer equilibration times, leading to increased run times. This observation was attributed to the use of methanol as the organic mobile phase component. Due to its lower eluotropic strength compared to acetonitrile, compounds are eluted at higher % organic. Also, in reversed phase chromatography, non-polar compounds are retained more strongly than polar compounds, hence require increased amounts of organic solvent [38]. Therefore, to obtain lower retention times as desired, the starting condition of the mobile phase gradient elution was optimized at 0.1% (v/v) formic acid/ methanol (50:50 v/v). Methanol was selected as the organic component of the mobile phase as opposed to acetonitrile because it provides for better ESI sensitivity, better peak shapes and it is inexpensive. The retention times and order of elution under the optimized conditions are shown in **Table 6.2.**

Table 6.2: Optimized MS/MS parameters for the multiple reaction monitoring analysis

Compound	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	Dwell time (ms)	CE (v)	ESI mode
MePB	2.2	151.1	92.1	50	23	(-)
		151.1	136	50	14	
EthPB	3.1	165.1	91.9	50	23	(-)
		165.1	136.2	50	20	
ProPB	5.0	179.1	92.1	50	25	(-)
		179.1	136	50	18	
Azinphos methyl	5.8	317.9	132.1	50	-15	(+)
		317.9	159.9	50	-9	
Parathion-methyl	6.0	264.1	232.1	50	-18	(+)
		263.8	125.1	50	-21	

m/z: mass-to-charge, CE=collision energy

6.3.3 Univariate optimisation: Elution solvent selection

To ensure the highest obtainable extraction efficiencies, different organic solvents required for elution of the target analytes were investigated for their effectiveness in desorbing the analytes from the CNDs. The solvents studied were methanol, acetonitrile, 10 % (v/v) acetic acid in methanol and 10 % (v/v) acetic acid in acetonitrile. The experiments were conducted by maintaining fixed parameters of other variables which comprised of 50 mL model sample solution at 100 µg L^{-1}, mass of adsorbent (170 mg), constant flow rate (1 mL min^{-1}) and eluent

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volume of 6 mL. From the result obtained in **Figure 6.2**, the 10 % acetic acid in acetonitrile was observed to give high % recoveries (>70%) for all the target analytes. On the other hand, 10 % acetic acid in methanol gave relatively low recoveries (50-60 %). Based on the evaluation of the % recovery results obtained, the optimum elution solvent selected was 10 % acetic acid in acetonitrile. These phenomena can be attributed to enhanced hydrogen bonding capacity of the acetonitrile in the presence of an acid, thus inducing competition with the retained analytes for hydrogen binding on the sites of the CNDs [39]. In addition, the polarity of this solution was more favourable in the desorption of the analyte components from the sorbent material. The experiments were conducted in triplicate with % RSDs of <2, which is acceptable. The addition of acetic acid to obtain optimum analyte desorption is vital, hence 10 % acetic acid in acetonitrile was used for further experimental runs in the study.
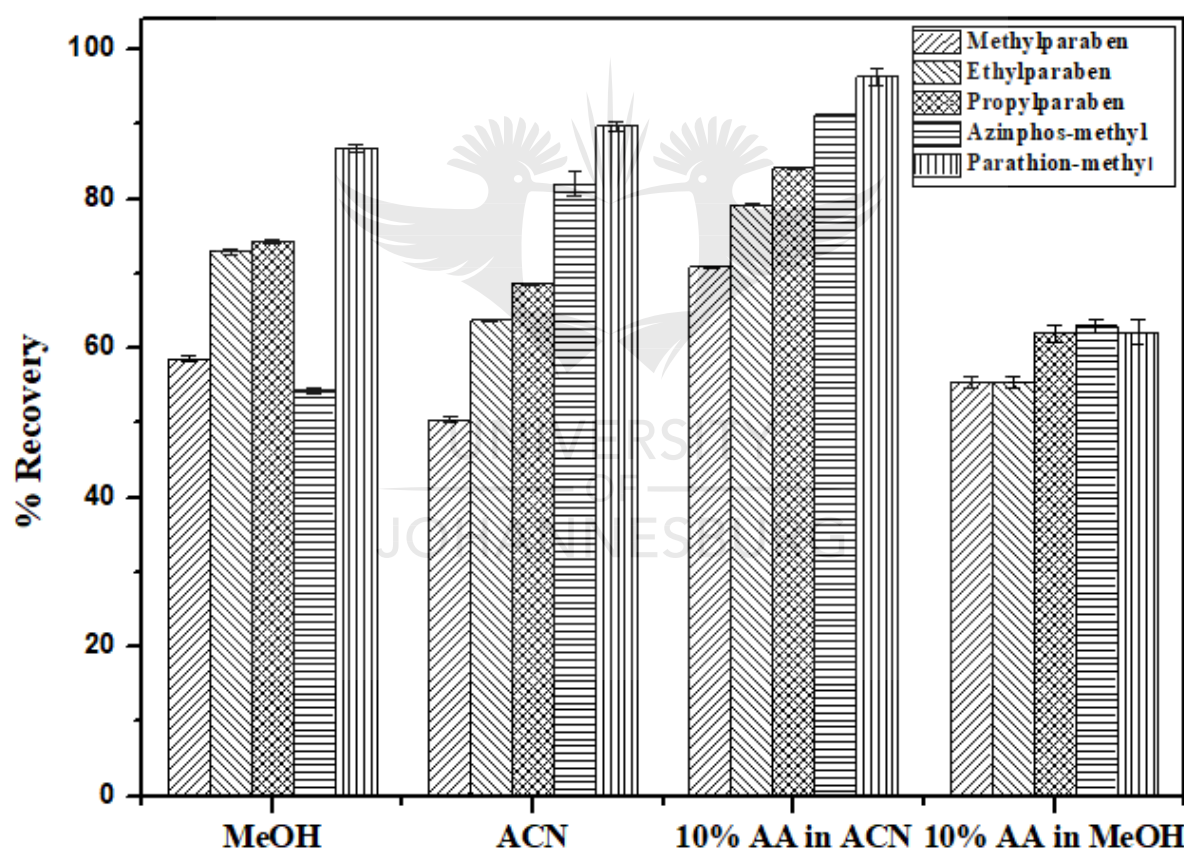

Figure 6.2: Optimisation of the elution solvent for CNDs based SPE: - the error bars correspond to the RSD of the mean recovery for n = 3 replicates. AA: acetic acid, MeOH: methanol, ACN: acetonitrile

6.3.4 Multivariate optimisation of SPE procedure

The optimisation of the other experimental factors was performed using a multivariate approach. The advantage of using a multivariate approach as opposed to one-variable-at-a-time (OVAT) is that it provides for the variation of factors simultaneously, thus saving time and resources [40]. The experimental design undertaken, together with the results obtained reported in terms of % recoveries are shown in **Table 6.3**. To evaluate the outcome of the design of experiments, analysis of variance (ANOVA) was employed to decipher the main interactions and influences that the factors selected had in achieving the higher recoveries. The results obtained from ANOVA are shown in **Figure 6.3** in terms of standardized Pareto charts. The bars on the Pareto chart are assigned factors such as pH, MA, EV and SV or the interactive effect of factors. The vertical line parallels the 95 % confidence level. A factor that exceeds this line is considered significant at the 95% confidence level [41]. Based on the Pareto charts, the predominant significant parameter that impacted positively on the analytical response across all the five analytes was EV. Other factors that also had a significant effect on the analytical response included sample pH, and the interactive effect of MA-EV for EthPB, ProPB, azinphos-methyl and parathion-methyl. This was also evidenced by the coefficient values for each factor on the Pareto chart. For MePB, only EV was the most significant parameter at the confidence level. All these factors affected the analytical response positively as denoted by the positive sign on the coefficient values. The positive sign is an indication that as the factor is increased, the analytical response is expected to increase [40]. It can also be observed that out of the four parameters under investigation using factorial design, only SV had a relatively low effect on the outcome of the analytical response, for all the analytes, except azinphos-methyl. In this regards pH, MA and EV were carried forward for further optimisation using response surface methodology.

Table 6.3: Analytical responses corresponding to full factorial design (2^4) matrix optimisation

Exp Runs	pH	MA (mg)	SV (mL)	EV (mL)	MePB	EthPB	ProPB	Azinphos-methyl	Parathion-methyl
							% Recoveries		
1	3.0	90	50	3	24	32	34	25	40
2	9.0	90	50	3	29	38	45	36	52
3	3.0	150	50	3	20	27	29	19	35
4	9.0	150	50	3	28	37	44	40	53
5	3.0	90	150	3	31	38	41	30	46
6	9.0	90	150	3	24	32	41	46	60
7	3.0	150	150	3	23	31	34	20	40
8	9.0	150	150	3	26	32	41	35	49
9	3.0	90	50	6	45	55	57	41	68
10	9.0	90	50	6	49	56	67	56	82
11	3.0	150	50	6	50	61	66	45	79
12	9.0	150	50	6	54	66	73	56	87
13	3.0	90	150	6	50	56	61	49	72
14	9.0	90	150	6	40	44	57	64	76
15	3.0	150	150	6	49	60	63	42	76
16	9.0	150	150	6	64	73	84	67	93

Sample pH, mass of adsorbent (MA), eluent volume (EV) and sample volume (SV)
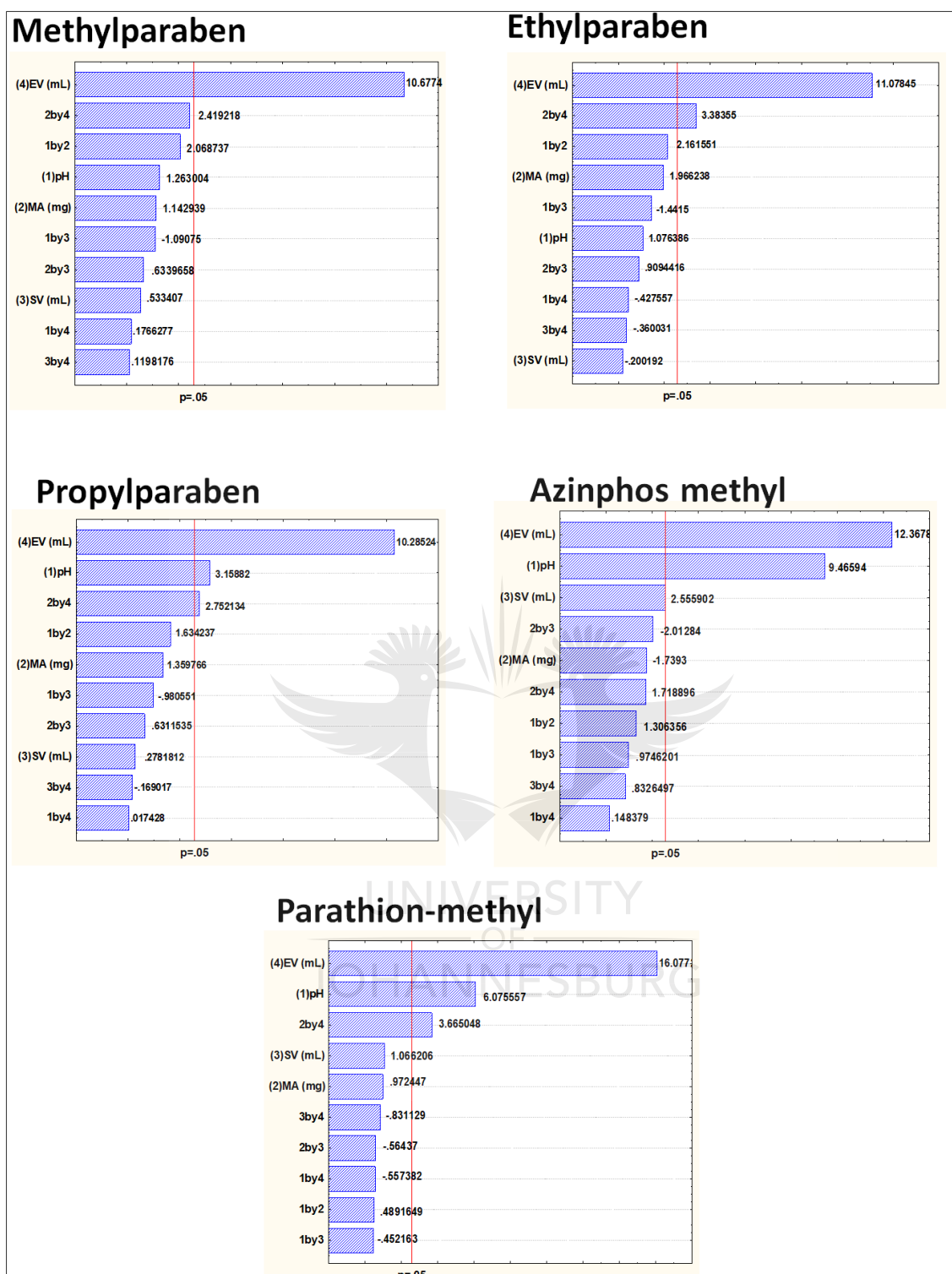
Figure 6.3: Standardized Pareto charts of parabens and organophosphorus pesticides

Further optimisation was conducted using CCD based on response surface methodology (RSM). RSM provides details on the significance and magnitude of the main effects, interactive and quadratic effects of the independent variables on the extraction recovery of the target analytes [42]. Twenty experiments were generated using CCD for optimising the experimental

factors including 6 centre points. The matrix design of the CCD along with the % recoveries of the target analytes is detailed in **Table 6.4.** The results obtained using CCD were analysed using three-dimensional (3D) surface plots as shown in **Figure 6.4**. The 3D plots were determined as a function of pH, MA and EV. Responses were mapped against two independent variables while keeping the other factor constant at its central value. To obtain the optimum conditions, an approximation can be performed visually by extrapolating the surface plots. However, it was vital to obtain synchronous optimum conditions for the three parameters, for the simultaneous extraction of the five target analytes. Therefore, by using the global optimisation function in Minitab 17 statistical software, the optimum conditions could be derived. The optimum experimental conditions were obtained as pH=4.5, MA=170 mg and EV=6 mL. The sample loading volume was maintained at 50 mL. This was beneficial in terms of reduced extraction times and potential matrix effects. These optimum conditions were then employed for further method validation and optimisation of the method.

Table 6.4: Experimental variables* and levels of central composite design matrix with analytical responses

				MePB	EthPB	ProPB	Azinphos-methyl	Parathion-methyl
EXP	**pH**	**MA**	**EV**			% Recoveries		
Run								
1	3.0	90	3.0	78	73	71	58	63
2	3.0	90	6.0	97	87	84	60	77
3	3.0	150	3.0	59	64	64	48	66
4	3.0	150	6.0	101	74	93	62	89
5	9.0	90	3.0	37	44	48	48	61
6	9.0	90	6.0	58	64	70	70	77
7	9.0	150	3.0	44	55	58	53	58
8	9.0	150	6.0	60	69	72	64	62
9	1.0	120	4.5	20	24	24	58	61
10	11	120	4.5	16	19	27	40	55
11	6.0	70	4.5	54	62	67	67	53
12	6.0	170	4.5	60	72	74	61	55
13	6.0	120	2.0	50	67	70	59	55
14	6.0	120	7.0	58	64	66	56	54
15 (C)	6.0	120	4.5	66	76	80	70	70
16 (C)	6.0	120	4.5	51	64	64	53	62
17 (C)	6.0	120	4.5	68	80	80	68	72
18 (C)	6.0	120	4.5	53	63	63	51	58
19 (C)	6.0	120	4.5	61	74	75	66	63
20 (C)	6.0	120	4.5	60	72	74	65	63

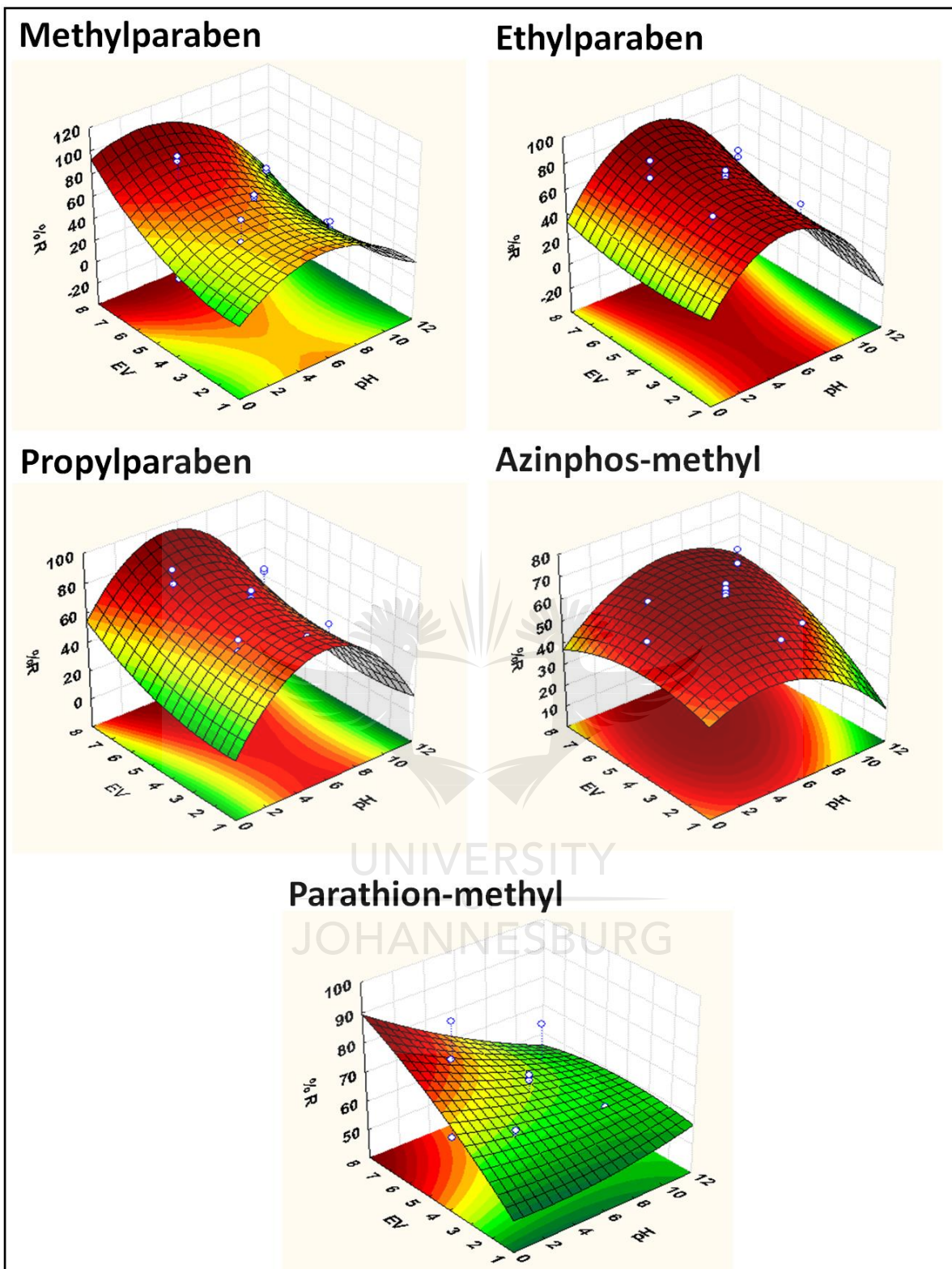*Sample pH, mass of adsorbent (MA), eluent volume (EV)

Figure 6.4: Response surface plots of the interactive effects of extraction volume (EV) vs sample pH with mass of adsorbent (MA) at a constant value

6.3.5 Method performance characteristics

The method performance of the SPE based on CNDs was evaluated based on determination coefficient (R^2), method precision (% RSD), limit of detection and quantification (LOQ & LOQ), linear dynamic range (LDR) as well as % matrix effect (ME). The LDR was between 5-100 µg L^{-1} for each of the analytes with R^2 values (>0.995) which is indicative of good linearity of the method. The LOD and LOQ were evaluated as 3xSD/*b* and 10xSD/*b* respectively, where SD is the residual standard deviation of the linear regression and *b* is the slope of the calibration curve. As observed in **Table 6.5,** the LOD and LOQ values ranged between 0.02-0.13 µg L^{-1} and 0.05-0.42 µg L^{-1}, respectively. The method precision was evaluated in terms of % relative standard deviation (% RSD) for each analyte at a concentration of 50 µg L^{-1} at (n=8). The % RSD obtained were much lower than 10 %. To evaluate the matrix effect (ME), slopes obtained after analysing two different sets of calibration standards were compared. The first set of standards were prepared in solvent (mobile phase) while the second set of standards were prepared in a matrix blank sample (effluent wastewater). The slopes were compared using **equation 6.1** [43]. The calibration range levels were 5-100 µg L^{-1}.

$$\% ME = \frac{\text{Slope(martrix.matched)}}{\text{slope(solvent)}} \text{ x } 100 \qquad\qquad (6.1)$$

Where ME is the matrix effect. The importance of conducting matrix effect is because the ionisation of the analytes can be compromised when using ESI source. ME can be dependent on the sample matrix or type of analyte. ME value of <100 % signifies ionisation suppression of the analyte, ME value of >100% is indicative of analyte ionisation enhancement, while ME of 100 % indicates similar response in the mobile phase and matrix solvent [44]. As shown in **Table 6.5**, differences in ME were observed. Azinphos-methyl showed ionisation enhancement (143 %) while the parabens demonstrated ionisation suppression (50-66 %). The signal suppression and enhancement as observed herein could be attributed to coeluting endogenous matrix components, that strongly compete with or are suppressed by, the presence of analytes of interest at the ESI source as has been widely reported in the literature [45, 46]. The naturally occurring organic matrix components in wastewater samples such as humic and fulvic acids can be co-extracted during SPE, resulting in signal ionisation or enhancement of the target analyte [47]. To alleviate ME, matrix-matched calibrations were employed in all the quantitative analysis using blank matrix samples as has been reported in previous studies [48]. The un-spiked matrix samples (effluent wastewater) were qualified as blanks by running

triplicate sample extracts alongside quality control samples that included laboratory blanks, instrument blanks and spiked ultrapure water (5 ug L^{-1}), to ensure no analyte detection in the blanks.

Table 6.5: Analytical features of method performance characteristics (n=8)

Analyte	LOD (µg L^{-1})	LOQ (µg L^{-1})	LDR (µg L^{-1})	R^2	Reproducibility % RSD	%Matrix effect
Methylparaben	0.13	0.42	5-100	0.9991	3.5	65.6
Ethylparaben	0.10	0.32	5-100	0.9985	3.7	51.3
Propylparaben	0.08	0.25	5-100	0.9976	4.1	66.1
Azinphos-methyl	0.04	0.13	5-100	0.9993	7.0	147
Parathion-methyl	0.02	0.050	5-100	0.9995	4.3	103

6.3.6 Method validation and application to real wastewater samples

The accuracy of the developed CNDs based SPE procedure was validated by spiking influent and effluent wastewater samples containing none of the parabens or OPPs at detectable levels. The spiking was performed at two concentration levels, 10 and 100 µg L^{-1} in four replicates (n=4) for each level. The results are detailed in **Table 6.6**. The spiking procedure was adopted due to the unavailability of certified reference material with the organic contaminants in the study. As observed in **Table 6.6**, the recoveries obtained for the two spike levels ranged between 63-102 % and 71-123 % for influent and effluent wastewater samples respectively with <10 % RSDs for all the analytes. These results are proof that developed CNDs-SPE method achieved acceptable quantitative recoveries with good repeatability making it suitable for routine analysis and monitoring of these organic contaminants in wastewater simultaneously.

The developed method was further applied in application to real wastewater samples obtained from a domestic municipal WWTP analyzed in four replicates (n=4). The concentrations obtained are as shown in **Table 6.7**. The three parabens (MePB, EthPB and ProPB) were found in the studied wastewater samples albeit at low concentrations (0.13-3.5 µg L^{-1}). This is similar to what has been reported by other studies in the literature [10, 49]. The two OPPs pesticides studied were not detected in both the influent and effluent wastewater samples. The presence of trace amounts of MePB, EthPB and ProPB can be attributed to the fact that the WWTP in this study mostly treats domestic wastewater. Parabens are preservatives used in consumer

products used on a daily basis such as shampoos, body lotion toothpaste. They are therefore easily susceptible to be washed off down the drainage systems that are connected to the WWTPs. **Figure 6.5** shows the total ion chromatogram of the spiked and un-spiked (blank) effluent wastewater samples. From the blank chromatogram, it can be deduced that the method was free from any interferences that could hinder the correct identification and quantification of the multi-class analytes in wastewater samples.

Table 6.6: Compound matrix recoveries of two OPPs and three parabens in wastewater matrices (n=4)

| | Influent water | | | | Effluent water | | | |
| | 10 (µg L⁻¹) | | 100 (µg L⁻¹) | | 10 (µg L⁻¹) | | 100 (µg L⁻¹) | |
Analyte	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%
Methylparaben	67	8.0	69	6.7	105	5.1	123	6.9
Ethylparaben	73	6.7	63	6.6	75	4.9	71	6.7
Propylparaben	87	6.8	85	5.7	12	3.5	80	5.3
Azinphos-methyl	66	7.3	71	7.0	91	3.0	82	3.5
Parathion-methyl	71	7.1	67	3.2	85	4.4	101	6.3

Table 6.7: Application of the proposed method on unspiked wastewater samples (n=4)

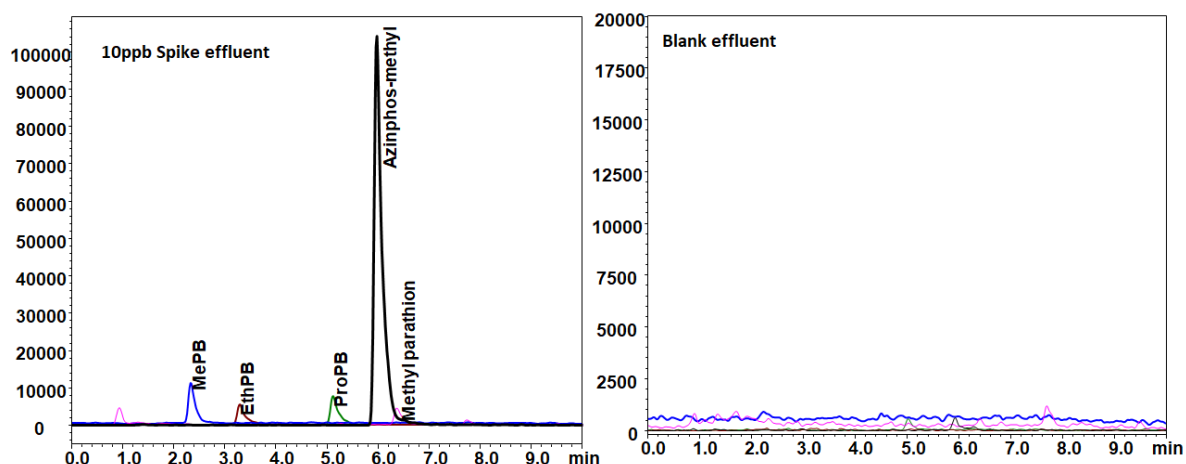| | Influent water | | Effluent water |
	Conc (µgL⁻¹)	RSD %	Conc (µgL⁻¹)
Methylparaben	3.51	2.6	<LOD
Ethylparaben	0.13	3.4	<LOD
Propylparaben	1.46	5.4	<LOD
Azinphos-Methyl	<LOD		<LOD
Parathion-methyl	<LOD		<LOD

Figure 6.5: Typical total ion chromatogram (TIC) of blank (unspiked) and spiked effluent wastewater sample spiked at 10 µg L^{-1}

6.3.7 Comparison of commercial based adsorbent with synthesized CNDs

The performance of the developed method in terms of extraction recovery was compared with the commercially based SPE sorbents (Oasis HLB). The experiments were performed concurrently in replicas of four, (n=4) using the same optimized conditions at a spiking level of 25 µg L^{-1}. Looking at the data obtained in **Figure 6.6,** the % recoveries obtained with Oasis HLB cartridges ranged between 97-120% for all the analytes. With CNDs, the recoveries obtained were between 66-87 %. Although the experimental conditions were similar, the slightly lower recoveries observed with the CNDs could be because of the lower mass of sorbent with CNDs (170 mg) as compared to Oasis HLB (200 mg). However, lower % RSD <3 % were obtained for CNDs as compared to Oasis HLB.
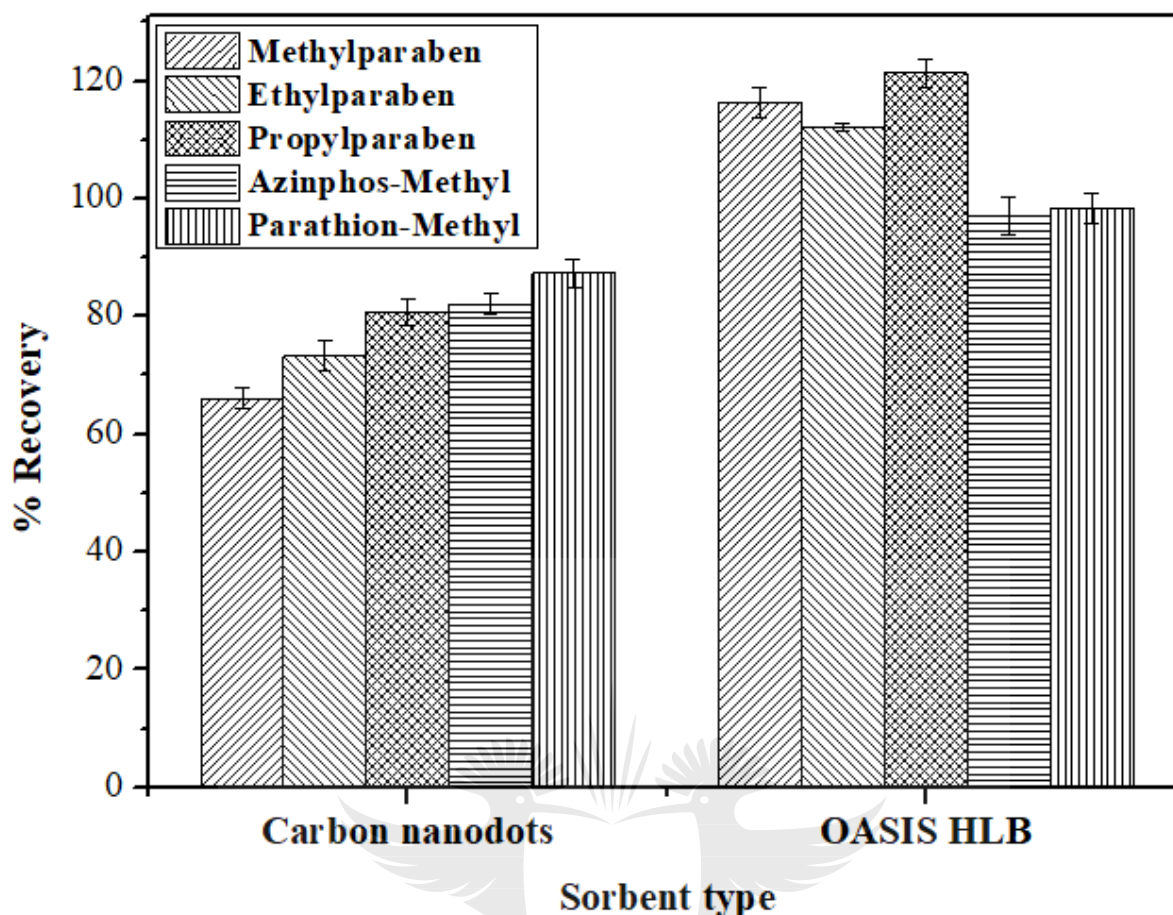
Figure 6.6: Sorbent type comparison between the synthesized CNDs and Oasis HLB. Experimental conditions: sample volume-50mL, elution volume-6 mL, pH=4.5, n=4 with standard deviation as error bars.

6.3.8 Comparison with other methods

A critical comparison of the developed method was also performed with various other sample extraction techniques reported in the literature. The results in **Table 6.8**, indicate that the performance characteristics of the developed method are comparable or better than other methods in the literature. This can be attributed to the CNDs used in this study that comprised small particles exhibiting small surface area and therefore incresing the extraction efficincy in determination of the analytes. In addition, good chromatographic peak shapes were obtained by use of the sensitive LC-MS/MS detection technque, as compared to the non MS methods reported

Table 6.8: Comparison of the developed method with other methods reported in the literature [40, 50-55]

Analyte	Matrix	Extraction method	Detection	LOD µg L^{-1}	LOQ µg L^{-1}	%RSD-R	%R	Reference
MePB, EthPB, ProPB,BuPB	Tap water, wastewater	RDSE	GC-MS	0.05	-	9.7	>80	[50]
MePB, EthPB,ProPB	Wastewater	µ-SPE	HPLC-UV	0.08-0.4	-	<7	82.8-108.3	[40]
OPPs	Drinking water	SPE	LC-MS	0.001-0.048	0.003-0.146	4.35–27.15	55.54-121.21	[51]
Parathion-methyl+ other EDCs	Sewage effluent, surface water	HF-LPME	LC-MS/MS	0.001-0.098	0.002-0.127	2.75-14.98	80.6-127	[52]
Azinphos-methyl, Parathion-methyl	Tap water, surface water, agricultural water	VLDS-SD-DLLME	HPLC-DAD	0.25-1	0.3-3.5	5.3	90-99	[53]
EthPB,ProPB,BuPB, BzPB, iBuPB	River	EME	HPLC-DAD	-	0.98-1.43	2.9-12.6	>80	[54]
MePB, ProPB & pesticides	Surface water	SD-DLLME	LC-MS/MS	-	0.0125-1.25	2-29	61-130	[55]
MePB, EthPB, ProPB, Azinphos methyl, Parathion-methyl	Wastewater	SPE-CNDs	LC-MS/MS	0.015-0.125	0.05-0.415	<10	62-123	This work

RSDE=rotating disk Sorptive extraction; µ-SPE=micro-solid phase extraction; HF-LPME=hollow fibre liquid phase microextraction; VLDS-SD-DLME=vortex assisted low density solvent based demulsified dispersive liquid-liquid extraction; EME= electromembrane extraction

6.3.9 Regeneration studies of CNDs as SPE sorbent

Further experiments were performed to establish the reusability of the synthesised CNDs under the optimised experimental conditions. This was done using a model solution of 25 µg L^{-1}. An evaluation of the mean % recoveries using two-tailed t-tests revealed no significant differences at 95% confidence limit, up to the 5th cycle. Therefore, the adsorbent could be re-used at a minimum of approximately 5 times, without significant loss in recovery. The results are as shown in **Figure 6.7.**
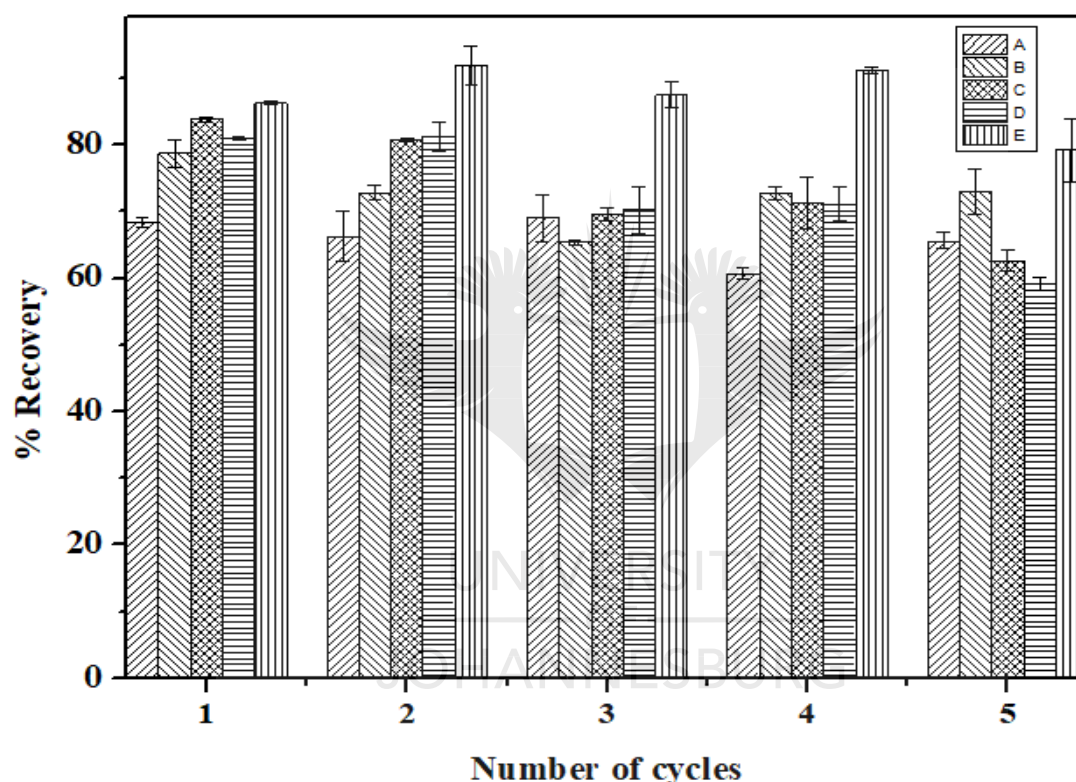
Figure 6.7: Reusability of carbon nanodots tested with a model solution of 25 µg L^{-1}. A: Methylparaben, B: Ethylparaben, C: Propylparaben, D: Azinphos-methyl, D: Parathion-methyl

6.4 CONCLUSIONS

In this study, three parabens (MePB, EthPB and ProPB) and two OPPs (azinphos-methyl and parathion-methyl) were extracted and analysed in wastewater simultaneously using synthesised carbon nanodots, a technique employed for the first time. Detection was accomplished using LC-MS/MS which provided accurate and precise quantification using multiple reaction monitoring in both positive and negative modes. The CNDs used in the extraction employed a green synthesis protocol which gave good recoveries (63-123%) for all compounds studied in

the effluent water matrix. This demonstrates that the sorbent was highly effective in preconcentrating the studied multi-class analytes without much modification. The developed method also had acceptable method precision of <10% showing good method performance. The application of the method was also demonstrated in the analysis of real wastewater samples. Compared to other methods, the benefits of the proposed method employing green synthesis for the preparation of CNDs for the extraction is the ease of preparation, reusability, and cost-effectiveness. This renders them applicable for routine sampling procedures in environmental analysis.

6.5 REFERENCES

[1] I. Muñoz, M.J. Gómez, A. Molina-Díaz, M.A. Huijbregts, A.R. Fernández-Alba, E. García-Calvo, Ranking potential impacts of priority and emerging pollutants in urban wastewater through life cycle impact assessment, Chemosphere 74 (2008) 37-44.

[2] W. Gwenzi, N. Chaukura, Organic contaminants in African aquatic systems: Current knowledge, health risks, and future research directions, Science of The Total Environment 619-620 (2018) 1493-1514.

[3] L. Gusmaroli, S. Insa, M. Petrovic, Development of an online SPE-UHPLC-MS/MS method for the multiresidue analysis of the 17 compounds from the EU "Watch list", Analytical and Bioanalytical Chemistry 410 (2018) 4165-4176.

[4] S.J. Varjani, M. Chaithanya Sudha, Treatment Technologies for Emerging Organic Contaminants Removal from Wastewater, in: S. Bhattacharya, A.B. Gupta, A. Gupta, A. Pandey (Eds.), Water Remediation, Springer Singapore, Singapore, 2018, pp. 91-115.

[5] E.A. Songa, J.O. Okonkwo, Recent approaches to improving selectivity and sensitivity of enzyme-based biosensors for organophosphorus pesticides: A review, Talanta 155 (2016) 289-304.

[6] M.l. Farré, S. Pérez, L. Kantiani, D. Barceló, Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment, TrAC Trends in Analytical Chemistry 27 (2008) 991-1007.

[7] I. Sibiya, O. Olukunle, O. Okonkwo, Seasonal variations and the influence of geomembrane liners on the levels of PBDEs in landfill leachates, sediment and groundwater in Gauteng Province, South Africa, Emerging Contaminants 3 (2017) 76-84.

[8] L. Maldaner, I.C.S.F. Jardim, Determination of some organic contaminants in water samples by solid-phase extraction and liquid chromatography–tandem mass spectrometry, Talanta 100 (2012) 38-44.

[9] X. Ma, Y. Wan, M. Wu, Y. Xu, Q. Xu, Z. He, W. Xia, Occurrence of benzophenones, parabens and triclosan in the Yangtze River of China, and the implications for human exposure, Chemosphere 213 (2018) 517-525.

[10] G. Mashile, A. Mpupa, P. Nomngongo, In-Syringe Micro Solid-Phase Extraction Method for the Separation and Preconcentration of Parabens in Environmental Water Samples, Molecules 23 (2018) 1450.

[11] S. Esplugas, D.M. Bila, L.G.T. Krause, M. Dezotti, Ozonation and advanced oxidation technologies to remove endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents, Journal of Hazardous Materials 149 (2007) 631-642.

[12] M.J. Bebianno, M. Gonzalez-Rey, Chapter 16 - Ecotoxicological Risk of Personal Care Products and Pharmaceuticals, in: C. Amiard-Triquet, J.-C. Amiard, C. Mouneyrac (Eds.), Aquatic Ecotoxicology, Academic Press2015, pp. 383-416.

Bebianno, M.J. and Gonzalez-Rey, M., 2015. Ecotoxicological risk of personal care products and pharmaceuticals. In *Aquatic Ecotoxicology* (pp. 383-416). Academic Press

[13] J.-J. Jiang, C.-L. Lee, M.-D. Fang, Emerging organic contaminants in coastal waters: anthropogenic impact, environmental release and ecological risk, Marine pollution bulletin 85 (2014) 391-399.

[14] S. Montesdeoca-Esponda, L. Checchini, M. Del Bubba, Z. Sosa-Ferrera, J.J. Santana-Rodriguez, Analytical approaches for the determination of personal care products and evaluation of their occurrence in marine organisms, Science of The Total Environment 633 (2018) 405-425.

[15] A.G. Frenich, R. Romero-González, M. del Mar Aguilera-Luiz, Comprehensive analysis of toxics (pesticides, veterinary drugs and mycotoxins) in food by UHPLC-MS, TrAC Trends in Analytical Chemistry 63 (2014) 158-169.

[16] N.H. Tran, H. Chen, T.V. Do, M. Reinhard, H.H. Ngo, Y.Z. He, K.Y. Gin, Simultaneous analysis of multiple classes of antimicrobials in environmental water samples using SPE coupled with UHPLC-ESI-MS/MS and isotope dilution, Talanta 159 (2016) 163-173.

[17] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, Ultra-high-performance liquid chromatography–tandem mass spectrometry for determining the presence of eleven personal care products in surface and wastewaters, Journal of Chromatography A 1216 (2009) 6994-7000.

[18] R. Celano, A.L. Piccinelli, L. Campone, L. Rastrelli, Ultra-preconcentration and determination of selected pharmaceutical and personal care products in different water matrices by solid-phase extraction combined with dispersive liquid–liquid microextraction prior to ultra high pressure liquid chromatography tandem mass spectrometry analysis, Journal of Chromatography A 1355 (2014) 26-35.

[19] A. Spietelun, Ł. Marcinkowski, M. de la Guardia, J. Namieśnik, Recent developments and future trends in solid phase microextraction techniques towards green analytical chemistry, Journal of Chromatography A 1321 (2013) 1-13.

[20] J.M. Jiménez-Soto, S. Cárdenas, M. Valcárcel, Evaluation of single-walled carbon nanohorns as sorbent in dispersive micro solid-phase extraction, Analytica chimica acta 714 (2012) 76-81.

[21] T. Anumol, S.A. Snyder, Rapid analysis of trace organic compounds in water by automated online solid-phase extraction coupled to liquid chromatography–tandem mass spectrometry, Talanta 132 (2015) 77-86.

[22] B. Constantin, Carbon nanotubes as a new solid phase extraction sorbent for analysis of environmental pollutants, Carbon Nanotubes, InTech2010.

[23] M.R. Hadjmohammadi, M. Peyrovi, P. Biparva, Comparison of C18 silica and multi-walled carbon nanotubes as the adsorbents for the solid-phase extraction of Chlorpyrifos

and Phosalone in water samples using HPLC, Journal of separation science 33 (2010) 1044-1051.

[24] A. Azzouz, S.K. Kailasa, S.S. Lee, A. J. Rascón, E. Ballesteros, M. Zhang, K.-H. Kim, Review of nanomaterials as sorbents in solid-phase extraction for environmental samples, TrAC Trends in Analytical Chemistry 108 (2018) 347-369.

[25] K.K. Karali, L. Sygellou, C.D. Stalikas, Highly fluorescent N-doped carbon nanodots as an effective multi-probe quenching system for the determination of nitrite, nitrate and ferric ions in food matrices, Talanta 189 (2018) 480-488.

[26] P. Shen, Y. Xia, Synthesis-modification integration: one-step fabrication of boronic acid functionalized carbon dots for fluorescent blood sugar sensing, Anal. Chem. 86 (2014) 5323-5329.

[27] V.N. Mehta, S. Jha, R.K. Singhal, S.K. Kailasa, Preparation of multicolor emitting carbon dots for HeLa cell imaging, New Journal of Chemistry 38 (2014) 6152-6160.

[28] M. Zheng, S. Liu, J. Li, Z. Xie, D. Qu, X. Miao, X. Jing, Z. Sun, H. Fan, Preparation of highly luminescent and color tunable carbon nanodots under visible light excitation for in vitro and in vivo bio-imaging, Journal of Materials Research 30 (2015) 3386-3393.

[29] S.N. Baker, G.A. Baker, Luminescent carbon nanodots: emergent nanolights, Angewandte Chemie International Edition 49 (2010) 6726-6744.

[30] N. Wang, Y. Wang, T. Guo, T. Yang, M. Chen, J. Wang, Green preparation of carbon dots with papaya as carbon source for effective fluorescent sensing of Iron (III) and Escherichia coli, Biosensors and Bioelectronics 85 (2016) 68-75.

[31] B. Yin, J. Deng, X. Peng, Q. Long, J. Zhao, Q. Lu, Q. Chen, H. Li, H. Tang, Y. Zhang, S. Yao, Green synthesis of carbon dots with down- and up-conversion fluorescent properties for sensitive detection of hypochlorite with a dual-readout assay, Analyst 138 (2013) 6551-6557.

[32] X. Yang, Y. Zhuo, S. Zhu, Y. Luo, Y. Feng, Y. Dou, Novel and green synthesis of high-fluorescent carbon dots originated from honey for sensing and imaging, Biosensors and Bioelectronics 60 (2014) 292-298.

[33] P. Deme, T. Azmeera, S. Kanjilal, P. Jonnalagadda, V.S. Upadhyayula, LC-MS/MS determination of organophosphorus pesticide residues in coconut water, Food analytical methods 6 (2013) 1162-1169.

[34] S. Song, Z. Zhang, N. Zou, R. Chen, L. Han, C. Pan, Y. Sapozhnikova, Determination of six paraben residues in fresh-cut vegetables using QuEChERS with multi-walled carbon nanotubes and high-performance liquid chromatography–tandem mass spectrometry, Food analytical methods 10 (2017) 3972-3979.

[35] L. Shi, X. Li, Y. Li, X. Wen, J. Li, M.M. Choi, C. Dong, S. Shuang, Naked oats-derived dual-emission carbon nanodots for ratiometric sensing and cellular imaging, Sensors and Actuators B: Chemical 210 (2015) 533-541.

[36] S. Gwebu, P. Nomngongo, P. Mashazi, T. Nyokong, N. Maxakato, Platinum Nanoparticles Supported on Carbon Nanodots as Anode Catalysts for Direct Alcohol Fuel Cells, Int. J. Electrochem. Sci 12 (2017) 6365-6378.

[37] C.J. Raj, B.C. Kim, B.-B. Cho, W.-J. Cho, S.-J. Kim, S.Y. Park, K.H. Yu, Electrochemical supercapacitor behaviour of functionalized candle flame carbon soot, Bulletin of Materials Science 39 (2016) 241-248.

[38] R. Kostiainen, T.J. Kauppila, Effect of eluent on the ionization process in liquid chromatography–mass spectrometry, Journal of Chromatography A 1216 (2009) 685-699.

[39] K. Demeestere, M. Petrović, M. Gros, J. Dewulf, H. Van Langenhove, D. Barceló, Trace analysis of antidepressants in environmental waters by molecularly imprinted polymer-based solid-phase extraction followed by ultra-performance liquid chromatography coupled to triple quadrupole mass spectrometry, Analytical and bioanalytical chemistry 396 (2010) 825-837.

[40] K.M. Ara, S. Pandidan, A. Aliakbari, F. Raofie, M.M. Amini, Porous-membrane-protected polyaniline-coated SBA-15 nanocomposite micro-solid-phase extraction followed by high-performance liquid chromatography for the determination of parabens in cosmetic products and wastewater, Journal of separation science 38 (2015) 1213-1224.

[41] R. Abdelmajid, M.R. L., E.S. Salah, E.H. Mohammadine, Use of response factorial design for process optimization of basic dye adsorption onto activated carbon derived from Persea species, Microchemical Journal (2017) 129-136.

[42] A. Asati, G.N.V. Satyanarayana, D.K. Patel, Comparison of two microextraction methods based on solidification of floating organic droplet for the determination of multiclass analytes in river water samples by liquid chromatography tandem mass spectrometry using Central Composite Design, Journal of Chromatography A 1513 (2017) 157-171.

[43] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography–electrospray tandem mass spectrometry, Analytical and Bioanalytical Chemistry 391 (2008) 1293-1308.

[44] T. Benijts, R. Dams, W.E. Lambert, A. De Leenheer, Countering matrix effects in environmental liquid chromatography–electrospray ionization tandem mass spectrometry water analysis for endocrine disrupting chemicals, Journal of Chromatography A 1029 (2004) 153-159.

[45] I. González-Mariño, J.B. Quintana, I. Rodríguez, R. Cela, Simultaneous determination of parabens, triclosan and triclocarban in water by liquid chromatography/electrospray ionisation tandem mass spectrometry, Rapid Communications in Mass Spectrometry 23 (2009) 1756-1766.

[46] J. Aceña, D. Rivas, B. Zonja, S. Pérez, D. Barceló, Liquid Chromatography–Mass Spectrometry: Quantification and Confirmation Aspects, Fast Liquid Chromatography–Mass Spectrometry Methods in Food and Environmental Analysis2015, pp. 347-377.

[47] L. Kharbouche, M.G. García, A. Lozano, H. Hamaizi, M.M. Galera, Solid phase extraction of pesticides from environmental waters using an MSU-1 mesoporous material and determination by UPLC-MS/MS, Talanta 199 (2019) 612-619.

[48] M.P. Vázquez, P.P. Vázquez, M.M. Galera, M.G. García, A. Uclés, Ultrasound-assisted ionic liquid dispersive liquid–liquid microextraction coupled with liquid chromatography-quadrupole-linear ion trap-mass spectrometry for simultaneous analysis of pharmaceuticals in wastewaters, Journal of chromatography A 1291 (2013) 19-26.

[49] J. Regueiro, E. Becerril, C. Garcia-Jares, M. Llompart, Trace analysis of parabens, triclosan and related chlorophenols in water by headspace solid-phase microextraction

with in situ derivatization and gas chromatography–tandem mass spectrometry, Journal of Chromatography A 1216 (2009) 4693-4702.

[50] M. Becerra-Herrera, V. Miranda, D. Arismendi, P. Richter, Chemometric optimization of the extraction and derivatization of parabens for their determination in water samples by rotating-disk sorptive extraction and gas chromatography mass spectrometry, Talanta 176 (2018) 551-557.

[51] T.G. Schwanz, C.K. Carpilovsky, G.C.C. Weis, I.H. Costabeber, Validation of a multi-residue method and estimation of measurement uncertainty of pesticides in drinking water using gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry, Journal of Chromatography A 1585 (2019) 10-18.

[52] V. Salvatierra-stamp, R. Muñiz-Valencia, J.M. Jurado, S.G. Ceballos-Magaña, Hollow fiber liquid phase microextraction combined with liquid chromatography-tandem mass spectrometry for the analysis of emerging contaminants in water samples, Microchemical Journal 140 (2018) 87-95.

[53] K. Seebunrueng, Y. Santaladchaiyakit, S. Srijaranai, Vortex-assisted low density solvent based demulsified dispersive liquid–liquid microextraction and high-performance liquid chromatography for the determination of organophosphorus pesticides in water samples, Chemosphere 103 (2014) 51-58.

[54] M. Villar-Navarro, M.d.C. Moreno-Carballo, R. Fernández-Torres, M. Callejón-Mochón, M.Á. Bello-López, Electromembrane extraction for the determination of parabens in water samples, Analytical and Bioanalytical Chemistry 408 (2016) 1615-1621.

[55] S.S. Caldas, C. Rombaldi, J.L. de Oliveira Arias, L.C. Marube, E.G. Primel, Multi-residue method for determination of 58 pesticides, pharmaceuticals and personal care products in water using solvent demulsification dispersive liquid–liquid microextraction combined with liquid chromatography-tandem mass spectrometry, Talanta 146 (2016) 676-688.

CHAPTER 7:

ULTRASONIC ASSISTED MAGNETIC SOLID PHASE DISPERSIVE EXTRACTION FOR PRECONCENTRATION OF CHLORPYRIFOS AND TRICLOSAN IN WASTEWATER SAMPLES PRIOR TO LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRIC DETECTION

ABSTRACT

In the present study, carbon nanodots (CNDs) were successfully anchored on magnetite (Fe_3O_4) under magnetic stirring and the nanocomposite prepared was assessed as new adsorbent for ultrasonic-assisted magnetic solid phase dispersive extraction of chlorpyrifos and triclosan in water samples. Detection was achieved using liquid chromatography tandem mass spectrometry (LC-MS/MS). The prepared magnetic CNDs were characterised by transmission electron, microscopy (TEM), scanning electron microscopy (SEM), Fourier transform infra-red (FTIR) and X-ray diffraction (XRD). The investigation and optimisation of the main experimental variables having an influence on the analytical response was performed using multivariate approach. The factors studied were sample pH, mass of adsorbent and extraction time. The selection of desorption solvent and desorption time were examined and optimised univariately. By appropriating the optimum experimental conditions, the developed method was validated for accuracy using real environmental water samples. The average percentage recoveries obtained using influent and effluent spiked wastewater samples ranged between 76-108 % and 79-96 % for CPF and TCS, respectively. A good linearity ($R^2 > 0.995$) was established ranging between 5-100 µg L^{-1}. The limit of detection (LOD) and limit of quantification (LOQ) were in the range of 0.024-0.081 µg L^{-1} and 0.057-0.192 µg L^{-1}, respectively. The repeatability and reproducibly expressed as % relative standard deviation (%RSD) were less than 4.7 %. The developed method exhibited good method performance, is rapid, simple, inexpensive and environmentally friendly.

Key words: Magnetic carbon nanodots, chlorpyrifos, triclosan, wastewater, LC-MS/MS

7.1 INTRODUCTION

The ubiquitous occurrence of emerging organic contaminants such as triclosan (TCS) an antimicrobial agent and chlorpyrifos (CPF) an organophosphate pesticides, in global aquatic environment has raised a great deal of concern in the scientific community and regulatory authorities [1, 2]. CPF is used in the agricultural sector as an insecticide and TCS is incorporated in personal care products such as body lotions, toothpaste, soaps, and sunscreens. The massive and continuous use of these compounds has led to their incessant release into the aquatic environment mostly via wastewater treatment plants (WWTPs), posing a threat to human population and aquatic life. This could be attributed to their resistance towards the wastewater treatment procedures resulting to their incomplete removal [3]. They have been found in surface and ground water in trace levels ranging between ng-μg L^{-1} levels as reported in previous studies [4]. Surface and ground water can be used for drinking purposes, hence the presence of these pollutants in the aquatic environment needs constant monitoring to mitigate their long term health effects in the human and aquatic life [5].

Due to the low concentrations of these compounds present in the environmental water matrices, sensitive and selective sample extraction procedures are required [6]. In addition, the presence of potential matrix interfering species or high concentrations of competing analyte components may hinder accurate analyte identification and quantitation [7]. Enrichment of these analytes in the sample and removal of matrix components is therefore a prerequisite prior to gas chromatographic or liquid chromatographic determination [8]. As such, it is of necessity to develop sample preparation procedures that are robust, employ minimal amount of organic solvents, are less laborious, fast and sensitive for applicability in routine monitoring and high sample throughput [9]. Methods that have been developed hitherto that embody such characteristics include solid phase microextraction (SPME) [10], dispersive solid phase extraction (DSPE) [11], dispersive liquid-liquid microextraction (DLLME) [12], microwave assisted solid phase extraction (MASPE)[13] and magnetic solid phase extraction (MSPE)[14]. In the recent past, MSPE has gained much popularity as a technique whereby the analyte is adsorbed onto a magnetic adsorbent dispersed in aqueous solution and the sorbent is separated from the solution by application of an external magnetic field, after the completion of extraction. The analyte is thereafter recovered by desorption using a suitable solvent from the adsorbent, prior to analysis, in a similar manner [15]. The main advantages of this technique is simple and quick separation of analytes without filtration or centrifugation steps, less laborious,

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reduced sample preparation time, high sample throughput and a wide diversity of materials that can be employed or synthesized as adsorbents [15].

In MSPE, various materials such as carbon nanomaterials, have been purchased or synthesized and employed as sorbents by coating magnetic cores such as maghemite or magnetite to inorganic or organic substrates [16]. Some of these carbon nanomaterials that have been used include carbon nanotubes, carbon nanofibers, graphene sheets, graphene oxide and activated carbon. However, some of these materials are toxic and require complicated synthesis procedures. Carbon nanodots have emerged as a relatively new class of carbon nanomaterials applied as sorbents for various applications [17]. The CNDs have unique properties such us relative stability, biocompatibility, eco-friendliness and low toxicity and large surface area to volume ratio [18, 19]. Due to the presence of functional groups, the magnetic (Fe_3O_4) nanoparticles can be easily anchored on the surface of the CNDs, resulting in a composite nanomaterial with magnetic properties [20]. Researchers have reported the use of magnetic CNDs in multifaceted applications such as photocatalysis [21], sensors [22], bioimaging [23] and fluorescent detection [20].

To the best of our knowledge, only one publication has employed the use of magnetic CNDs in environmental applications, for the purposes of degradation [21]. However, the report has limited or no reports on the application of magnetic CNDs as sorbents for the simultaneous extraction and preconcentration of organic pollutants in wastewater. Therefore, the aim of this work was to synthesize magnetic CNDs for the simultaneous extraction of CPF and TCS in wastewater prior to detection with LC-MS/MS.

7.2 EXPERIMENTAL

7.2.1 Chemicals and reagents

Pesticides analytical standards (chlorpyrifos and triclosan), with purity of 98-99 % were purchased from Merck (Darmstadt, Germany). Individual stock solutions of each of the compounds (1000 mg L^{-1}) were prepared in methanol. A mixed standard working solution of 10 mg L^{-1} was prepared in methanol and stored at -18 °C. Calibration standards containing the two analytes were prepared prior to the analytical runs by appropriate dilution of 10 mg L^{-1} stock standard solution in water/acetonitrile (80:20 v/v). HPLC grade methanol (MeOH), acetonitrile (ACN) and acetic acid (96 %) and formic acid (98 %) were supplied by Merck (Darmstadt, Germany). Hydrochloric acid (37 %), sodium hydroxide (NaOH) and ethanol (97 %) were also supplied by Merck (Darmstadt, Germany) were used to adjust the pH of the model

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solutions and environmental water samples. Ethanol (97 % *v/v*), Iron (III) chloride (FeCl$_3$·6H$_2$O), Iron (II) and chloride (FeCl$_2$·4H$_2$O) were obtained from Sigma Aldrich (St. Louis, MO, USA). Deionized water was obtained from a Millipore filtration system with a specific resistance of 18 mΩ.

7.2.2 Preparation of Fe$_3$O$_4$ nanoparticles

The magnetic iron oxide nanoparticles (m-Fe$_3$O$_4$-NPs) were prepared using a chemical co-precipitation procedure as reported in literature with minor modifications [24, 25]. Briefly 16 g of FeCl$_3$·6H$_2$O and 7 g of FeCl$_2$·4H$_2$O were dissolved in deionized water (150 mL) under nitrogen atmosphere with vigorous magnetic stirring under a heated oil bath at 90 °C. Subsequently, 50 mL of ammonia solution (25 % v/v) was added quickly into the above solution. The mixture was stirred for another 30 minutes under the same conditions. After the reaction finalized, the solution was cooled to room temperature. The resulting black m-Fe$_3$O$_4$-NPs were collected by magnetic decantation and washed severally with de-ionized water and ethanol with centrifugation (7000 rpm). The m-Fe$_3$O$_4$-NPs were then dried at 60 °C for 6 hrs. in an oven then further ground to finer particles.

7.2.3 Preparation of CNDS@Fe$_3$O$_4$ nanoparticles (CNDs@Fe$_3$O$_4$ NPs)

Firstly, the CNDs were prepared using a method from literature [26]. The CNDs@Fe$_3$O$_4$ NPs were prepared according to a previously reported method with slight modification [27]. In summary a simple co-mixing method with magnetic stirring was employed. 250 mg of pristine CNDs was dissolved in ethanol (50 mL) under sonication. Then 1 g of m-Fe$_3$O$_4$ NPs was dispersed into the prepared CNDs solution and the mixture was subjected to overnight stirring at room temperature. After this process, the obtained crude product was separated by an external magnet and washed with ultrapure water numerous times, and then dried at 60 °C for 6 hrs. for further use.

7.2.4 Characterization of CNDs@Fe$_3$O$_4$ NPs

Morphologies of the prepared nanocomposite were studied using transmission electron microscope (JEOL JEM-2100F) instrument equipped with a LaB6 source. Prior to TEM analysis, the samples were prepared by dissolving the nanoparticles in ethanol under ultrasonication for 10 minutes, then afterwards placing a drop of the solution onto a coated copper grid (200 mesh size Cu-grid). X-ray diffraction (XRD) was measured using a

PANalytical X'Pert PRO X-ray diffractometer in a ranging at 4–90° of 2θ at room temperature. A Pelmer Spectrum Fourier transform infrared spectrometer (FTIR) (MA, USA) was used to determine the functional groups of the nanocomposite ranging from 400 to 4000 cm^{-1}. The sample was prepared by mixing potassium bromide (KBr) and the nanocomposite in a ratio of 100:1 respectively, then compressed with a hydraulic press to from 1 mm discs, prior to the analysis. The magnetic behavior of the nanocomposite was characterized by vibrating sample magnetometer (VSM, Lakeshore cryotronics 730, USA) at room temperature. The identification and quantitative analysis were acquired using a Shimadzu ultra-high-performance liquid chromatograph (UHPLC), equipped with two LC-30AD pumps, DGU-20A5R degasser unit, a SIL-30AC Nexera autosampler, a CTO-30A column oven, and a CBM-20A communication module. This was coupled to a triple quadrupole mass spectrometer (LC-MS 8040) having an electrospray ion source (ESI).

7.2.5 LC-MS/MS operating conditions

The analysis of the MSPE of the triclosan and chlorpyrifos was carried out in multiple reaction monitoring mode (MRM) and gradient elution programme was utilized for the chromatographic separation using raptor ARC-18 column (100 mm x 2.1 mm, 3 µm) (RESTEK, USA), column. The mobile phase comprised of 0.1% FA in de-ionized water (A) and methanol (B). The column oven temperature was maintained at 40 °C and autosampler was kept at 4 °C. Sample injection volume was 30 µL. The flow rate was maintained at 0.2 mL/min and the column was equilibrated with 50% B prior to injection. The gradient elution programme began at 50% B and was increased to 75% B in 4min, increased to 100 % B in 1 min, maintained at 100 % B for 4 min, and re-equilibrated at 50% B for 5 min. Lab solutions software (Tokyo, Japan) was used in peak detection, instrument control, data analysis and method optimization.

7.2.6 Environmental water sampling and preparation

Water samples were sampled in triplicate from a local wastewater treatment plant (WWTP) located in Pretoria South Africa. The WWTP receives effluent discharge from both domestic and industrial sectors. The samples were obtained from different stages of the WWTP. Firstly, primary effluent was collected after undergoing processing in the primary settling tank. The secondary effluent was collected after the chlorination process, and lastly, the final effluent was collected after ultra-violet (UV) treatment as the water flowed into the nearby river. The samples were collected in precleaned glass bottles and placed in a cooler box with ice and

transported to the laboratory where they were filtered using 0.45 um PVDF syringe and stored in a fridge at 4 °C prior to subsequent extraction and analysis procedures.

7.2.7 UA-MSPDE analytical procedure and optimization

The extraction procedure was conducted as follows: 60 mg of the adsorbent ($Fe_3O_4@CNDs$) was placed in a glass bottle followed by the addition of a sample solution of 20 mL at pH 4.5. The extraction and preconcentration step were achieved by dispersing the adsorbent in the sample via ultrasonication for 20 minutes. The adsorbent containing the adsorbed analytes was separated from the aqueous solution by application of an external magnet at the base of the glass bottle and the supernatant was discarded. Thereafter, 2 mL of methanol was used to desorb the analytes from the adsorbent via ultrasonic dispersion for 15 minutes. Similarly, the eluent containing the analytes was retrieved by magnetic decantation. 200 µL was diluted 5x with mobile phase ready for LC-MS/MS analysis.

A multivariate approach was employed in optimization of the experimental factors that have an impact on the analytical response. The factors include, sample pH, amount of sorbent (AS) and extraction time (ET). Central composite design (CCD) was used in the optimization of the factors that affect the extraction and preconcentration of target analytes. The levels and independent variables used in setting up the experimental matrix are highlighted in **Table 7.1.** The experimental design was achieved using Minitab 17 software.

Table 7.1: Variables and levels selected for central composite design used in setting up the experimental matrix.

Variable	Low level (-1)	Central point (0)	High Level (+1)
Sample pH	4.5	7.0	9.5
Amount of sorbent (mg)	20	50	80
Extraction time (min)	10	15	20

7.3 RESULTS AND DISCUSSION

7.3.1 Characterization

The morphology of the synthesized CNDs, and CNDs@Fe_3O_4 are depicted in **Figure 7.1a** as characterized by TEM measurements. The TEM image of the CNDs (**Figure 7.1b**) revealed a dot-like structure while the image of CNDs@Fe_3O_4 nanocomposite was observed to be

spherical and well dispersed. CNDs were homogenously aggregated on iron oxide nanoparticles. The XRD patterns of the CNDs, Fe_3O_4 and $CNDs@Fe_3O_4$ are shown in **Figure 7.1c**. The patterns provide information on the phase and crystal structure of the synthesized nanoparticles. The diffraction pattern of the CNDs shows a broad peak 2θ values of $24.63°$ which relates to amorphous carbon. The diffraction peaks observed in Fe_3O_4 and $CNDs@Fe_3O_4$ can be assigned to (220), (311), (400), (422), (511) and (440) crystal planes. These results also indicate the successful coating of the amorphous CND layers onto iron oxide nanoparticles resulting in the formation of $Fe_3O_4@CNDs$, without altering the crystal phase and properties of iron oxide [20]. The functional groups present in the synthesized CNDs, Fe_3O_4 and $Fe_3O_4@CNDs$ were investigated using FTIR as shown in **Figure 7.1d** between 400-4000cm^{-1}. The strong absorption band positioned at 3430 cm^{-1} for CNDTs and $Fe_3O_4@CNDs$ is ascribed to the stretching vibration of the –OH. Also, from the FTIR spectrum of Fe_3O_4 and $Fe_3O_4@CNDs$, the absorption at 601 cm^{-1} corresponds to the Fe-O stretching bond, which confirms that the nanocomposite contains magnetite. The peak at 1625 and 1401 cm^{-1} present in all the three spectra can be ascribed to C=O (amide I band) and C-N stretching vibrations respectively. The peak at 1125 cm^{-1} corresponds to the C-O-C vibration. The characterization results obtained here-in are in agreement to those reported in literature [20, 28].

The magnetic properties of the samples were investigated using vibrating sample magnetometer (VSM) at room temperature. The hysteresis loops of the Fe_3O_4 and $Fe_3O_4@CNDs$ nanocomposite as shown in **Figure 7.2** indicate that the samples exhibited ferromagnetic behaviors. The magnetic saturation (Ms) for Fe_3O_4 and $Fe_3O_4@CNDs$ were 50.84 and 47.86 (emu/g), respectively. The results show only a slight decrease in the magnetic saturation between the magnetite and the composite due to the non-magnetic property of the CNDs in the nanocomposite. Despite of this, the nanocomposite did exhibit enough magnetization which allowed for the rapid separation from aqueous solution after extraction by applying an external magnet as well as quick dispersion back to the aqueous solution without the application of external magnet. This observation renders the synthesized magnetic composite a suitable sorbent for UA-MSPDE.
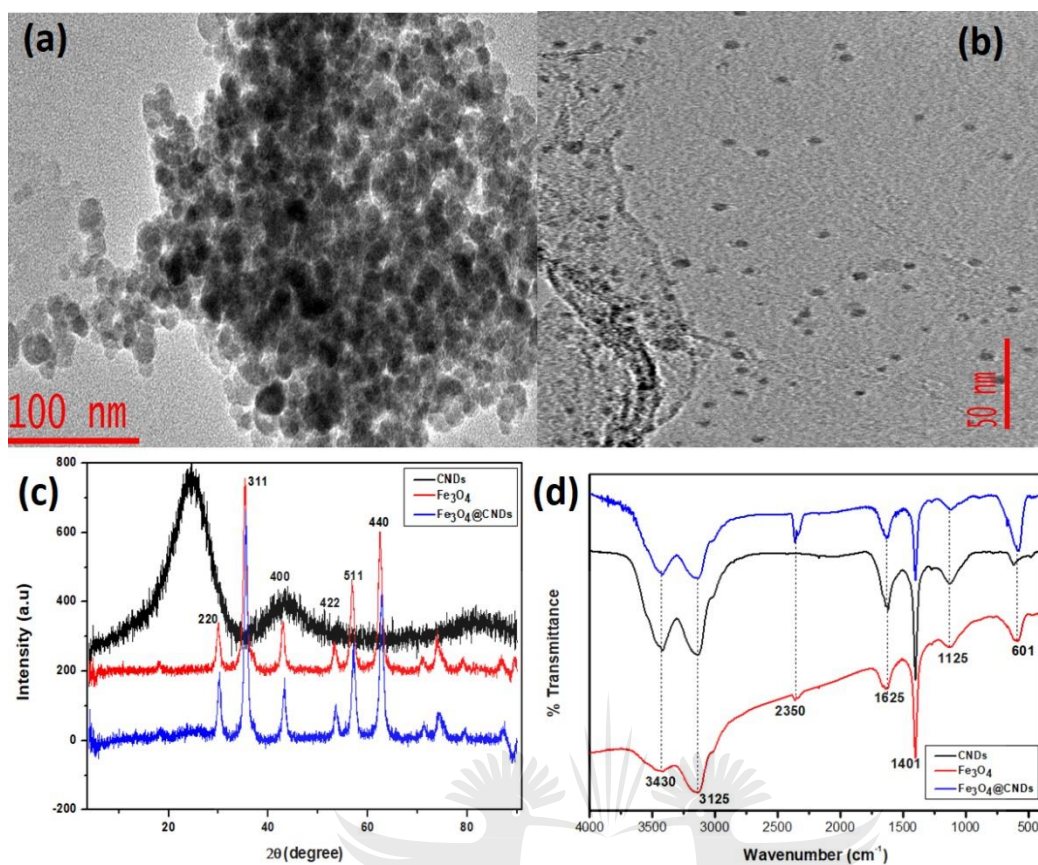
Figure 7.1: a) TEM image of Fe_3O_4@CND, **b)** TEM image of CNDs, **c)** XRD patterns of CNDs, Fe_3O_4 and Fe_3O_4@CNDs, **d)** FTIR spectra of CNDs, Fe_3O_4 and Fe_3O_4@CND
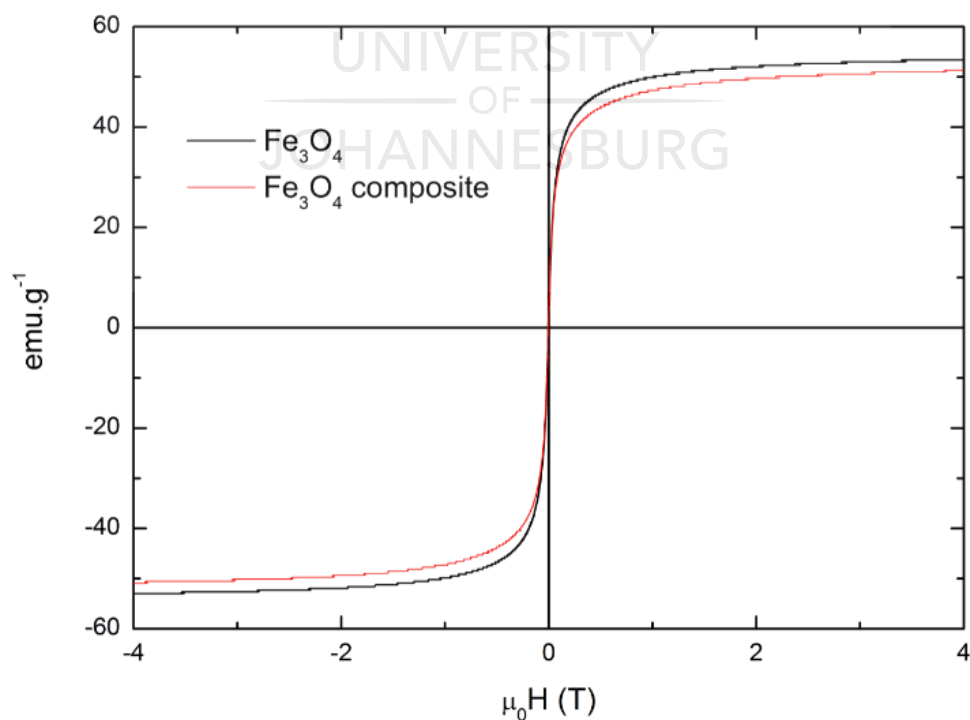

Figure 7.2: Magnetic hysteresis loops of pristine Fe_3O_4 (black) and magnetized CNDs (red)

7.3.2 Development of LC-MS/MS method

The analytical determination of CPF and TCS was developed by optimizing the mass spectrometry (MS) parameters for optimum sensitivity. The chromatographic separation is as described in section 2.5. Analysis was performed using multiple reaction monitoring (MRM) mode. The MRM transitions were used for the identification and quantitation of analytes in the samples. The most intense transition was selected as the target ion (quantifier m/z) while the least intense transition was selected for qualification **(Table 7.2).** The mass spectrometry parameters were obtained by infusing separately 1 µg mL^{-1} of CPF and TCS prepared in methanol directly into the ESI at 0.2 mL min^{-1}. The mobile phase used was 50:50, 0.1 % formic acid in water: methanol. CPF ionized in the positive mode [M+H]$^{+}$ while TCS ionized in the negative mode [M-H]$^{-1}$. The collision induced dissociation (CID) of CPF yielded an abundant product ion at m/z of 198 which corresponds to the loss of phosphonothioate group ($C_4H_{10}O_2PS$) [29, 30]. For TCS the CID yielded a product ion at m/z 35 as the target peak, which corresponds to chloride anion (Cl^{-}), which only required very low collision energy of 8ev. These results agree with previously published data [31, 32]. A dwell time of 50 ms was optimum in providing enough data points to obtain good chromatographic peaks. A summary of the optimization conditions is detailed in **Table 7.2**.

Table 7.2: Optimized mass spectrometry conditions for chlorpyrifos and triclosan

Analyte	Retention time (min)	Ion polarity	Precursor ion (m/z)	Product ions (m/z)	Collision energy (ev)
Chlorpyrifos	9.1	Positive	351.9	96.90 (T)	37
				197.90	22
Triclosan	8.6	Negative	287	35.00 (T)	8
			289	36.90	12

T=target ion

7.3.3 Selection of desorption solvent

The nature of the desorption solvent determines the elution of the adsorbed analytes from the sorbent material. Due to the polarity of the target analytes, different solvent and their combinations were investigated as potential desorption solvents for the elution of TCS and CPF spiked in aqueous solution at 100 µg L^{-1}, from the magnetic sorbent. From the results in **Figure 7.3,** it can be observed that methanol gave the relatively higher recoveries for both target analytes, as compared to the other solvents. The polarity of methanol was more favorable in

extracting the two analytes than acetonitrile. This observations coincide with the results reported in previous studies [32, 33]. However, it was observed that when methanol was acidified with acetic acid, the recovery of CPF increased while that of TCS reduced drastically. Due to the need for simultaneous extraction of the two target analytes, a compromise had to be reached on the most appropriate elution solvent and as such, pure methanol was selected as the desorption solvent and used for further experimental procedures in the study.
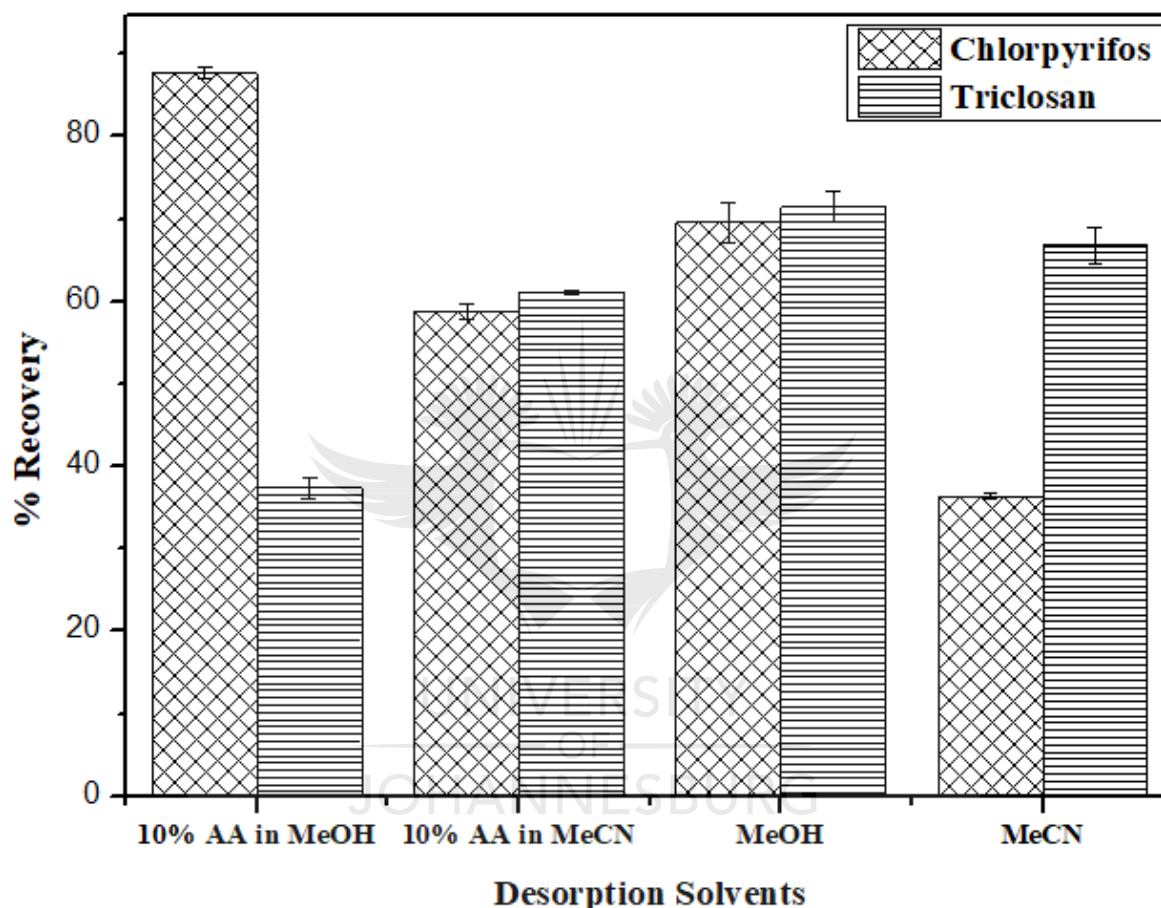

Figure 7.3: Univariate optimisation of desorption solvent of CPF and TCS from magnetic CNDs

7.3.4 Optimization of the analytical procedure

The optimization of the analytical procedure was performed to investigate the variables and the interactions that have an influence on the analytical response. The influential independent variables selected include sample pH, mass of sorbent (MA), and extraction time (ET). The analytical response for each compound was expressed as percent recovery (% R) as detailed in **Table 7.3**. Ultra-pure water spiked with $100\,\mu g\,L^{-1}$ of the mixed standard was used as the model sample solution. The data in **Table 7.3** was analyzed using ANOVA and probability (P) values.

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Pareto charts of standardized effects (**Figure 7.4**) were drawn from the ANOVA results, to examine the main effects and their interactions. The length of the bar charts also indicate the magnitude and the relevance of each effect [34]. The red reference line on the chart, gives an indication if a parameter is significant or insignificant at $\alpha = 0.05$ on the extraction efficiency, such that if a factor is below this line, it is deemed insignificant [35]. Sample pH as seen from the pareto charts, was the most significant parameter for both compounds. Other factors such as mass of adsorbent and extraction time did not exhibit much statistical significance on the extraction recovery of CPF and TCS. A response surface methodology based on CCD, was used to evaluate and optimize the main, interactive and quadratic effects of (i) sample pH (4.5-9), (ii) mass of the adsorbent (20-80 mg) and (iii) extraction time (10-20 min) with 4 central points, yielding a total of 18 experimental runs. The CCD matrix design and the results obtained expressed as percent recoveries of CPF and TCS in water are shown in **Table 7.3.** The empirical relationship between the studied variables was described by fitting the quadratic regression function (**equations 7.1 and 7.2)** to the experimental data. Response surface plots were also generated that give a visual representation of the interactive effects on the extraction recovery of the target analytes. The 3D plots were determined as a function of pH, MA and ET (**Figure 7.5**). By using the global optimization function in Minitab 17 statistical software, the optimum conditions could be derived. An approximation of these optimum conditions can equally be visually extrapolated from the surface plots. Therefore, the optimum experimental conditions were found to be 4.5, 60 mg and 20 minutes for pH, ET and ER, respectively. The sample volume was maintained at 20 mL.

$$\%R \ (CPF) = 72.9 \ - 10.5 \ pH \ + 0.571 \ MA \ + 0.73 \ ET \ + 0.383 \ pHxpH \ - 0.00332 \ MAxMA + 0.010 \ ETxET + 0.0090 \ pHxMA \ - 0.001 \ pHxET \ - 0.0097 \ MAxET \qquad \textbf{(7.1)}$$

$$\% \ R \ (TCS) = 92.1 \ - 25.4 \ pH \ + 0.671 \ MA \ + 6.54 \ ET \ + 1.574 \ pHxpH \ - 0.00633 \ MAxMA - 0.181 \ ETxET + 0.0220 \ pHxMA \ - 0.038 \ pHxET \ - 0.0054 \ MAxET \qquad \textbf{(7.2)}$$
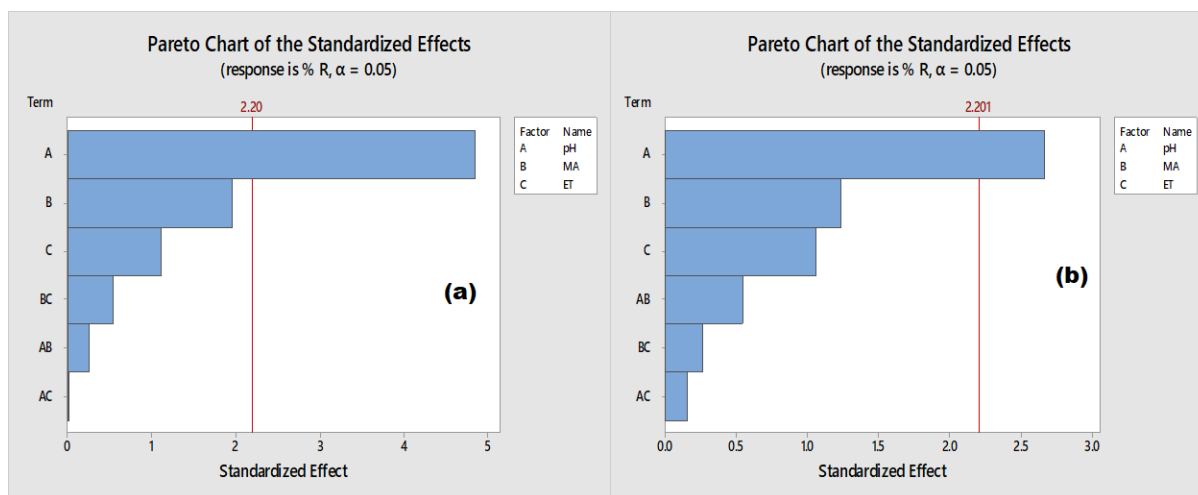
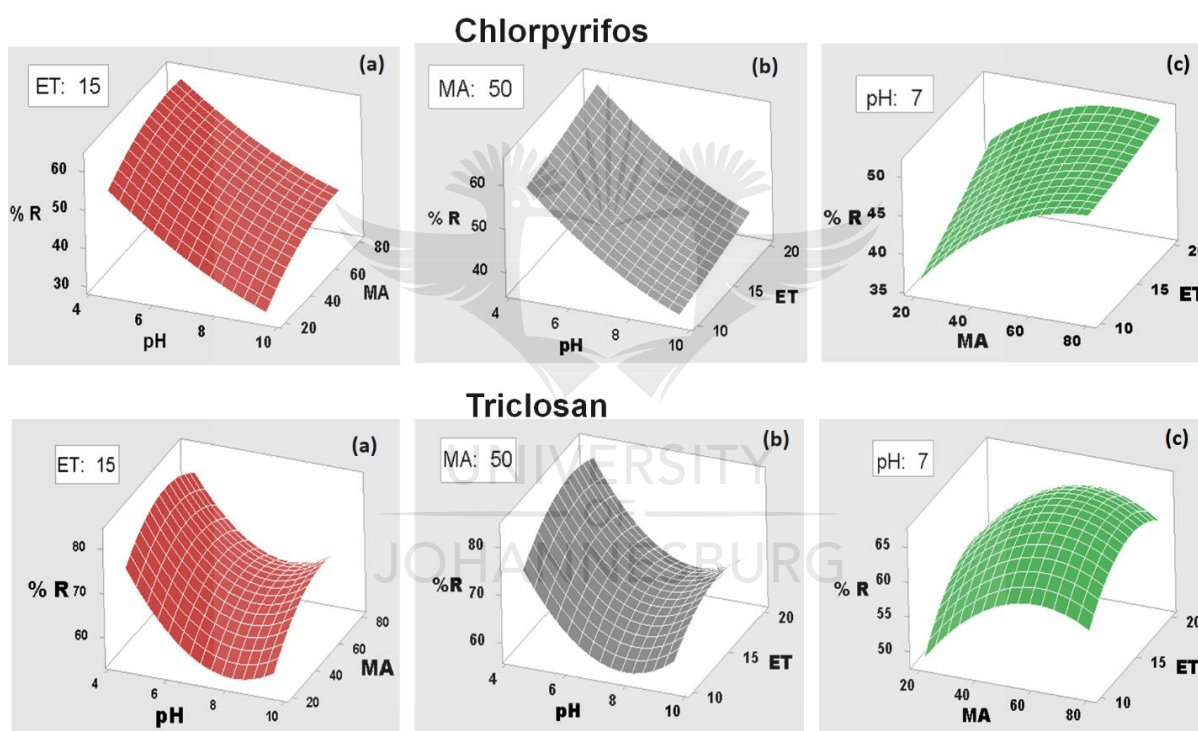**Figure 7.4**: Pareto charts of standardized effects of a) CPF and b) TCS.

Figure 7.5: Surface response to optimize the variables pH, MA and ET. (a) Effect of pH and MA on the extraction efficiency. (b) Effect of pH and ET on the extraction efficiency. (c) Effect of MA and ET on the extraction efficiency.

Table 7.3: Variable and factors of the CCD for extraction of chlorpyrifos and triclosan

				Chlorpyrifos	Triclosan
Standard Runs	pH	MA (mg)	ET (min)	%Recoveries	
1	4.5	20	10	50	65
2	4.5	20	20	65	78
3	4.5	80	10	69	74
4	4.5	80	20	61	75
5	9.5	20	10	29	51
6	9.5	20	20	27	53
7	9.5	80	10	33	57
8	9.5	80	20	42	65
9	4.5	50	15	52	82
10	9.5	50	15	50	77
11	7.0	20	15	40	59
12	7.0	80	15	52	69
13	7.0	50	10	43	63
14	7.0	50	20	55	67
15 (C)	7.0	50	15	37	48
16 (C)	7.0	50	15	51	65
17 (C)	7.0	50	15	48	66
18 (C)	7.0	50	15	50	70

7.3.5 Analytical figures of merit

The performance of the developed method was evaluated by investigating analytical parameters such as linearity, correlation coefficient, precision (within and between day) as a function of relative standard deviation (%RSD), limit of detection (LOD) and limit of quantification (LOQ). External calibration curves for the quantitative analysis of extracted target analytes were generated by plotting peak areas versus the initial concentrations in aqueous solution. As summarized in **Table 7.4**, the coefficient correlation (R^2), was >0.995 for both CPF and TCS, showing good linearity in the concentration range studied (5-100 µg L^{-1}). The LOD and LOQ determination were obtained by calculation using **equation 7.3** and **7.4** below.

$$3.3(S\,y/x)/b \qquad\qquad\qquad (7.3)$$

$$10(S\,y/x)/b \qquad\qquad\qquad (7.4)$$

where $S_{y/x}$ is the standard deviation of blank measurements and b is the slope of the calibration curve. From **Table 7.4**, the LOD and LOQ obtained were in the range of 0.02-0.08 and 0.06-0.19 µg L^{-1} respectively for both analytes. Method precision evaluated as intraday and inter-day varied from 3.28 to 4.18 % for seven repeated experiments. The matrix effect was also

evaluated by comparing slopes obtained by running calibration standards in solvent and in matrix (effluent wastewater) under the optimized conditions. The results in **Table 7.4** revealed that there was negligible matrix effect 94 % and 108 % for CPF and TCS, respectively. These overall results demonstrated excellent reproducibility and sensitivity of the developed method.

Table 7.4: Analytical features of method performance characteristics (n=7)

Analyte	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	LDR ($\mu g\ L^{-1}$)	R^2	Intra-day % RSD	Inter-day %RSD	% ME
Chlorpyrifos	0.02	0.06	5.0-100	0.9976	3.3	4.2	94
Triclosan	0.08	0.19	5.0-100	0.9950	4.7	4.4	108

7.3.6 Validation and application of MSPE to real environmental samples

The feasibility of the developed method was evaluated by extracting CPF and TCS in wastewater samples drawn from a local wastewater treatment plants (WWTP). Two wastewater samples (influent and effluent) were spiked with standard solutions at two concentration levels (25 and 100 $\mu g\ L^{-1}$) as summarized in **Table 7.5**. The relative recoveries of the target compounds expressed as the mean values (n=7) ranging between 76-108 % with a precision of 5.3-11%. The results obtained demonstrate that the method exhibits good practicability in the analysis of CPF and TCS in wastewater. Unspiked wastewater samples from different sampling points of the WWTP were also extracted and analyzed using the developed method, as revealed in **Table 7.6**. From the results, triclosan was present in the all the samples, albeit at low concentrations, while chlorpyrifos was detected at very trace levels, below the quantification limit of the method. The detection of TCS can be attributed to the frequent use of personal care products care products that contain TCS as an antimicrobial agent such as soaps, toothpastes, body lotions among others [36]. **Figure 7.6** shows the total ion chromatogram (TIC) of spiked (5 $\mu g\ L^{-1}$) and unspiked (blank) water samples obtained using the developed method under MRM mode. The results indicate negligible method interferences. Overall, results obtained reveal that the present method was suitable for the trace determination of CPF and TCS in real environmental water samples.

Table 7.5: Compound matrix recoveries of chlorpyrifos and triclosan using the UA-MSPDE method, in wastewater matrices (n=7)

Analyte	Spiked Level (µg L^{-1})	Influent water		Effluent Water	
		R %	RSD %	R %	RSD%
Chlorpyrifos	25	95	10	76	5.3
	100	109	8.2	78	6.2
Triclosan	25	91	11	97	8.0
	100	95	7.5	79	7.0

Table 7.6: Application of developed UA-MSPDE method on unspiked wastewater samples (n=6) for extraction of chlorpyrifos and triclosan

Analyte	Triclosan		Chlorpyrifos
	Conc (µg L^{-1})	%RSD	Conc (µg L^{-1})
Raw influent	1.45	3.28	<LOQ
Secondary influent	1.06	5.79	<LOQ
Final effluent	1.02	3.57	<LOQ
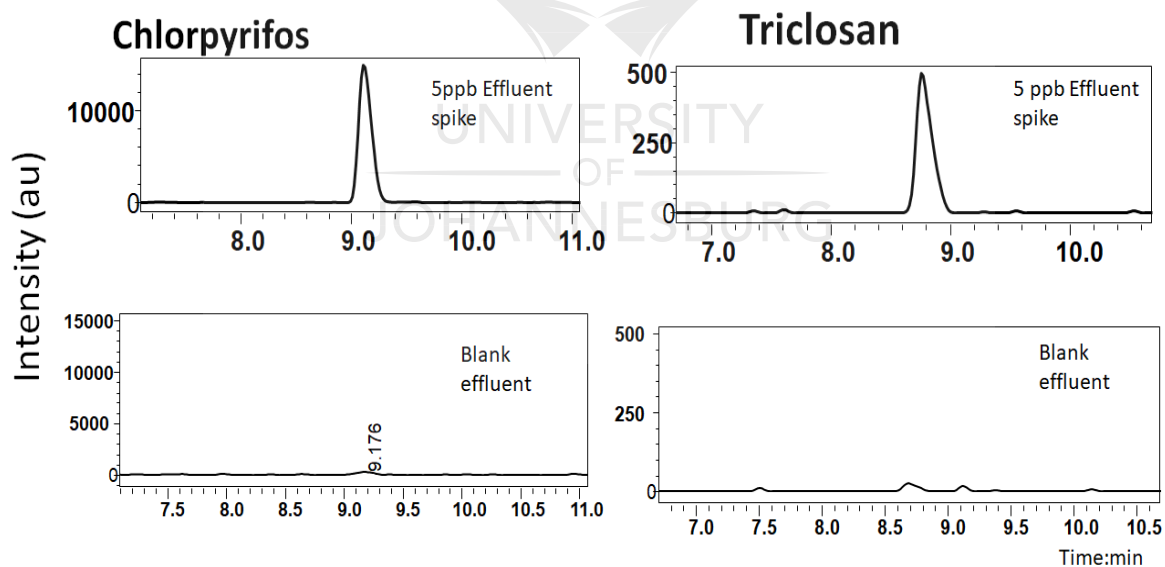
Figure 7.6: Extracted ion chromatogram (EIC) of spiked effluent (5 µg L^{-1}) water and blank water samples

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7.3.7 Comparison of MSPE with other extraction methods

Different methods reported in the literature were compared with the developed method as detailed in **Table 7.7**. From the results, we observed that the developed method had much lower LODs than other methods reported in the literature except one [37]. The obtained results in this study can be attributed to the use of magnetic CNDs applied in the extraction procedure that comprised small particles exhibiting small surface area and thereby increasing the extraction efficiency in determination of the target analytes. Also, good chromatographic peak shapes were obtained by use of the sensitive LC-MS/MS detection technique, compared to the non-MS methods reported. In general, the sensitivity and repeatability attained in the developed method showed relatively better extraction performance than the methods reported in previous studies.

Table 7.7: Comparison with other methods proposed for chlorpyrifos and triclosan analysis using UA-MSPDE for extraction and preconcentration in water samples

Method	Analyte	Linearity ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	Recovery (%)	RSD%	References
IL/IL-DLLME-HPLC-MS/MS	Triclosan	2.50-500	0.230-0.350	88-110	6.41	[8]
SPE-HPLC	Triclosan	5-1000	0.01-0.08	73-104	8-15	[33]
HF-LPME-LC-MS/MS	Chlorpyrifos, Triclosan	1-100	0.006-0.018	80-123	3.30-9.70	[37]
SSME-UV	Triclosan	0.95-400	0.280	103-118	2.40-5.50	[38]
LLME-HPLC-UV	Chlorpyrifos	0.50-400	0.100-0.350	92-110	2.00-5.70	[39]
WE-DLLME-GCFID	Chlorpyrifos	3-10000	0.920	91-98	6.20	[40]
SPE-LCMS/MS	Chlorpyrifos	0.02-1000	0.01	>70	<15	[41]
UA-MSPDE-LC-MS/MS	Chlorpyrifos, Triclosan	5-100	0.024-0.081	76-108	3.28-4.36	**This work**

SSME: Supramolecular microextraction, LLME: liquid-liquid microextraction: WE: water emulsion, DLLME: dispersive liquid-liquid microextraction, IL: ionic liquid, HF-LPME: hollow fibre liquid phase microextraction, UV : Ultraviolet, GCFID: gas chromatography flame ionisation detector, SPE: solid phase extraction.

7.4 CONCLUSIONS

In summary, Fe_3O_4@CNDs was successfully prepared by a simple co-mixing method under magnetic stirring. The combination of the rapid UA-MSPDE based on the synthesized magnetic CNDs, coupled with LC-MS/MS was used as a sensitive and efficient analytical method for the simultaneous determination of chlorpyrifos and triclosan in wastewater samples. The experimental variables (pH, adsorbent mass, and extraction time) affecting the analytical response using the developed UA-MSPDE method were optimized. The prepared magnetic CNDs nanocomposite was characterized and applied as a viable nanomaterial to extract the target analytes from the water samples with relatively good method accuracy (76-108 %) and precision of less than 10% in the spiked sample matrices. The method also is highly advantageous in that it avoids the laborious and time-consuming procedures that are synonymous to the conventional solid phase extraction. Furthermore, the use of an external magnetic field prevented any centrifugation and/or filtration steps. The collected samples from the WWTP, were quantitatively analyzed using the developed method. Overall, the results obtained in this study in terms of method precision, accuracy, LODs and LOQs, reveal that the method can be employed for routine magnetic solid phase dispersive extraction prior to instrumental analysis of chlorpyrifos and triclosan, in complex environmental matrices such as wastewater.

7.5 REFERENCES

[1] J. Guo, H. Iwata, Risk assessment of triclosan in the global environment using a probabilistic approach, Ecotoxicology and Environmental Safety 143 (2017) 111-119.

[2] R.U. Halden, On the need and speed of regulating triclosan and triclocarban in the United States, ACS Publications, 2014.

[3] S.S. Caldas, C. Rombaldi, J.L. de Oliveira Arias, L.C. Marube, E.G. Primel, Multi-residue method for determination of 58 pesticides, pharmaceuticals and personal care products in water using solvent demulsification dispersive liquid–liquid microextraction combined with liquid chromatography-tandem mass spectrometry, Talanta 146 (2016) 676-688.

[4] R. Celano, A.L. Piccinelli, L. Campone, L. Rastrelli, Ultra-preconcentration and determination of selected pharmaceutical and personal care products in different water matrices by solid-phase extraction combined with dispersive liquid–liquid

microextraction prior to ultra high pressure liquid chromatography tandem mass spectrometry analysis, Journal of Chromatography A 1355 (2014) 26-35.

[5] A. Asati, G.N.V. Satyanarayana, D.K. Patel, Comparison of two microextraction methods based on solidification of floating organic droplet for the determination of multiclass analytes in river water samples by liquid chromatography tandem mass spectrometry using Central Composite Design, Journal of Chromatography A 1513 (2017) 157-171.

[6] S.S. Caldas, C.M. Bolzan, J.R. Guilherme, M.A.K. Silveira, A.L.V. Escarrone, E.G. Primel, Determination of pharmaceuticals, personal care products, and pesticides in surface and treated waters: method development and survey, Environmental Science and Pollution Research 20 (2013) 5855-5863.

[7] A. Demoliner, S.S. Caldas, F.P. Costa, F.F. Gonçalves, R.M. Clementin, M.R. Milani, E.G. Primel, Development and validation of a method using SPE and LC-ESI-MS-MS for the determination of multiple classes of pesticides and metabolites in water samples, Journal of the Brazilian Chemical Society 21 (2010) 1424-1433.

[8] R.-S. Zhao, X. Wang, J. Sun, C. Hu, X.-K. Wang, Determination of triclosan and triclocarban in environmental water samples with ionic liquid/ionic liquid dispersive liquid-liquid microextraction prior to HPLC-ESI-MS/MS, Microchimica Acta 174 (2011) 145-151.

[9] A. Prieto, R. Rodil, J.B. Quintana, I. Rodríguez, R. Cela, M. Möder, Evaluation of low-cost disposable polymeric materials for sorptive extraction of organic pollutants in water samples, Analytica Chimica Acta 716 (2012) 119-127.

[10] I. Domínguez, F.J. Arrebola, R. Romero-González, A. Nieto-García, J.L. Martínez Vidal, A. Garrido Frenich, Solid phase microextraction and gas chromatography coupled to magnetic sector high resolution mass spectrometry for the ultra-trace determination of contaminants in surface water, Journal of Chromatography A 1518 (2017) 15-24.

[11] P. Rocío-Bautista, C. Martínez-Benito, V. Pino, J. Pasán, J.H. Ayala, C. Ruiz-Pérez, A.M. Afonso, The metal–organic framework HKUST-1 as efficient sorbent in a vortex-assisted dispersive micro solid-phase extraction of parabens from environmental waters, cosmetic creams, and human urine, Talanta 139 (2015) 13-20.

[12] Y. Assadi, M.A. Farajzadeh, A. Bidari, 2.10 - Dispersive Liquid–Liquid Microextraction, in: J. Pawliszyn (Ed.), Comprehensive Sampling and Sample Preparation, Academic Press, Oxford, 2012, pp. 181-212.

[13] K.M. Dimpe, A. Mpupa, P.N. Nomngongo, Microwave assisted solid phase extraction for separation preconcentration sulfamethoxazole in wastewater using tyre based activated

carbon as solid phase material prior to spectrophotometric determination, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 188 (2018) 341-348.

[14] M. Wierucka, M. Biziuk, Application of magnetic nanoparticles for magnetic solid-phase extraction in preparing biological, environmental and food samples, TrAC Trends in Analytical Chemistry 59 (2014) 50-58.

[15] C. Herrero-Latorre, J. Barciela-García, S. García-Martín, R.M. Peña-Crecente, J. Otárola-Jiménez, Magnetic solid-phase extraction using carbon nanotubes as sorbents: A review, Analytica Chimica Acta 892 (2015) 10-26.

[16] K. Aguilar-Arteaga, J. Rodriguez, E. Barrado, Magnetic solids in analytical chemistry: a review, Analytica Chimica Acta 674 (2010) 157-165.

[17] M.S. Mannoor, H. Tao, J.D. Clayton, A. Sengupta, D.L. Kaplan, R.R. Naik, N. Verma, F.G. Omenetto, M.C. McAlpine, Graphene-based wireless bacteria detection on tooth enamel, Nature communications 3 (2012) 763.

[18] Y. Guo, D. Wang, X. Liu, X. Wang, W. Liu, W. Qin, Synthesis and characterization of the nickel@ carbon dots hybrid material and its application in the reduction of Cr (VI), New Journal of Chemistry 38 (2014) 5861-5867.

[19] G. Gedda, S. Pandey, M.L. Bhaisare, H.-F. Wu, Carbon dots as nanoantennas for anti-inflammatory drug analysis using surface-assisted laser desorption/ionization time-of-flight mass spectrometry in serum, RSC Advances 4 (2014) 38027-38033.

[20] M.L. Bhaisare, G. Gedda, M.S. Khan, H.-F. Wu, Fluorimetric detection of pathogenic bacteria using magnetic carbon dots, Analytica chimica acta 920 (2016) 63-71.

[21] A.-C. Sun, Synthesis of magnetic carbon nanodots for recyclable photocatalytic degradation of organic compounds in visible light, Advanced Powder Technology 29 (2018) 719-725.

[22] S. Ahmadian-Fard-Fini, M. Salavati-Niasari, D. Ghanbari, Hydrothermal green synthesis of magnetic Fe 3 O 4-carbon dots by lemon and grape fruit extracts and as a photoluminescence sensor for detecting of E. coli bacteria, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy (2018).

[23] H. Wang, J. Shen, Y. Li, Z. Wei, G. Cao, Z. Gai, K. Hong, P. Banerjee, S. Zhou, Magnetic iron oxide–fluorescent carbon dots integrated nanoparticles for dual-modal imaging, near-infrared light-responsive drug carrier and photothermal therapy, Biomaterials Science 2 (2014) 915-923.

[24] Z. Liu, Y. Liu, K. Yao, Z. Ding, J. Tao, X. Wang, Synthesis and magnetic properties of Fe 3 O 4 nanoparticles, Journal of materials synthesis and processing 10 (2002) 83-87.

[25] A.H. Lu, E.e.L. Salabas, F. Schüth, Magnetic nanoparticles: synthesis, protection, functionalization, and application, Angewandte Chemie International Edition 46 (2007) 1222-1244.

[26] S. Gwebu, P. Nomngongo, P. Mashazi, T. Nyokong, N. Maxakato, Platinum Nanoparticles Supported on Carbon Nanodots as Anode Catalysts for Direct Alcohol Fuel Cells, Int. J. Electrochem. Sci 12 (2017) 6365-6378.

[27] Y. Guo, J. Qian, A. Iqbal, L. Zhang, W. Liu, W. Qin, Pd nanoparticles immobilized on magnetic carbon dots@Fe3O4 nanocubes as a synergistic catalyst for hydrogen generation, International Journal of Hydrogen Energy 42 (2017) 15167-15177.

[28] Y. Guo, J. Qian, A. Iqbal, L. Zhang, W. Liu, W. Qin, Pd nanoparticles immobilized on magnetic carbon dots@ Fe3O4 nanocubes as a synergistic catalyst for hydrogen generation, International Journal of Hydrogen Energy 42 (2017) 15167-15177.

[29] S.N. Sinha, Liquid chromatography mass spectrometer (LC-MS/MS) study of distribution patterns of base peak ions and reaction mechanism with quantification of pesticides in drinking water using a lyophilization technique, American Journal of Analytical Chemistry 2 (2011) 511.

[30] H.-T. Liao, C.-J. Hsieh, S.-Y. Chiang, M.-H. Lin, P.-C. Chen, K.-Y. Wu, Simultaneous analysis of chlorpyrifos and cypermethrin in cord blood plasma by online solid-phase extraction coupled with liquid chromatography–heated electrospray ionization tandem mass spectrometry, Journal of Chromatography B 879 (2011) 1961-1966.

[31] S.W.C. Chung, B.T.P. Chan, Validation and use of a fast sample preparation method and liquid chromatography–tandem mass spectrometry in analysis of ultra-trace levels of 98 organophosphorus pesticide and carbamate residues in a total diet study involving diversified food types, Journal of Chromatography A 1217 (2010) 4815-4824.

[32] J.B. Quintana, T. Reemtsma, Sensitive determination of acidic drugs and triclosan in surface and wastewater by ion-pair reverse-phase liquid chromatography/tandem mass spectrometry, Rapid communications in mass spectrometry 18 (2004) 765-774.

[33] L.M. Madikizela, S.F. Muthwa, L. Chimuka, Determination of Triclosan and Ketoprofen in river water and wastewater by solid phase extraction and high performance liquid chromatography, South African Journal of Chemistry 67 (2014) 0-0.

[34] P.N. Nomngongo, J.C. Ngila, Multivariate optimization of dual-bed solid phase extraction for preconcentration of Ag, Al, As and Cr in gasoline prior to inductively coupled plasma optical emission spectrometric determination, Fuel 139 (2015) 285-291.

[35] A. Mpupa, G.P. Mashile, P.N. Nomngongo, Ultrasound-assisted dispersive solid phase nanoextraction of selected personal care products in wastewater followed by their determination using high performance liquid chromatography-diode array detector, Journal of hazardous materials (2018).

[36] J.V. Rodricks, J.A. Swenberg, J.F. Borzelleca, R.R. Maronpot, A.M. Shipp, Triclosan: a critical review of the experimental data and development of margins of safety for consumer products, Critical reviews in toxicology 40 (2010) 422-484.

[37] V. Salvatierra-stamp, R. Muñiz-Valencia, J.M. Jurado, S.G. Ceballos-Magaña, Hollow fiber liquid phase microextraction combined with liquid chromatography-tandem mass spectrometry for the analysis of emerging contaminants in water samples, Microchemical Journal 140 (2018) 87-95.

[38] A. Mpupa, G.P. Mashile, P.N. Nomngongo, Vortex assisted-supramolecular solvent based microextraction coupled with spectrophotometric determination of triclosan in environmental water samples, Open Chemistry 15 (2017) 255-262.

[39] P. Zohrabi, M. Shamsipur, M. Hashemi, B. Hashemi, Liquid-phase microextraction of organophosphorus pesticides using supramolecular solvent as a carrier for ferrofluid, Talanta 160 (2016) 340-346.

[40] M.A. Farajzadeh, M.R.A. Mogaddam, L. Esrafili, Surfactant-less water emulsion based dispersive liquid–liquid microextraction for determination of organophosphorus pesticides in aqueous samples, Analytical Methods 7 (2015) 7899-7906.

[41] H. Zhang, D. West, H. Shi, Y. Ma, C. Adams, T. Eichholz, Simultaneous Determination of Selected Trace Contaminants in Drinking Water Using Solid-Phase Extraction-High Performance Liquid Chromatography-Tandem Mass Spectrometry, Water, Air, & Soil Pollution 230 (2019) 28.

CHAPTER 8:
CONCLUSIONS AND RECOMMENDATIONS

8.1 GENERAL CONCLUSIONS

This thesis reports on the development of sample extraction and preconcentration techniques of organic contaminants in wastewater, prior to their determination using UHPLC-MS/MS. The benefits of preconcentrating the samples are to aid in elimination of potential complex matrix interferences and to obtain low detection limits of the target analytes that occur in trace levels in the environment. Development of these techniques is therefore a vital step in the accurate and precise determination of these organic contaminants. In this study, solid phase extraction (SPE), vortex assisted dispersive liquid-liquid microextraction (VA-DLLME) and ultrasonic-assisted magnetic solid phase dispersive extraction (UA-MSPDE) were employed. Experimental parameters such as sample pH, sample volume, elution volume, mass of adsorbent and extraction time, were optimized using multivariate approach. Samples were collected from local wastewater treatment plant (WWTP) at different treatment stages, i.e. primary, secondary and tertiary stages.

The SPE procedure was successfully developed and optimized for the analysis of single class compounds (parabens) and multi-class compounds (parabens and organophosphorus pesticides). The first set of extraction employed the use Oasis HLB cartridges for extraction of parabens in wastewater samples prior to UHPLC-MS/MS analysis. Spike recoveries experiments resulted in satisfactory recoveries ranging between 70-120% in three different matrices with % RSD of <10 %. The concentration of the analytes from the various sampling points indicated presence of methylparaben, ethylparaben and propylparaben at trace levels (<3 $\mu g\ L^{-1}$) in the WWTP. Synthesized CNDs were also evaluated as novel SPE adsorbents for the simultaneous extraction and preconcentration of organophosphorus pesticides and parabens. Under the optimized conditions, satisfactory extraction recoveries (63-123%), low LOD and LOQs were obtained for the five target analytes which exhibited diversity in physicochemical properties. Percentage recoveries obtained using the synthesized CNDs for SPE were lower than the commercial based SPE cartridges Oasis HLB. However, only 170 mg of the CNDs was employed compared to the 200 mg in the commercial based cartridges. These results indicate the applicability of the synthesized CNDs in extraction of multi-class organic compounds in environmental water samples.

Magnetic solid phase dispersive extraction based on ultrasonic dispersion of magnetic CNDs, was used for simultaneous extraction and preconcentration of chlorpyrifos and triclosan in water samples prior to UHPC-MS/MS analysis. This method permitted quick and simple extraction technique by the application of an external magnet for separation of sorbent material from the aqueous solution. Method performance characteristics showed excellent precision (<4%) with low LODs and LOQs. The accuracy of the developed method was established with % recoveries ranging between 76.19-108 % and 78.88-96.33% for CPF and TCS, respectively. The method developed was thereafter applied for the determination of CPF and TCS, in environmental water samples. The concentration levels found for TCS ranged between 1.02-1.45 (μg L^{-1}) while that of CPF were below the LOD.

Dispersive liquid-liquid microextraction method was applied in the extraction of organophosphorus pesticides in wastewater samples. This method was successfully optimized using factorial design and central composite design. The advantages of this method include small sample volume (5 mL), ease of operation, high sample throughput, cost effective and very small amounts of organic solvents used for extraction. Relatively low LODs and LOQs were attained with relatively good precision (<10 %).

The results obtained in this PhD study showcase the viability of using UHPLC-MS/MS coupled with chemometric optimization approach in determining the occurrence of the organic contaminants in environmental samples.

8.2 RECOMMENDATIONS

The sample preparation techniques reported in this study were appropriate for extraction and preconcentration of organic contaminants in this water samples with applicability for routine sample analysis. However, the following aspects are recommended for future studies.

- ➢ Due to the diversity and occurrence of many other toxic organic contaminants in the environment, screening of the water samples using with detection techniques such as time of flight mass spectrometers (TOF-MS) for the determination of other contaminants present in the water samples that may be present at elevated concentrations, is required.
- ➢ Other solid phase extraction system with various adsorbents could be investigated to improve sample clean up in a complex sample matrix in WWTPs.

- The current study was carried out in wastewater samples from a WWTP. Therefore, the analysis of other water matrices such as drinking water, surface water, ground water and river water are recommended.

- Widening the number of target analytes in multi-residue analysis using LC-MS/MS and GC-MS methods,

- Testing the methodology for lower analyte concentrations (sub ppb levels) to validate the method at concentrations close to those present in samples.

- Applying higher pre-concentration factors for sample pretreatment methodologies, by utilizing using lower sample volumes for reconstitution after taking the eluate to dryness. This would enhance sensitivity in the methods developed.

- Applying a stricter criterion such as ion ratio intensity for confirmation of positive samples, following the current international guidelines.

- This study was also carried out in one geographic region, that is, Gauteng province in South Africa. Collection of samples to include municipalities from other provinces to will give a more elaborate and holistic overview of the occurrence of these organic contaminants in the water systems in the entire country is therefore recommended.

APPENDIX

Here-in are the extracted ion chromatograms and the mass spectra of 5 µg L^{-1} matrix standard of the personal care products (parabens and triclosan) and organophosphorous pesticide compounds in this study. The matrix matched calibatrion standards (5-100 µg L^{-1}) used for quantification purposes are also highlighted.
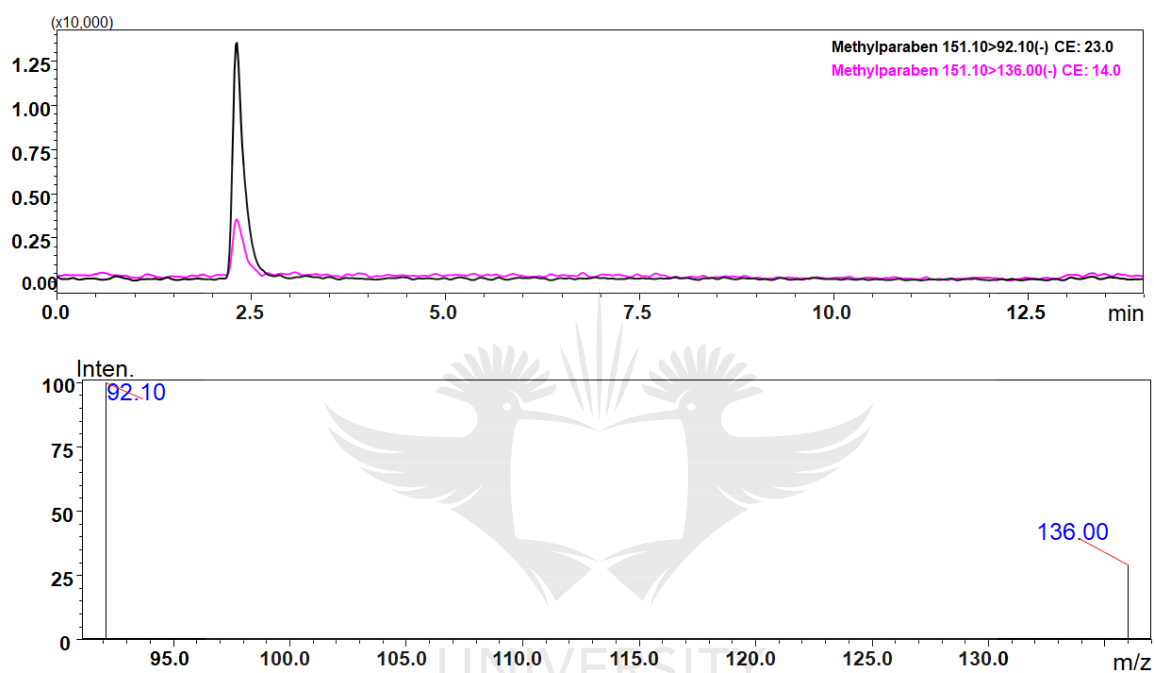
Figure A1: Extracted ion chromatogram and mass spectrum of methylparaben
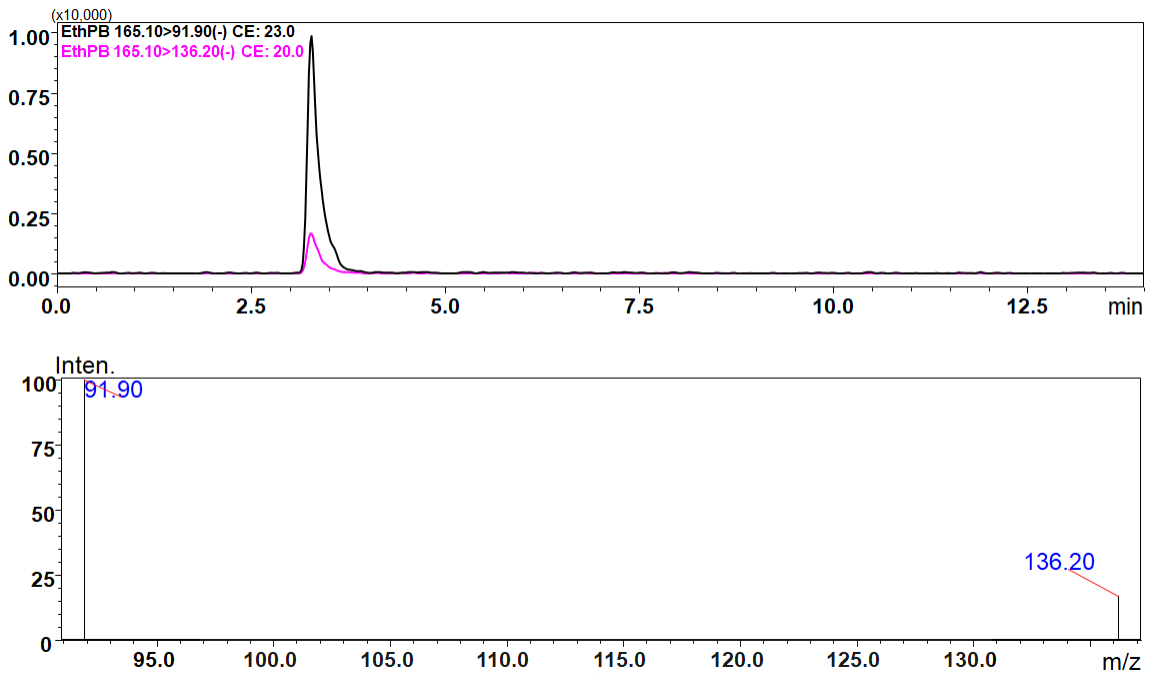
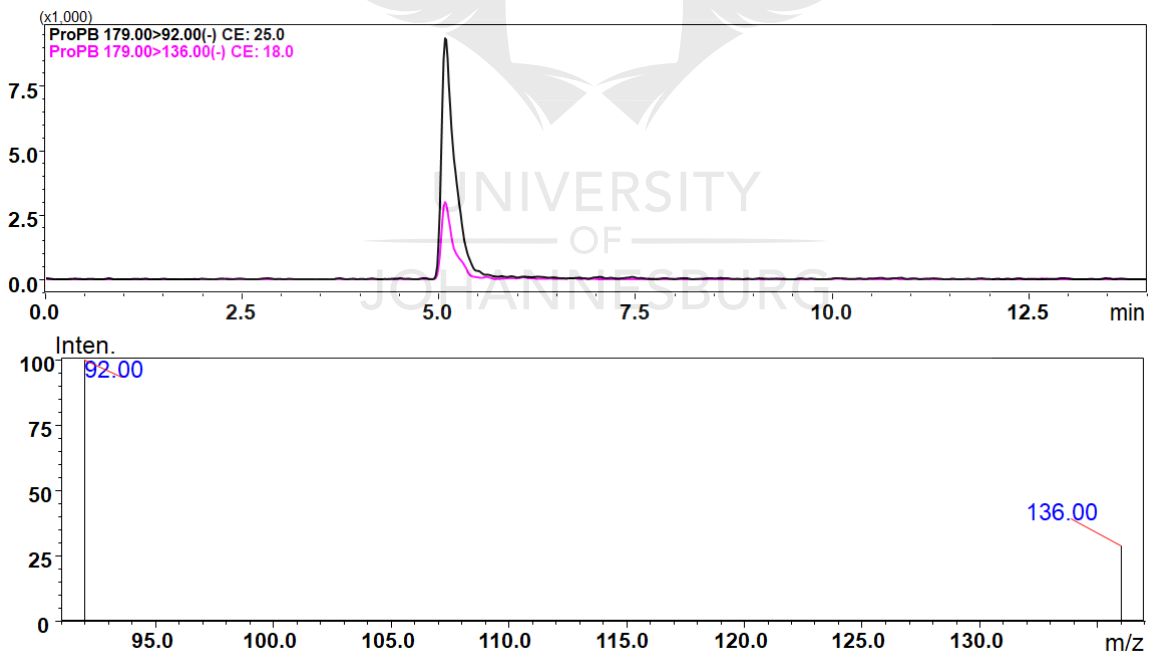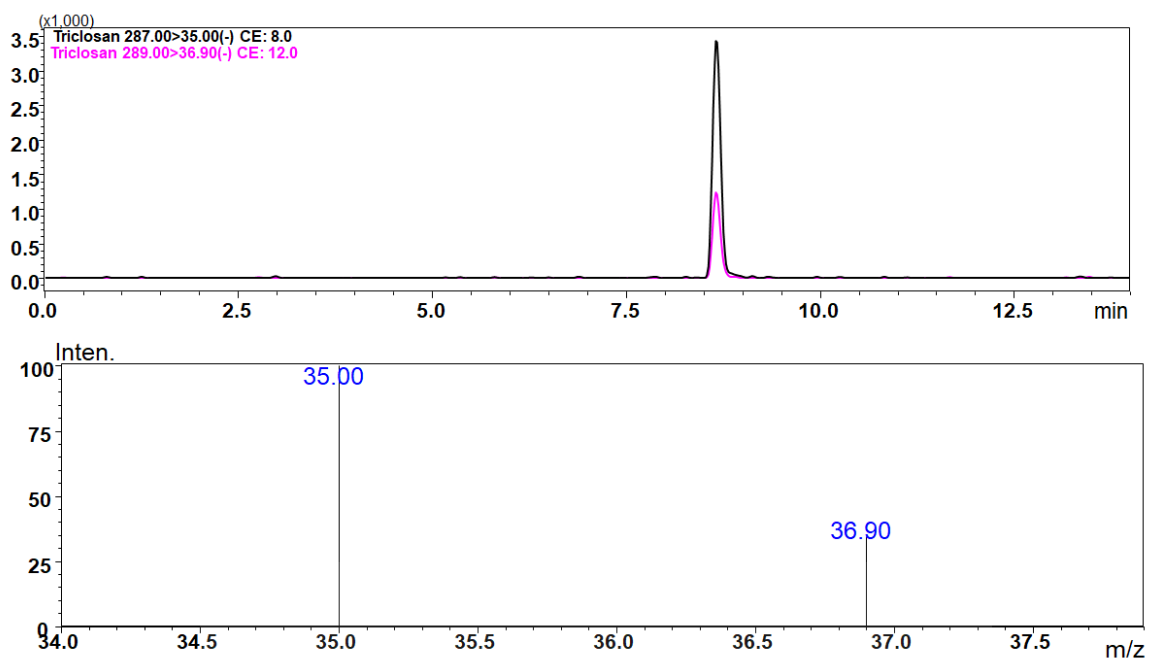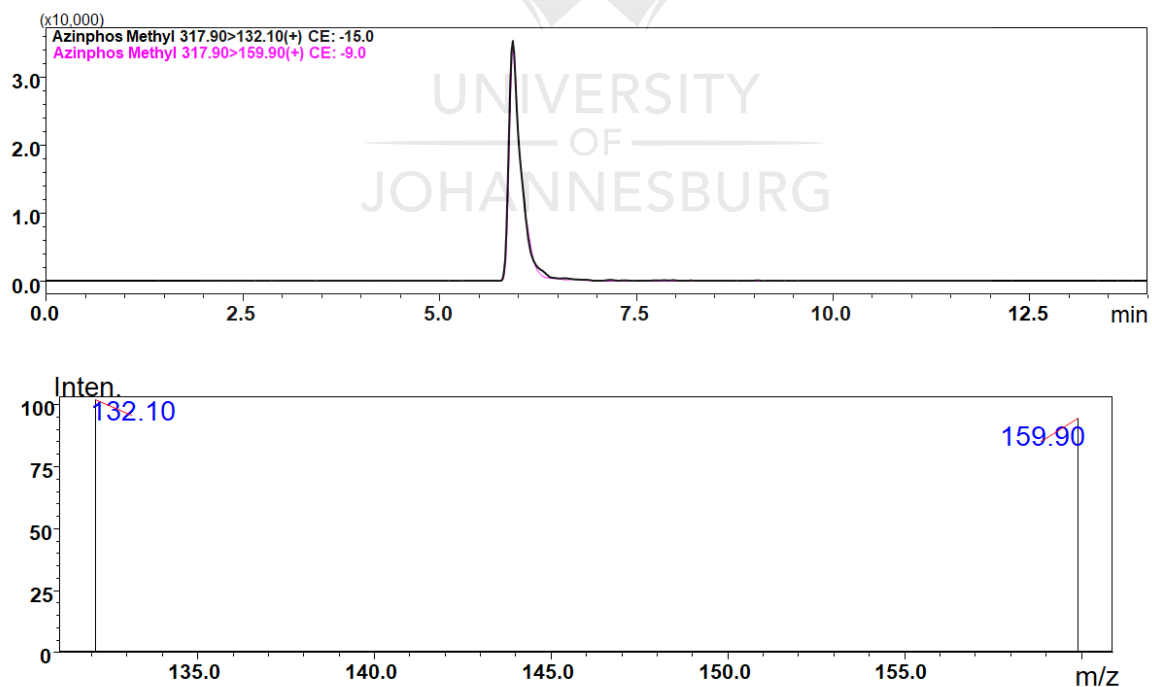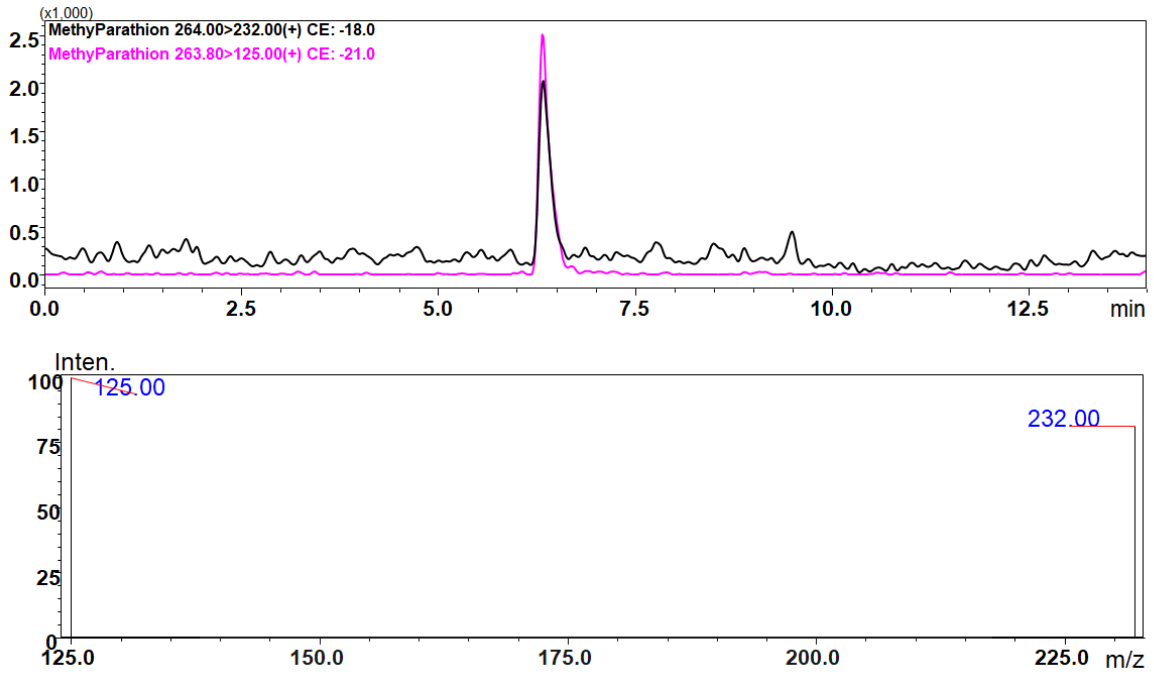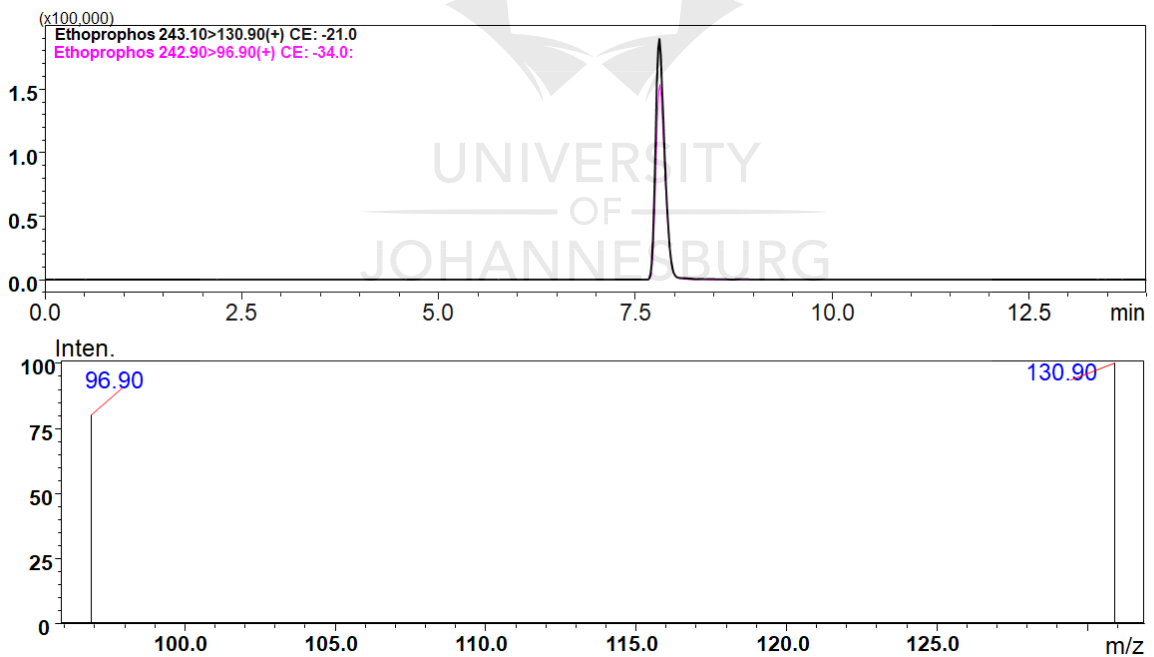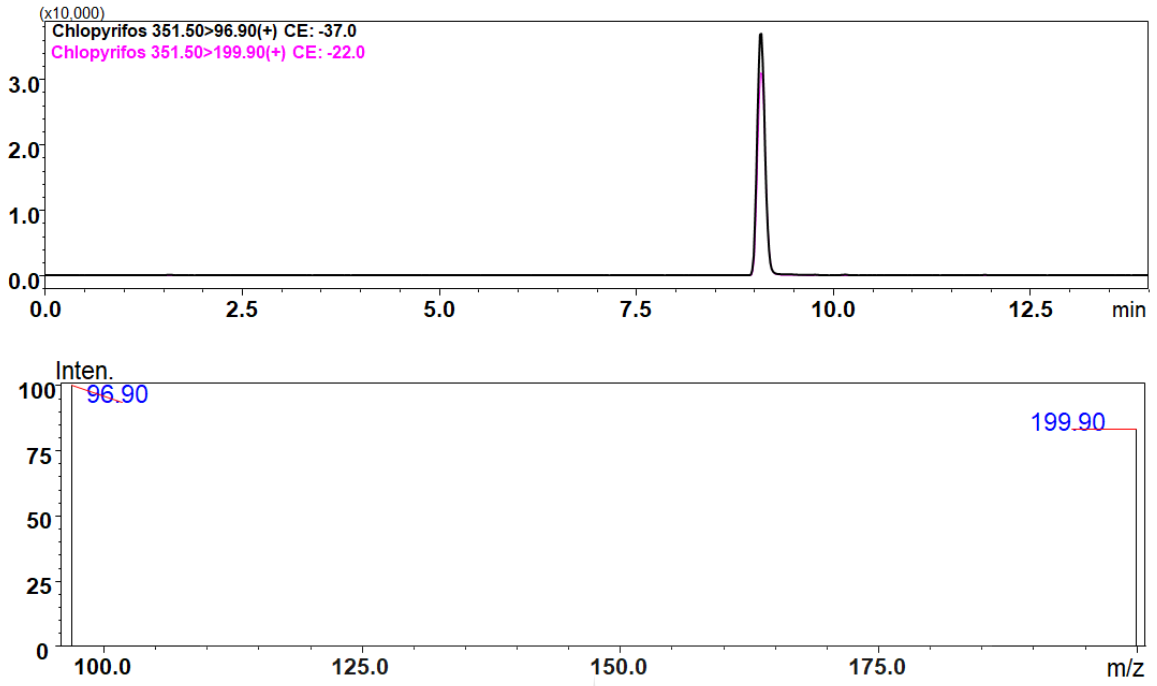**Figure A2:** Extracted ion chromatogram and mass spectrum of ethylparaben

Figure A3: Extracted ion chromatogram and mass spectrum of propylparaben

Figure A4: Extracted ion chromatogram and mass spectrum of triclosan

Figure A5: Extracted ion chromatogram and mass spectrum of azinphos-methyl

Figure A6: Extracted ion chromatogram and mass spectrum of parathion-methyl

Figure A7: Extracted ion chromatogram and mass spectrum of ethoprofos

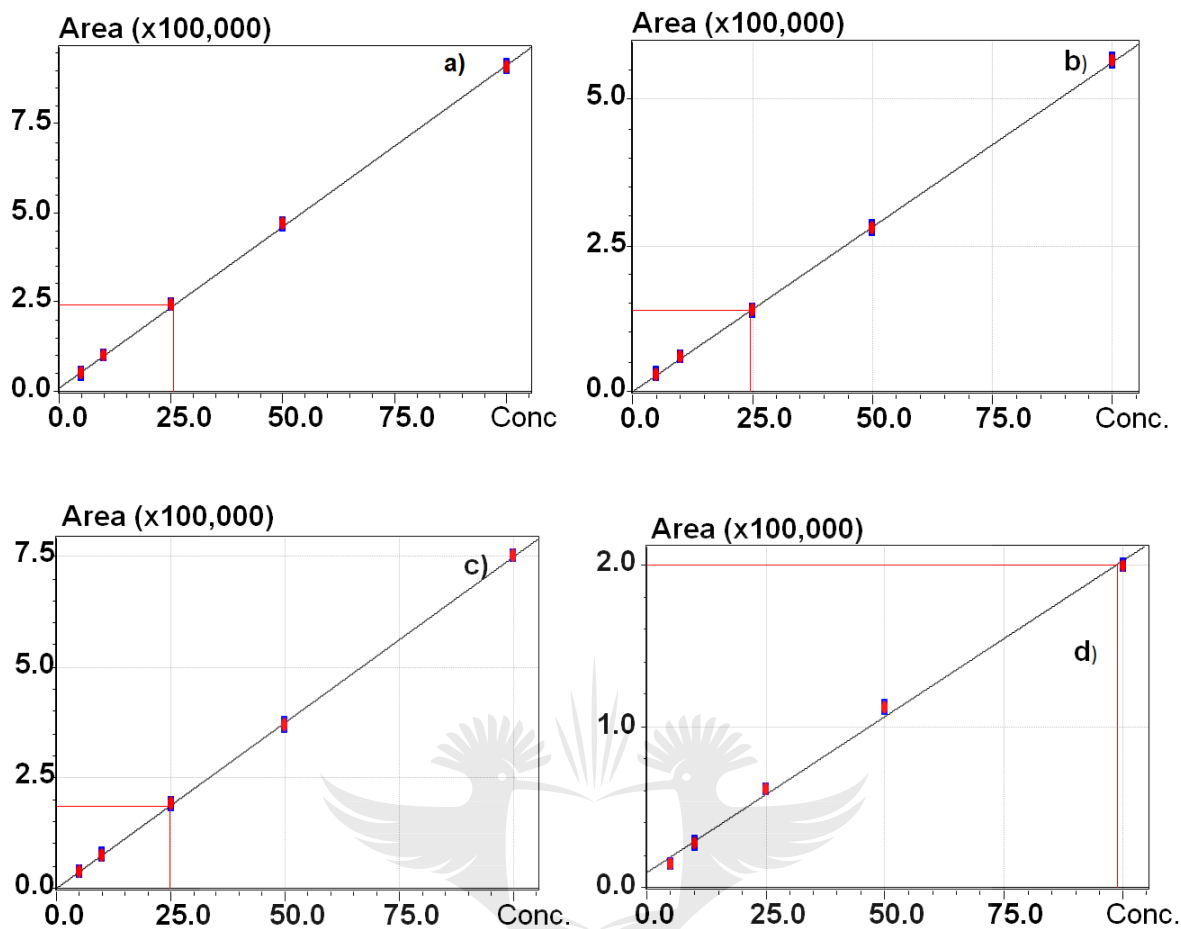**Figure A8**: Extracted ion chromatogram and mass spectrum of chlorpyrifos
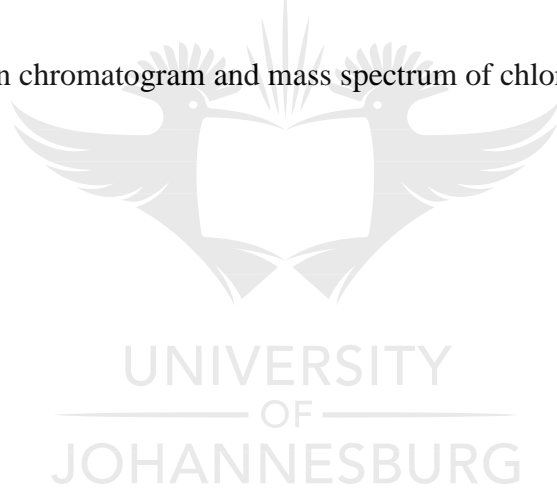
Figure A9: Matrix-matched calibration curves (5-100 µg L^{-1}) of personal care products prepared in effluent wastewater: a) Methylparaben, b) Ethylparaben, c) Propylparaben and d) Triclosan
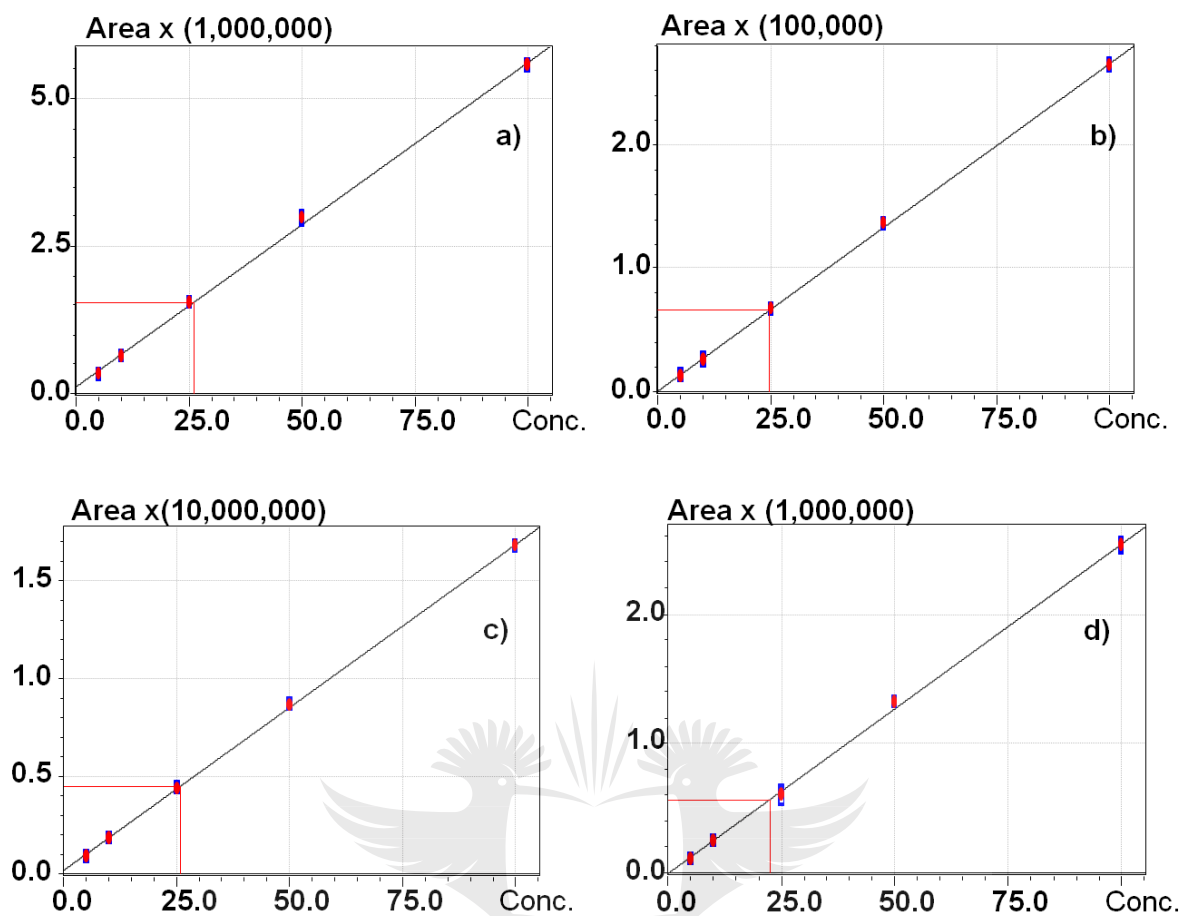
Figure A10: Matrix-matched calibration curves (5-100 µg L^{-1}) of personal care products prepared in effluent wastewater: a) Azinphos methyl, b) Parathion-methyl, c) Ethoprofos and d) Chlorpyrifos