



**OPTIMIZATION OF EXTRACTION TECHNIQUES FOR THE ISOLATION
AND PRE-CONCENTRATION OF PHARMACEUTICALS IN AQUATIC
ENVIRONMENTS**

BY

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DECLARATION

I hereby certify that this dissertation for the fulfilment of the MASTER'S DEGREE IN APPLIED SCIENCE in Durban University of Technology, Durban, South Africa is entirely my own work, therefore I declare that I have implemented rational care to ensure that the work is novel and does not to the best of my knowledge breach any law of copyright and has not been taken from the work of others nor submitted in any other institution. Brief quotations from this dissertation are permissible without special authorization, on condition that accurate acknowledgements of the source are made.

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DEDICATION

To myself for constantly choosing me and pushing myself to achieving all that is deemed impossible for me against all odds through Christ that strengthens me.

To my daughter Lunathi Ayolise Sigonya, the source of my encouragement and the reason to my existence.

To my parents Bulelwa Babalwa Sigonya and Vuyani Victor Sigonya, for their continued support and prayers

And lastly to my siblings Asavela, Kaulele, Likuye, Mihlali, you guys are my life support.

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My lord and saviour, if it wasn't for the lord I wouldn't have made it this far in anything in life and more over this work, the lord has been my strength, my guide and the love of my life, and therefore I thank you God for giving me the serenity to accept the things I cannot change, the courage to change the things that I can and most importantly the wisdom to know the difference. *Ubemhle nkosi empilweni yam.*

I thank my daughter Lunathi for having the heart that she has, for allowing me to not be a physically parent to achieve my dreams, all that I do I do it for you mntanam and I thank the lord for giving you an accepting and supportive heart.

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May the grace of the lord Jesus Christ, the love of God, the fellowship of the holy spirit be with you all throughout the days of your life. Stay blessed.

ABSTRACT

The occurrence of pharmaceuticals in South African aquatic environments has been reported in several studies. However, most of these reports focused on the occurrence of organic compounds in wastewater and surface water. There are very few studies reporting the presence and concentration of these compounds in seawater and coastal areas. Further, most studies have looked at only on one season. This study focussed on the optimisation of a SPE extraction method using Bond Elut Plexa cartridges for the identification and quantification three non-steroidal anti-inflammatory drugs (NSAIDs), three antiretroviral drugs (ARVs) and a lipid regulator in coastal area of Durban city, South Africa covering four seasons. The optimised SPE conditions were as follows: 500 mL sample volume and at pH 5.8, 5 and 5 mL as conditioning and elution volumes, respectively. The flow rate ranging from 5 to 10 mL/min 10 and 5 mL/min as sample and elution flow rates. The extracted compounds were qualitatively and quantitatively detected by a high-performance liquid phase chromatographic instrument coupled to a photodiode array detector (HPLC-PDA). The recoveries ranged from 62 -102% with RSD values of 0.56 to 4.68% respectively for the determination of emtricitabine, tenofovir, naproxen, diclofenac, ibuprofen, efavirenz, and gemfibrozil. The analytical method was validated by spiking estuarine water samples with 5 $\mu\text{g L}^{-1}$ of a mixture containing the target pharmaceuticals and the matrix detection limits (MDL) were established to be 0.62- 1.78 $\mu\text{g L}^{-1}$ for the target compounds. The optimized method was applied to seasonal monitoring of pharmaceuticals at chosen study sites from winter and spring of 2019 and summer and autumn of 2020. The sum of emerging pollutants (ΣEP) were calculated based on each study site. The influent of the Kingsburgh WWTP (EFK) had the highest ΣEP of 144.88 $\mu\text{g L}^{-1}$ in winter between the two wastewater treatment plants area in this study. The Northern WWTP influent (INN) had a total ΣEP of 117.11 $\mu\text{g L}^{-1}$ in autumn, the Kingsburgh WWTP effluent (EFK) had a concentration 63.8 $\mu\text{g L}^{-1}$ in autumn and a concentration 63.8 $\mu\text{g L}^{-1}$ in summer and the

Northern (EFN) had a total Σ EP of $43.97 \mu\text{g L}^{-1}$ in winter. A comparison between UMgeni (UR) and Kingsburgh river (KR) showed that the KR had the highest concentration of total Σ EP of $22.66 \mu\text{g L}^{-1}$ and UR with the total Σ EP of $18.3 \mu\text{g L}^{-1}$ both in winter and spring, respectively. The seawater EPs Blue Lagoon (BL) had the highest Σ EP of $46.75 \mu\text{g L}^{-1}$ in spring, subsequently Warner Beach bottom (WBB), Glen Ashley (GA) and Warner Beach top (WBT) with concentrations of $24.96 \mu\text{g L}^{-1}$ in summer, $13.29 \mu\text{g L}^{-1}$ in spring and $6.94 \mu\text{g L}^{-1}$ in autumn, respectively. Estuarine EPs had concentrations of $37.9 \mu\text{g L}^{-1}$ and $20.97 \mu\text{g L}^{-1}$ for Warner beach estuary (WE) and UMgeni estuary (UE) in winter. WBE having the highest concentration between the two. This showed a significant variation on the presence of these pharmaceuticals in different season.

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ANNEXURES

LIST OF ABBREVIATIONS

ARV: Antiretroviral

BL: Blue Lagoon

COX: Cyclooxygenase

CQC: Catchment quality control

DBPs: Disinfection by-products

DICLO: Diclofenac

EFK: Effluent in Kingburgh

EFN: Effluent in Northern

EFV: Efavirenz

EMI: Emtricitabine

GA: Glen Ashley

GC-MS: Gas chromatography- Mass spectrometer

GEM: Gemfibrozil

HIV: Human immune virus

HLB: Hydrophilic and lipophilic balance

HPLC-PDA: High pressure liquid chromatography- photodiode array detector

IBU: Ibuprofen

INK: Influent in Kingsburgh

INN: Influent in Northern

KR: Kingsburgh River

LOD: Limit of detection

LOQ: Limit of quantification

MAX: Mixed mode anionic exchange

MCL: Maximum contaminant level

MDL: Method detection limit

NAP: Naproxen

NNRTI: Non-nucleoside reverse transcriptase inhibitors

NOM: Natural organic matter

NRTI: Nucleoside reverse transcriptase inhibitor

NSAIDs: Non-steroidal anti-inflammatory drugs

PAC: Powder activated carbon

RSD: Reproducibility standard deviation

ΣEP: Sum of emerging pollutants

SPE: Solid phase extraction

STR: Single tablet regimen

TENOD: Tenofovir Disoproxil

U.S: United States

UR: UMgeni River

WBB: Warner beach bottom

WBT: Warner Beach Top

WHO: World health organization

WWTP: Wastewater treatment plant

CHAPTER ONE

1 Introduction

1.1 Background

The presence of pharmaceuticals was initially determined by Thames Water Authority (TWA) in 1977 during the catchment quality control (CQC) in London. (Glassmeyer et al., 2005; Matongo et al., 2015a; Tran et al., 2018; Dearden & Nicholson, 1985), it was assumed that the drug residues would survive various wastewater treatment processes. Over the years, the fate and occurrence of these pharmaceuticals in aquatic environment has been of great interest to scientists and the scientific community to evaluate the effectiveness of the ecological policy (Zhang et al., 2013). The increase in the presence of these pharmaceuticals in water system is a problem and a possible health risk to human life and aquatic life as they are classified as hazardous and intractable bioaccumulative. This means they are likely to enter the food chain and result in their excessive consumption. Excessive exposure to pharmaceuticals can lead to hostile side effects such as nausea, vomiting, abdominal pain and drowsiness (Lagha et al., 2011). These pharmaceuticals find their way into the water system via excretion or metabolites from households, hospitals, clinics into the wastewater treatment works or direct disposal of the effluent from pharmaceutical producing industries into the river water or sea (Amdany et al., 2015; Matongo et al., 2015c; Zunngu et al., 2017). They are further discharged as part of the effluents into the river from the wastewater treatment sectors and this water flows into the estuary and feeds into the sea. Also, the use of wastewater treatment plant (WWTP) sludge for manure and river water from where the effluent is discharged for crop irrigation may serve as a source of transportation for these pharmaceuticals into the environment. A great concern is that many South Africans especially those residing in informal and rural settlements still depend on water from the rivers and dam which suggests that they are directly exposed to these pharmaceuticals.

Pharmaceuticals are considered as intractable and easily transported into the water system. Wastewater treatment are unsuccessful in completely removing these pharmaceuticals (Madikizela & Chimuka, 2016; Mlunguza et al., 2019; Wasilewska et al., 2011). The successful removal of these pharmaceuticals depends on the removal and purification technologies used as well as the physiochemical properties exhibited by these pharmaceuticals (Tran et al., 2018).

Thus far, several classes of pharmaceuticals have been detected in aquatic environments globally, these classes include steroid hormones (Aris et al., 2014; Yang et al., 2017), antibiotics (Balakrishna et al., 2017; Lapworth et al., 2012; Yang et al., 2017), ARV drugs that (Abafe et al., 2018a; K'oreje et al., 2016; K'oreje et al., 2012; Mahlambi et al., 2019; Mosekiemang et al., 2019; Ngumba et al., 2016; Ramaswamy & Dhas, 2014; Schoeman et al., 2015; Wood et al., 2015; Wooding et al., 2017), NSAIDs (Feng et al., 2013; Gilart et al., 2013; Gumbi et al., 2017; Madikizela et al., 2017; Radke et al., 2010; Shanmugam et al., 2014; Vergeynst et al., 2015; Wang and Wang, 2016). A number of review articles deliberating various pharmaceuticals detected in the environment have been published (Feng et al., 2013; Leal et al., 2010; Madikizela et al., 2017; Modi et al., 2012; Rivera-Utrilla et al., 2013; Sophia and Lima, 2018). Pharmaceuticals that have been reported in South African water bodies are given in Table 1.1.

Table 1-1.1 Pharmaceuticals found across South African water systems.

Class of pharmaceuticals	Target compounds	Used technique	Sampling sites	Town/city	References
ARV drugs	Emtricitabine, Efavirenz	Using SPE Strata SDB-L cartridges	WWTP	Cape Town	(Mosekiemang et al., 2019a)
	Efavirenz	Using SPE Oasis HLB cartridges	WWTP	Durban	(Abafe et al., 2018)
	Efavirenz & Tenofovir disoproxil	Using SPE Bond elute Plexa cartridges	Surface water	Johannesburg	(Rimayi et al., 2018)

Efavirenz	Using SPE bond elute cartridge, sonication	WWTP	Johannesburg	(Schoeman et al., 2015)
Efavirenz	Using SPE MIP	WWTP	Durban	(Mahlambi et al., 2019)
Efavirenz	Direct sorption	Surface water	Johannesburg	(Wooding et al., 2017)
Efavirenz	HF-LPME	Surface water, WWTP Aquatic plants	Durban	(Mlunguza et al., 2020)
Tenofovir	Using SPE OASIS HLB cartridges	Surface water	Johannesburg	(Wood et al., 2015)

NSAIDs	Diclofenac, naproxen, ibuprofen	Using OASIS cartridges	SPE, HLB and water	Aquatic plants and surface water	Durban	(Sibeko et al., 2019) (Gumbi et al., 2017a, 2017b)
	Diclofenac, naproxen, ibuprofen, ketoprofen	Using OASIS MAX as well as MIPs	SPE, HLB and cartridges	Surface water and WWTP	Durban Lady smith	(Madikizela et al., 2016; Madikizela & Chimuka, 2017b, 2017a; Zunngu et al., 2017)
	Diclofenac, naproxen, ibuprofen	Using OASIS cartridges	SPE, WWTP	WWTP estuarine water, seawater	Durban	(Ngubane et al., 2019a)

Diclofenac, naproxen, HF-LPME	WWTP,	Durban	(Mlunguza et al., 2019b)
ibuprofen, fenoprofen	aquatic plants, surface water		
Fenoprofen	Using SPE, MIP	WWTP	Durban
			(Mbhele et al., 2018)

NB: The NSAIDs and ARVs are those selected for investigation in this study as shown in the table

These pharmaceuticals comprise of ARVs, NSAIDs and antibiotics. Pharmaceuticals such as, ARVs are the least examined in the world. ARVs were only integrated into the human community in 1987 with zidovudine/lamivudine delivered as the first ARV drug (Walker et al., 2008). A study by Ncube et al.(2018) reported that there were 20 million people administering ARV drugs in 2018 with the research predicting 21.1 tons of Atripla administered worldwide in 2016 alone. Various researchers have shown that these ARV drugs are released into the natural environment as part of drainage from household wastewater(Kimosop et al., 2016) . The environmental status of these pharmaceuticals especially ARVs is not well known. There is increasing concern in tracking the occurrence of ARV drugs in aquatic systems. To date, several ARV drugs have been found in surface water from two African countries, Kenya (K'oreje et al., 2016; K'oreje et al., 2012; Ngumba et al., 2016; Langenhove et al., 2018) and South Africa (Mlunguza et al., 2020; Rimayi et al., 2018; Schoeman et al., 2017; Wood et al., 2015; Wooding et al., 2017). Contrary to other countries South Africa has recently gained interest in monitoring the presences of ARV drugs in the past 6 years. Provinces that have reported on ARV drugs in water are Gauteng, KwaZulu-Natal, and the Western Cape.

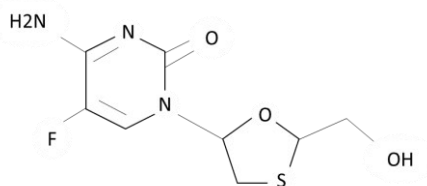
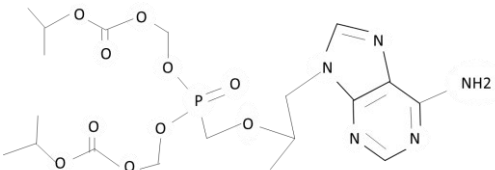
In 2019, the South African statistics reported a growth in the number of diagnosed citizens infected with human immune virus (HIV), (StatsSA, 2019). The number of people living with virus was estimated to be approximately 7.79 million in 2019 which is an increase of 790 thousand from the 2018 mid-year statistics which suggested that there were 790 thousand people who were ingesting the antiretroviral drug. KwaZulu-Natal is the region with second highest population after Gauteng, with an approximate 11.3 million residents residing in the province.

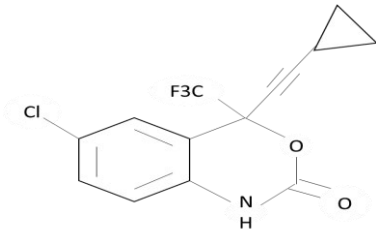
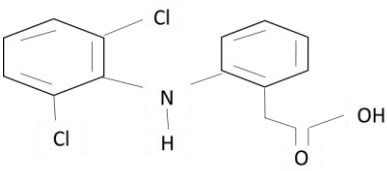
ARV drugs have pKa values varying from 2.6, 10.2 and 18.59 for EMI, EFV and TENOD and with poor water solubility at 25 °C as illustrated in Table 1.2. However, their polarity

contributes to the inability of WWTPs to totally extract them during the water purification process. A study by Abafe et al. (2018) reported on the presence of ARV drugs in various WWTP around Durban, KZN. However, there has not been any record of the presences of ARVs in estuarine water and seawater, the purpose of this study is to report on the occurrence of these drugs in wastewater from WWTP and from estuaries and seawater.

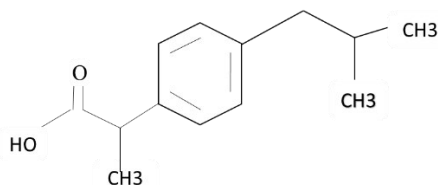
NSAIDs such as diclofenac, ibuprofen, and naproxen are one of the pharmaceuticals that are frequently detected in South African water bodies due to the easy access to humans as an over-the-counter drugs and free distribution from clinics and hospitals and use in the veterinary application. These NSAIDs are classified as weak organic acids, used to treat inflammation, arthritis, pain and fever (Mbhele et al., 2018; Saleh et al., 2011; Strauch et al., 2011; Tran et al., 2018). Their popularity in the water system is due to their properties as shown in Table 1.2 which include high water solubility, polarity, and human excretion rates as un-metabolized drugs (Dahane et al., 2013; Kermia et al., 2016; Madikizela & Chimuka, 2017). The presence of NSAIDs in the aquatic systems have proven to be a great environmental concern over the years, a high number of these pharmaceutical residues are continuously detected in wastewater treatment plants, rivers, sediments and plants (Archer et al., 2017; Madikizela et al., 2018; Matongo et al., 2015b).

Table 1.2 physiochemical properties and molecular structures of the selected pharmaceuticals (ARVs and NSAIDs)

Compound	Structure	Compound type	Water solubility (mg L ⁻¹)	pKa	Use and side effects on humans
Emtricitabine		Non-nucleoside reverse transcription inhibitor	1.12x10 ⁶	2.65	Used to prevent the virus from multiplying. Renal failure, nephrogenic diabetes insipidus, Fanconi syndrome, lactic acidosis, liver damage, skin reaction
Tenofovir disoproxil		Nucleoside reverse transcription inhibitor	13400	3.8	Used to prevent the virus from multiplying. Severe liver problems, severe lactic acidosis, renal failure, bone loss,

					nephrogenic diabetes insipidus, Fanconi syndrome
Efavirenz		Nucleoside reverse transcription inhibitor	0.093	10.2	Used to prevent the virus from multiplying. Moderate to severe liver problems, rash and hepatotoxicity, central nervous system adverse effects, lipoatrophy, hypertriglyceridemia
Diclofenac		Non-steroidal anti-inflammatory drug	10	4.15	Used as an anti-inflammatory agent. Indigestion, nausea, headaches, pain, and increased blood pressure

Ibuprofen



Non-steroidal 58

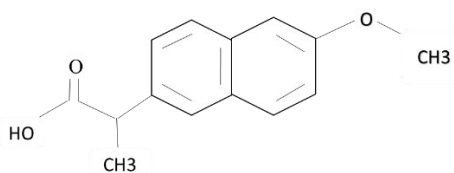
anti-inflammatory drug

4.85 Anti-inflammatory drug and chronic

arthritis

Bloating, nausea, dizziness, decreased appetite, and headaches

Naproxen



Non-steroidal 44

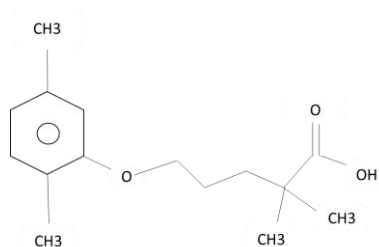
anti-inflammatory drug

4.15 Used for pain and cramps, an anti-

inflammatory drug.

Bruising. indigestion, headaches, swelling, drowsiness

Gemfibrozil



Lipid regulator

4.42 Used to lower lipid level.

Stomach pain, numbness, blurred vision, joint pain, rash

As shown in Table 1.1 there are various techniques that have been reported for the study of ARV drugs and NSAIDs. It is eminent that solid phase extraction (SPE) is the most popular method. Despite the high extraction efficiency recorded for this procedure, there is still no method accepted globally. The SPE method that is utilizing HLB, or MAX cartridges is commonly considered to be non-selective. Of the two HLB cartridges that are frequently used, they have been demonstrated to extract both hydrophilic and lipophilic compounds (Agunbiade & Moodley, 2014; Gumbi et al., 2017; Ngubane et al., 2019). The idea in this study was therefore to investigate the applicability of Agilent bond elute Plexa cartridges with a SPE technique that could offer selectivity and sensitivity in the analysis of both NSAIDs and ARV drugs in complex matrices using a common HPLC-PDA instrument. Nevertheless, this method has been mainly explored for single analytes, while, in most situations, NSAIDs are observed concurrently in water bodies. Rarely has there been a technique that is used to investigate both these classes of pharmaceuticals in all aquatic spheres including the estuaries and sea. The purpose of this investigation is to detect and quantify pharmaceuticals with different polarities and physiochemical behaviours from the initial source of contamination (WWTP) to rivers, estuaries and sea using a single technique in HPLC-PDA.

CHAPTER TWO

2 Literature review

2.1 Source of Pharmaceuticals in the aquatic environment

Source of pharmaceuticals can be in two forms namely, point source and diffuse source pollutants. Polluted point sources are a common recognizable single source that originates from different locations and can be determined in statistical modelling (Lapworth et al., 2012). Industrial effluents, hospital effluent and wastewater treatment facilities, for example, as well as the septic tanks, are the primary outlet for the surface region and the water supplies. Contrary to that, diffusion pollution is difficult to classify the distinct position on the spatial scale (Li, 2014). One example is runoff from livestock waste and manure, which is similar to agricultural runoff, industrial runoff from domestic waste as well as leakage from sewage management systems and plants. (Bueno et al., 2012). Compared with point source pollutants, diffuse pollutants typically have lower environmental impacts as it has greater natural soil and subsurface attenuation capacity (Murray et al., 2010).

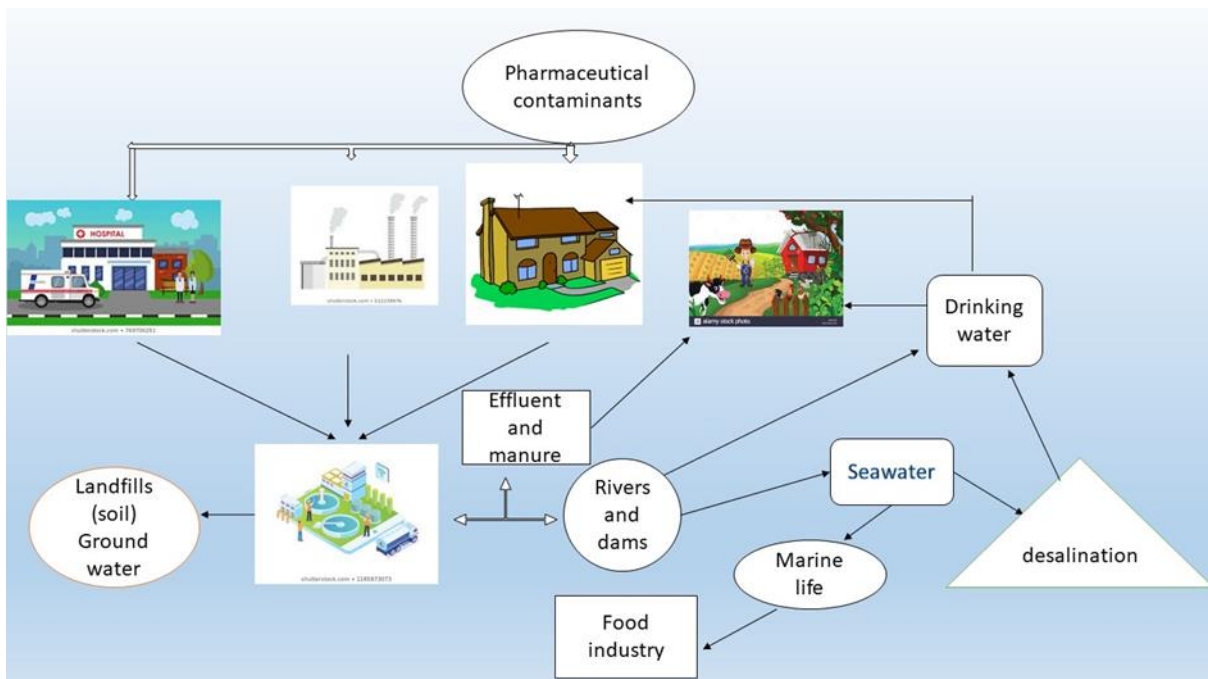


Figure 2.1: sources of Pharmaceuticals in Aquatic environments.

Figure 2.1. demonstrates the mechanisms how the pharmaceuticals discharged from the sources are passed to the receptors. There are six key sources, including manure, animal waste, freshwater aquaculture waste, agricultural waste and domestic waste, outlined and condensed by many literature reviews (Eggen et al., 2010; Heberer, 2002; Lapworth et al., 2012; Pal et al., 2010). For instance, soil field, groundwater and surface water, receptors of pharmaceuticals are classified as the three main classes in the natural environment.

2.1.1 *Point source pollutants*

Municipal landfills are sources of a large variety of biological, biodiversity and human health compounds (Eggen et al., 2010). The waste stored in the landfill may contain chemicals that may have detrimental impact on the environment's safety. In addition, urban landfills that generate leachate that contains substantial amounts of dissolved organic matter, heavy metals and other pollutants (Li, 2014). According to a study by Buszka et al.(2009), it was reported that wastewater that was infiltrating water that mixes with the submerged waste products. Furthermore, it was found that wells collecting many forms of pollutants, such as hormones, pharmaceuticals, and fire retardants, which are located down from the landfill gradient (Peng et al., 2014). Another study reported that the wells connected to the landfill had large accumulation of pharmaceuticals that were continuously flowing into the groundwater as the landfills produced toxic wastewater that passes into the drainage network and infiltrates through the soil and discharges into the surface water Barnes et al. (2004) .

Wastewater bodies were deemed one of the most significant supply points of pharmaceuticals in aquatic environment (Glassmeyer et al., 2005; Matongo et al., 2015a; Tran et al., 2018). Several surveys in numerous nations, such as United States, Algeria, United Kingdom, South Africa, Europe , indicated that the vast number of pharmaceuticals ranging from 16 to 70 were present in wastewater (Bueno et al., 2012; Kermia et al., 2016; Lapworth et al., 2012;

Madikizela et al., 2017b; Nikolaou et al., 2007; Wilkinson et al., 2017). There are reports that had suggested that most untreated water which is pumped into the sewer system to the sewage treatment plant could lead to the release of pharmaceuticals and personal care products (PPCP) items (Ebele et al., 2017; Kanama et al., 2018; Papageorgiou et al., 2016; Wang & Wang, 2016a). Despite the alarmingly high presence and consumption rates of these pharmaceuticals, many antiviral drugs have been discovered in aquatic environments but are seldom carefully examined. (Nannou et al., 2020; Nikolaou et al., 2007; Ouyang et al., 2017). Other outlets, such as hospitals and factory drainage, are also essential drivers of pharmaceuticals reaching the community. Several reports have studied the presence of pharmaceuticals in effluent and influent of water treatment facilities and showed that the elimination of pharmaceuticals was insufficient (Abafe et al., 2018a; Funke et al., 2016; K'oreje et al., 2016; Madikizela et al., 2017). For instance several studies found antiretroviral drugs, antibiotics and trihalomethanes in wastewater facilities that could not be entirely eliminated with a mean removal efficiency varying from 34 to 93 % (Kamba et al., 2017; Mlunguza et al., 2020; Mosekiemang et al., 2019; Ngumba et al., 2016; Rimayi et al., 2018). Various studies have shown that septic tanks contain common forms of pharmaceuticals such as ibuprofen, naproxen, diclofenac and gemfibrozil Pal et al. (2010), This suggested the leakage of these septic systems to be a high risk contributor to the contamination of the soil and water with pharmaceuticals in the environment.

2.1.2 *Diffusion source pollutants.*

Organic chemicals, such as pharmaceuticals, are joining the land and water supplies in a number of areas, with waste sludge being one of the more significant causes (Cortés et al., 2013; Saleh et al., 2011). It was reported that by applying waste sludge to the ground surface could significantly lead to diffusion of soil and water pharmaceutical products (Lapworth et al., 2012). Sewage sludge is frequently disposed in two main strategies, which are reusable for agricultural

and/or landscaping purposes or final disposal. In countries like the U.S and Poland, reports indicated that it could be used as soil amendment because it contained organic matter and nutrients which could improve the quality of soil (Kinney et al., 2008). The largest concentration of pharmaceuticals present in the sludge is thiabendazole (about 5000 mg/kg) and other forms of pharmaceuticals, such as caffeine, carbamazepine ibuprofen, and diclofenac. Furthermore, because of the high concentration of these pharmaceuticals and low solubility of halogenated hydrocarbons in the sludge, there is a risk of groundwater deterioration through sludge application in the soil and surface runoff (Lapworth et al., 2012).

Another type of diffusion source pollution is groundwater surface layer, the interface is the indirect route by which surface water and ground water are shared by precipitation and downward movement due to attenuation processes in the soil and the unsaturated region (Lapworth et al., 2012).

Pharmaceuticals including ARVs and NSAIDs have been detected in various water systems across the globe, but there is little information of these two classes of pharmaceuticals in the coastal area. Another study revealed the presence of NSAIDs such as ibuprofen, naproxen and diclofenac in the Kwa-Zulu Natal region of South Africa, to our knowledge there is no information about the presence of ARV drugs in the coastal area across South Africa and Africa at large. This study will be the first to report on the presence of these class of pharmaceuticals in seawater. Few studies have documented the presence of NSAIDs and ARV along the UMgeni River from its point of origin to the estuary where the river flows into the Indian ocean (Agunbiade & Moodley, 2014; Gumbi et al., 2017b; Matongo et al., 2015c; Rimayi et al., 2018). This was an indication that ARVs and NSAIDs were transported along the UMgeni River and disposed of into the Durban coastal zone. This was reported to raise an issue since seawater is released as an acceptable response to shortage of drinking water in Africa (Goga et al., 2019).

2.2 Toxic effects of pharmaceuticals in the aquatic environment

Most pharmaceutical compounds exist for longer periods at low concentrations in aquatic environments. Their lipophilicity and the toxic effects were recently reported to be most likely chronic than acute (Khan et al., 2020). *In vitro* and *in vivo* are mainly used to assess the toxic activity of pharmaceuticals compounds, although the latter is superior. Development, mortality, and fertility were typical endpoints for toxicity research. However, only recently considerable attention has been given to studying possible impacts on molecular, behavioural, and genetic reactions (Godoy & Kummrow, 2017). A report by Gunnarsson. and co-workers showed that the toxicity of pharmaceutical substances differed depending on the type of contaminant, exposure time, developmental time frame, and concentration in which test organisms are exposed (Gerbersdorf et al., 2015). Organisms such worms, molluscs, water crustaceans and fish were recently used to investigate the toxic effects of pharmaceuticals.

The toxic effects of analgesics were recorded for ibuprofen, diclofenac, naproxen, and paracetamol (Lonappan et al., 2016). Environmental contact to diclofenac was reported to induce modification to gill and renal lesion in rainbow trout at concentration of $5 \mu\text{g L}^{-1}$ (Köhler et al., 2004). Moreover, acute renal failure occurred in vultures feeding on diclofenac treated livestock, which was the reason for the significant decrease in their population recorded in Pakistan and India (Oaks et al., 2004). There is little to no evidence of any reproductive abnormalities caused by analgesics at concentrations of environmental importance. However, one study found diclofenac had concentrations ranging from ($1000 -2000 \mu\text{g L}^{-1}$) to induce a suspension in Zebrafish hatching (Hallare et al., 2004). Commonly reported effects were linked to inhibition of growth in cyanobacteria and green algae and development of antibiotic resistance (Khan et al., 2020). The presence of other contaminants, especially heavy metals may increase the toxicity of pharmaceuticals for example oxytetracycline and ciprofloxacin

complexes with copper, cadmium, and zinc, which showed maximum inhibition of algal growth relative to individual antibiotics. Ignoring those correlations could underestimate the risk thus, studies by Zhang and co-workers has indicated that these effects of these pharmaceuticals must be considered in the environmental assessments. Beta-blockers and blood lipid receptors are known to have effects in non-target and target organisms. For example, a widely administered blood lipid regulator atorvastatin prescribed at $1.2 \mu\text{g L}^{-1}$, contributed to the degradation of lipid, carbohydrates, and protein reservoirs, and inhibited transcription of main enzymes involved in mitochondrial biogenesis and fatty acid metabolism in the digestive gland of keystone species (Falfushynska et al., 2019). Much as in human beings, the presence of beta-2 receptors in fish reproductive, cardiac, and liver tissues raises the probability of an ecotoxic activity triggered by beta-blockers. Reproductive abnormalities for blood lipids and beta-blockers are usually considered to pose little danger to aquatic species due to their high sorption affinity to sediments reducing bioavailability (Maszkowska et al., 2014). Just recently, a study by Kristensen et al.(2018) confirmed that the over the counter drug ibuprofen caused reproductive disorder in human males leading to compensated hypogonadism. This finding might have served as a basis for further studies on the consequences of human health on environmental exposure to such pollutants. A study by Köhler et al. (2004) showed that the exposure to diclofenac display concentrations in tissues such as muscles, kidneys, gills, and liver. In general, the highest concentration was found in the liver followed by the kidneys and gills. Very small concentrations of diclofenac have been observed in the muscle tissue. After being exposed to $1 \mu\text{g L}^{-1}$ diclofenac levels of the compound were found in the kidney at $1024.8 \pm 67.5 \text{ ng/g}$, the liver and gills at $2882.4 \pm 159.1 \text{ ng/g}$, and the muscular tissue at $72.8 \pm 23.3 \text{ ng/g}$.

A study in Pakistan reported the presence of diclofenac, ibuprofen, naproxen in wastewater and surface water, with concentration of 905.03, 41.9, 14 ng L^{-1} for each compound

respectively in surface water and 72710.6, 219147.8, 140819 ng L⁻¹ respectively in wastewater (Ashfaq et al., 2019). Gemfibrozil had concentration of 100 ng L⁻¹ in wastewater in study from India (Cahn et al., 2017).

2.3 Detailed description of target compounds

2.3.1 Antiretroviral drugs

ARVs are drugs used in the treatment of retroviral infections, especially the type 1 human immune virus (HIV-1). However, HIV-1 antiretroviral therapy does not cure or destroy the virus, but only inhibits or slows down its replication (Page & Taylor, 2018). Typically, a person who requires HIV medication takes two or three pills a day, each affects the virus differently, and keeping the balance is important. There were benefits that were shown by reported studies of a single tablet regimen (STR) (Hodder et al., 2010; Plosker, 2013). The STR is easy to administer and track and works well for the patient taking the medication. Lower pill pressure and high adherence were associated with increased virologic control, and STR could increase patient's satisfaction (Nachege et al., 2014).

Emtricitabine (EMI) is a nucleoside reverse transcriptase inhibitor drug (NRTI) with the chemical formula 5-fluoro-1(2R,5S)-[2-(hydroxymethyl)-1,3 oxathiolan-5-ylcytosine]. EMI is the equivalent of cytidine, which varies from other cytidine equivalents since fluorine is in 5th position. The mechanism of interaction of EMI was reported to involve the intracellular phosphorylation to form emtricitabine 5- triphosphate and integrated into HIV-1 DNA, resulting in chain termination and inhibition of HIV-1 reverse transcriptase function (Plosker, 2013).

Tenofovir disoproxil (TENOD) is chemically known as [(2R)-1-(6-aminopurin-9-yl)oxymethyl-(propan-2-yl)oxycarbonyloxymethoxy]phosphoryl] oxy methylpropane-2-yl

carbonate (Ramaswamy & Dhas, 2014). TENOD is a tenofovir pro-drug that belongs to the NRTI group, it is used in conjunction with other ARV drugs like EMI, EFV.

Efavirenz (EFV) is chemically defined as (S)-6-chloro- 4-(cyclopropyl ethynyl)-1, 4-dihydro-4-(trifluoromethyl)-2H-3 ,1 benzoxazin-2-one, EFV is a HIV type 1 belonging specifically to the non-nucleoside reverse transcriptase inhibitors group (NNRTIs). Drugs in the NNRTI class prohibit HIV from replicating within the cells by binding the active site near reverse transcriptase and inhibiting the activity of polymerase.

2.3.2 *Non-steroidal anti-inflammatory drugs*

NSAIDs are the most used medication that can be bought over the counter for curative purposes. They were reported to be responsible for about 5- 10 percent of all drugs prescribed annually (Onder et al., 2004). These medications are used to treat and alleviate pain and inflammation in various post-operatives and arthritic conditions. These drugs were reported to contribute to major therapeutic activities, which were defined as either analgesic, anti-pyretic or the most common anti-inflammatory drugs (Kress et al., 2016; Modi et al., 2012). NSAIDs like diclofenac, naproxen, and ibuprofen was found as weak organic acids with pKa values extending from 4.15 to 4.85 (Lindqvist et al., 2005). Properties of these compounds include high water solubility at neutral pH as well as their polar orientation , the properties could likely create strain in their removal performance in the wastewater treatment process (Dahane et al., 2013; Larsson et al., 2009).

NSAIDs work by inhibiting the pathway of cyclooxygenase (COX) by hindering the production of prostaglandins. Out of the three existing COX enzymes, two are associated with the biological activity of the NSAIDs. In this case, the COX-1 enzyme forms part of the production of prostaglandins that act as an intestinal shield and gastric liner. The enzyme COX-2 is linked with the development of prostaglandins associated with inflammation. COX-2 was found to be

subsequently caused by endotoxins, mitogens and cytokines (Moreno et al., 2009). A report by Auriel and co-workers indicated that the popular side effects of NSAIDs could include heartburn, headaches, stomach pain, stomach ulcer and dizziness). In some cases the propensity to bleed and have complications with the liver or kidney could occur (Hörl, 2010). In extremely rare cases, NSAIDs can cause glomerular disease such as membrane nephropathy, which clinically complicated by nephrotic syndrome. Each NSAIDs has its own typical side effect as further elaborated in the paragraphs below.

Diclofenac (DICLO) is the most used NSAID in the world and was first developed in 1973 alongside a variety of experimental diclofenac containing drugs to boost safety, tolerability, and patient comfort (McGettigan & Henry, 2013). DICLO compared to other NSAIDs, is associated with an elevated risk of severe dose related gastrointestinal, renal and speech impairment side effects (Altman et al., 2015).

Ibuprofen (IBU) was the first propionic acid derivatives to be approved as a safer substitute for Aspirin in 1969 (Abraham et al., 2005). The most frequent side effects of this drug are vomiting, nausea, gastric uneasiness, the intensity is less than that of aspirin or indomethacin (Moreno et al., 2009). IBU is one of the most widely available and often administered NSAID. Also, it is a non-selective COX-1 and COX-2 receptor. While its anti-inflammatory properties may be poor than certain other NSAIDs, it was reported to have important antipyretic and analgesic function (Moreno et al., 2009). It has a wide variety of side effects, including coronary (Huang et al., 2006), renal and hepatic disruption associated with long term therapy (Moreno et al., 2009).

Naproxen (NAP) is also the propionic acid group and is brought under the common brand name Naprosyn, among others. It was developed in 1967 and approved for commercial use in the United States of America in 1976 and remained a prescription drug until 1994 when it was made an over-the-counter drug (Giménez et al., 2014). It is commonly used for treatment of

rheumatoid arthritis, fever, pain and inflammation (Island et al., 2018). Naproxen is thoroughly metabolized in the liver. Around 95% of the drug is excreted in the urine.

The physiochemical properties and uses of the drugs are in summary as illustrated in Table 1.2 above.

2.3.3 Occurrence of antiretroviral drugs in aquatic environments

WWTPs are the first recipients of pharmaceuticals and antiviral drug residues. In WWTPs, they are partially or completely eliminated through various treatment processes such as adsorption on activated carbon, oxidation, chlorination, ozonation, and reverse osmosis, before being released to the aquatic environment as parent compounds or biotic transformation (Nannou et al., 2020). WWTPs may influence the path and fate of these pharmaceuticals after they have been consumed by humans. The technologies used are largely aimed at removing carbon compounds, and to a lesser extent, biogenic pollutants, and new contaminants such as antiviral drugs, as evidenced by the high concentration of these drugs in wastewater (Tijani et al., 2014). Despite advancement in water treatment technology, effluents continue to be a significant source of antiviral drug load, owing to occasionally low or negative removal efficiency (Funke et al., 2016; Prasse et al., 2010).

EFV concentration levels suggest that it is a refractory antiviral drug. In a research study in South Africa, efavirenz was discovered at low quantities, although the removal efficiency ranged from 33 to 100 percent, depending on the area of interest (Mosekiemang et al., 2019a). In Kenya lower EFV concentrations have been recorded (K'oreje et al., 2012). EFV's concentrations were often found to be greater in raw wastewater, and in dry seasons compared to rainy season (Mosekiemang et al., 2019a). Emtricitabine (EMI) was found to only partially metabolize in the human body from 10-30% (Funke et al., 2016). Funke et al.(2016) found EMI in municipal WWTP influents at quantities of up to 980 ng L⁻¹. Wooding et al.(2017)in a study

reported 148 ng L⁻¹ efavirenz in surface water followed by Rimayi et al.(2018) where they reported 303 ng L⁻¹ in the dam and 354 ng L⁻¹ in the river. EMI was detected in the Juskei River and UMgeni River with concentrations ranging from 8-13 ng L⁻¹.

2.3.4 Occurrence of non-steroidal anti-inflammatory drugs in aquatic environments

NSAIDs are amongst the most detected organic pollutants in various water bodies, and they are regarded as emerging pollutants. Emerging contaminants are substances that have been detected at trace amounts in drinking water sources across the world, but the risk to the aquatic environment and human health is unknown. While these pollutants have been present in our drinking water for as long as people have used them, improvements in analytical techniques and instruments have only lately enabled them to be detected (Li, 2014).

Carmona et al.(2014) reported that the NSAIDs could find their way into drinking water through WWTPs effluents and surface run-off. Furthermore, pharmaceuticals discharged into groundwater from sources such as pipeline and sewage leaks may contribute to the presence of NSAIDs in drinking water (Caban et al., 2015). This is significant because it raises awareness about the state of contamination in aquatic environments, which might lead to unmonitored human intake of these drugs. According to the study by Madikizela and Chimuka, the mean naproxen concentration identified in African wastewater influent, effluent, and surface water varied from 1.1-55.8 µg L⁻¹, 0.33-20.48 µg L⁻¹, and 0.688 µg L⁻¹ (Madikizela & Chimuka, 2017). The average amounts of diclofenac, ibuprofen and naproxen found in drinking water in Spain were 11, 39, and 18 ng L⁻¹ (Carmona et al., 2014). In Iran, the maximum measured amounts of diclofenac, ibuprofen and naproxen in tap water were 47, 39 and 24 ng L⁻¹ respectively (Eslami et al., 2015). Table 2.1 summaries the quantities of these pharmaceuticals in various aquatic environments around the world. A lower concentration of ibuprofen recorded

in South African wastewaters was $221 \mu\text{g L}^{-1}$ compared to the levels of NSAIDs detected in water systems in developed nations such as Europe which were higher. For instance, diclofenac concentrations measured in the Turkey WWTP did not surpass 1.4 g L^{-1} (Sari et al., 2014) but the highest diclofenac concentration reported in South African WWTP influent was $104 \mu\text{g L}^{-1}$ as shown in (Table 2.1). The disparities in NSAID levels in the environment between nations might be explained by changes in sanitation systems. In many African nations, for instance, a lack of adequate infrastructure leads to the direct disposal of NSAIDs into the surface water. As a result, the concentration of ibuprofen across the African continent reached a maximum of $85 \mu\text{g L}^{-1}$ in surface water (Matongo et al., 2015). In contrast in a study conducted in Brazil and China, the maximum ibuprofen concentrations in surface water were 20. and $5.4 \mu\text{g L}^{-1}$ (Peng et al.(2017).

Table 2.1 Maximum concentrations detected in various sampling sites around the world.

Sample Site	Location	Concentration (ng/L and µg/L)			References
		Diclofenac	Ibuprofen	Naproxen	
WWTP influent	Canada	50-2450	4100-10,210	1730-6030	(Lee et al., 2005)
	Germany	Nd-1230	600-1660	-	(Vergeynst et al., 2015)
	Belgium	507-1450	5711-7847	2374-4110	(Weigel et al., 2004)
	Italy	1020	6624	1079	(Vergeynst et al., 2015)
	Sweden	110.7	751.3	5153	(Patrolecco et al., 2013)
	Sweden	160	3590	3650	(Zorita et al., 2008)
	Spain	1490	39,800	3580	(Bendz et al., 2005)
	Spain	<LOQ-561	<LOQ-4113	1196-5228	(Gracia-Lor et al., 2012)
	Poland	460	280	240	(Rosal et al., 2010)
	Portugal	69.7-89.4	1536-1715	741-522	(Migowska et al., 2012)
	Algeria	991-2319	1608-8613	1220-9585	(Kermia et al., 2016b)
	South Africa	2.3,0.99,22.3-104 µg L ⁻¹	1.6, 8.6,6.02-221, 39.6, 111.9, 5.2,	9.6, 1.2, 55, 52, 2.3, 1.22-39.6 µg L ⁻¹	(Agunbiade & Moodley, 2016; Amdany, 2013; Amdany et al., 2015; Lawrence
	Kenya	0.93-1.51 µg L ⁻¹			

	Spain	0.85 $\mu\text{g L}^{-1}$	7.2, 62.8, 5.8 $\mu\text{g L}^{-1}$	-	Mzukisi Madikizela & Chimuka, 2017b; Matongo et al., 2015b, 2015c)
	South Africa	0.59, 0.81 $\mu\text{g L}^{-1}$	6.46-10.6 $\mu\text{g L}^{-1}$ 1.36 $\mu\text{g L}^{-1}$ <LOQ-26.1 $\mu\text{g L}^{-1}$ <LOQ-35.5 $\mu\text{g L}^{-1}$	2.2 $\mu\text{g L}^{-1}$ <LOQ-5.21 $\mu\text{g L}^{-1}$ <LOQ-3.56 $\mu\text{g L}^{-1}$	
WWTP effluent	Canada	70-250	110-2170	360-2540	(Lee et al., 2005)
	Uk	459	2203	856	(Guitart & Readman, 2010)
	Germany	2100	3400	520	(Prasse et al., 2010)
	France	210-486.4	17.7-219	42.1-289.1	(Togola & Budzinski, 2008)
	Belgium	542-1391	<LOQ	<LOQ	(Vergeynst et al., 2015)
	Italy	507	1003	526	(Patrolecco et al., 2013)
	Sweden	33.3	14.1	32.1	(Zorita et al., 2008)
	Sweden	120	150	250	(Bendz et al., 2005)
	Spain	0.74	<LOQ	0.72	(Gracia-Lor et al., 2012)
	Spain	6-431	<LOQ	359-2208	(Rosal et al., 2010)
Serbia	1338	20.13	208	(Petrović et al., 2014)	

	Poland	120	110	70	(Migowska et al., 2012)
	Portugal	42.9-19.5	119-136	303-275	(Pereira et al., 2016)
	Taiwan	<2-30	<12-34	-	(Chen et al., 2008)
	Algeria	1616-2711	341-431, 0.34, 0.43 $\mu\text{g L}^{-1}$	Nd-334, 0.33 $\mu\text{g L}^{-1}$	(Kermia et al., 2016b)
	South Africa	1.6, 2.7, 19, <LOQ-20.8	0.04-8.84 $\mu\text{g L}^{-1}$	13.5, 20.4, 0.4, 0.8, <LOQ-534 $\mu\text{g L}^{-1}$	(Amdany, 2013; Amdany et al., 2015; Lawrence Mzukisi Madikizela & Chimuka, 2016a; Matongo et al., 2015b, 2015c)
	Nigeria	-	12.6, 24.6, 1.1, 1.6, <LOQ-20.8 $\mu\text{g L}^{-1}$	-	(Oluwatosin et al., 2016)
	Kenya	0.03-0.06 $\mu\text{g L}^{-1}$	-	-	(K.O. K'oreje et al., 2016)
	Spain	0.74 $\mu\text{g L}^{-1}$	Nd-17	0.098 $\mu\text{g L}^{-1}$	(Dahane et al., 2013)
	South Africa	0.58-2.02, 2.098-5.42 $\mu\text{g L}^{-1}$	1.98-5.42, 0.52-2.02 $\mu\text{g L}^{-1}$	<LOQ-5.95, nd-6.06 $\mu\text{g L}^{-1}$	This study
	South Africa	<LOQ, <LOQ			
Surface water	UK	305	52	104	(Guitart & Readman, 2010)
	France	<LOQ-41	Nd-24	Nd-24	(Idder et al., 2013)
	Germany	1200	530	390	(Prasse et al., 2010)
	Italy	Nd-120	95-210	200-264	(Patrolecco et al., 2013)
	Spain	313-3363	2234-16,886	387-3140	(Valcárcel et al., 2011)

	Spain	49	830	278	(Carmona et al., 2014)
	Serbia	<LOQ-324	<LOQ-346	<LOQ-74.2	(Petrović et al., 2014)
	China	13.6	23.3	12.3	(Wen et al., 2014)
	China	63	54	72	(Lin et al., 2005)
	South Korea	0.87-30	1.2-51	5.3-100	(Yoon et al., 2010)
	Algeria	85	373, 0.31 $\mu\text{g L}^{-1}$	334	(Kermia et al., 2016b)
	South Africa	Nd, 12.4, 0.73 $\mu\text{g L}^{-1}$	85, 0.23-6.2, <LOQ-11.4 $\mu\text{g L}^{-1}$	<LOQ-0.68 $\mu\text{g L}^{-1}$	(Hlatshwayo et al., 2016; Matongo et al., 2015b, 2015c)
	Nigeria	0.03-0.27 $\mu\text{g L}^{-1}$		0.03-0.06 $\mu\text{g L}^{-1}$	(Oluwatosin et al., 2016)
	Kenya	Nd-0.73 $\mu\text{g L}^{-1}$	0.04-8.84, nd-17.4 $\mu\text{g L}^{-1}$	-	(K.O. K'oreje et al., 2016)
	Spain	Nd-0.036 $\mu\text{g L}^{-1}$		Nd-<LOQ	(Dahane et al., 2013)
	South Africa	0.37-0.99, Nd- 7.39 $\mu\text{g L}^{-1}$	Nd-<LOQ 1.98-5.42, 0.52-2.02 $\mu\text{g L}^{-1}$	<LOQ-5.95, nd-6.06 $\mu\text{g L}^{-1}$	This study
Seawater	Europe	<LOD	0.0007 $\mu\text{g L}^{-1}$	-	(Weigel et al., 2004)
	Brazil	0.02 $\mu\text{g L}^{-1}$	2.09 $\mu\text{g L}^{-1}$	-	(Pereira et al., 2016)
	Singapore	0.04 $\mu\text{g L}^{-1}$	0.12 $\mu\text{g L}^{-1}$	0.03 $\mu\text{g L}^{-1}$	(Wu et al., 2010)

	Eastern Mediterranean Sea	0.02 µg L ⁻¹	<LOD	0.0008 µg L ⁻¹	(Alygizakis et al., 2016)
	Portuguese coast	0.24 µg L ⁻¹	0.22 µg L ⁻¹	0.18 µg L ⁻¹	(Lolić et al., 2015)
	South Africa	<LOD	0.17 µg L ⁻¹	0.16 µg L ⁻¹	(Ngubane et al., 2019)
	South Africa	<LOQ, <LOQ	<LOQ-7.39	1.4-6.73	This study
Drinking water	USA	-	510-1350	-	(Loraine & Pettigrove, 2006)
	Canada	-	25	-	(Kleywegt et al., 2011)
	France	Nd- 2.5	Nd-0.6	Nd-0.2	(Togola & Budzinski, 2008)
	Spain	18	39	11	(Carmona et al., 2014)
	Serbia	-	-	-	(Petrović et al., 2014)
	China	80	77	96	(Lin et al., 2005)
	Algeria	-	312	-	(Carmona et al., 2014)

2.4 *Modern sample extraction techniques of pharmaceuticals in aqueous samples*

2.4.1 *Solid phase extraction technique*

2.4.1.1 *Principles of Solid phase extraction*

The basic principles of SPE tend to be like those of liquid-liquid extraction. Both methods involve the distribution of dissolved species between two phases. However, SPE involves the dispersion of the analyte between a liquid sample medium and a solid phase which is the adsorbent instead of the two liquid phases that cannot be mixed in liquid-liquid extraction. This technique allows the enrichment and purification of the analyte on a solid adsorbent through adsorption from the solution. SPE is a well-established sample preparation technique with an ever widening applications base.

Many organic compounds are found in trace amounts in water at $\mu\text{g L}^{-1}$ and sometimes below. As a result, very low detection limits are required to monitor the fate and transportation of pollutants in the environment (Pichon, 2000). To address the limitations of for direct detection of pollutants using gas or liquid chromatography systems, a sample pre-treatment with the purpose of providing a sample fraction enriched with all target analytes and as free as possible from all matrix components is required. There is no doubt that solid phase extraction (SPE) has become the preferred process for the simultaneous isolation and pre-concentration of a variety of compounds in aqueous samples. SPE is the most used process for the extraction and alteration of solvents, cleaning the matrix, extraction, and fractionation of many types of organic compounds (Dimpe & Nomngongo, 2016). Many new SPE applications for multiresidue analysis were mainly focusing on the sample volume and the choice of sorbent (Ngumba et al.,

2016). SPE is well suitable for a variety of studies including compounds with a wide range of polarity or that are distinguished by distinct physiochemical properties.

2.4.1.2 Advantages and limitations of solid phase extraction

One of the key advantages of SPE is that the analyte adsorbed on the SPE cartridge/ column can be stopped from decomposing and hence retained for a certain time without any modification in the concentration or identity. SPE is also said to be faster and less labour intensive compared to other extraction techniques such as liquid-liquid extraction (LLE) (Andrade-Eiroa et al., 2016). Another significant benefit of SPE is that it can extract a wide range of organic analytes (from very polar to non-polar analytes) from a large variety of samples. In actuality, SPE could remove a wide range of organic compounds from biological, agricultural, and environmental samples such as pharmaceuticals; steroids, xenoestrogens, PPCs (Ebele et al., 2017) and some endocrine disrupting compounds; methyl tert-butyl ether and associated compounds; surfactants and their metabolites; sulphophenyl carboxylates; fuel additives; brominated flame retardants; pesticides and drinking water disinfection by-products and other chemicals such as caffeine or cholesterol (Rodriguez-Mozaz et al., 2007), polycyclic aromatic hydrocarbons (PAHs) (Andrade-Eiroa et al., 2010). One of the limitations of SPE methods is that it does not form emulsion like the LLE.

2.4.1.3 Choice of sorbent for the extraction of acidic and basic organic compounds

The comparison between LC and SPE has been largely defined and demonstrated. The possibility of predicting and optimizing the key SPE parameters from the data produced by LC was reported (Hennion et al., 1995; Mayer et al., 1995). The design of a SPE method includes the knowledge of the interaction between the analyte and sorbent and the knowledge of the retention activity of the extraction sorbent in the LC instrument. The breakthrough curves and the recovery curves are based on the sample volume (Hennion et al., 1995) and a study showed

that, with a sorbent value of 500 mg, recovery in the range of 90-100 % for a percolated volume of 500 mL, it requires $\log k_w > 3$ for sorbent which could improve adsorption of the analyte which is the basis of the choice of sorbent (Pichon, 2000).

2.5 *Identification and determination technique of pharmaceuticals in aqueous samples*

2.5.1 *High Pressure Liquid Chromatography-Photo Diode Array*

HPLC-PDA can be used for non-volatile, thermally unstable compounds and applicable to inorganic ions too. The photodiode array (PDA) detector is an analytical technique that can be used to evaluate the purity of an analyte or corresponding peak impurity eluting during HPLC separation. PDA detector utilizes the same operating concepts as the variable wavelength (VWD). However, the array of diodes allows simultaneous detection across several wavelengths rather than just one. Spectral acquisition used in combination with chromatographic isolation is to obtain several spectra over chromatographic peak and to exploit the advantages of both.

2.6 *Sampling approaches of pharmaceuticals in aqueous environments*

2.6.1 *Sampling of pharmaceuticals*

Each analytical technique for determining the concentration of pharmaceuticals requires a variety of interrelated operations, which should be carefully coordinated to achieve results that represent the actual quality of the analyte in the sample being analysed. Two specific methods are preservations of the entire samples prior to pre-treatment and analysis; and the separation or enrichment of analytes from samples and extracts (Kot-Wasik et al., 2007). Improper sampling or storage of samples longer than recommended can result in false measurements. I

Water samples for the assessment of trace and ultra-trace concentrations of pharmaceuticals should only be collected in glass containers. If compounds are vulnerable to photodegradation, collecting vessels must be made of non-transparent materials (Andreozzi et al., 2003). Sampling site should be carefully selected with respect to their representativeness for a given study (Kot-Wasik et al., 2007). Samples can typically be obtained at sites subject to significant variation in water quality for example, wastewater outlets and locations downstream and upstream of urban areas, WWTP and industrial plants. Samples of river water can be obtained at depth of 20-50 cm from midstream. Samples should be collected from a distance equal to one third of the average depth in freshwater rivers.

In analyses of the effect of tributaries or effluents on the river, samples should be obtained upstream from the source of the inflowing waters, beyond effluent zone at 50-150 m, and downstream from where the drainage combines with the inflowing waters. Such sampling technique facilitates the detection of distribution routes and the fate of the pharmaceuticals in aquatic environment.

CHAPTER: THREE

3 Research Context

3.1 *Justification*

Reports on the concentration of pharmaceuticals in the environment have risen significantly in past few decades (Koba et al., 2018; Matongo et al., 2015a; Patel et al., 2019; Wood et al., 2016). As analytical methods become more sensitive and frequently used, more of these pharmaceuticals are being detected in wastewater samples. The removal of these pharmaceuticals in the marine environment has been reported (Aminot et al., 2016; Feng et al., 2013; Mlunguza et al., 2019a). The performance of various extraction techniques, such as, SPE, HF-LPME, MIPs have been evaluated. The SPE approach has proven to be the most effective extraction method. Therefore, the complex sample matrix such as surface water, estuaries, and seawater in South African water are evaluated thoroughly in this study using the SPE methods. To understand the danger of marine life and water from experiencing the health effects caused by these contaminants, a full evaluation of occurrence of these pharmaceuticals is required. This performance is important; hence this study investigates these pharmaceuticals on a seasonal bases to study their frequency after water treatment.

Developing highly sensitive and selective approaches for trace determination of these compounds in complex samples is important. One of the most suitable techniques for the study of these drugs is the used of chromatographic methods coupled to extremely sensitive detectors. It is necessary to use cheaper and readily available detectors such as UV-Visible detectors. Sensitivity of the UV-Visible detector is typically enhanced with the use of solid-phase extraction for pre-concentration, clean-up, and removal of interference.

3.2 *Aim*

The aim of this study was to develop suitable analytical methods which was used quantify selected pharmaceuticals in estuarine water, river water and seawater. Objectives of the study are to:

- Optimize SPE methods for the extraction of selected pharmaceuticals and volatile compounds from river water, estuarine water, and seawater.
- Validate the optimized methods using recovery studies and statistical methods.
- Apply the optimized extraction methods for isolation and pre-concentration of pharmaceuticals prior to the quantitative analysis using HPLC.

3.3 *Problem statement*

Many South Africans are exposed to pharmaceuticals like NSAIDs and ARVDs due to their easy access and number of infections by HIV disease in the country, this leads to high excretion rates which contain these pharmaceuticals. WWTPs have been found to have poor removal of these pharmaceuticals due to their solubility and some properties which are not catered for during the water treatment process. The water discharged into the rivers and dams contain these pharmaceuticals which flow into the sea, even though they are found in low concentrations (Matongo et al., 2015a; Amdany et al., 2015; Gwenzi & Chaukura, 2018). In most South African rural areas, people and animals still depend on water from these rivers and dams, and the effects of prolonged intake of contaminated water is not well understood. Their distribution in the environment is also not well known.

3.4 *Dissertation outline*

Chapter one: Introduction

The introduction of this dissertation basically states the background to the research and the problem at hand. This section covers the objectives of the research and states the hypothesis as well as the extent or scope of the research.

Chapter two: Literature review

This section includes the analysis of environmental laws, theories and fundamental concepts which will be applied during the research.

Chapter three: Methodology

The techniques and apparatus used during the research are described in this section. A clear description of the equipment and materials used are also included herein. Sufficient information is provided to enable a successful repetition of the experiment.

Chapter four: Results and discussion

Experimental results obtained are clearly presented in a manner which allows for easy study and comparison. Statistics are used in the analysis of results and estimation of errors. This chapter also covers extensive discussion of the results obtained.

Chapter five: Conclusion and recommendation

This chapter states the objectives that were accomplished, the research findings. Recommendations of any further work are discussed in this chapter.

CHAPTER FOUR

4 Methodology

4.1 *Experimental*

4.1.1 *Chemicals and materials*

High purity (>98%) standards of target pharmaceutical compounds (diclofenac (DICLO) salt, ibuprofen (IBU), naproxen (NAP), emtricitabine (FTC), efavirenz (EFV), and tenofovir disoproxil (TENO) were purchased from Sigma-Aldrich (Steinheim, Germany). Gemfibrozil (GEM) was purchased from J&H Chemicals Co. Ltd (Hangzhou, China). Hydrochloric acid (HCl) and formic acid (>98%) were also purchased from Sigma-Aldrich (Steinheim, Germany). The HPLC-grade solvent acetonitrile (>99%), methanol (99, 5%), and acetic acid (>98%) were purchased from Merck (Darmstadt, Germany) and they were used for the SPE as well as HPLC mobile phase. Sodium chloride was purchased from Associated Chemical Enterprise (Johannesburg, South Africa). Ultrapure water was obtained using a Purite select HP 40 system (Purite Ltd.'s, Oxfordshire, UK). A 100 mg L⁻¹ stock solution containing a mixture of the 7 target pharmaceutical compounds was prepared by dissolving the drugs in acetonitrile. A series of working standards in a concentration range of 0.001-10 mg L⁻¹ were prepared in a mixture of acetonitrile and 0.2 % formic acid (60:40). The stock solution was stored at 3°C in a refrigerator and used for the preparation of working standards for the duration of the experiments.

4.2 *Instrumentation*

Water samples were filtered using a 0.45 µm hydrophilic polypropylene membrane filter papers and Whatman (70 mm x 100 circles) filter papers purchased from GE Healthcare UK Ltd Supplies (South Africa). The pH of water samples was measured with a Bante900P portable

multi-parameter water quality meter obtained from Bante instruments (Shanghai, China). For the extraction of the target compounds from the water, a vacuum pump manifold sourced from Phenomenex (California, USA) attached to a vacuum pipe lining purchased from Pall Corporations (Fribourg, Switzerland) was used. Various SPE cartridges were utilized in this study, namely, mixed-mode anionic exchange (MAX) sorbent (OASIS MAX, 6cc, 200 mg), hydrophobic-lipophilic balance (HLB) sorbent (OASIS HLB, 6cc,200 mg) from Waters Corporation (Milford, MA, USA) and Agilent Bond Elute Plexa, (3ml, 200 mg) purchased from Chemetrix (Johannesburg, South Africa). The HPLC instrument, purchased from Shimadzu Corporations (Kyoto, Japan), contained an online mobile phase degasser unit (DGU-20A3), ternary pump (LC-20AB) was used. All samples and standards were injected onto a Rheodyne 7010 injector equipped with a 20 μ L sample loop (California, USA). The compounds were separated using a C₁₈ Kromasil column (150 mm x 4.60 mm x 5 μ m) obtained from Phenomenex (California, USA) and detected using a PDA detector (SPD-M20A).

4.3 Optimization of HPLC method

An HPLC coupled to a photodiode (PDA) detector was employed for the separation and analysis of the target pharmaceuticals. TAll samples and standards were injected onto a Rheodyne 7010 injector equipped with a 20 μ L sample loop. Chromatographic separation was achieved by applying a mobile phase containing 0.2% formic acid in water (solvent A) and Acetonitrile (solvent B). A multi-step gradient elution program was employed by using 15% solvent B for the first 3 minutes at a flow rate of 1 mL min⁻¹ and at 3.01 minutes ramped up to 60% solvent B until 12 minutes at a flow rate of 2 mL min⁻¹ and 12.01 minutes changed to 15% solvent B and held at a flow rate of 2 mL min⁻¹ to 19.50 minutes and changed back 1 mL min⁻¹ for equilibration of the system. The total run time for the analysis was 20 min. The PDA

wavelengths were set with regards to each compound, naproxen, ibuprofen, and gemfibrozil, tenofovir disoproxil and efavirenz were monitored at 254 nm and emtricitabine and diclofenac were monitored at 280 nm.

A diagram of the chromatographic separation is illustrated below in figure 4.1.

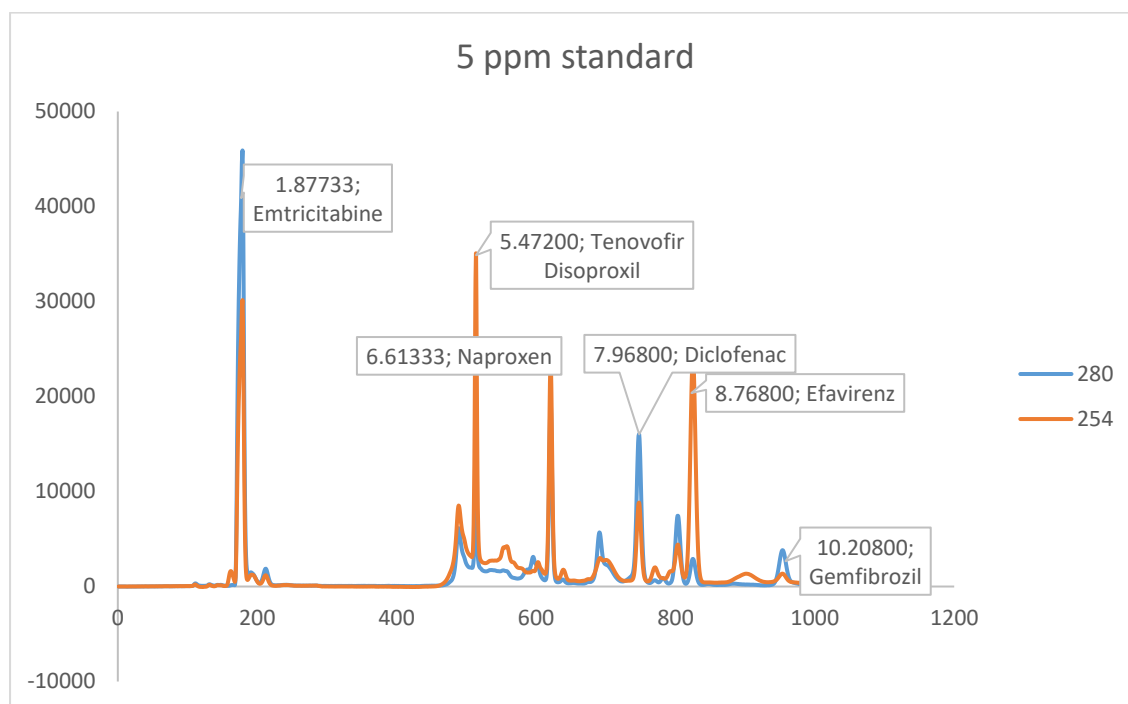


Figure-4.1: Retention time

4.4 Sample collection and pre-treatment

Water samples were collected from 12 different sampling sites located around Durban, KwaZulu-Natal province, South Africa (Table 4.1). These sites included wastewater treatment plants (Northern WWTP and Kingsburgh WWTP) where both influent and effluent were collected, surface water (UMgeni river and Kingsburgh river), seawater (Blue lagoon beach, Glen Ashley beach and Warner beach (top and bottom at 2 km apart) and estuary (Warner beach estuary and UMgeni estuary). Samples were collected by filling pre-cleaned 3.5 L amber

glass bottles and kept in a cooler box with ice during transportation to the laboratory. They were filtered immediately for the removal of solid matter and thereafter stored in the refrigerator at 4°C until analysis.

The selected sampling sites and their corresponding GPS coordinates are shown in Table 3.

Table 4.1 Geographical location of sampling sites and corresponding GPS co-ordinates

Sampling sites	GPS co-ordinates
Northern WWTP	S29.81546° E30.986515°
Kingsburgh WWTP	S30.07110° E30.882368°
UMgeni River	S29.48415° E31.061242°
Kingsburgh River	S29.68516° E31.977135°
Blue lagoon beach	S29.80929° E31.041494°
Glen Ashley beach	S29.77442° E31.083275°
Warner Beach A	S30.50112° E30.525811°
Warner beach B	S30.81227° E30.981271°
UMgeni Estuary	S29.50499° E31.062893°
Warner Estuary	S30.08501° E30.862712°

4.5 SPE extraction of water samples

The SPE extraction method reported by Rimayi et al., 2018 was optimized to get maximum recovery. The parameters that were optimized are the amount of salt in the water sample, sample volume and its flow rate. The rest of the parameters were taken from Rimayi et al.,

2018. Optimizations was done by varying one parameter while keeping others constant. At optimum conditions, the Bond Elut Plexa SPE cartridges (3 mL, 200 mg Styrene divinyl benzyl) were conditioned with 5 mL methanol and equilibrated with 5 mL ultrapure water adjusted to pH 5.8, before loading the 500 mL water samples at a flow rate of 10 mL min⁻¹. The cartridges were dried under a gentle vacuum for 30 minutes. Elution of the retained compounds was performed with 3 mL methanol followed by 3 mL acetic acid in methanol (20:80) at a flow rate of 5 ml min⁻¹. The extracts were evaporated under a gentle steam of nitrogen gas and temperature of 40°C and then reconstituted with 1 mL mixture of acetonitrile and 0.2% formic acid in water (60:40).

CHAPTER: FIVE

5 Results and discussion

5.1 Seasons and climate of the study site

KZN is of the provinces of South Africa. It is characterized by a subtropical coastline, grasslands in the east, and the Drakensberg Mountain range in the west. KZN's climate is strongly influenced by the Indian Ocean, and particularly the warm Agulhas current, hence accounting for the high humidity, temperatures, and summer rainfalls. Tropical temperate troughs, east coast low-pressure systems, and southeast coast ridging high-pressure systems dominate during austral summer and spring. (Lakhraj-Govender & Grab, 2019). During winter, moisture from the tropics is reduced by the northward migration of the Intertropical Convergence Zone (ITCZ) and the intensification of the anticyclonic circulation, hence limiting precipitation. However, perturbations in the westerly circulation, in the form of anticyclones, as well as ridging high-pressure systems behind cold fronts, dominate during winter (Roffe et al., 2019). Table 5.1 shows the sampling dates and season of sampling.

Table 5.1 Sampling seasons and periods

Seasons	Calendar dates	Sampling dates
Summer	1 Dec – 28/29 Feb	17 February 2020
Autumn	1 Mar – 31 May	15 March 2020
Winter	1 Jun – 31 Aug	6 August 2019
Spring	1 Sept – 30 Nov	1 November 2019

5.2 Optimization of Solid-phase extraction

5.2.1 Salting Effect

It is essential to investigate the effects of salt as the samples collected from the estuaries and sea may contain high levels of salt. According to Wasilewska et al, 2011 and Ngubane et al, 2019, the ionic strength of the water samples has slight or no effect on the adsorption of the target compounds. The salt content is directly related to the ionic strength in water samples. The extraction efficiency of the SPE cartridge therefore had to be evaluated for the target samples. Therefore, 500 mL deionised water with sodium chloride content ranging from 0 to 4% w/v were spiked with $5 \mu\text{g L}^{-1}$ mixture of the target compounds. The range of the salt content was selected because sea water is estimated to contain 3.5% of salt (Cadotte et al., 1980). The range therefore accommodated the disparities of salt expected in the marine environment. The analysis was done in triplicate, and the spiked samples were adjusted to pH 5.8 before SPE extraction as this pH gave optimum results as opposed to a neutral or any other pH. Thereafter, the water samples were loaded onto a pre-conditioned Agilent Bond Elut Plexa SPE cartridges at a flow rate of 10 mL min^{-1} . The results reported in Fig. 5.1. suggested that the presence of salt has minimal effect on the percent recovery. At 0% NaCl content, the recoveries were in the range of 64 - 93% and at 4% content, it was 63 - 92% as shown in Figure 5.1 below. The sea had the recoveries that indicated a decreased by 8% for tenofovir, 4% for naproxen, 2% for diclofenac, and increased by 3% for ibuprofen and 4% for gemfibrozil. A slight difference was observed for efavirenz, while emtricitabine had very low recoveries hence it was not shown in the graph. The recoveries were still within the acceptable range which suggested that the presence of salt has little or no effect on the percent recoveries. The standard deviation error bars overlap in the graph below suggesting that there is no statistical difference between the results in figure 5.1 except for one of the samples of ibuprofen which showed no

overlap suggesting that there was a significant difference between the 0% and the rest of the NaCl percentages.

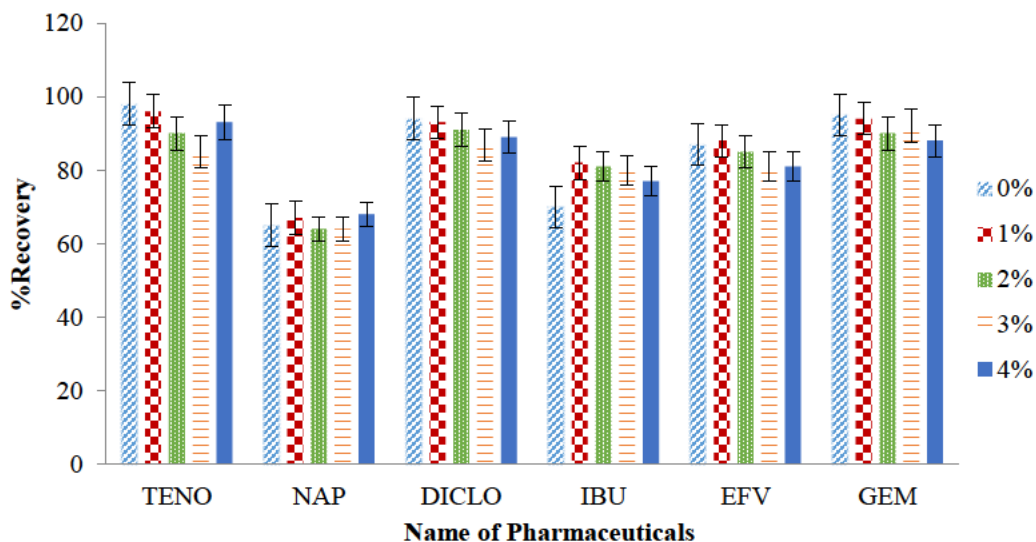


Figure 5.1. The effects of salt content on the percentage recovery

5.2.2 Effects of sample volume

The effect of sample volume on recoveries was investigated by extracting 500 mL and 1000 mL deionized water samples that were spiked with $5 \mu\text{g L}^{-1}$ mixture of the target pharmaceuticals at pH 5.8. Figure 5.2 shows the results obtained. The 500 mL sample volume was chosen as the optimum because at 1000 mL, the recovery decreased for most compounds. Recoveries ranging from 79-121% were obtained for the 500 mL sample volume while for 1000 mL ranged from 63 to 94%.

The decrease in recovery at high sample volume showed that the sample breakthrough volume was exceeded. This observation has been noted by other researchers as well (Debska et al., 2005; Paíga & Delerue-Matos, 2013; Madikizela & Chimuka, 2017)

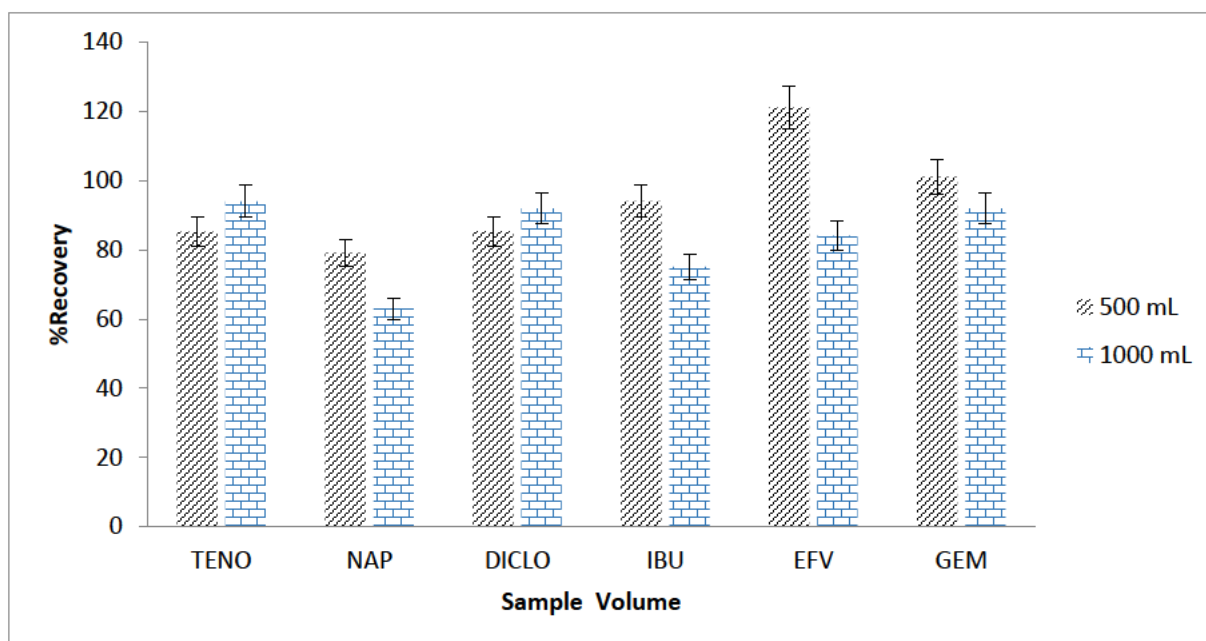


Figure 5.2 The effects of sample volume on percentage recovery

5.2.3 Effects of flow rate

The results for the elution flow rate studies are shown in Figure 5.3. In this case, the elution flow rate was investigated from 1 – 10 mL min⁻¹. Sample flow rate from Rimayi et al., 2018 was used. The results showed that the lower the elution flow rate, the higher was the recovery. This was expected as slow flow rate allowed for more time to desorb more analytes from the sorbent. The results of the flow rates agreed well with previously reported study which indicated that the flow rate at which the sample was percolated and eluted into the SPE cartridge was one of an important parameters that influenced the retention and elution of target compounds from the cartridge (Ngubane et a., 2019a). Even though 1 mL min⁻¹ gave good results, 5 mL min⁻¹ elution flow rate was selected to shorten analysis time as it had no significant effect on recoveries except for EMI and IBU. TENO has a significant difference between the 1 and 5mL/min showing no overlap in the error bars. Suggesting that a slower flow might be eluting the compound as the compound partitions faster with the sorbent.

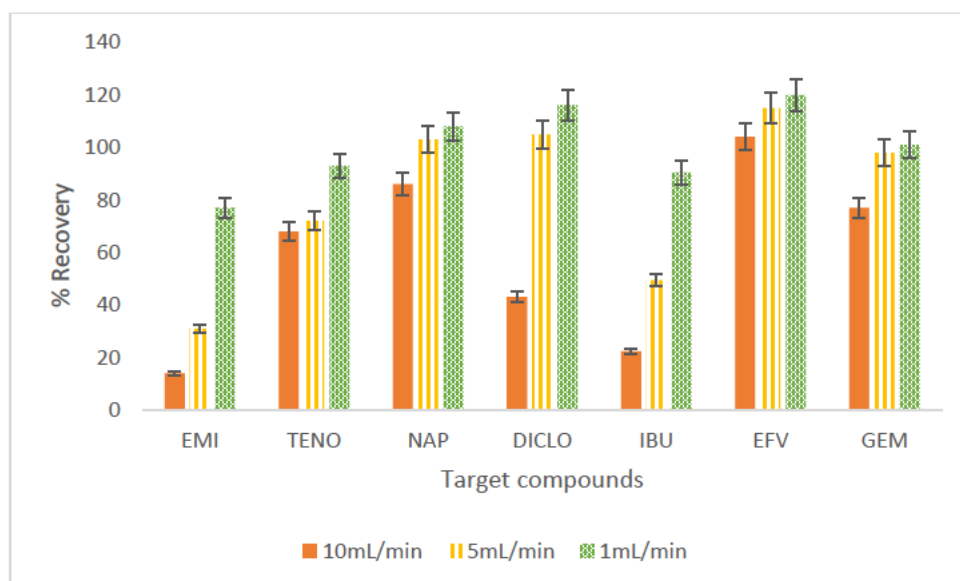


Figure 5.3 The effects of flowrate on the percentage recovery

5.3 Quality parameters of the method

The linearity was calculated by plotting the concentration of each analyte against its peak area attained in the HPLC analysis. The instrument detection limit was calculated as the limit of detection (LOD) and limits of quantification (LOQ) using a statistical equation of 3.3- and 10-times standard error of y-intercept divided by the slope of the graph.

$$LOD = \frac{3.3 \times SE}{m} \quad \text{and} \quad LOQ = \frac{10 \times SE}{m}$$

To study the influence of sample matrix on recovery, both deionized water and estuary river water was spiked with $5 \mu\text{g L}^{-1}$ concentration of target compounds and thereafter extracted with SPE before analysis. The results in Table 5.2 showed that the extraction of samples had a better sensitivity and was demonstrated by the low LODs in environmental samples. The recoveries of the deionised water and estuary water were more less similar and slightly higher than expected. This showed that the developed method was not influenced by the sample's matrix

found in river water. The number of samples analysed for the accuracy and precision of the method was $n=6$ as well as to find the reproducibility. The method detection limits were higher than the instrument detection limits which validated the method.

Table 5.2 Limits of detection, Limits of quantification, Linearity, and recoveries

Compound	Instrumental parameters		Deionized water as sample		Estuary river water as sample		
	LOD (mg L ⁻¹) ¹⁾	LOQ (mg L ⁻¹)	Linearity (R ²)	% Recovery	MDL (µg. L ⁻¹)	Linearity (R ²)	%Recovery± RSD
Emtricitabine	0.08	0.23	0.9982	62±0.12	0.62	0.9997	62 ± 0.20
Tenofovir	0.48	1.45	0.9993	91±0.343	0.56	0.9986	80 ± 0.18
Disoproxil							
Naproxen	0.45	1.35	0.9991	88.4±0.09	0.70	0.9981	68 ± 0.22
Diclofenac	0.07	0.22	0.998	86 ±0.08	1.27	0.9974	105 ± 0.41
Ibuprofen	0.49	1.47	0.999	79±4.93	4.68	0.9995	110 ± 1.49
Efavirenz	0.09	0.28	0.9994	102± 0.51	0.85	0.9995	104 ± 0.27
Gemfibrozil	0.67	2.03	0.9961	96.7±0.32	1.78	0.9981	101.4 ± 0.57

5.4 *Environmental monitoring of target compounds based on the seasons.*

5.4.1 *Seasonal comparison on the concentrations found in samples.*

The sum of concentrations of each compound obtained per given season at each sampling site is given in Figure 5.4 and Table 5.3 for each study site and season. Samples were collected during summer which is a wet season (December to February), Winter a dry season (June to August) and autumn and spring rational seasons (March to May) and (September to November). Summer is accompanied by warm temperatures in the 32–40 °C range and significant rains, while the winter is humid and cold, with temperatures ranging from 11 to 24 °C and limited rainfall. Autumn and spring interchange between being windy and dry and sometimes have rainfalls. The concentration of the total emerging pollutants in Figure 5.3 showed that the NWWTP influent had high concentration which were observed in autumn and spring ranging from 117.11 to 114.4 $\mu\text{g L}^{-1}$, respectively, where there is minimal to no rainfall, similarly to its effluent site, high concentrations were observed in winter and spring with concentrations ranging from 43.97 to 42.59 $\mu\text{g L}^{-1}$, respectively. The KWWTP had high concentration of 144.88 $\mu\text{g L}^{-1}$ in the winter season. Its effluent site had high concentrations during the summer season with a concentration of 63.8 $\mu\text{g L}^{-1}$.

The UMgeni and Kingsburgh river had a sum of the total emerging pollutants concentrations of 18.3 $\mu\text{g L}^{-1}$ Umgeni river and 22.66 $\mu\text{g L}^{-1}$ Kingsburgh river during the spring and winter seasons, respectively. Because rainfall was limited throughout these seasons, the high levels during the dry season may be attributed to the lack of dilution. Blue lagoon and Glen Ashely had high concentration in spring with ΣEP concentration of 46.75 and 13.3 $\mu\text{g L}^{-1}$. Warner beach Top and bottom had a ΣEP concentration of 6.94 and 24.96 $\mu\text{g L}^{-1}$ in autumn and

summer. The UMgeni and Warner beach estuaries had high concentration observed in winter with concentrations of 20.97 and 37.9 $\mu\text{g L}^{-1}$, respectively.

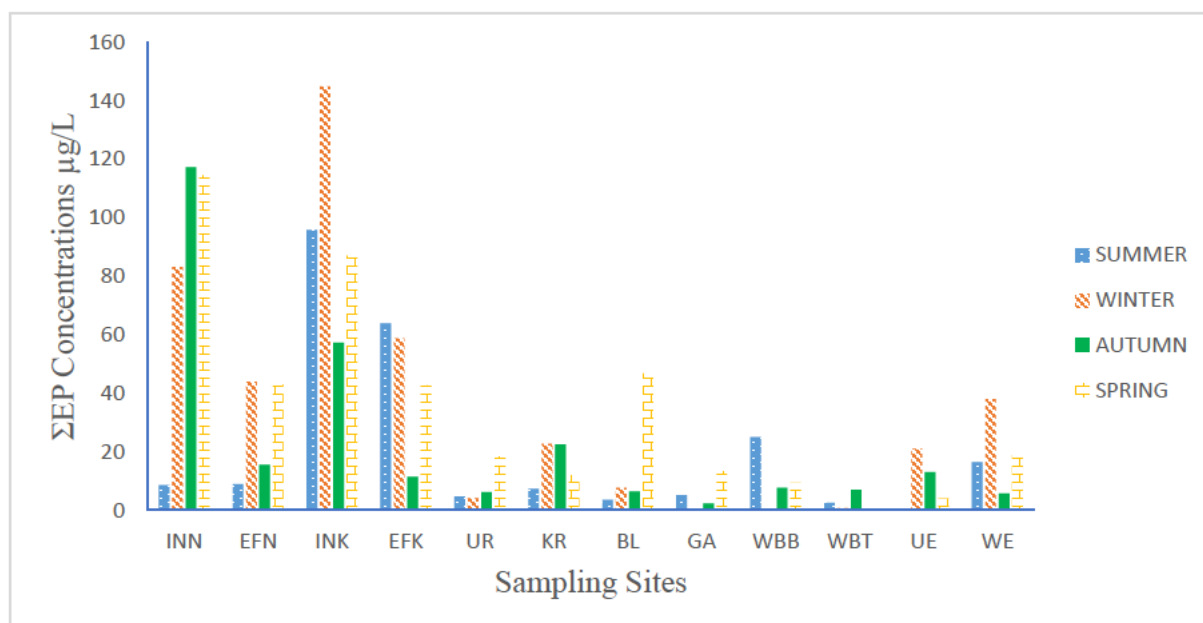


Figure 5.4: Seasonal variation and ΣEP concentrations in $\mu\text{g/L}$ around Durban aquatic sites

5.4.2 Analysis of pollutants in wastewater treatment plants

The concentrations of pharmaceuticals in the influent and effluent of the Northern WWTP and Kingsburgh WWTP varied considerably in between the two wastewater treatment plants (Table 5.3). Higher concentration levels of all the target compounds were detected in the Northern WWTP influent. Spring had the most frequently detected pharmaceuticals with concentrations ranging from 1.98 to 44.7 $\mu\text{g L}^{-1}$ diclofenac being the least detected compound in this season. Similar trends in the concentrations of 1.24 $\mu\text{g L}^{-1}$ for diclofenac were detected by Madikizela et al., 2018 in the Lady Smith wastewater treatment plant. The emtricitabine was the most frequently detected compounds in this site in all seasons with concentrations higher than that detected by (Mosekiemang et al., 2019a) where the concentration of 172 ng/mL was observed. The total ΣEP in this study site was found to be 114.4 $\mu\text{g L}^{-1}$ in Spring (Figure 5.4). This could

be due to the pre-exposure to prophylaxis drug that are prescribed over the counter, which are freely handed in clinics, these contains a large dose of all these compounds (Plosker, 2013). Also, the NWWTP receives both industrial and domestic waste as well as runoff. These compounds were found to be more dominant in the Autumn with the Σ EP of $117.11 \mu\text{g L}^{-1}$ with concentrations ranging from $1.81- 101.2 \mu\text{g L}^{-1}$, Diclofenac being the least recoverable compound, this is in line with previous studies which reported poor recovery of diclofenac (Lindqvist et al., 2005; Madikizela & Chimuka, 2017; Ngubane et al., 2019). Emtricitabine was found to have the highest concentration in this water sample. Winter was found to have a total Σ EP of $83.14 \mu\text{g L}^{-1}$ with concentration in range of $5.07- 37.5 \mu\text{g L}^{-1}$, again diclofenac and emtricitabine being the least and most detected compounds in all these seasons. In summer, the concentrations was found to range between 0.591 to $26.4 \mu\text{g L}^{-1}$ compared to $24000 \pm 1400 \text{ ng L}^{-1}$ determined by Abafe and co-workers in the same site (Abafe et al., 2018b). This suggested that these compounds were not effectively removed after the treatment process. The NWWTP effluent site only diclofenac and efavirenz were detected in summer with concentrations of 2.098 and $6.83 \mu\text{g L}^{-1}$ respectively, which was lower than what each compound was found to be in the inlet. In autumn, concentrations of 4.17 , 5.56 , and $5.70 \mu\text{g L}^{-1}$ were determined for diclofenac, efavirenz and gemfibrozil, respectively. In winter, concentration in the range of 5.42 to $25.03 \mu\text{g L}^{-1}$ were determined, naproxen with the lowest concentration and tenofovir disoproxil with the highest concentration. In spring, concentrations ranged from 0.82 to $36.75 \mu\text{g L}^{-1}$ with efavirenz being the lowest detected compound and emtricitabine being the highest detected compound. The trend showed that after water treatment some of these compounds were removed but some traces remain in the effluent. This might be due to the wastewater purification processes, or settling of the compound in sludge.

The Kingsburgh WWTP was found to have lower concentrations of these pharmaceutical than the NWWTP as it is an industrial sewage plant. Winter was found to have the highest total Σ EP of $144.88 \mu\text{g L}^{-1}$, the concentration ranged from 2.94 to $48.4 \mu\text{g L}^{-1}$, with emtricitabine being the least detected and highest being Tenofovir disoproxil. In Spring, the total Σ EP of $95.8 \mu\text{g L}^{-1}$ was observed, with the concentration ranging from 1.18 to $36.3 \mu\text{g L}^{-1}$ for diclofenac and the highest concentration detected being emtricitabine. In Summer, it was further observed that the total Σ EP was $95.81 \mu\text{g L}^{-1}$, with concentrations ranging from 7.25 - $39.38 \mu\text{g L}^{-1}$, with gemfibrozil being the lowest detected compound and Tenofovir disoproxil being the highest detected compound. In autumn as well, this season showed the concentrations ranging from 1.55 - $16.52 \mu\text{g L}^{-1}$, with ibuprofen which had higher concentrations of 22.8 , 35.5 , $6.06 \mu\text{g L}^{-1}$ respectively for each season compared to those determined by Madikizela & Chimuka(2017) of $2.1 \mu\text{g L}^{-1}$ in the same study site. These pharmaceuticals were frequently detected in winter, which is flu and cold season leading to frequent consumptions of these pharmaceuticals by patients with arthritis for pain relief. The effluent of this site showed low concentrations of these pharmaceuticals except for emtricitabine with the highest concentrations of 32.7 , 44.7 and $45.8 \mu\text{g L}^{-1}$ in spring, winter, and summer, respectively. Up to $63.8 \mu\text{g L}^{-1}$ total Σ EP of these pharmaceuticals were detected in summer in this site. The percentage removal efficiencies for individual pharmaceuticals in all seasons are shown in (Table 5.3). In the NNWTP, these pharmaceuticals were almost completely removed except for tenofovir, diclofenac, gemfibrozil which accumulated in autumn. Spring was the only season with low removal efficiency for all pharmaceuticals. In KWWTP, emtricitabine and efavirenz were two pharmaceuticals that had accumulated in the effluent except in autumn, but low removal was observed with only 36.7% efavirenz being removed, these results were comparable to those found by Schoeman et al., (2015) where these drugs accumulated after the chlorination process. Emtricitabine had 93% accumulation after treatment this suggested that the compound was

accumulative or was not being effectively removed during the purification process. The Northern and Kingsburgh employ similar treatment technologies, hence low removal efficiency of the ARV drugs were observed in both sites.

The result of this section suggested that wastewater treatment plants are still incapable of effectively removing these pharmaceuticals in the chlorination process, High quantities of bioactive pharmaceuticals, such as ARV drugs, have been reported to impair the efficiency of WWTPs (Márta et al., 2018; Mbhele et al., 2018).

5.4.3 *Analysis of surface water pollutants*

The UMgeni River flows through several distant and rural areas that are little populated, therefore the potential of pollution is low. However, when the UMgeni River reaches Durban, pharmaceuticals were detected along the various sampling areas. UMgeni River and Kingsburgh River were the sites of interest. The UMgeni river had low concentrations of these pharmaceuticals (Table 6). Diclofenac, Ibuprofen and efavirenz were the only compounds detected in the summer season with concentrations of 0.73, 1.60, 2.34 $\mu\text{g L}^{-1}$, respectively. Efavirenz concentration of this study had a higher concentration of 3.56 and 2.54 $\mu\text{g L}^{-1}$ compared to efavirenz with the reported concentration of 138 ng L^{-1} (Rimayi et al., 2018). Ibuprofen had lower concentrations compared to those reported in literature of 68.14, 0.100 in summer, 12.94 and 0.36 $\mu\text{g L}^{-1}$ respectively in the same sampling site (Amos Sibeko et al., 2019; Gumbi et al., 2017a; Matongo et al., 2015a; Ngubane et al., 2019a). Autumn had most of these pharmaceuticals detected ranging from 0.40- 2.71 $\mu\text{g L}^{-1}$, with efavirenz which had the lowest concentration and tenofovir with highest concentration.

Higher concentrations of ibuprofen, gemfibrozil, and efavirenz of 1.4, 5.55, 0.32 $\mu\text{g L}^{-1}$ were detected in the Kingsburgh River, respectively. In summer, similar results were found by Madikizela & Chimuka, (2017) in the Kingsburgh river. In autumn most of these pharmaceuticals were detected with concentrations ranging 0.53-12.2 $\mu\text{g L}^{-1}$, with diclofenac being the lowest detected drug and emtricitabine being the highest detected drug. Winter had concentrations ranging from 2.14-9.89 $\mu\text{g L}^{-1}$, ibuprofen being the lowest detected compound and efavirenz with the highest detected concentration in this season. Ibuprofen and gemfibrozil were the only detected pharmaceuticals in spring with concentration in the general order of 6.73, 5.19 $\mu\text{g L}^{-1}$. Tenofovir was not detected in all seasons, naproxen was found to be below the quantification limit. This could be due to the large sampling area instigated resulting in the reduction of the target pharmaceutical. These pharmaceuticals were frequently detected in autumn 2020 with the total ΣEP being 22.5 $\mu\text{g L}^{-1}$ which showed a 0.16 $\mu\text{g L}^{-1}$ difference in the concentration of these pharmaceuticals in these water bodies as the years progress. This is supported by considering that the winter of the 2019 season, the first sampling was taken, which yielded the ΣEP of 22.66 $\mu\text{g L}^{-1}$ which slightly remained the same in the following seasons.

5.4.4 *Analysis estuarine water pollutants*

The UMgeni and Warner Beach estuaries were areas of interest in this study. In the summer only efavirenz was detected with a concentration of 0.55 $\mu\text{g L}^{-1}$. In Autumn low concentrations of these pharmaceuticals were found ranging from 0.394- 6.69 $\mu\text{g L}^{-1}$, with emtricitabine being the lowest recoverable compound and gemfibrozil having higher concentration, this trend was expected considering the accumulation of gemfibrozil in this season in the NWWTP. The concentrations of these drugs in winter were found ranging from 0.95- 9.6 $\mu\text{g L}^{-1}$, with diclofenac having the lowest concentration and gemfibrozil with highest concentration. The concentration of gemfibrozil found in this study were lower compared to those found elsewhere

in ground water where they reported the concentration of $19.4 \mu\text{g L}^{-1}$ for gemfibrozil (Fang et al., (2012)). In Spring, the concentrations of 0.22, 0.14, $3.04 \mu\text{g L}^{-1}$ were detected for emtricitabine, diclofenac, and efavirenz, respectively. In this study it was found that diclofenac was detected in all seasons compared to the study by Ngubane et al., (2019) where they did not report these compounds in the same site. The presence of these pharmaceuticals in the estuarian water are not surprising as the Msunduzi and UMgeni river flow into this stream and they have been reported to have high concentrations of these compounds (Sibeko et al., 2019; Gumbi et al., 2017; Matongo et al., 2015; Mlunguza et al., 2019).

Warner Beach estuary was one of the study sites of interest, where naproxen was only detected in autumn season with a concentration of $1.82 \mu\text{g L}^{-1}$ and efavirenz with a concentration of $3.92 \mu\text{g L}^{-1}$. Tenofovir disoproxil was not detected in all seasons in this study area. In summer, concentrations ranging from 0.21- $11.95 \mu\text{g L}^{-1}$ were detected, diclofenac having the lowest concentration and emtricitabine having the highest concentration. Concentration of emtricitabine detected in this site was found to be higher than those detected by (Funke et al., 2016; Mlunguza et al., 2020) in their influent site, with concentrations of 980 ng.L^{-1} and $0.033 \mu\text{g L}^{-1}$, respectively. In spring, emtricitabine and diclofenac were the only drugs detected with concentrations of $18.2 \mu\text{g L}^{-1}$ and $0.55 \mu\text{g L}^{-1}$, respectively. In Winter, diclofenac, efavirenz, and gemfibrozil were the only drugs detected with concentrations of 01.26, 12.3, $24,35 \mu\text{g L}^{-1}$. Warner Beach estuary was found to contain higher concentrations of these pharmaceuticals compared to the UMgeni estuary. The total ΣEP in UMgeni was found to be $20.97 \mu\text{g L}^{-1}$, where winter was the season that had the highest concentrations and the total ΣEP in Warner beach estuary being $37.9 \mu\text{g L}^{-1}$ in the same season. This is due to the size of this estuary which

is smaller than the UMgeni estuary hence lower recoveries are detected at UMgeni due to the large water dilution.

5.4.5 *Analysis of pollutants in the seawater*

The presences of these pharmaceuticals in seawater have been reported (Ngubane et al., 2019, Nikolaou et al., 2007). However, there is lack of information regarding the presence of these pharmaceuticals in South African seawater, therefore, Blue Lagoon, Glen Ashley, and Warner beach at the top and bottom were areas of interest. In Blue Lagoon over the summer season, concentrations of 2.01, 0.68, 0.81 $\mu\text{g L}^{-1}$ were detected for emtricitabine, diclofenac, and efavirenz, respectively. In autumn concentration of 2.67, 0.21, 3.54 $\mu\text{g L}^{-1}$ were detected for diclofenac, efavirenz, and gemfibrozil. Notable so, in winter the same compounds were detected with the concentrations of 0.43, 0.64, 6.52 $\mu\text{g L}^{-1}$, respectively. In spring, the concentrations of 6.1, 0.31, 38.3, 2.04 were detected for emtricitabine, diclofenac, ibuprofen, and efavirenz, respectively. Naproxen and ibuprofen were found to have concentrations below the quantification limit. Compared to results reported by Ngubane et al., (2019) in the same study site, diclofenac was detected in all seasons, naproxen and ibuprofen were found to be lower than the concentrations they detected. The presence of these pharmaceuticals was expected as the NWWTP discharges into the UMgeni river which flows into the UMgeni estuary and feeds into the Indian ocean within a couple of meters from the Blue Lagoon.

In Glen Ashley, the detected concentration in summer for diclofenac, ibuprofen, and efavirenz were 1.51, 1.92, 1.72 $\mu\text{g L}^{-1}$, respectively. Gemfibrozil was detected but could not be quantified in all the seasons. In Autumn, the concentrations of 1.39, 0.67, 0.21 $\mu\text{g L}^{-1}$ for emtricitabine, diclofenac and efavirenz, respectively were detected. In winter, only emtricitabine was detected with a concentration of 0.22 $\mu\text{g L}^{-1}$. In spring, concentrations ranging from 0.47- 5.44 $\mu\text{g L}^{-1}$

were detected with efavirenz having the lowest concentration and ibuprofen with the highest concentration. The concentrations of ibuprofen in seawater in this area are higher than those reported in Tromso, Norway of $0.007 \mu\text{g L}^{-1}$ and Santos, Brazil $2.09 \mu\text{g L}^{-1}$, respectively (Pereira et al., 2016; Weigel et al., 2004). Naproxen was not detected in these sea waters all the seasons.

Top and bottom of Warner beach were found to have higher concentrations of these pharmaceuticals. The bottom of Warner Beach, situated 1 km away from the top, had higher concentrations compared to the top as it is the most frequently used beach for swimming and fishing. In summer concentrations of 0.22 , 24.3 , $0.44 \mu\text{g L}^{-1}$ for diclofenac, ibuprofen, and efavirenz were detected, respectively. In autumn, concentrations of 0.36 - $3.63 \mu\text{g L}^{-1}$, with efavirenz being the only drug with the lowest concentration and the highest concentration being gemfibrozil. The concentrations of gemfibrozil that was detected are higher than those in Singapore which found to be ranging from 1 - 9 ng/L (Wu et al., 2010). In winter, only efavirenz was detected with a concentration of $0.31 \mu\text{g L}^{-1}$. In spring concentrations ranged from 0.78 - $3.38 \mu\text{g L}^{-1}$, where efavirenz was found to have the lowest concentration and tenofovir disoproxil with the highest concentration.

The top of Warner beach in summer had $2.56 \mu\text{g L}^{-1}$ of emtricitabine, the rest of the compounds were below the detection limit. In autumn, the concentration ranged from 0.402 - $2.92 \mu\text{g L}^{-1}$ where efavirenz found to have the lowest concentration and gemfibrozil with highest concentration. Concentrations detected in seawater were low due to the high dilution of seawater. Comparing all these seawater sites, summer and winter had the largest ΣEP of 24.96 and $37.9 \mu\text{g L}^{-1}$ respectively.

Table 5.3 Seasonal concentration of NSAIDs and ARVs detected in aquatic environments.

		Concentration ($\mu\text{g L}^{-1}$)							
	Sampling sites	Seasons	Emtricitabine	Tenofovir disoproxil	Naproxen	Diclofenac	Ibuprofen	Efavirenz	Gemfibrozil
Wastewater Treatment Plant	NWWTP Influent	Summer	15.2	26.4	<LOQ	0.591	26.1	10.1	7.7
		Autumn	101.2	nd	2.6	1.81	<LOQ	6.6	4.9
		Winter	5.07	7.77	<LOQ	37.5	13.76	13.66	5.38
		Spring	44.65	32.25	5.21	1.98	15.31	7.44	7.51
	NWWTP Effluent	Summer	nd	nd	nd	2.098	nd	6.83	nd
		Autumn	nd	nd	nd	4.17	nd	5.56	5.70
		Winter	nd	25.03	<LOQ	5.42	5.95	7.57	nd
		Spring	36.8	nd	Nd	3.83	<LOQ	0.82	1.19
	KWWTP Influent	Summer	39.4	16.9	<LOQ	0.81	22.8	8.67	7.25
		Autumn	10.6	16.5	<LOQ	1.55	<LOQ	14.2	14.4
		Winter	2.94	48.4	3.56	23.4	35.5	8.25	22.8
		Spring	36.3	28	2.51	1.18	6.06	2.88	9.99
	KWWTP Effluent	Summer	45.8	nd	<LOQ	0.52	5.48	8.81	3.19
		Autumn	1.84	nd	<LOQ	0.58	nd	8.99	<LOQ
		Winter	44.7	nd	<LOQ	2.02	nd	12	<LOQ
		Spring	32.7	nd	<LOQ	0.89	nd	9.04	<LOQ
Surface water UMgeni River		Summer	nd	nd	<LOQ	0.73	1.60	2.34	<LOQ
		Autumn	nd	2.71	<LOQ	0.99	2.07	0.40	2.55
		Winter	nd	nd	<LOQ	0.49	<LOQ	3.56	<LOQ
		Spring	17.9	nd	<LOQ	0.37	nd	nd	<LOQ

		Concentration ($\mu\text{g L}^{-1}$)							
Sampling sites	Seasons	Emtricitabine	Tenofovir disoproxil	Naproxen	Diclofenac	Ibuprofen	Efavirenz	Gemfibrozil	
Seawater	Kingsburgh River	Summer	nd	nd	nd	nd	1.4	0.32	5.55
		Autumn	12.2	nd	<LOQ	0.58	4.79	4.93	<LOQ
		Winter	nd	nd	<LOQ	7.39	2.14	9.89	3.24
		Spring	nd	nd	<LOQ	<LOQ	6.73	nd	5.19
	Blue Lagoon	Summer	2.01	nd	<LOQ	0.68	nd	0.81	<LOQ
		Autumn	nd	nd	<LOQ	2.67	<LOQ	0.21	3.54
		Winter	nd	nd	<LOQ	0.43	<LOQ	0.643	6.52
		Spring	6.1	nd	nd	0.31	38.3	2.04	<LOQ
	Glen Ashely	Summer	nd	nd	nd	1.51	1.92	1.75	<LOQ
		Autumn	1.39	nd	nd	0.67	nd	0.21	nd
		Winter	0.22	<LOQ	nd	nd	nd	nd	<LOQ
		Spring	1.96	4.53	nd	0.89	5.44	0.47	<LOQ
	Warner Beach Bottom	Summer	nd	nd	<LOQ	0.22	24.3	0.44	<LOQ
		Autumn	nd	nd	<LOQ	1.14	2.46	0.36	3.63
	Warner Beach Top	Winter	nd	nd	<LOQ	nd	nd	0.31	<LOQ
		Spring	1.84	3.38	nd	1.53	1.78	0.78	<LOQ
		Summer	2.56	nd	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
		Autumn	nd	2.17	nd	1.44	<LOQ	0.402	2.93
	Warner Beach Top	Winter	nd	nd	nd	0.36	nd	0.33	<LOQ
		Spring	<LOQ	nd	nd	<LOQ	nd	nd	<LOQ
		Concentration ($\mu\text{g L}^{-1}$)							
Sampling sites	Seasons	Emtricitabine	Tenofovir Disoproxil	Naproxen	Diclofenac	Ibuprofen	Efavirenz	Gemfibrozil	

Estuary	UMgeni estuary	Summer	nd	nd	<LOQ	<LOQ	nd	0.55	<LOQ	
		Autumn	0.39	1.46	nd	1.57	2.89	<LOQ	6.69	
		Winter	3.08	nd	3.79	0.95	1.46	2.09	9.6	
		Spring	0.22	nd	nd	0.74	<LOQ	3.04	<LOQ	
	Warner estuary	Beach	Summer	11.9	nd	<LOQ	0.21	1.59	2.71	<LOQ
			Autumn	nd	nd	1.82	nd	nd	3.92	nd
			Winter	nd	nd	nd	1.26	nd	12.3	24.4
			Spring	18.2	nd	<LOQ	0.55	<LOQ	nd	<LOQ

5.5 *Removal efficiencies from wastewater treatment plants*

Based on the raw influent and effluent concentrations, the removal efficiency of each pharmaceutical in the WWTPs was determined. As shown in (Table 5.4), the percentage removal for target compounds varied with each season for each WWTP. In the NWWTP, summer had a percent removal of 32.3-100%, with efavirenz having the lowest percentage removal. The removal efficiency in autumn ranged from 15.8-100%, with efavirenz being the lowest compound that was removed, however, gemfibrozil and tenofovir disoproxil had an accumulation 16.3% after treatment compared to their initial concentrations. In winter, diclofenac was 85.6% accumulated after treatment compared to its initial concentration, poor removal of diclofenac in WWTP has been reported in various studies (Lindqvist et al., 2005; Madikizela et al., 2018). The removal efficiency in Spring ranged from 17.7-100%, with emtricitabine which had the lowest removal efficiency. Naproxen was 100% effectively removed in all seasons. The KWWTP had an accumulation of several compounds in various season after the treatment process. In summer, emtricitabine and efavirenz had an accumulation of 16 and 1.62% respectively, and in winter it was 93 and 45.5%, respectively. In spring, only efavirenz had a negative result of 68.14% after the treatment process compared to the influent. Accumulation of the ARV drug emtricitabine and efavirenz have been reported in other studies in Africa (Mosekiemang et al., 2019; Schoeman et al., 2017; Wood et al., 2016). The KWWP had a poor removal efficiency compared to the NWWTP and yet they use similar treatment technologies. Wastewater treatment plants are the primary facilities for removing or degrading of contaminants from wastewater. The activated sludge system is the standard biological treatment procedure used in WWTPs. The removal efficiency varies depending on the physiochemical properties of the compounds as well as environmental conditions such as biological reactor configuration and operational parameters such as retention time and pH. Further work is required to effectively remove these pharmaceuticals and rectify this issue.

Table 5.4 Percent removal efficiencies for wastewater treatment plant

Compound	NWWTP				KWWTP			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
Emtricitabine	100	100	100	17.7	-16	82.7	-93	9.92
Tenofovir Disproxil	100	-16.32	-100	22.4	100	100	100	100
Naproxen	100	100	100	100	100	100	100	100
Diclofenac	72	56.6	-85.6	48.3	35.8	62.5	91.4	24.6
Ibuprofen	100	100	56.8	82.8	75.9	100	100	100
Efavirenz	32.3	15.8	44.6	89	-1.62	36.7	-45.5	-68.14
Gemfibrozil	100	-16.3	100	84.2	56	18.7	91	93

CHAPTER: SIX

6 Conclusion and recommendation

6.1 Conclusion

The determination of selected NSAIDs, ARVs and a lipid regulator in various aquatic environments was performed using an SPE technique employing Bond Elut Plexa cartridges, followed by quantitation using HPLC-PDA. Tenofovir and naproxen were the least detected pharmaceuticals in these study sites across all seasons. This study presents a survey of NSAIDs and ARV drugs in various aquatic sites. Generally, the treatment technique employed in these study sites appeared to be effective in the removal of most the pharmaceuticals, apart from emtricitabine, efavirenz and tenofovir disoproxil. High concentrations and recoveries of ARV drugs were detected across all studied water bodies. Most of these pharmaceuticals were detected in high concentrations in summer and autumn 2020. The discharge points of the Northern and Kingsburgh wastewater treatment plants could be the source of the presence of these drugs in the rivers, estuaries, and sea. The degradation kinetic and breakdown of these pharmaceuticals need to be further investigated. The accumulation of efavirenz concentration after treatment is a concern especially in the FDC therapy that includes this drug in a single tablet. As a result, the prevalence of this resistant drug is anticipated to rise. The presence of these pharmaceuticals suggested incomplete removal of these compounds by the wastewater treatment sectors as well as the state of contamination in the KwaZulu-Natal water bodies. The high concentration of these pharmaceuticals serves as motivation for continuous monitoring of these acidic compounds seasonally, and highly effective removal techniques to be studied in the future. The Agilent bond elut plexa cartridges is recommended for retaining more of these pharmaceuticals compared to OASIS HLB, and OASIS MAX used by other researchers. It is

recommended that more rapid and sensitive technique such as the LC/MS be employed in future.

6.2 *Recommendation*

The presence of ARV drugs and NSAIDs in the aquatic environment has substantial differences, making it difficult to observe a specific trend. This is due to factors such as varying consumption and prescription rate, as well as various treatment processes and environmental and geographical circumstances. There is very little information on the presence of antiviral drugs in industrial wastewater. Antiviral drug removal; from WWTPs should be improved. This may be achieved by optimization of current WWTP technologies, addition of new technology, and the control of pollution at the source. According to the regional distribution of the published studies, African aquatic environments and wastewater are the most studied, with the subclass of antiretroviral drugs being the most targeted. The occurrence of pharmaceuticals in various water samples in this study is concerning as this indicates the need for more research into their presence in water resources since there are people who still rely on untreated surface water for their daily living. Furthermore, because the number of HIV-1 infected people is increasing daily, comprehensive monitoring of these pharmaceuticals especially ARV drugs to understand their removal patterns in various nations. Environmental monitoring studies should be mandatory, especially in rural areas where most inhabitants still use pit toilets and drink river water that may be contaminated with different contaminants. They also utilize surface water for irrigation since they rely on agricultural cultivation to support their families. Long term exposure to these contaminants in human systems is not well understood.

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