Detection and quantification of emerging contaminants in Mgeni and Msunduzi Rivers by gas chromatography-mass spectrometry in KwaZulu-Natal, South Africa

by

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PREFACE

The research contained in this dissertation was completed by the candidate while based in the Discipline of Chemistry, School of Chemistry and Physics of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, South Africa. The research was financially supported by Water Research Commission and National Research Foundation of South Africa.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

Signed: Patrick Ndungu

Date: 12 February 2018

Signed: Grace Birungi

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Signed: Brenda Moodley

Date:

DECLARATION 1: PLAGIARISM

I, Bhekumuzi Prince Gumbi, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

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Signed: Bhekumuzi Prince Gumbi

Date:

DECLARATION 2: PUBLICATIONS

My role in each paper and presentation is indicated. The * indicates corresponding author.

Chapter 3

1. Gumbi BP*, Moodley B, Birungi G, Ndungu P. Qualitative and quantitative analysis of selected drugs in the Mgeni River by gas chromatography – mass spectrometry. Paper presented at 42nd National Convention 2015, South African Chemical Institute, 29th Nov to 4th Dec, 2015, Durban, South Africa. Presented by BP Gumbi

2. Gumbi BP, Moodley B, Birungi G, Ndungu P* 2017. Detection and quantification of acidic drug residues in South African surface water using gas chromatography – mass spectrometry. Chemosphere 168 (2017) 1042 – 1050

The research reported on is based on the data obtained from seasonal analysis of environmental samples, I collected from the Mgeni and Msunduzi Rivers between 2013 and 2016. I designed the experiments, developed methods, collected and processed the data, and wrote the paper.

Chapter 4

3. Gumbi BP*, Moodley B, Birungi G, Ndungu P. Assessment of nonsteroidal anti-inflammatory drugs in Mgeni and Msunduzi River sediments by ultrasonic assisted extraction and gas chromatography – mass spectrometry. Paper presented at College Research Day 2016, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, 1st Nov, 2016, Durban, South Africa. Presented by BP Gumbi

4. Gumbi BP*, Moodley B, Birungi G, Ndungu P. Assessment of nonsteroidal anti-inflammatory drugs in Mgeni and Msunduzi River sediments by ultrasonic assisted extraction and gas chromatography – mass spectrometry. Paper presented at ChromSA Seminar 2017, The Chromatography Division of the South African Chemical Institute, 5th April 2017, Durban, South Africa. Presented by BP Gumbi

5. Gumbi BP, Moodley B, Birungi G, Ndungu P*. 2017. Assessment of nonsteroidal anti-inflammatory drugs by ultrasonic – assisted extraction and GC-MS in Mgeni and Msunduzi river sediments, KwaZulu-Natal, South Africa. Environmental Science and Pollution Research 24 (2017) 1614 – 7499

The research reported on is based on the data obtained from seasonal analysis of environmental samples, I collected inform the Mgeni and Msunduzi Rivers between 2013 and 2016. I designed the experiments, developed methods, collected and processed the data, and wrote the paper.

Chapter 5

6. Gumbi BP*, Moodley B, Birungi G, Ndungu P. 2017. Determination of personal care products and pharmaceuticals in river sediments, KwaZulu-Natal, South Africa. Paper presented at SETAC North America 2017, Society of Environmental Toxicology and Chemistry, 12th to 16th Nov, 2017, Minneapolis MN, United States of America. Presented by BP Gumbi.

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Date:

ABSTRACT

This work concerns the development, optimization and validation of simple and affordable analytical methods for determination of different classes of emerging contaminants in environmental waters and solids. Occurrence of emerging contaminants in the African environment has received much attention recently. However, there is paucity of detailed analytical methods for determination and regulation of emerging contaminants in the environment and wastewater effluents in South Africa today some parts of the world. The developed methods included, extraction with solid phase (clean-up), ultrasound-centrifuge assisted extraction (sediments), detection with gas chromatography-mass spectrometry (after derivatization of polar analytes) and ecological risk assessment technique associated with emerging contaminants. For all developed methods, recoveries (60% - 130%), R^2 (> 0.99) and precisions (< 25%) within acceptable limits were achieved. This study was undertaken to determine the occurrence and concentration of major classes of emerging contaminants (pharmaceuticals, personal care products and stimulants) between 2014 and 2016 in Mgeni and Msunduzi Rivers, KwaZulu-Natal, South Africa. Surface water, wastewater (influent and effluent), sediment and biosolid samples were collected from these rivers and wastewater treatment plants along both rivers. The developed methods were combined and applied to qualitative and quantitative analysis of pharmaceuticals (acidic/ non-steroidal anti-inflammatory drugs, antibiotic and hormones), stimulants (caffeine) and personal care products (paraben and triclosan). Approximately 50 emerging contaminants of different classes were detected and only 15 were quantified. Environmental concentration of contaminants were found to range from 0.02 µg L^{-1} to 68 µg L^{-1} and 0.12 to 220 ng g^{-1} in water and sediments respectively. Acidic drugs, antibiotic and hormones were detected in all samples analysed in both water and sediments, however, stimulant and PPC were not detected in some of the samples. Wastewater treatment plants were recognised as one of the main routes of emerging contaminants into the aquatic environment. The developed methods can be used to monitor emerging contaminants in the environment.

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Abbreviations

Brominated flame retardants	BFRs
Chemicals of emerging concerns	CECs
Emerging contaminants	ECs
Gas chromatography - mass spectrometry	GC-MS
Liquid chromatography - mass spectrometry	LC - MS
Mass spectrometry	MS
Nonsteroidal anti-inflammatory drugs	NSAIDs
nucleoside reverse transcriptase inhibitors	NRTIs
Over the counter	OTC
Personal care products	PCPs
Pharmaceutical active compounds	PhACs
Pharmaceuticals and personal care products	PPCPs
Polybrominated diphenyl esthers	PBDEs
Single ion monitoring	SIM
Solid phase extraction	SPE
Wastewater treatment plants	WWTPs
Water Research Commission	WRC

Chapter 1: General Introduction

1.1 Introduction

Emerging contaminants (ECs) are defined as new pollutants that are not regulated and which have the potential to affect the environment and human wellbeing (Gavrilescu et al., 2015). Chemicals of emerging concern (CECs) can include pharmaceuticals, personal care products, nanoparticles, natural and synthetic hormones, flame retardants and other industrial chemicals with potential significant adverse effects on human health and aquatic life (Deblonde et al., 2011). These compounds have long been existing in the environment but have not gained scientific or public attention until 2 decades ago (der Beek et al., 2016). ECs have been detected in wastewater (Bartelt-Hunt et al., 2009), surface waters (Agunbiade and Moodley, 2016) and sediments (Chen and Zhou, 2014) throughout the world (Sarmah et al., 2006). An increase in population in the world has produce a similar increase in the need for the planet earth's restricted supply of freshwater (Arria and Compton, 2017). Thus, preserving our water reservoirs is one of the most important environmental problems of the recent decades (Arria and Compton, 2017).

There are concerns about what level of risk may be associated with the presence of ECs in the environment, as many sources of water are affected by wastewater. The municipality wastewater treatment plant systems are considered as the main entry point for ECs and their residues into the surface water cycles (Bolong et al., 2009). The lack of accepted worldwide analytical methods, non-uniform monitoring data and lack of accurate information about the fate and effects of these compounds and/or their metabolites and transformation by-products in the surface water make risk assessment problematic. It is now known that ECs enter the environment and persist and, can make their way back to humans through the food chain or drinking water, as shown Figure 1.1.

1.2 Sources of Emerging Contaminants

A variety of household, commercial, or industrial chemicals are used by humans for personal health, cosmetic purposes, agricultural purposes and various other reasons as shown in Figure 1.1. The major classes of ECs include: Pharmaceuticals, Personal care products, hormones, flame retardants and nanoparticles, but pharmaceutical and personal care products (PPCPs) are an important class of ECs.

PPCPs group of compounds can include over-the-counter medication as well as medication prescribed by physicians, and illicit drugs. These compounds are used for cure, treatment of diseases and pleasure. Personal care products are used for skin and body treatment, and include chemicals such as soaps, detergents, shampoos, cosmetics, sun-screen products, fragrances, insect repellents and antibacterial compounds.

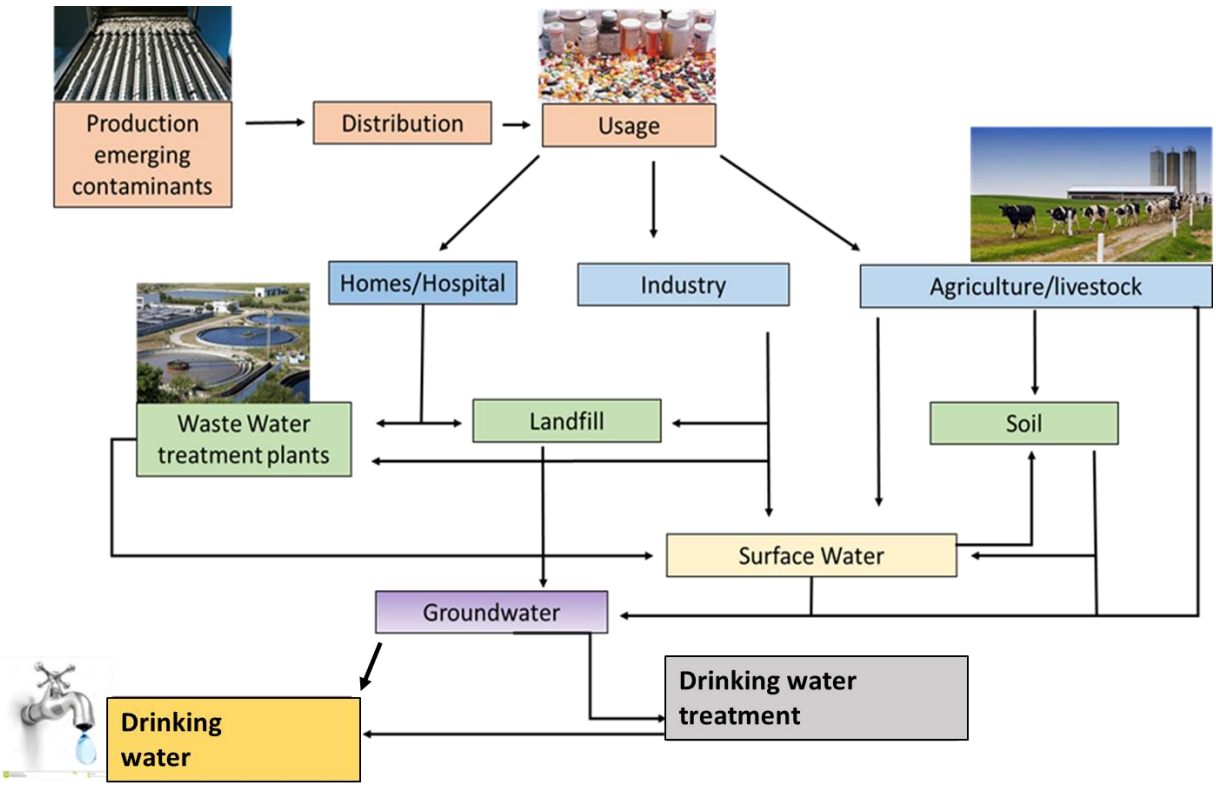

Figure 1.1 Representative sources, distribution and fate of emerging contaminants in the environment (Aali et al, 2014, Petrie et al, 2015).

Hormones are categorised into two groups as naturally occurring and synthetic. Analogues of steroids and hormones are structurally related to one another (Gunatilake et al., 2014). The most common sterol in vertebrates is cholesterol, which is found in cell membranes and serves as a central intermediate in the biosynthesis of many biologically active steroids such as sex hormones (Gunatilake et al., 2014). Hormones are intercellular chemical messengers, which act in low concentrations by binding to stereospecific target cell receptors (Maggioni et al., 2013). Many studies focus is on the synthetic hormones, such as birth control pills, which are often prescribed by medical doctors.

Nanoparticles are utilised in various applications because of their unique structures that give them exceptional electrical, chemical and physical properties (Aitken et al., 2006). For example, polymeric materials containing nanoparticles are increasingly used in consumer products and for construction, aerospace and medicine (Aitken et al., 2006). In addition to classification of nanoparticles as ECs, they are also used in analytical chemistry to aid in the measurements of other ECs.

Previously, environmental agencies have focussed on regulating polybrominated diphenyl ethers (PBDEs), which belong to a group of brominated flame retardants that have been used extensively to lower the flammability of many products sold to general public (Brits et al., 2016). As a result of banning and restricting the use of PBDEs worldwide, the incorporation of new flame retardants is expected to rise due to efforts to abide by fire safety standards (Covaci et al., 2011). All of these chemicals, and various others, are continuously discharged into air, water and soil through municipality and industrial sewage (Giger et al., 2003). Once in the environment, the concentrations, fate and toxicity are largely unknown, and hence can be considered as emerging pollutants or contaminants of concern.

1.3 Fate of Emerging Contaminants in the Environment

Most ECs are believed/suspected to enter the environment through effluent of wastewater treatment plants (WWTPs) which treats sewage waste from household cleaning (bathing, laundry and kitchen washing), toilets flushing, and the disposal of unused pharmaceuticals into sinks, and industrial waste. Other sources of CECs include runoff water from agricultural fields, concentrated animal feeding food and water from landfill leachates and urban runoff (Sarmah et al., 2006). WWTPs treats sewage waste by employing biological and disinfection processes, and in some instances hydroxyl radicals or membrane technology is used as a form of advanced treatment (Marcelino et al., 2016). WWTP systems are designed to remove solid materials and reduce pathogens in the treated wastewater by conventional activated sludge and membrane biological reactor (MBR) mechanisms (Jones-Lepp and Stevens, 2007). However, the efficiency of a WWTP depends on the physicochemical characteristics of the contaminants, the treatment process employed with as well as weather and temperature conditions. Most conventional WWTPs partially remove CECs, making the treatment process vulnerable to polluting the receiving water bodies (K'oreje et al., 2016). In this context, the non-degradable or remaining CECs in the effluent from WWTPs are likely to be detected in dams (Matongo et al., 2015), rivers and drinking water. The fate and distribution of CECs in the environment depends on the n-octanol-water distribution ratio (D_{ow}) which is pH-dependent. The low volatility of many CECs means that their variation through the environment will primarily occur *via* aqueous transport (Chen and Zhou, 2014). The polar, non-volatile properties of most drugs prevents their migration from the aquatic system (Stumpf et al., 1999).

1.4 Public Concern on the Presence of Emerging Contaminants in the Environment

It is now known that ECs may pose hazards to a variety of organisms including humans as indicated by observables effects (Batscher, 2006, Bazin et al., 2010, Celiz et al., 2009). Most CECs are designed with the purpose of causing a biological effect. They often have the same

type of physicochemical behaviour, for example are lipophilic they are able to pass through membranes. Also, they are stable which allows curing effects on the target before substance inactivity (Jacob et al., 2016). Thus, the CECs have many of the necessary properties to bio accumulate, enter the food chain cycle and provoke adverse effects in the aquatic or terrestrial ecosystems (Dietrich and Hitzfeld, 2004, Tomy et al., 2004). Pharmaceutical residues in the environment are suspected of inducing deleterious effects such as resistance in bacterial strains causing a threat to people's health as infectious diseases can no longer be treated with the presently known antibiotics (Aali et al., 2014, Amador et al., 2015, Bergeron et al., 2015). The level to which this problem has drawn interest across organizations is exemplified by the voices of concern originating from medical practitioners, environmental agencies, district municipalities, government bodies, and the public in general (Deblonde et al., 2011, Jin et al., 2014). Knowing the occurrence of ECs in the river, establishing links between biological effects observed, environment and water quality has been the goals of many studies and will continue to be so in the near future in order to study the ecological status of surface waters (Altenburger et al., 2015, Petrie et al., 2015). Moreover, the problem related to ECs in surface waters may be solved using various socioeconomic measures and not only science.

Bodies such as government agencies and industrial chambers may undertake mitigation measures leading to minimization of release of CECs into surface waters. Proper management of pollutant flows, new disposal measures and regulation of effluent from WWTPs may be required. This is important because it is now known that at WWTPs CECs are being transformed into dangerous by-products other than the parent compound (Diniz et al., 2015). The chemicals present in treated wastewater can persist and travel through surface and ground water, which can potentially be a source of water for another community (Haman et al., 2015).

The American Environmental Protection Agency (EPA) initiated studies in the environment to evaluate the occurrence of CECs in surface waters since 2005. Frequent occurrence of CECs in their country's environment and inefficiency of conventional WWTPs to remove such compounds. Also, further recommendations promoted the United States of America (USA) to amend its environmental laws to cover a large set of hazardous compounds, as well as further recommendations of wastewater treatment steps or even new treatment methods. The USA revised its contaminant candidate list (CCL) released in 2008, listing 104 contaminants. In addition, there is also another list for unregulated contaminant monitoring regulation (UCMR) of 30 compounds of emerging concerns issued in 2006.

In Europe, the directive 2000/60/EC was the first mark in the European water policy, which set up a strategy to prioritise high risk substances (Migowska et al., 2012). Four years ago, the European Union directive 2013/39/EU recommended attention be given to the monitoring and treatment options of a group of 45 pollutants, to ensure the protection of aquatic life and health. In that Directive (2013) two classes of ECs were included; pharmaceuticals (diclofenac) and hormones (17-alpha-ethylestradiol-EE2). Diclofenac has been the subject of investigation in many countries including Asian states (Green et al., 2004). Diclofenac has been responsible for the disappearance of vulture populations in India (Green et al., 2004, Swan et al., 2006). As a result, Indian authorities have passed a law that banned the use of diclofenac in their country (Green et al., 2004).

1.5 Problem Statement

Most existing analytical methods are not developed enough to study the behaviour, toxicity and fate of CECs in the environment. Thus, there is a demand to develop and optimize new analytical protocols (extraction and detection techniques) for new studies of CECs in the environment.

1.6 Research Motivation

In South Africa, the drought has prompted the country to speed up the indirect re-use of wastewater, but there are no guidelines for the presence of emerging contaminant compounds in wastewater. The South African Department of Water Affairs and Forestry is the steward of South Africa's water resources and issues water quality guidelines. The South Africa's water quality guidelines is a primary source of information used in determining the water quality requirements for different water uses and for the health of aquatic ecosystems, South Africa. The South African government acknowledge that water quality guidelines of 1996 need to be changed and will be updated and modified on regular basis, as per recommendations of the ongoing researches, and views of local and international scientists in ecosystems. The existing

South African water quality guidelines do not include CECs nor have they been revised since 1996. For these reasons, this project was undertaken through funding from the Water Research Commission (WRC) of South Africa to inform Government structure about occurrence of emerging contaminants in South African waters and sediments.

When deciding to regulate, the international law stipulated by the World Health Organization (WHO) requires that three areas are considered; projected adverse effects from the contaminants, the extent of occurrence of the contaminant in drinking water, and whether regulation of the contaminant would present a meaningful opportunity for reducing risks to health. Thus, this study provides important information regarding the current status of ECs in the province of KwaZulu-Natal.

1.7 Organization of Dissertation

The presented Ph.D. dissertation is organized in chapters, each one dealing with a different topic and including its own introduction, experimental, results and discussion, and references.

In chapter 1, "General Introduction'' a broad overview is presented concerning emerging contaminants in the environment and analytical protocol for their determination. Focus is directed to the sources, pathways and fate of chemicals of emerging contaminants in the environment. Moreover, the public point of view towards emerging contaminants is considered: American Environmental Protection Agency's list of pollutants, European Directive on emerging contaminants and South African policies on wastewater issues are visited. The motivation for undertaking this work is outlined.

In Chapter 2, "Literature Review" this literature review focuses more on the main classes of emerging contaminants, namely pharmaceuticals, personal care products, hormones and flame retardants. Details are provided on selected types, their examples, occurrence, toxicity and suitable analytical methods for specific types of pollutants. In addition, the review highlights new trends, and discuses only a few and quality research publications. Special emphasis is given to detection, fate and environmental concentration levels.

In Chapter 3, a paper on "Detection and quantification of acidic drug residues in surface water of South Africa by gas chromatography-mass spectrometry'' is presented. In this Chapter a development and application of the method for simultaneous detection and quantification of acidic pharmaceutical drug residues (salicylic acid, acetylsalicylic acid, nalidixic acid, ibuprofen, phenacetin, naproxen, ketoprofen, meclofenamic acid and diclofenac) in surface water using chromatography-mass spectrometry, associated with solid phase micro-extraction for the sample preparation is presented. The developed method is applied in the analysis of acidic drugs in the Mgeni River system, KwaZulu-Natal South Africa. Quantification results of the sampling and a conclusion are described. Five of nine analysed acidic drugs are found to range between 0.02 and 8.14 ng mL^{-1}.

In Chapter 4, "Assessment of nonsteroidal anti-inflammatory drugs by ultrasonic-assisted extraction and GC-MS in Mgeni and Msunduzi River sediments, KwaZulu-Natal, South Africa" the occurrence of eight nonsteroidal anti-inflammatory (NSAIDs) drugs are monitored during four seasons (spring, summer, autumn and winter) along a 250 km stretch of the Msunduzi and Mgeni Rivers in KwaZulu-Natal, South Africa.

Chapter 4, describes an optimized method for the determination of drugs in sediments. The method combines ultrasonic, centrifugation and gas chromatography mass spectrometry for detection of these drugs in solid samples. Most of the parameters that affect the extraction step are optimized. Satisfactory recoveries are obtained ranging from 66% to 130% depending on the analyte. Precision expressed as RSD (%) (n = 3) are less than 20% for all analytes. The LODs and LOQs are in the range of 0.024 to 1.90 ng g^{-1}, which allowed the developed method to be applied in the determination of selected drugs in sediment samples.

In Chapter 5, "Determination of personal care products and pharmaceuticals in river sediments, KwaZulu-Natal, South Africa" in this chapter a quantitative method is described for ultrasonic-assisted solid-phase extraction (SPE) followed by GC-MS after derivatization for the simultaneous analysis of personal care products and pharmaceuticals (PCPPs); propyl paraben, triclosan, caffeine, carbamazepine and chloramphenicol.

Ultrasonic-assisted extraction combined with centrifuging is used to extracts sediment samples collected from Mgeni and Msunduzi Rivers. An SPE procedure is used for clean-up and to concentrate selected compounds from diluted aqueous extracts. The recoveries of the analytes

ranged from 66% to 108%. The method detection limits were 0.08 – 1.82 ng g^{-1} and quantification limits 0.42 – 5.51 ng g^{-1}. The proposed method was applied in the evaluation of two rivers over a three month period in KwaZulu-Natal. To our knowledge, this is a first report on the simultaneous determination of these PCPPs by GC-MS in Africa.

In Chapter 6, "Target, suspects and non-target analysis of emerging pollutants – a new spectral library for derivatives" There has been an interest recently to determine the unidentified peaks within a sample spectrum with intensities higher than the analytes of interest. Availability of reference standards and hyphenated instruments has been a key and limitation factor on studying the occurrence of emerging contaminants in the environment. In this chapter, we developed the library to be used in GC-MS (single ion monitoring mode (SIM)) for detection of emerging pollutants in the environment. Suspect and non-target analysis were also performed to identify more novel contaminants in the Mgeni and Msunduzi Rivers, which were previously not reported in South Africa.

In chapter 7. "Final conclusions and remarks: work novelty, knowledge gaps and future research", the key achievements of the work are presented, as well as the main knowledge and missing evidences which may be filled in and enlightened during some future experiments' proposals are discussed.

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Chapter 2: Literature Review

2.1 Abstracts

This review describes emerging contaminants (ECs) and their metabolites. Furthermore, it covers their occurrence in the environment, fate, distribution, toxicity, and their analytical method developments in environmental matrices. In addition, the review highlights new trends, with a focus on quality research publications. This review highlights emerging contaminant groups as they pertain to those within the thesis, and discuss several representative papers that originate from Africa. Also, studies of analytes similar to those use in this study and those that use analysed using gas chromatography-mass spectrometry (GC-MS) are discussed.

2.2 Classification of Emerging Contaminants

The extensive development of resources and technology has produced more chemicals and materials, which are released into the environment. This consequently increases the number of contaminants that are identified as possessing potential threats to both humans and various other organisms (Deblonde et al., 2011). This review focuses more on the main classes of emerging contaminants namely pharmaceuticals, personal care products, hormones and flame retardants. Details on selected types, examples, occurrence, toxicity, and analytical methods of these compounds in the environment as shown in Figure 2.1.

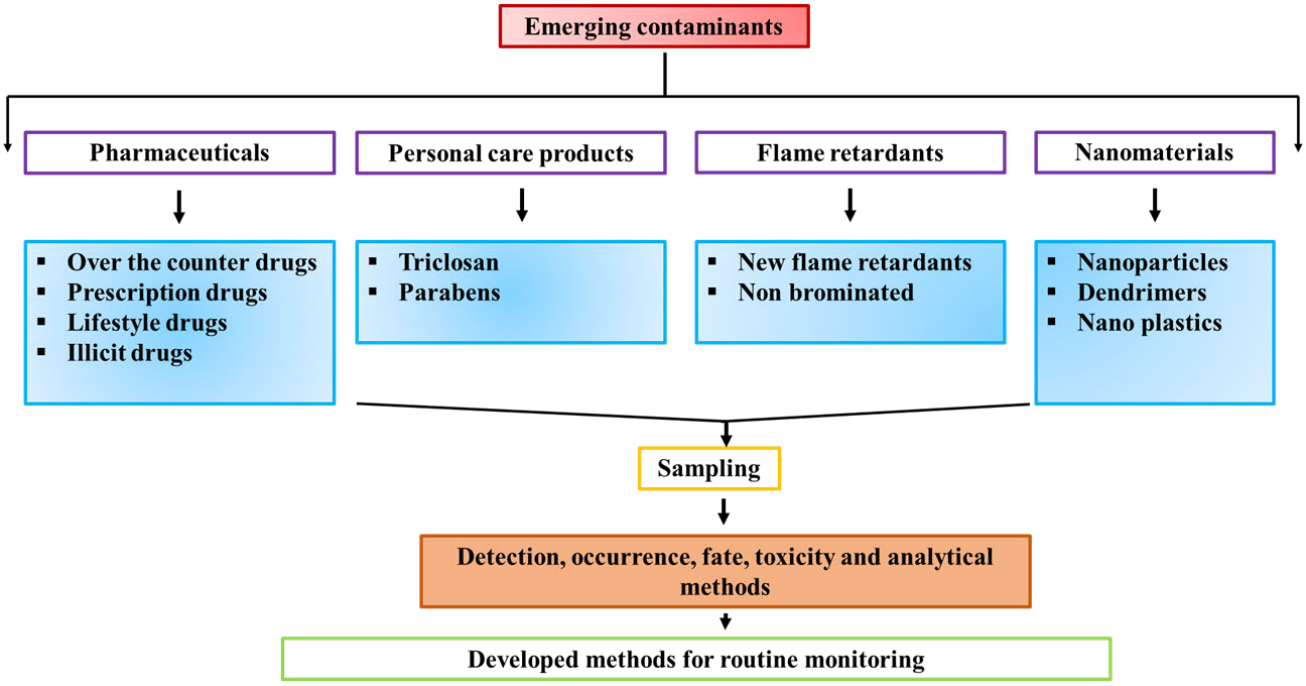


Figure 2.1: Classification of emerging contaminants and and flowdiagram for emerging contaminants analysis.

2.2.1 Pharmaceuticals

Pharmaceuticals are synthetic or natural compounds that can be found in medicine and therapeutic drugs (Bottoni et al., 2010). Pharmaceuticals comprise of more than 3000 active compounds with quite different structures, functional groups and, physical-chemical properties, which all have been accepted for human consumption (Ankley et al., 2007). Pharmaceutical active compounds (PhACs) are produced to have pharmacological effects on target organisms and bestow a significant welfare to society, but they may pose threat on non-target organisms in the environment (Cleuvers, 2003). Many investigations and research studies have corroborated the existence of pharmaceuticals in municipality wastewater influent and effluent, and these pathways have been established as the major source of PhACs in the environment (Stefanakis and Becker, 2015). Paiga and Delerue-Matos, (2016) reported the occurrence of 33 pharmaceuticals and their metabolites along Lis River in Portugal. The increase in detection is largely attributed to the advances in analytical protocols and instrumentation. Most studied or detected pharmaceutical classes in the environment are over the counter drugs, prescription drugs, lifestyle drugs and illicit drugs as depicted in Figure 2.1.

2.2.1.1 Over the Counter Drugs

Over the counter (OTC) drugs are defined as those drugs that are available to the public without prescription such as ibuprofen, diclofenac, acetylsalicylic acid, naproxen, ketoprofen and acetaminophen (Kennedy, 1996). Their structural examples are shown in Figure 2.2.

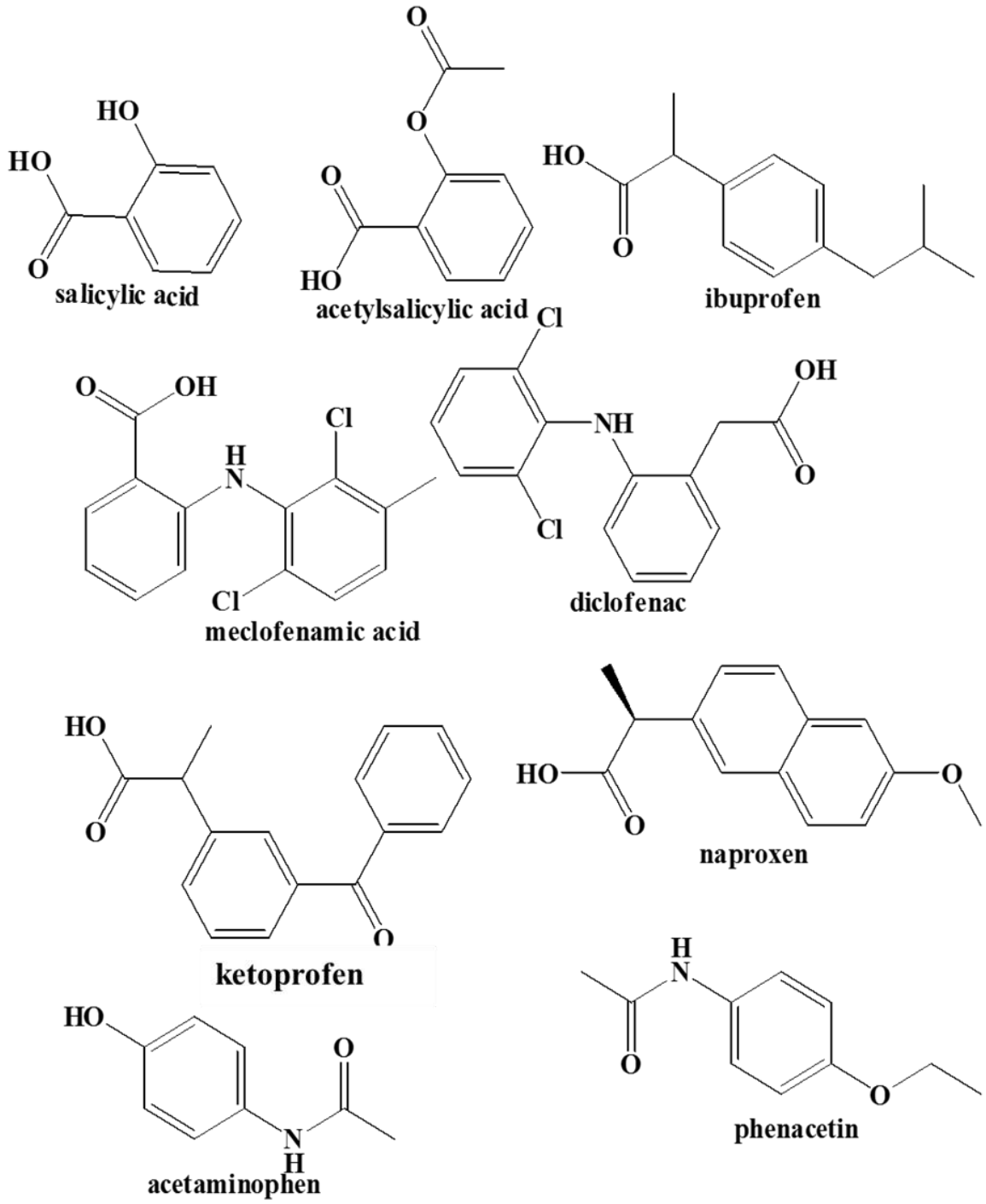


Figure 2.2: Structural examples of over the counter drugs.

Policies on OTC drugs vary globally, and many governments prefer to transfer a share of the expanding health cost burden to consumers by promoting policies that allow greater direct access to self-medication (Beausse, 2004). In the European Union (EU), OTC drugs are available through pharmacies, but in the USA and South Africa, all OTC drugs can be sold in general retail outlets (Gracia-Vasquez et al., 2015, Kennedy, 1996). Most OTC drugs are non-steroidal anti-inflammatory drugs (NSAIDs) which are employed in human and veterinary medicine. They are used due to their analgesic, antipyretic, anti-inflammatory activities due to inhibition of cyclooxygenases such as COX-1 and COX-2 in the prostaglandin formation pathway, and are one of the most commonly used pharmaceutical classes (Vane and Botting, 1998). NSAIDs are acidic with *pKa* generally ranging between 3.0 and 6.0 (Lee et al., 2014). These acidic drugs are not extensively degraded and are often stable in water after excretion or disposal by humans or animals (Carmona et al., 2014). A portion of the free excreted active drugs and metabolites can escape elimination in the sewage treatment process and enter the environment. Due to their polar structures, these acidic compounds are not adsorbed in the subsoil and may percolate into the groundwater aquifers from the polluted surface water and soil or sediments (Fairbairn et al., 2015). Despite their potential threat to the public, there is limited information available on their presence in African water bodies and WWTPs.

2.2.1.1.1 Detection, Occurrence, Fate and Environmental Concentration of OTC drugs

Almost two decades ago, there was evidence that significant quantities of OTC drugs have the potential to be discharged into the environment. Quantities of OTC drugs found in the environment probably exceeds other classes of pharmaceuticals (Ternes, 1998). In South Africa, the occurrence of OTC drugs specifically in KwaZulu-Natal has been shown by (Agunbiade and Moodley, 2016, 2014). They studied the occurrence and distribution pattern of OTC drugs in water, wastewater and sediment, along the Msunduzi River (South Africa), using liquid chromatography-mass spectrometry (LC-MS). They found that acetylsalicylic acid in wastewater was 118 µg L^{-1}, which was much higher than other drugs in their study (Agunbiade and Moodley, 2016). They also concluded that WWTP did not remove pharmaceuticals after detecting OTC drugs in the effluent. Thus, these compounds are likely to partition to sediments in the Msunduzi River, South Africa (Agunbiade and Moodley, 2016). Matongo et al., (2015) also studied the occurrence of OTC drugs in the Msunduzi River,

Table 2.1: Literature evaluation on sampling, extraction, detection and environmental concentration of over the counter drugs globally.

Selected drugs	Sampling method	Extraction method	Detection method	Environmental concentration	Country	Reference
Naproxen, acetylsalicylic acid	Grab	SPE - Strata	Derivatization GC-MS	ND - 27,0 µg/L	Brazil	(Ide et al., 2017)
Diclofenac, ketoprofen,ibuprofen	Grab	SPE - HLB cartridges	Derivatization GC-MS	155,5 - 6552 ng L^{-1}	Algeria	(Kermia et al., 2016)
Naproxen, ibuprofen	Grab	SPE imprinted polymer	HPLC	ND – 221 µg L^{-1}	South Africa	(Madikizela and Chimuka, 2016)
Ibuprofen, naproxen and diclofenac	Grab	SPE - Strata C18	Derivatization GC-MS	ND - 489 ng g^{-1}	Poland	(Kumirska et al., 2015)
Diclofenac, ketoprofen, ibuprofen,	Grab	SPE - HLB cartridges	Derivatization GC-MS	ND - 12,8 µg L^{-1}	Poland	(Nosek et al., 2014)
Diclofenac, naproxen	Grab	Assisted Ultrasound	LC-MS	ND - 200 ng g^{-1}	Spain	(Garcia-Rodríguez et al., 2014)
Diclofenac, ibuprofen, naproxen	Grab	SPE	Derivatization GC-MS	ND - 177 ng L^{-1}	Canada	(Uslu et al., 2013)
Ibuprofen and naproxen	Grab	SPE - Oasis HLB	GC-MS	ND - 60000 ng L^{-1}	Greece	(Stamatis et al., 2013)
Acetaminophen, diclofenac, naproxen,	Grab	SPE- Strata X	Derivatization GC-MS	ND - 1,4 µg L^{-1}	Poland	(Migowska et al., 2012)
Salicylic acid	Grab	Assisted sonication	Derivatization GC-MS	ND - 20 ng g^{-1}	Spain	(Sánchez-Brunete et al., 2011)
Ibuprofen, paracetamol, ketoprofen, diclofenac	Grab	SPE - WXC	Derivatization GC-MS	ND - 983,3 ng L^{-1}	Italy	(Giandomenico et al., 2011)
Ibuprofen, naproxen and diclofenac	Grab	SPE - Oasis HLB	GC-Ms	ND - 77 µg L^{-1}	USA	(Yu and Wu, 2011)
Ibuprofen, naproxen, meclofenamic acid, diclofenac	Grab	SPE - C18	Derivatization GC-MS	ND - 704 ng L^{-1}	Greece	(Samaras et al., 2010)
Ibuprofen, ketoprofen	Grab	Microwave-assisted	Derivatization GC-MS	1 ng g^{-1} - 1000 ng g^{-1}	USA	(Rice and Mitra, 2007)
Ibuprofen, naproxen, ketoprofen, naproxen	Grab	SPE - Oasis HLB and strata X	Derivatization GC-MS	Qualitative	Canada	(Lajeunesse and Gagnon, 2007)
Ibuprofen, diclofenac, naproxen,	Grab	SPE - HLB cartridges	GC	ND - 68 700 ng L^{-1}	USA	(Trenholm et al., 2006)
Ibuprofen, ketoprofen, diclofenac	Grab	SPE - ENV-18 and Oasis HLB	Derivatization GC-MS	30 - 420 ng L^{-1}	Taiwan	(Lin et al., 2005)
Diclofenac, ibuprofen	Grab	SPE - Oasis HLB	Derivatization GC-MS	ND - 101 ng L^{-1}	Germany	(Weigel et al., 2004)
Ibuprofen, diclofenac and clofibrates	Grab	SPE - C18	Derivatization GC-MS	ND - 100 ng L^{-1}	Greece	(Koutsouba et al., 2003)

South Africa and found the concentration of ibuprofen to be the highest at 117 µg L^{-1} and 659 ng g^{-1} in water and sediments, respectively.

Paiga and Delerue-Matos, (2016) reported the occurrence of OTC drugs in Lis River and two WWTPs located along the River in Portugal. The highest concentration of OTC drugs was found near the river mouth and downstream of WWTPs. They identified the possible sources of pollution of Lis River as WWTPs, untreated effluent wastewater and livestock production. NSAIDs were the therapeutic class with high contribution to the total mass load of pharmaceuticals entering the Lis River, followed by psychiatric drugs and antibiotics with concentrations up to 1.3 µg L^{-1} analysed with LC-MS. Although 27 pharmaceuticals were detected in the Lis River, they concluded that it was impossible to determine seasonal variation of these pharmaceuticals (Paiga and Delerue-Matos, 2016). Tauxe-Wuersch et al., (2005) studied the occurrence and fate of five acidic drugs (ketoprofen, diclofenac, mefenamic acid, ibuprofen and clofibric acid) in WWTPs. Their results showed that these drugs were persistent in wastewater effluent after treatment at WWTPs. They concluded that the concentration of ibuprofen, diclofenac and mefenamic acid were relatively high in the effluent (0.15 – 2 µg L^{-1}). Many other researchers also reported the occurrence of OTC drugs in the environment as shown in Table 2.1.

2.2.1.1.2 Toxicity and Risk Assessment of OTC drugs

NSAIDs mostly inhibit the cyclooxygenases, the key enzymes catalysing the biosynthesis of prostaglandins, which are inter alia responsible for inflammations (Vane and Botting, 1998), but these compounds act unspecific by non-polar narcosis (Diniz et al., 2015). Thus, toxicity may be more associated with the log k_{ow} of the drugs rather than with any specific toxic action (Jjemba, 2006). Pharmaceuticals in the environmental waters occur as mixture of compounds, an precise prediction of the mixtures toxicity is essential for environmental risk evaluation. Cleuvers, (2003) evaluated ecotoxicity of the NSAIDs diclofenac, ibuprofen, naproxen and acetylsalicylic acid using acute daphnia and algal tests. Key findings included that the toxicity was relatively low with half-maximal effective concentration (EC_{50}) values obtained using *Daphnia* in the range 68 to 166 mg L^{-1} and 72 to 626 mg L^{-1} in the algal test. Diclofenac was found to be the most toxic and naproxen the least toxic compound. Diclofenac has been reported to be toxic to vultures and contributed to their population decrease on the Asian continent (Naidoo et al., 2010, Swan et al., 2006, Green et al., 2004).

Ginebreda et al., (2010) reported on the environmental risk assessment of pharmaceuticals in Llobregat River, Spain. Their study was carried out through the determination of hazard quotient indices, calculated from measured values using three different bioassays, namely, fish, Daphnia and algae. Ibuprofen and diclofenac had an impact in all three bioassays as compared to other classes of pharmaceuticals studied (Ginebreda et al., 2010). Many researchers have also reported projected toxicity and risk assessment of acidic drugs in the environment (Celiz et al., 2009, Diniz et al., 2015).

2.2.1.1.3 Analytical Methods for Analysis of OTC Drugs

Most OTC drugs are polar, and when present in the environment, they normally exist at part per billion or part per trillion concentration levels. There are many factors in the environment that present analytical challenges such as matrix effects. Sensitive and selective analytical procedures for OTC drugs are important to determine the environmental occurrence and quantity of these compounds in sediment and water. To date, many analytical methods for the determination of OTC drugs have been described (Antonic and Heath, 2007, Dasenaki et al., 2016). Madikizela and Chimuka, (2016) developed a method for the analysis of acidic drugs naproxen, ibuprofen and diclofenac in wastewater and river water from South Africa. In their method, they synthesised a multi-template molecular imprinted polymer (MIP) as a sorbent in the solid phase extraction (SPE) of these compounds. Upon extraction, they detected acidic drugs in water samples with high-performance liquid chromatography (HPLC). Ibuprofen was the most frequently detected compound at concentrations up to 221 µg L^{-1} and 11.0 µg L^{-1} in wastewater and river water, respectively (Madikizela and Chimuka, 2016). Shin and Oh, (2012) reported the development of a GC-MS assay method for the determination of 13 NSAIDs in rivers. The method was used to analyse 10 river water samples from various regions in Korea. Diclofenac, indoprofen and loxoprofen were detected at concentration levels up to 1.29 µg L^{-1} in river water samples. They found their method to be selective and sensitive with no interferences from other coexisting substances in water after extraction and derivatization. Antonic and Heath, (2007) optimised an extraction method for the determination NSAIDs in river sediment in Slovenia and Central Europe with GC-MS. Their optimised analytical method consisted of microwave-assisted extraction, a clean-up step of the extract with SPE, derivatization, and determination with GC-MS. Research output regarding NSAIDs in the environment not only show that they are toxic to many animal species, but also highlights the

need for robust analytical methods for monitoring the concentration level of OTC drugs in the environmental matrices of greater complexity (Buchberger, 2007, Jindal et al., 2015).

2.2.1.2 Prescription Drugs

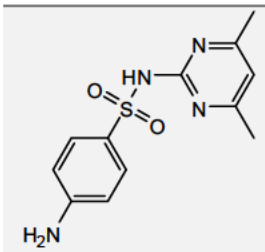
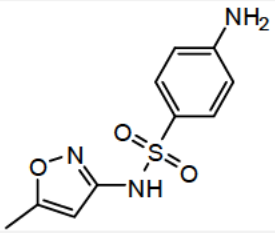
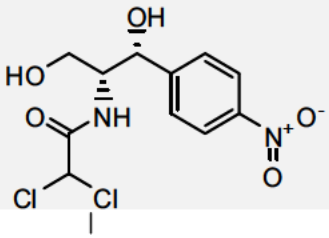
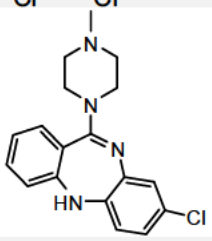
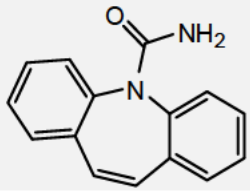
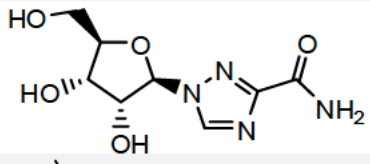
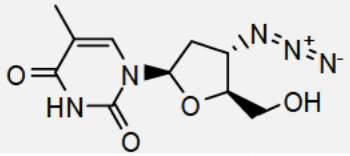
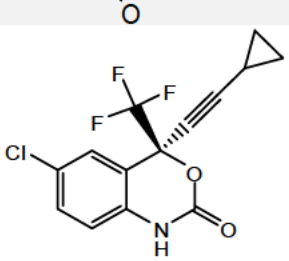
Prescription drugs refer to those drugs that can be dispensed to the public only with an order given by an authorized person. The most prescribed prescription drugs in South Africa are antibiotics and antiretrovirals (Arria and Compton, 2017, Ilyas and Moncrieff, 2012, Morgan et al., 2011). This section will cover antibiotic, psychiatric and antiretroviral drugs, since they are some of the most prescribed prescription drugs as listed by South African health department (Laxminarayan et al., 2013). Some of these drugs are shown in Table 2.2. Most of these drugs are used in combination, in order to reduce side effects and to prevent drug resistance development (Amador et al., 2015).

Prescription drugs are bioactive substances and effective inactivation of microorganisms, viruses, and eukaryotic cells (Lancini and Parenti, 2013). Today, a large percentage of naturally occurring and synthetic prescription drugs are used in the therapy of infectious diseases in humans as well as veterinary medicine (Barbosa et al., 2016). Prescription drug consist of a non-polar and polar functional groups. They are responsive to strong acid and base, and dissociate or protonate with respect to pH of the medium.

The class of antibiotic compound primarily administered in veterinary medicine are tetracyclines, sulphonamides, aminoglycosides, β-lactams and macrolides. In human medicine β-lactams, tetracyclines and macrolides are mostly prescribed (Adamek et al., 2016). There are more than 20 anti-HIV active drugs licensed for human consumption worldwide and the licensed anti-HIV drugs are classified into five categories: nucleoside reverse transcriptase inhibitors, protease inhibitors, non-nucleoside reverse transcriptase and fusion inhibitors.

The most prescribed psychiatric classes of drugs used in England are antipsychotics (thioridazipine, olanzapine, quetiapine and risperidone), antidepressants (fluoxetine, venlafaxine, amitriptyline and paroxetine), mood stabilizers (sodium valproate and lithium), hypnotics (diazepam), stimulants (methylphenidate) and anti-dementia drugs (Ilyas and Moncrieff, 2012) but clozapine and carbamazepine are the most used drugs in South Africa

Table 2.2: Structural examples of prescription drugs

STRUCTURES	NAME	DRUG TYPE	PKA
	Sulfamethazine	Antibiotic	7.5
	Sulfamethoxazole	Antibiotic	5.7
	Chloramphenicol	Antibiotic	5.5
	Clozapine	Psychiatric	7.4
	Carbamazepine	Psychiatric	13.9
	Ribavirin	Antiretroviral	11.9
	Zidovudine	Antiretroviral	10.0
	Efavirenz	Antiretroviral	12.5

(Matongo et al., 2015). Pharmacokinetics of these drugs are optimised to prevent their accumulation in the body (An et al., 2011). After administration, they are mainly excreted as bioactive parent compounds and metabolites.

2.2.1.2.1 Detection, Occurrence, Fate and Environmental Concentration of Prescription Drugs

Detection of prescription drugs in the environment depends on the drugs pharmacokinetic behaviour (half-life, metabolism, urinary and faecal excretion). Previous work on prescription drugs in the environment is shown in Table 2.3. A national investigation of pharmaceutical compounds in United States of America (USA) rivers revealed that 27% of 139 samples analysed contain veterinary and human antibiotics at concentration levels up to 0.7 µg L^{-l} (Kolpin et al., 2002). The adsorption and fixation of prescription drugs are strongly controlled by the compounds ability to ionize, depending on the medium pH. The degradation of pharmaceuticals in the environment is mostly *via* microbial processes and some prescription drugs are prone to enzymatic transformation reactions like oxidative decarboxylation and hydroxylation (Cheng et al., 2015, Aali et al., 2014)). Cheng et al., (2015) reported on the determination of 32 antibiotics in Jiulong River, China. The concentration of antibiotics in the sediments samples was found to range from not detected to 17.5 ng g^{-1}, the samples were analysed with LC-MS/MS. Peng et al., (2009) investigated occurrence, behaviour and fate of several antibiotics in Pearl River, South China. They found that in the urban part of the Pearl River sulfonamides and macrolides were detected at a concentration between 22 and 725 ng L^{-1}, fluoroquinolones were only occasionally detected at 2 – 152 ng g^{-1} and they suggested that the latter tends to partition to sediment and/or photodegrade. They concluded that rain plays a key role in the seasonal distribution of prescription drugs in the Pearl River.

Table 2.3: Literature evaluation on sampling, extraction, detection and environmental concentration of prescription drugs globally.

Analytes	Sampling method	Extraction method	Detection method	Environmental concentration	Country	Reference
Carbamazepine and fluoxetine	Grab	SPE - Strata X	LC-MS	ND - 1.3 µg L^{-1}	Germany	(Paiga and Delerue-Matos, 2016)
Carbamazepine and fluoxetine	Grab	SPE - Strata X	LC-MS	ND 20.0 - 22.3 ng L^{-1}	Portugal	(Paiga et al., 2016)
Sulfamethoxazole, ciprofloxacin, trimethoprim, zidovudine	Grab	SPE - Oasis HLB	LC-MS/MS	ND - 5430 ng L^{-1}	Kenya	(Ngumba et al., 2016)
Lamivudine, stavudine, nevirapine, tenofovir	Grab	SPE - Oasis HLB	LC-MS	26.5 - 430 ng L^{-1}	South Africa	(Wood et al., 2015)
Carbamazepine, clofibric acid	Grab	SPE - HLB cartridge	Derivatization GC-MS	ND - 12,8 ng L^{-1}	Poland	(Nosek et al., 2014)
Sulfamethoxazole, sulfamethazine	Grab	Assisted Ultrasound	LC-MS	ND - 200 ng g^{-1}	Spain	(Garcia-Rodríguez et al., 2014)
Carbamazepine and trimethoprim	Grab	SPE - Oasis HLB	HPLC	ND - 0.21 µg L^{-1}	Switzerland	(Singh et al., 2014)
Sulfonimides, tetracyclines, chloramphenicol	Grab	SPE - Oasis HLB	UHPLC-MS/MS	ND - 859 ng L^{-1}	China	(Chen and Zhou, 2014)
Sulfamethoxazole, Sulfamethazine, tetracycline	Grab	SPE	Derivatization GC-MS	ND - 610 ng L^{-1}	Canada and USA	(Uslu et al., 2013)
Carbamazepine	Grab	SPE - Oasis HLB	GC-MS	ND - 250 ng L^{-1}	Greece	(Stamatis et al., 2013)
Carbamazepine	Grab	SPE - Oasis HLB	GC-MS	ND - 77 µg L^{-1}	USA	(Yu and Wu, 2011)
Carbamazepine	Grab	SPE - Strata X	GC x GC/TOF - MS	Qualitative	Spain	(Samaras et al., 2010)
HIV drugs (limovudine, stavudine, zidovudine and nevirapine)	Grab	SPE - Envi+	LC-MS	370 - 1800 g d^{-1}	Germany	(Prasse et al., 2010)
Carbamazepine, sulfamethaxazole	Grab	SPE - HLB cartridges	GC	ND - 274 ng L^{-1}	USA	(Trenholm et al., 2006)
Carbamazeoine	Grab	SPE - Envi-18 and Oasis HLB	Derivatization GC-MS	30 - 420 ng L^{-1}	Taiwan	(Lin et al., 2005)
Carbamazepne	Grab	SPE - Oasis HLB	Derivatization GC-MS	ND - 101 ng L^{-1}	Germany	(Weigel et al., 2004)

Paiga and Delerue-Matos, (2016) reported on the analysis of psychiatric drugs in groundwater passing in Portugal and their concentration ranged from not detected to 22 ng L^{-1} in water samples. Camacho-Munoz et al., (2013) also determined the distribution of psychiatric drugs in river sediments collected from Donana Park, Spain. Prasse et al., (2010) analysed antiviral drugs in wastewater and surface water in Germany and their concentration ranged from not detected to 170 ng L^{-1}. Ngumba et al., (2016) studied the occurrence of antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. All the selected drugs (sulfamethoxazole, trimethoprim, ciprofloxacin, lamivudine, nevirapine and zidovudine) were detected in the Nairobi River and they found the maximum concentrations in the river of the selected drugs to be 13800 ng L^{-1}. Wood et al., (2015) reported the occurrence of antiretroviral (ARV) drugs used for HIV treatment in South Africa water and their average concentration in the country was found to range from not detected to 430 ng L^{-1}. This was the first reported study of HIV drugs in the South African environmental and further research describing their biotransformation and toxicity in the environment was recommended by the researchers.

2.2.1.2.2 Toxicity and Risk Assessment of Prescription Drugs

Once in the environment prescription drugs and their residues in both water and sedimentary phases may cause potential risk to the food chain, including curbing the capability of soil to decompose, the hazardous effect to aquatic organisms, and promoting the development of bacterial resistant genes (Gao et al., 2012, Bouki et al., 2013). Researchers have reported that wastewater/treated water, contains higher proportions of various resistant bacterial populations in relation to the respective proportions contained in surface water. Aali et al., (2014) reported the fate of antibiotic resistance genes and removal from wastewater. Dirany et al., (2011) studied the toxicity of sulfamethoxazole, which is a widely used antibiotic. This drug together with its metabolites was found to be toxic. Bottoni et al., (2010) reported that zidovudine, an antiretroviral drug, has carcinogenic potential and has been classified in Group 2B which constitutes possible human carcinogens. Reynolds, (2011) reported that most WWTPs will experience inhibition of microbial growth in plant operation and will lead to WWTPs unable to biologically treat wastewater. Many of these prescription drugs are toxic and relatively persistent in water bodies.

2.2.1.2.3 Analytical Methods for Prescription Drugs

Prescription drugs are mostly analysed by liquid chromatography attached to a diode-array detector (DAD), fluorescence detector and/or mass spectrometry due to their polar nature. It has been reported that an interaction between antibiotics and sediment component cause their extraction from these environmental solids problematic (Christian et al., 2003, Kay et al., 2004, Lalumera et al., 2004). A lots of extraction methods have been tried to date, including mechanical shaking, pressurised liquid extraction (PLE) and ultrasonic extraction with various solvents (Blackwell et al., 2004, Christian et al., 2003). Cheng et al., (2015) reported method development for the quantitative analysis of antibiotics in aquifer sediments. They used microwave-assisted solvent extraction (MASE) and SPE for sample pre-concentration and purification. Ultra-high performance liquid chromatography coupled to hybrid quadrupole-high resolution Orbitrap mass spectrometry (UHPLC-Q-Orbitrap) was used for detection and quantification. Drljača et al., (2016) compared four extraction methods for the determination of veterinary drugs in sediment and concluded that SPE was a better extraction method for emerging contaminants. Wei et al., (2014) developed a method for the simultaneous quantification of different classes of antibiotics in water, sediments and fish muscles, and samples were cleaned with SPE cartridges and analysed with LC-MS. They applied their developed methods in Dianachi Lake, China and found concentrations in water as not detected – 0.713 μg L^{-1}, and 344.8 μg L^{-1} in sediment. They concluded that the number of detected antibiotics and overall antibiotic concentrations were higher in an urban area than the rural area. Ngumba et al., (2016) reported a multiresidue analytical method for trace level determination of antiretroviral drugs in wastewater and surface water SPE-LC-MS/MS. Homem et al., (2014) developed a modified acetonitrile-based extraction procedure UHPLC-MS for the analysis of psychiatric drugs in sediments. Their developed method was applied to sediments of two Portuguese rivers (Douro and Lima Rivers) and nine out of eleven psychiatric drugs were detected in sediments at concentrations up to 26.4 ng g^{-1} dry weight. Since prescription drugs are found in trace levels in the environment, powerful hyphenated techniques are necessary for their reliable identification and quantification.

2.2.1.3 Lifestyle Drugs

The term ‘lifestyle drug’ is not easy to define absolutely. In the current review, it will be defined as those drugs that have non-medical or non-health-related goals or modify or change

conditions at the margins of health and welfare (Gupta et al., 2010). These drugs can be used fashionably to alter physical and mental capabilities of the person using it. The lifestyle drugs commercial market today is worth 435 billion Rand from its starting value. Alcohol is one of the lifestyle drugs that have been used by humankind for ages. Sildenafil citrate (Viagra) represents a novel class of emerging lifestyle drugs, a historical change in the era of modern lifestyle drugs that have change the lifestyle of many people (Atkinson, 2002, Rahman et al., 2010). Caffeine, nicotine and alcohol are the most used lifestyle drugs worldwide due to their stimulant activities (Weintraub, 2007). Structures of these are shown in Figure 2.3. In addition, drugs that are used to treat lifestyle illness or diseases arising from lifestyle of the an individual such as alcoholism, smoking, addiction and unbalance diet are regarded as lifestyle drugs (Flower, 2004, Gilbert et al., 2000, Møldrup, 2004). The use of tobacco, alcohol and other stimulant drugs is the predominant cause of the burden of disease in developed countries, including mortality, and has wide-ranging effects on personal safety, mental health, and social well-being.

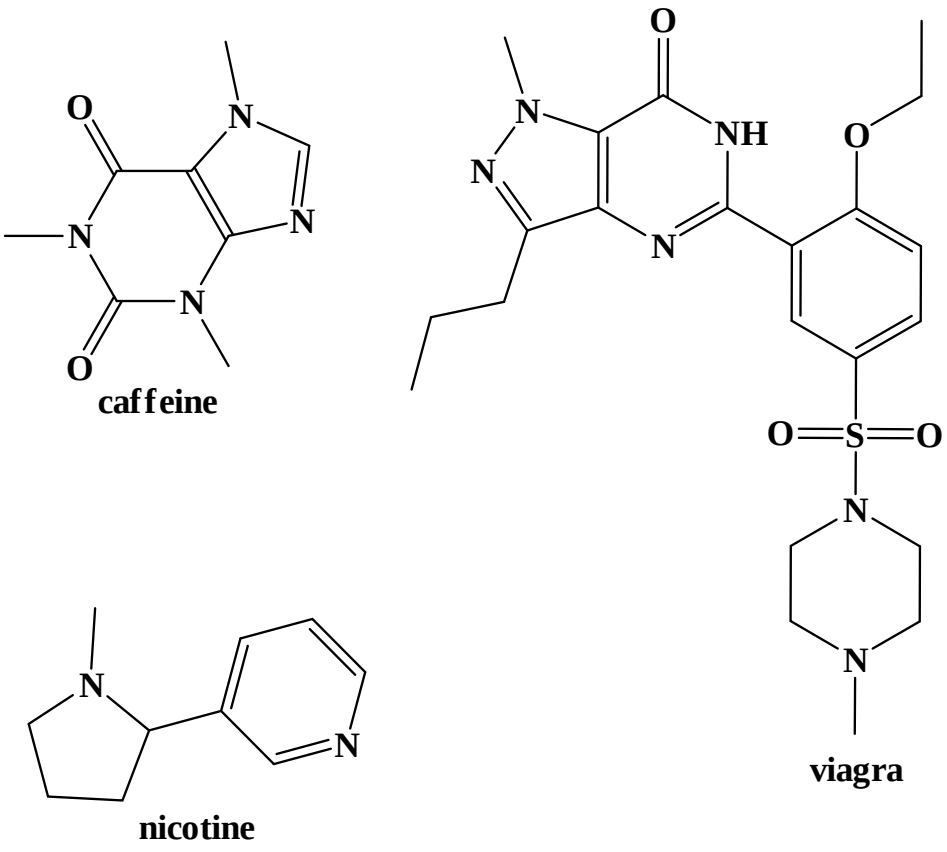


Figure 2. 3: Structural examples of lifestyle drugs

2.2.1.3.1 Detection, occurrence, fate and environmental concentration of lifestyle drugs

Garrido et al., (2009) studied the seasonal occurrence, temporal-spatial distribution and potential sources of caffeine and other pharmaceutically active compounds in Guadiamar River, Spain. They reported that non-prescription drugs were more frequently detected. The highest median concentration of caffeine was found to be 558 ng L^{-1} and showed highly significant seasonal variation. Pharmaceuticals in the environment depend on the daily load added by WWTP effluents, which makes it difficult to deduce a meaningful seasonal variation data from their studies. Wanda et al., (2017) reported the occurrence of caffeine in water systems in Gauteng, Mpumalanga, and North West provinces of South Africa. They found the mean concentration levels of caffeine ranging from not detected to 82.41 ng L^{-1}, the highest concentration determined was at Mkomazane River. They also observed that concentration results varied spatially and no significant temporal variation in the occurrence of the analyte was observed in water. The concentration of pharmaceuticals in the environment was not evenly distributed along the rivers, but was more concentrated near the WWTP effluents.

Seckar et al., (2008) studied the environmental fate of lifestyle drugs, by using octanol-water partition coefficient they showed that these drugs would be expected to be found predominantly in water (93%), followed by soil (4%), air (3%) and sediment (0.4%). The concentration of lifestyle drugs varies per region as shown in Table 2.4.

2.2.1.3.2 Toxicity and Risk Assessment of Prescription Lifestyle Drugs

Most lifestyle drugs that are continuously entering the aquatic environment are more likely to cause chronic rather than acute toxic effects. However, most studies have focused on short-term experiments and high concentrations that are unlikely to be found in aquatic environments. Pires et al., (2016) studied the long-term exposure of polychaetes to caffeine, to evaluate the biochemical alterations induced by environmentally relevant concentrations of caffeine on the polychaete species, *Diapatra neapolitana and Arenicola marina*. Despite both antioxidant and pro-oxidant properties of caffeine, in this it was demonstrated that an exposure to caffeine induces oxidative stress in tested species.

Table 2.4: Literature evaluation on sampling, extraction, detection and environmental concentration of lifestyle drugs globally.

Analytes	Sampling method	Extraction method	Detection method	Environmental concentration	Country	Reference
Steroid	SPE	SPE	Derivatization GC-MS	ND - 286 ng L^{-1}	Belgium	(Ben et al., 2017)
Caffeine	Grab	SPE - Oasis HLB	LC-MS	ND - 195 ng L^{-1}	Spain	(Baena-Nogueras et al., 2016)
Caffeine	Composite grab	SPE – Oasis HLB	LC -MS	ND – 639 ng L^{-1}	Spain	(Garrido et al., 2016)
Caffeine	Passive	-	LC-MS/MS	4.2 – 23.0 ng L^{-1}	Italy	(Mirasole et al., 2016)
Caffeine	Grab	SPE - Oasis HLB	GC-MS	ND - 60000 ng L^{-1}	Greece	(Stamatis et al., 2013)
Caffeine	Grab	SPE - Strata X	GC x GC/TOF - MS	Qualitative	Spain	(Samaras et al., 2010)
Musk ketone, caffeine, steroid	Grab	Microwave-assisted solvent extraction, silica column clean-up	Derivatization GC-MS	1 - 1000 ng g^{-1}	USA	(Rice and Mitra, 2007)
Steroid	Grab	SPE	GC-MS	1.61 – 4.58 µg L^{-1}	China	(Yang et al., 2006)
Caffeine	Grab	SPE - Oasis HLB	Derivatization GC-MS	98 - 176 ng L^{-1}	Germany	(Weigel et al., 2004)
Caffeine	Grab	SPE - Oasis HLB	GC-MS	18 - 72 ng L^{-1}	USA	(Thomas and Foster, 2004)

Cruz et al., (2016) reported the biochemical alterations in the clam species *Ruditapes Philippinarum* after exposure to caffeine at an environmentally relevant concentration of 0.3 µg L^{-1} up to 18 µg L^{-1}. They also reached the same conclusion that an exposure to caffeine causes oxidative stress to species. Caffeine is consumed in South Africa by drinking coffee and energy boosters, and its daily use and disposal is unregulated. The above studies strengthen the need to analyse caffeine in the environment as a potential toxic substance.

Nicotine is an alkaloid and its toxic effects are well known. Since 1994 EPA has classified nicotine as a toxic substance (Seckar et al., 2008) and it is classified by European Union Regulation as hazardous waste when its dry weight exceeds 500 mg kg^{-1} (Jacob et al. 2000). However, Seckar et al., (2008) reported that nicotine is readily biodegradable and is unlikely to bioaccumulate independent of pH medium. Their toxicity tests with microorganisms, algae, and terrestrial plants showed that nicotine has low toxicity to typical organisms found in the environment. Gomez et al., (2007) found the concentration of nicotine and caffeine in wastewater to be up to 1573 ng L^{-1} and 833 215 ng L^{-1} respectively. Ruan and Liu, (2015) found that nicotine inhibited the growth of some bacterial species in sediment.

2.2.1.3.3 Analytical Methods for Lifestyle Drugs

Garrido et al., (2016) optimized and validated a method for the monitoring of caffeine and other emerging pollutants in Guadiamar River, Spain. Their analytical method was based on solid-phase extraction followed by LC-MS/MS and caffeine was amongst the highest analysed contaminants with a concentration of up to 623 ng L^{-1}. Baena-Nogueras et al., (2016) reported the optimized SPE-LCMS method for the analysis of caffeine and other pharmaceuticals, and they found that caffeine was the most commonly detected substance with a concentration up to 195 ng L^{-1}. Ruan and Liu, (2015) also reported an analysis of nicotine in sediment using polymerase chain reaction and denaturing gradient gel electrophoresis. The unavailability of these instruments (LC-MS/MS) in the African laboratories hinders the investigations of these compounds in the environment. Development of cheaper GC-MS methods, which are capable of detecting caffeine and nicotine in the environment, will lead to an increase in data on occurrence of these compounds in the African water bodies.

2.2.1.4 Illicit Drugs

Illicit drugs are those that are illegal to make, sell, or the national laws prohibit nonmedical use (Binelli et al., 2012). They are categorised into: cocaininics, amphetamines, tryptamines, piperazines, pyrrolidinophenones, arylcyclohexylamine, opioids, cannabinoids and hallucinogens (Castiglioni et al., 2006). Many illicit drugs are highly addictive, psychoactive and pose serious risks on non-target organisms (Karolak et al., 2010). Illicit-drug-users are estimated to be around 165 – 315 million around the world, and thousands of tons of illicit drugs are consumed annually (Van Nuijs et al., 2011). Most illicit drugs are associated with the stroke based on their mechanism of action and pharmacological evidence reported by medical scientists (Esse et al., 2011). Cocaine is the most powerful addictive known compound (Esse et al., 2011). Cocaine prevents neurotransmitter reuptake at presynaptic nerve terminals, causes the amounts of neurotransmitters present for stimulation of sympathetic nerves to increase (Esse et al., 2011). The euphoria connected to cocaine use result from increased concentration of dopamine and serotonin in the mesolimbic and mesocortical respectively, within the brain (Rosi-Marshall et al., 2015).

Methamphetamine is the most powerful amphetamine and is readily available; it metabolized by the liver within 12 hours of administration into active metabolite, which is a powerful hallucinogen (Rosi-Marshall et al., 2015). Amphetamines block the presynaptic reuptake of the dopamine, norepinephrine, and serotonin, permitting these neurotransmitters to stay the same in the synapse to trigger and saturate the postsynaptic receptors. Amphetamines impact every organ system in any ways (Wang et al., 2016).

Heroin is synthesised from opium. Heroin binds to endogenous opiate receptors positioned throughout the body, including the spinal cord and brain. Heroin crosses the blood-brain barrier very easily (Esse et al., 2011). Marijuana is most abused in South Africa due to the ability of most people to cultivate cannabis plants. The proposed mechanism for marijuana-associated cerebral infarction includes vasospasm, hypotension, and arrhythmia resulting in a cardioembolic stroke (Esse et al., 2011). Today investigation are needed to evaluate how each of these compounds act on the non-target organism in the environment; such studies are limited by the availability of analytical methods. Examples of illicit drugs are presented in Figure 2.4.

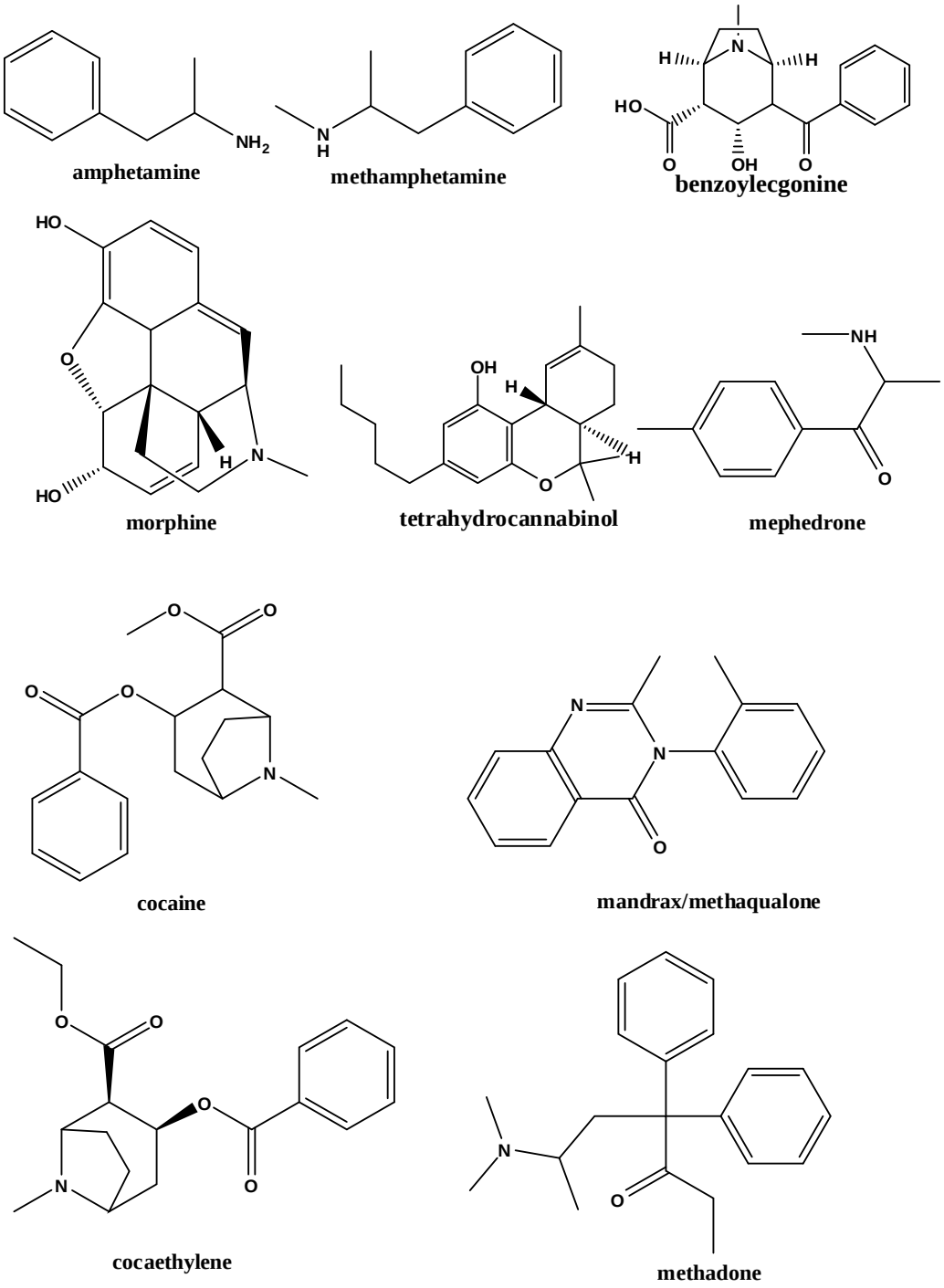


Figure 2.4: Structural examples of illicit drugs

2.2.1.4.1 Detection, Occurrence, Fate and Environmental Concentration of Illicit Drugs

In contrast to permitted pharmaceutical drugs, information on their presence in the environment is still limited in South Africa. However, they have been found in the environmental waters and sediments in some parts of the world. Pal et al., (2011) recently published a review based on illicit drugs in the environment, looking to their pathways, metabolites, occurrence, concentration levels and impact on the ecosystem. Andres-Costa et al., (2016) reported suspect

screening and quantitative determination of 42 illicit drugs of different classes and their metabolites, in WWTPS and surface waters from Turia River in Spain. Huerta-Fontela et al., (2008) also reported the occurrence of illicit drugs in water reservoir from north-eastern Spain. They detected cocaine and its metabolite at a concentration up to 4 ng L^{-1} while concentrations of amphetamine was found to range from 2 to 688 ng L^{-1}. Van Nuijs et al., (2009) investigated the occurrence of illicit drugs in wastewater in a yearlong study in Brussels, Belgium. Langford et al., (2011) reported an analysis of illicit drugs in Scottish sediments; however, cocaine and amphetamine were not detected in any of the samples they analysed. Detection of illicit drugs in different regions is presented in Table 2.5. Emerging drugs of abuse, belonging to many different chemical classes in the environment waters and solids, can only be realized if a suitable analytical screening method exists to detect, quantify and classify them in these matrices. Since results of standard methods are unavailable, the occurrence of illicit drugs in South Africa is not documented.

2.2.1.4.2 Toxicity and Risk Assessment of Illicit Drugs

The existence of illicit drugs and their metabolites needs attention based on their toxicological point of view because their possible negative effects on aquatic organisms, biota and the ecosystem might be on par with licit pharmaceutical drugs. The information on the toxicity of illicit drugs in the scientific literature is limited. Recently, Rosi-Marshall et al., (2015) reviewed ecological impact and environmental destination of illicit drugs in the environment. They concluded that illicit drugs exist in the environment and present ecological effects on bacteria, algae, invertebrates and vertebrates.

Table 2.5: Literature evaluation on sampling, extraction, detection and environmental concentration of illicit drugs globally.

Analytes	Sampling method	Extraction method	Detection method	Environmental concentration	Country	Reference
Amphetamine, methamphetamine, benzoylecgonine, heroine	Grab	Microwave	LC-MS	ND - 200 ng g^{-1}	The United Kingdom	(Petrie et al., 2017)
Methamphetamine	Grab	SPE - Cation exchange	LC-MS/MS	27 - 60 ng L^{-1}	USA	(Boles and Wells, 2016)
Ethylamphetamine, ephedrine, methadone, ketamine	Composite grab	SPE - Strata X	UHPLC - QTOF-MS	ND - 1693 ng L^{-1}	Spain	(Andres-Costa et al., 2016)
Cocaine, opiods, cannabis and ketamine	Grab	SPE - Oasis HLB	LC-MS	0.4 - 100 ng L^{-1}	Spain	(Bijlsma et al., 2014)
Methamphetamine, amphetamine, benzoylecgonine, methyl ester	Automated sampling	SPE	LC-MS/MS	ND - 1841 ng L^{-1}	Belgium	(van Nuijs et al., 2011)
Benzoylecgonine and cocaine	Grab	SPE - Oasis HLB	GC-MS	Qualitative - detected	Spain	(Gonzalez-Marino et al., 2010)

They further suggested that the concentration necessary to exact ecological impact requires more investigations. Félix et al., (2017) reported that morphological, physiological and behavioural effects may reflect neurodevelopment vulnerabilities of fish after exposure to ketamine concentration at 70 – 90 mg L^{-1}. Parolini et al., (2015) investigated the effects of a mixture of illicit drugs (cocaine 50 ng L-1; benzoylecgonine 300 ng L^{-1}; amphetamine 300 ng L^{-1}; morphine 100 ng L^{-1}; 3.4-methylenedioxymethamphetamine 50 ng L^{-1}) to zebra mussel. Their results showed that a mixture of illicit drugs at environmentally relevant concentrations can impair the oxidative status of the zebra mussel, posing a serious hazard to the health status of this bivalve species. Illicit drugs are present in the environment as a multi-residue mixtures of compounds. Many of these drugs are chiral and as a result may reveal different environmental persistence, fate and toxicity, which is enantiomeric ratio dependent. Unfortunately, the phenomenon of chirality, despite its necessity to the pharmaceutical industry, is often ignored by environmental researchers (Kasprzyk-Hordern, 2010). Current methods to determine chirality are complicated and development of new techniques is necessary to evaluate the impact of illicit drugs in the African water bodies (Petrie et al., 2015, Kasprzyk-Hordern et al., 2010).

2.2.1.4.3 Analytical Methods for Illicit Drugs

Advances in analytical method development have permitted determination at trace level of different illicit drugs and metabolites from various environmental matrices. The detection and quantification of illicit drugs in the environment have become popular among environmental researchers since the early on detection of methamphetamine and cocaine by (Jones-Lepp and Stevens, 2007). Zhang et al., (2016) developed a simultaneous method for the determination of five illicit drugs (amphetamine, methamphetamine, ketamine, ephedrine and hydroxylamine) in water samples. Illicit drugs were extracted with SPE and detected with LC-MS/MS. The analysed drugs were detected in all sampling sites and concentrations ranged from 1.00 to 99.51 ng L^{-1}. The performed risk assessment showed that the studied illicit drugs are unlikely to exert biological effects on the aquatic ecosystem at the current concentration in the surface water of Beijing. De Boer and Backer, (1956) also reported a multi-residue analytical protocol for the monitoring of 65 stimulants in the environment, opioid, morphine derivatives, benzodiazepines, dissociative anaesthetics, antidepressants, drug precursors, human urine indicators and their metabolites in wastewater and surface water based on SPE-LC-MS/MS.

Alvarez-Ruiz et al., (2015) developed and validated a method for analysis of cocaine, tryptamines, amphetamines, arylcyclohexylamines, cathinone, morphine derivatives, pyrrolidinones derivatives, entactogens, piperazines and other psychostimulants in particulate matter, sludge and sediment. The samples were ultrasound extracted, cleaned with SPE and analysed with LC-MS. To study illicit drugs in the South African environment it is necessary to develop methods that provide a high percentage of recovery, specificity and high sensitivity.

2.2.2 Personal Care Products

Personal care products (PCPs) are a different group of chemical compounds used in toothpaste, fragrances, soaps, lotions, and sunscreens. PCPs are differentiated as follows; disinfectants (triclosan), fragrances (musks), insect repellants (DEET), preservatives (parabens) and UV filters (methylbenzylidene camphor) as shown in Figure 2.5. PCPs are used in large quantities externally on the human body; they are not subjected to a metabolic degradation and enter the environment unaltered. Recent studies have proved that many of them are bioactive, persistent, and have the potential to bioaccumulate in the environment (Dietrich and Hitzfeld, 2004). Triclosan a biphenyl ether used as an antimicrobial in disinfectant products is among the top ten detected compounds in wastewater (Halden and Paull, 2004). Fragrances are extensively studied class of PCPs because they are believed to be omnipresent in the environment (Ternes, 1998). The most commonly used fragrance in deodorants, soap and detergents are synthetic musks (musk xylene and musk ketone). N, N-diethyl-m-toluamide (DEET) is an active compound in insect repellent and has been widely detected in the environment (Costanzo et al., 2007, Costanzo et al., 2005, Glassmeyer et al., 2005). DEET function by interfering with insect ability to detect lactic acid. However, it has a low bio-accumulation factor (BCF) and thus is unlikely to accumulate in aquatic organisms (Kim and Choi, 2014). Parabens are antimicrobial active compounds used as preservatives in toiletries, cosmetics, pharmaceuticals and food (Daughton and Ternes, 1999). There are 7 different types of parabens accepted for human use in nowadays (isopropyl, methyl benzyl, butyl, propyl, and isobutyl) (Golden et al., 2005). UV filters are used in cosmetics and sunscreen products for protection from sun radiation, and can be either organic or inorganic; however, in this section only organic UV filters will be discussed.

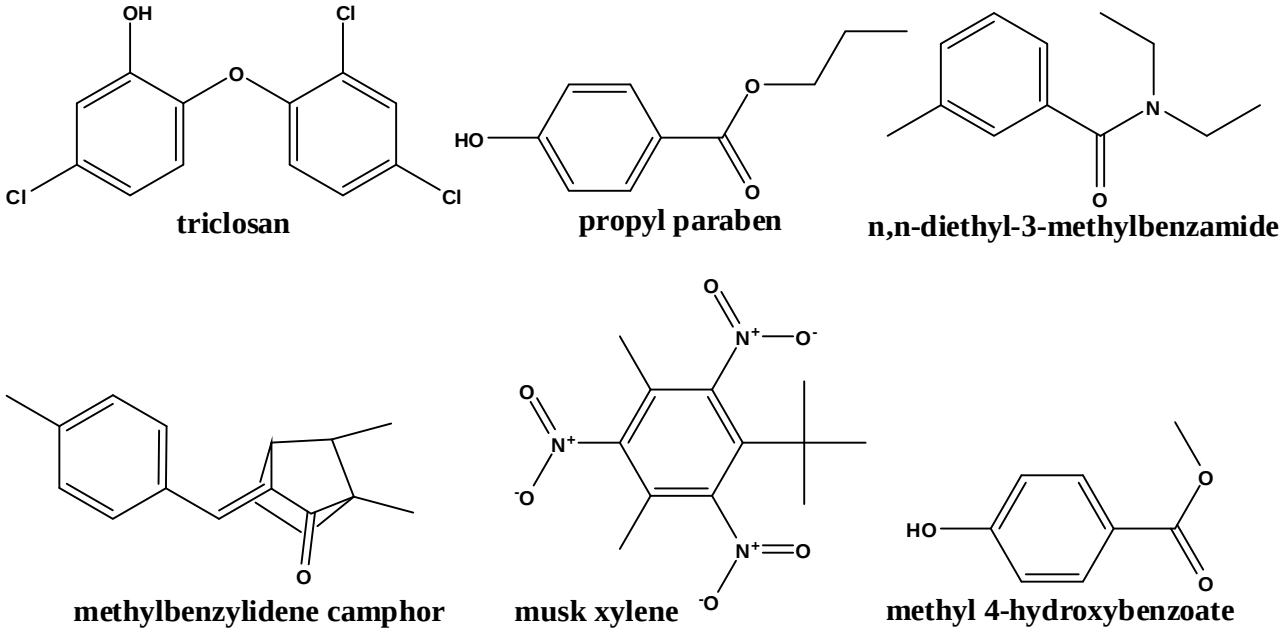


Figure 2.5: Chemical structures of personal care products.

Despite their widely detection in other region PCPs, as shown in Figure 2.5, have not been reported in the South African environment.

2.2.2.1 Detection, Occurrence, Fate and Environmental Concentration of PCPs

PCPs are among the widely found compounds in surface water globally as shown in Table 2.6. Souchier et al., (2015) reported the detection of disinfectants in sediments in France. Chen et al., (2012) also reported the determination of PCPs in different environmental matrices, their concentration in water ranged from 0.4 to ng L^{-1} and in sediment it ranged from 1.1 to 887 ng g^{-1}. In wastewater, disinfectant concentration levels have been found to be up to 650 ng L^{-1}. Disinfectants are relatively lipophilic and stable, and therefore are likely to bio-accumulate in biota. The highest concentration of disinfectants was found in fish with levels as high as 2100 ng g^{-1} (Buser et al., 2006, Buser et al., 1998). However, bioaccumulation of disinfectants has not been frequently observed in aquatic plants and studies contradict each other (Coogan et al., 2007).

Table 2.6: Literature evaluation on sampling, extraction, detection and environmental concentration of personal care products globally.

Analytes	Sampling method	Extraction method	Detection method	Environmental concentration	Country	Reference
Methylparaben and propylparaben	Grab	Microwave	LC-MS	ND - 200 ng g^{-1}	United Kingdom	(Petrie et al., 2017)
Salicylic acid	Grab	SPE - Strata X	LC-MS	ND - 1.3 µg L^{-1}	Germany	(Paiga and Delerue-Matos, 2016)
Triclosan	Grab	SPE HLB	Derivatization GC-MS	ND - 12,8 µg L^{-1}	Poland	(Nosek et al., 2014)
Propylparaben, camphor, DEET	Grab	SPE – Liquid-liquid microexctraction	UHPL-MS/MS	ND – 1818 ng L^{-1}	Italy	(Celano et al., 2014)
Triclosan	Grab	SPE	Derivatization GC-MS	ND	Canada and USA	(Uslu et al., 2013)
Triclosan	Grab	SPE - Oasis HLB	GC-MS	ND - 5 ng L^{-1}	Greece	(Stamatis et al., 2013)
Triclosan	Grab	SPE - Strata	Derivatization GC-MS	Qualitative	Columbia	(Martinez and Penuela, 2013)
Triclosan	Grab	SPE - Oasis HLB	GC-Ms	ND - 77 µg L^{-1}	USA	(Yu and Wu, 2011)
Salicylic acid	Grab	Assisted sonication	Derivatization GC-MS	ND - 20 ng g^{-1}	Spain	(Sánchez-Brunete et al., 2011)
Triclosan	Grab	SPE - Oasis HLB	Derivatization GC-MS	0,67 - 347 ng g^{-1}	China	(Zhao et al., 2009)
Triclosan	Grab	Microwave-assisted solvent extraction, silica column clean-up	Derivatization GC-MS	1 ng g^{-1} - 1000 ng g^{-1}	USA	(Rice and Mitra, 2007)
Triclosan and salicylic acid	Grab	SPE - Oasis HLB and strata X	Derivatization GC-MS	Qualitative	Canada	(Lajeunesse and Gagnon, 2007)
Triclosan	Grab	SPE - HLB cartridges	GC	ND - 3780 ng L^{-1}	USA	(Trenholm et al., 2006)
Triclosan	Grab	SPE - Oasis HLB	Derivatization GC-MS	ND - 4,1 ng L^{-1}	Germany	(Weigel et al., 2004)
Triclosan	Grab	SPE - Oasis HLB	GC-MS	18 - 72 ng L^{-1}	USA	(Thomas and Foster, 2004)

Fromme et al., (2001) investigated fragrances for the first time in 1983 and conducted the first major monitoring study of these compounds in different matrices. Dietrich and Chou, (2001) and Dietrich and Hitzfeld, (2004) reported a median concentration of fragrances in biota from 100 to 3000 ng g^{-1} of lipid. Wang and Kelly, (2017) studied the occurrence and distribution of synthetic musk in the urban catchment. Musk's were found to range from 0.0056 to 42.9 ng L^{-1} in water, and 0.01 to 108 ng g^{-1} in sediments. Balmer et al., (2005) evaluated the presence of four UV filters in surface water, fish tissue, and wastewater effluent in Switzerland. Huang et al., (2016) studied the occurrence and distribution of UV filters from riverine and coastal sediments in the Pearl River estuary of China. The concentration of UV filters and other PCPs ranged from 0.35 ng g^{-1} to 456 ng g^{-1} in these sediments. Zhang et al., (2011) investigated the occurrence of benzophenone and benzotriazole UV filters in sewage sludge and sediment. Moreover, Kim and Choi, (2014) reviewed the occurrence, ecological risks and toxicities of benzophenone-3, a widely known ingredient of organic sunscreen products in the environment. In addition, Haman et al., (2015) published a review on occurrence, fate and behaviour of parabens in the aquatic environment. There is little information on the environmental occurrence of insect repellent in sediments. However, Swedish Environmental Protection Agency has performed a screening study of DEET in several matrices in Sweden. Many researchers have focused on the occurrence of DEET in aquatic media (Costanzo et al., 2007, Yu et al., 2017). However, data from the African continent on occurrence of PCP in the environment has been scarce compared to other regions as shown in Table 2.6.

2.2.2.2 Toxicity and Risk Assessment of PCPs

The presence of PCPs in the environment is also a major concern, but their by-product or transformation presents serious environmental health issues. For instance, biotransformation of triclosan forms a more persistent by-product: methyl-triclosan (Batscher, 2006). Most PCPs in the environmental medium transform into dioxin (Hagenmaier et al., 1992, Daughton and Ternes, 1999, Caracciolo et al., 2015). Macedo et al., (2017) studied the impact of methyl-triclosan and triclosan in the embryonic development of *Danio rerio and Paracentrotus lividus*. Park et al., (2017) investigated the mixture toxicity of three organic UV-filters, ethylhexyl methoxycinnamate, octocrylene, and avobenzone on *Daphnia Magna*. In addition, Fong et al., (2016) reported on the toxicity of common UV filter, benzophenone-2 toward zebrafish embryos. Furthermore, Torres et al., (2016) developed a screening method to study the toxicity

of three different classes of PCPs using embryo bioassays. Song et al., (2006) studied the effect of butyl paraben on the development and microbial composition of periphyton and found that environmental residues of butylparaben have a very low risk to *periphyton* in aquatic ecosystems.

The above studies have shown that PCPs adversely affect untargeted species in the environment. Methods to perform similar studies on the African continent are needed to increase the awareness to government officials and the public about the danger posed by PCPs in water bodies.

2.2.2.3 Analytical Methods for PCPs

The determination of PCPs in various matrices in the environment frequently requires extraction and detection techniques that are capable of high efficiency, unique selectivity and sensitivity. Direct quantification of these compounds is often complex. For this reason, development of a rapid, inexpensive, simple and modern analytical method is always evolving. Zhang et al., (2011) developed a multi-residue method for the investigation of PCPs in sediments and biota, involving co-extraction and detection with GC-MS/MS and LC-MS/MS. Souchier et al., (2015) developed and validated a method for the target screening of triclosan in river sediment using LC-MS with a detection limit of 0.01 to 0.12 ng g^{-1}. Pintado-Herrera et al., (2016) also developed a method for analysis of triclosan using a pressurised liquid technique and stir bar for extraction, and GC-MS for detection. Furthermore, Montaseri and Forbes, (2016) published a review of the monitoring methods for triclosan and its occurrence in surface waters. Tarazona et al., (2014) reported a method for the invistigation of UV filters in beach sediments based on a leaching process of target compounds from sand samples using vortex mixer agitation and further centrifugation, followed by dispersive liquid-liquid extraction and detection with GC-MS.

Most analytical methods employed in the analyses of PCPs in the environment are LC-MS based, but GC-MS methods do exist as shown in Table 2.6. The existence of such methods will be useful to study the occurrence of PCPs in the African continent.

2.2.3 Flame Retardants

Flame retardant compounds are a structurally diverse group of chemicals that are mixed with polymers which makes plastic used in textiles, electronic circuitry, and other materials to reduce the risk of fire (Abou-Elwafa Abdallah, 2016). One of this group of compounds are the brominated flame retardants (BFRs). They have been detected in biota, sediments, air, water, marine mammals and even in human milk (Law et al., 2006). Many studies have focused on polybrominated diphenyl ethers (PBDEs) due to their persistence and hydrophobicity, two characteristics that make them amenable to bioaccumulation and bio-magnification (Eljarrat and Barceló, 2004). Due to their toxicity impact, production and use of BFRs are banned in many countries. To comply with increasing international regulations on BFR, novel additive flame retardants are needed. And to comply with commercial product fire safety standards are being produced and are now present in the environment (Verreault et al., 2007). Some of these non-BFRs are pentabromoethylbenzene (PBEB), hexabromobenzene (hexaBBz), organophosphorus esters (OPs) and decabromodiphenylethane (debDethane) (Covaci et al., 2011). These new flame retardants are being found in environmental samples from Africa, North America and Europe (Leonards et al., 2008, Lopez et al., 2009, Hoh et al., 2005). This review discusses non-BFRs as a flame retardant as they are shown in Figure 2.6.

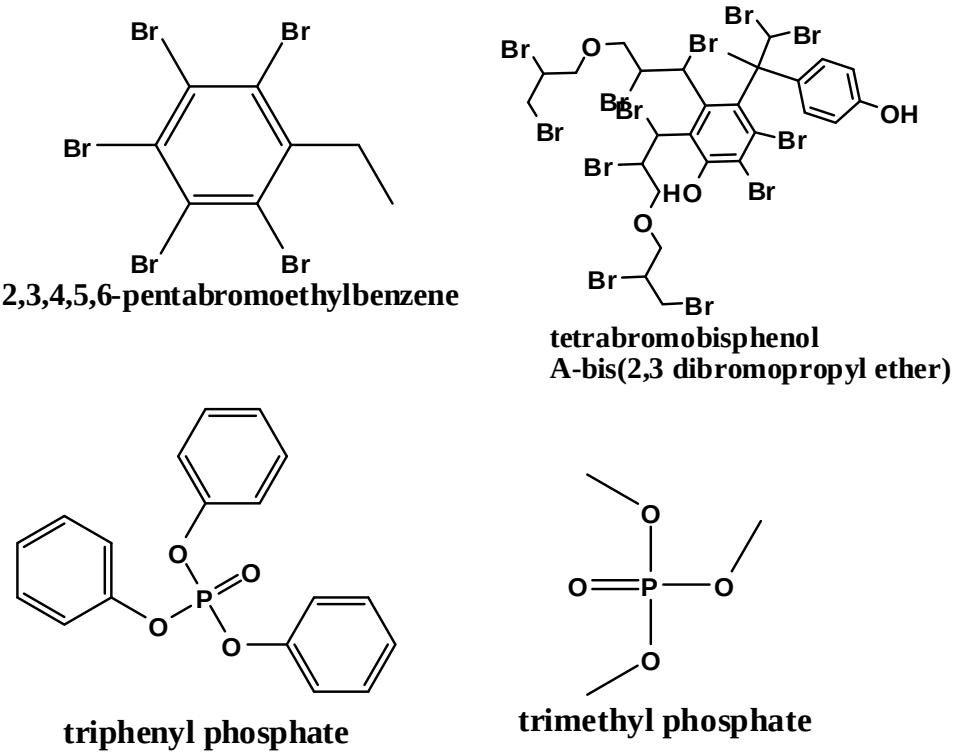


Figure 2. 6: Structural examples of flame retardants

Non-BFRs have not been reported in South Africa, yet South Africa is leading in banning materials that contain BFRs which has affected the mining sector. The use of non-BFRs is expected to rise in South Africa as new regulation in building materials commence.

2.2.3.1 Detection, Occurrence, Fate and Environmental Concentration of Flame Retardants

Emerging flame retardants have been detected in the environment in quantifiable levels in both aquatic and solid media. Guardia et al., (2010) determined the occurrence of flame retardants in Llobregat basin, Spain. They detected these compounds in all sediment samples analysed, at a concentration ranging from 3.1 to 9.6 ng g^{-1} for PBED, from 0.4 to 2.4 ng g^{-1} for hexaBBz, and from 4.8 to 23 ng g^{-1} for deBDethane. Organophosphate esters are now employed as flame retardants more often, with an annual production volume of 91 000 tons in Western Europe alone, and their occurrence in the environment is now ubiquitous. Reemtsma et al., (2008) published a review on organophosphorus used in flame retardants and plasticizers based on their occurrence and fate, in water. Regnery and Püttmann, (2010) reported the occurrence and fate of organophosphorus in Germany's surface waters and quantified at concentration levels between 17 and 126 ng L^{-1}. Zeng et al., (2014) investigated the occurrence and distribution of

the widely used OPs in sludge samples collected from WWTPs along Pearl River Delta, South China. Barón et al., (2014) reported the occurrence of emerging halogenated flame retardants in sediment and sludge from Ebro and Llobregat Rivers (Spain) with a concentration range from 2.7 to 340 ng g^{-1}. Covaci et al., (2011) published a review on novel brominated flame retardants, reviewing their analysis, environmental fate and behaviour. The detection of non-BFRs was mostly as collateral information resulting from the analysis of major PBDs. Only few analytical methods have been optimised and validated for detecting non-BFRs in environmental samples. Therefore, the occurrence of non-BFRs in African water bodies are not studied.

2.2.3.2 Toxicity and Risk Assessment Flame Retardants

Flame retardants bear many physicochemical and ecotoxicological similarities with legacy organic contaminants such as polychlorinated biphenyls (PCBs) and chlorinated pesticides. However, studies have shown that flame retardants are absorbed poorly in the body and huge amount maybe excreted in faeces (Guerra et al., 2011). Furthermore, upon cleavage these compounds may yield metabolites that are known neurotoxins (Covaci et al., 2011). Hardy et al., (2011) investigated the potential toxicity of decarbromodiphenyl ethane to five aquatic and sediment organisms, and concluded that this compound presents little risk to sediment organisms. Li et al., (2013) studied the accumulation distribution of dechlorane plus and associated biological impact on rats. After 90 days exposure, they found that this compound preferred to bio-accumulate in liver than in the muscle and there was no observable-effect in histopathology or death during the study. These results warrantee the need to investigate the occurrence of BFRs in the South African environment.

2.2.3.3 Analytical methods

As the number and the amount of PBDE replacement halogenated fire retardants (HFRs) increases, more of these compounds will be detected in the environment. It is important that methods are available to detect and accurately monitor them. Lopez et al., (2009) developed a method for determination of flame retardants and their metabolites in water based on SPE-GC-MS technique. Cristale and Lacorte, (2013) developed and validated a multi-residue method for the determination of new brominated and organophosphorus flame retardants in sludge,

dust and sediment. Their analytical method was based on ultrasound-assisted extraction with ethyl acetate/cyclohexane, clean-up with florisil cartridges and detection by GC-MS. They detected 24 out of 27 studied compounds in the environment. These developed methods are simple and can be easily optimized, which can enable detection of non-BFRs in South Africa.

2.3 Sampling of Emerging Pollutants

Acquiring a representative sample of the matrix sometimes in not given enough attention but vital component of any environmental analysis study (Wright, 2009). Failure to properly collect a representative sample of the original matrix: air, water, sediment, biota, food, dust, biosolid and also preventing further contamination arising during collection and transport to the laboratory may invalidate any results subsequently obtained (Sorensen et al., 2015). The collection of a representative sample starts in the laboratory by creating a sampling strategy that covers the following; sampling method, determination of sampling quantity, preserving the sample, suitable sample log book, and sampling objective must be clear (Söderström et al., 2009). Water quality parameters such as temperature, flow, pH, turbidity, total dissolved solids, oxygen demand and salinity are usually recorded on site (Storey et al., 2011). The sampling logbook must include the location of the sampling sites, sample collection procedures, time and date samples were collected. Additional information on weather conditions or site conditions and photographs of the sampling site are helpful. ECs must be sampled with tools made of stainless steel, aluminium, amber glass, or Teflon containers (Richardson, 2009). Materials manufactured from rubber, polyethylene or other plastics should not be used due to the tendency of these materials to absorb or desorb most ECs (plasticizers and flame retardants) from/into the collected sample (Xu et al., 2011).

The importance of sample preservation is necessary for quality of the collected data and depend on chemicals classes being tested. Mostly requires the addition of preservative chemicals to sample matrixes being tested, but this is practice is not recommended for a number of ECs. However, if high concentration levels of chlorine residue are present in a sample, sodium thiosulfate is normally added to prevent the breakdown of analyte of interest to chlorinated by-products (Vanderford et al., 2011). To prevent alteration and degradation, samples are transported cooled (4 to 6 ºC).

2.3.1 Surface Water

Surface water is a heterogeneous matrix. The distribution pattern of waterborne chemical compounds in a water matrix are controlled by the sorption partition coefficients of the chemicals, the hydrodynamics of the water, and the amount of organic/inorganic materials (dissolved organic carbon, and suspended sediment) present. In addition, occasional phenomenon from surface runoff, spills, and other point source contamination can result in isolated and/or short-lived chemical pulses in the water, sampling sites and methods (Ort et al., 2010).

The widely used method for collecting surface water is grab or spot sampling. This includes picking a single sample or a composite sample representative of a depth- and width-integrated profile. Another method is automated sampling systems used to collect composite samples over time. These are commonly used in remote sampling sites (streams, storm, ephemeral, small drains, and effluent discharges) where the availability of water is sporadic.

2.3.2 Sediments and Pore Water

Sediments have been studied for years to determine their nature and properties for different purposes. Recently, sediments contaminated with emerging contaminants have become one of the most important environmental issues (Antonic and Heath, 2007). They appear to become a regulatory issue with important scientific implications, as they are the fate of several compounds. Contaminants in surface water can sink and contaminate sediments. Exposure to contaminants in sediment would be by dermal contact through playing in water. Contaminated sediments may be collected with a stainless steel auger or spoon if the conveyance is dry adapted (Dimpe and Nomngongo, 2016). Inside the water body, the sediment sample is obtained by wading into the middle of the surface water body and scooping up the sediment whilst allowing the excess water to drain off. Care should be taken to minimize the loss of fine-grained materials. Pore water samples, especially from sediments, are a vital constituent in examining toxicity to microorganisms and understanding the potential trophic transfer of pollutant. Pore water can also be a marker of chemicals, which may be released into the

overlying water column. Pore water can be obtained *in situ* using passive sampling techniques or in the laboratory (Lohmann et al., 2017, Söderström et al., 2009). Acquiring of pore water from sediment samples in the laboratory can be done by squeezing, centrifugation, and vacuum filtration. Sediments are normally collected by using grab and core sampling methods. Centrifugation involves putting a soil/sediment sample in a centrifuge tube and centrifuging until the soil/sediment separated from the pore water and settles in the bottom. Guo et al., (2016) determined the distribution of ECs in the great lakes region by means of core sampling method. Pintado-Herrera et al., (2014) collected surface sediment samples (0 – 5 cm) using Van Veen grabs for the analysis of ECs.

Collection of seasonal samples for sediment analysis is challenging because of changing river width during wet and dry season. Thus, the data collected in different seasons does not fit to any pattern or distribution model, as result of significant different concentration levels. New technologies allow equipment (cameras and pH meters) used in the field to accurately record globally position system (GPS) coordinates of the sampling point. Hence, the same point can be sampled and compared over two or more seasons.

2.4 Extraction Techniques and Clean-up

Among several issues challenging analytical chemistry societies is the development of standardized and robust analytical protocols, and techniques that can be reproduced to laboratories globally for the investigation of ECs in the environment. Although there are many methods published in the literature that can detect up to ng L^{-1} or ng g^{-1} concentration of various classes of ECs in different matrices, the proper analytical method must still be followed. The concentration of ECs detected in the water and sediment samples are normally below µg L^{-1} range making extraction, pre-concentration, and clean-up prior to instrumental analysis an important step. New trends in sampling and extraction include increased use of stir bar sorptive extraction and hollow-fibre membrane microexctraction. Dimpe and Nomngongo, (2016) described current sample preparation methodologies used in the determination of ECs in different environmental matrices in detail. Some perspective developments that could be easily adapted in resource-limited labs include ultrasound-assisted extraction, pressurised hot water extraction, solid phase extraction and sorptive extraction.

2.4.1 Ultrasound-Assisted Extraction (Sonication)

In recent times, ultrasound-assisted extraction (UAE) has received great interest to overcome the disadvantage of classical solvent extraction such as low yields and prolonged extraction times (Albero et al., 2015). UAE is based on the production of ultrasound waves and their transmission throughout the solvent, which results in the formation of cavitation bubbles. When the cavitation bubbles collapse, there is a generation of liquid, circulation currents, and turbulence that improve the mass transfer rate (Vila et al., 2016). To perform an extraction based on sonochemistry, the choice of solvent becomes an important parameter because its physical properties like polarity, viscosity, vapour pressure and surface tension influence the cavitation phenomena. Important parameters are the frequency and the power of the ultrasound horn or bath. De Sousa et al., (2015) developed an ultrasound-assisted extraction method for the simultaneous determination of ECs in freshwater sediments. Albero et al., (2015) reviewed ultrasound-assisted extraction methods for the extraction of ECs from environmental samples. Vakondios et al., (2016) extracted emerging contaminants from sludge using ultrasound-assisted extraction followed by solid-phase microextraction (SPME) and GC for detection. Several parameters affecting the extraction steps were investigated, including the type of organic solvent used, duration of the ultrasound extraction step, and purification of the extract. In addition, De Sousa et al., (2015) developed an ultrasound-assisted extraction method followed by SPE and LC-MS for simultaneous determination of emerging contaminants. The developed method was applied to freshwater sediment samples collected from different sites in Jundiai River basin of Sao Paulo, Brazil.

2.4.2 Solid Phase Extraction

Solid phase extraction (SPE) is a common method reported for separating ECs from environmental samples (Huerta et al., 2015). SPE was invented as a better option compared to liquid-liquid extraction (LLE) which is laborious, possessing challenges to automate, and requires a large amount of analytical grade, expensive solvents such as acetonitrile. Nevertheless, LLE has been useful in extracting ECs comprise of hydroxyl groups (e.g, nonylphenol ethoxylates, bisphenol A, alkylphenol, hormones and steroid) from water (Li et al., 2001). Because a large number of hydrophilic ECs do not partition into an organic solvent,

resulting in poor recoveries, SPE is preferred over LLE. SPE offers minimal solvent usage, high throughput, easy to convert into automatic process, and simplified procedures than LLE.

SPE is commercially available in three basic formats: thin flat discs, small cylindrical cartridges and well plates. Each type of format can employ a wide-variety of sorbents such as silica (C_{18}) hydrophilic-lipophilic balanced (HLB), mixed cation exchange (MCX), and mixed anionic exchange (MAX). SPE sorbents are selected for their ability to retain the ECs of interest, based upon a variety of physicochemical properties of both the SPE phase and analytes. Another type of method, which is similar to SPE, is hollow-fibre microextraction, which uses a polypropylene hollow fibre membrane attached to the tip of a syringe that contains an extraction solvent. Celano et al., (2014) developed a SPE method using methanol/water as conditioning and/or eluting solvents and Oasis HLB cartridge for the analysis of pharmaceutical and personal care products. Capriotti et al., (2014) reported multiresidue determination of UV filters in water samples by SPE. Oasis HLB are recommended for extraction of emerging contaminants because of they can to extract various compounds in different matrices by changing the pH medium of the matrix.

2.4.3 Pressurized Hot Water Extraction

In pressurized hot water extraction (PHWE), water is used as the extraction solvent at elevated temperature and under defined pressure. Temperature primarily affects extraction efficiency during PHWE, since at elevated temperature high diffusion, low viscosities and less surface tension are achieved. Besides, the vapour pressure of the compounds increases at high temperatures and thermal desorption from the sediments and soil matrix occurs. However, breakdown, hydrolysis or oxidation of analytes can also occur at higher temperatures. Another variable studied during PHWE is the pH of the water phase when analytes with acid-base properties are studied, since the charged species are more soluble in the water-phase and increased extraction efficiency can be obtained under those circumstances. Pintado-Herrera et al., (2016) developed, optimized and validated a new method for the simultaneous determination of ECs in the environment. PHWE is an alternatively cheaper and greener method over Oasis HLB cartridges and have the potential to replace these cartridges.

2.4.4 Sorptive Extraction

Sorptive extraction involves the use of sorbent-coated materials such as stir bar, which is normally assisted by stirring the aqueous sample to extract the analytes of interest. Montesdeoca-Esponda et al., (2015) reported a fast and sensitive sample preparation strategy using fabric phase sorptive extraction for the analysis of UV stabilizers. Pintado-Herrera et al., (2013) optimized the stir bar sorptive extraction (SBSE) for analysis of 102 ECs in water samples. Gilart et al., (2013) developed novel coatings for the SBSE to determine pharmaceutical and personal care products in environmental waters. Magi et al., (2013) extracted UV filters from urban wastewater treatment plants using SBSE and LC-MS/MS. The coating of the stir bar in this method has been a limiting factor, as the layer must be reproducible for the results to be accurate and comparable. Amount of sorbent deposited on the surface of stir bar must be known, however this is a time consuming process and it is often not possible to produce the same amount of the deposit.

2.4.5 Separation and detection techniques used in emerging contaminants analysis

Although Soxhlet is time-consuming, labour intensive and requires the use of large volumes of organic solvents, it has so far been applied for organic compound extraction from solid matrices due to its high extraction efficiency. Despite the disadvantages mentioned above, there are still many applications of this extraction technique for the determination of ECs in environmental solids. Yao et al., (2011) reported a selective extraction of ECs from aqueous samples by dispersive liquid-liquid microextraction. Yao and Yao, (2017) also developed a magnetic ionic liquid an aqueous two-phase system for the extraction of chloramphenicol. Cunha et al., (2015) developed a dispersive liquid-liquid microextraction of bisphenol A and UV filters in wastewaters. These methods are accurate and efficient, but the world is moving toward developing sustainable and green methods for analysis of contaminants in the environment. The latter methods are easy to adapt into routine analysis and monitoring.

2.5 Separation techniques and instruments used in emerging contaminants analysis

Chromatographic separation of ECs from environmental matrix extracts is normally achieved by both liquid chromatography (LC) and gas chromatography (GC). Even though the latter is more suited for determination of volatile compounds and non-polar; non-volatile compounds such as surfactants, pharmaceuticals, personal care products and hormones can be analysed after a derivatization step. Rapid development in the area of mass spectrometry (MS) has changed it into an important technique to perform environmental detections, substituting other detectors commonly used in the previously, such as UV and fluorescence detectors for LC, and electron capture (ECD), flame ionization (FID) and photoionization (PID) detectors for GC. Tandem MS/MS today is most used in combination with LC, because of its higher, accuracy, sensitivity, precision and selectivity. Single MS detector is normally attached to GC, equipped with quadrupole, ion trap (IT) and time of flight (TOF) analysers. TOF is commonly employed when working with GC x GC chromatography.

2.5.1 GC-MS

GC-MS combination high resolution and selectivity allows better precision and accuracy, wide mass ranges and reasonable sensitivity. Separating chromatography columns commonly employed in GC comprise of narrow-bore capillary columns. There about 3 most commonly employed injection systems in GC-MS; splitless, on-column and programmable temperature vaporization. The common ionization sources used in GC-MS are electron impact (EI) or chemical ionization, either in negative or positive mode. Derivatization is normally employed when GC-MS is applied in determination of ECs in the environment.

2.5.2 LC-MS

Reversed-phase HPLC is the most use technique for the separation of many polar ECs. Gradient elution present an important strategy in separation of polar compounds. The mobile phases commonly employed are methanol, acetonitrile or mixtures of both solvents. When acetonitrile is used shorter retention time and improved resolution of the analytes is achieved. Because acetonitrile has higher elution strength and at low pressures. To get better retention time of analytes and improved sensitivity when MS detector is used, mobile phase modifiers, buffers and acids are added. Maggioni et al., (2013) used LC-ESI-MS/MS for the screening of ECs in the environmental samples.

2.6 Validation Protocols

Analytical method development and validation are continuous and an inter-dependent task associated with the advancement in research and development, quality and monitoring environmental agencies. Analytical procedure plays a critical role in the study of occurrence, quantification and risk assessment of ECs in the environment. Analytical methods could be spectral, chromatographic, electrochemical, hyphenated or miscellaneous. Development is the process of selecting an accurate procedure to determine the extent of contamination in the environment. The validation of an analytical method demonstrates the scientific soundness of the measurement or characterization of analytes. The validation practice demonstrates that an analytical method measures the correct analytes, in the correct amount, and in the appropriate range for the intended samples. It allows the environmental chemist to understand the behaviour of the method and establish the performance limits of the method using parameters listed in Table 2.7.

Table 2.7: Parameters used in validation and their definition

Parameter	*Definition*
Accuracy	An assessment of the difference between the measured value and the real value.
Precision	A measure of the agreement for multiple measurements on the same sample
Limits of detection and quantification	The lowest amounts of analyte that can be detected/determined accurately, respectively
Linearity and range	The proportionality of the measurement to the concentration of the analyte within a specified range
Robustness	A check of the deliberate small changes to method on the result

2.7 Summary of the literature Review

Detection, identification and quantification of ECs and their transformation products in the various environmental compartments are essential for gaining knowledge on their occurrence and fate. This is highly challenging for several reasons: the number of currently known potential ECs (transformation products) is very high (> 3000 worldwide but only a few known in the South African environment). Their relevance changes over time due to changes in production, use and disposal of pharmaceuticals, and new information on their occurrence, fate and hazards. Certain ECs such as pharmaceuticals, hormones, steroids and nanoparticles affect the ecosystem at extremely low concentrations and need analytical methods with correspondingly low detection limits. Not all ECs, during sampling and analysis are known; non-target methods are still needed. Different types of ECs with widely varying physical/chemical properties exist: pharmaceuticals, personal care products and flame retardants. Current state-of-the-art methods for sampling and analysis, are regional-based and vary amongst environmental agencies (EPA and EU directive) yet South Africa or Africa have no adapted standard methods that suit their environment. They are typically dedicated to certain EC classes and by far do not cover the full range of ECs of potential concern present in South

Africa. Moreover, for a number of known highly hazardous ECs that are prioritized elsewhere, their environmental concentration levels are inadequate to allow proper risk assessment. Furthermore, for EC-types of more recent concern such as flame retardants, methods for sampling and environmental analysis are in the initial state of development or virtually non-existent.

In conventional methods targeting of specific ECs, advanced ultra-sensitive instrument techniques (e.g. GC-MS/MS, LC-MS/MS and ICP-MS/MS) have become available but so far, they are seldom used due to higher costs. Development of validated methods with simplified sample preparation, offer improved possibilities for simultaneous determination of multiple ECs, and/or significantly improved detection limits for ECs will lead to a new paradigm for developing countries to study the occurrence of these classes of compounds in their environment. Data processing software and associated databases are still hindering progress made so far.

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Chapter 3: Detection and quantification of acidic drug residues in South African surface water using gas chromatography-mass spectrometry

3.1 Abstract

A method was optimised for derivatisation, isolation, detection and quantification of salicylic acid, acetylsalicylic acid, nalidixic acid, ibuprofen, phenacetin, naproxen, ketoprofen, meclofenamic acid and diclofenac in surface

.0water using gas chromatography-mass spectrometry. For most of the acidic drugs, recovery was in the range of 60 to 110% and the percent standard deviation was below 15% for the entire method, with limits of detection ranging from 0.041 to 1.614 µg L^{-1}. The developed method was applied in the analysis of acidic drugs in Umgeni River system, KwaZulu-Natal South Africa. All of the selected acidic drugs were detected and quantified, with concentrations ranging from 0.0200 to 68.14 µg L^{-1} in Umgeni River system.

Keywords: acidic drugs, method validation, derivatization, GC-MS, surface water, Umgeni River

3.2 Introduction

In recent years, pharmaceutical contaminants have been reported to be present in wastewater effluent, drinking water, rivers and dams in Asia (Jindal et al., 2015, Saravanan et al., 2014, Shanmugam et al., 2014, Li et al., 2015, Qin et al., 2015, Chen et al., 2015), America (Qin et al., 2015, Kümmerer, 2009, Caracciolo et al., 2015, Sarmah et al., 2006), Australia (Sarmah et al., 2006, Watkinson et al., 2009) and Europe (Frederic and Yves, 2014, Net et al., 2015, Kümmerer, 2009, Sarmah et al., 2006, Valcárcel et al., 2013). However, within Africa, there is limited information concerning the occurrence of pharmaceuticals in the environment (Matongo et al., 2015, Agunbiade and Moodley, 2014, Madikizela et al., 2014, Shanmugam et al., 2014). The reason for this is in part, due to the lack of suitable methods that can be used along with the limited analytical facilities available.

Pharmaceuticals play an important role in safeguarding people's health (Hotez and Kamath, 2009). However, some, like acidic drugs do not completely degrade in wastewater treatment plant processes (Lacey et al., 2012), and their presence in the environment can be hazardous towards humans, terrestrial and aquatic organisms, and can disrupt ecosystems (Celiz et al., 2009). For example, Lacey et al., (2012) reported that diclofenac caused vitellogamin in male Japanese medaka fish, and Diniz et al., (2015) reported on the toxicity of pharmaceuticals to zebrafish. Furthermore, some drugs have been found to inhibit seed germination, and crop growth (Caracciolo et al., 2015).

Analytical methods for the quantification and monitoring of pharmaceutical compounds, so as to elucidate the fate and behaviour within the environment are relatively complicated, time consuming and expensive (Iglesias et al., 2012). This is more so in developing countries; yet routine analysis of pharmaceuticals at ng L^{-1} levels is of paramount importance (Qiu et al., 2016, Ji et al., 2014, Rozet et al., 2007). Liquid chromatography-mass spectrometry (LC-MS) and gas chromatography (GC-MS) are widely used for environmental analysis of pharmaceuticals (Petrović et al., 2005, Zhao et al., 2014, Cheng et al., 2015, Maggioni et al., 2013). LC-MS is preferred for analysis of polar, non-volatile and acidic analytes, but it can be expensive when used for routine analysis and few organisations can afford such instruments (Kumirska et al., 2015). In contrast, GC-MS is sensitive, selective, cheaper to maintain and is

more readily available. In addition, GC-MS may be superior to LC-MS for trace analysis of organic compounds in matrices of greater complexity, but is limited when used for non-volatile compounds in aqueous matrices (Hao et al., 2007). Derivatization methods are often used to increase volatility, reduce polarity and enhance detectability of acidic drugs by GC-MS (Lin et al., 2008). The choice of derivatizing reagents is crucial, and more so when developing a routine analytical method (Kumirska et al., 2013).

This work presents optimised methods for the quantification of acidic drugs in water samples using GC-MS. The method developed reduces the retention times 2 – 3 folds shorter than published methods in literature (Togola and Budzinski, 2007, Togola and Budzinski, 2008, Samaras et al., 2010, Samaras et al., 2011, Giandomenico et al., 2011, Migowska et al., 2012, Kumirska et al., 2013). In addition, a silylation-GC-MS method has been developed for analysis of acidic drugs in South African waters.

3.3 Experimental

3.3.1 Chemicals and Reagents

Aspirin, salicylic acid, nalidixic acid, ketoprofen, Ibuprofen, diclofenac, meclofenac, phenacetin, naproxen, 4,4-Di-tert-butylbiphenyl, dichlorodimethylsilane, sodium thiosulfate, 99% N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylsilyl chloride (TMSCLl) were of analytical purity and were purchased from Sigma-Aldrich (South Africa). Cinnamic acid was purchased from BDH Chemical Ltd (South Africa). Analytical grade hydrochloride acid (HCl, 37%) was bought from Merck (South Africa). Acetone, acetonitrile, dichloromethane, toluene methanol and ethyl acetate were chromasolv® grade (99.9%) purchased from Sigma-Aldrich (South Africa). Double distilled water was obtained using an Aquation Biby A4000D water purification system (Biby Sterlin LTD (UK)). All carrier gases, including those used for extraction, were of high purity (99.999 %) and were purchased from Afrox (Durban, South Africa). All chemicals were used without further purification.

3.3.2 Apparatus, Materials, and Instruments

All glassware, including amber bottles used for sampling, were washed with phosphate free soap Dynachem (South Africa) and soaked in an acid bath for 24 hrs. After the acid bath, they were then rinsed with 5% dichloromethylsilane in toluene and methanol respectively, and then heated at 60 ºC for 12 hrs (except the sampling bottles). Small volumes were measured by a micropipette plus kit Dragon lab (China) ranging from 0.5 to 1000 µL. All glass fibre Millipore filters were bought from Pall Corporation (South Africa). The extraction manifold and sorbents used for extraction (Oasis HLB 20 cc (1 g) LP, sepak-pak plus CN cartridge and tC18 environmental cartridge sepak-pak) were purchased through Microsep from Waters (United State of America (USA)). The GC-MS used was a Shimadzu QP2010 SE equipped with an auto injector (AOC-20i) and auto sampler (AOC-20s) (South Africa, Kyoto Japan, respectively). The GC was equipped with a capillary column, Crossbond 5% diphenyl/95% dimethyl polysiloxane (intercap 5 Sil MS 0.25 mml. D x 30 m df = 0.25 µm, non-polar) bought from Restek (USA). Both glassware and instrument were kept at a laboratory temperature of 20 ºC.

3.3.3 Preparation of Stock Solutions

Stock solutions of each compound, internal standard (IS) 2-chlorobenzoic acid, surrogate standard 3-phenylprop-2-enoic acid and injector standard 4,4-di-tert-butylbiphenyl (1000 µg L^{-1}) were prepared in methanol and stored at 4 °C. Working solution of the standards containing 10000 µg L^{-1} of each target analyte and IS were also prepared and used in optimizing the derivatization procedure. For the corresponding calibration curves, standard solutions (10 µg L^{-1} to 5000 µg L^{-1}) were prepared by diluting a working stock solution that contained all of the target compounds in the appropriate amounts of acetonitrile and stored in the dark at 4 ºC. All solutions including samples were evaporated to dryness under a gentle stream of nitrogen, and subjected to derivatization and GC-MS analysis under optimal conditions.

3.3.4 Sampling

Sampling was carried out (January 2015 and July 2016) along the Umgeni River situated in Kwa-Zulu Natal province, South Africa. The Umgeni River has a 4418 km^2 catchment, 257 km long, contains four large dams, and supports over 4 million people. Figure 3.1 presents the locations of the various sampling sites.

Samples were collected from Midmar dam (1), Albert Falls (2), Henley dam (3), influent (4) and the effluent (5) of the Darvill wastewater treatment plant (WWTP), Nagle dam (6), Inanda dam (7, 8), inlet (9) and outlet (10) of the Northern wastewater treatment works, and the joining point between the wastewater discharge point and the Umgeni estuary (11). Some communities and animals source water directly from both the Nagle and Inanda dams (Figure 1). All composite water samples (5 x 500 mL) were collected from an area of 2 m^2, into 2.5 L amber bottles. Environmental parameters (pH, temperature, total dissolve solid, salinity, redox, dissolved oxygen and conductivity) were measured on site and all water samples were preserved at 4 °C. Sodium thiosulfate was added in wastewater samples to prevent further degradation of samples due to residual chlorine. Samples were transported to the laboratory and stored in a freezer for later analysis.

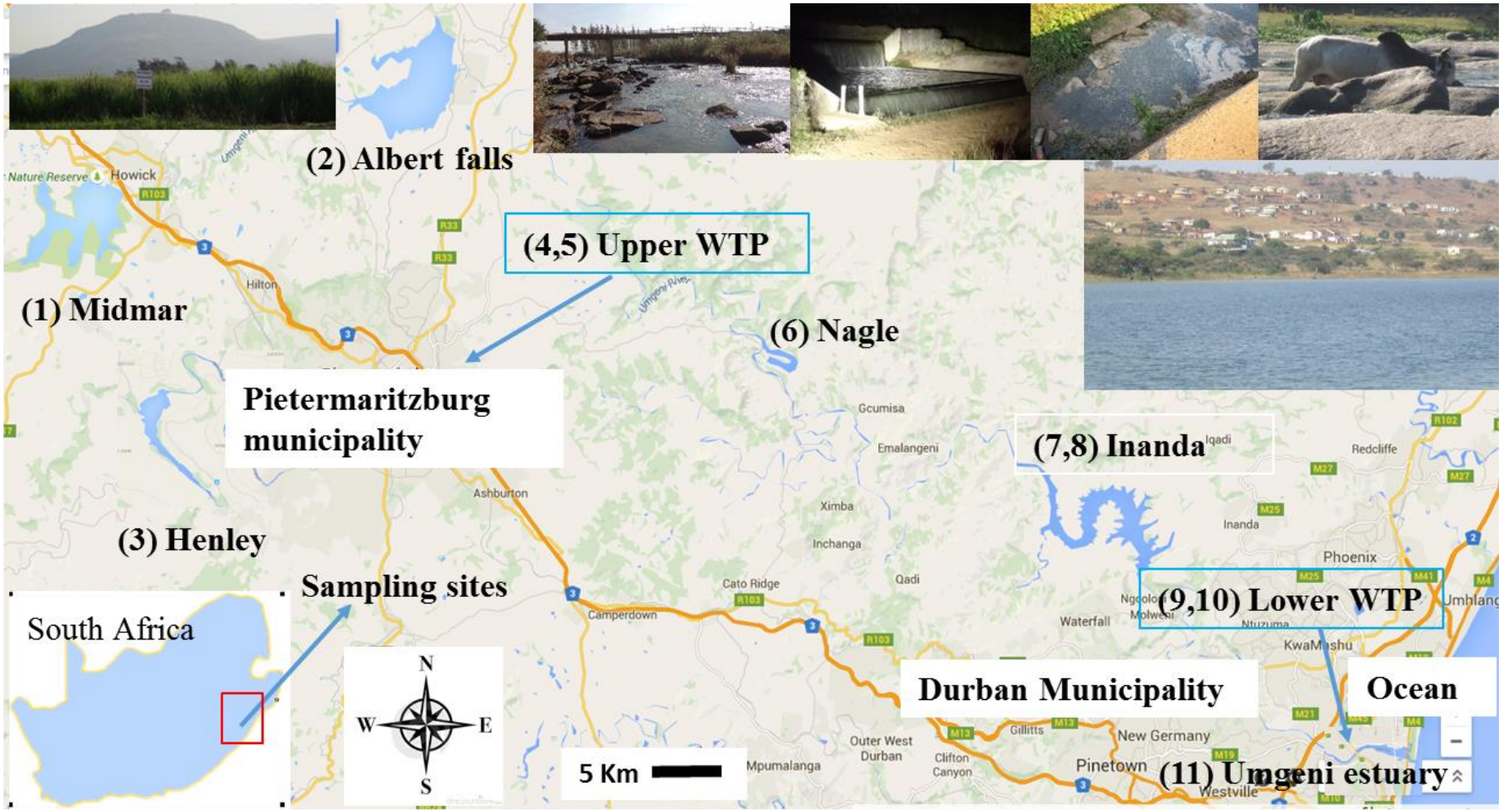

Figure 3.1: Location of the sampling sites along the Umgeni River system in Kwazulu-Natal, South Africa with the photographs showing the activities along the river (global positioning system (GPS) coordinate Appndix A5).

3.3.5 Sample Preparation

Samples were filtered through 0.45 µm glass fibre (Millipore) filters, and then 1 L of each sample was mixed with a solution of cinnamic acid in acetonitrile, as a surrogate standard (final concentration 100 ng L^{-1}), and then acidified to pH ≤ 2 with HCl. Target analytes were extracted using Oasis HLB SPE cartridges. Cartridges were conditioned with 8 mL methanol and then 10 mL of distilled water (pH ≤ 2) at flow rates of 3 - 6 mL min^{-1}. A 1 L of water sample was passed through the cartridge at a flow rate of 6 – 8 mL min^{-1} for approximately two hours. Thereafter, cartridges were left under vacuum for 30 min, and then a gentle stream of nitrogen was passed through for 5 min. Analytes were eluted with 8 mL of acetone:ethyl acetate in a ratio of 1:1 and thereafter 1 mL of acetonitrile, at a flow rate of 0.5 - 1 mL min^{-1}. The sample eluate was then mixed with a 10 µL solution of o-chlorobenzoic acid in acetonitrile (10 mg L^{-1}), and then dried under a gentle stream of nitrogen. Samples were derivatized by adding 100 µL of BSTFA and 10 µL TMSCl, in a vial sealed with Teflon lined septa and held at 70 °C in a water bath for 30 min. The derivatized sample was partially dried under nitrogen and finally re-dissolved in 1 mL acetonitrile.

3.3.6 GC-MS Analysis

Samples were analysed using a GC-MS (QP2010SE Shimadzu) system and separation was performed on a capillary column. The initial column oven temperature was 70 °C, injection port temperature was kept at 250 °C and 2 µL samples were auto-injected in splitless mode. The carrier gas was helium at a constant flow rate of 8.0 mL min^{-1} and 61.5 KPa pressure. The oven temperature was kept at 70 °C for 1 min, then ramped at 30 °C min^{-1} to 190 °C, held for 1 min, followed by ramping at 15 °C min^{-1} to 230 °C, held for 3 min, and finally ramping at 30 °C min^{-1} to 270 °C which was held for 1 min. The transfer line was set at 200 °C and the ion source at 200 °C. The electron energy for the filament was set at 70 V. The ion trap detector (ITD) setting was as follows: mass range 50 – 850 *m/z* (full scan only) with a start time of 4 min and end time of 14 min. For quantification of analytes ITD was operated in the selected ion monitoring (SIM) mode to enhance detectability of the selected drugs in water. The retention times, main fragment ions that were detected, and the two *m/z* peaks (one for

quantification, and the other for qualitative information) chosen for selected ion monitoring of the analytes are presented in the Table 6.1.

An independent injector standard 4,4'-Di-tertbutylbiphenyl of 100 µg L^{-1} was prepared and auto-injected into the GC-MS 5 times to evaluate the stability of the entire instrument. Peak areas obtained from each of the 5 injections were constant with a relative standard deviation of less than 2%.

3.3.7 Validation Protocol

To determine the limit of detection (LOD), limit of quantification (LOQ), and inter-day and intra-day precision of the entire method, six independent solutions were prepared in triplicate by spiking river water (Midmar dam, 1 L). The final concentration of the solutions ranged from 0.05 – 5 µg L^{-1}. These solutions were analysed with the developed method; extracted, derivatised and detected by GC-MS in SIM mode. Blank samples were also analysed (non-spiked Midmar sample) and subtracted from the spiked sample to determine absolute recoveries. The LOD and LOQ were estimated using equations 1 and 2 respectively (Thompson et al., 2002; (Gustavo González and Ángeles Herrador, 2007).

$$LOD = 3\,s / b \qquad \text{Equation 1}$$

$$LOQ = 10\,s / b \qquad \text{Equation 2}$$

$$\%R = ((\text{Spiked peak area} - \text{blank peak area})/\text{standard peak area}) \times 100\% \qquad \text{Equation 3}$$

Where's' is the standard deviation of ten independent blank samples, and '*b*' is the slope of the calibration curves. The intra-day and inter-day precision were evaluated at three concentration levels (low: 0.5 µg L^{-1}, medium: 1 µg L^{-1} and high: 5 µg L^{-1}) in triplicate.

3.4 Results and Discussion

3.4.1 Optimization of Derivatization of Target Compounds

The derivatization protocol was optimized, since it is a crucial step in GC-MS analysis of acidic analytes (Helenkar et al., 2010). A solution of 100 µg L^{-1} containing all target analytes including internal and surrogate standards were prepared and used to optimize the temperature, time, solvent, GC-MS conditions, and concentration of BSTFA. Only one parameter was investigated at a time, while the others were kept constant. Peak areas of the analytes were plotted against each parameter, and the optimum condition was taken at a point where the peak area no longer showed significant changes.

3.4.1.1 Effect of Derivatization Solvents

Methanol is usually used as a solvent, because most acidic compounds dissolve completely in protic solvents (Shareef et al., 2006). However, upon derivatization in methanol, a labile hydrogen atom on methanol was found to compete with an active hydrogen of the analytes for silylation, which resulted in the incomplete formation of analyte silyl derivatives, and this observation is similar to previous reports (Shareef et al., 2006, Zhou et al., 2007, Verenitch et al., 2006). In some cases, methanol formed esters with the analytes, which resulted in additional peaks (impurities) and affected the integration of analyte peaks. Hence methanol was removed completely using nitrogen. Analyte residues were reconstituted in BSTFA + 10% trimethylsilyl chloride (TMSCL), because many organic substances are readily soluble in this mixture (Kumirska et al., 2013, Basaglia and Pietrogrande, 2012). After silylation, acetonitrile was then used as a diluent.

3.4.1.2 Effect of Temperature

Heating the solution after addition of the silylation reagent is necessary for complete derivatization of analytes (Basaglia and Pietrogrande, 2012, Schummer et al., 2009, Yu et al., 2007). The temperature was varied from 30 ºC to 100 ºC (10 ºC interval) to find the optimum conditions. The results obtained (Figure 3.2 (A)) showed that there was an increase in the derivative formation from 30 ºC to 70 ºC, however, no significant difference observed after 70

ºC to 100 ºC. This indicated that the derivatization was complete at 70 ºC and hence it was taken as the optimum derivatization temperature.

3.4.1.3 Effect of Reaction Time

The reaction time is an equally important parameter (Yu et al., 2007, Basaglia and Pietrogrande, 2012), and was varied from 10 - 50 min (10 min interval). There was no change observed between 10 - 50 minutes, but 30 minutes was chosen for good repeatability as recommended other researchers (Basaglia and Pietrogrande, 2012).

3.4.1.4 Optimisation of BSTFA concentration

The amount of the derivatizing reagent is important in quantification analysis, and should be in excess (Basaglia and Pietrogrande, 2012). Special attention is needed to prevent silylation reagents from becoming impurities in a chromatogram, which may be mistaken as analyte peaks (Zhou et al., 2007, Yu et al., 2007). The amount needed for derivatization was optimized as shown in Figure 3.2 (B), and there was no significant difference in the silyl derivative formation between 100 µL to 120 µL for all analytes. Thus, 100 µL of BSTFA + 10 % TMSCl was then used as an optimum amount. But derivatization started occurring at 25 µL, however, derivatizing agent was not in excess. For all standards and samples, a gentle stream of nitrogen was used to remove the excess silylation reagent followed by dilution with acetonitrile to 1 mL prior to GC-MS analysis.

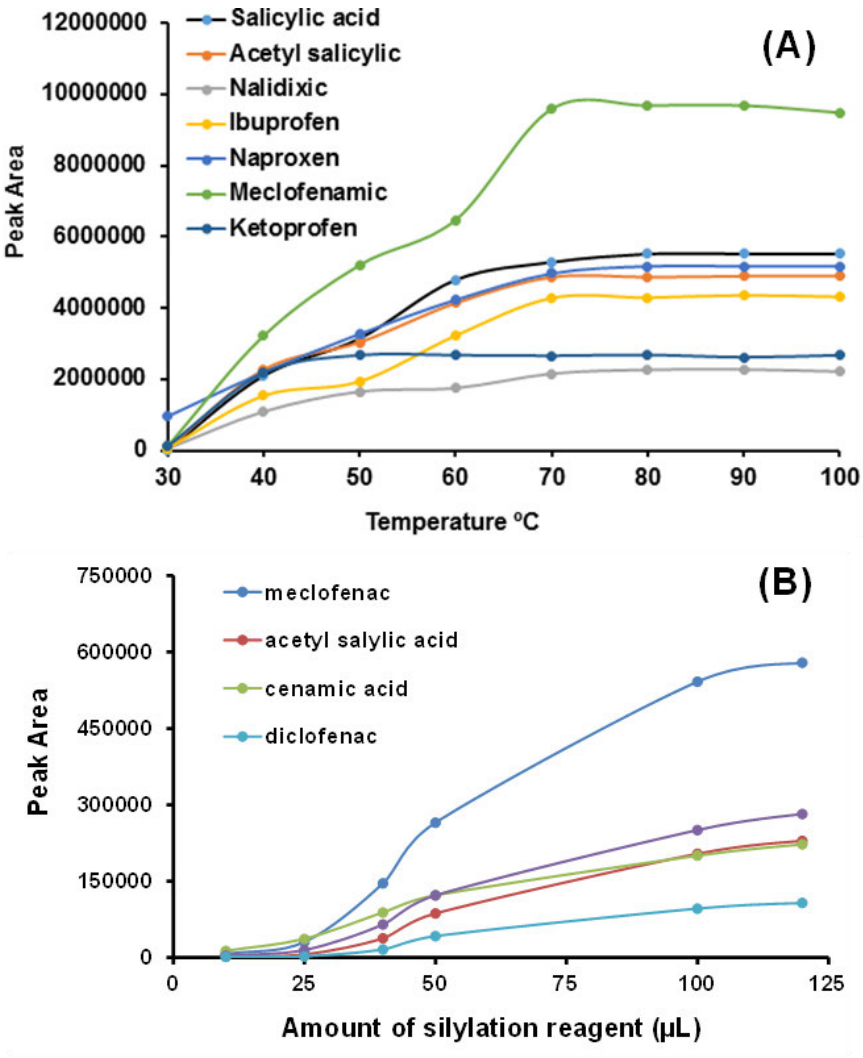

Figure 3.2: (A) Temperature optimization for the derivatization of the various drugs using a concentration of 10 mg L^{-1}. (B) Peak area response of the respective derivatized compounds, after using varying amounts of the silylation reagent.

3.4.2 Method Development

To obtain maximum detection sensitivity and specificity, the base peak chromatogram and distinct peaks of silylated acidic drugs were chosen and used in SIM mode except the TMSCL $[73]^+$ peak. The $[M+H]^+$ ion was detected for the silylated acetylsalicylic acid, cinnamic acid, nalidixic acid, ibuprofen, phenacetin, naproxen, meclofenamic, and diclofenac, but not for ketoprofen. The ion at *m/z* 263 of silylated ibuprofen represented loss of the methyl fragment ion (Figure 3.3 A). For silylated ketoprofen (Figure 3 B) the base ion was at *m/z* 282, presumably the loss of methyl and the ring opening, silyl derivatives are very strong and likely to be the last to fragment (Li et al., 2001). Also silylated diclofenac (Figure 3.3 C) has a similar ring opening that results in the *m/z* 308 ion, before TMSCl $[73]^+$ fragment. This is a good

indication that the silylated acidic compounds can pass through the chromatographic column without degradation. The retention times of each of the compounds were also determined. The *m/z* peaks used for quantification are presented in Appendix A2.

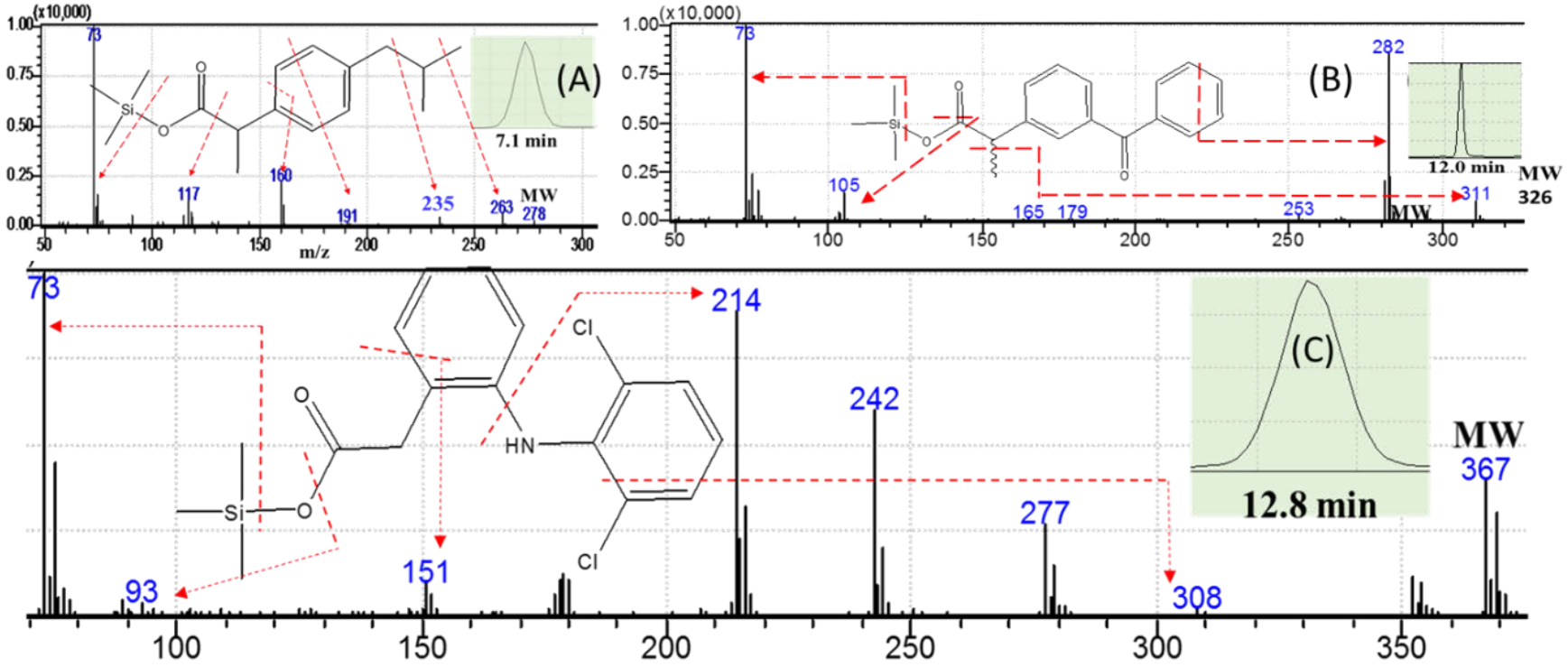

Figure 3.3: The profiles of EI mass spectra and tentative fragmentation of the silylated derivatives of (A) Ibuprofen, (B) Ketoprofen and (C) Diclofenac.

The parameters used in the identification of silylated acidic drugs are presented in Table 3.1. Retention times and selected ion were used to develop the method. As predicted, molecular ion $[M]^+$ peak was equal to the theoretical molar mass of the silylated compounds.

Table 3.1: Detection characteristics, retention time and fragmentation pattern of all acidic drugs.

No. Fig	Acidic analytes	Molecular weight *m/z*	Retention time/ Minutes	Main ion fragment *m/z*	SIM ion *m/z*
1	2-Chlorobenzoic acid	156 + 72	5.8	50, 111, 139, 169, 213	169, 213
2	Salicylic acid	138 + 72 +72	6.3	73, 155, 193, 200, 267	135, 267
3	Acetylsalicylic acid	180 + 72	6.4	65, 73, 120, 195, 210, 268	120, 195
4	Cennamic acid	148 + 72	6.6	75, 131, 161, 205, 221	131, 205
5	Nalidixic acid	232 + 72	6.9	73, 162, 180, 236, 251, 301	162, 236
6	Ibuprofen	206 + 72	7.1	73, 117, 160, 191, 263, 278	117, 160
7	Phenacetin	179 + 72	7.7	53, 109, 137, 179, 209	109, 179
8	Naproxen	230 + 72	10.6	73, 141, 185, 243, 287, 302	185, 243
9	4.4’ D-Tertbutylbipheynl	266	10.8	90, 251, 266	251, 266
10	Meclofenamic	296 + 72	11.9	73, 152, 208, 223, 298, 313	223, 313
11	Ketoprofen	254 + 72	12.0	73, 165, 179, 251, 282, 311	282, 311
12	Diclofenac	296 + 72	12.8	73,151, 214, 242, 277, 367	214, 367

Figure 4.4 displays the chromatogram of the multi-drug standard solution. All silylated acidic drugs were present and their retention times did not change, indicating that there were no interferences among selected acidic drugs. Separation of symmetric peaks was achieved in less than 13 minutes, and this is shorter than the 25 - 50 minutes time frames that were reported previously (Migowska et al., 2012, Kumirska et al., 2013, Togola and Budzinski, 2008). This can be attributed to the different temperature program applied in this developed method.

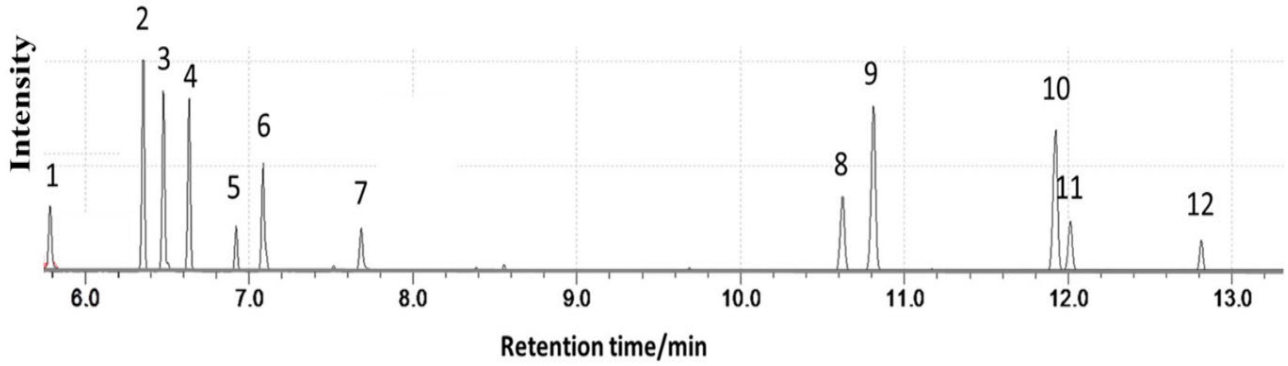

Figure 3.4: Chromatogram of multi-drug spiked standard solution of 10 µg L^{-1}, derivatised and analysed by GC-MS in SIM mode, numbers correspond to column 1 in Table 3.1.

3.4.3 Method Validation

The developed method was validated with reference to internationally accepted guidelines for single laboratory validation of method of analysis (Trullols Soler, 2006, Thompson et al., 2002, Peters et al., 2007, Rozet et al., 2007). Linearity, limit of detection (LOD), limit of quantification (LOQ), intra- and inter-day precision, and recoveries were established. All parameters were calculated using the peak area ratio of the target analyte to the internal standard (IS).

3.4.3.1 Validation of Linearity

To determine the linearity of the method, a maximum of 12 different concentrations were analysed in triplicate over a concentration range of 1 – 8000 µg L^{-1}. Linearity was established within 5 – 5000 µg L^{-1} for all silylated derivatives, all calibration curves had a minimum of six points and correlation coefficients (R^2) were higher than 0.99 for all analytes.

3.4.3.2 Limits of Detection of the Instrument

Lower and upper detection limit of the instrument was determined by taking low and upper concentration standards with percent standard deviation less than 20% (supplementary material

Table S2). Upper detection limit of the instrument for all target analytes were the same as the highest point on the calibration curves.

3.4.3.3 Optimization and Validation of the Solid Phase Extraction Conditions

The solid phase extraction (SPE) step was optimized by studying pH and sorbent material independently. In each study, 1 L double-distilled water was spiked to provide a final concentration of 1 µg L^{-1}. Each study was carried out in triplicate, thus, enabling an estimation of repeatability.

3.4.3.4 pH and elution solvents

Prior to extraction, water samples were adjusted to pH 2 with HCl (4 M). The chosen pH 2 was recommended by manufacturer. Results obtained in Figure 3.5 showed that pH 2 favoured the extraction of acidic drugs. The pH of 2, is in agreement with previous work in the literature (Matongo et al., 2015, Agunbiade and Moodley, 2014). Ethyl acetate has been reported to be suitable for extraction of pharmaceuticals where silylation is employed. However, this step was optimised by comparing methanol, acetone/ethyl acetate (1:1) and gradient elution (acetone 4 mL, ethyl acetate 4 mL and methanol 1 mL) as the solvent systems. A mixture of acetone/ethyl acetate (1:1) was found to have higher recoveries than the other solvents, and thus the results using an optimum pH of 2, and elution with acetone/ethyl acetate are presented in Figure 3.5.

3.4.3.5 Type of Cartridge and Matrix

Three different types of cartridges; specifically, cyno, environmental C_{18} and Oasis HLB, were compared using optimum pH and elution solvents. The results presented in Figure 3.5 show the extraction recoveries for the three different cartridges under optimum conditions. Recoveries ranged from 0 to 140% (for cyno) and 0 to 160% (for environmental C_{18}). In addition, chromatograms of blank solutions from the cyno and environmental C_{18} cartridges had some peaks, which directly interfered with integration of target analytes peaks. Hence, recoveries of these cartridges were not acceptable according to acceptable guidelines (Thompson et al., 2002). Furthermore, salicylic, nalidixic and naproxen were not recovered by

the cyno cartridge. The cyno cartridge is recommended by the manufacturer to be suitable for extraction of pharmaceutical drugs in blood samples. Since it is cheaper than the Oasis HLB cartridge, it was tested as an alternative cartridge for environmental samples. However, the optimization procedures used in this study were unable to adapt the cartridge for environmental work. Environmental C_{18} cartridges did show high recoveries for ibuprofen and ketoprofen, and maybe due to interferences. Oasis HLB recoveries ranged from 60 – 120% for all target acidic drugs, and there were no extraneous peaks observed. Therefore, it was selected for the extraction of acidic drugs, at pH 2 with flow rate between 4 – 10 mL min^{-1} and eluted with 9 mL (1:1, v/v) mixture of acetone/ethyl acetate. Matrix effect was determined by spiking deionised water, a river water sample (final concentration 1 µg L^{-1}) and a wastewater sample (final concentration 50 µg L^{-1}) followed by extraction with optimum conditions. The obtained results were not significantly different in terms of recoveries and percent standard deviations.

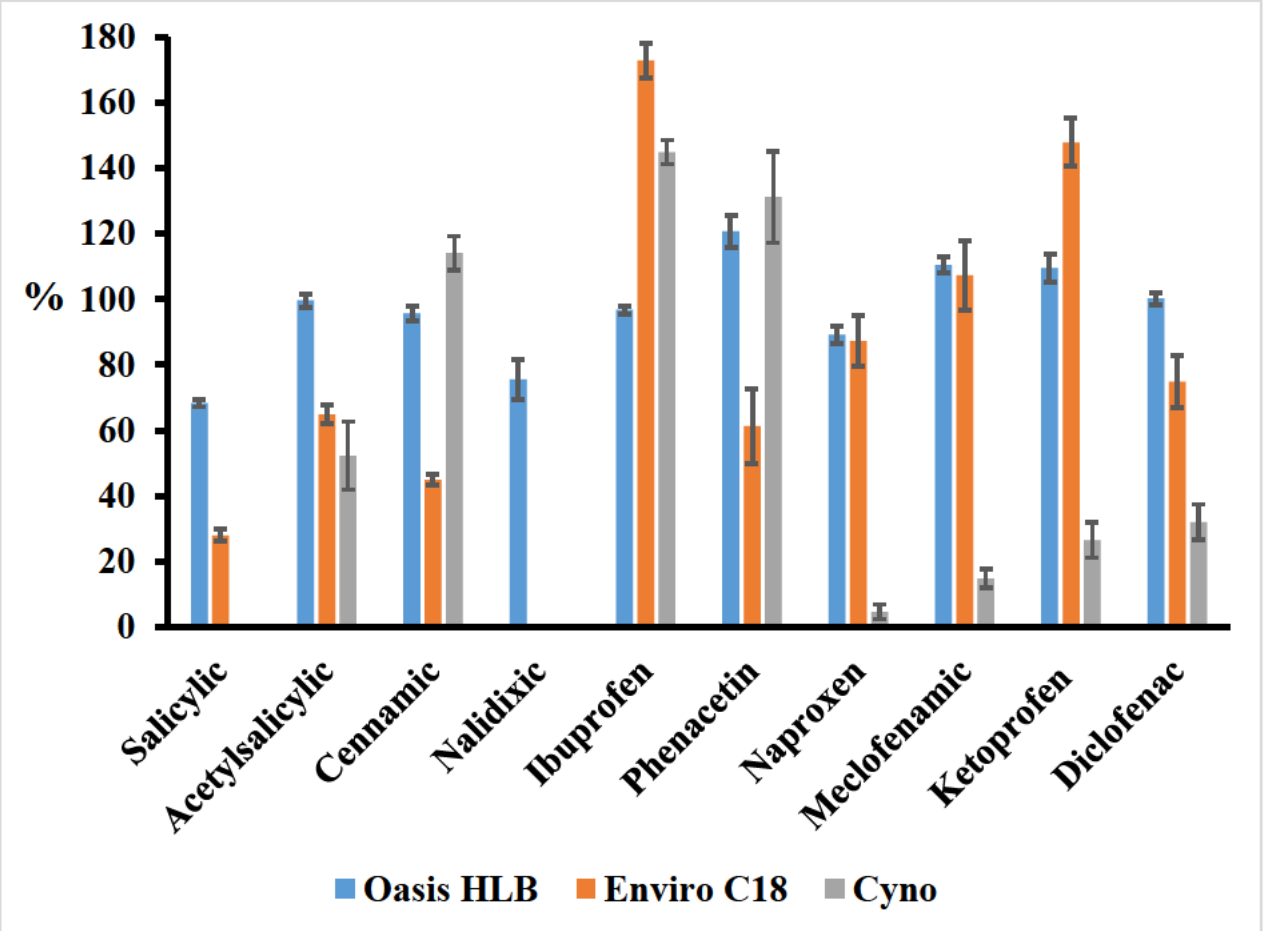

Figure 3.5: Extraction recovery percentages obtained with different SPE cartridges using pH 2 and eluted with 9 mL of acetone/ethyl acetate (1:1). River water samples were spiked and the final concentration of the solutions were 1 µg L^{-1}.

3.4.3.6 LOD, LOQ, inter- and intra-day Studies

The LOD and LOQ of the target analytes presented in Table 3.2 were similar to those reported in the literature for GC-MS and LC-MS techniques (Lolic et al., 2015, Helenkar et al., 2010, Hao et al., 2007, Lin et al., 2005), and slightly better when compared to published HPLC methods (Agunbiade and Moodley, 2014, Madikizela et al., 2014, Weigel et al., 2004, K'Oreje et al., 2016, Hao et al., 2007). For all target analytes, precision results had a percent standard deviation lower than 15%, as seen in Table 3.2.

Table 3.2: LOD, LOQ, intra-day precision and inter-day for the entire method.

Acidic analytes	LOD µg L^{-1}	LOQ µg L^{-1}	Intra-day precision %RSD			Inter-day precision %RSD		
			low	med	high	low	med	high
Salicylic acid	0.041	0.135	1.16	3.63	3.25	5.52	11.98	0.06
Acetylsalicylic acid	0.285	0.950	0.08	4.02	2.95	1.11	8.26	0.50
Cinnamic acid	0.117	0.390	0.12	3.15	2.07	14.74	9.80	2.42
Nalidixic acid	0.186	0.620	2.19	11.58	20.00	11.23	4.56	1.71
Ibuprofen	0.143	0.477	1.04	3.52	1.35	8.20	10.59	0.97
Phenacetin	0.345	1.151	20.00	20.00	9.75	7.10	2.86	6.10
Naproxen	0.075	0.248	8.66	1.88	4.42	8.21	14.17	4.04
Meclofenamic	0.082	0.272	3.79	1.03	3.25	6.39	8.40	1.76
Ketoprofen	0.130	0.400	6.27	2.80	6.64	6.47	14.47	8.49
Diclofenac	0.484	1.614	6.68	3.63	4.39	6.13	11.73	2.88

Low (0.5 µg L^{-1}), med (1 µg L^{-1}) and high (5 µg L^{-1})

The results presented in Table 3.2 showed that the developed method is suitable for the analysis of environmental samples. Table 3.3 shows the environmental concentration levels of pharmaceuticals in African countries. These concentration levels reported by other authors are within the detection range of our developed method. South Africa has high concentrations of pharmaceuticals compared to African countries. The South African municipalities' conventional wastewater treatment plants do show partial removal of pharmaceuticals but a fair amount is returned back to rivers thus leading to environmental exposure. Zambia had the

least concentration of pharmaceuticals when compared to Kenya and Nigeria. This trend is attributed to the number of homes connected to municipality sewage pipes, and the ability to recycle and treat the sewage (infrastructure).

Table 3.3: Environmental concentration levels of pharmaceuticals in African waters.

Pharmaceutical	Extraction / matrix	Country	Instruments	Environmental concentration µg L^{-1}	Reference
Acidic and antibiotic	SPE/water	Kenya	HPLC – MS	ND – 30.0	(Beausse, 2004)
Acidic and antibiotic	SPE/water	South Africa	HPLC – MS/MS	ND - 117	(Matongo et al., 2015)
Acidic and antibiotic	SPE/water	South Africa	HPLC – DAD	ND – 61.0	(Agunbiade and Moodley, 2014, Kennedy, 1996)
Acidic and antibiotic	SPE/water	Nigerian	HPLC – MS/MS	ND – 8.84	(Halling-Sørensen et al., 1998)
Acidic/personal care products	SPE/water	South Africa	HPLC – PDA	ND - 221	(Madikizela et al., 2014, Ternes, 1998)
Stimulants/personal care products	Liquid – liquid/water	Zambia	GC – MS	ND – 1	(Stumpf et al., 1999)
Acidic drugs	SPE/water	South Africa	Derivatization – GC – MS	ND – 68.3	Current study

3.5 Analysis of Samples from the Umgeni River system

All selected acidic drugs were detected in the Umgeni River system (Table 3.4). Meclofenamic was not frequently detected, perhaps due to its almost complete degradation prior to excretion (Metcalfe et al., 2003). However, in winter it was detected in four sites, in South Africa people use more medication in winter, and they eventually dump the unused medicine into their sinks. These pharmaceuticals will eventually reach the environment through wastewater treatment plants. None of the selected acidic drugs were quantifiable at the Midmar dam during the summer season, and maybe due to the decentralised sanitation exist in rural/farms surroundings it, as well as it is far from other possible sources of contamination and partitioning to sediments. However, three drugs were quantified during the winter, which was attributed to the drought in South Africa being experienced at the time of sampling which has led to reduced dam capacity. Hence, concentrations of pharmaceuticals in the environment will concentrate under these conditions (dam capacity reduction). Ibuprofen was frequently detected at all sites but was only quantified at Pietermaritzburg (PMB) STP inlet in summer, where it was found at a concentration of 3 µg L^{-1}. During summer, the Durban STP inlet showed high concentrations of ibuprofen, which is indicative of the variation in effluent received at wastewater treatment plants at any given time. Phenacetin concentrations increased as the river flows toward the ocean, i.e. 1.95 µg L^{-1}, 2.35 µg L^{-1}, and 8.14 µg L^{-1} at the PMB STP inlet, Inanda dam and the Umgeni estuary, respectively during summer.

The Inanda dam inlet was found to be more polluted compared to the other sites, with high concentrations of phenacetin. It has been shown in other studies that Inanda dam is vulnerable to pollutants other than pharmaceuticals (Papu-Zamxaka et al., 2010). These results maybe of serious concern because the bulk of drinking water consumed by the Durban municipality comes from this dam. Most selected drugs were detected in winter compared to summer. This trend is similar to what has been observed by other researchers in African waters, in Table 3.2. Furthermore, the environmental concentration levels found in this work are within the same range as what has been found elsewhere in the African continent as shown in Table 3.2.

The presence of salicylic acid in the Durban STP outlet sample can be related to the presence of the drug acetylsalicylic acid. Approximately, 10% of a low dose of acetylsalicylic (aspirin) is released as salicylic acid in the urine (Huerta et al., 2015). In addition, acetylsalicylic acid under STP conditions degrades to salicylic acid and can result in the observed increase at the inlet. The present results are also comparable to world-wide studies (Helenkar et al., 2010, Lee et al., 2014, Carmona et al., 2014, Kumirska et al., 2015). The results show that the sensitivity of the developed analytical method is sufficient to detect most of the compounds studied and is able to quantify them in the environmental seasonal samples.

Table 3.4: Application of the developed method to real water samples collected in Umgeni system.

	Summer Season								
	Salicylic Acid	Acetylsalicylic acid	Nalidixic Acid	Ibuprofen	Phenacetin	Naproxen	Meclofenamic	Ketoprofen	Diclofenac
sampling site	µg L^{-1}	µg L^{-1}	-	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}
Midmar dam	D	-	-	-	-	-	-	-	-
Albert falls dam	D	-	-	D	-		-	-	-
Henley dam	-	-	-	-	D	-	-	-	-
PMB STP inlet	**-**	**-**	**-**	**3.00 ± 0.07**	**1,95 ± 0.01**	**D**	**-**	**D**	**-**
PMB STP outlet	**-**	**-**	**-**	**D**	**D**	**-**	**-**	**-**	**-**
Nagle dam	-	-		D	-	-	-	-	-
Inanda dam inlet	D	1.13± 0.07	2.53 ± 0.43	D	2 34 ± 0.20	D	-	0.62 ± 0.09	D
Inanda dam outlet	-	-	-	-	-	-	-	-	-
Durban STP inlet	**D**	**D**	**-**	**D**	**-**	**-**	**-**	**D**	**-**
Durban STP outlet	**D**	**-**	**-**	**D**	**-**	**-**	**-**	**-**	**-**
Umgeni Estuary	-	-	-	D	8 14 ± 0.47	-	-	-	-
	Winter Season								
Midmar dam	-	D	-	D		D	0.84 ± 0.09	0.44 ± 0.00	-
Albert falls	D	D	-	D	D	D	-	-	-
Henly dam	-	D	-	2.13 ± 0.01	-	-	2.38 ± 0.58	9.22 ± 1.81	-
PMB STP inlet	**0.82 ± 0.13**	**D**	**-**	**7.38 ± 0.46**	**-**	**-**	**-**	**D**	**D**
PMB STP outlet		**D**	**-**	**D**	**-**	**-**	**-**	**-**	**-**
Nagle dam	-	D	-	0.87 ± 0.06	-	-	1.62 ±0.12	0.44 ± 0.00	1.01 ± 0.37
Inanda dam inlet	-		-	D	68.3 ± 7.0	-	-	D	-
Inanda dam outlet	-	D	-	0.52 ± 0.045		-	-	D	-
Durban STP inlet	**-**	**D**	**-**	**17.6 ± 0.85**	**D**	**59.30 ± 2.30**	**D**	**D**	**10.20 ± 0.25**
Durban STP outlet	**6.60 ± 0.00**	**-**	**-**	**D**	**D**	**-**	**-**	**D**	**-**
Umgeni Estuary	-	-	-	2.57 ± 0.41	-	-	-	-	-

D – detected but below quantification limit, - below detection limit, bolded raws – wastewater

3.6 Conclusion

The developed method was able to detect target analytes at the µg L^{-1} level, and all selected acidic drugs were detected in surface water and wastewater samples. Sample preparation and derivatization was fast and simple, and analyses of derivatives was achieved in less than 13 minutes. As demonstrated in the method validation for linearity, LOD, LOQ, precision and confirmation of the sorbent, the developed method was sensitive and repeatable over the established calibration ranges. Winter had high concentrations of pharmaceutical compared to the summer season. The detection of the selected acidic drugs in the Inanda dam needs further study, and the findings add to the growing data on pharmaceuticals in the environment on the African continent.

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Chapter 4: Assessment of nonsteroidal anti-inflammatory drugs by ultrasonic-assisted extraction and GC-MS in Mgeni and Msunduzi River sediments and biosolids, KwaZulu-Natal, South Africa

4.1 Abstracts

The occurrence of 8 pharmaceuticals were monitored during four seasons (spring, summer, autumn and winter) along a 250 km stretch of Msunduzi and Mgeni Rivers in KwaZulu-Natal, South Africa. This paper describes an optimized method for the determination of nonsteroidal anti-inflammatory drugs (NSAIDs) in sediments. The method combines ultrasonic, centrifuge and gas chromatography-mass spectrometry for detection of these drugs in solid samples. Most of the parameters that affect the extraction step were optimized. Sediment samples were placed in a centrifuge tube and extracted with ethyl acetate/acetone (1:1, two cycles) followed by cleaning with Oasis HLB cartridge and derivatization with N,O-bins(trimethylsilyl) trifluoroacetamide (BSTFA). Satisfactory recoveries were obtained ranging from 66% to 130% depending on the analyte. Precision expressed as RSD (%) (n = 3) were less than 20% for all analyte. The LODs and LOQs were in the range of 0.024 to 1.90 ng g^{-1}, which allowed for the method to be applied in the analysis of sediment samples in Msunduzi and Mgeni Rivers. In the sediment samples analysed, NSAIDs concentration ranged from not detected to 221 ng g^{-1}.

Keywords: GC-MS, NSAIDs, ultrasonic-assisted extraction, emerging pollutants and sediments

4.2 Introduction

The persistence of a drug in sediment or soil mostly depends on its photostability, its binding and sorption capability, degradation rate and leaching in water (Caracciolo et al., 2015; Gavrilescu et al., 2015; Halling-Sørensen et al., 1998). Strong sorbing pharmaceuticals tend to accumulate in soil or sediment. By contrast, highly mobile pharmaceuticals tend to leach into groundwater and get transported with groundwater (Fairbairn et al., 2015; Jindal et al., 2015; K'Oreje et al., 2016). The environmental persistence of some commonly prescribed drugs is longer than one year, for instance the lifetime of clofibric acid in the environment is 21 years (Buser et al., 1998; Saravanan et al., 2014). But for most drugs its environmental fate and risk is poorly understood, because of the lack of analytical standards for their metabolites, the high cost of analysis and the lack of suitable methods for routine monitoring (Petrie et al., 2015). Pharmaceutical drugs have been confirmed to exist in the environment to a greater extent than anticipated (Carmona et al., 2014; Shanmugam et al., 2014). There are over 3000 pharmaceutical substances accepted for use by humans (Jones et al. 2001). The fate of each substance may be very different, depending on their physicochemical properties and the technology of the wastewater treatment plant (WWTP) receiving sewage (Fairbairn et al., 2015; Qin et al., 2015; Sarmah et al., 2006).

Humans (for treatment and prevention of illness) use pharmacologically active substances regularly. In the animal and fish farming industries there is a greater dependence on drugs, used to prevent diseases and as growth promoters or parasite suppressors (Qin et al. 2015). Most of the drugs available on the market are not for a cure, but are used to limit or control symptoms, with some exceptions including antibiotics and antineoplastic. As a result, the consumption of a number of pharmaceuticals can be continuous and over a long period. These substances can be excreted as partially metabolized or as active metabolites, which eventually enter WWTPs (Brewer and Lunte, 2015). In addition, the unused medicine might be a source of sewage contamination. Kuspis and Krenzelok, (1996) reported that 35% of people in the USA dump medication down the toilet or sink. At WWTPs, pharmaceuticals are partially removed and escape into the environment (Carmona et al., 2014). The continual input of pharmaceuticals through sewage, and partitioning to sediments or sludge, may lead to long-term exposure of

aquatic and terrestrial organisms to harmful substances (Caracciolo et al., 2015; Celiz et al., 2009; Halling-Sørensen et al., 1998).

The acute and chronic toxicities of pharmaceuticals in the environment have been studied by researchers and widely acknowledged by the public (Carmona et al., 2014; Morgan et al. 2011). Because pharmaceuticals are designed to cause biological effects, their occurrence in the environment is no longer only a scientific interest but also a public interest (Sarmah et al. 2006; Trouiller et al., 2002). Currently, a decreasing vulture population in Asia, which is associated with the presence of diclofenac in the environment, has created public awareness about the danger these substances pose in untargeted organisms (Ankley et al., 2007; Swan et al. 2006). Diclofenac is an anti-inflammatory drug that enters vulture eco-systems through the ordinary method of disposing the carcass in Asia. European and Asian lawmakers banned some of the pharmaceuticals drugs because when released into the environment, it causes ecological disturbance and risks to organisms (Kümmerer, 2003; Rico et al., 2012). South Africa has also recorded a significant disappearance of bearded vultures around Maloti-Drakensberg mountain range, which has not been linked to a pharmaceutical drug (Simmons et al. 2006). However, the determination of these compounds in the environment specifically sediments is still scarcely documented in South Africa, due to a lack of suitable analytical methods and government awareness about their occurrence in the environment.

Environmental samples contain high amounts of interferences as well as low levels of analyte which require the development of reliable robust analytical methods (Hao et al., 2007). However, most developed methods are not economical for monitoring large and long rivers with several activities. Sample preparation is an important step in the analytical method; liquid-liquid extraction (LLE) and soxhlet extraction are common techniques still widely used for extraction of liquid ans solid environmental samples, respectvely (Gakuba et al., 2015; Olutona et al., 2016; Vallecillos et al., 2015). Because of the conditions required for extraction of analytes and clean-up procedures needed in both techniques, most analytes are lost, and this together with solvent waste generated leads to low recoveries (Xing et al., 2015). Recently, researchers have focused their efforts to simplify solid-liquid extraction techniques involving ultra-sonication of solid sample with an appropriate organic solvent (Chen et al., 2015). Ultra-sonication techniques are then normally followed up with clean-up steps with sorbents such as Oasis HLB, C18 and silica gel cartridges (Lacey et al., 2012). Sonication provides efficient contact between the solid and extractant, resulting in the good recovery of most analytes (Chen

et al., 2015; Gomez et al., 2007). In the extraction of pharmaceuticals, contaminants from sediments, the different physicochemical characteristics of the compounds and matrix may have a significant influence on the extraction parameters.

The selection of extraction conditions is critical, especially in the development of multi-drug residues method. Moreover, pharmaceutical compounds present widely differing polarities and pKa values. In this Chapter, a sensitive multi-residue method is proposed for the simultaneous extraction of 8 commonly used pharmaceutical drugs from sediments, with many different polarities and pKa values. We opted for these drugs because they belong to non-steroidal anti-inflammatory drugs class of compounds frequently detected in many countries.
The specific objectives were:

- Optimization of the extraction from sediment using sonication followed by solid phase extraction for the clean-up.
- Validation of the entire analytical method for 8 drugs in sediments.
- Application of the method to the occurrence and seasonal variation of these drugs in sediments.

4.3 Experimental

4.3.1 Chemicals and Reagents

All analytical standards were of high purity purchased from Sigma-Aldrich (South Africa). 37% hydrochloride acid (HCl) of analytical grade bought from Merck (South Africa). Organic solvents: acetone, acetonitrile, dichloromethane, methanol and ethyl acetate were chromasolv® gradient grade (99.9%) purchased from Sigma-Aldrich. Doubly distilled water was obtained using an Aquation Biby A4000D water purification system in our laboratory bought from Biby Sterlin LTD (UK). All carrier gases including that used for extraction were of high purity bought from Afrox. Derivatization reagent 99% N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylsilyl were bought from Sigma-Aldrich. Unless stated, all chemicals and gases were supplied by South African Companies.

4.3.2 Apparatus and Instruments

All glassware, were washed with phosphate free soap dynachem and soaked in the acid bath for 24 hrs. Thereafter the glassware was rinsed with dichloromethane, acetone and methanol, and baked at 60 ºC for 12 hrs. Small volumes were measured by micropipette plus kit Dragon lab (China) ranging from 0.5 to 1000 µL. All glass fiber Millipore filter papers were bought from Pall Corporation. The mesh used for sieving sediments after grinding were bought from KingTest laboratory test. Sediment was sonicated with UMC C20 bought from Ultrasonic Manufacturing Company (South Africa) and then centrifuged (Germany (Hettich Zentrifugen) Rotofix 32 A bought from Labotec). Extraction vacuum manifold and sorbents used for extraction: Oasis HLB 6 cc (60 mg) LP bought from Microsep, Waters. GC-MS used for detection was a Shimadzu QP2010 SE equipped with an auto injector (AOC-20i) and Auto sampler (AOC-20s) (South Africa, Kyoto). GC was equipped with a capillary column (intercap 5 Sil MS 0.25 mml. D x 30 M df = 0.25 µm, non-polar) bought from Restek supplies manufactured China. Both glassware and instrument were kept at laboratory temperature. Unless stated all apparatus and instruments were supplied by South African Companies.

4.3.3 Preparation of Stock Solution

A standard stock solution of the target compounds (1000 µg mL^{-1}) were prepared by dissolving 50 mg of each compounds into 50 mL of methanol and the solutions stored at 0 ℃. A pipette was used to transfer 10 mL of all target analytes stock solution into 100 mL volumetric flask and diluted with acetonitrile to the mark, for the preparation of multi-drugs working stock solution (100 µg mL^{-1}).

4.3.4 Preparation of Spiked Solid Samples

Sediment samples were dried at room temperature, homogenized with a porcelain mortar, and then sieved with a mesh (600 µm to 100 µm). The solid sample was accurately weighed (10 g) into a 50 mL screw-top Teflon centrifuge tube, mixed with 10 mL of acetonitrile/methanol containing an appropriate amount of analyte mixture (100 µg mL^{-1}) to give a final concentration

in the solid sample at 100 ng $g^{-}1$ level. It was left for 24 hrs in order to evaporate the acetonitrile/methanol. The non-spiked samples were also prepared in order to correct absolute recoveries.

4.3.5 Optimization of Extraction Parameters

Solvent optimization; 10 mL of acetonitrile, methanol, ethyl acetate, acetone, water and their mixtures were added to separate Teflon centrifuge tubes containing 10 g portions of the spiked and non-spiked sediments, respectively. The vials were closed and agitated for 5 minutes using a vortex system. The vials were sonicated for 25 minutes and thereafter the samples were centrifuged for 25 minutes. The content of the vial was decanted into a glass vial and then the sample was extracted twice with the above mentioned solvents. A mixture of acetone/ethyl acetate (1:1) gave high recoveries and cleaner extracts compared to methanol. Sonication and centrifuge parameters were optimized by varying time in 5 minutes intervals from 0 to 30 minutes. In all cases, the extraction was carried out in triplicate.

4.3.6 Clean-up Step

Oasis HLB extraction cartridges were used for cleaning up the sediment extracts. In each case, the organic layer, was evaporated to less than 0.5 mL under a nitrogen stream, and diluted to 200 mL with water adjusted to pH 2 using 1 M sulfuric acid. Each cartridge was preconditioned with 5 mL methanol and 5 mL double distilled water adjusted to pH 2. Then a diluted sample extract (pH 2) was passed through the cartridge at a flow rate of approximately 5 mL min^{-1} to 7 mL min^{-1} under the vacuum manifold system. The cartridge was allowed to dry under a stream of nitrogen and the analytes, eluted with 8 mL of acetone/ethyl acetate (1:1).

4.3.7 Derivatization Procedure

Derivatization performed using the optimized derivatization procedure described in detail in the previous Chapter 3. Briefly, extracts from SPE were evaporated to dryness under a gentle

stream of nitrogen. To the dry residues, 50 µL of a mixture of BSTFA + 1% TMCS were added. The vials were closed and mixed for 2 min. Derivatization was performed at 70 °C for 30 minutes. The derivatives were then cooled to room temperature, and diluted to 0.5 mL with acetonitrile and subjected to gas chromatography-mass spectrometry (GC-MS).

4.3.8 Instrument Analysis

The samples were analysed using a GC-MS (QP2010SE Shimadzu) developed from earlier work in water analysis (Chapter 3). Upon injection of extracts in the GC-MS system, the capillary column separated the analytes. The initial column oven temperature was 70 °C, injection port was temperature kept at 250 °C and auto-injected 2 µL in splitless mode. The carrier gas was helium at a constant flow rate of 8.0 mL min^{-1} and 61.5 KPa pressure. The oven temperature initially kept at 70 °C for 1 min, was then ramped at 30 °C min^{-1} to 190 °C, held for 1 min, followed by ramping at 15 °C min^{-1} to 230 °C, held for 3 min, and finally ramping at 30 °C min^{-1} to 270 °C which was held for 1 min. The transfer line was set at 200 °C and the ion source at 200 °C. The electron energy for the filament was set at 70 eV. The ion trap detector (ITD) setting was as follows: mass range 50 – 850 *m/z* (full scan only) with a start time of 4 min and end time of 14 min. ITD was operated in the selected ion monitoring (SIM) mode to enhance detectability of the selected drugs in sediment and biosolids for the quantification of analytes. All target compounds were eluted under 13 minutes and fragmentation were reported in Table 4.1.

Table 4.1: Target compounds retention time, molecular fragmentation and ion monitored.

Target compounds	Retention time/ Minutes	Main ion fragment *m/z*	SIM *m/z*
Salicylic acid	6.30 – 6.36	73, 155, 193, 200, 267	135, 267
Acetylsalicylic acid	6.40 – 6.55	65, 73, 120, 195, 210, 268	120, 195
Ibuprofen	6.95 – 7.15	73, 117, 160, 191, 263, 278	117, 160
Phenacetin	7.6 – 7.7	53, 109, 137, 179, 209	109, 179
Acetaminophen	7.99 – 8.10	109, 181, 223	181, 223
Naproxen	10.58 – 10.65	73, 141, 185, 243, 287, 302	185, 243
Meclofenamic acid	11.90 – 11.95	73, 152, 208, 223, 298, 313	223, 313
Diclofenac	12.80 – 12.85	73,151, 214, 242, 277, 367	214, 367

4.4 Results and Discussion

4.4.1 Optimization of sample extraction techniques

The main advantage of GC-MS is its high selectivity and ability to fragment pollutants in complex matrices. Thus, this makes the mass spectrometry library very useful in identification of these compounds in the environment. To confirm the presence of the acidic drugs, two parameters are normally employed, the retention and relative abundance of molecular ion peak of the selected drugs shown in Table 4.1. These parameters were studied in detail and reported elsewhere (Chapter 3). With regard to the presence of the metabolites (salicylic and acetylsalicylic acid) and isomers (meclofenamic acid and diclofenac) retention time and fragmentation pattern were used to separate these analytes as shown in Figure 4.1, since they all have similar molecular ion peaks.

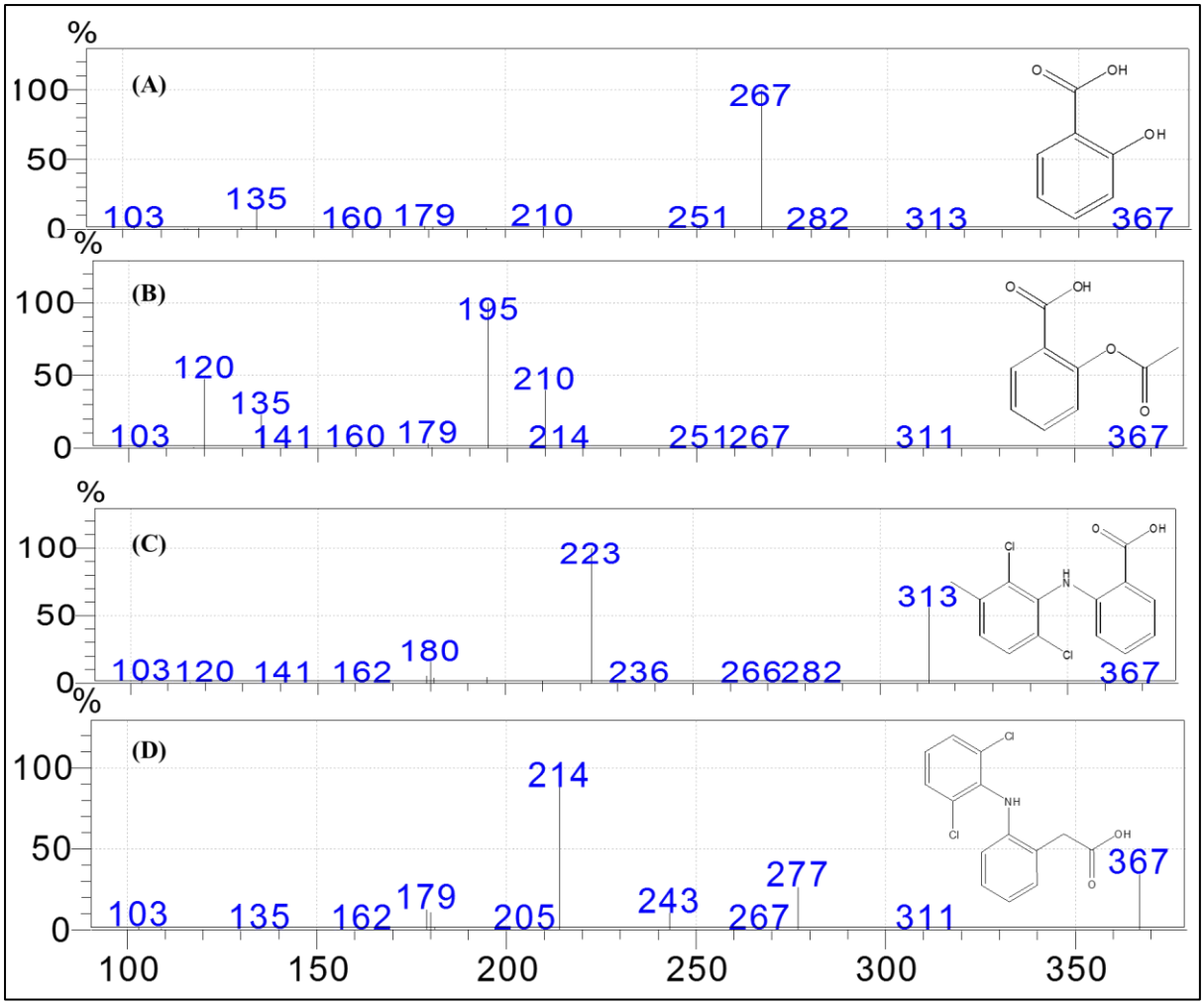

Figure 4.1: Mass fragmentation of selected compounds (A) salicylic acid, (B) acetyl salicylic acid, (C) meclofenamic acid and (D) diclofenac. Obtained by injecting extracted biosolid samples spiked with 300 ng g^{-1} of standard solution.

4.4.2 Optimization of Sample Extraction

The effects of the extraction solvent were firstly studied using sediments (10 g) spiked with all studied analytes at 100 ng g^{-1} concentration level. Ethyl acetate, methanol, acetone and dichloromethane were selected for this study because these have been used before, for the extraction of pharmaceuticals from solid samples. Extractions were initially performed using 10 mL of extracting solvent and extraction time of 25 minutes at room temperature. Recoveries below 50% were obtained for all studied analytes. In order to improve the recoveries, sonication and centrifugation time were optimized. Extracting the sample twice with solvents was found to increase the recoveries.

A mixture of solvents gave high recoveries compared to single solvents. Because acetone and ethyl acetate have different properties. The results revealed that the use of a double 25 minute cycle of ultrasonic extraction followed by centrifuging with 10 mL (1:1, ethyl acetate:acetone) solvent each time clearly improved the extraction efficiency with recoveries between 66% and 130% depending on the analyte. This was attributed to the facts that the second cycle was able to extracts strong held analytes within the sediments samples. In addition, the sample weight could be increased or decreased by 2 g without observing a decrease in the recoveries, and does not affect the sensitivity of the developed procedure in the environmental sample of complexity. Sample clean-up was performed with Oasis HLB as described in the previous chapter (Chapter 3).

From the obtained results, it was concluded that acidic drugs can be successfully extracted from the studied samples by ultrasonic-centrifugation assisted extraction with ethyl acetate:acetone, using two cycles of 25 minutes with 10 mL volume of extraction solvent each time. This extraction method can be used for sediments and biosolids because acceptable recoveries were obtained as shown in Figure 4.2. Methanol and and dichloromethane are not suitable for derivatization.

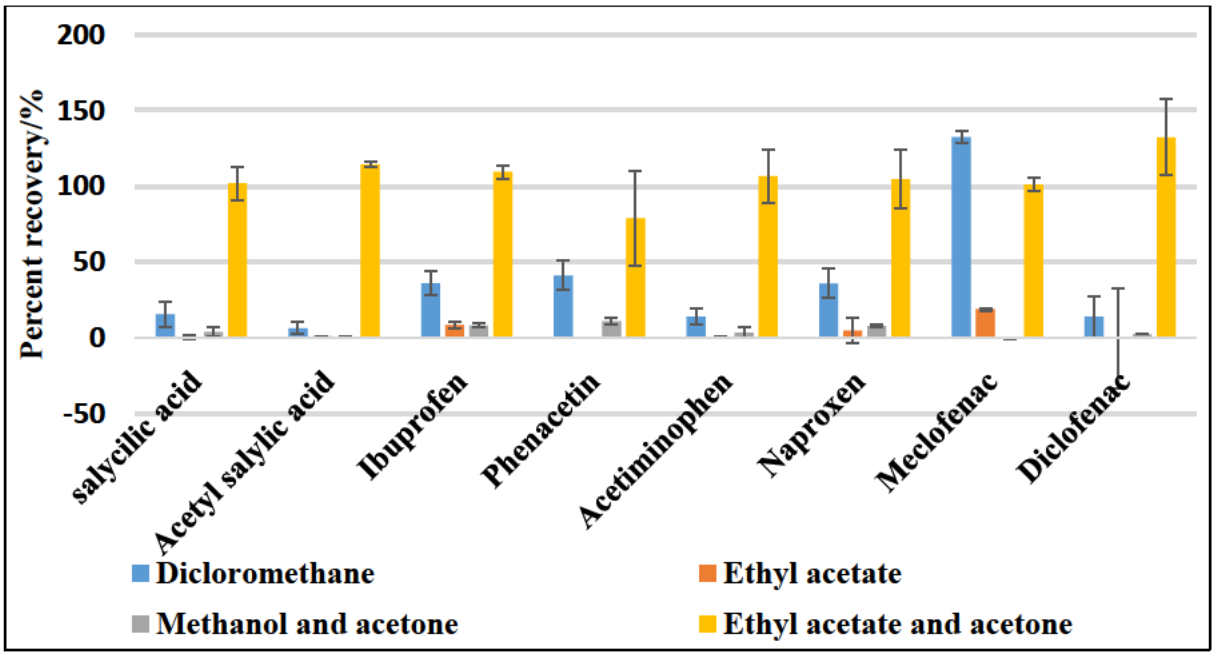

Figure 4.2: Selection of extraction solvent for extraction of selected (n = 3), dichloromethane, ethyl acetate, methanol: acetone and ethyl acetate: acetone. Experimental conditions: sample mass, 10 g; solvent volume, 10 mL (two cycles); analytes concentration.

4.4.3 Method Validation

After optimization, the developed analytical method was evaluated in terms of linearity, precision, accuracy and detection limits before it was applied in the determination of pharmaceutical drug residues in sediments and biosolids, as per published analytical guidelines (Thompson et al. 2002). The method was validated according to procedure described in the experimental. Non-spiked soil samples were also extracted.

4.4.3.1 Linearity

To determine the linearity of the method, a minimum of six different concentration levels were analyzed in triplicate over a wide range of concentrations from 1 to 500 ng g^{-1}. The linearity of the calibration curves was estimated using a linear mode, least-square regression in the concentration range studied. For all calibration curves, the correction coefficient was above 0.990, and the results are presented in Table 4.2.

Table 4.2: Molecular ion peaks of the compounds derivatized with BSTFA, their properties and linearity.

Analytes	MM g mol^{-1}	Derivatives	p*K*a	R^2	Linearity ng g^{-1}
Salicylic acid (SA)	138	282	2.97	0.9972	0.1 - 500
Acetylsalicylic acid (ASA)	180	252	3.5	0.9956	1 - 500
Ibuprofen (IB)	206	278	4.91	0.9934	0.1 - 500
Phenacetin (PN)	179	251	2.2	0.9923	5 - 500
Acetaminophen (AN)	151	223	9.9	0.9952	0.1 - 250
Naproxen (NP)	230	302	4.22	0.9901	0.1 - 500
Meclofenamic acid (MA)	296	368	3.8	0.994	10 - 250
Diclofenac (DC)	296	368	4.15	0.9957	1 - 300

4.4.3.2 Recovery/Accuracy and Precision

Recoveries in the different matrices were evaluated by extracting fortified samples of sand, sediment and biosolid with standard solutions (5, 50 and 250 ng g^{-1}) containing all target analytes in triplicate under optimum conditions. These fortified samples were allowed to stand for 4 hrs to allow solvent evaporation under stream of nitrogen gas, then they were analyzed following the GC-MS method described in section 2.8. Non-spiked blank samples previously analyzed were subtracted. Recoveries for sand ranged between 92% and 105%, between 66% and 120% for sediments and between 98% and 130% for biosolid (Table 4.3). The range of the recoveries achieved is similar to that obtained by other authors in sand, sediments and biosolid (Chen et al. 2015; Kumirska et al. 2015). The precision of the method expressed by relative standard deviation (RSD) of mean recovery values, when triplicate spiked sand, sediments and biosolid samples were analyzed (within and between days) ranged from 1% to 20% for all matrices (Table 4.3). The RSD for most targeted drugs were lower than 15% as Table 4.3 shows, which confirms the good repeatability (Thompson et al. 2002).

4.4.3.3 Limits of Detection and Quantification

The limits of detection (LOD) were calculated as three times the signal of the background noise obtained in the lowest spiked sample (1 ng g^{-1}) at the retention times of the corresponding analytes, and limit of quantification (LOQ) were determined considering a value of 10 times the background noise (Thompson et al. 2002). These results are summarized in Table 4.3, LOQs lower than 0.10 ng g^{-1} were obtained, and therefore the proposed method shows very good sensitivity and selectivity for the determination of target drugs in studied matrices. Hence, the method was suitable for the environmental application.

Table 4.3: Results for Recoveries, limits of detection and quantification and precision of spiked different matrices.

Analyte	Recoveries			LOD ng g^{-1}			LOQ ng g^{-1}			Precision %
	Sand	Sediment	Biosolid	Sand	Sediment	Biosolid	Sand	Sediment	Biosolid	All matrices
Salicylic acid (SA)	101	100	105	0.055	0.044	0.170	0.183	0.145	0.565	2.0 - 13
Acetylsalicylic acid (ASA)	97.0	91.0	102	0.078	0.020	0.090	0.260	0.065	0.030	1.0 - 10
Ibuprofen (IB)	102	92.0	102	0.133	0.048	0.024	0.443	0.161	0.079	1.0 – 7.0
Phenacetin (PN)	75.0	120	98.0	0.069	0.077	0.176	0.231	0.258	0.585	1.0 - 15
Acetaminophen (AN)	105	92.0	106	0.087	0.017	0.479	0.291	0.058	1.595	5.0 - 20
Naproxen (NP)	93.0	66.0	112	0.312	0.084	0.031	1.039	0.280	0.104	7.0 - 20
Meclofenamic acid (MA)	93.0	85.0	121	0.482	0.114	0.137	1.606	0.380	0.456	7.0 - 15
Diclofenac (DC)	92.0	103	98.0	0.592	0.092	0.546	1.973	0.305	1.820	3.0 – 9.0

4.5 Application of the Developed Method in Msunduzi and Mgeni Rivers

The developed method was used to assess the occurrence and concentration levels of pharmaceutical drugs in various environmental solid samples collected over four seasons along the economically important rivers in KwaZulu-Natal, South Africa. Sampling points are indicated in Figure 4.3.

The Mgeni and Msunduzi Rivers both consist of five dams and these dams supply over five million people with water within the Durban and Pietermaritzburg municipalities. Activities found along these rivers may introduce pharmaceutical drugs from hospital effluents, discharges from wastewater treatment plants, informal settlements, farming or animal husbandry. Two WWTPs were also included in this study to determine the occurrence of pharmaceuticals in biosolids. These South African WWTPs use conventional processes such as biological and mechanical means to clean wastewater from municipal sewage pipes. The identification of the target compounds in the environmental samples was based on optimised parameters and are presented in Table 4.1 and Table 4.2, and the quantitative analysis was carried in SIM mode for high sensitivity.

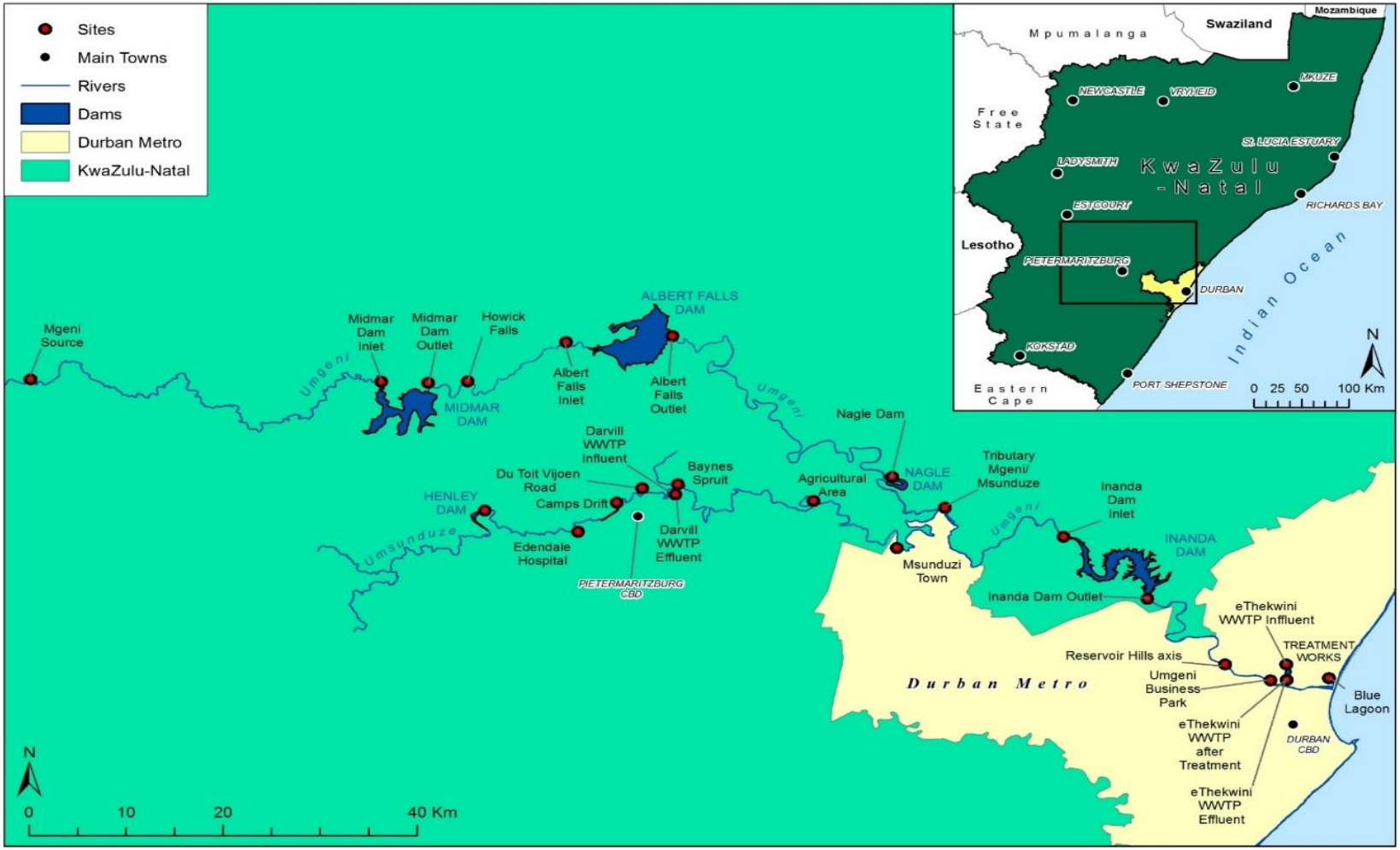

Figure 4.3: Sampling sites from Mgeni and Msunduzi Rivers, KwaZulu-Natal South Africa (developed using GIS software).

4.5.1 Msunduzi River

4.5.1.1 Occurrence of Pharmaceuticals

All target pharmaceuticals were detected in both sediments and biosolids in the Msunduzi River. In spring and summer, all drugs were detected in the stream passing through the Edendale Hospital as shown in Table 4.4. But no drugs were detected in autumn and winter. This was attributed to the fact that during rainy seasons hospital drains can overflow and contaminated runoff water might enter close by streams and contaminants and are likely to partition onto sediments (Jones Simmons et al. 2006). Another site that had most detection of target pharmaceuticals was Msunduzi Town, which lies near the informal town in the rural area between Durban and Pietermaritzburg Municipalities with no proper sanitation. Henley Dam showed least detection of target analytes. This site is before the Pietermaritzburg Municipality and the results suggest that residents of the Pietermaritzburg Metro are the major contributors of this pharmaceutical in the Msunduzi River.

Table 4.4: Detection and quantification of targeted pharmaceuticals in Msunduzi River.

Season	Analytes	Pharmaceutical concentration along Msunduzi River ng g^{-1}							
		Henley Dam	Hospital (stream)	Baynes spruit	Du toit	Camp Drift	Darvill WWTP biosolid	Agriculture (Rural area)	Msunduzi town
Spring	SA	ND	3.43 ± 0.28	BQL	ND	ND	6.89 ± 0.71	ND	ND
	ASA	ND	94.50 ± 9.50	163 ± 10.0	ND	BQL	124.00 ± 11.10	BQL	8.00 ± 0.15
	IB	ND	BQL	BQL	ND	ND	1.96 ± 0.50	ND	BQL
	PN	BQL	BQL	ND	ND	ND	0.120 ± 0.030	BQL	BQL
	AN	BQL	BQL	ND	ND	10.12	6.90 ± 0.58	BQL	BQL
	NP	ND	BQL	BQL	ND	ND	2.52 ± 0.350	ND	BQL
	MA	ND	BQL	BQL	ND	ND	ND	ND	BQL
	DC	ND	BQL	BQL	ND	ND	9.53 ± 0.83	ND	BQL
Summer	SA	ND	1.01 ± 0.01	0.240 ± 0.110	ND	0.447 ± 020	48.7 ± 5.01	ND	ND
	ASA	ND	0.32 ± 0.05	BQL	BQL	0.31 ± 0.03	33.20 ± 2.10	BQL	ND
	IB	ND	1.32 ± 0.15	0.505 ± 0.122	0.30 ± 0.01	0.74 ± 0.08	27.20 ± 3.02	0.18 ± 0.23	ND
	PN	ND	0.65 ± 0.12	0.32 ± 0.11	0.30 ± 0.01	0.58 ± 0.00	35.00 ± 5.03	0.32 ± 0.05	0.29 ± 0.01
	AN	0.26 ±0.01	0.67 ± 0.01	0.33 ± 0.01	0.318 ± 0.010	0.70 ± 0.01	2.15 ± 0.09	0.27 ± 0.01	0.35 ± 0.01
	NP	BQL	BQL	BQL	BQL	BQL	12.9 ± 0.520	BQL	BQL
	MA	BQL	2.83 ± 0.10	1.78 ± 0.01	BQL	1.05 ± 0.01	46.20 ± 6.03	BQL	BQL
	DC	BQL	8.10 ± 0.99	3.77 ± 0.09	BQL	2.17 ± 0.034	181.00 ± 10.00	BQL	BQL
Autumn	SA	ND	ND	BQL	ND	BQL	2.84 ± 0.35	ND	BQL
	ASA	ND	ND	BQL	BQL	ND	ND	ND	ND
	IB	ND	ND	BQL	ND	BQL	16.6 ± 0.7	ND	BQL
	PN	ND	ND	ND	ND	BQL	ND	ND	BQL
	AN	BQL	BQL	2.60 ± 0.52	BQL	BQL	3.67 ± 0.19	ND	1.70 ± 0.10
	NP	ND	ND	ND	ND	ND	ND	ND	ND
	MA	ND	ND	ND	ND	ND	ND	ND	ND
	DC	ND	ND	BQL	ND	ND	ND	ND	ND
Winter	SA	ND	ND	ND	ND	ND	ND	ND	BQL
	ASA	ND	ND	ND	ND	11.5 ± 0.28	221 ± 23	ND	ND
	IB	ND	ND	6.87 ± 0.19	ND	BQL	3.08 ± 0.21	BQL	BQL
	PN	ND	ND	ND	ND	BQL	ND	BQL	BQL
	AN	ND	ND	0.303 ± 0.016	0.26 ± 0.06	3.24 ± 0.29	BQL	1.29 ± 0.029	BQL
	NP	ND	ND	BQL	BQL	ND	ND	BQL	ND
	MA	ND	ND	BQL	BQL	ND	54.86 ± 4.15	BQL	ND
	DC	ND	ND	ND	ND	ND	ND	ND	ND

ND not detected and BQL detected but below quantification limit

4.5.1.2 Quantification

The high concentration of targeted pharmaceuticals was observed in biosolids at the Pietermaritzburg WWTP (Darvill) and more drugs were quantified at this site followed by Baynes spruit sampling site. Acetylsalicylic was found to range from not detected to 221 ng g^{-1} in biosolid, while in sediment it was found between not detected to 3.42 ng g^{-1} (Table 4.4). The partitioning of pharmaceuticals is influenced by humic acid, pharmaceuticals are likely to sorb to sludge because of it high organic matter content (Jones et al. 2006). The highest concentrations were found in winter and most target pharmaceuticals were quantified in the summer season.

4.5.2 Mgeni River

4.5.2.1 Occurrence

Targeted pharmaceuticals were not frequently detected in Midmar Dam, except acetaminophen, which was detected in summer, autumn and winter as presented in Table 4.5. Midmar Dam is located in the mountainous area away from most sources of contamination, and near the source of the Mgeni River. Most target analytes were detected after the joining of Msunduzi River to Mgeni River, which might suggest that Msunduzi River is also a source of contamination into the Mgeni River. It should be noted that Mgeni River is located away from people and the possibility of contamination is minimal. However, when the Mgeni River approaches the Durban Municipality there was a detection of pharmaceuticals in many sampling sites. The sampling site showing frequent occurrence of target pharmaceuticals is the joining point of the Msunduzi tributary to the Mgeni River. Most target pharmaceuticals were detected in biosolids at the Durban WWTPs and the Mgeni River estuary. Six drugs were detected in the estuary, this area is used for recreational activities (fishing and boat sports) and vulnerable to contamination through dumping during major events. In addition, the Mgeni Estuary is located after the Durban WWTP.

4.5.2.2 Quantification

Most targeted pharmaceuticals in the Mgeni River were quantified in the summer season and the results are presented in Table 4.5. The results showed high concentrations at WWTPs with diclofenac ranging from not detected to 206 ng g^{-1}. Acetylsalicylic acid was quantified in most sites in winter, but its highest concentration was found at the tributary joining point at 178 ng g^{-1} during the spring season. This further confirmed the contribution of the Msunduzi River toward the concentration levels of pharmaceuticals in Mgeni River. In general winter showed high concentrations of pharmaceuticals and this was attributed to the scenario that in winter the source of water for this river is likely to be recycled water from WWTPs with high concentration levels of pharmaceuticals entering the river system. Naproxen, meclofenamic acid and diclofenac were not quantified in spring and autumn in the Mgeni River.

Table 4.5: Detection and quantification of targeted pharmaceuticals in Mgeni River, seasonal concentration variation in Mgeni River (ng g^{-1}).

Season	Analyte	Sampling Sites												
		Midmar Inlet	Midmar Outlet	Howick Falls	Albert Falls Inlet	Albert Falls Outlet	Tributary Mgeni/ Msunduzi	Nagle Dam	Inanda Dam Inlet	Inanda Dam Outlet	Reservoir Hills	Business Park	Durban WWTP Biosolid	Umgeni Estuary
Spring	SA	ND	ND	ND	BQL	ND	2.57	1.01	BQL	BQL	1.36 ± 0.31	ND	BQL	BQL
	ASA	ND	ND	53.70 ± 8.88	ND	ND	191 ± 27	31.70 ± 4.10	200 ± 35	ND	178 ± 14	ND	40.90 ± 3.80	92.70 ± 10.00
	IB	ND	ND	ND	ND	ND	BQL	BQL	ND	BQL	BQL	ND	BQL	2.29 ± 0.59
	PN	ND	ND	ND	BQL	ND	BQL	BQL	ND	BQL	BQL	ND	BQL	ND
	AN	ND	ND	ND	BQL	0.80 ± 0.10	BQL	BQL	17.30 ± 3.41	ND	0.333 ± 0.024	ND	0.39 ± 0.05	ND
	NP	ND	ND	ND	ND	ND	BQL	BQL	ND	BQL	ND	ND	BQL	ND
	MA	ND	ND	ND	ND	ND	BQL	BQL	ND	BQL	BQL	ND	BQL	ND
	DC	ND	ND	ND	ND	ND	BQL	BQL	ND	BQL	BQL	ND	BQL	ND
Summer	SA	ND	ND	ND	0.26 ± 0.01	ND	ND	ND	ND	ND	ND	BQL	55.3 ± 10.8	ND
	ASA	ND	ND	ND	0.18 ± 0.01	ND	0.18 ± 0.01	2.10 ± 0.33	0.37 ± 0.00	BQL	ND	ND	24.4 ± 1.0	ND
	IB	0.23 ± 0.02	0.23 ± 0.01	0.47 ± 0.08	0.32± 0.02	ND	ND	BQL	0.35 ± 0.05	ND	ND	0.31 ± 0.00	20.20 ± 0.10	0.10 ± 0.02
	PN	BQL	BQL	BQL	BQL	BQL	BQL	ND	BQL	ND	ND	0.32 ± 0.01	40.81 ± 8.03	0.31 ± 0.00
	AN	0.20 ± 0.01	0.27 ± 0.04	0.27 ± 0.05	0.48 ± 0.01	BQL	0.34 ± 0.07	ND	0.22 ± 0.01	0.36 ± 0.01	0.37 ± 0.01	0.36 ± 0.01	6.00 ± 0.14	0.27 ± 0.07
	NP	ND	ND	ND	ND	ND	ND	ND	15.10 ± 1.00	1.40 ± 0.02	ND	ND	ND	ND
	MA	ND	ND	ND	ND	ND	ND	4.01 ± 0.30	0.98 ± 0.12	ND	ND	ND	46.70 ± 1.00	ND
	DC	ND	ND	ND	BQL	ND	ND	0.91 ± 0.05	3.75 ± 0.01	BQL	BQL	1.07 ± 0.15	206.00 ± 15.00	BQL

Season	Analyte	Sampling Sites												
		Midmar Inlet	Midmar Outlet	Howick Falls	Albert Falls Inlet	Albert Falls Outlet	Tributary Mgeni/ Msunduzi	Nagle Dam	Inanda Dam Inlet	Inanda Dam Outlet	Reservoir Hills	Business Park	Durban WWTP Biosolid	Umgeni Estuary
Autumn	SA	1.92± 0.05	BQL	BQL	BQL	BQL	3.99 ± 0.12	BQL	BQL	ND	ND	BQL	ND	BQL
	ASA	ND	ND	ND	ND	ND	ND	BQL	BQL	93.30 ± 5.00	ND	ND	ND	ND
	IB	BQL	BQL	ND	BQL	BQL	2.25 ± 1.17	BQL	BQL	2.52 ± 0.32	ND	BQL	ND	ND
	PN	ND	BQL	ND	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	ND	BQL
	AN	0.13 ± 0.07	BQL	ND	0.24 ± 0.06	0.52 ± 0.13	4.60 ± 1.20	BQL	BQL	BQL	BQL	6.18 ± 1.56	ND	1.03 ± 0.15
	NP	BQL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	MA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	DC	ND	ND	ND	ND	ND	ND	ND	BQL	ND	ND	ND	ND	ND
Winter	SA	ND	ND	ND	BQL	BQL	ND	1.15 ± 0.17	ND	ND	ND	ND	1.81± 0.29	43.2 ± 5.0
	ASA	ND	ND	ND	BQL	BQL	64.80 ± 14.40	ND	32.90 ± 0.94	97.09 ± 11.50	129 ± 4.81	ND	ND	BQL
	IB	ND	ND	ND	BQL	BQL	0.16 ± 0.01	ND	0.23 ± 0.03	0.47 ± 0.10	0.30 ± 0.07	ND	ND	13.40 ± 0.40
	PN	ND	ND	ND	BQL	BQL	ND	ND	ND	ND	ND	ND	ND	BQL
	AN	8.30 ± 0.40	ND	ND	BQL	BQL	BQL	ND	BQL	9.12 ± 1.13	1.25 ± 0.139	ND	0.670 ± 0.014	BQL
	NP	ND	ND	ND	BQL	BQL	ND	ND	BQL	ND	ND	ND	ND	4.31 ± 0.40
	MA	ND	ND	ND	BQL	BQL	BQL	ND	BQL	BQL	ND	ND	ND	5.40 ± 0.98
	DC	ND	ND	ND	BQL	ND	ND	ND	ND	ND	ND	ND	ND	ND

4.6 Comparison of the results between rivers and the literature

Considering the number of pharmaceuticals detected per season, most drugs were detected in summer followed by winter, and spring, and autumn had the least occurrence of pharmaceuticals in the environment in both rivers as shown in Table 4.4 and 4.5. Salicylic acid and acetaminophen were detected in all seasons; and acetaminophen was frequently detected in autumn. More drugs were detected in the Msunduzi River than in the Mgeni River with the exception of Inanda dam and Mgeni estuary sampling sites.

Table 4.6: Comparison of the proposed method environmental results and the literature method results.

Analytes	Spring ng g^{-1}	Summer ng g^{-1}	Autumn ng g^{-1}	Winter ng g^{-1}	References
Salicylic acid	ND BQL – 6.89	- BQL – 55.3	ND BQL – 3.99	- BQL – 43.2	Moreno-Gonzalez et al. 2015 Proposed method
Acetyl acetylsalicylic	- BQL - 200	- BQL – 33.2	212 – 427 BQL – 93.3	- BQL - 221	Agunbiade and Moodley 2016 Proposed method
Ibuprofen	ND - BQL – 2.29	- - ND – 20.1	- 4.76 – 9.56 BQL – 16.6	- - BQL – 13.4	Antonic and Heath 2007 Agunbiade and Moodley 2016 Proposed method
Phenacetin	- BQL – 0.109	- BQL – 40.8	- BQL - ND	- BQL - ND	No study Proposed method
Acetaminophen	ND – 222 BQL – 10.13	- BQL – 6.00	- BQL – 6.18	- BQL – 9.13	Paiga et al. 2016 Proposed method
Naproxen	ND – 60 ND – LOQ ND – 12.0 BQL – 2.52	- - 4.00 – 20.0 BQL – 15.1	- - 4.0 – 10.0 BQL - ND	- - 9.00 – 20.0 BQL – 4.31	Atomic and Heath 2007 Paiga et al. 2016 Varga et al. 2010 Proposed Method
Meclofenamic acid	8.00 – 13.0 BQL - ND	35.0 – 45.0 BQL – 47.0	- BQL – ND	18.0 – 28.0 BQL – 5.40	Zhou and Broodbank 2014 Proposed method
Diclofenac	10.0 – 15.0 ND BQL – 2.65 5.00 – 12.0 BQL – 9.53	55 – 65 - - 14.0 – 24.0 BQL - 206	- - - ND – 22.0 BQL – 222 BQL - ND	15.0 – 25.0 - - 10.0 – 38.0 BQL - ND	Zhou and Broodbank 2014 Atomic and Health 2007 Paiga et al. 2016 Varga et al. 2016 Agunbiade and Moodley 2016 Proposed method

ND not detected, - not considered in study and BQL detected but below quantification limit

The concentrations of these drugs were found to be higher in biosolid than in sediments. For example, the highest concentration of diclofenac in biosolid was 209 ng g^{-1} whereas in sediment

it was 8.1 ng g^{-1} at the sampling site closest to the hospital. Generally, the Msunduzi River exhibited higher concentrations of the selected pharmaceutical drugs than the Mgeni River. The Msunduzi River flows through Pietermaritzburg city and past informal settlements, while the Mgeni River flows through small towns and sparsely populated areas. Towards the ocean, the Mgeni River enters the Durban Metropolitan area, where the level of pharmaceutical drugs started to increase and more were detected. Salicylic acid, naproxen and meclofenamic acid were not frequently detected within the Mgeni River sediments except in spring, and the salicylic acid concentrations ranged from not detected to 50 ng g^{-1}. Salicylic acid is a metabolite of acetylsalicylic acid, and is prone to hydrolysis. Meclofenamic acid is known to be completely degraded by our bodies (Samaras et al. 2010). The high concentration of pharmaceuticals detected in Msunduzi River can be attributed to the scenario that pharmaceuticals enter Msunduzi River through various pathways, such as waste disposal or directly from human excretion because of the close proximity of the river to these activities.

However, malfunctioning sanitation systems have been reported to cause outbreaks all over the world (Kristina Blom, 2015). Sanitation is generally inadequate in rural areas and developing countries. Because of poor sanitation in rural areas, failure of hospital drainage system during rainy season and conventional method used by South African WWTPs pharmaceutical load enters Mgeni and Msunduzi Rivers constantly. WWTPs are focusing mainly on stabilizing biologically active substances and heavy metals while chemical compounds such as emerging contaminants are ignored. New technologies or methods are needed to manage sanitation better in South Africa. Occurrence of pharmaceuticals in sites that are in close proximity to a hospital highlights the need for physical barriers such as water seals for drainage that can prevent storm water from entering the drainage during rainy seasons. Proper faecal management and installation of sanitation facilities in rural areas might help to prevent the pollution of these rivers in these areas.

The concentration of these drugs in the matrix studied is similar to other studies done elsewhere using different analytical methods (Carmona et al. 2014) and also in comparison with Table 4.6. Table 4.6 shows that most studies focus on one or two seasons specifically winter and summer. There is little information available on four seasonal variations of the year for better comparison of our proposed method. However, Varga et al., (2010) did four seasons and their results showed similar patterns with our current work that occurrence of pharmaceutical is frequently in winter and summer with respect to diclofenac and naproxen drugs which they

studied. Moreover, our proposal showed better recovery of these drugs in the environment. In winter they found high concentration levels of these drugs compared to summer. Spring was the season of choice for most researchers reviewed as depicted in Table 4.6. The developed method was able to detect targeted drugs in different matrices in the environment and WWTP biosolids.

4.7 Conclusion

In this study, we developed an analytical method for the detection and quantification of eight pharmaceutical drugs in sediments and biosolids matrices, based on a derivatized-GC-MS method. The use of ultrasonic and centrifugation for extraction followed by derivatization provides low LOQs and therefore this developed method is useful for determination of acidic drugs in sediments and biosolids at trace levels. The validation results showed that the method can be used to study pharmaceuticals in the environmental solid samples. This method was successfully applied to the determination of eight pharmaceutical drugs in environmental samples collected along Mgeni and Msunduzi Rivers in a yearlong study, in KwaZulu-Natal, South Africa. These pharmaceuticals were found in higher concentrations in biosolids than in sediments, and the Msunduzi River had higher concentration levels of targeted drugs compared to Mgeni River. Acetylsalicylic and ibuprofen were frequently detected in most sites, while diclofenac was found to be predominantly at WWTPs (206 ng g^{-1}) in summer.

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Chapter 5: A simple method for occurrence and risk assessment of personal care products, pharmaceuticals and stimulant in Mgeni and Msunduzi Rivers, KwaZulu-Natal, South Africa

5.1 Abstract

In this work, a simple method to study the environmental occurrence and risk assessment of pharmaceutical and personal care products (PCPPs) is presented. A quantitative technique is described for ultrasonic-assisted solid-phase extraction (SPE) followed by GC-MS after derivatization for the simultaneous analysis of PCPPs; propyl paraben, triclosan, caffeine, carbamazepine and chloramphenicol. Ultrasonic assisted extraction together with centrifuge were used to extract sediment samples collected from the Mgeni and Msunduzi Rivers. An SPE procedure was used for clean-up and to concentrate selected compounds from diluted aqueous extracts. Final extracts were derivatized and analysed with GC-MS in selected ion monitoring (SIM) mode. The recoveries of the analytes ranged from 66% to 108%. The method detection limits were (0.08 – 1.82 ng g^{-1} solids and 0.08 – 10 μg L^{-1}) and liquid quantification limits (0.42 – 5.51 ng g^{-1} and 0.25 – 25 μg L^{-1}). The proposed method was applied in the evaluation of two rivers over a three month period in KwaZulu-Natal, South Africa. All targeted compounds were present in the environment at concentration levels between not detected to 174 ng g^{-1} and not detected to 30 μg L^{-1} for solids and aqueous environmental samples, respectively. A comparison of predicted no environmental effect concentration (PNECs) with measured environmental concentration (MECs) showed that these PCPPs present a high ecological risk to the receiving environment (agricultural lands or household). Our work is close to reality because we used MECs as opposed to using predicted environmental concentration (PECs) values, which is normally calculated from consumption, production of compound per year and various estimated factors. To our knowledge, this is a first report on the simultaneous assessment of occurrence and risk of PCPPs by GC-MS in Africa.

Keywords: ultrasonic, derivatization, GC-MS, personal care products, pharmaceutical and ecological risk assessment.

5.2 Introduction

The presence of emerging contaminants in the environment has raised concerns worldwide. Due to their increased usage and their pharmacokinetic properties, personal care products and pharmaceuticals (PCPPs) can be excreted in the parent form or as metabolites (Albero et al., 2012, Dai et al., 2014). PCPPs exposure assessments may be conducted by means of either laborious or exhaustive monitoring programs, which result in measured environmental concentrations (MECs), or by means of predicted environmental concentrations (PECs) (Ferrari et al., 2004, Bradbury et al., 2004, Celle-Jeanton et al., 2014). The need to know the levels of PCPPs in the environment is critical in understanding limits on water quality, and planning for suitable interventions to maintain or improve domestic and agricultural water supplies.

The primary source of these contaminants in the environment is through discharge of effluent from wastewater treatment plants (Albero et al., 2012, Giger et al., 2003, Haman et al., 2015). Other pathways are through sludge disposal into landfills and use of sludge for agricultural purposes (Albero et al., 2012, Muñoz et al., 2009, Uggetti et al., 2012). Leaching and runoff water containing these contaminants from such fields end up in rivers and dams. Fate modelling suggests that triclosan and parabens tend to sorb onto soil or sediment in the environment and from a biological perspective do not degrade at a fast-enough rate (Ying et al., 2007, Miller et al., 2008, Liao et al., 2013). Recently studies have been reported on the occurrence of these contaminants in environmental waters, soil and sediments (Santos et al., 2016, Al-Khazrajy and Boxall, 2016, Halling-Sorensen et al., 1998, Butkovskyi et al., 2017). Ramaswamy et al., (2011) reported a novel method for the trace analysis of triclosan and parabens in environmental water samples by using solid phase extraction (SPE) gas chromatography-mass spectrometry (GC-MS) detection (Ramaswamy et al., 2011). Gasperi et al., (2014) reported the first assessment of paraben and triclosan performed in France at a larger scale in 2014 using various analytical methods. Earlier studies showed that the concentration of these compounds in environmental solids ranged from 0.27 to 130 μg kg^{-1}. The public impression is that in recent decades the situation has become alarming since PCPPs have been reported to exist in the environment at a greater concentration than first anticipated. This impression does not take into account that many of the compounds that are currently reported to be present in the

environment were approved for human use three to four decades ago. However, their occurrence in the environment went unnoticed until the last decade, due to lack of analytical methods exhibiting sufficiently low detection limits and robustness in complex matrices.

An evaluation of the impact of PCPPs on the environment requires the availability of reliable data in all countries, so there is a demand for new analytical methods that can be used for extensive monitoring programs (Ocaña-González et al., 2015). Methods that can analyze parabens and triclosan simultaneously have attracted some interest. González-Mariño et al., (2011) reported the microextraction of parabens and triclosan in wastewater by GC-MS, and Nieto et al., (2009) developed a pressurized liquid extraction method for analysis of triclosan and parabens in sewage sludge. Environmental risk assessment is defined as an attempt to address the concern for potential impact of individual substances on non-target organisms. This is done by examining both exposure pathways resulting from discharges and/or application of impacted water and the consequences of such contamination on the food chain and function of the ecosystem (Agerstrand et al., 2015, Bound and Voulvoulis, 2004, Ferrari et al., 2004, Pereira et al., 2017). In addition, due to the lack of work done on evaluation of PCPPs in the environment, there is a methodological gap in the environmental assessment of surface waters (found in dams and rivers) affected by wastewater used for irrigation and drinking in South Africa. Guidelines usually consider only 2 possible pathways for a pollutant to enter the soil compartment (aerial and application of wastewater sludge), and WWTPs in South Africa discharge effluents into rivers which eventually feed into dams. Thus, there is a need to perform risk assessments on the various compartments that come into direct contact with effluent from WWTPs, and to predict the impact on agricultural soils. In South Africa, surface waters are used for irrigation without further purification. Thus, the introduction of emerging contaminants in agricultural lands through irrigation entails another pathway whereby these compounds can also enter the food chain.

The main objective of the present work was the development of a simple and rapid method suitable for risk assessment of PCPPs in the environment in four compartments: sediments, biosolids, and wastewater and freshwater, simultaneously. This method is based on sample preparation by sonication-assisted extraction followed by detection with GC-MS. Experimental parameters were optimized to achieve the maximum efficiency during analyte extraction and detection. The validated method was applied to the analysis of these compounds in various freshwaters, wastewater effluent, sediments and biosolid collected from the Mgeni and

Msunduzi Rivers. Risk characterization was performed by calculating the ratio between measured environmental concentrations (MECs) and predicted no-effect concentrations (PNEC) (MEC/PNEC ratio), where a value above one means that adverse effects are likely to occur, thus calling for risk-reduction measures (Inam et al., 2015).

5.3 Experimental

5.3.1 Chemicals and Reagents

Standards triclosan (irgasan), propyl 4-hydroxy-benzoate, caffeine, carbamazepine and chloramphenicol of high purity were used. Derivatizing reagent N, O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylsilyl chlorides (TMCs) were used without further treatment. All organic solvents were HPLC grade. Unless stated otherwise, all chemicals were purchased from Sigma-Aldrich (Germany) through Capital Lab Supplies CC (South Africa). All carrier gases, including those used for extraction, were of high purity and were bought from Afrox (South Africa). Acids and bases used in pH adjustment were of analytical grade and were purchased from Merck (South Africa). Doubly distilled water was obtained from a Bibby Sterilin LTD (United Kingdom) water still. Individual solutions of each analyte were dissolved in methanol. Further dilutions and mixtures of two compounds were made in ethyl acetate. A working standard mixture with a concentration of 10 µg mL^{-1} was prepared weekly by dilution of the stock solutions and used to spike solid samples. All the standard solutions were prepared gravimetrically and kept under 4 °C in the refrigerator.

Internal and surrogate standards: 4,4-di-tert-butylbiphenyl and phenoxyphenol were bought from Sigma-Aldrich (South African). *o*-chlorobenzoic acid and cinnamic acid were purchased from BDH chemical Ltd (South Africa).

5.3.2 Apparatus and Instrumentation

5.3.2.1 Extraction Equipment

Mesh used for sieving sediments after grinding were bought from KingTest laboratory (South Africa). Sediments were sonicated with a UMC C20 ultrasound bath bought from Ultrasonic

Manufacturing Company (South Africa). A Hettich Zetrifugen Rotofix centrifuge was bought from Labotec (South Africa). Extraction manifold and sorbents used for preconcentration and cleaning step (Oasis HLB 6 cc (60 mg) LP were bought from Microsep (South Africa).

5.3.2.2 Detection Instrument

All measurements were performed in a GC-MS system from Shimadzu QP2010 SE equipped with an autoinjector (AOC-20i) and autosampler (AOC-20s) (Japan). The GC was equipped with a capillary column (intercap 5 Sil MS 0.25 mmL. D x 30 M df = 0.25 µm, non-polar) (China).

5.3.3 Sampling

Water and sediments samples were collected along Mgeni and Msunduzi Rivers located in Kwa-Zulu Natal, South Africa (Figure 5.1). These two rivers pass through towns, agricultural fields, wastewater effluent discharge sites, landfills, hospitals and villages. Biosolids were collected from two wastewater treatment plants located approximately 80 km apart in two municipalities, Pietermaritzburg and Durban. These are the two biggest cities in the province of Kwa-Zulu Natal.

Sediments were sampled from the bank of the river with a stainless-steel spade and transported to the laboratory where samples were dried under air and sieved through a mesh (600 - 50 µm). Composite water samples (0.5 L) were taken 1 metre apart and collected in 2.5 L amber bottles, kept under 4 ℃and transported in the laboratory. While biosolids samples were collected from the inlet and outlet of the wastewater treatment plant grid. Solid samples were homogenized and stored in a cool place below 15 °C. The solid samples used in the recovery studies were sediments, biosolids and acid washed sand. No preservatives were added except samples collected after chlorination where sodium thiosulfate was added to prevent further degradation by residual chlorine in the samples. The samples were stored in a cooler box at 4 – 6 °C and transferred to the laboratory for further analysis.

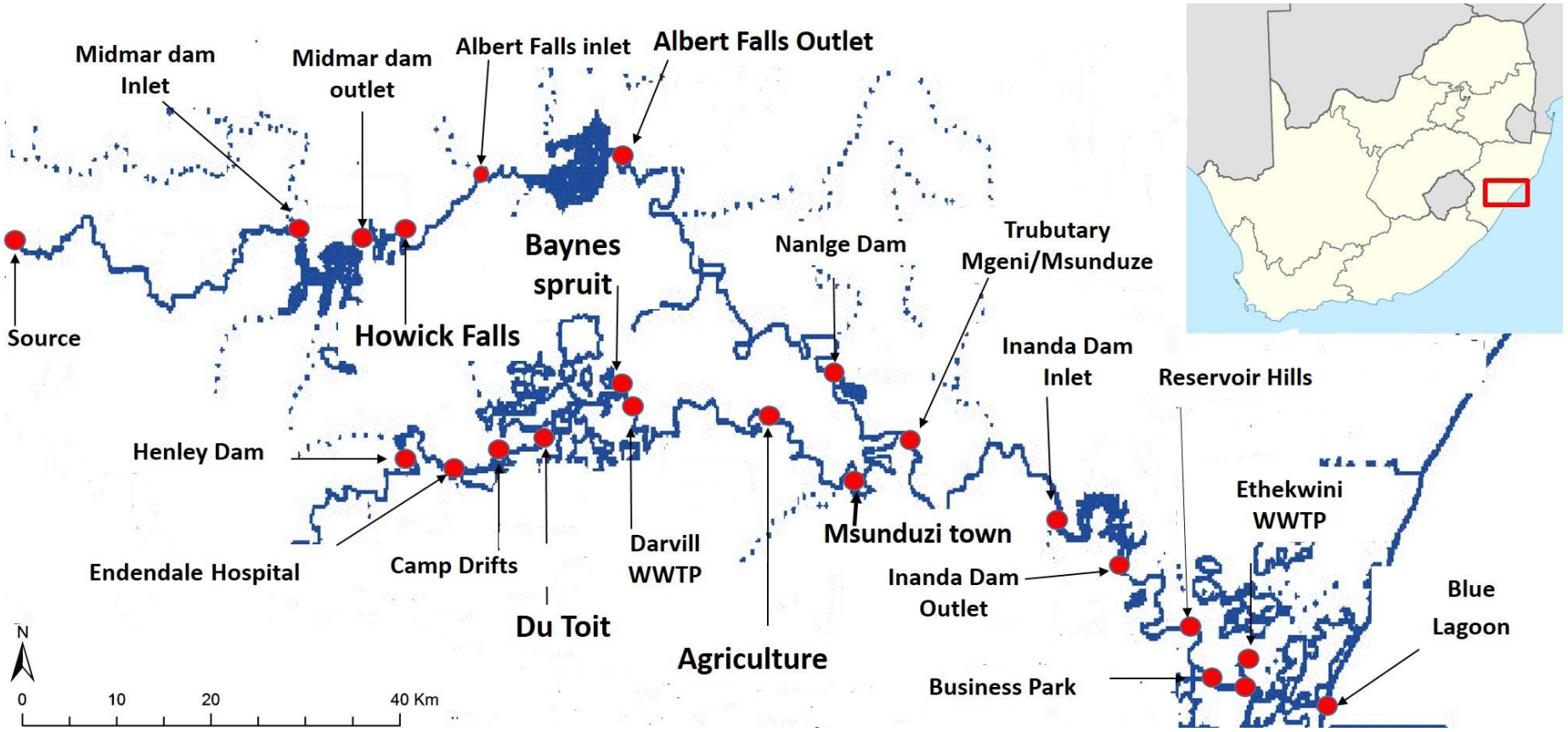


Figure 5.1: Sampling area of the Mgeni and Msunduzi Rivers in KwaZulu-Natal, South Africa. Grab samples were collected in all points and using the developed method described in this work (Map was drawn using GIS software shape file). .

5.3.4 Extraction Procedure

5.3.4.1 Extraction of River Water and Wastewater Samples

Surface water and wastewater were extracted with SPE using Oasis HLB cartridge (1 g) at pH 2 adjusted by adding 1 M of diluted sulphuric acid dropwise (final pH was determined using a pH meter). The cartridges were preconditioned with successive additions of 6 mL methanol, and ultra-pure water (pH 2 or pH 7). Subsequently, 1 L of the sample was extracted/pre-concentrated using a vacuum manifold at a flow rate of 5 mL min^{-1}. After air-drying the cartridge for 30 min under a gentle stream of nitrogen, the sample was eluted with a total of 9 mL of extraction solution (acetone/ethyl acetate 1:1 6 mL; methanol 1 mL; acetonitrile 1 ml; 1 dichloromethane 1 mL). The eluate was concentrated to dryness under nitrogen at 40 °C, and then the residues were re-dissolved with 100 µL of BSTFA derivatizing agent and heated at 70 °C for 30 minutes. Finally, the samples were diluted with acetonitrile and then analysed with GC-MS. To perform recoveries 1 L of separate river water, wastewater and distilled water sample was spiked with 100 µL and 1000 µL (10 mg L^{-1}) of to make final concentrations of 1 µg L^{-1} and 10 µg L^{-1}.

5.3.4.2 Ultrasonic-Assisted Extraction of Sediments and Biosolids

Spiked samples were prepared by adding 10 µL solution (10 mg L^{-1}) of triclosan, caffeine, chloramphenicol, carbamazepine and propyl paraben in ethyl acetate to an accurately weighed sample (10 g) and solvents were evaporated in dark conditions overnight below 15 °C to prevent degradation of the compounds by light. The concentration of analytes in the samples after drying was 10 ng g^{-1}. The non-spiked samples were also prepared in order to correctly assess absolute recoveries. Extraction of compounds from sediments, biosolids and acid washed sand was carried out by ultrasonic extraction in Teflon centrifuge tubes. Spiked and dried solid samples were transferred into clean 50 mL centrifuge tubes and 10 mL of acetone:ethyl acetate (1:1) was added. Tubes were immersed in an ultrasonic water bath for 20 min and centrifuge for 15 min, in two consecutive extraction steps (10 mL x 2). Fractions were combined and solvents were reduced to 1 mL with a gentle stream of nitrogen. Extracts were diluted with double distilled water and adjusted to pH 2 with sulphuric acid. The sample extracts were passed through an Oasis HLB cartridge at a flow rate of 5 mL min^{-1}, previously

conditioned with 3 mL methanol and doubly distilled water as mentioned in method section 5.3.4.1. Finally, extracts were evaporated to dryness under a nitrogen stream and followed by derivatization. Samples from the environment were extracted following the same method used to analyse spiked samples.

5.3.4.3 Derivatization

Extracts were redissolved into 100 µL of BSTFA + 1% TMCS, gently mixed while the vial was closed and allowed to react at room temperature for 2 minutes. Then the mixture was transferred into an oven to react for 30 min at 70 °C. After the derivatization process, extracts were diluted up to 0.5 mL volume with ethyl acetate and 2 µL of the derivatized sample extract was auto-injected into the GC-MS.

5.3.4.4 GC-MS Analysis

The samples were analyzed using a GC-MS (QP2010SE Shimadzu) system and separation was performed on a capillary column. The initial column oven temperature was 70 °C, injection port temperature kept at 250 °C and 2 µL samples were auto-injected in splitless mode. The carrier gas was helium at a constant flow rate of 8.0 mL min^{-1} and 61.5 KPa pressure. The oven temperature was kept at 70 °C for 1 min, then programmed at 30 °C min^{-1} to 190 °C (held for 1 min), followed by 15 °C min^{-1} to 230 °C (held for 3 min) and finally 30 °C min^{-1} to 270 °C, which was held for 1 min. The transfer line was set at 280 °C and the ion source at 200 °C. Electron energy for the filament was set at 70 eV. The ITD setting was as follows: mass range 50 – 850 *m/z* (full scan only) with start time of 4 min and end time of 14 min. SIM mode was used for quantification of analytes. Retention times, major fragment ions, and quantification ions were used to identify compounds of interest in the environmental matrices.

5.3.5 Risk Assessment

A preliminary risk characterization in the different environmental compartments was based on a tiered system outlined by the European Medicine Evaluation Agency (EMEA) and the Food and Drug administration (Stuer-Lauridsen et al., 2000, Bound and Voulvoulis, 2006, Commission, 1996). The risk quotient (RQ) method is the basic principle globally accepted in the development of environmental risk assessment (ERA) (Commission, 1996). A very important information needed in ERA is the concentration range at which a compound studied causes no effect on non-target organisms. These concentrations known as PNEC for the aquatic compartment is estimated from EC_{50} values obtained with acute toxicity test (algae, daphnia, and fish) and by application of an assessment factor. The evaluation of whether a substance poses a risk to organisms in the environment is based on the comparison of the detected contaminant concentration MEC with its PNEC organism in the environmental compartment.

The presented potential ecological risk is based on the estimation of RQ using the concentration of drug residues found in each compartment. A standard assessment factor (1000) introduced by Hernando et al., (2006) has been adopted because it accounts for all species in the environment compared to the factors (10 or 100) used by the other researchers. The PNEC in aqueous and solid compartments have been determined following the formula (1) and (2) formulated by Hernando et al., (2006) or obtained from the literature.

$$PNEC_{aq} = \frac{EC_{50}}{1000} \qquad 1$$

$$PNEC_{s} = \left[\frac{PNEC_{aq} \times K_p}{d}\right] \times 1000 \qquad 2$$

Where $PNEC_{aq}$ is the PNEC calculated for aqueous compartment, K_p is the solid – water partition coefficient and d is the density of solid.

When the RQ equals or exceeds 1 (MEC/PNEC ≥ 1) then ecological risk is suspected. In addition, a more descriptive criterion (RQ < 0.1 means low risk, 0.1 < RQ < 1 means medium risk, RQ > 1 means high risk) that has been employed in main studies was also used to interpret obtained RQ values.

5.4 Results and Discussion

5.4.1 Sample Preparation

The recovery of analytes from environmental samples in reasonable percentages depends on several factors, such as the type of extraction, clean-up, solvents, ionic strength or the pH and instruments for detection. Often most factors require evaluation and optimization prior to determination of contaminants in the environment. Extraction and clean-up of extracts for separation and preconcentration of analytes of interest are one of the most important steps in multi-residue detection and quantification of emerging contaminants in environmental matrices (Berlioz-Barbier et al., 2014). The applicability of solid phase extraction (SPE) in the clean-up step for the determination of emerging organic contaminants from environmental samples has been previously demonstrated in previous chapters. The effect of extract clean-up was briefly demonstrated in this current work in Figure 5.2. Sonication effect in the extraction process prior to SPE was initially assayed. This preliminary evaluation was undertaken using sediments samples spiked at 10 ng g^{-1} fortification level. The samples were prepared according to the method described above in the experimental section and extracts were obtained with and without sonication. These extracts were derivatized and analyzed with GC-MS. Optimum conditions include sonication and clean-up steps carried out simultaneously. Carbamazepine and chloramphenicol were not recovered when the sonication was not applied during the extraction. Although caffeine, propyl paraben and triclosan were extracted without sonication their yield was very low, actually lower than acceptable recoveries as shown in Figure 5.2. Low recoveries were attributed to the timeframe required to soak the solid samples in the solvents when extracting without the assistance of ultrasound. In some literature, 24 hours was needed for the extraction without sonication (Núñez et al., 2008). In this work, 2 hours was used and was comparable to our optimum conditions of extracting these compounds. Also without clean-up, only propyl paraben and triclosan were recovered. These two analyses showed that some compounds can be extracted independently of sonication or a clean-up step. High recoveries, when using ultrasound, has been attributed to the propagation of ultrasound pressure waves through the solvent and resulting cavitation phenomena (Núñez et al., 2008).

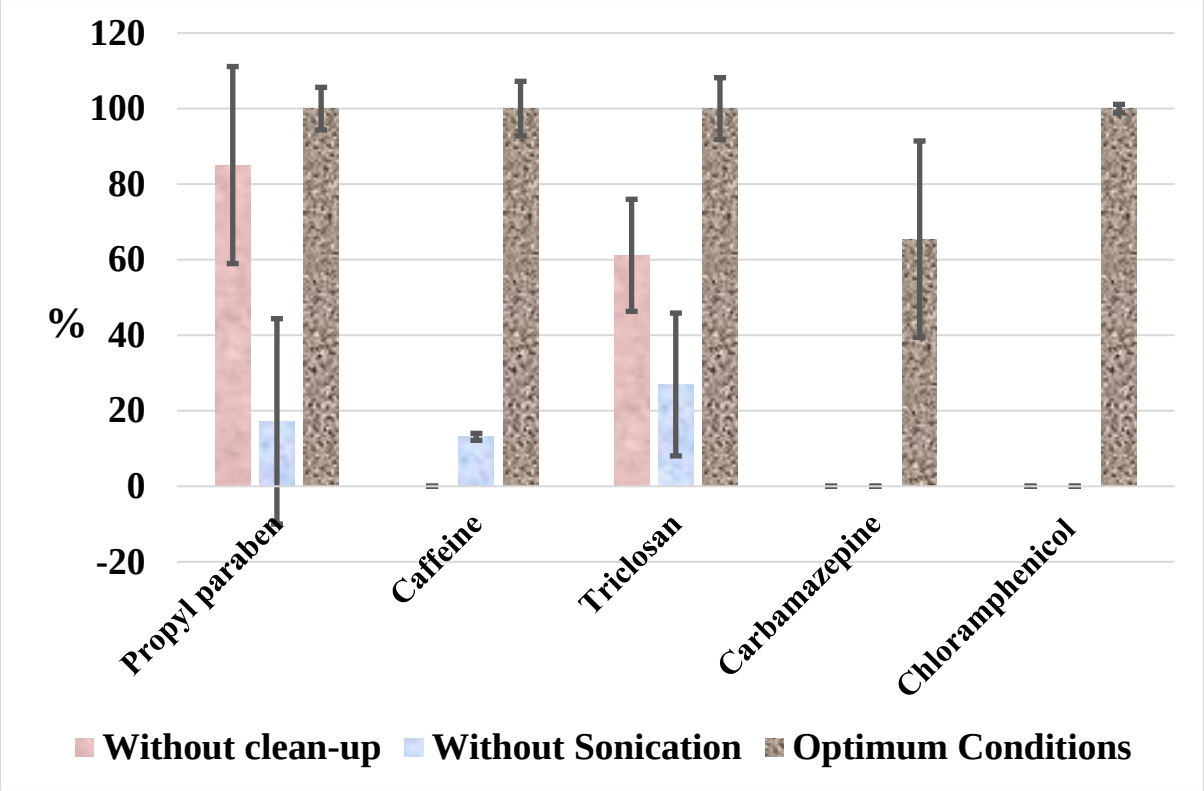


Figure 5.2: Influence of sonication and clean-up steps in the simultaneous extraction target compounds from spiked sediments samples at 10 ng g^{-1}.

Mechanical impact of sonic wave can also increase the interaction on the surface area between solid and liquid phases due to the possibility of size decrease in the solid matrix (Tang et al., 2009). The increased rates of mass transfer will also enhanced rate of solvent being transferred to the solid surface, and the transfer of the soluble component into the organic solvents will be enhanced. However, these proposed mechanisms depend on the ability of the extraction solvent to dissolve and keep all the analytes of interest in the soluble form (Albero et al., 2015). Recoveries can be affected if the analytes can precipitate and partition to solids during centrifugation after sonication. The selection of the most appropriate solvent for extracting the analytes of interest from the matrix of the sample is a basic step in the development of any method of extraction (Albero et al., 2015). To find the optimum extraction solvent, three solvents were chosen; methanol, dichloromethane and optimum solvents mixture (ethyl acetate:acetone 6 mL, methanol 1 mL, acetonitrile 1 mL and water 2 mL) based on literature and assayed as shown in Figure 5.3. Moreover, all these solvents can dissolve all the analytes.

Methanol was able to extract propyl paraben and triclosan with reasonable recoveries. However, methanol showed low recoveries for chloramphenicol and high recoveries for

carbamazepine up to 250%. Methanol has normally been avoided as an extraction solvent because of its ability to extract a lots of compounds in matrices and even the untargeted compounds, which are difficult to clean (Morales et al., 2005). Methanol extracts might have interfered with derivatization because of the complicated extracts. Dichloromethane showed low recoveries less than 50% except for triclosan with 95%.

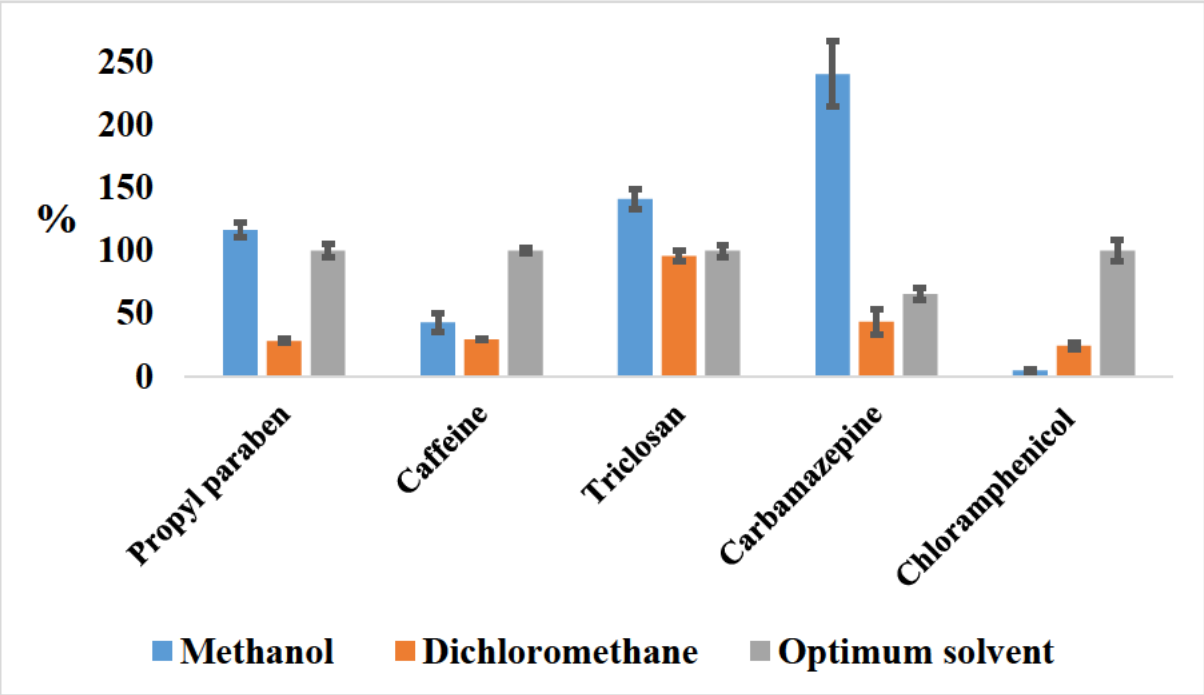


Figure 5.3: Influence of extraction solvent, methanol, dichloromethane and optimum solvent (acetone:ethyl acetate).

Compounds involved in this study had varying pK_a values ranging from 7.5 to 14.0. Therefore, their isolation at different pH values needed to be optimized. The aqueous distribution of compounds was investigated using water adjusted at three different pH values (2, 7 and 9) with HCl or NaOH as shown in Figure 5.4. Ethyl acetate:acetone (1:1), acetonitrile and methanol were used as elution solvents. Spiked double distilled water (10 µg L), aliquots of 200 mL, adjusted to pH 2, 7, and 9 were passed through Oasis HLB at flow rates between 5 – 8 mL min^{-1}. After elution, compounds were silylated and analysed by GC-MS. Recoveries were determined from the standard solution of the same concentration as corresponding aliquots of different pH values. The recovery values at pH 7 were low for caffeine and triclosan, while recoveries for propyl paraben and carbamazepine were observable above 120%. Propyl

paraben and triclosan had low recoveries at pH 9. These compounds were found to exist in two forms as silyl derivatives and methyl esters at pH 9, which in turn affected the recoveries because only peak areas from silyl derivatives were used for calculations of percent recoveries. Recoveries at pH 2 were acceptable and ranged from 65% to 108%.

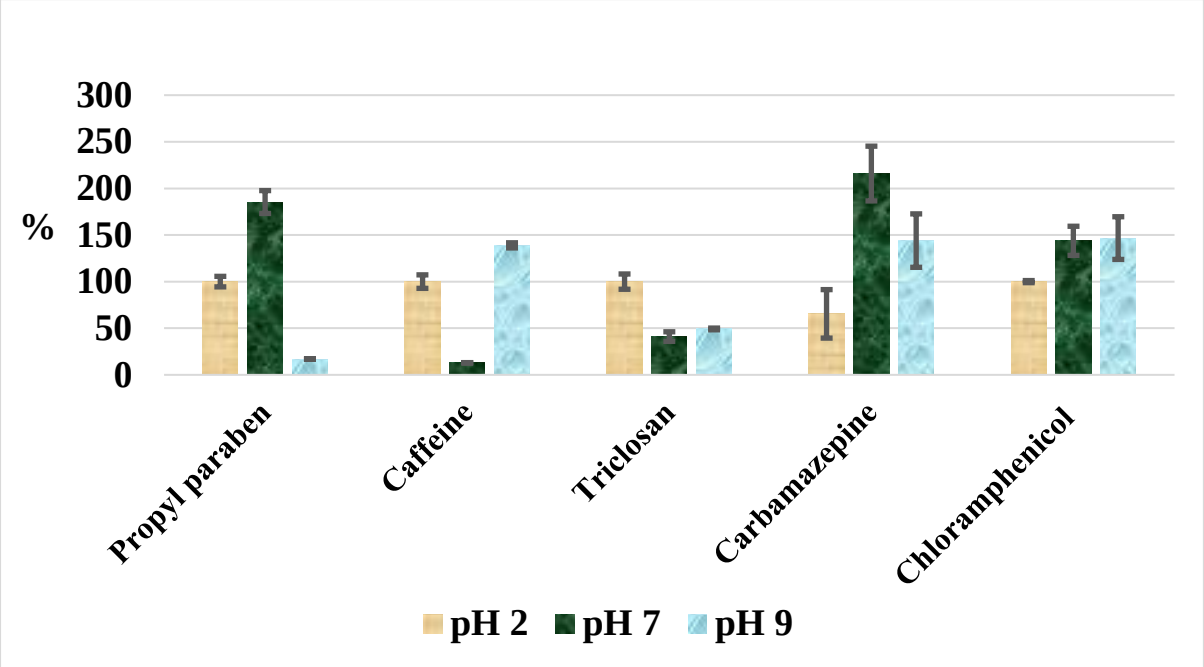


Figure 5.4: Influence of pH in the water extraction and clean-up step.

5.4.2 Method Validation

5.4.2.1 Recovery

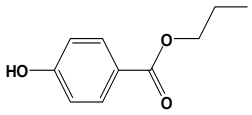
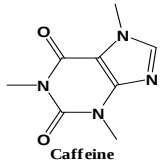
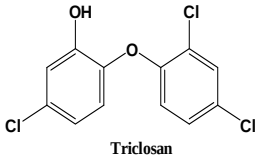
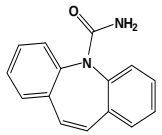
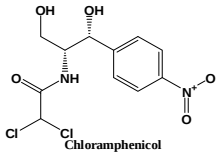
Recovery of the method was tested by adding known amounts of analytes (1, 10 and 20 ng g^{-1}) in triplicate sediment samples. These fortified samples were allowed to stand for 4 hours under a gentle stream of nitrogen to allow solvent evaporation before sonication and centrifuge extraction and extracts were subjected to GC-MS analysis after derivatization. Good recoveries of the compounds were obtained and ranged from 66% to 108% for all compounds. These results are reported in Table 5.1. The range of recoveries achieved is similar to that obtained by other authors in sediments as shown in Table 5.7, in line with IUPAC and EPA recommendation (Thompson et al., 2002).

5.4.2.2 Limits of Detection and Quantification

Limits of detection (LODs) and quantification (LOQs) were determined following IUPAC recommendation which considers these limits as the minimum amount of target analytes concentration that produces a signal-to-noise ratio (S/N) of 3 and 10 times the background chromatographic noise, respectively (Thompson et al., 2002). The S/N was measured at the lowest spiked level in the validation studies. Low limits were obtained due to the selectivity and high sensitivity of the GC-MS-SIM technique, allowing the determination of these PPCPs compounds at environmentally relevant concentration levels. Table 5.1 shows the LOD and LOQ values corresponding to the extraction by optimized method followed by derivatization-GC-MS analysis. These limits range from 0.14 to 1.9 ng g^{-1} for LODs and from 0.45 to 5.5 ng g^{-1} for LOQ. Relatively high limits for chloramphenicol were observed compared to other compounds, this was attributed to the fact that the labile hydrogen was shielded from possible easy derivatization. Nevertheless, the range of LODs and LOQs achieved in this proposed method are less than those reported by other authors (Núñez et al., 2008)

.

Table 5.1: Validation data (n = 3), Retention time, calibration data, linearity, recovery, detection limits (LOD), quantification limits (LOQ) and repeatability of the studied compounds.

Analytes	Sample matrix	Regression analysis	Linearity	Recovery %	LOD	LOQ	Retention Time	Precision %
 Propyl paraben	Solid ng g^{-1}	0.9971	0.1 – 50	94 - 105	0.14	0.42	7.2 – 7.45	3.0 – 20
	Aqueous µg L^{-1}	0.9972	1 – 500	98 – 102	1.5	4.0		
 Caffeine	Solid ng g^{-1}	0.9970	0.1 – 200	92 - 107	0.35	1.07	8.75 – 9.25	0.85 – 20
	Aqueous µg L^{-1}	0.9969	1 – 2000	96 – 104	4.0	11.0		
 Triclosan	Solid ng g^{-1}	0.9927	0.1 – 100	91 - 108	0.08	0.25	11.20 - 11.25	0.33 – 10
	Aqueous µg L^{-1}	0.9965	1 – 1000	94 – 100	0.89	2.7		
 Carbamazepine	Solid ng g^{-1}	0.9940	0.1 – 20	66 - 91	0.11	0.32	13.75 – 13.85	4.0 – 25
	Aqueous µg L^{-1}	0.9956	1 – 200	80 – 95	1.4	2.9		
 Chloramphenicol	Solid ng g^{-1}	0.9997	2.5 – 50	98 - 102	1.82	5.51	13.95 – 14.25	4.1 - 11
	Aqueous µg L^{-1}	0.9998	10 – 500	98 – 102	10	25		

5.4.2.3 Linearity

A multipoint calibration curve was obtained from a minimum of five extracted and derivatized standard solutions at different concentrations. A good linearity was obtained in the range within 1 to 200 ng g^{-1} with correlation coefficients higher than 0.99 for all compounds. These calibration results are summarized in Table 5.1.

5.4.2.4 Repeatability or Precision

The precision was determined by analysing the spiked sediment samples at three different concentration levels. These samples were analysed within given days. The RSD calculated between days ranged from 0.3 to 25%, less than 32% is recommended by the EPA.

5.5 Application to Real Samples

To the best of our knowledge, this is the first work reporting the simultaneous determination of these compounds in sediments, biosolids, freshwater and wastewater by a derivatization-GC-MS method in South Africa. Recently, GC-MS has emerged as a useful analytical technique because of its availability to most environmental laboratories in many developing countries. On the other hand, LC-MS/MS, which is a preferred technique for the determination of PPCPs in environmental samples, is still scarce in these countries. The LOQs obtained with the proposed method are within the range of those established with LC-MS/MS (Chu and Metcalfe, 2007, Stolker et al., 2004, Yu et al., 2011). Although the derivatization step is normally required in GC-MS analysis of PCPPs, this step was optimized in our work described in Chapter 3. The proposed method was applied for the analysis of sediments, biosolids, wastewater and freshwater samples from two rivers in South Africa.

5.5.1 Sediments and Biosolids

The sediment samples were collected over a three month period (May, July and August) from the Mgeni and Msunduzi Rivers. PCPP concentrations were determined in sediment samples, the levels found expressed as ng g^{-1} dry weight, are given in Table 5.2, 5.3 and 5.4.

Table 5.2: Concentration of the studied compounds (ng g^{-1}) in sediments collected in Mgeni and Msunduzi Rivers in the Month of May.

Mgeni River

	Propyl paraben	Caffeine	Triclosan	Carbamazepine	Chloramphenicol
Midmar inlet	0.41 ± 0.06	-	1.73 ± 0.05	0.46 ± 0.01	-
Midmar Dam outlet	-	-	D	D	30.75 ± 4.49
Albert Falls Dam Inlet	-	D	-	D	-
Albert Falls Dam outlet	-	4.13 ± 0.07	1.32 ± 0.39	-	_
Howick Falls	-	-	-	-	_
Joining point	0.58 ± 0.02	-	38.81 ± 7.93	-	_
Nagle Dam	-	-	-	1.17 ± 0.23	_
Inanda dam inlet	-	-	-	-	_
Inanda dam outlet	-	-	-	0.82 ± 0.06	-
Reservoir hills	-	--	-	-	-
Business Park	D	-	-	-	-
DWS inlet	0.88 ± 0.35	-	-	-	-
DWS outlet		-	-	-	-
Umgeni Estuary	D	-	-	-	-

Msunduzi River

	Propyl paraben	Caffeine	Triclosan	Carbamazepine	Chloramphenicol
Henley Dam	-	-	-	D	-
Hospital	-	-	-	D	-
Bay Spruit	-	D	7.02 ± 1.2	D	-
Du Toit	D	D	D	D	-
Camp Drift	-	-	-	D	-
Darvill WWTP outlet	-	-	1.18 ± 0.25	D	D
Agriculture	-	-	1.32 ± 0.32	D	-
Msunduzi Town	-	-	-	D	-

- Not detected, D detected but not quantified

In all of the analysed samples, propyl paraben was the predominant contaminant and this is expected since it is a water based preservative commonly used in food, personal care and

pharmaceutical products. In addition, propyl paraben occurs naturally and is found in some plants and insects. Propyl paraben was found at all of the sampling sites at levels from not detected to 31.81 ng g^{-1}. The highest concentration of was detected in July and the lowest levels were observed at 3 of the 22 sites in May and 6 of the 22 sites in July (Table 5.3).

Propyl paraben was found to occur more in the Mgeni River compared to the Msunduzi River. Given the path of Mgeni River, this might suggest even natural sources contribute to a load of propyl paraben in these rivers, while Msunduzi River passes mostly through urbanised areas. The concentration level of propyl paraben found in the study is higher than those found in Japan (nd – 2.84 ng g^{-1}) and US (nd – 3.52 ng g^{-1}), and lower than those reported in Korea (nd – 64.5 ng g^{-1}) (Liao et al., 2013, Yu and Wu, 2011).

Caffeine concentration levels were higher than the other compounds analysed, approaching 174 ng g^{-1} in the sample collected at Durban WWTPs in August (Table 5.4), followed by Mgeni estuary 128 ng g^{-1} and Henley Dam 90.00 ng g^{-1} (Table 5.3). The results of the present study with showed high concentrations of unmetabolized caffeine in both rivers. The increase concentration levels of caffeine may be associated with high consumption of coffee, tea and soft drinks as well as the improper disposal of these items. The relevance of these findings is that if caffeine is present in water, numerous pathogens and biologically active PPCPs are likely to be present as well. Determination of many pathogens is both difficult and technologically not feasible to monitor in environmental samples.

Triclosan is employed as an antimicrobial agent in various medical and consumer care products. Triclosan was found in both rivers at many sites and its concentration ranged from not detected to 79.10 ng g^{-1}. The triclosan compound had the second highest concentration recorded in this study. The incorporation of triclosan in a vast array of products resulted in it being discharged to WWTPs (Durban, 42.00 ng g^{-1} in July) and then into sediments (Mgeni Estuary, 79.00 ng g^{-1} in July). Triclosan is a relatively stable, hydrophobic and non-volatile compound, (K_{ow} 4.9) expected to adsorb in particulate matter and accumulate into sediments. However, low concentration levels were observed in May and August, which was attributed to photochemical degradation of triclosan. Because of reduced daylight and amount of light reaching the South African environment in July compared to other months, with temperatures below 20 ºC and not favouring degradation of triclosan, triclosan was detected in high concentration in July.

Carbamazepine was not found in most sites but was detected in all sites in Msunduzi River in May, (Table 5.2). This trend was attributed to the fact that carbamazepine undergoes extensive metabolism and thirty-three metabolites of carbamazepine have been identified from human and rat urine. Carbamazepine is not stable in its current form (Scheytt et al., 2005). Since it is used exclusively by humans, the anthropogenic drug in the environment represents human pollution (Ramaswamy et al., 2011). Therefore, it has been targeted in rivers and found to occur in many parts of the world. In this work it was found to range from not detected to 12.31 ng g^{-1}, lowest concentration quantified among selected compounds.

Table 5.3: Concentration of the studied compounds (ng g^{-1}) in sediments collected in Mgeni and Msunduzi Rivers in the Month of July.

	Mgeni River				
	Propyl paraben	Caffeine	Triclosan	Carbamazepine	Chloramphenicol
Midmar inlet	0.44 ± 0.01	-	-	-	D
Midmar Dam outlet	D	-	2.55 ± 0.94	3.45 ± 0.43	-
Albert falls Dam inlet	D	-	-	-	-
Albert falls Dam outlet	D	-	-	-	-
Howick Falls	0.45 ± 0.04	-	-	-	-
Joining point	D	D	D	-	-
Nagle Dam	D	-	-	-	-
Inanda dam inlet	D	-	-	-	-
Inanda dam outlet	1.73 ± 0.18	-	-	4.46 ± 0.21	-
Reservoir hills	0.62 ± 0.01	D	3.22 ± 0.50	12.31 ± 0.65	-
Business Park	-	-	-	-	-
DWS inlet	D	2.27 ± 0.09	-	-	-
DWS outlet	D	D	3.22 ± 0.94	-	-
Umgeni Estuary	13.50 ± 2.01	128.40 ± 18.64	79.06 ± 16.43	-	-
	Msunduzi River				
	Propyl paraben	Caffeine	Triclosan	Carbamazepine	Chloramphenicol
Henley Dam	31.81 ± 5.93	89.09 ± 15.93	-	-	-
Hospital	-	-	-	-	-
Bay Spruit	D	-	-	0.53 ± 0.02	-
Du Toit	D	-	-	-	
Camp Drift	-	-	-	-	-
Darvill WWTP outlet	-	D	42.24 ± 14.11	-	10.13 ± 1.27
Agriculture	D	D	1.19 ± 0.02	-	D
Msunduzi Town	D	-	10.56 ± 1.65	-	D

- not detected, D – detected but not quantify

Chloramphenicol was quantified in few sites compared to other compounds since approximately 80% of the compound is metabolized and excreted as the glucuronide, after administration. Some studies have shown that the metabolite glucuronide can convert back to the bioactive chloramphenicol in manure (Lai et al., 1995). Moreover, chloramphenicol is used as broad-spectrum antibiotic by humans in exceptional cases only such as meningitis (Tang et al., 2009). Nevertheless, its toxicity has led many studies to target it in the environment. The use of chloramphenicol in animal medicine is completely forbidden by the European Union because it causes damage to bone marrow in humans. In this study chloramphenicol ranged from not detected to 19.93 ng g^{-1}, these results are presented in Tables 5.2, 5.3 and 5.4.

Table 5. 4: Concentration of the studied compounds (ng g^{-1}) in sediments collected in Mgeni and Msunduzi Rivers in the Month of August.

	Mgeni River				
	Propyl paraben	Caffeine	Triclosan	Carbamazepine	Chloramphenicol
Midmar inlet	-	-	-	-	-
Midmar Dam outlet	D	-	1.97 ± 0.17	1.97 ± 0.07	D
Albert falls Dam inlet	-	-	-	-	_
Albert falls Dam outlet	-	-	-	-	_
Howick Falls	D	-	-	2.61 ± 0.18	D
Joining point	D	-	-	1.31 ± 0.68	_
Nagle Dam	D	-	-	0.54 ± 0.06	_
Inanda dam inlet	D	-	-	-	-
Inanda dam outlet	0.53 ± 0.04	-	-	5.07 ± 0.17	-
Reservoir hills	D	-	-	D	-
Business Park	D	-	-	D	-
DWS inlet	28.62 ± 3.61	173.90 ± 23.97	-	D	
DWS outlet	_		-	D	-
Umgeni Estuary	_	-	-	D	-
	Msunduzi River				
	Propyl paraben	Caffeine	Triclosan	Carbamazepine	Chloramphenicol
Henley Dam	-	-	-	-	-
Hospital	D	-	-	2.04 ± 0.54	-
Bay Spruit	-	-	4.38 ± 0.61	D	D
Du Toit	-	-	-	-	-
Camp Drift	5.59 ± 0.59	9.72 ± 0.40	-	D	19.93 ± 1.19
Darvill WWTP outlet	5.02 ± 0.50	1.92 ± 0.01	43.51 ± 4.97	4.70 ± 0.84	16.45 ± 1.86
Agriculture	3.97 ± 0.89	-		-	-
Msunduzi Town	D	-	0.39 ± 0.06	2.40 ± 0.06	-

- not detected, D detected but quantified

5.5.2 Fresh Water and Wastewater

Freshwater samples were collected from Mgeni and Msunduzi Rivers in KwaZulu-Natal, South Africa during the month of May, July and August. Wastewater was collected from two WWTPs in Durban and Pietermaritzburg municipalities. The presented mean concentrations and standard deviations of fresh water and wastewater are results of triplicate extraction and analysis of each sample with the developed method. Tables 5.5 and 5.6 present these mean concentrations of PCPPs found in water (dams and river water) and wastewater, the concentration ranged from a very quantifiable level to below the LOD. Measurable levels of propyl paraben, caffeine and triclosan observed in the Mgeni and Msunduzi Rivers and demonstrate the contamination of fresh (natural) waters in South Africa by PCPPs.

In Mgeni River and Ethekwini WWTP, all target PCPPs compounds were detected in the month of July as presented in Table 5.5. Most PCPPs were quantified in Ethekwini wastewater WWTP compared to the Mgeni River. The highest concentration of propyl paraben was found in the month of August at EThekwini WWTP influent at 12.30 μg L^{-1}. There was no significant variation observed for propyl paraben concentration. Caffeine was detected in almost all sampling sites except for the business park. The highest concentration of caffeine was also found in Ethekwini WWTP at 15.30 μg L^{-1} in July. Triclosan was detected in few sampling sites but was the only compound found in quantifiable level at the sampling point after Msunduzi River joined Mgeni River and at Mgeni Estuary. Triclosan was found at 5.3 μg L^{-1} and 4.5 μg L^{-1} concentration levels at the Joining point and Mgeni Estuary, respectively. Msunduzi River might have contributed to a load of triclosan at the Joining point and caused it to be high. The Mgeni Estuary is a recreational site where many people get together for braais and some national sports events are held at the site, which makes it vulnerable to contamination. Concentration levels of carbamazepine and chloramphenicol were found to be below quantification limit at all sites. These compounds belong to the group of prescription drugs and consumption of antibiotic is controlled in South Africa. However, their detection in the environmental surface waters used as a source in agricultural irrigation pose a threat for development and spread of antibiotic resistance genes. In Msunduzi River and Darvill WWTP, PCPPs were found in high concentration compared to Mgeni River. Msunduzi is directly exposed to contaminantion because is surrounded by informal settlements.

Table 5. 5: Concentration (µg L^{-1}) of personal care products and pharmaceuticals in fresh water and wastewater along Mgeni River.

	Environmental concentration of selected compounds µg L^{-1}														
	Propyl paraben			Caffeine			Triclosan			Carbamazepine			Chloramphenicol		
	May	July	August	May	July	August	May	July	August	May	July	August	May	July	August
Midmar inlet	-	-	-	-	D	-	-	-	-	-	-	-	-	D	-
Midmar Dam outlet	-	-	-	-	D	-	-	-	D	-	-	D	D	-	-
Albert falls Dam inlet	-	-	-	-	D	-	-	-	-	-	-		-	-	_
Albert falls Dam outlet	-	-	-	D	D	-	-	-	-	-	-	-	_	-	_
Howick Falls	-	-	-	D	D	-	-	-	-	-	-	-		-	-
Joining point	D	D	-	-	D	-	-	5.30 ± 0.45	-	-	-	-	_	-	_
Nagle Dam	-	-	-	-	D	-	-	-	-	-	-	D	_	-	_
Inanda dam inlet	-	D	-	-	-	D	-	-	-	-	-	-	_	-	-
Inanda dam outlet	-	D	D	D	D	-	-	-	-	D	-	-	-	-	-
Reservoir hills	-	D	D	D	D	D	-	-	-	-	-	D	-	-	-
Business Park	D	-	D	-	-	-	-	-	-	-	D	-	-	-	-
Wastewater influent	D	D	12.30 ± 1.06	< LOQ	15.30 ± 0.23	D	D	D	-	-	-	D	-	D	
Wastewater effluent	D	D	D	D	D	D	D	-	D	-	-	D	D	D	-
Umgeni Estuary	< LOQ	D	D	D	< LOQ	D	D	4.50 ± 0.04	-	D	-	-	D	D	-

- not detected, D – detected but not quantify

Propyl paraben highest concentration was found in Camp drift at 22.00 µg L^{-1}, followed by Henley Dam with 10.20 µg L^{-1} and the lowest quantifiable concentration of 4.00 µg L^{-1} was found at the Agricultural area sampling site as shown in Table 5.6. At this point subsistence farming is taking place, villagers irrigate their gardens and farms by collecting water directly from the Msunduzi River.

Table 5. 6: Concentration (µg L^{-1}) of personal care products and pharmaceuticals in fresh water and wastewater along Msunduzi River.

Selected compounds concentration µg L^{-1}						
Sampling site	Months	Propyl paraben	Caffeine	Triclosan	Carbamazepine	Chloramphenicol
Henley Dam	May	-	D	-	-	-
	July	10.20 ± 1.30	15.30 ± 2.30	D	-	-
	August	D	< LOQ	-	-	-
Hospital Stream	May	-	-	-	-	-
	July	-	D	D	-	D
	August	D	-	-	D	-
Bay Spruit	May	-	D	-	-	-
	July	D	-	20.0 ± 0.52	< LOQ	-
	August	-	-	< LOQ	D	D
Du Toit	May	-	-	-	-	
	July	D	-		-	D
	August	D	D	-	-	-
Camp Drift	May	-	-	-	-	-
	July	22.00 ± 0.03	< LOQ	D	-	-
	August	4.20 ± 0.35	< LOQ	-	D	< LOQ
Wastewater Effluent	May	-	< LOQ	5.2 ± 0.23	-	D
	July	D	9.00 ± 2.30	30.00 ± 5.60	D	< LOQ
	August	< LOQ	< LOQ	11.50 ± 0.05	< LOQ	D
Agriculture Area	May	-	-	< LOQ	-	-
	July	D	D	1.80 ± 0.32	-	D
	August	4.00 ± 0.89	-		-	-
Msunduzi Town	May	-	-	-	-	-
	July	D	10.50 ± 1.20	5.60 ± 0.35	D	D
	August	D	-	D	D	-

- not detected, D – detected but not quantify

The highest concentration of caffeine was found at Henley Dam at 15.30 μg L^{-1} followed by Msunduzi Town sampling site 10.50 μg L^{-1}. Triclosan occurred at a high concentration at the Darvill wastewater effluent. Carbamazepine and chloramphenicol were not quantified in the Msunduzi River as well. These drugs are not seasonal like flu medication, depend on the population of people prescribed for these compounds.

5.6 Risk Assessment Investigation

The Mgeni and Msunduzi Rivers receives a variety of organic waste from the informal settlement, urban areas, farms, industries and municipality sewage. Both subsistence and commercial farms rely on the surface water stored in the five dams found along these rivers for irrigation. In addition, villagers along these rivers use surface water without further treatment for their daily needs. Wastewater effluent discharged from WWTPs in South Africa is not tested or regulated for the levels of emerging contaminants. Biosolids in WWTPs is normally pre-concentrated, and put through a process of biological inactivation and later used for agricultural purposes or land rehabilitation. This has been widely recognised as another pathway for introduction of PCPPs into the environment (Thomaidi et al., 2016, Uggetti et al., 2012). This work was undertaken to develop a rapid risk assessment method for evaluation of PCPPs in the four compartments of the environment (fresh water, wastewater, biosolid and sediments). Most classes of PCPPs have been represented as antibiotic drugs (chloramphenicol), antiepileptic drugs (carbamazepine), personal care products (propyl paraben and triclosan) and stimulants drugs (caffeine). Recently, caffeine has been used as an indicator of environmental pollution by human excreta. In addition, the presence of caffeine in the environment indicates the potential existence of other emerging contaminants including pathogenic compounds.

The highest measured concentration of PCPPs residues in the environmental compartment was used to predict the ecological risk (Table 5.7). For compounds that were not quantified but were detected, the detection limits were used as their MECs. Hazard quotient or RQ values for the present study (0.01 – 600) were within the ratio range reported elsewhere (0.001 – 123628) (Aguirre-Martínez et al., 2013, Ramaswamy, 2015). All studied contaminants showed potential ecological risk in the environment when this freshwater or biosolids are used for human

consumption purposes. Since this is a preliminary study, further ERA studies are recommended.

Table 5.7: Potential ecological risk (in terms of RQ (MECs/PNECs)) of selected PCPPs in water, wastewater, biosolids and sediments using maximum MEC detected.

Risk assessment parameters	PNEC				MEC Highest measured environmental concentration			
Analytes	Water µg L^{-1}	Wastewater µg L^{-1}	Biosolids ng g^{-1}	Sediments ng g^{-1}	Water µg L^{-1}	Wastewater µg L^{-1}	Biosolids ng g^{-1}	Sediments ng g^{-1}
Propyl paraben	0.20 (Yamamoto et al., 2011)	0.26 (Molins-Delgado et al., 2016)	20.00 (Ramaswamy, 2015)	0.5206 (Harada et al., 2008)	22.00	12.30	28.62	5.59
Caffeine	87.50 (Zhou et al., 2010)	(Dai et al., 2014)	3.00 (Thomaidi et al., 2016)	2.37 (Aguirre-Martínez et al., 2013)	15.03	9.00	173.9	128.4
Triclosan	0.500 (Singer et al., 2002)	0.05 (Zhou et al., 2010)	0.399	50 (Zhou et al., 2010)	20.00	30.00	43.00	79.06
Carbamazepine	0.42 (Zhou et al., 2010)	139 (Dai et al., 2014)	46.00 (Butkovskyi et al., 2017)	0.32 l(Aguirre-Martínez et al., 2013)	< LOD (1.4)	< LOD (1.4)	4.67	3.45
Chloramphenicol	0.64 (Choi et al., 2008)	1.60 (Dai et al., 2014)	1.80 (Thomaidi et al., 2016)	0.267 (Zhou et al., 2014)	< LOD (1.8)	< LOD (1.8)	16.45	30.75
Risk Quotient or hazard quotient					Potential ecological risk			
	Water	Wastewater	Biosolids	Sediments	Water Risk	Wastewater Risk	Biosolids Risk	Sediments Risk
Propyl paraben	110.0	47.30	1.43	10.73	High	High	High	High
Caffeine	0.171	0.05	57.96	54.17	Medium	High	High	High
Triclosan	40.00	600	108.0	1.58	High	High	High	High
Carbamazepine	3.33	0.01	0.101	10.78	High	Low	Low	High
Chloramphenicol	2.81	1.12	9.14	115.0	High	high	High	High

5.7 Conclusion

A method for the determination of personal care products and pharmaceuticals, propyl paraben, triclosan, caffeine, carbamazepine and chloramphenicol, in four environmental compartments, namely: water, wastewater, biosolids and sediments, based on ultrasonic-assisted extraction followed by GC-MS after derivatization was developed. The use of ultrasonic extraction followed by sample clean-up with SPE provides low LOQs in sediments at trace levels. The proposed method has been properly validated, and the suitability of the procedure for analysis of PCPPs in compartments has been shown by established precision less than 25%. Several environmental samples from different parts of KwaZulu-Natal were analyzed and all the targeted PCPPs were detected in the environment. The levels ranged from not detected to 174 ng g^{-1} in and 22 μg L^{-1} in solid and aqueous samples, respectively. Risk assessment of these contaminants in the environment showed high potential ecological risk. More ERA studies are recommended to determine the extent of ecological risk in these compartments, as they are important for agricultural security in the region and to prevent further human exposure. Detection of an antibiotic (chloramphenicol) shows that there is need to regulate irrigation water and fertilizers (compost or biosolids) to minimize the risk of antibiotic resistance. This is the first simultaneous detection and risk assessment of PCPPs in Africa by GC-MS.

5.8 Reference

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Chapter 6: Analysis of emerging contaminants – a new spectral library for silylated derivative in single ion monitored mode

6.1 Abstract

There is growing interest to determine the unidentified peaks within a sample spectrum having intensities higher than the analytes of interest. Availability of reference standards and hyphenated instruments has been a key and limiting factor on the rapid determination of emerging contaminants in the environment. In this work, we developed a spectral library of derivatized compounds to be used on targeted GC-MS single ion monitored (SIM) mode for detection of pharmaceutical and personal care products in Mgeni and Msunduzi River. We also performed, suspect and non-target analysis to identify more novel contaminants in these rivers, which have previously not been reported or identified.

Keywords: Target analysis, suspect analysis, non-target analysis, GC-MS, emerging contaminants.

6.2 Introduction

Many polar micro-contaminants such as pharmaceuticals, personal care products, illicit drugs, plasticizers and flame retardants are present in the environment as evidence from various studies (Agunbiade and Moodley 2014; 2016, Albero et al., 2015, Dafouz et al., 2018, Sopilniak et al., 2018). Additionally, several transformation products can be formed from these various micro-contaminants, of which just a few have been identified (Wise, 2002, Bartels and von Tümpling Jr, 2008, Gavrilescu et al., 2015). Transformation products may be toxic, or even more toxic than the parent compound (Haakh, 2010). The level of toxicity maybe further exacerbated by the presence of potentially harmful unknown compounds that are simultaneously present in the environment together with priority contaminants. Methods on how to account for these various micro-contaminants and include such compounds in the analysis of environmental samples is of growing interest. Moreover, the presence of natural organic matter such as humic acid can interfere with extraction, ionization and identification of anthropogenic compounds.

In the literature, there are three approaches that are normally used for the detection and/or identification of compounds: target, suspect/post-target and non-target analytes (Gago-Ferrero et al., 2015, Sursyakova et al., 2017, Hernández et al., 2014). Target methods are limited to the restricted number of compounds, which have readily available (and sometimes costly) reference standards, and therefore the identification of new contaminants or problematic compounds within the environment may be delayed. Suspect screening takes advantage of databases with known structural compounds properties, including molecular formulae, fragmentation patterns, and retention times, which can then be computationally correlated to spectral mass spectrometry data to give potential similarities to the compound of interest. The third approach, non-target analysis, is of increasing interest but more challenging to undertake since no prior information is usually available (Gosetti et al., 2015, Hauler and Vetter, 2015, Plassmann et al., 2016). Even with the help of automated peak-picking software, thousands of peaks can be detected in an individual sample (Schymanski et al., 2015). Consequently steps must then be taken to reduce the number of peaks to more manageable numbers, deduce suitable molecular formulae, isotopic pattern distributions, mass defect analysis, and retention time prediction (Bade et al., 2016, Hauler and Vetter, 2015, Solliec et al., 2015).

Due to its selectivity and accuracy, mass spectrometry is a method of choice to screen and identify unknown compounds (Jernberg et al., 2013). It was initially based on the comparison of the identification of the fragmentation pattern identification of mass spectra acquired with gas chromatography (GC-MS). However, application of GC-MS in full-scan screening methods leads to low sensitivity/detection limits, poor selectivity and many peaks are detected in a single sample leading to false reporting (Baduel et al., 2015). Moreover, most emerging pollutants are polar and GC-MS is not suitable for their analysis in the environmental matrices. More recently, the evolution of accurate high-resolution mass spectrometry (HRMS) initiated a new trend in analytical data processing towards non-target analytical methods. Multi-residue analytical methodologies are powerful tools, as they may provide greater knowledge about overall contamination and fast-track the identification of unknown compounds in the environment (Gago-Ferrero et al., 2015, Solliec et al., 2015, Tang et al., 2016, Castro et al., 2016).

Combination of GC-MS, derivatization of samples and detection in single ion monitoring (SIM) mode methods may offer some advantages when compared to HRMS methods in terms of selectivity, sensitivity, simplicity, low-cost and ion suppression due to matrix effects. However, the lack of libraries for the derivatized compounds in their SIM mode compared to full-scan mode has resulted in this method being overlooked for the screening of environmental samples in favour of HRMS. GC-MS methods are known to be cheap and robust if they are developed properly. They can be reliable in our technological society where more than 100 000 chemical substances are registered, with several hundred new chemicals being introduced and registered every year. Because of the potential adverse environmental and/or health outcomes associated with exposure to some of these chemicals, data concerning the presence of known and unknown compounds in the environment must be provided timeously.

In this paper, we propose a new spectral library of derivatized micro-pollutants based on SIM mode and a non-target method for the analysis of emerging pollutants in the environment. The developed method was used to screen portable water, wastewater, biosolid and sediment samples from Mgeni and Msunduzi Rivers. We discovered new molecules in the environment that have not been reported in South Africa or elsewhere in the world.

6.3 Experimental

6.3.1 Chemicals and Reagents

Analytes: salicylic acid, ketoprofen, ibuprofen, diclofenac, meclofenamic acid, naproxen, sulfamethazine, clozapine, carbamazepine, triclosan and propylparaben were purchased from Sigma-Aldrich (South Africa). Phenacetin, procaine, chlorpromazine and chloramphenicol were purchased from BDH chemical Ltd (South Africa). Caffeine was purchased from Fluka (South Africa). Acetylsalicylic acid was purchased from Merck (South Africa). Cocaine, methamphetamine, morphine and cocaethylene were purchased from the Industrial analytical laboratory (South Africa).

Internal and surrogate standards: 4,4-di-tert-butylbiphenyl and phenoxyphenol were bought from Sigma-Aldrich (South African). *o*-chlorobenzoic acid and cinnamic acid were purchased from BDH chemical Ltd (South Africa).

Reagent and solvents: dichloromethylsilane, 99% N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylsilyl chloride (TMSCl) were of analytical purity and were purchased from Sigma-Aldrich (South Africa). Analytical grade hydrochloride acid (HCl, 37%) was bought from Merck (South Africa). Hexane, acetone, acetonitrile, dichloromethane, toluene methanol and ethyl acetate were chromasolv® grade (99.9%) purchased from Sigma-Aldrich (South Africa). Double distilled water was obtained using an Aquation Biby A4000D water purification system (Biby Sterlin LTD (UK)). All carrier gases, including those used for extraction, were of high purity (99,999%) and were purchased from Afrox (Durban, South Africa). All chemicals were used without further purification.

6.3.2 Apparatus, Materials, and Instruments

All glassware, including amber bottles used for sampling, were washed with phosphate-free soap dynachem (South Africa) and soaked in an acid bath for 24 hrs. After the acid bath, all glasswares were then rinsed with 5% dichloromethylsilane in toluene and methanol respectively, and then heated at 60 ºC for 12 hrs. Small volumes were measured by micropipette

plus kit Dragon lab (China) ranging from 0.5 to 1000 µL. All glass fibre Millipore filters were bought from Pall Corporation (South Africa). Extraction manifold and sorbents used for extraction (Oasis HLB 20 cc (1 g and 60 mg) LP, sepak-pak plus CN cartridge and tC18 environmental cartridge sepak-pak) were purchased through Microsep from Waters (United State of America (USA)). The GC-MS used was a Shimadzu QP2010 SE equipped with an autoinjector (AOC-20i) and autosampler (AOC-20s) (South Africa, Kyoto Japan, respectively). The GC was equipped with a capillary column, Crossbond 5% diphenyl/95% dimethyl polysiloxane (intercap 5 Sil MS 0.25 mml. D x 30 M df = 0.25 µm, non-polar) bought from Restek (USA). Both glassware and instrument were kept at a laboratory temperature of 20 ºC.

6.3.3 Software and Databases

GC-MS solution software in postrun mode and assisted by online databases: ChemSpider, Drugbank, PubChem, National Institute of Standards and Technology (NIST) and metabolomics (for identification of possible drug metabolites) were used.

6.3.4 Preparation of Stock Solutions

Stock solutions of each compound, internal standard (IS) 2-chlorobenzoic acid, surrogate standard 3-phenylprop-2-enoic acid, cinnamic acid and injector standard 4,4-di-tert-butylbiphenyl (1000 µg L^{-1}) were prepared in methanol and stored at 4 ºC. Working solutions of the standards containing 10 000 µg L^{-1} of each target analyte and internal standards were also prepared and used in optimizing the derivatization procedure. All solutions including samples were evaporated to dryness under a gentle stream of nitrogen and subjected to derivatization and GC-MS analysis in optimal conditions.

6.3.5 Sampling

Sampling was carried out along the Mgeni and Msunduzi Rivers situated in the Kwa-Zulu Natal province catchment area, South Africa. Additionally, information on sample sites can be found in the previous Chapter 3, 4 and 5.

6.3.5.1 Water Sampling and Sample Treatment

The samples of river water and wastewater were collected in 2.5 L amber glass bottles using a grab sampling method. No preservatives were added, the samples were stored in a cooler box at 4 – 6 °C and transferred to the laboratory for further analysis.

Solid phase extraction (SPE) of the liquid samples was carried out on Oasis HLB cartridge (1 g) at pH 2 and pH 7; pH was adjusted by adding 1 M of diluted sulphuric acid dropwise (final pH was determined using a pH meter). The cartridges were preconditioned with successive additions of 6 mL methanol, and ultra-pure water (pH 2 or pH 7). Subsequently, 1 L of the sample was extracted/pre-concentrated using a vacuum manifold at a flow rate between 5 to 10 mL min^{-1}. After air-drying the cartridge for 30 min under a gentle stream of nitrogen, the sample was eluted with a total of 9 mL of extraction solution (acetone/ethyl acetate 1:1 6 mL; methanol 1 mL; acetonitrile 1 ml; 1 dichloromethane 1 mL). The eluate was concentrated to dryness under nitrogen at 40 °C, and then the residues were re-dissolved with 100 µL of BSTFA derivatizing agent and heated at 70 °C for 30 minutes. Finally, the samples were diluted with acetonitrile and then analysed with GC-MS.

6.3.5.2 Sediment sampling and sample preparation

Sediment samples were collected from different sampling sites using the grab method and were covered with aluminium foil. Sediments were air dried at 30 °C for 3 days, then ground by hand in a ceramic mortar and pestle, and sieved through different layers of mesh to obtain a final particle size of 53 µm (600, 400, 300, 200, 100, 75 and 53 µm) to ensure consistency and normalisation of the sample.

Sediment samples were subjected to an ultrasound extraction procedure, and each sediment sample was treated twice. In a typical treatment step, a mass of 10 g of sediment (dry weight) was placed in a 50 mL centrifuge tube (PTFE) with 10 mL of solution (acetone/ethyl acetate (1:1) 8 mL and water/acetonitrile (1:1) 2 mL). The samples were initially shaken vigorously and then placed in an ultrasonic bath for 20 minutes at room temperature. Samples were centrifuged at 6000 rpm for 20 minutes and the extraction solvent was decanted into a silylated glass bottle. After centrifugation, extracts from two-treatment steps (on 1 sediment sample) were mixed before the clean-up/SPE procedure.

The mixed extracts obtained in the ultrasound-centrifugation step were evaporated to 0.5 mL in a glass vial under nitrogen stream. The contents of the vial were transferred into a 250 mL glass bottle *via* rinsing with 0.5 mL methanol and diluted to 200 mL with ultrapure water and the pH adjusted to pH 2 or pH 7 with 1 M sulphuric acid. This solution was percolated through the Oasis HLB cartridge (60 mg) previously conditioned and eluted as in the above-mentioned procedure in section 6.3.5.1.

6.3.6 Biosolid Sampling

Biosolid samples were collected at the influent of the wastewater treatment plants, and are partially concentrated solids from the sewage. These were dried under air and processed using the same procedure used to treat and extract analytes from the sediment samples as described in section 6.3.5.2 above.

6.3.7 GC-MS Analysis

Analysis of target, suspect and non-target compounds were detected by Shimadzu QP2010 SE equipped with an auto-injector (AOC-20i) and autosampler. The initial column oven temperature was 70 °C, injection port temperature was kept at 250 °C and 2 µL samples were auto-injected in splitless mode. The carrier gas was helium at a constant flow rate of 8.0 mL min^{-1} and 61.5 KPa pressure. The oven temperature was kept at 70 °C for 1 min, then ramped at 30 °C min^{-1} to 190 °C (held for 1 min), followed by ramping at 15 °C min^{-1} to 230 °C (held

for 3 min) and finally ramping at 30 °C min^{-1} to 270 °C which was held for 1 min. The transfer line was set at 200 °C and the ion source at 200 °C. Electron energy for the filament was set at 70 eV. The ITD setting were as follows: mass range 50 – 850 *m/z* (full scan only) with start time of 4 min and end time of 30 min. and was also operated in single ion monitoring (SIM) mode for analysis of target analytes. This GC-MS method was based on initial work, and quantification of acidic drugs in surface waters (Chapter 3)

6.3.8 Data Analysis

The GC-MS system was controlled using GC-MS Solution software from Shimadzu. The analysed data were processed using Postrun, which is an application manager of GC-MS Solution. Postrun software allows for peak detection with automatic library searching and comparison features after integration. Integration is based on the peak area, height and rejection parameters such as signal to noise ratio, slope, and drift. The instrument was operated in both full scan and single monitoring (SIM) mode, only compounds with an *m/z* between 30 and 850 were monitored. The peaks recorded by the instrument were matched with library spectra based on these parameters: minimum similarity, search depth, hit number and retention index. The number of compounds registered in a public library is so vast that some suggested compounds were to be rejected using similarity index. The similarity index is a quantitative expression of the difference between the spectrum of an unknown sample and spectrum recorded in a library as shown in Figure 6.1.

The similarity index (SI) is calculated using the equation below.

$$SI = \left(1 - \frac{\sum_{\frac{m}{z}} \left\| lu\left(\frac{m}{z}\right) - lt\left(\frac{m}{z}\right) \right\|}{\sum_{\frac{m}{z}} \left\{ lu\left(\frac{m}{z}\right) + lt\left(\frac{m}{z}\right) \right\}}\right) \times 100$$

lu(*m/z*): relative spectrum intensity of the *m/z* of the unknown mass spectrum.

lt(*m/z*): relative spectrum intensity of the *m/z* of a mass spectrum recorded in a library.

An SI of 100 indicates mass spectra that are identical, while an SI of 0 indicates spectra that are completely different as shown in Figure 6.1.

The search for a list of potential positive compounds was done by either using the database based on trimethylsilyl (TMS) derivatives or ChemSpider, DrugBank, PubChem and NIST. The results of the external database were compared with the GC-MS Solution embedded library. If the compound commercially existed, hits, which only represented chemical formulas, were excluded.

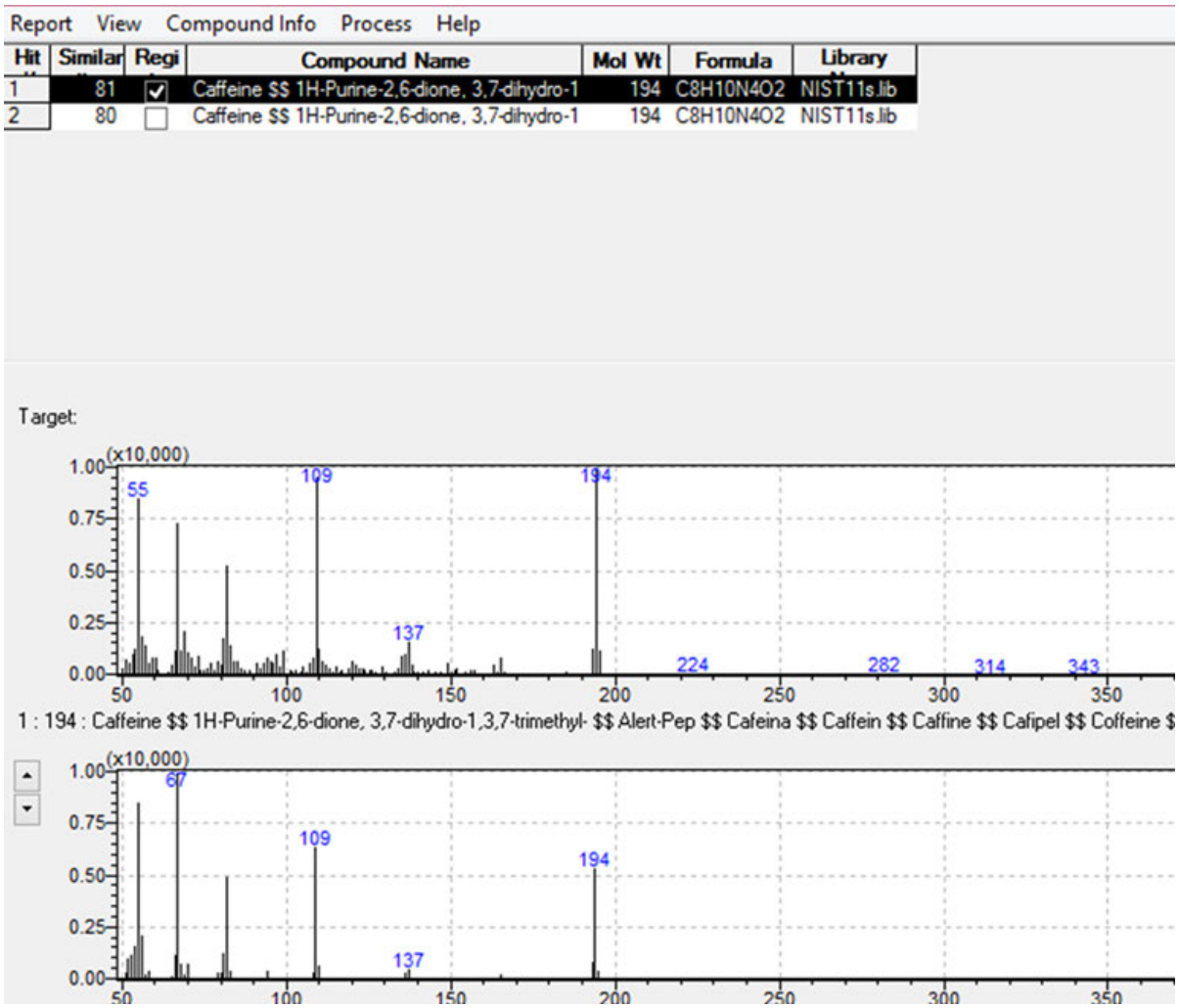

Figure 6.1: Analysis of environmental sample showed the positive presence of and similarity index was used with an identical factor of 80, which give an indication that caffeine was present in the sample.

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6.4 Results and Discussion

6.4.1 Recoveries, confirmation and validation

The extraction method was derived from our previous work, which was based on the analysis of acidic drugs and PCPs in both solid and water samples taken along the Mgeni and Msunduzi Rivers (Chapter 3, Matongo et al., 2015, Agunbiade and Moodley, 2014). Aqueous extraction solvents showed poor recoveries for semi-polar antibiotics, and organic solvents revealed low recoveries. Many studies have used acetonitrile and methanol to extract different drug residues (Alvarez-Ruiz et al., 2015, Antonic and Heath, 2007, Blackwell et al., 2004, Castiglioni et al., 2008, Archana et al., 2016). Moreover, acetone/ethyl acetate was preferred over methanol or acetonitrile, since methanol and acetonitrile extracts a large number of matrix components, which can complicate identification of known or unknown compounds in the sample (Gonzalez-Marino et al., 2010, Wu et al., 2013). Clean-up is an important step for compounds with different physicochemical properties, and SPE methodologies are amongst the most widely used. Analyte loss can occur with an excessively selective SPE clean-up, but this can be significantly reduced with a hydrophilic/lipophilic phase sorbent (such as Oasis HLB cartridges) with pH adjusted and loading steps that are carefully controlled. Since a large number of emerging contaminants are polar and contain heteroatoms such as oxygen, this type of cartridge is able to capture a broad class of polar compounds. For example, veterinary antibiotics, PCPs (such as triclosan and propylparaben) and pharmaceuticals, may create favourable hydrophilic and/or π-π bonding interactions with the cartridge, thus allowing for the retention of these various analytes. In addition, this type of cartridge also retains a large number of polar compounds, such as primary or secondary amines, which could result in several other compounds, besides the target analytes being retained on the cartridge (Togola and Budzinski, 2007).

The compounds remaining after statistical treatment were first confirmed by visually checking their presence and their chromatogram peak shape in the TIC spectrum. In addition, their exact masses were associated with their supposed chemical formulas *via* the peak picking tool and the in-house library as shown in Table 6.1.

Table 6.1: New library for derivatized pharmaceutical and personal care products, physicochemical properties of targeted analytes. (Library built from reference standards)

Target analytes	**Type**	**Chemical formula**	**Molar mass** **g mol^{-1}**	**p*Ka*** **(25 °C)**	**LogK_{ow}**	**Retention time** **Minutes**	**Fragment pattern** ***m/z***	**SIM** ***m/z***
Methamphetamine	Illicit drug	$C_{10}H_{15}N$	149.24	9.87	2.23	5.450	58, 91, 134, 148	58, 91
Salicylic acid	NSAID	$C_7H_6O_3$	138.12	2.97	2.26	6.345	73, 135, 193, 209, 267	135, 267
Acetylsalicylic acid	NSAID	$C_9H_8O_4$	180.16	3.50	1.19	6.467	65, 73, 120, 195, 210, 268	120, 195
Nalidixic acid	Antibiotic	$C_{12}H_{12}N_2O_3$	232.23	5.95	1.50	6.914	73, 116, 162, 180, 236, 301	180, 236
Ibuprofen	NSAID	$C_{13}H_{18}O_2$	206.29	4.85	3.50	7.105	73, 117, 160, 191, 263, 278	117, 160
Propyl paraben	Antifungal agent	$C_{10}H_{12}O_3$	180.20	8.50	3.04	7.458	73, 116, 162, 180, 236, 301, 251	162, 236
Phenacetin	Analgesic	$C_{10}H_{13}NO_2$	179.22	14.98	1.57	7.800	53, 109, 137, 179, 209	109, 179
Acetaminophen	NSAID	$C_8H_9NO_2$	151.16	9.46	0.51	8.000	73, 106, 166, 181, 223	181, 223
Phenoxyphenol	Standard	$C_{12}H_{10}O_2$	186.21	6.96	4.76	8.250	73, 122, 150, 185, 258	150, 258
Morphine	Opioid analgesic	$C_{17}H_{19}NO_3$	285.33	10.26	0.99	8.450	75, 103, 119, 174, 204, 232, 285	232, 204
Caffeine	Stimulant	$C_8H_{10}N_4O_2$	194.19	14.00	-0.07	8.912	109, 194	109, 194
Naproxen	NSAID	$C_{14}H_{14}O_3$	230.26	4.19	3.26	10.685	173, 41, 185, 243, 302	185, 243
procaine	Anaesthetic	$C_{13}H_{20}N_2O_2$	236.21	8.05	1.92	10.950	58, 86, 164	58, 86
Triclosan	Disinfectant	$C_{12}H_7Cl_3O_2$	289.54	7.68	5.53	11.250	109, 185, 200	109, 200
Meclofenamic acid	NSAID	$C_{14}H_{11}Cl_2NO_2$	296.15	3.79	5.11	11.990	73, 152, 208, 223, 313, 180	223, 313
Ketoprofen	NSAID	$C_{16}H_{14}O_3$	254.28	4.45	3.12	12.105	73, 105, 165, 179, 253, 282, 311	282, 311

Target analytes	**Type**	**Chemical formula**	**Molar mass** **g mol^{-1}**	**p*Ka*** **(25 °C)**	**LogK$_{ow}$**	**Retention time** **Minutes**	**Fragment pattern** ***m/z***	**SIM** ***m/z***
Diclofenac	NSAID	$C_{14}H_{11}Cl_2NO_2$	296.15	4.15	4.51	12.806	73, 93, 151, 214, 277, 367	214, 367
Carbamazepine	Anticonvulsant	$C_{15}H_{12}N_2O$	236.26	13.9	2.32	13.654	63, 96, 165, 193, 236	193, 236
Chloramphenicol	Antibiotic	$C_{11}H_{12}Cl_2N_2O_5$	323.13	7.49	1.14	13.921	73, 93, 147, 208, 225, 361, 451	208, 225
Cocaine	Illicit drug	$C_{17}H_{21}NO_4$	303.35	8.61	2.30	14.530	77, 82, 152, 182, 272, 303	82, 182
Procainamide	Transformation	$C_{11}H_{26}NO_2$	267.00	-	-	15.450	85, 99, 192	86, 99
2-phenylindolizine	Metabolite	$C_{14}H_{11}N$	193.00	-	-	15.960	63, 96, 165, 193	165, 193
Sulfamethoxazole	Antibiotic	$C_{10}H_{11}N_3O_3S$	253.28	6.16	0.89	16.500	65, 92, 156, 189, 253	92, 156
Chlorpromazine	Antipsychotic	$C_{17}H_{19}ClN_2S$	318.86	9.30	5.41	17.605	58, 214, 232, 272, 315	58, 214
Lactose	Metabolite	$C_{36}H_{86}O_{16}$	918.00	-	-	18.560	73, 103, 147, 204, 243, 319, 521	204, 243
Sulfamethazine	Antibiotic	$C_{12}H_{14}N_4O_2S$	278.33	7.59	0.28	20.052	92, 108, 156, 213, 277	92, 213
Clozapine	Antipsychotic	$C_{18}H_{19}ClN_4$	326.82	7.50	3.67	21.750	70, 99, 164, 192, 243, 268, 326	192, 243

Depending on their availability, the reference standards of the targeted analytes were used to determine retention times, fragmentation patterns and abundant ion for SIM mode. The isotopic abundance patterns of the identified compounds were also checked to confirm the identification. Product ions of derivatized drug molecular weights were compared with calculated molar masses (drug plus TMS) and those found in the sample for confirmation purposes. Each targeted compound was associated with its $\log K_{ow}$ and p*K*a available from readily accessible databases and was correlated to the recoveries and with their expected retention times (Figure 6.2). Finally, the entire method was evaluated on water spiked with emerging contaminants from different families/classes (100 µg L^{-1}).

The method was validated by determining the limit of identification (LOI) of each target analyte. The LOI of a qualitative screening method is the lowest concentration of which it has been demonstrated that a compound can be detected and identified by the identification procedure method. The LOIs were determined by spiking at different concentration levels, extracted and injected on the GC-MS.

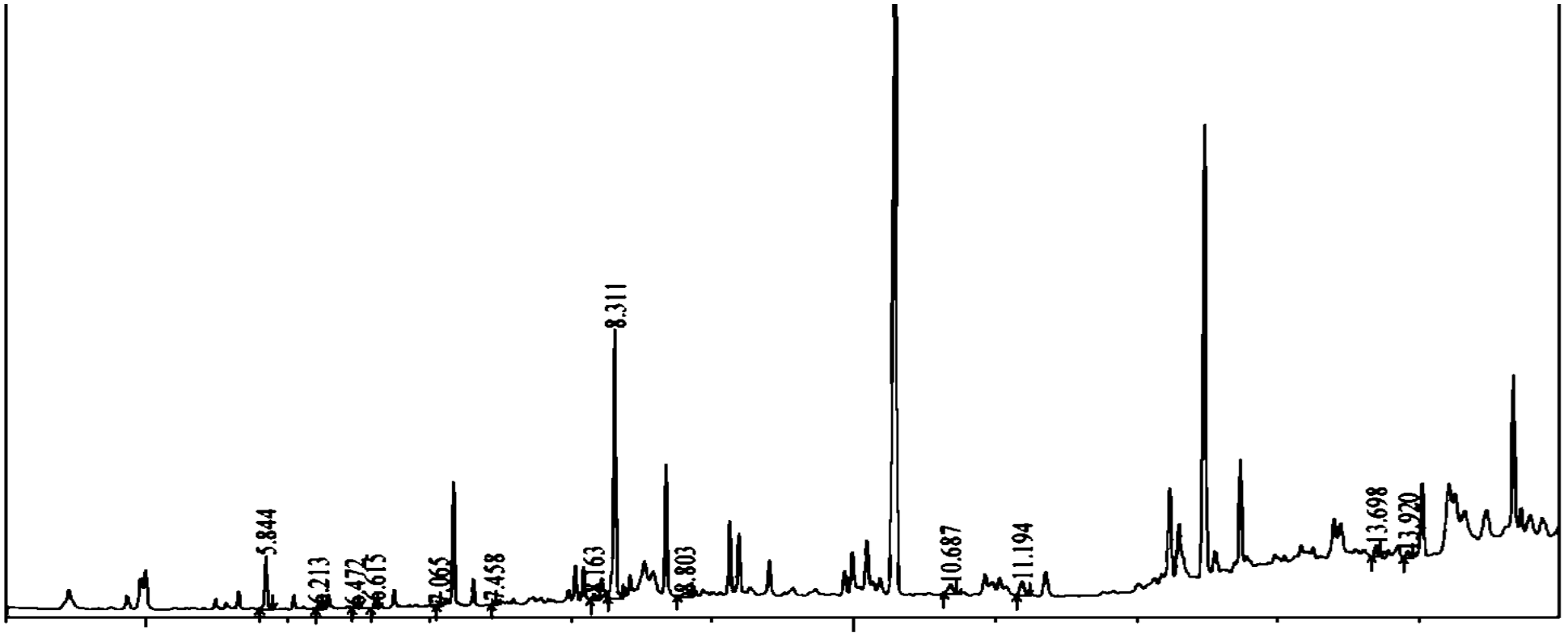

Figure 6.2: TIC chromatogram showing analysis sediment samples, with some peaks being identified using their retention time.

6.5 Environmental Analysis

6.5.1 Target Analysis

The identification and confirmation of contaminants at low concentrations requires both a high sensitivity and selectivity against a complex background matrix. For a large range of compounds, selected ion monitoring in a GC-MS provides a way to lower detection and improve selectivity comparable to MS/MS. A range of studies have shown that monitoring only one ion fragment might result in false positive identifications for individual compounds and in this work, two ions were selected to be monitored for each individual (Basaglia and Pietrogrande, 2012, Helenkar et al., 2010, Molina-Fernandez et al., 2016, Lee et al., 2014, Baduel et al., 2015). In addition, retention times were also used to further confirm the presence of targeted analytes in the environmental sample matrix. Databases and libraries contain lots of compounds from different classes. Nevertheless, other important classes of contaminants are not contained in these search engines, as is the case with many pharmaceutical and personal care products (PCPs). Furthermore, there are only a few libraries that contain spectra of derivatized emerging pollutants, and do not have SIM data ion spectra. Without reference standards, it is impossible to determine the SIM ion to be monitored. Our work has therefore led to the creation of a library with SIM ion, which is correlated with retention times and other physicochemical properties.

Samples from various surface water, wastewater, sediments and biosolids along Msunduzi and Mgeni Rivers were analysed in SIM mode and contaminants were determined by using the established database as shown in Table 6.1. Table 6.2 shows the results of the targeted analysis. NSAIDs and PCPs were detected in all studied matrices. Illicit drugs were mostly detected in wastewater and biosolids. Antibiotics were not readily detected owing to the absence of a labile hydrogen on most of the compounds for easy derivatization (Solliec et al., 2015). This method could detect known metabolites such as salicylic acid. Furthermore, the method could selectively detect structural isomers diclofenac and meclofenamic acid in all matrices. Transformation (formation of new compounds from parent drug) products were not found in any of the matrices.

Table 6.2: Target analysis of emerging contaminants in Mgeni and Msunduzi Rivers.

Target analytes	Type	River water	Wastewater	Sediments	Biosolids
Pharmaceutical					
Over the counter drugs					
Acetylsalicylic acid	NSAID	Detected	Detected	Detected	Detected
Ibuprofen	NSAID	Detected	Detected	Detected	Detected
Phenacetin	Analgesic	Detected	Detected	Detected	Detected
Acetaminophen	NSAID	Detected	Detected	Detected	Detected
Naproxen	NSAID	Detected	Detected	Detected	Detected
Meclofenamic acid	NSAID	Detected	Detected	Detected	Detected
Ketoprofen	NSAID	Detected	Detected	Detected	Detected
Diclofenac	NSAID	Detected	Detected	Detected	Detected
Prescription drugs					
Sulfamethoxazole	Antibiotic	Detected	-	-	-
Nalidixic acid	Antibiotic	Detected	Detected	-	-
Chloramphenicol	Antibiotic	Detected	Detected	Detected	Detected
Sulfamethazine	Antibiotic	-	-	-	Detected
Chlorpromazine	Antipsychotic	-	-	-	Detected
Clozapine	Antipsychotic	-	-	-	Detected
Carbamazepine	Anticonvulsant	-	-	-	Detected
procaine	Anaesthetic	-	-	-	Detected
Illicit drug					
Cocaine	Illicit drug	-	Detected	-	-
Methamphetamine	Amphetamine	Detected	Detected	Detected	Detected
Morphine	Opioid	Detected	Detected	Detected	Detected
Lifestyle drugs					
Caffeine	Stimulant	Detected	Detected	Detected	Detected
Personal care products					
Propyl paraben	Antifungal agent	Detected	Detected	Detected	Detected
Triclosan	Disinfectant	Detected	Detected	Detected	Detected
Metabolites					

Target analytes	Type	River water	Wastewater	Sediments	Biosolids
Salicylic acid	Metabolite	Detected	Detected	Detected	Detected
2-phenylindolizine	Metabolite	-	Detected	-	Detected
Lactose	Metabolite	-	-	-	Detected
Transformation products					
Procainamide	Transformation	-	-	-	-

- not detected

6.5.2 Suspect Analysis

In contrast to target analysis, the suspects screening approach (see Table 6.3) does not rely on reference standards for quantification and confirmation. However, compound-specific information for suspects was available, such as molecular formula and structure, which was efficiently used in the identification and confirmation process. First, the molecular formula allowed us to calculate exact *m/z* of the expected molecular ion. The large number of suspects required an efficient filtering approach, comprising rather straightforward and obvious criteria such as absence in analytical blanks and the match of the observed isotope pattern with theoretically predicted ones for the molecular formula of the suspect (Hauler and Vetter, 2015). In this work, the use of external databases to obtain fragmentation patterns of the suspect analytes and comparison with the one found in the sample helped to identify molecules with a high level of accuracy. Furthermore, comparison of our in-house database (Table 6.1) with fragmentation patterns predicted by NIST, DrugBank, PubChem and ChemSpider with respect to our reference standard gave an indication that these external databases can be used to identify contaminants in the sample. It is not possible to prove from the outset whether a compound present in a sample will be identified in the chromatogram, as it could get lost during any step of the analytical procedure or is not ionized as anticipated. However, external databases provided more information on the physicochemical properties, predicted retention times and fragmentation of the suspects. Using the obtained information, we were able to identify the suspected analytes in the environment, and the results are presented as shown in Table 6.3. Plasticizers were easy to identify because of their distinct peak 149 *m/z* and were detected in all matrices. Different classes of emerging contaminants that were suspected to be present in the environment were found (Agunbiade and Moodley, 2014, Matongo et al., 2015, Agunbiade and Moodley, 2016, Gakuba et al., 2015, Wood et al., 2015).

Table 6.3: Suspect analysis of emerging contaminants in Mgeni and Msunduzi River. (Library built from external database).

Suspect analytes	Type	Chemical formula	Molar mass g mol^{-1}	Fragment pattern *m/z*	River water	Wastewater	Sediments	Biosolids
Clofibric acid	Pharmaceutical	$C_{10}H_{11}ClO_3$	214	39, 99, 128, 130, 214	Detected	Detected	-	Detected
Codeine	Pharmaceutical	$C_{18}H_{21}NO_3$	299	115, 162, 214, 229, 299	-	Detected	-	-
Oxazepam	Pharmaceutical	$C_{15}H_{11}ClN_2O_2$	286	77, 205, 233, 239, 268	-	Detected	-	-
Trimethoprim	Pharmaceutical	$C_{14}H_{18}N_4O_3$	290	123, 200, 243, 259,	-	-	-	Detected
Nicotine	Pharmaceutical	$C_{10}H_{14}N_2$	162	42, 84, 161	-	-	-	Detected
Amphetamine	Pharmaceutical	$C_9H_{13}N$	135	44, 65, 91, 120	-	-	-	-
Benzyolecgonine	Pharmaceutical	$C_{16}H_{19}NO_4$	289	77, 82, 94, 124, 138,	-	Detected	-	-
Benzicaine	Pharmaceutical	$C_{16}H_{11}NO_2$	165	65, 92, 120, 137, 165	-	-	Detected	-
Cotinine	Pharmaceutical	$C_{16}H_{19}NO_4$	176	98, 176	-	-	-	-
Propranolol	Pharmaceutical	$C_{16}H_{21}NO_2$	259	30, 72, 115, 144, 331	Detected	-	-	-
Azelaic acid	PCP	$C_9H_{14}Cl_2O_2$	225	55, 83, 124, 152, 367	Detected	-	-	-
4-Oxoisophorone	PCP	$C_9H_{12}O_2$	152	39, 68, 96, 152	-	Detected	-	-
Musk xylene	PCP	$C_{12}H_{15}N_3O_6$	297	43, 282	-	-	Detected	-
Dibutyl phthalate	Plasticizer	$C_{16}H_{23}O_4$	278	149, 205	Detected	Detected	Detected	Detected
Bisphenol A	Plasticizer	$C_{15}H_{16}O_2$	228	119, 213, 228, 372	-	-	-	-
Triethyl phosphate	Plasticizer	$C_6H_{15}O_4P$	182	81, 99, 109, 155, 182	Detected	Detected	Detected	Detected

Suspect analytes	**Type**	**Chemical formula**	**Molar mass** $g\ mol^{-1}$	**Fragment pattern** ***m/z***	**River water**	**Wastewater**	**Sediments**	**Biosolids**
Triethyl citrate	Plasticizer	$C_{12}H_{20}O_7$	276	115, 157, 203, 348	Detected	Detected	Detected	Detected
Oxybenzone	UV filters	$C_{14}H_{12}O_3$	228	51, 77, 151, 227, 300		Detected	-	Detected
Tris(2-chloroethyl) phosphate	Flame retardant	$C_6H_{12}Cl_3O_4P$	285	63, 143, 205, 249, 253	Detected	-	-	-
Triphenyl phosphate	Flame retardant	$C_{18}H_{15}O_4P$	326	77,169, 233, 233, 326	-	-	-	Detected

6.5.3 Non-Target Analysis

In contrast to suspect screening, non-target (unknown compound) screening in a strict sense starts without any prior information on the compounds to be detected or its occurrence has not previously been reported in the environment. The number of chemically meaningful structures, which can be assigned to an unknown peak detected, is limited to structures showing a close relationship with the parent compound. Various compounds were tentatively identified in our samples using databases mentioned above. In this case, DrugBank database was mostly used because it has an option to insert a fragmentation pattern, and then predict structures. From the structure name, a search on the NIST database was carried out for fragmentation prediction, and then these patterns were compared with those arising from the sample. If more than three fragments matched, the compound was accepted as the unknown discovered. Metabolomics database was used to predict parent forms of any metabolite discovered, and subsequently the family/classes. Different classes of metabolites were found in the environment and results are presented in Table 6.4. For example, oxindole was predicted by our databases as a lipid metabolite and nicotinic acid as a metabolite of vitamin B12. Moreover, it was interesting that the method was able to identify metolachlor, a herbicide, in river water. This shows that the present method can be adapted to multi-residue analysis and employed to detect other pollutants in the environment. It should be noted that most databases use IUPAC names to report on various compounds, and known health threatening compounds might be not recorded to be present in the environment because only certain researchers know the IUPAC names. However commercial/industrial workers routinely involved with water analysis may only know certain commercial names. Thus, some of these external databases were found to be very useful in converting IUPAC names into familiar/commercial or trade names of the contaminants, which may not have been reported to exist in the environment. For example, detection of 2,6-dimethylphenol isocyanide in river water and wastewater, of which is predicted by these databases to be highly toxic, and different transformation products of phthalic acid were also identified in the samples.

Table 6.4: Non-target analysis of emerging contaminants in Mgeni and Msunduzi Rivers.

non-target analytes	Source of origin	Chemical formula	Molar mass g mol^{-1}	Fragment pattern *m/z*	River water	Wastewater	Sediments	Biosolids
Butyldiglycol	Paints	$C_8H_{18}O_3$	162	57, 100, 132	Detected	Detected	-	-
2-propanol, 1-[1-methyl-2-(properyloxy)ether	-	$C_9H_{18}O_3$	174	59, 103, 174	detected	-	-	-
Nicotinic acid	Vitamin	$C_6H_5NO_2$	123.10	51, 91, 136, 195	-	Detected	-	-
Phenylmalonic	-	$C_9H_8O_4$	180	69, 91, 136	-			-
2-ethyl-3-hydroxyhexyl 2-methyl propanoate	-	$C_{12}H_{24}O_3$	216	71, 95, 99, 143, 174	Detected	Detected	-	-
Oxindole	Human metabolite	C_8H_7NO	133	78, 104, 133	Detected	Detected	-	-
2,6-Dimethylphenyl isocyanide	Cyanobacteria	C_9H_9NO	147	51, 118, 147	Detected	Detected	-	-
Phthalic anhydride	Plasticizer	$C_8H_4O_4$	148	76, 104, 148	Detected	Detected	Detected	Detected
2-Pyrrolidone	Pharmaceutical Metabolite	C_4H_7NO	85	73, 142, 157	-		Detected	-
Dioctyl Phthalate	Plasticizer	$C_{24}H_{38}O_4$	390	57, 149, 167, 279	-	Detected	-	-
Obtusifoliol	Hormone	$C_{30}H_{50}O$	426	75, 215, 355, 370, 429	-	Detected	-	-
Cholesterol	Hormone	$C_{27}H_{46}O$	386	129, 329, 353, 368, 458	-	Detected	-	-
Metolarchlor	Herbicide, pesticide	$C_{15}H_{22}ClNO_2$	283	91, 162, 238	Detected		-	-

non-target analytes	**Source of origin**	**Chemical formula**	**Molar mass** **g mol^{-1}**	**Fragment pattern** ***m/z***	**River water**	**Wastewater**	**Sediments**	**Biosolids**
2-Phenoxyethanol	Pharmaceutical/PCPs	$C_8H_{10}O_2$	138	77, 94, 138	Detected		-	-
Diethyl phthalate	Plasticizer	$C_{12}H_{14}O_4$	222	149, 177, 222	Detected		-	-

6.6 Comparison of Approaches

The obtained results were compared with each other, a total of over 50 compounds were detected in the four compartments and the results are presented in Table 6.5. Most targeted compounds were detected in biosolids while only one non-targeted compound was detected. This was attributed to the complexity of the biosolid matrix, which hindered the identification of compounds without prior knowledge of spectral behaviour. However, most non-target compounds were detected in river water because of its simpler matrix for easy matching of fragment peaks with low background noise. There were no typical trends observed with suspected compounds across the four compartments.

Table 6.5: Total number of compounds detected in each compartment.

	RIVER WATER	WASTEWATER	SEDIMENT	BIOSOLID
TARGET	16	17	15	23
SUSPECT	7	9	5	8
NON- TARGET	10	9	2	1
TOTAL	33	35	22	32

6.7 Conclusions

A new library based on pharmaceutical and personal care products and trimethylsilyl derivatives was developed and successfully used to detect over 50 compounds in Mgeni and Msunduzi Rivers. Two approaches, suspect and non-target analysis were undertaken to determined unidentified peaks in the sample. Some peaks were correctly assigned to compounds, by the use of the external databases whose compounds together with their metabolites classified and their sources were predicted. With the world rapidly implementing new technology innovation, more compounds enter the environment simply because they are

new or no longer needed as raw materials. These approaches will fast forward the determination of these new compounds at the environmentally relevant levels, where matrix complexity plays a major role limiting many research studies on emerging contaminants.

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Chapter 7: General Conclusion and Recommendations for Future Work

7.1 Conclusion

The conclusions obtained throughout this research are:

The overall aim of this research was to develop, optimize and validate simple analytical methods for determination of emerging contaminants in the environment for application in developing countries laboratories (**Chapter 1**). Also, their application in determination of emerging contaminants in environmentally samples has been demonstrated. This aim was achieved by developing and optimizing various analytical steps including extraction, clean-up, derivatization, separation and detection methods to develop an analytical method. The methods were applied to study the occurrence of selected emerging contaminants in four compartments of the aquatic environment (River water, wastewater, sediments and biosolids) in Mgeni and Msunduzi river-systems, KwaZulu-Natal, South Africa. Most of the selected emerging contaminants were found in both Mgeni and Msunduzi River-systems and wastewater treatment plants found along these rivers.

Literature review findings (**Chapter 2**), has shown that the extensive development of resources and technology has produced more chemicals and materials which in turn enters the environment mostly through effluent from wastewater treatment plants. There is lack of analytical data concerning the occurrence of emerging contaminants in South Africa and Africa as whole. The existing analytical methods are too expensive and complicated for developing countries resulting in the often lacking state of art equipment to perform environmental analysis or regulation of these new contaminants. Hence, accepted methods for determination of emerging contaminants are seldom used in developing countries, and as result many contaminants in these countries remain unidentified/undocumented and their existence in the environment is not known. Development of validated methods with simplified sample preparation, offer improved possibilities for simultaneous determination of multiple emerging contaminants with significantly improved detection limits. This lead to a new paradigm for developing countries to study occurrence of these classes of compounds in their environment.

Solid phase extraction, derivatization, separation and GC-MS detection methods were successfully optimized for detection and quantification of acidic drug residues in South African surface water (**Chapter 3**). The developed method was validated and applied for the determination of selected acidic drugs in the Mgeni River system (dams, WWTPs and estuary), KwaZulu-Natal, South Africa. All selected acidic drugs were detected and quantified, their concentration in the Mgeni River system ranged from 0.020 to 68.1 µg L^{-1}. Seasonally, winter had higher concentrations of pharmaceuticals compared to summer. The findings add to the growing environmental data on pharmaceuticals in the African continent.

An ultrasonic-assisted extraction method was developed and optimized for assessment of nonsteroidal anti-inflammatory drugs in Mgeni and Msunduzi River sediments and biosolids (**Chapter 4**). Satisfactory recoveries obtained ranged between 66% – 130% and precision was less than 2%, which allowed the developed method to be applied to environmental samples. The ultrasonic-assisted method was used to monitor the occurrence of 8 pharmaceuticals during four seasons (spring, winter, autumn and summer) along a 250 km stretch of Msunduzi and Mgeni Rivers in KwaZulu-Natal, South Africa. The concentration of pharmaceuticals in the environment ranged from detected to 220 ng g^{-1}. The studied pharmaceuticals were higher in biosolids than in the sediments. The Msunduzi River had high concentration levels of these compounds compared to the Mgeni River. Acetylsalicylic acid and ibuprofen were frequently detected in most sites, while diclofenac was found to be predominantly at the WWTP (209 ng g^{-1}) in January. The developed method has high throughput and is cost-efficient, and can be used in routine monitoring of pharmaceuticals in the environment on a large scale. In addition, this method will be useful in developing countries to study the occurrence of pharmaceuticals in solids and sediments where there is scarcity of data worldwide.

A simple method for occurrence and risk assessment of personal care products, pharmaceuticals and stimulants in Mgeni and Msunduzi River is presented (**Chapter 5**). The proposed method was applied in the evaluation of two rivers (Mgeni and Msunduzi Rivers) over a three month period in KwaZulu-Natal, South Africa. All targeted compounds were present in the environment at concentration levels between not detected to 174 ng g^{-1} and not detected to 30 µg L^{-1} for solids and aqueous environmental samples, respectively. A comparison of predicted no environmental effect concentration (PNECs) with measured

environmental concentration (MECs) showed that these PCPPs present a high ecological risk to the receiving environment. To our knowledge, this is a first work on the simultaneous assessment of occurrence risk of PCPPs by GC-MS in Africa. Detection of an antibiotic (chloramphenicol), shows that there is need to regulate irrigation water and fertilizers (compost or biosolids) to minimize the risk of antibiotic resistance.

There is growing interest to determine the unidentified peaks within a sample spectrum having intensities higher than analytes of interest. Availability of reference standards, internal standard and hyphenated instruments has been a key and limiting factor on the rapid determination of emerging contaminants in the environment. A new spectral library for silylated derivatives in single ion monitored mode was successfully developed **(Chapter 6**). In addition, non-targeted emerging contaminants present in the sample were also identified and assigned to relevant classes. The developed library was used to detect over 50 compounds in Mgeni and Msunduzi Rivers. This method will allow the detection of new toxic compounds in the environment.
The developed and optimized methods can be used to contribute data on the studies of occurrence of emerging contaminants in the environment and also for the rapid identification of unknown compounds in the environment. Emerging contaminants are of particular concern, both because of their ubiquity in the aquatic environment and potential impacts.

7.2 Recommendations for Future Work

7.2.1 Equipment and Technique

The availability of equipment and technique is a limiting factor to determine the occurrence of emerging contaminants in the environment. Since the costs of extraction method and instrumentation is an important factor in determining the overall budget of the entire method, these two steps can be investigated further. The methods presented in this work have eliminated the use LC-MS technique, which is expensive and complex. However, the use of Oasis HLB cartridge (non-reusable) in this developed method was found to be another factor that needs to be eliminated in order to reduce the burden of costs in developing countries. It is recommended that the solid phase extraction must be eliminated, two approaches are proposed below:

Pressurised hot water extraction, which has become a popular green extraction method for different classes of compounds present in numerous kinds of matrices such as environmental, food and botanical samples. This method is recommended to be optimized and used as a standard method for extraction of emerging contaminants instead of solid phase cartridges. The second approach recommended is the use of on-line pre-concentration of water samples. This method uses two columns combined with Ultra-performance liquid chromatography system (UPLC) to delay analyte, pre-concentrate and bring them above the instrument detection level.

7.2.2 Prioritisation and Addition of Emerging Contaminants

Synthetic emerging contaminants are being prioritized over unknown natural emerging contaminants; this trend however does not address all potential health risk compounds in the environment. Cyanobacteria (Blue-green algae) blooms for example were observed in the last stages of wastewater treatment plants we sampled in KwaZulu-Natal. These are prokaryotes that were among the first photosynthesizing organisms on earth. Under the proper conditions, cyanobacteria can grow and form dense blooms that also produce harmful secondary metabolites known as cyanotoxins, which pose significant health risks to humans and animals through multiple exposure pathways. Presence of these compounds in water may be deleterious hence there is a need for researchers to investigate the presence and identification of toxins production by cyanobacteria present in water, algal cells, and fisheries. This would ensure the high quality of food and water for consumption and recreation. In addition, South Africa has a high number of people living with HIV, and therefore antiretroviral drugs must be prioritised in the South African environmental studies. The presence of emerging contaminants in the environment may lead to drug resistance genes.

Appendixes

Appendix A1: Target Compounds Calibration Curves

$$Ratio = \frac{Peak\ Area\ of\ Analyte}{Peak\ Area\ of\ internal\ Standard}$$

Pharmaceutical Drugs

Nonsteroidal Anti-inflammatory Drugs.

Acetylsalicylic aid

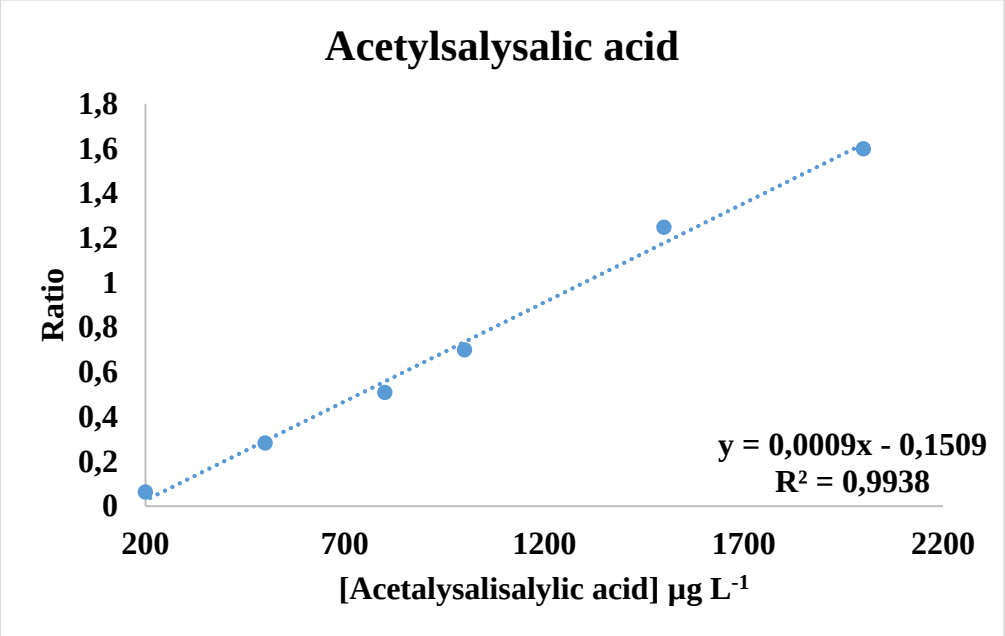

Figure A.1. 1: Calibration curve of acetylsalicylic acid constructed by auto injecting 2 µL of standard solution into GC-MS after derivatization.

Ibuprofen

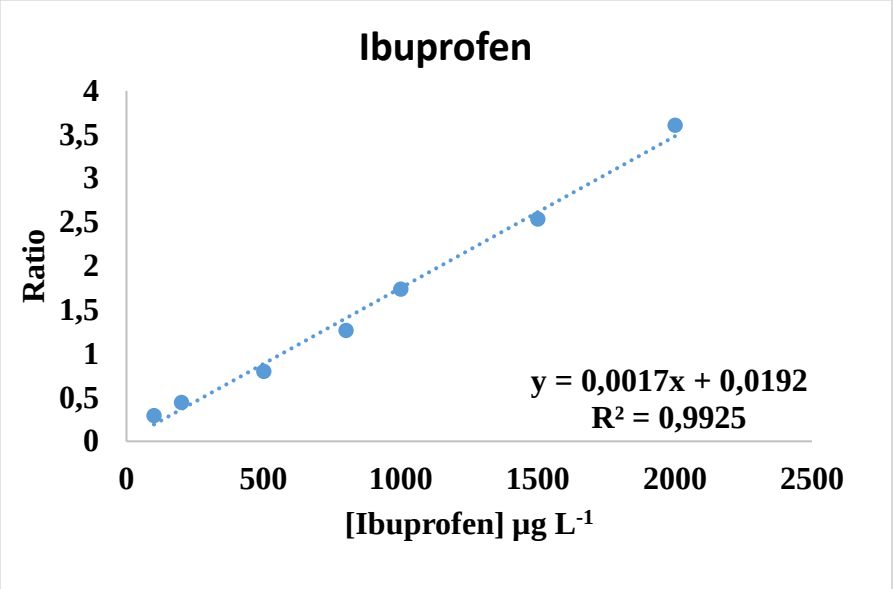

Figure A.1. 2: Calibration curve of ibuprofen constructed by auto injecting 2 µL of standard solution into GC-MS after derivatization.

Phenacetin

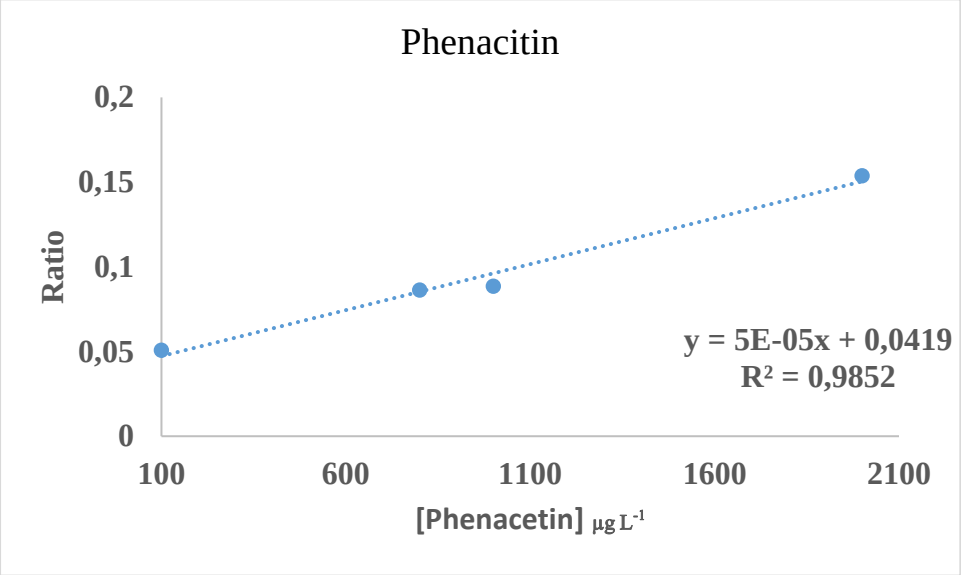

Figure A.1. 3: Calibration curve of phenacetin constructed by auto injecting 2 µL of standard solution into GC-MS after derivatization.

Naproxen

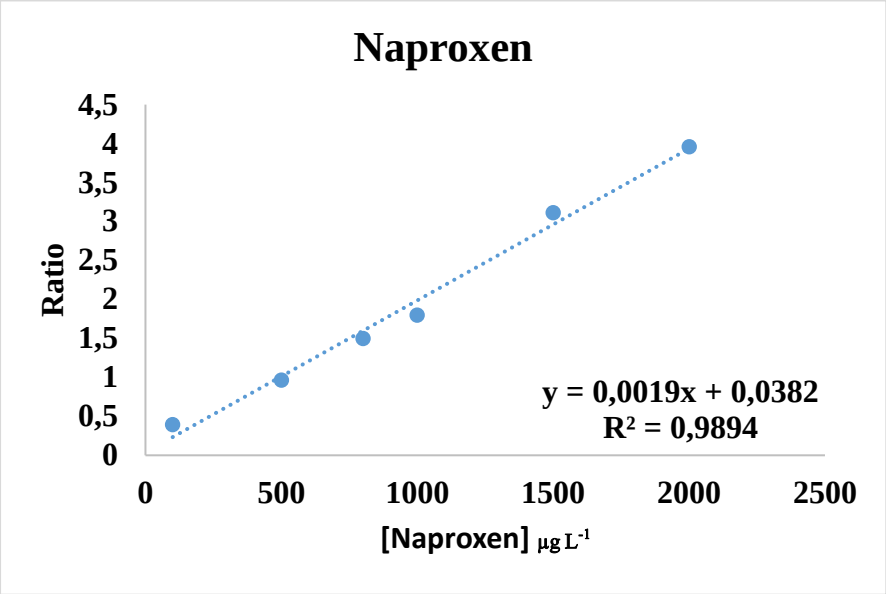

Figure A.1. 4: Calibration curve of naproxen constructed by auto injecting 2 µL of standard solution into GC-MS after derivatization.

Meclofenamic acid

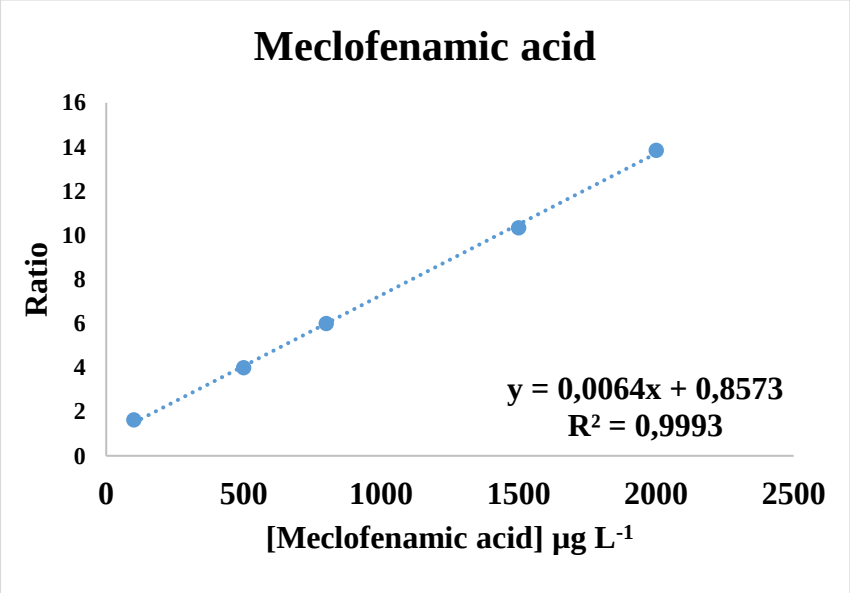

Figure A.1. 5: Calibration curve of meclofenamic acid constructed by auto injecting 2 µL of standard solution into GC-MS after derivatization.

Ketoprofen

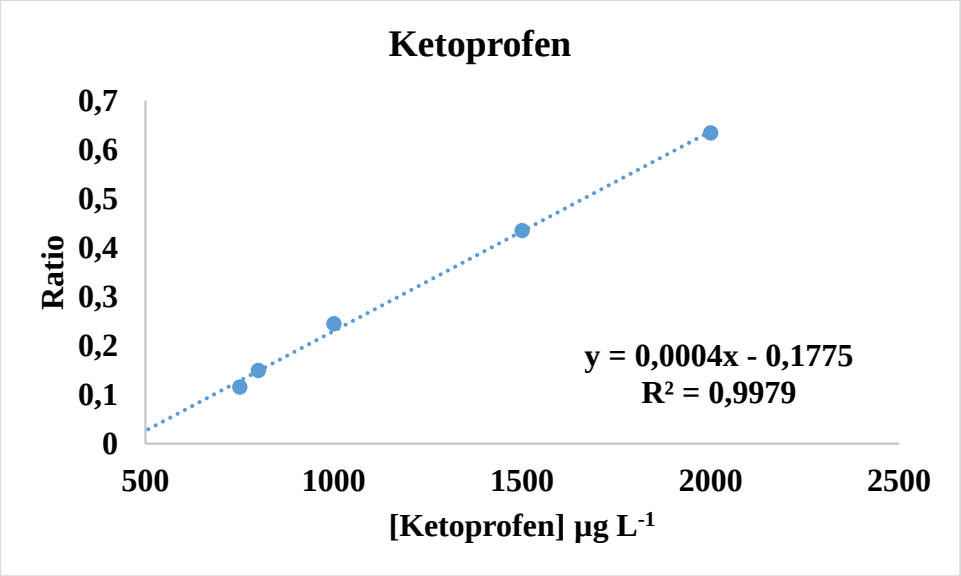

Figure A.1. 6: Calibration curve of Ketoprofen constructed by auto injecting 2 µL of standard solution into GC-MS after derivatization.

Diclofenac

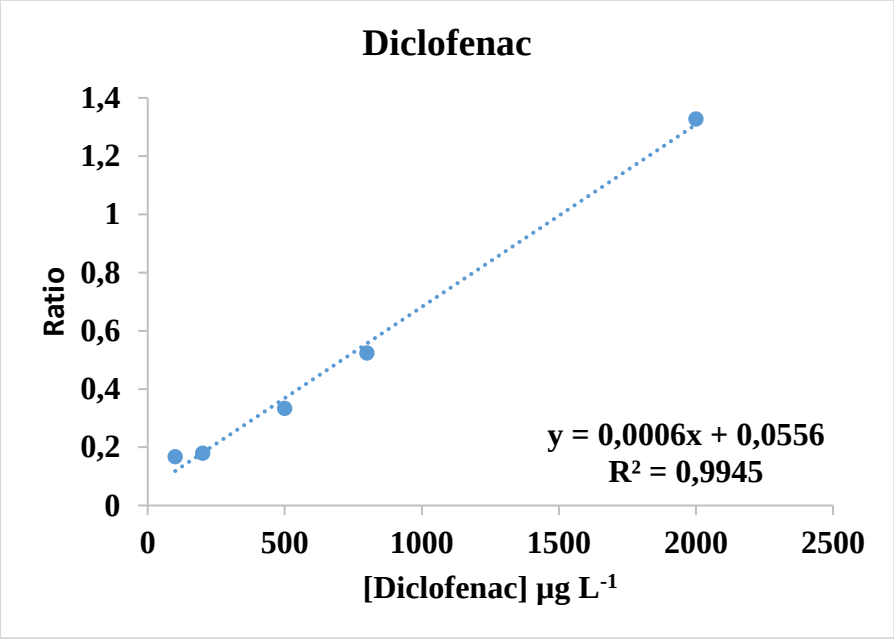

Figure A.1. 7: Calibration curve of diclofenac constructed by auto injecting 2 µL of standard solution into GC-MS after derivatization.

Psychiatric Drug

Carbamazepine

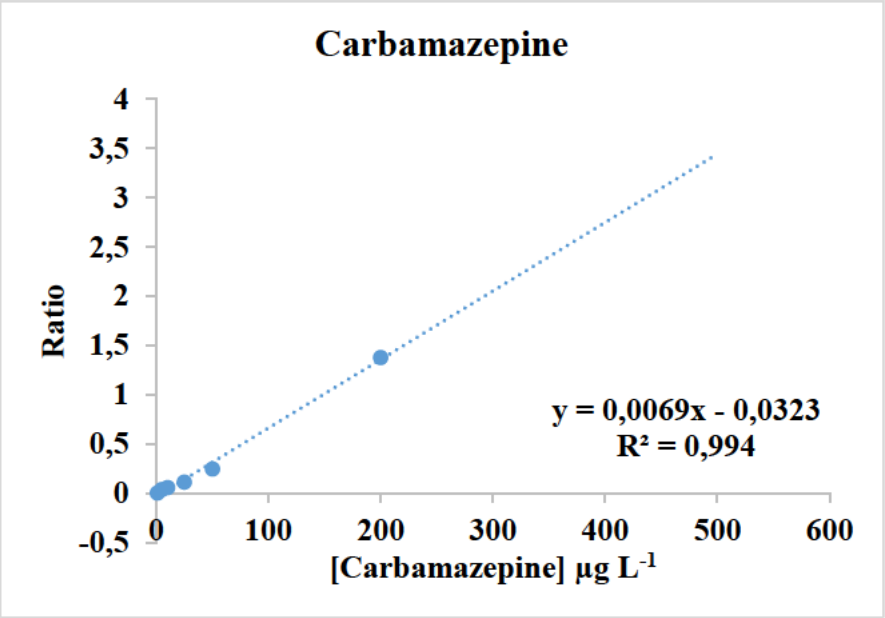

Figure A.1. 8: Calibration curve of carbamazepine constructed by auto injecting 2 µL of standard solution into GC-MS after derivatization.

Antibiotic Drug

Chloramphenicol

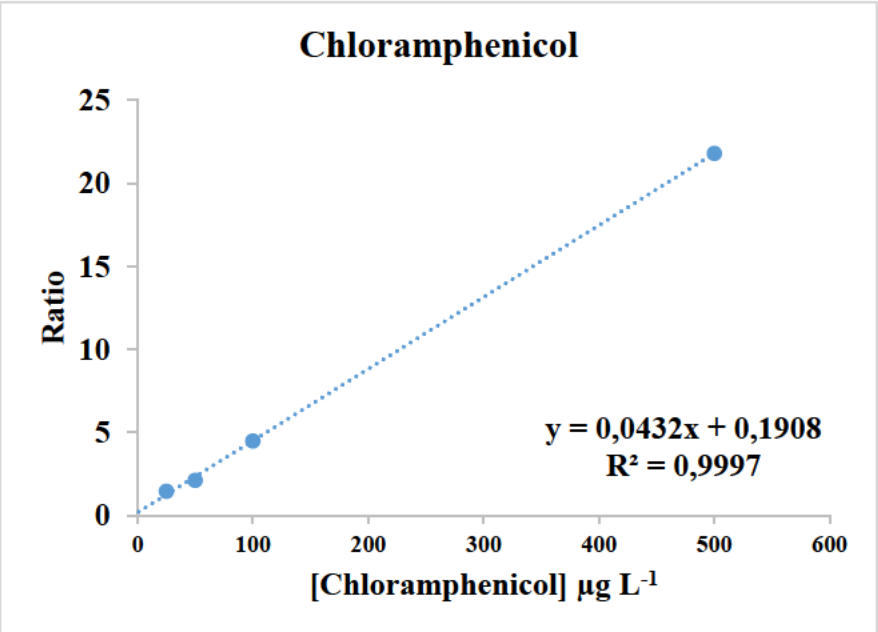

Figure A.1. 9: Calibration curve of chloramphenicol constructed by auto injecting 2 µL of standard solution into GC-MS after derivatization.

Personal Care Product Drugs

Propylparaben

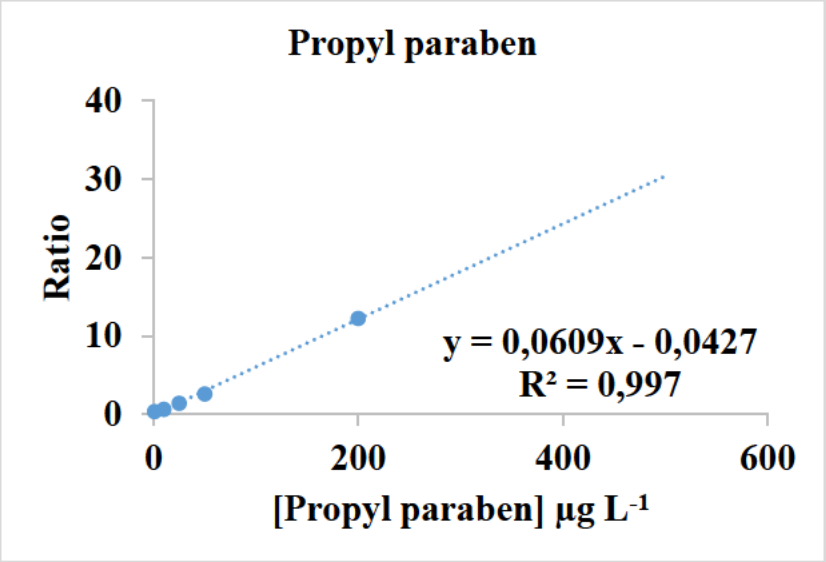

Figure A.1. 10: Calibration curve of propyl paraben constructed by auto injecting 2 µL of standard solution into GC-MS after derivatization.

Triclosan

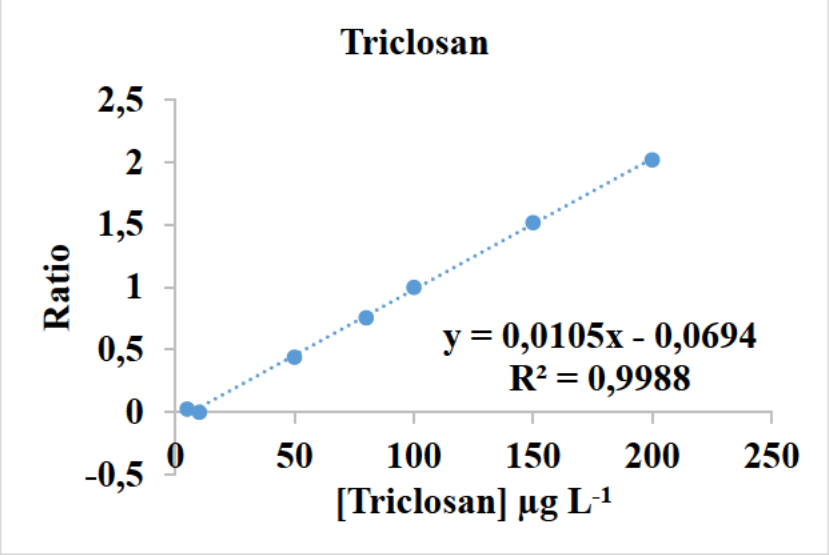

Figure A.1. 11: Calibration curve of triclosan constructed by auto injecting 2 µL of standard solution into GC-MS after derivatization.

Stimulant

Caffeine

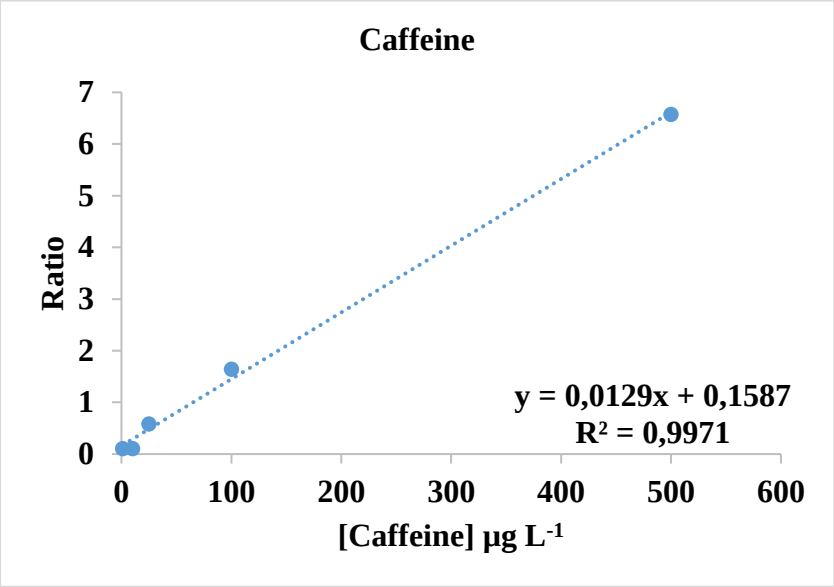

Figure A.1. 12: Calibration curve of acetylsalicylic acid constructed by auto injecting 2 µL of standard solution into GC-MS after derivatization.

Appendix A2: Instrumental Analysis

Column Conditioning

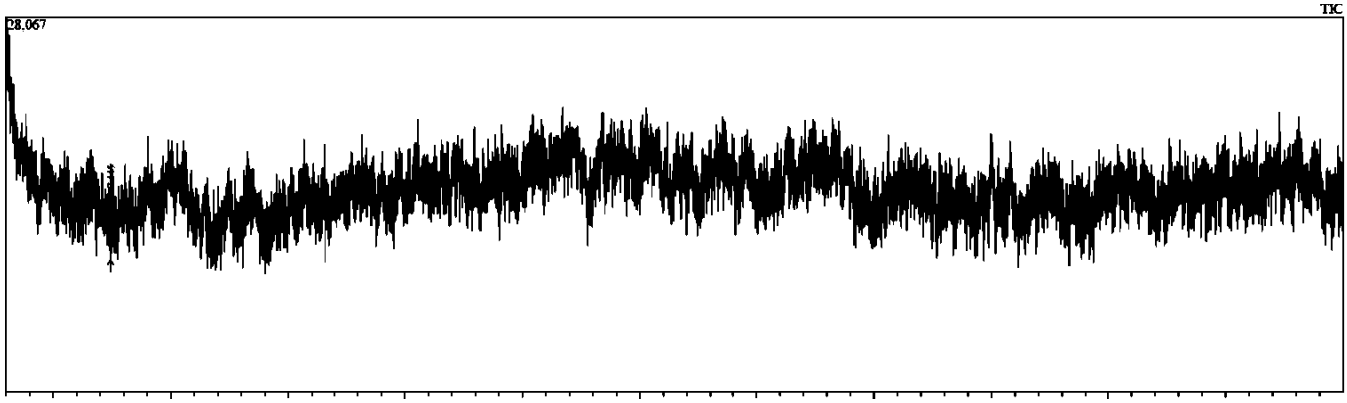

Figure A2. 1: Chromatogram was obtained by injecting 2 µL of acetonitrile into GC-MS and was set in scan mode (50 to 850 *m/z*). Column was condition by heating the column at 280 °C for 3 hours.

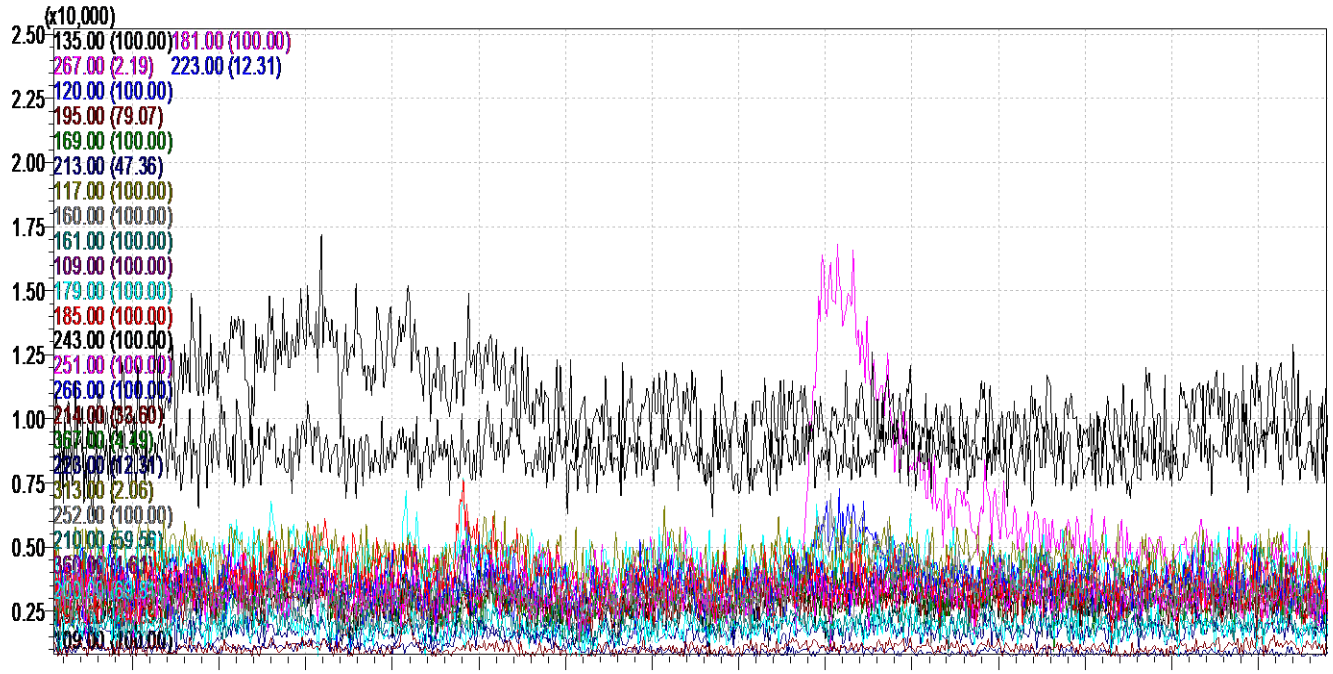

Figure A2. 2: Chromatogram was obtained by injecting 2 µL of acetonitrile into the GC-MS and was operated in SIM mode (selected channels are shown in the chromatogram). The employed method is explained in Chapter 3.

Appendix A3: Target compounds mass spectrometry spectrums

Chlorobenzoic acid compound was used as an internal standard.

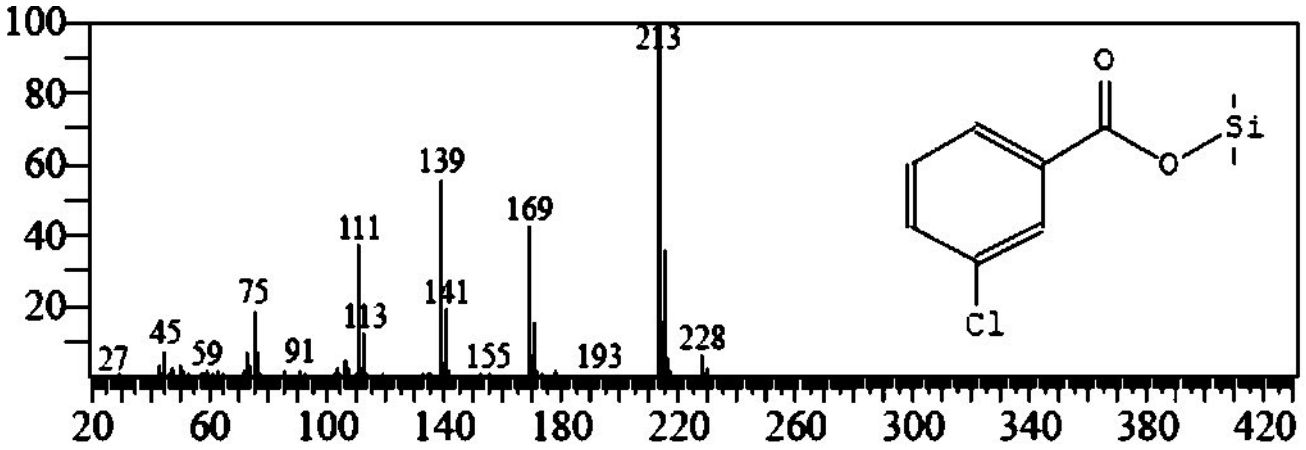

Figure A3. 1: Spectrum of derivatized chlorobenzoic acid. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Cinnamic acid was used as a surrogate standard.

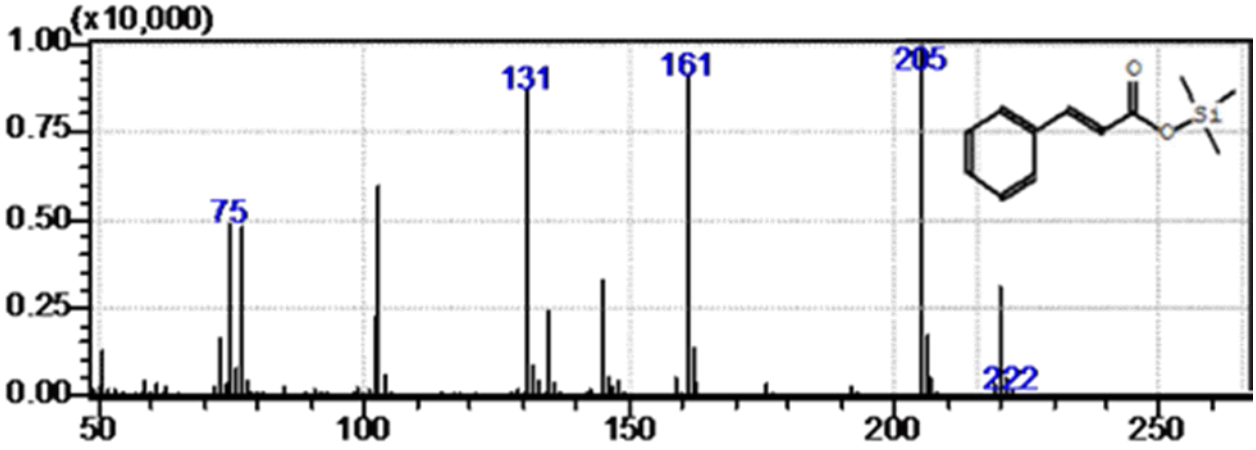

Figure A3. 2: Spectrum of derivatized cinnamic acid. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

4-Phenoxyphenol was used as an internal standard.

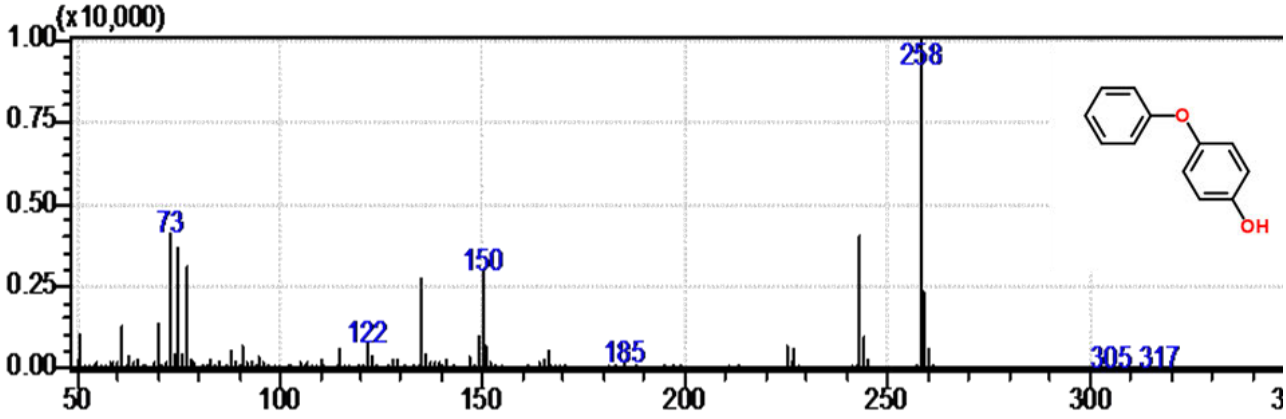

Figure A3. 3: Spectrum of derivatized 4-phenoxyphenol. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Acetylsalicylic acid (aspirin) is a pharmaceutical drug under anti-inflammatory group**.**

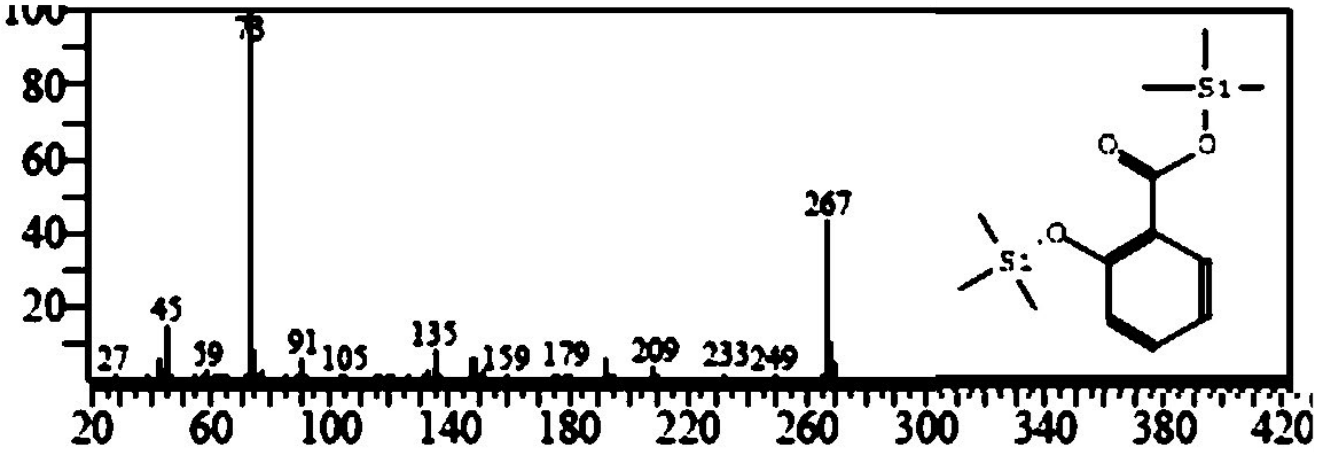

Figure A3. 4: Spectrum of derivatized acetylsalicylic acid. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Ibuprofen is a pharmaceutical drug under group anti-inflammatory group.

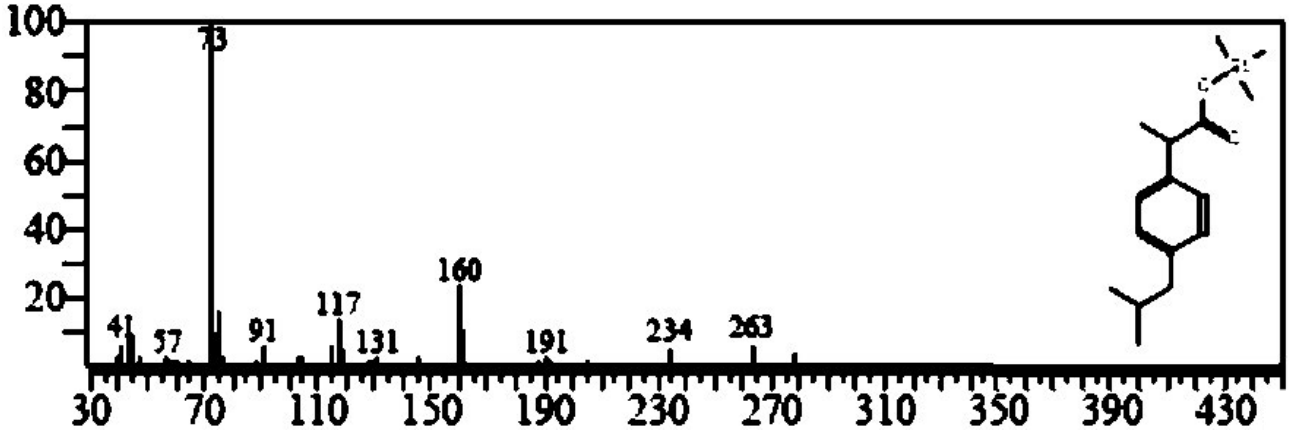

Figure A3. 5: Spectrum of derivatized ibuprofen. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Phenacetin a pharmaceutical drug under group anti-inflammatory group which was banned by USA government, but is still detected in the environment until today.

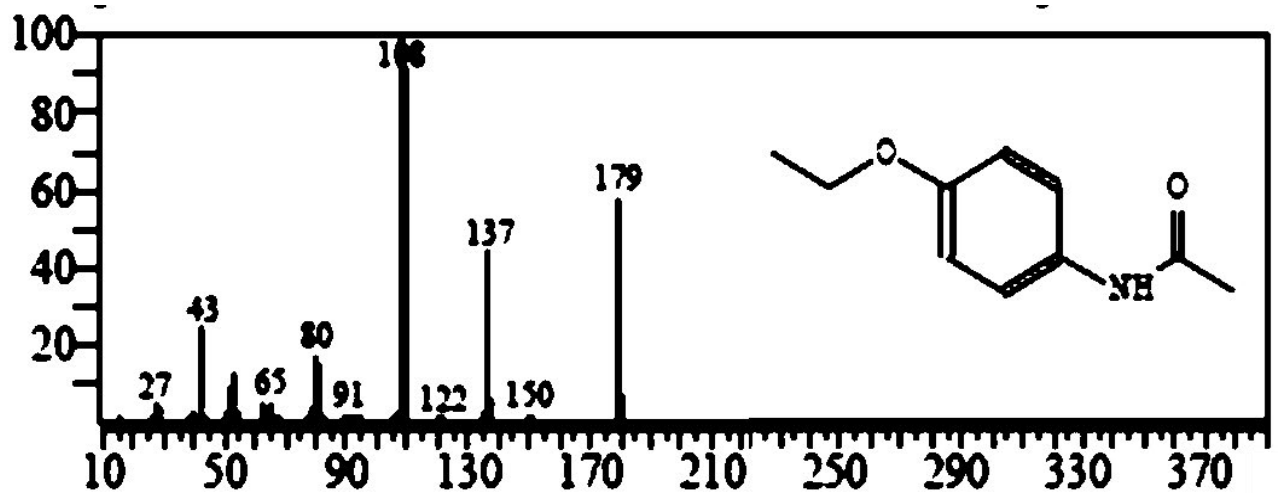

Figure A3. 6: Spectrum of phenacetin. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Acetaminophen (Paracetamol) is a pharmaceutical drug use to treat pain and fever.

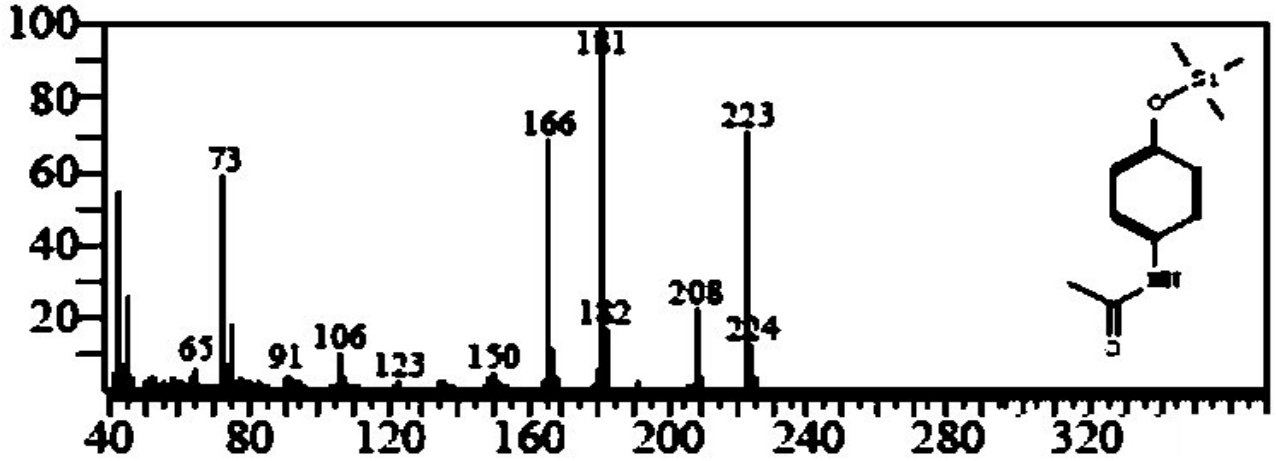

Figure A3. 7: Spectrum of derivatized acetaminophen. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Naproxen is a pharmaceutical drug under the anti-inflammatory drugs group.

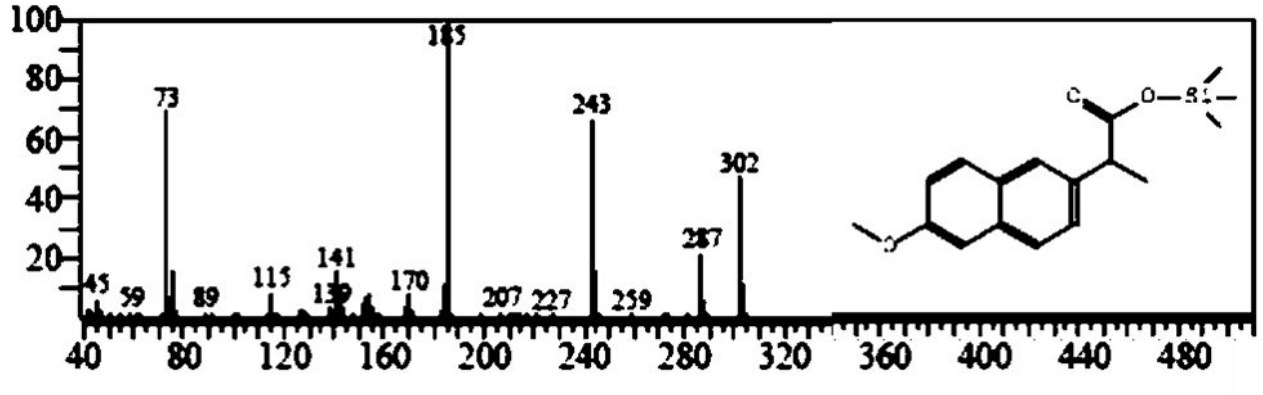

Figure A3. 8: Spectrum of derivatized acetaminophen. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Caffeine is a stimulant belong to lifestyle drug found in various beverages and coffee.

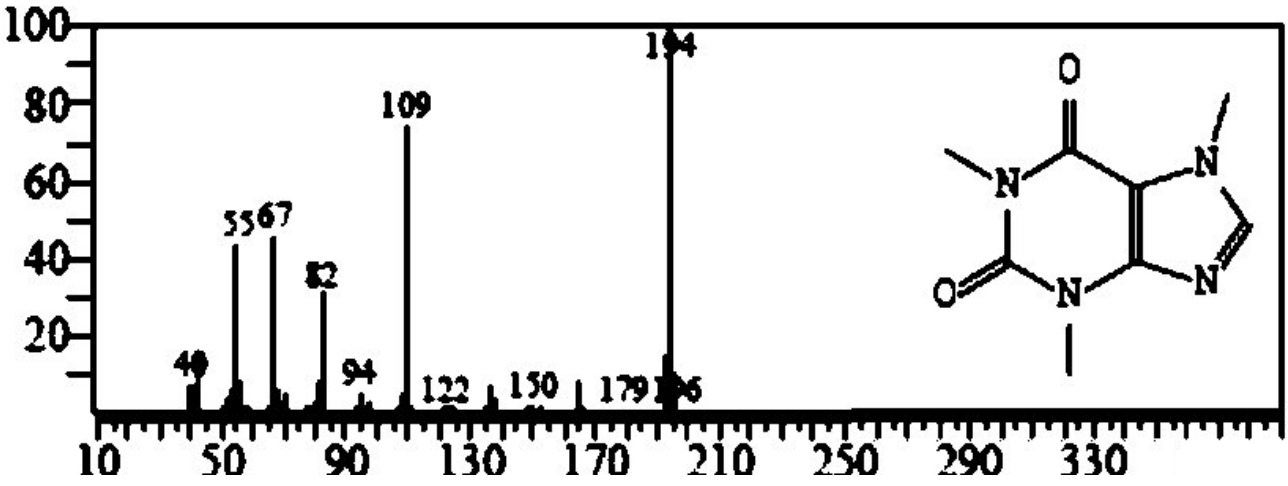

Figure A3. 9: Spectrum of caffeine. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Carbamazepine is a prescription drug belong to psychiatric group.

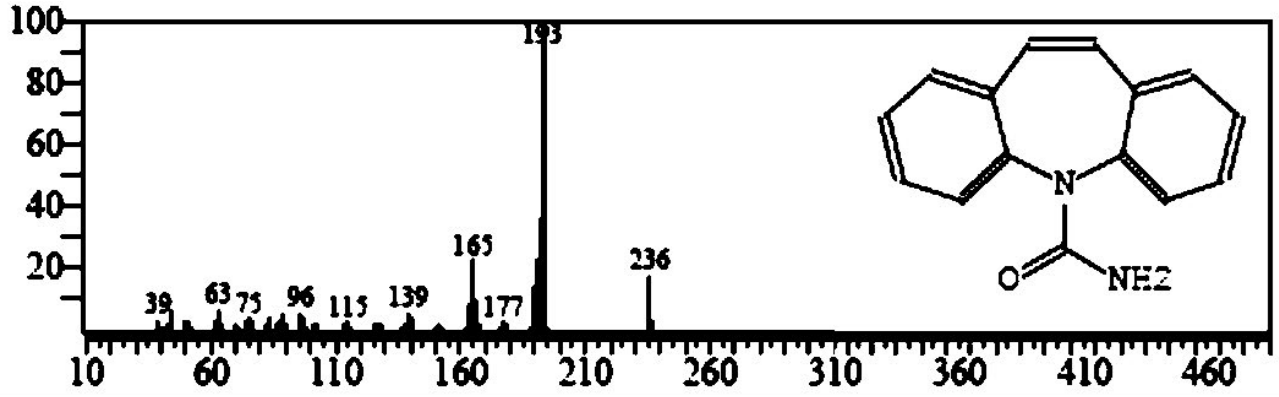

Figure A3. 10: Spectrum of derivatized carbamazepine. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Clozapine is a prescription drug belong to psychiatric group.

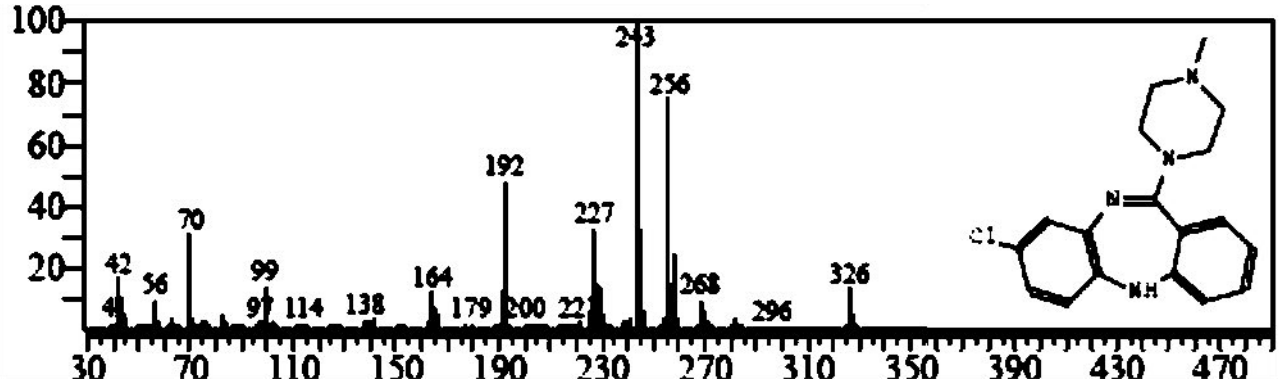

Figure A3. 11: Spectrum of derivatized clozapine. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Chlorpromazine is prescription drug belong to psychiatric group.

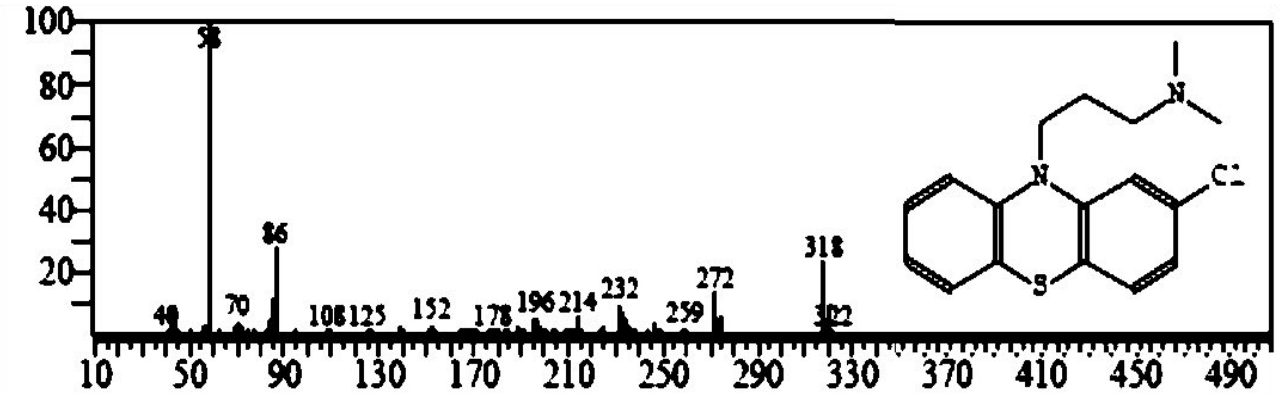

Figure A3. 12: Spectrum of derivatized chlorpromazine. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Sulfamethoxazole is prescription drug belong to antibiotic group.

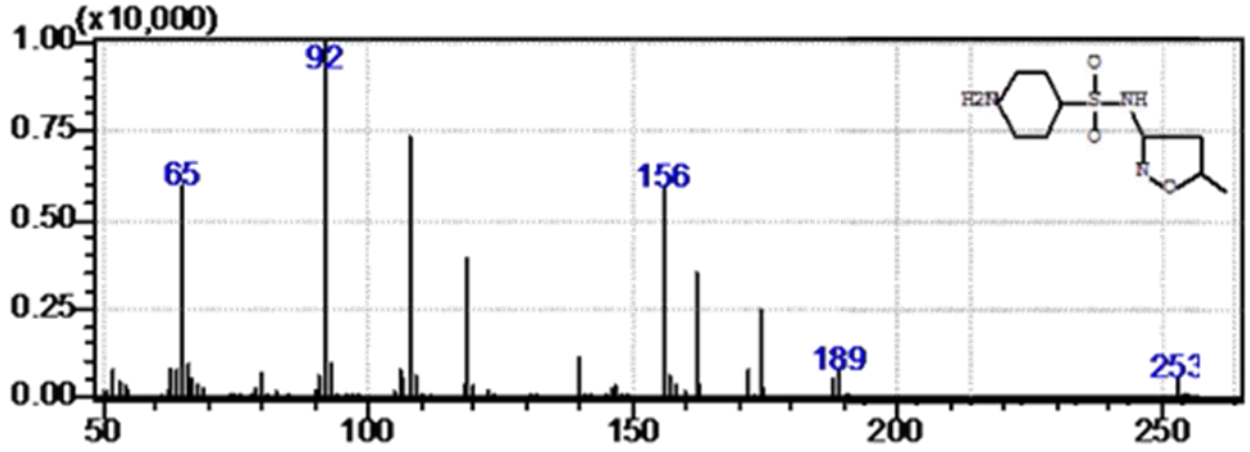

Figure A3. 13: Spectrum of sulfamethoxazole. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Sulfamethazine is prescription drug below to antibiotic group.

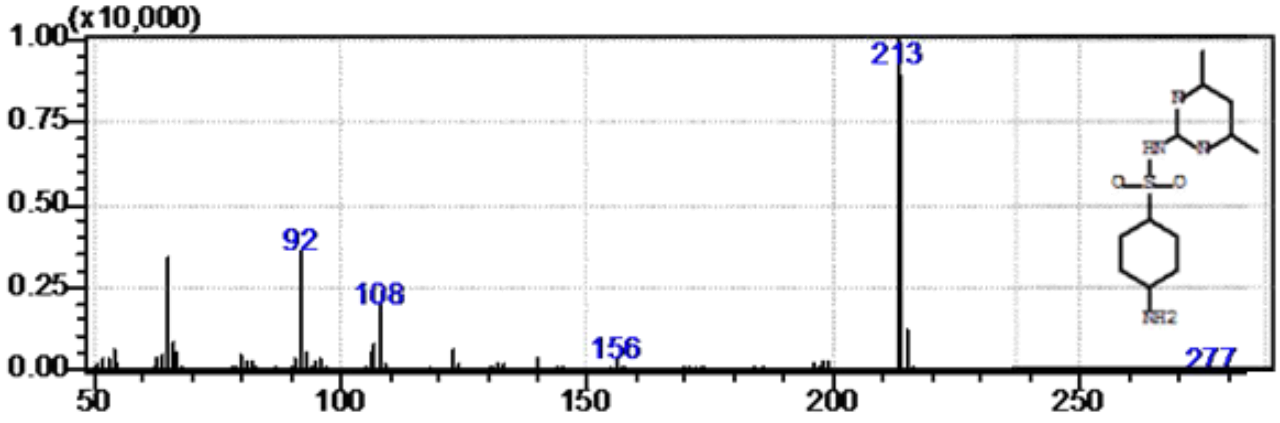

Figure A3. 14: Spectrum of sulfamethazine. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Chloramphenicol is a prescription drug belong to antibiotic group.

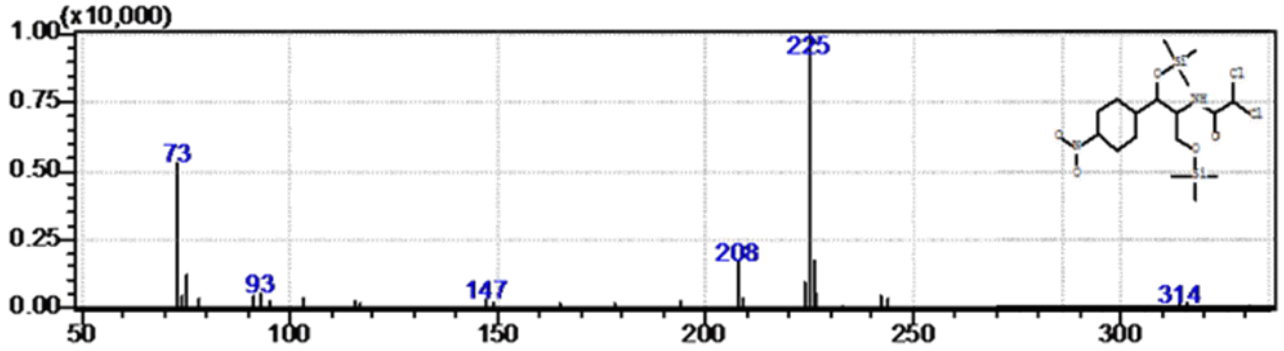

Figure A3. 15: Spectrum of derivatized chloramphenicol. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization.

Cocaine is an illicit drug.

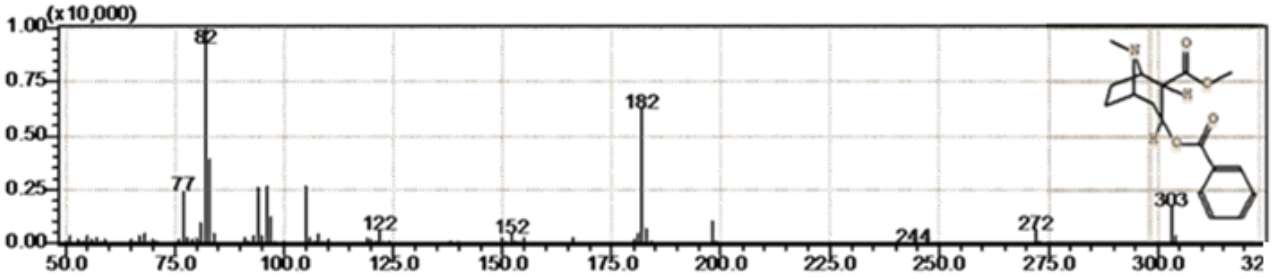

Figure A3. 16: Spectrum of cocaine. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization

Methamphetamine is an illicit drug.

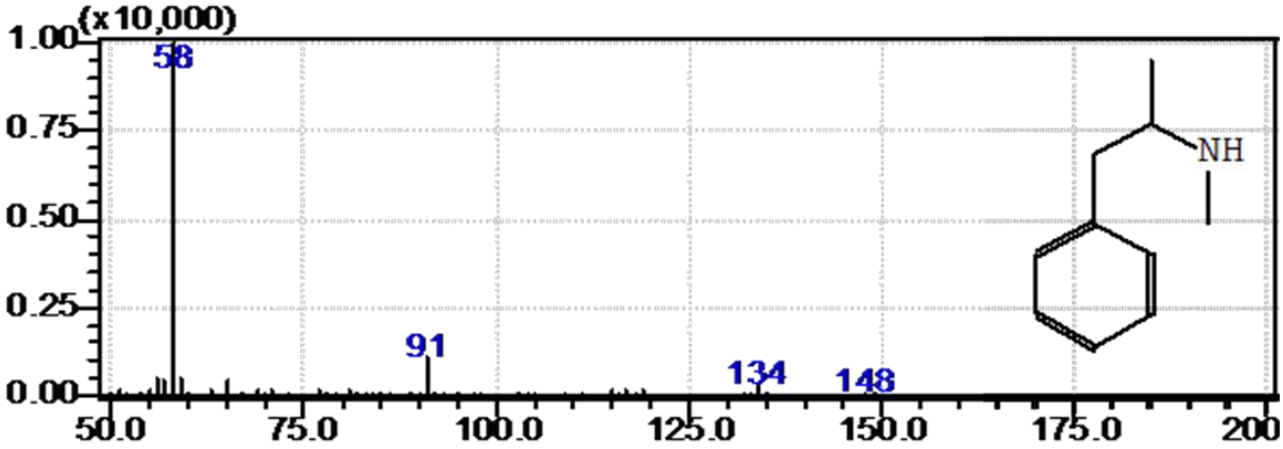

Figure A3. 17: Spectrum of methamphetamine. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization

Morphine is an illicit drug.

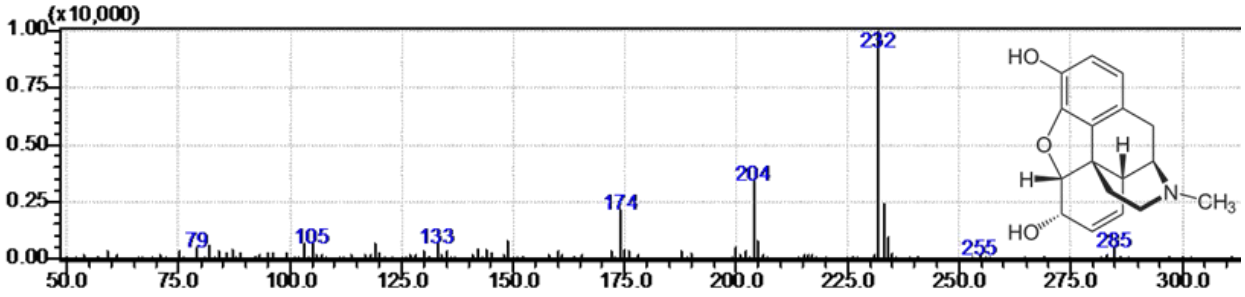

Figure A3. 18: Spectrum of morphine. Obtained by injecting 2 µL of standard solution into GC-MS after derivatization

Appendix A4: Sampling Sites Description

Water and Sediments Samples were Collected Along Mgeni and Msunduzi Rivers.

Mgeni River

Midmar Dam Inlet and Outlet

Midmar Dam is located within the farming area and proximity to the source of Mgeni River. The surrounding villagers and stock farming in the area contribute the load of emerging contaminants into the dam. This sampling site was rocky and it was difficult to collect sediments samples.

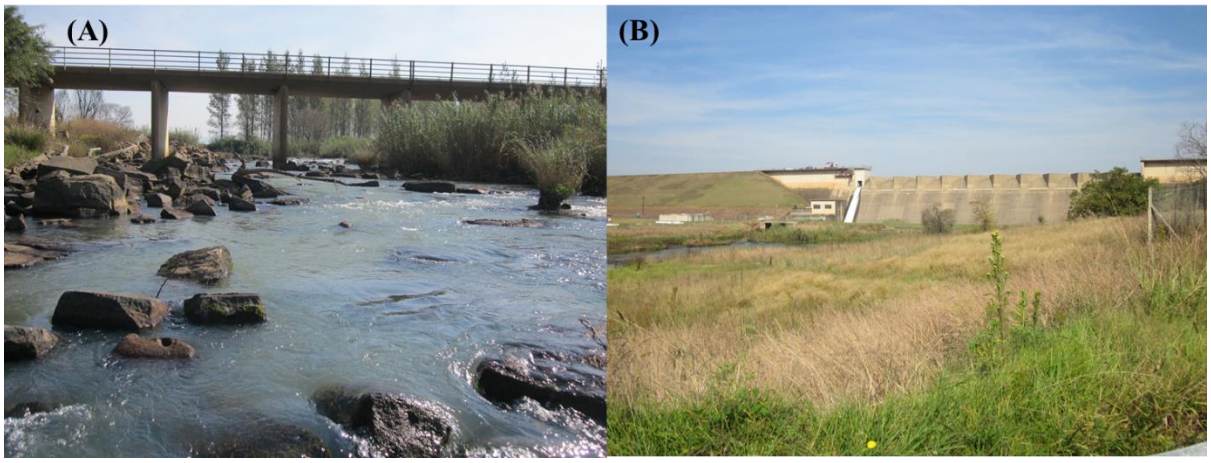

Figure A4. 1: The picture was taken during the sampling campaign, (A) Midmar Dam Inlet and (B) Midmar Dam Outlet.

Howick Falls

This sampling site is located on the cliff in Howick area. The villagers uses this place for washing cloths and bathing. Personal care products drugs were expected to be dominants in this location.

Figure A4. 2: The picture was taken during the sampling campaign showing the cliff of Mgeni River at Howick Falls.

Albert Falls Dam Inlet and Outlet

Albert Falls Dam is surrounded by cane field farms. And villagers staying in these farms uses the dam for fishing and bathing. Since the area is sparsely populated there no proper sanitation in place. Runoff water during rainy season exposes the dam to emerging contaminants.

Figure A4. 3: The picture was taken during the sampling campaign showing the sugarcane farm surrounding Albert Falls Dam.

Nagle Dam

Nagle dam is located in the mountainous areas of Pietermaritzburg surrounded by conservancy area. But the dam is not fenced which constantly exposes the dam to contamination from domestic and wild animals. This is dam was less contaminated compare to other dams.

Figure A4. 4: The picture of Nagle Dam was taken during the sampling campaign.

The Joining Point

This point is located immediately after Msunduzi River has joined the Mgeni River. After this point concentration of emerging contaminants increases in the Mgeni River. Msunduzi River contributes to the load of contaminants in the Mgeni River. In addition, the slope is steep ground water passes through pit latrines might contribute to the load of pharmaceuticals.

Figure A4. 5: The picture taken during the sampling campaign showing pit latrine used by villagers close to the river.

Inanda Dam Inlet and Outlet

Inanda is an important water source in Durban. However, this dam because of its geographical location, experiences higher concentration levels of contaminants. Water in this dam moves slowly and it allows the contaminants to settle to the sediment.

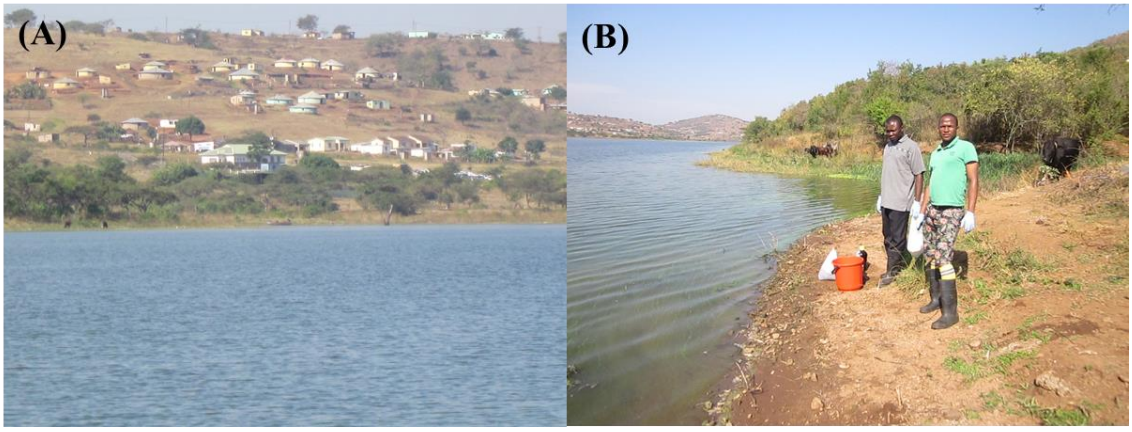

Figure A4. 6: Pictures taken during sampling campaign in Inanda Dam.

Reservoir Hills

Reservoir hills is an informal suburban area, where people occasional build houses and divert their sewage pipe in Mgeni River. This area is contaminated and the water at this site smells like sewage from a wastewater treatment plant.

Figure A4. 7: The picture taken during the sampling campaign showing the River covered with vegetation due to nutrients from diverted sewage.

Mgeni Business Park

Mgeni Business Park site is surrounded by factories including pharmaceutical industries.

Figure A4. 8: The picture taken during the sampling campaign showing parked industrial truck on the bank of the Mgeni River.

Ethekwini Wastewater Treatment Plant

This wastewater treatment plant receives influent from domestic sewage pipes and uses conventional method of cleaning wastewater. Pharmaceutical were detected in the final effluent from this site.

Figure A4. 9: the picture taken during sampling campaign, showing point after chlorination, taking pictures is not allowed in the main plant.

Blue Lagoon

Blue lagoon is the estuary of the Mgeni River and used as recreational site. People have direct access to this site and is often littered.

Figure A4. 10: The picture taken during the sampling campaign showing cars near Mgeni River, this sites is used recreational area by Ethekwini Municipality.

Msunduzi River

Camp drift, Baynes Spruit, Edendale Hospital and Du Toit

These sites are proximity to each other, surrounded by informal settlements and located within Pietermaritzburg town. They are exposed to contamination in many route runoff water from city centre and through illegal dumping.

Figure A4. 11: Pictures taken during our sampling campaign; (A) Camp Drift, (B) Baynes Spruit River and (C) Du Toit.

Pietermaritzburg Wastewater Treatment Plant

This plant treats water from both domestic and industrial waste. After cleaning its discharges mega litres of effluent directly into the Msunduzi River. Emerging contaminants were detected in the effluents.

Figure A4. 12: The picture taken during the sampling campaign showing the point of discharge at Pietermaritzburg Wastewater Treatment Plants.

Agricultural area

Agriculture area is located in rural area of Pietermaritzburg where villagers practise subsistence farming.

Figure A4. 13: Picture taken during our sampling campaign in Mkhambathini district, Agricultural area. Water in this site is of poor quality but villager's uses it for irrigation.

Henley Dam

Henley Dam is only dam found in the Msunduzi River and before Pietermaritzburg Town. Surrounded by informal build homes with no proper sanitation.

Figure A4. 14: The picture taken during the sampling campaign showing outlet of Henley Dam.

Msunduzi Town

Msunduzi Town is surrounded by villagers, people wash cars and tents in this point. Personal care products were expected to be at higher concentration in this site. Generally, this site was among the highest contaminated site investigated.

Figure A4. 15: The picture taken during the sampling campaign showing tents being washed on the bank of the Msunduzi River.

Appendix A5. Geographical Location of Points – Global Positioning System (GPS)

Table A4. 1: GPS coordinates of the sampling sites.

Mgeni River		
Site	Latitude	Longitude
Midmar Dam Inlet	-29.484444	30.158333
Midmar Dam Outlet	-29.485278	30.201944
Howick Falls	-29.483889	30.238611
Albert Falls Inlet	-29.441944	30.329722
Albert Falls Outlet	-29.435578	30.428338
Nagle Dam	-29.585556	30.631944
Tributary Umgeni/Msudunzi	-29.61816	30.680651
Inanda Dam Inlet	-29.649422	30.790629
Inanda Dam Outlet	-29.715278	30.868611
Reservoir Hills axis	-29.785556	30.940278
Umgeni Business Park	-29.8025	30.982778
eThekwini WWTP Influent	-29.785278	30.997222
eThekwini WWTP Effluent	-29.801944	30.9975
eThekwini WWTP after Treatment	-29.801944	30.9975
Blue Lagoon	-29.800278	31.036667
Umgeni Source	-29.481444	29.833537
Msunduzi River		
Site	Latitude	Longitude
Camp Drift	-29.613056	30.376667
Du Toit Vijoen Road	-29.597778	30.400278
Darvill WWTP Inlet	-29.604167	30.431111
Darvill WWTP Outlet	-29.604167	30.431111
Agricultural Area	-29.611111	30.558889
Msunduzi Town	-29.661111	30.636111
Baynes Spruit	-29.593473	30.433513
Edendale Hospital	-29.64431	30.340719
Henley Dam	-29.621116	30.25452