Spectroscopic and Chromatographic Analysis of Nevirapine, Lamivudine and Zidovudine Anti-Retroviral Compounds in Wastewater Samples from Selected Wastewater Treatment Plants, Cape Town

by

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Declaration

I, **Gbolahan Sunday Olabode**, declare that the contents of this dissertation represent my own unaided work, and that the dissertation has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

18 November, 2021

Signed **Date**

Abstract

The HIV/AIDs pandemic is still prevalent and the consumption of antiretroviral (ARV) compounds is on the increase. After ingestion, the compounds are not completely consumed in the human system, they flow as parent compounds or metabolites into wastewater treatment plants (WWTPs) via urine and faeces. Unfortunately, the compounds are not completely removed in WWTPs and subsequently end up in aquatic ecosystems. The occurrence of these compounds in aquatic ecosystems constitute a health risk to aquatic life, human and the environment at large which requires adequate removal approach. In South Africa, three pharmaceuticals compounds called lamivudine (3TC), zidovudine (AZT) and nevirapine (NVP) are commonly used for the treatment of HIV/AIDs. This study collected monthly grab wastewater samples from two WWTPs in Cape Town metropole, South Africa, from autumn of 2018 to autumn of 2019. Raw influent (RI), final effluent before UV treatment (BUVT) and final effluent after UV treatment (AUVT) samples were collected from WWTP 1, while final effluent (FE) was collected from WWTP 2. To assess the prevailing physical and chemical conditions of the wastewater from the two plants, ten physicochemical parameters from the wastewater samples were evaluated using approved standard methods. Also, a UV-visible spectrophotometer was validated and used to determine residues of 3TC, AZT and NVP in the wastewater samples from both plants. Two novel advanced oxidation processes (AOPs) namely sunlight(UV)/Cl_2 and sunlight (UV)/$KMnO_4$ were developed. Operating parameters/conditions such as solar irradiance, pH, reaction time and oxidant to analyte ratio (OAR) were optimised and used to degrade the three pharmaceutical compounds in Milli-Q water, while the residual concentrations were measured by UV Visible spectrophotometer. The optimised method was applied to degrade the three target pharmaceuticals residues in real wastewater samples from both plants and monitored with high-performance liquid chromatography (HPLC). The result of the physicochemical investigation in the two plants showed that temperature, pH and SO_4^{2-} levels comply with the regulatory limit for wastewater discharge by both national (Department of Water Affairs and Forestry (DWAF), South Africa and international (United State Environmental Protection Agency (USEPA) regulatory standards. Nevertheless, the remaining seven parameters evaluated did not completely conform with the set limits prescribed by both bodies. Method validation confirmed that the UV-vis spectroscopy method is an accurate, precise, quick and less expensive way of determining the selected ARVs in wastewater samples. Similarly, the result emanating from the spectroscopic determination of the three pharmaceutical compounds in the AUVT sample in WWTP 1 and FE in WWTP 2 showed that 3TC ranged between (11.80 – 52.90 µg/L), AZT (0.90 – 66.98 µg/L) and NVP (54.70 – 207.70 µg/L). The percentage removal of the ARVs compounds in WWTP 1 was found to be 70%, 65% and 55% for AZT, 3TC, and NVP, respectively. Though our target pharmaceutical compounds are not yet regulated; the results however indicated that effluents originating from the two WWTPs are probable sources of ARVs and physicochemical pollutions to the receiving water bodies, which require adequate improvement of the WWTPs operations to guarantee safe effluent discharge. The HPLC method used was able to resolve the three compounds of interest within 7 minutes, and the validation of the method showed that the method is suitable for the analysis of 3TC, AZT and NVP in wastewater samples. For the degradation studies,the optimised conditions for (sunlight)UV/chlorine process was at pH 7, OAR 7:1, solar irradiance 250 ± 25 mW/m^2,and 90 minutes and the % removal recorded were 3TC: 7.31 ─ 70.79%, AZT:

29.64 ─ 91.69% and NVP: 15.60 ─ 67.77%. For UV/KMnO4, the optimised conditions were pH 5, OAR 7:1, solar irradiance 250 ± 25 mW/m^2,and 45 minutes and the % removal recorded ranged between 2.70 ─ 100.00%, 6.48 ─ 100.00% and 15.41– 100.00% for 3TC, AZT and NVP. The % removal obtained in both methods were higher than those recorded for the two control experiments which involve chlorine alone or $KMnO_4$ alone and sunlight (UV radiation) alone.

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Dedication

This work is dedicated to my parents late Special Apostle Solomon Taiwo Ojebode and Senior Mother in Israel Alice Abeke Ojebode.

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Abbreviations and Acronyms

3TC	Lamivudine
AIDS	Acquired Immunodeficiency Syndrome
AMT	Amandatine
AOPs	Advanced Oxidation Processes
ART	Antiretroviral Therapy
ARV	Antiretroviral
AZT	Zidovudine
CCL	Contaminant Candidate List
CCL-4	Contaminant Candidate List 4
CE	Capillary Electrophoresis
CIP	Ciprofloxacin
CMV	Cytomegalovirus
COD	Chemical Oxygen Demand
DBPs	Disinfection by Products
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DTG	Dolutegravir
DWAF	Department of water affairs and forestry, South Africa
EC	Electrical Conductivity
ECs	Emerging Contaminants
EFV	Efavirenz
FAC	Free Available Chlorine

FDA	Food and Drug Administration
GAC	Granular Activated Carbon
HAAs	Halo Acetic Acids
HAART	Highly Active Antiretroviral Therapy
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HPLC	High-Performance Liquid Chromatography
HRMS	High-Resolution Mass Spectrometry
HRT	Hydraulic Retention Time
HSRT	High Sludge Retention Time
HSV	Herpes Simplex Virus
IDUs	Injection Drug Users
LC/MS/MS	Liquid Chromatography/Tandem Mass Spectrometry
LPV	Lopinavir
MS	Mass Spectroscopy
MSM	Men Sex Men
NDMA	N-nitrosodimethylamine
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
NOM	Natural Organic Matter
NPDWR	National Primary Drinking Water Regulations
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NVP	Nevirapine
OARs	Oxidant to Analyte Ratios
PIs	Protease Inhibitors

PLHIV	People Living with HIV/AIDS
PMTCT	Prevention of Mother-to-Children Transition
RIM	Rimantadine
RNA	Ribonucleic Acid
ROS	Radical Oxygen Species
RQ	Risk Quotient
RSV	Respiratory Syncytial Virus
RT	Reverse Transcriptase
RTV	Ritonavir
SDWA	Safe Drinking Water Act
SMX	Sulfamethoxazole
SRT	Sludge Retention Time
TAF	Tenofovir
TDSs	Total Dissolved Solids
THMs	Trihalomethanes
TLD	Tenofovir disoproxil fumarate, Lamivudine, and Dolutegravir
TMP	Trimethoprim
TOF	Time-of-Flight
TPs	Transformational Products
UHPLC	Ultra-High-Performance Liquid Chromatography
USEPA	United State Environmental Protection Agency
VZV	Varicella-Zoster Virus
WSPs	Waste Stabilisation Ponds
WWTPs	Wastewater Treatment Plants

List of Publications and Conference Presentations

Published Papers

- **Olabode, G.S.,** Olorundare, O.F. & Somerset, V.S. (**2020**). Physicochemical properties of wastewater effluent from two selected wastewater treatment plants (Cape Town) for water quality improvement. *International Journal of Environmental Science and Technology*. doi.org/10.1007/s13762-020-02788-9.

Submitted Journal Papers

- **Olabode, G.S**. & Somerset, V.S. (**2021**). UV-visible spectroscopic determination of antiretroviral compounds in wastewater samples. (**In Review**).

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Book Chapters

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- **Olabode G.S**., Somerset V.S.Advanced oxidation treatment and characterization of nevirapine in effluent from a wastewater treatment plant. The Society of Environmental Toxicology and Chemistry (SETAC) 9th African Biennial Conference/Society of Risk Analysis (SRA) 5th World Congress, **6- 8th May 2019**, Cape Town, South Africa.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter introduced the need, peculiarity and reasons for this research work. It considered findings from the survey of literature that wastewater treatment plants (WWTPs) belong to one of the major sources of antiretroviral pollution in aquatic ecosystems. These compounds are reported to be hazardous in aquatic environments. This work hypothesised a high concentration of the compounds in WWTPs in South Africa because of the constant consumption of the compounds due to the high HIV pandemic in the country.

Consequently, this chapter then aimed to investigate the presence of three commonly used antiretroviral drugs lamivudine (3TC), zidovudine (AZT) and nevirapine (NVP) in the wastewater samples from two wastewater treatment plants (WWTPs) in Cape Town metropole. The work also proposed two advanced oxidation processes with sunlight(Cl_2) and sunlight($KMnO_4$) to remove the antiretrovirals (ARVs) contaminants in the wastewater samples.

In summary, the highlights of this chapter include background to the study, the occurrence of antiviral compounds as emerging contaminants in the aquatic ecosystem, the problem statement, hypothesis, aims of the study, objective of the study, novelty and expectations of the study and delimitations of the study.

1.2 Background to study

In 1981, Human Immunodeficiency Viral/Acquired Immunodeficiency Syndrome (HIV/AIDS) first appeared as a fatal disease in men who had sex with men (MSM) and injection drug users (IDUs) in the United States (Beyrer et al., 2012; Lange and Ananworanich, 2014). Then, it was first diagnosed as an incurable disease or simply put “death sentence” (Broder, 2010; Naeger et al., 2010). This motivated diverse research groups to develop antiretroviral (ARV) agents (Broder, 2010). 3′-azido-3′-deoxythymidine (Retrovir later called zidovudine (AZT)) was the first approved antiretroviral drug on March 19, 1987, by the Food and Drug Administration (FDA). This shows that effective control of HIV-1 was possible, and disproving the earlier prediction that death is the usual result to the contrary (Broder, 2010; Lange and Ananworanich, 2014; De Clercq, 2019).

Due to the market incentive for pharmaceutical companies, other ARV such as nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs) appeared on the market. Later in 1996, non-nucleoside reverse transcriptase inhibitors (NNRTIs) came as a cheaper alternative to PI-based highly active antiretroviral therapy (HAART), which make it possible to extend ARV therapy in resource-poor settings, and a tripartite ARV drug therapy called HAART was announced (Naeger et al., 2010; Broder, 2010). Today, the FDA has approved

about ninety antiviral drugs, and about fifty products are antiretroviral. The remaining antivirals are mainly used for the treatment of herpes virus (herpes simplex virus (HSV), hepatitis B virus (HBV), varicella-zoster virus (VZV), and cytomegalovirus (CMV)), influenza virus, respiratory syncytial virus (RSV) and hepatitis C virus (HCV) infections (De Clercq, 2004; Broder, 2010; Naeger et al., 2010; De Clercq, 2019).

1.3 The occurrence of antiviral compounds as emerging contaminants in the aquatic ecosystem

The occurrence of antiviral compounds has been reported in raw water, treated wastewater, surface and groundwater, and drinking water in many nations of the world (Prasse et al., 2010; Swati et al., 2011; Jain et al., 2013; Ngumba and Gachanja, 2015). Wastewater treatment plants (WWTPs) have been reported as the major source of these compounds (Prasse et al., 2010; Swati et al., 2011; Jain et al., 2013; Ngumba and Gachanja, 2015; Abafe et al., 2018; K'oreje et al., 2018). After being used, the antiviral drugs that were not completely used up in the patient's system are excreted in faeces and urine in their original forms or as metabolites from where they got to the conventional WWTPs where they are partially degraded and discharged with the effluent (Prasse et al., 2010; Swati et al., 2011; Jain et al., 2013; Ngumba and Gachanja, 2015).

Notwithstanding, there is an increase in the demand for antiviral drugs. They are prescribed in large quantities every year, as well as continuous usage and constant administration on daily basis because of the rising occurrence of viral infections in the world (Oguntibeju, 2012; Lange and Ananworanich, 2014). This automatically increases their concentrations in the aquatic ecosystems (An et al., 2011; Jain et al., 2013). Although, these compounds are unregulated (Oller et al., 2011; Richardson and Ternes, 2014). On November 17, 2016, EPA included pharmaceuticals among the lists of contaminant candidate list 4 (CCL- 4) for future regulation under the Safe Drinking Water Act (SDWA) (Richardson and Kimura, 2016; Glassmeyer et al., 2017).

1.4 Problem statement

Access to qualitative and quantitative water is essential for human life, but, residues of ARV compounds used for the suppression of HIV loads in the human body were reported in the various aquatic environment in South Africa and different parts of the world (Ngumba and Gachanja, 2015; Abafe et al., 2018; K'oreje et al., 2018; Mosekiemang et al., 2019; Mlunguza et al., 2020). Effluent discharged from WWTPs was reported as the major source of these compounds (K'oreje et al., 2018; Mosekiemang et al., 2019; Mlunguza et al., 2020). This is because the ARV drugs that were not completely metabolised in the patient's body system but excreted via faeces and urine, flowed into the WWTPs where they were not also completely removed (Abafe et al., 2018; Mlunguza et al., 2020). Other routes include direct dumping into aquatic bodies, effluent from pharmaceutical industries and hospital effluent (Jain et al., 2013). These compounds were reported to be hazardous to aquatic life, and potentially harmful to human health (Swanepoel et al., 2015; Schoenfuss et al., 2016). In South Africa, the HAART

recipe containing NRTIs (3TC and AZT) and NNRTIs (NVP) has been in operation as the first-line regimen for the treatment of HIV infection due to its effectiveness against HIV infection and secondly for its cost-effectiveness when compared to other ARV drugs (Swanepoel et al., 2015; Stinson et al., 2017). In that case, this study is motivated based on the need to effectively eliminate these ARVs from wastewater using improved technologies of sunlight/Cl_2 and sunlight/$KMnO_4$ advanced oxidation processes (AOPs). This is because of the inefficient removal of the compounds by the conventional WWTPs.

1.5 Hypothesis

Based on the prevalence of HIV/AIDs in South Africa and the continual consumption of 3TC, AZT and NVP to suppress the HIV infection, we hypothesised that the compounds are present in the two WWTPs in Cape Town metropole and the proposed AOPs with sunlight(UV)/Cl_2 and sunlight/(UV)$KMnO_4$ will effectively remove the compounds in the wastewater samples to prevent the pollution of the aquatic ecosystems with these ARV compounds and ensure a safe environment.

The questions in the course of this research are:

- Can 3TC, AZT and NVP be detected and quantified in wastewater at Khayelitsha and Bellville WWTPs?
- How effectively do the two WWTPs remove 3TC, AZT and NVP during treatment?
- What are the prevailing physicochemical parameters (temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), $SO4^{2-}$, chemical oxygen demand (COD), biochemical oxygen demand (BOD), Cl^-, and PO_4^{3-} in the wastewater and their impacts on the target ARVs?
- Can the use of sunlight(UV)/Cl_2 and sunlight(UV)/$KMnO_4$ as new advanced oxidation processes (AOPs) effectively remove 3TC, AZT and NVP than the conventional method already in use in the two WWTPs?
- Can the conventional wastewater treatment method in Khayelitsha and Bellville be improved by the sunlight(UV)/Cl_2 and/ or sunlight(UV)/$KMnO_4$ as the new AOPs method?

1.6 Aim of the study

The main aim of this study was to investigate whether the commonly used ARVs in South Africa 3TC, AZT and NVP are present in the wastewater from two selected wastewater treatment plants in the Cape Town Metropole and to remove them with advanced oxidation processes involving sunlight(UV)/Cl_2 and sunlight(UV)/$KMnO_4$.

To achieve the main aim of this study, the following objectives were identified.

1.7 Objectives of the study

(i) The **first** objective was to determine the monthly and seasonal levels of the ARVs of 3TC, AZT and NVP contaminants in wastewater samples from the Khayelitsha and Bellville WWTPs.

(ii) The **second** objective was to evaluate the efficiency of Bellville WWTP in eliminating 3TC, AZT and NVP contaminants through the quantitative determination of the ARVs residues in influent and effluent wastewater samples using UV/visible spectrophotometer analysis.

(iii) The **third** objective was to determine the level of specific physicochemical parameters in the wastewater samples, which included temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), chemical oxygen demand (COD), biochemical oxygen demand (BOD), SO_4^{2-}, Cl^-, and PO_4^{3-} concentrations in the wastewater and investigate their co-influence on the target ARVs.

(iv) The **fourth** objective was to develop novel advanced oxidation treatment technologies using sunlight(UV)/Cl_2 treatment and sunlight(UV)/$KMnO_4$ for the degradation of 3TC, AZT and NVP from wastewater samples.

v) The **fifth** objective was to optimise degradation time, pH and oxidant to analyte ratio for the selected ARVs

1.8 Novelty and expectations of the study

The novelty and expectations of the study are highlighted below:

(i) Provide novel and efficient treatment method(s) for the removal of ARVs contaminants in wastewater to ensure good ecological status in the aquatic ecosystem and a healthy environment to plant, animals, and human life.

(ii) At the investigated optimised conditions for the novel proposed treatment methods, the advantages of the readily available sunlight radiation and the existing chlorination process operation in wastewater treatment can be easily maximized in the two plants to effectively remove 3TC, AZT and NVP contaminants in wastewater at a reduced cost.

(iii) The use of greener and environmental friendly $KMnO_4$ and sunlight radiation will enhance environmental sustainability by removing 3TC, AZT and NVP contaminants.

(iv) Prevent the transmission of ARVs contaminants in recycled water used in irrigation.

(v) Eradicate the ‘consumption’ of ARVs in the domestic water supply.

1.9 Delimitations

Out of several ARVs used in South Africa, three ARVs were selected because they are the most frequently used ARVs since 1999 to treat HIV in the studied areas (Levy et al., 2005; Boulle et al., 2010; Stinson et al., 2014; Stinson et al., 2017). The choice of UV-visible Spectroscopy and High-Performance Liquid Chromatography (HPLC) as analytical methods were based on the use of low-cost analytical technologies that show-cased its feasibility and accessibility in developing countries. The work was carried out on the samples taken over 12 months, which covered the entire seasons in Cape Town.

1.10 Outline of the Thesis

The outline of the thesis chapters is described in the following paragraphs.

Chapter 1: This chapter is a general introduction to the importance and the need to embark on this work. The highlights of this chapter include background to the study, the occurrence of antiviral compounds as emerging contaminants in the aquatic ecosystem, the problem statement, hypothesis, aims of the study, objective of the study, novelty and expectations of the study and delimitations of the study. From the survey of the literature, the chapter began with the emergence of HIV/AIDs in 1981 as a deadly disease that motivates rapid development and production of different antiretroviral (ARV) compounds. Later, residues of the compounds were found in several aquatic environments majorly via WWTPs and they are reported to be hazardous. Thus, the need to eliminate them particularly the 3TC, AZT and NVP which are the commonly used ARV in South Africa.

Chapter 2: This chapter presents a review of literature on the pollution of the environment by emerging contaminants which encompasses different compounds including pharmaceuticals, personal care products and numerous other classes of compounds. The chapter discussed extensively the rate of HIV infection and consumption of ARV compounds, occurrence/fate of ARVs in the aquatic environment, their hazardous effects, need to monitor and eliminate them in the aquatic environment and most especially in South Africa where water is scarce and wastewater is being used in agriculture and some other few areas. Different methods for the elimination of ARVs in the aquatic sample and several analytical methods for their determination were also reviewed.

Chapter 3: This chapter describes the different scientific methods and protocols used to:

a) Determine the monthly and seasonal levels of the ARVs of 3TC, AZT and NVP contaminants in wastewater samples from the Khayelitsha and Bellville WWTPs.
b) Evaluate the efficiency of Bellville WWTP in eliminating 3TC, AZT and NVP contaminants through the quantitative determination of the ARVs residues in influent and effluent wastewater samples using UV/visible spectrophotometer analysis.
c) Determine the level of specific physicochemical parameters in the wastewater samples, which included temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), chemical oxygen demand (COD), biochemical oxygen demand (BOD), SO_4^{2-}, Cl^-, and PO_4^{3-} concentrations in the wastewater and investigate their co-influence on the target ARVs.
d) Develop novel advanced oxidation treatment technologies, including exposure to combined sunlight(UV) and oxidation treatment practices, for the degradation or removal of 3TC, AZT and NVP from wastewater samples using:
    - Sunlight(UV)/Cl_2 treatment;
    - Sunlight(UV)/ KMnO4 tratment.

Chapter 4: This chapter presents the results of physicochemical properties of wastewater effluent from the two WWTPs. The chapter also describes the impact of these physicochemical parameters on the ARV compounds.

Chapter 5: This chapter describes the results of the determination of selected antiretroviral compounds (3TC, AZT and NVP) in wastewater samples. Likewise, the chapter evaluates the efficiency of Bellville WWTP in eliminating 3TC, AZT and NVP contaminants through the quantitative determination of the ARVs residues in influent and effluent wastewater samples using UV/visible spectrophotometer analysis. The effect of UV treatment in the elimination of the target ARVs was also discussed in this chapter.

Chapter 6: This chapter discusses the influence of determinant factors such as pH, the oxidant to analyte ratio, and the contact time to optimise the degradation conditions for the selected ARVs. The control experiments involving degradation with Cl_2 alone; $KMnO_4$ alone and sunlight (UV) radiation alone which investigated the novel impact of the advanced oxidation treatment technologies involving the combination treatments were also discussed. Finally, the chapter presents the results of degradation studies on the selected anti-retroviral compounds in wastewater subjected to sunlight(UV)/cl2 and sunlight(UV)/KMnO4 treatment.

Chapter 7: In this chapter conclusions were drawn based on all findings in this research work. Some recommendations were made on the need to upgrade the two WWTPs and the great potential in the application of the new methods as they will both ensure a save environment. Also, the chapter identified some future work that could expand the coast of these findings to enhance a good environment.

CHAPTER 2

LITERATURE REVIEW[α]

2.1 Introduction

This chapter presents a review of the literature. It contained the pollution of aquatic ecosystems by yet to be regulated contaminants called emerging contaminants. These include residues of pesticides, algal toxin, biocides, pharmaceutical compounds, constituents of personal care products, sunscreen agents, microplastics and numerous others.

Out of this, pharmaceutical and personal care products were discussed and emphasis was on antiretroviral compounds. Subjects reviewed in this chapter include occurrence/fate of ARVs in the world and South Africa, level of pollution in aquatic ecosystems, toxic effects of the compounds which necessitate more work on them, analytical methods for their determination in the aquatic sample, and so on.

The ARV of interest 3TC, AZT and NVP chose based on the level of availability and consumption in South Africa, particular reference was made to the locations of this study which are Khayelitsha and Bellville in Cape Town metropole were also reviewed.

Furthermore, advancements in water treatment technologies were reviewed. The chapter emphasised on AOPs which show enhancement in the removal of antiretroviral compounds and other emerging contaminants. Finally, the potential and benefits of the two AOPs sunlight (UV)/Cl_2 and sunlight (UV)/$KMnO_4$ proposed for the elimination of the target compounds from the wastewater samples were discussed.

2.2 Emerging contaminants

Long ago, chemicals generated from industrial and agricultural operations are regarded as major sources of environmental contamination (Jabeen et al., 2015; Osorio et al., 2016). Human activities are not static, however, advances in environmental evaluation reveal that this range of chemicals constitutes a small proportion of the numerous contaminants occurring globally. However, advancement in the environmental assessment and evaluation exposed other groups of new bioactive contaminants in the environment, called emerging contaminants (ECs) (Sauvé and Desrosiers, 2014). Emerging contaminants are defined as any natural or synthetic substance that has not been regulated nationally or internationally in the environment, but with the ability to penetrate the soil and aquatic ecosystems, producing known or suspected adverse ecological

[α] **Gbolahan Olabode** and Vernon Somerset. (**2019**). Chapter 3: Advances in Chromatographic Determination of Selected Anti-Retrovirals in Wastewater. In Elvis Fosso-Kankeu (Ed.), *Nano and Bio-Based Technologies for Wastewater Treatment: Prediction and Control Tools for the Dispersion of Pollutants in the Environment.* (pp 105-127). New York, USA: Wiley-Scrivener Publishers. ISBN: 9781119577096.

and/or human health effects (Brack et al., 2012; Chander et al., 2016). They are caused by increased population, urbanisation, variation in the way of life, climatic change and other various anthropogenic activities (Chigor et al., 2013; Matongo et al., 2015; Jabeen et al., 2015; Osorio et al., 2016). These substances include numerous synthetic chemicals such as pesticides, algal toxin, biocides, residues of drugs/pharmaceutical compounds used for human and animal health (as well as prescription/nonprescription drugs and biologics, diagnostic agents, "nutraceuticals," hormones, active constituents of personal care products, sunscreen agents, microplastics and numerous others. Based on the NORMAN network report, about 700 compounds grouped into 20 classes, are included in the European aquatic environment as ECs (Brack et al., 2012; Geissen et al., 2015; Richardson & Kimura, 2016).

Though these compounds have been reported in several environmental assessments in many nations of the world, they are not yet included in the routine monitoring programmes at the international or national level, and their fate, characteristics and toxic impact on the environment are not yet clear (Oller et al., 2011; Brack et al., 2012; Postigo and Richardson, 2014; Richardson and Kimura, 2016). This group of contaminants has hitherto received little attention as potential environmental pollutants (Daughton and Ternes, 1999; Thomaidi et al., 2016). The need to focus on these contaminants is important, because of their possible harmful consequences (Jabeen et al., 2015; Osorio et al., 2016). Paying attention to these earlier unrecognised compounds is very vital due to the increasing rate of technological advancement enlarging the coasts of synthetic chemicals designed for use, along with many others inadvertently formed by-products (Nikolaou, 2013).

These compounds spread to the environment through several anthropogenic sources, which are from this point distributed throughout different environmental matrices (Richardson and Ternes, 2014; Richardson and Kimura, 2016). Furthermore, the significances of unfamiliar, anonymous, unanticipated, or unpredicted contaminants in the environment are of foremost concern to the environmentalists and in the area of public health (Jabeen et al., 2015; Osorio et al., 2016). The importance of identifying such emerging contaminants is essential and should be a priority to protect human health and the general environment (Rana et al., 2017; Glassmeyer et al., 2017). This involves the cooperation of all, including the sundry to the essence that future generations receive a cleaner and healthier environment (Dore, 2015).

2.3 Pharmaceuticals and personal care products

Pharmaceuticals and personal care products (PPCPs) constitute one of the major groups of EC. Pharmaceuticals belong to a class of chemical substances that possess medicinal properties. They include all prescriptions, non-prescription, over-the-counter therapeutically, prophylactic drugs, including some sex hormones and steroid hormones used for human and animal health (Gogoi et al., 2018). They have been categorised as emerging contaminants because they possess different damaging elements with potentially harmful effects. Residues of these substances, used by humans and animals, have been detected in several environmental matrices at a very minute quantity. A greater portion of consumed pharmaceuticals is expelled into the WWTPs as a mixture of original compounds, and various metabolites through faeces and urine (Jain et al., 2013; Petrie et al., 2014; Chander et al., 2016; Gogoi et al., 2018). Other sources of pharmaceutical compounds into the environment include effluents from pharmaceutical

industries, hospitals, including improper disposal of excess and expired drugs (Jain et al., 2013; Schoeman et al., 2015; Ngumba and Gachanja, 2015; Wood et al., 2015; Schoeman et al., 2017).

Varieties of pharmaceutical compounds have been reported in aquatic ecosystems. Ibuprofen, sulfamethoxazole, atenolol, carbamazepine (CBZ), ciprofloxacin, erythromycin, ofloxacin were detected in sewage treatment plants (STPs) and the river Po, in Italy (Prasse et al., 2010; Prasse et al., 2011; Matongo et al., 2015; Ngumba and Gachanja, 2015; Al Qarni et al., 2016). Antibiotics compounds including sulfamethoxazole, trimethoprim and ciprofloxacin, together with some commonly used antiretroviral drugs (lamivudine (3TC), NVP and AZT) have been reported in some of Kenya's WWTPs, river water in Nairobi River Basin, and Kenya at nanogram levels. Some river waters were reported to have higher contaminants than the WWTP effluents levels (Ngumba and Gachanja, 2015). According to Petrie et al. (2014), about 200 different pharmaceuticals residues including ciprofloxacin an antibiotic as high as 6.5 mg/L have been reported internationally in river waters. However, among the concerns relating to their occurrence in the environment include their persistency in the surface water, development of antimicrobial resistance and likely toxicity to aquatic organisms (Kolpin et al., 2002; Postigo and Richardson, 2014). The growing resistance of bacteria to some antibiotics recorded in the environment globally is a result of exposure of microorganism to sub-lethal concentrations of antimicrobial compounds; and this is becoming a worldwide health challenge (Gaw et al., 2014; Gogoi et al., 2018). For some pharmaceuticals compounds such as acetaminophen, bezafibrate, carbamazepine, clofibric acid – metabolite, diclofenac (DIC), ibuprofen, Metoprolol (MTP), naproxen and trimethoprim, their toxicity test showed that they are detrimental to aquatic organisms. Residues of antibiotics such as erythromycin, sulfamethoxazole, ofloxacin and oxytetracycline in aquatic environments equally fall within the toxic groups (Petrie et al., 2014; Rubirola et al., 2014; Richardson and Kimura, 2016)

The synergistic impact of pharmaceuticals has been reported. For instance, the exposure of *Daphnia magna* to the mixture of CBZ and clofibric acid for a longer period during immobilisation tests showed a higher toxic effect on the organism than the control experiment performed with individual compound at the same concentration (Dietrich et al., 2010; Petrie et al., 2014). Similarly, acute toxicity of a mixture of some pharmaceuticals including DIC, ibuprofen, naproxen and aspirin were documented, whereas an insignificant effect was detected for the same chemicals at the equal concentration that exists individually (Rubirola et al., 2014; Gaw et al., 2014). Also, the impact of CBZ, DIC, 17a -ethinylestradiol (EE2) and metoprolol (MET) at both individual pharmaceutical and drug mixture level was investigated at environmentally relevant concentrations on some morphologies and life-history of *D. magna* over six generations (Dietrich et al., 2010; Rubirola et al., 2014). According to the report, a combination of pharmaceuticals at environmentally relevant concentration negatively affects the organism than the individual substance at the same concentration (Dietrich et al., 2010). A major reduction in embryo production was recorded in a female *D. magna* when it was subjected to a pharmaceutical mixture of acetaminophen (ACE), CBZ, gemfibrozil (GEM), and venlafaxine (VEN) and WWTP effluents over six weeks (Dietrich et al., 2010; Rubirola et al., 2014; Gaw et al., 2014). Similarly, it has been reported that the contamination of water with pharmaceuticals mixture could be of great adverse effect compared to a single pharmaceutical contaminant (Schoenfuss et al., 2016).

Despite all the problems associated with pharmaceutical in the environment, the production and consumption of pharmaceutical are increasing due to factors such as increasing population, expanding potential market, increase in the number of old people, advancement in health care policies, and long-life expectancy of the people (Ngumba and Gachanja, 2015; Chander et al., 2016). Therefore, the imminent danger of this class of environmental pollutants, to which antiviral compounds belong, should be considered critically.

2.4 Antiviral drugs

Antiviral drugs are pharmaceutical compounds that can reduce or retard the action of viruses. They are classified into two groups:

i. Antiretroviral compounds (ARV), and
ii. General antiviral compounds (non-antiretroviral drugs) (Broder, 2010; Naeger et al., 2010; De Clercq and Li, 2016; De Clercq, 2019).

ARVs are a specific group of antiviral compounds administered for the management and suppression of HIV load (Swati et al., 2011; Jain et al., 2013; Swanepoel et al., 2015). Some of these compounds, including NVP and efavirenz (EFV), have been reported to persist in the environment and they are possible risks to water resources (Vaňková, 2010; Jain et al., 2013; Ngumba and Gachanja, 2015; Schoeman et al., 2017).

2.5 Rate of HIV infection in the world and consumption of ARV compounds

The universal danger of HIV infection and AIDS is pandemic in scopes. In the year 2002, about 300,000 people in developing countries (out of which people in sub-Saharan Africa was significant) were people living with HIV (PLHIV) receiving ARV treatment (Lange and Ananworanich, 2014; Steinbrook, 2016). Some regions in the world including Asia, Latin America, the Caribbean and Eastern Europe were seriously infected with HIV/AIDS (Avert, 2018; Stanley and Kaplan, 2018). In 2010, approximately 33.3 million people all over the world were infected by HIV out of which 17.5 million received antiretroviral therapy (ART) (Avert, 2018; Stanley and Kaplan, 2018).

Oguntibeju (2012) reported that the population of HIV patients was on the increase, due to the annual occurrence of new HIV infections. To support the above statement, Stanley and Kaplan (2018) reported new infections of HIV in 2016 which was close to 2 million. Nonetheless, there has been a consistent increase in HIV/AIDS infection; for instance, a total amount of 28.9, 31.8 and 33.3 million people were infected in 2000, 2005 and 2010, respectively (Boulle et al., 2010; Stinson et al., 2014; Stinson et al., 2017; Avert, 2018; Stanley and Kaplan, 2018). WHO and UNAIDS reported that close to 37 million people were HIV positive in 2016 (Avert, 2018; Stanley and Kaplan, 2018). And the report further stated that less than 60% of these HIV patients had access to ART therapy globally at the end of that year. This is a drastic increase in contrast to the 28.9 million people affected in 2000, wherein only 770,000 had access to ART (Avert, 2018; Stanley and Kaplan, 2018).

AIDS was the principal source of death for people between the age group of 25 to 44 years old in the U.S. Statistically, a total of 33,590 U.S. residents who were within the age-group died of HIV infection in 1992 (Naeger et al., 2010). Globally, about 1.7 million people died of AIDS-related diseases in 2001, while the number increased to about 2.0 million in 2007 (Oguntibeju, 2012). Sub-Saharan Africa is seen as the central for HIV/AIDS pandemic because reports disclosed that the region alone accommodates about 67.0% of all PLHIV 9 (Idele et al., 2014; Avert, 2018). East Africa and South Africa regions account for more than 64.0% of all fresh HIV infections (WHO, 2017). For instance, HIV/AIDS infection increased globally from 0.6% in 2008 to 12.2% in 2012; most especially in Sub-Saharan Africa, the rise of the HIV/AIDS infection is worrisome as the infection increased from 50,000 in 2002 to 7.5 million within a decade (Idele et al., 2014).

To date, there is a steady production increase in the demand for ARV drugs across the world, apparently because of a steady increase in the infection rate (Broder, 2010; Oguntibeju, 2012; Stanley and Kaplan, 2018). Secondly, HIV still constitutes a therapeutic task and burden universally (Jain et al., 2013). Essentially, the best approach to deal with HIV infection would have been the development of an effective vaccine to reduce the continual consumption of an antiviral drug. Although, till today, there is yet to be any vaccine to prevent HIV/AIDs, which makes ARV therapy a life-long one (Naeger et al., 2010; Broder, 2010). Moreover, the development and approval of antiviral agents have experienced rapid progress in recent years (Naeger et al., 2010; De Clercq and Li, 2016). In 2010, Naeger et al. (2010) reported that up till 2010, about 40 antiviral compounds were approved by the FDA and more than half of the products are anti-retroviral. The report added that more than other forty antivirals were in clinical development for approval (Naeger et al., 2010; De Clercq and Li, 2016). With the recent report, more than forty new ones have been approved and today, ninety antiviral drugs are available for human use while about half of them are anti-retroviral (De Clercq and Li, 2016; De Clercq, 2019).

2.6 HIV/AIDS in South Africa

It is indexed that South Africa is among the countries with a high population affected by HIV/AIDS (Table 2.1) (Wood et al., 2015; Stinson et al., 2017; Abafe et al., 2018). The country had lost a high number of peoples to HIV infection; therefore, this effect continues to be a detriment to the fast-growing population of the country (Wood et al., 2015; Stinson et al., 2017; Cornell et al., 2017; Abafe et al., 2018). Due to this, the country annually spends hugely on the treatment of HIV, which continues to be a disadvantage because of the annual rise in the number of infected people and the death rate from the infection (Wood et al., 2015; Avert, 2018). In 2005, a total of 5.5 million people was infected, with an estimate of over 300,000 death recorded with HIV/AIDS cases in South Africa (Marconi et al., 2008). To curtail this health issue, ART therapy was made accessible to the general populace in several hospitals and clinics, this first started as an operational plan for comprehensive HIV/AIDS care treatment (Levy et al., 2005; Stinson et al., 2017). By the end of 2005, about 200,000 South Africans enjoyed ART therapy. This accounts for the large share of ARV therapy in Sub-Saharan Africa (Broder, 2010). In 2012, reports had it that more than 2 million individuals in South Africa needed ARV, and a

total of 470,000 new diagnoses were recorded within that same year (Schoeman et al., 2015; Steinbrook, 2016).

Recently, as part of the global efforts to reach and accelerate the treatment of HIV at an affordable price, dolutegravir (DTG), an integrase inhibitor, which is a combination of 3 drugs named tenofovir (TDF) disoproxil fumarate and 3TC, was also launched in South Africa (probably as one of the most affected countries), Kenya and some developing nations (Avert, 2018; Stanley and Kaplan, 2018).

Table 2.1: Ten top countries affected by HIV/AIDS (Avert, 2018; Stanley and Kaplan, 2018).

Country	Adult prevalence of HIV/AIDS (%)	Number of people infected with HIV/AIDS	Annual death from HIV/AIDS	Year of estimation
Swaziland	27.20	220,000	3,900	2016
Lesotho	25.00	330,000	9,900	2016
Botswana	21.90	360,000	3900	2016
South Africa	18.90	7,100,000	110,000	2016
Namibia	13.80	230,000	4,300	2016
Zimbabwe	13.8	1,300,000	30,000	2016
Zambia	12.40	1,200,000	21,000	2016
Mozambique	12.30	1,800,000	62,000	2016
Malawi	9.20	1,000,000	24,000	2016
Uganda	6.50	1,400,000	28,000	2016

As illustrated in the table, among the affected ten top countries almost 50% of infected people are from South Africa. This demonstrates a high consumption of HIV drugs by the high number of infected people in the country. Thus, the high consumption could cause more residue of ARVs that result in the different aquatic ecosystem (Wood et al., 2015; Avert, 2018).

2.7 The occurrence of antiviral drugs in the aquatic ecosystem

The presence of antiviral compounds has been discovered in the different aquatic environment that includes raw wastewater influent, effluents from WWTPs, different surface water like rivers, river basin, dam and also in groundwater in different countries of the world (Jain et al., 2013; Ngumba and Gachanja, 2015; Abafe et al., 2018). In the determination of nine antiviral compounds that include five ARVs (ABC, 3TC, NVP, d4T, AZT), four general antivirals OC, penciclovir (PCV), acyclovir (ACY), ribavirin (RBV) and one metabolite, oseltamivir carboxylate (OC- carboxylate) were detected in raw influent, final effluent, and surface water using LC/MS. Apart from RBV, the remaining 8 target antivirals were detected in raw wastewater. Also, the concentrations of NVP, AZT and OC reported in the effluent were similar to that of raw influent, but a substantial decrease in concentrations was detected for ACY, 3TC, and ABC. According to the report, concentrations of the antivirals in rivers and streams were in the ng /L level but OC and its metabolite, OC- carboxylate, were recorded nearly in all the surface water (Prasse et al., 2010; Slater et al., 2010; Picó and Barceló, 2015). The presence of OC- carboxylate was reported in Japanese waterways, the amount was found to be higher at a river downstream of a major WWTP (Prasse et al., 2010). Similarly, ritonavir (RTV), generally administered for ART in France have been reported in the effluent from a hospital (Jain et al., 2013).

Quantitative determination of 12 usually utilized ARV drugs in the South African surface was evaluated in water utilizing an LC-MS/MS protocol. The report uncovered that most of the target ARVs were found in the Roodeplaat Dam, which got discharge from 2 WWTPs. Out of these, NVP, lopinavir (LPV), and AZT frequently occurred and detected up to average concentrations of 0.360, 0.239, and 0.319 µg/L, respectively. However, d4T was reported to have a maximum average pollution of 0.431 µg/L (Wood et al., 2015; Richardson and Kimura, 2016). One investigation in Gauteng, South Africa, showed that the concentrations of EFV and NVP in wastewater raw influent were up to 17.40 and 2.10 µg/L, respectively. The authors documented that chlorination utilized in the WWTPs could dispose of just about half of the ARV medications, and result in contaminated effluent (Schoeman et al., 2015). In France, an investigation was carried out to determine some factors including suspended particles, sterilisation, the dilution that influence the degradation of 53 pharmaceuticals (including 8 ARV compounds) discharged into Garonne River (Aminot et al., 2018). It was found out that biodegradation which was enhanced by the increase in the suspended solid was majorly responsible for the removal of the pharmaceuticals (Aminot et al., 2018).

In a review of the level of pharmaceutical compounds in African water bodies, a detailed outline of different ARV compounds ever reported in the history of surface water and wastewater in African nations were reported by Madikizela et al. (2017). Their study indicated that EFV, 3TC, NVP and AZT, were the most common though at different concentrations in both surface water and wastewater. However, In addition to these compounds, some surface water also contains other seven ARV drugs which include TDF (TAF), zalcitabine (ddC), LPV, ddI, d4T, amandatine (AMT), and rimantadine (RIM) (Madikizela et al., 2017). This review which covers five years (2012-2016), reviewed only twenty-six scientific journals because of the scarcity of scientific articles focussing on the contamination of the African aquatic ecosystem by pharmaceuticals.

Interestingly, the number of research journals on ARV compounds in the aquatic environment in African countries are increasing (Madikizela et al., 2020). Recently, Madikizela et al. (2020) reviewed the occurrence of pharmaceuticals in African water bodies. The work focused on the current status of pharmaceutical compounds in African water bodies, the review based on the scientific publications between 2017 and 2019. From their survey, only researchers in less than twenty per cent of African countries participated in this critical research area of pharmaceuticals monitoring in the aquatic ecosystems. According to the review, NSAIDs and antibiotics are the most commonly studied pharmaceutical groups (Madikizela et al., 2020). The reason is not far fetched, NSAIDs is an over the counter drug that is easily accessible, antibiotics, on the other hand, belongs to one of the most consumed pharmaceutical products to prevent and treat a bacterial infection, most of which may or may not be prescribed in some African countries by health workers (Madikizela et al., 2020). The increasing number of research journals on ARV compounds in the aquatic environment from the African point of view varies from one country to another.

In Kenya, K'oreje et al. (2018) reported that the ARVs that occurred at high concentrations in wastewater samples were 3TC and NVP. In the river water, on the other hand, EFV had the highest detection frequency which was greater than 80 % while 3TC had the highest concentration followed by AZT.

Interestingly, the highest number of publications was from South Africa, this is because the country spends much on ARV drugs for the treatment of HIV infection (Abafe et al., 2018; Ncube et al., 2018; Madikizela et al., 2020). In KwaZulu-Natal province in South Africa for instance, Abafe et al. (2018) investigated thirteen ARVs from three WWTPs. The group reported that all the target ARVs were detected and quantified in at least one of the WWTPs, efavirenz was however detected in all the wastewater samples from the three WWTPs (Abafe et al., 2018). In the environmental monitoring conducted for some groups of emerging contaminants in the Hartbeespoort Dam catchment area in South Africa, ARV compounds (EFV and NVP) belongs to the pharmaceuticals that were detected in all the water samples in Hartbeespoort Dam (Rimayi et al., 2018). Other pharmaceutical including methocarbamol, bromacil and venlafaxine were quantified at lower percentages (Rimayi et al., 2018). The concentrations of the ARVs measured were reported to be at higher concentrations than the commonly used CBZ (Rimayi et al., 2018). The group also reported the highest concentration of EFV in the uMngeni River which was reported to account for the high pollution in the dam.

In another investigation, an LC-ESI-MS/MS was employed for the simultaneous quantification of six commonly used ARVs and their metabolites from wastewater samples from two WWTPs and surface water samples in the Western Cape, South Africa (Mosekiemang et al., 2019). A direct injection method was used for the wastewater samples while a solid phase extraction (SPE) method was first employed to concentrate the ARVs and their metabolites in the surface water before injection into LC-ESI-MS/MS (Mosekiemang et al., 2019). It was reported that all ARV compounds investigated were detected in wastewater samples from the two WWTPs but none was detected in the surface water. The absence of the ARVs in the surface water was attributed to their source being a mountain stream with negligible exposure to human activity (Mosekiemang et al., 2019).

A molecularly imprinted polymer was synthesised and used to selectively extract and concentrate an ARV drug EFV from surface water samples from Pietermaritzburg in KwaZulu-Natal and wastewater samples from Durban in South Africa. Thereafter, the concentrations of EFV in both samples were quantified by HPLC. The group reported that the concentrations ranges are 0.975 – 2.88 μg/L and 2.79 – 120.7 μg/L for the surface water and the wastewater, respectively (Mtolo et al., 2019).

Likewise, the occurrence of PPCPs was investigated in the influent and effluents samples from a WWTP and as well as the receiving river waters in Pretoria, South Africa using liquid chromatography (LC) coupled with high-resolution mass spectrometry (HRMS) Orbitrap™ HRMS. The samples were cleaned-up and preconcentrated with SPE cartridges (Mhuka et al., 2020). The group reported that about 70 compounds were quantified in the wastewater (influents and effluents) while about 60 compounds were detected in river water. Out of these compounds quantified in both influent and effluent samples, ARV compounds were reported as one of the compounds with high detection frequency and at an elevated concentration (> 1 μg/L) out of which efavirenz had the highest concentration (Mhuka et al., 2020). In another development, three ARV compounds emtricitabine (FTC), tenofovir disoproxil (TFD) and EFV were investigated from wastewater samples, surface water and aquatic plant *Eichhornia crassipes* in South Africa using LC-QTOF/MS after a microwave aided extraction and hollow fibre liquid-phase microextraction. The wastewater samples were collected from four different locations including Amanzimtoti, Northern, Umbilo, and Umhlathuzana WWTPs in Durban,

surface water was collected from Hartbeespoort dam near Pretoria and the aquatic plant was collected from Springfield Business Park in Durban and Hartbeespoort dam in Pretoria (Mlunguza et al., 2020). The result showed that the three ARVs investigated were detected in all the sample matrices and EFV was the most abundant compound in all samples (Mlunguza et al., 2020).

In another work in KwaZulu-Natal, South Africa, 3TC, an antiviral compound was one of the seven targetted pharmaceutical compounds investigated and separated from surface water and wastewater samples using LC-ESI-MS (Omotola and Olatunji, 2020). The maximum concentration of 3TC was 33.99 μg/L and its frequency of detection was 80 %. This was higher than the frequency of detections 50%, 50%, and 40% for VMC, CPX, and IVT, respectively and Diclofenac (DCF) which was not detected in the water samples (Omotola and Olatunji, 2020)

Logically, an increase in the infection rate intensifies the production and demand for ARV drugs, which causes an increase in discharge of drugs from urine and faeces into the WWTPs, and finally flows into the surface water (Swati et al., 2011; Jain et al., 2013; Swanepoel et al., 2015; Abafe et al., 2018; Stanley and Kaplan, 2018). Also, HIV still constitutes a therapeutic task and burden universally, and people seek solutions to live long (Swati et al., 2011; Oguntibeju, 2012; Chander et al., 2016; Stanley and Kaplan, 2018). In essence, the best approach to deal with HIV infection could have been the development of an effective vaccine (Naeger et al., 2010; Broder, 2010). Although, to date, there is yet to be any vaccine to prevent HIV/AIDs (Naeger et al., 2010; Broder, 2010). In that case, this makes ARV therapy a life-long one (Naeger et al., 2010; Broder, 2010). And the need to monitor them in water bodies

2.8 Antiviral drugs and their toxic effects in the aquatic ecosystem

Antiviral drugs are categorised as emerging contaminants because of their constant discharge into the environment via several routes such as WWTPs, pharmaceutical industries, and direct dumping where they impose toxicity, not only to the aquatic organisms but also on human (Prasse et al., 2010; Jain et al., 2013; Singer et al., 2014; Chander et al., 2016).

The complete environmental fortune and total (eco)toxicology of many organic contaminants including ARV is not fully known (Vergeynst et al., 2015; Russo et al., 2018; Ncube et al., 2018). However, the repeated antiviral problem on the environment which include bio-recalcitrance and the potential ability to adversely affect aquatic organisms has been a burden to the scientists and environmentalists (Ncube et al., 2018; Russo et al., 2018). The potential (eco)toxicological effect has been a risk to a healthy ecological condition of an aquatic environment, and likely to interfere with the endocrine system of higher organisms and human (Slater et al., 2010; Minguez et al., 2016). Several types of research evaluated the toxicity in fishes and algae predicted that ARV drugs belong to the class of hazardous therapeutic substances (Prasse et al., 2010; Ngumba and Gachanja, 2015; Minguez et al., 2016). Also, some studies assessing the chronic impact of ARVs mixtures have projected their probable negative significant impact (Fatta-kassinos et al., 2011; Picó and Barceló, 2015; Ngumba and Gachanja, 2015).

Concerning the ARVs, one of the problems facing scientists is their presence in the environment at a very low concentration (Prasse et al., 2010; Jain et al., 2013). Initially, their presence could appear insignificant but the foreseen danger could be that the compounds and their TPs/metabolites could accumulate in the aquatic environment, soil, plants and animals, where they could constitute more environmental and health risks, which include interfering with the endocrine system of higher animals which could result in cases like infertility and other reproductive defects (Camacho-mu et al., 2010; Chander et al., 2016). Most ARVs are recalcitrant due to their high molecular weight and chemical structure. Also, their removal in the WWTPs mostly results in the production of metabolites or other TPs, which may be more hazardous and problematic to eliminate during the WWTPs process (Jain et al., 2013; Swanepoel et al., 2015).

In essence, these drugs find their way into the food chain when discharged into the environment through various routes. And this effect could hamper or interfere with normal biological activities in living organisms. The risk quotient (RQ) reported for 3TC, AZT and NVP on algae, daphnia and fish show that each of them affects one organism or the other, and algae were found to be seriously susceptible with the highest RQ of 271.5 and 29.1 for AZT and NVP, respectively (Ngumba and Gachanja, 2015). In essence, such effects classify these compounds as potentially very toxic to aquatic organisms and the order of susceptibility of daphnia, algae and fish to ARV toxicity is algae < fish < daphnia. (Ngumba and Gachanja, 2015; Ncube et al., 2018).

Notwithstanding, professional finding on how to measure exact hazards like microbial resistance and antibiotics, sex hormones, and endocrine disruptors are requested because human health and ecological systems functionality are interrelated (Prasse et al., 2010; Swati et al., 2011; Jain et al., 2013; Sun et al., 2015; Osorio et al., 2016). A report showed that RTV has a toxic effect on the environment (Jain et al., 2013). The negative impact of antiviral on the population of viral existing in the aquatic ecosystem has been reported (Fatta-kassinos et al., 2011; Jain et al., 2013)

It has been postulated that high pollution of the aquatic environment with OC, an anti-influenza drug during influenza epidemics could have a negative impact such as the development of OC-resistance in wildfowl, which are the hosts of influenza viruses (Prasse et al., 2010; Jain et al., 2013; Singer et al., 2014). It has been reported that due to the high usage of OC in Japan, the aquatic organisms there are seriously exposed to OC which have been promoting resistance to OC (Singer et al., 2014). Likewise, morphological examinations of the liver of fish after being subjected to pharmaceutical doses in water revealed enlarged liver which could be linked to the fact that most ARV drugs could lead to liver damage and hepatotoxicity in humans (Schoenfuss et al., 2016).

In that case, this release of antiviral drugs into the aquatic environment is a critical concern, because numerous findings reported the potential disruption of the ecosystem and the development of viral resistance (Jain et al., 2013; Singer et al., 2014). The persistence of OC in the surface waters, due to the activity of OC-degrading bacteria in aquatic environments, could contaminate the natural habitats of waterfowl with OC-resistance influenza viruses (Jain et al., 2013; Singer et al., 2014). In due course, this might lead to the transfer of OC-resistance from avian to human influenza (Swati et al., 2011; Jain et al., 2013). More importantly, the occurrence of these chemicals in the environment is of greater concern because parent

compounds and their metabolites do not exist individually, but simultaneously occur as a complex mixture. In that case, this could lead to unwanted synergistic effects which could be of detrimental effect (Schoeman et al., 2017; Nováková et al., 2018).

2.9 Regulation concerning ARVs

Despite the environmental impact and toxicity of many ECs including ARV compounds, they have not been regulated but they may become candidates for future regulation depending on the level of research and information/orientation on them such as their occurrence, their effects on health and environment (Oller et al., 2011; Osorio et al., 2016; Barbosa et al., 2016; Barbosa et al., 2016; Schoeman et al., 2017). Most pharmaceuticals found in the diverse aquatic environment are characterised as emerging contaminants because they are not yet regulated or in the process of being regulated. They are not covered in the monitoring programs both at international and federal levels (Richardson and Ternes, 2014; Richardson and Kimura, 2016; Glassmeyer et al., 2017; Schoeman et al., 2017). However, their presence in the environment is of potential (eco) toxicological and health risk to wildlife and humans in the long term necessitates the need to include these chemicals in future regulations (Richardson and Kimura, 2016; Osorio et al., 2016). For many years, pharmaceuticals only exist as a draft in EPA CCL, which is the list of contaminants that are not yet subject to any regulation but may require future regulation (Richardson and Kimura, 2016; Barbosa et al., 2016). Recently, the national primary drinking water regulations (NPDWR) established by EPA, which legally enforce primary standards and treatment techniques, included pharmaceutical compounds among the final list of CCL- 4 on November 17, 2016 (Richardson and Kimura, 2016; Glassmeyer et al., 2017).

In South Africa, National Environment Management Waste Acts (Act 59 of 2008) of health and related laws, South Africa passed a law (Table. 2.2) on environmental and tourism in 2007 and 2008, founded on the general purpose of protecting the environment. The law, however, was a general one and not specific for ARVs (Rispel and Moorman, 2010). This means that there is yet to be any specific national law or regulation concerning ARVs contaminants in the aquatic ecosystem, and there is yet to be any limit (Schoeman et al., 2017).

Table 2.2: Health and related laws in South Africa in 2010 (Rispel and Moorman, 2010).

Act	Lead ministry	Brief descriptions
National environment management Waste Acts (Act 59 of 2008) 2	Environmental and Tourism	1) Aims to reform the management of domestic and industrial waste to protect health and the environment 2) Premised on: • Everyone has a constitutional right to an environment that is not harmful to health • The environment must be protected for the benefit of present and future generations • Promoting conservation and securing ecologically sustainable development and use of natural resources while promoting justifiable economic and social development 3) Addresses critical issues of waste disposal on riverbanks the creation of open dump disposal areas the general burning of waste and unauthorized waste processing involving hazardous substances 4) Targets waste minimization by promoting avoidance recovery reuse and recycling and using disposal of as a last resort 5) Sets out measures for the storage collection transportation recovery reuse recycling treatment disposal of waste and greater producer responsibility 6) Outlines the roles and responsibilities of three spheres of government and requirements for licensing of waste management activities

2.10 Need to monitor antivirals in aquatic environmental samples

There were previous studies conducted on the occurrence, fate, and toxicology of different groups of pharmaceuticals which include analgesics, anti-inflammatory, antibiotics, anaesthetics, anti-cancer agents, anticonvulsants, antidepressants, hormones, antihistamines, antihypertensive, lipid-regulators, psychiatric drugs, beta-blockers agents and so on in water bodies (Daughton and Ternes, 1999; Ferrer and Thurman, 2012; Petrie et al., 2014; Matongo et al., 2015; Al Qarni et al., 2016; Young et al., 2018). Jelic et al. (2011) investigated the fate of 43 pharmaceutical compounds in some conventional WWTPs. The researchers targeted some classes of pharmaceuticals, such as antibiotics, analgesics, anti-ulcer agent, psychiatric drugs, anti-inflammatory drugs, antiepileptic drug, ß-blockers, diuretics, antihistamines, cholesterol and lipid regulator.

Matongo et al. (2015) investigated the presence of some pharmaceutical residues in river water, sediment, and WWTP in the KwaZulu-Natal province of South Africa. This particular investigation was centred on the antipyretics, antibiotics, caffeine, antiepileptic and antipsychotic. Not until recently, information on the occurrence or determination of antivirals in the aquatic environment was not popular. The reason behind it could be attributed to the premises that little has been encountered in the past about ARV drugs in the aquatic environment because there are very limited publications in this area (Terzopoulou et al., 2016; Ncube et al., 2018; Russo et al., 2018). Also, this might be on the assumption of Ferrer and Thurman (2012) that about 40 different pharmaceutical compounds are frequently found in

waters polluted by wastewater effluent. This probably might be the reason for many types of research in this study domain were fashioned toward those limited classes of pharmaceuticals. Another possibility could be the opinion or belief that the use of ARVs is limited to a small percentage of the world population, or having the belief that HIV/AIDs is limited to a very small geographical boundary (Ncube et al., 2018; Russo et al., 2018).

Some of the previous studies considered specific groups of pharmaceuticals by monitoring probable elements that may be existing in the environment. Besides, some researchers based their investigation only on some past findings (Kolpin et al., 2002; Keen et al., 2014). Following this, Aminot et al. (2016) stated that carbamazepine, propranolol, diclofenac, naproxen, β-blockers, sotalol, caffeine and atenolol are the commonly studied pharmaceuticals. Other investigations focused on the presence of antiviral compounds' residues in biological matrices such as plasma, urine, hair (Duy et al., 2009; Kareuhanon et al., 2009; Krishna et al., 2012; Shekarchi et al., 2013; Pourfarzib et al., 2015).

Now that the environment and the environmental pollutions are becoming more complex due to more bioactive compounds that were constantly produced and discharged directly or indirectly into the environment (Brack et al., 2012; Chander et al., 2016). It is expedient to enlarge the coast of the environmental analysis, and particularly pharmaceuticals pollutants in the aquatic environment to other new areas like ARVs because antiviral drugs were predicted to be amongst the most hazardous therapeutic classes about their toxicity (Prasse et al., 2010; Terzopoulou, et al., 2016).

2.11 Choice of locations

2.11.1 Khayelitsha

Khayelitsha is a popular township in Western Cape Province (WCP) of South Africa, with a high population density of about 500,000 people is known for its HIV prevalent occurrence (Boulle et al., 2010; Stinson et al., 2017). The rate of prevalent occurrence of HIV infection in the aforesaid township is continually on the increase. The first HIV therapy conducted through a pilot demonstration scheme in May 2001, which only targeted 180 adults, but ended up treating over 7000 -naive adults by the end of 2007 (Boulle et al., 2010; Stinson et al., 2017). The level of HIV-1 recorded in government antenatal clinics in Khayelitsha alone was 24.9% of the total population (Boulle et al., 2010; Stinson et al., 2017). Levy et al. (2005) reported that HIV-positive patients in Khayelitsha were 5.2% of the entire province. Although it is reported that Khayelitsha township has the highest HIV infection in WCP (Stinson et al., 2017) Antenatal records of HIV prevalence in Khayelitsha were recorded as19.3%, 22.0%, 33.0% 32.7%, and 34.3% in the years 2000, 2001, 2006, 2007 and 2012, respectively (Levy et al., 2005; Boulle et al., 2010; Stinson et al., 2014; Stinson et al., 2017) (see Table 2.3).

Table 2.3: Summary of HIV infection rate in Khayelitsha from 1997 to 2012 (Levy et al., 2005; Boulle et al., 2010, Stinson et al., 2014; Stinson et al., 2017; Cornell et al., 2017).

Year	Percentage (%) HIV infection
1997	13.0
1999	20.0
2000	19.3
2001	22.0
2002	24.9
2006	33.0
2007	32.7
2012	34.3

2.11.2 Bellville

Bellville, a city built on the hills of the Tygerberg, situated east of Cape Town within the Cape Peninsula built-up area. It is formerly a village known as Twelfth Mile Stone. Bellville was established by the declaration of 1861 and named after Charles D. Bell, who was the surveyor-general of the Cape (Matsha et al., 2012; Matsha et al., 2013). It is formed in the late 1950s and comprises people of different origins which include Khoisan, 20–36%; African, 21–28%; European, 9–11% and Asian origin. Bellville developed into a town in 1940 and became a city in 1979 (Matsha et al., 2013; Vergotine et al., 2014). According to the 2001 population census, its population was approximately 26, 758 (Matsha et al., 2012; Matsha et al., 2013; Vergotine et al., 2014).

Bellville also contains institutions of higher learning such as The University of the Western Cape, Cape Peninsula University of Technology and Northlink College (Matsha et al., 2013; Vergotine et al., 2014). These educational institutions contain students within the age bracket of the sexually active group (Mturi et al., 2014; Rénette et al., 2015). The report has shown that the majority of these students engage in such activities which can increase the risk of contracting HIV such as non-use (or decreased use) of condoms during sexual activity, increased sexual activity under the influence of alcohol (Rénette et al., 2015). Cape Town International Airport is about 5 miles (8 km) southwest (Matsha et al., 2012; Matsha et al., 2013; Vergotine et al., 2014). Though HIV prevalence in the Western Cape Province is significantly lower compared to the national estimate, the province, however, is one of the few areas where the prevalence of HIV seems to be increasing (Reed et al., 2013; Rénette et al., 2015). This can be related to the high level of substance (alcohol, tobacco and cannabis) use (Pasche and Myers, 2012; Rénette et al., 2015). A report from a national survey showed that the high rates of substance use increase susceptibility to HIV mostly among women because several sexual risk behaviours including non-condom use and engaging in sex as a means of accessing substances or money to purchase substances are common (Reed et al., 2013).

2.12 The choice of ARVs for the study

The choice of ARV for this study is based on:

- The rate of administration of the drugs in the Khayelitsha, and South Africa at large (Marconi et al., 2008; Wood et al., 2015).
- Quantities being consumed (Boulle et al., 2010; Swanepoel et al., 2015).
- Presence and detection frequency of the compounds in the environment as conveyed in the literature (Boulle et al., 2010; Swanepoel et al., 2015; Stinson et al., 2017).

Figure 2.1 shows the chemical structures of the three antiretroviral compounds investigated in this work.

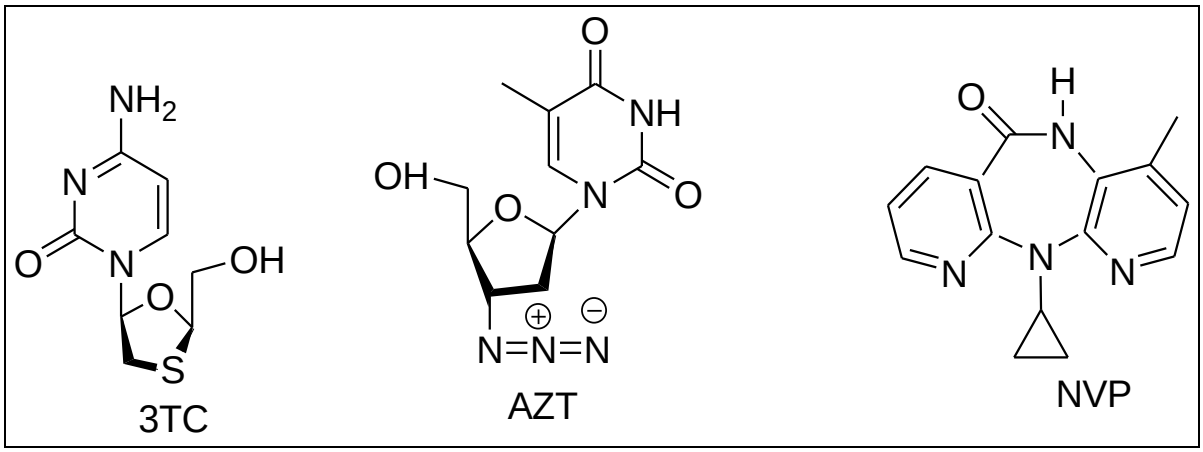

Figure 2.1: Chemical structures of the compound for the research work.

2.13 Analytes for the present research

2.13.1 Lamivudine

3TC, chemically known as is 2, 3-dideoxy-3-thiacytidine is a nucleoside reverse-transcriptase inhibitor (NRTI) with activity against HIV and HBV. It is used in HAART most especially in combination with other ARV, NRTI (AZT) and NNRTI (NVP) (Joshi and Adeyeye, 2012), 2012). In essence, it rapidly absorbed, and simultaneously distributed to extravascular sections. About 55.0% to 85.0% of the drug is eliminated unchanged through the urine, and it takes a terminal-phase half-life of 3 hours. In-vitro investigation of the drug-using cytochrome P 450 (CYP) enzyme proposes that 3TC has little potential to interact metabolically with other drugs (Beach, 1998; Sabo et al., 2002; Krishna et al., 2012). The 3TC molecule has two chiral centres, which are created as the unadulterated 2R, cis (–)-enantiomer. The racemic components out of which 3TC emerge possess antiretroviral activity but are less potent but significantly toxic than the unpolluted (–)-enantiomer. Compared with the (+)-enantiomer, the phosphorylated (–)-enantiomer resists cleavage from emerging RNA/DNA duplexes by cellular 3′-5′ exonucleases, which could contribute to its higher potency as a potent inhibitory activity against HIV reverse transcriptase (Deepali and Elvis, 2010; Shewiyo et al., 2011; Hübner et al., 2015).

2.13.1.1 Mechanism of action of lamivudine

3TC can be regarded as a derivative of dideoxycytidine wherein the 3' methylene group has been substituted with a sulphur atom. Besides, it is the unusual L isomer, unlike other nucleoside derivatives that possess the natural D configuration. After oral administration, 3TC is well absorbed as the DNA of the virus with an average bioavailability of 82.0% in a dosage of 8 mg/kg. It then undergoes phosphorylation in the cell of the patient to its triphosphorylated metabolite, 5-triphosphate. This inhibits HIV reverse transcriptase (RT) because they are incorporated into the DNA of the virus, where it functions as a chain terminator because prevents a complete transformation of virus DNA into a new virus (De Clercq and Li, 2016; Gautam et al., 2018; Zhang et al., 2018).

There is a reduction in bioavailability at a higher dose, which may be attributed to the saturation in the absorption process. The rate of absorption of 3TC is hindered by food consumption, however, the general bioavailability is not affected. More so poor penetration into the cerebrospinal fluid (CSF), with CSF-to serum ratios of 0.06 and 0.11, 2 hours after oral doses of 8 and 20 mg/kg per day, respectively. A total of 36.0% protein can bind to 3TC and is reported to associate with erythrocytes up to 50.0%. 3TC is principally excreted in the urine with 70.0% as an unchanged drug, but only 5% is found in the urine as triphosphate metabolite. (Beach, 1998; Shewiyo et al., 2011; Gautam et al., 2018).

2.13.2 Nevirapine

NVP(11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2b:2′,3′-e]diazepin-6-one) happened to be the first NNRTI approved in 1996 by the US FDA for the suppression of HIV-1 infection (De Clercq and Li, 2016; De Clercq, 2019). It was initially used during pregnancy and breastfeeding as single-dose therapy to prevent mother-to-children transition (PMTCT) of HIV-1 which caused drug resistance and hypersensitivity reactions (Boulle et al., 2010; Wood et al., 2016). The ARV combination containing NVP is considered to be one of the best choices for the treatment of HIV. It has been a major member of NNRTIs class used mainly in combination antiretroviral therapy (cART), with two of NRTIs as a first-line antiretroviral agent in HAART to suppress viral load (Boulle et al., 2010; Krishna et al., 2012). It is used mostly in low resource countries, because of its availability, effectiveness, favourable lipid profile and because it is comparatively cheaper than PIs (Hartmann et al., 2005; Pattarawarapan et al., 2007; Marconi et al., 2008; Boulle et al., 2010; Kavitha et al., 2012; Oluoch, 2016; Stinson et al., 2017).

It is an inducer of cytochrome P 450 isoenzymes CYP3A4 and CYP2B6. It was reported that treatment of HIV with the NVP dose of 200 mg per day for two-week can induce P 450 metabolic autoinduction of CYP3A and CYP2B6 pathways. This also increases NVP superficial clearance and reduction in NVP half-life from about 45 hours to 30 hours (Sabo et al., 2002; Sahoo et al., 2013). Pharmacokinetically, NVP is well absorbed orally (>90.0%) and dispensed well to almost all the tissues; about 60.0% of NVP bound to plasma proteins. It was reported that above 80.0% of NVP intake is biotransformed to hydroxylated metabolites and excreted as glucuronides from urine while only less than 3.0% is excreted as the original compound in the urine. (Sabo et al., 2002; Sahoo et al., 2013).

2.13.2.1 Mechanism of action of nevirapine

NVP is a dipyridodiazepinone inhibitor for the HIV-1 reverse transcriptase-polymerase. It is a highly potent non-competitive NNRTI for its inhibitory action toward deoxyguanosine triphosphate. This occurs through allosteric binding to the binary-RT: template-primer or ternary-RT: template-primer deoxyguanosine triphosphate enzyme complex (Oluoch, 2016; De Clercq, 2019). Instead, it fixes straight to the RT enzyme, which disrupts the catalytic site of the enzyme at the DNA polymerase active site of the enzyme and blocks retroviral reverse transcription via an allosteric mechanism action. This prevents HIV's RNA from translating to DNA, and from being replicated in the other original cell's DNA of the patient (Oguntibeju, 2012; De Clercq and Li, 2016).

2.13.3 Zidovudine

AZT was the first ARV drug approved by the FDA in 1987 for the treatment of HIV-1 infection. It belongs to the NRTI class of antiviral. The drug was first used in mono-therapy against HIV-1, but with the resultant effect of the development of viral resistance (De Clercq and Li, 2016; Stinson et al., 2016). Therapeutic procedures for HIV stipulated that a combination antiretroviral regimen should contain at least two nucleoside analogue reverse transcriptase inhibitors (NRTIs), and one non-nucleoside reverse transcriptase inhibitor (NNRTI) in a fixed-dose combination, which has helped to prevent the development of drug resistance but enhance ART effectiveness (Broder, 2010; Lange and Ananworanich, 2014; Stinson et al., 2017). The combination product that contains AZT and 3TC (both NRTIs) and NVP (NNRTI) in a single pill is one of the common and effective ART (Kavitha et al., 2012; Shamili et al., 2018).

The bioavailability of AZT after oral administration is between 60.0% and 70.0%. Its absorption rate in the system is usually impaired by food with a high level of fat, with 35.0% protein binding. AZT attains cerebrospinal fluid (CSF) levels with about 60.0% of plasma concentration demonstrated to cross the placenta, which yields fetal plasma levels that are approximately equal to the levels in maternal plasma. Because of its low half, AZT metabolises quickly to its glucuronide in the plasma and urine, which about 74.0% of it together with about 14.0% of the unchanged AZT is then excreted in urine (Beach, 1998; De Clercq, 2004; Clercq and Li, 2016).

2.13.3.1 Mechanism of action of zidovudine

When ingested by an HIV patient, AZT is phosphorylated by three deoxyribonucleoside kinases namely thymidylate kinase, nucleoside diphosphate kinase, and thymidine kinase. The triphosphorylated compound formed (AZT triphosphate) targets HIV reverse transcriptase (RT), and interferes with viral reverse transcription, which as well functions as a chain terminator in the RT reaction. This stops the subsequent production of viral DNA, including eventual viral replication. The action of AZT is governed by the azido group in the AZT molecule (Beach, 1998; De Clercq, 2004; Clercq and Li, 2016; De Clercq, 2019).

2.14 Analytical techniques used for the analysis of ARVs

From a study survey, various methods applied were relatively used in determining antiretroviral drugs in one matrix or the other. These methods include capillary electrophoresis/ electrochromatography (CE/CEC), liquid chromatography/tandem mass spectrometry (LC/MS/MS), high-performance liquid chromatography (HPLC), LC-MS, UV-visible spectrophotometric methods, gas chromatography-mass spectrometry(GS-MS) and mass spectrometry (Rezk et al., 2003; Anbazhagan et al., 2005; Sarkar et al., 2006; Sharada et al., 2010; Joshi and Adeyeye, 2012; Rathod et al., 2014; Ngumba and Gachanja, 2015; Schoeman et al., 2015; Schoeman et al., 2017). The choice of methods however depends on some parameters such as the sample matrix and the concentration of the ARVs in the matrix. However, some of the above methods suffer from some drawbacks including being laboratory-based, requiring a very high level of sophisticated equipment that can only be handled by well-trained personnel. Gas chromatography for example requires presenting the gaseous analytes into the instrument (Richardson and Kimura, 2016; Richardson and Ternes, 2018). In this case, many ARVs are not easily volatilised, thereby subjecting the target compounds to excessive heat. This effect may cause heat-labile compounds to break down when heated, thus losing the original target compounds. Similarly, some ARVs have higher boiling points that are beyond the operating temperatures of a GC system, while others require elaborate preparation/cleaning and analyte derivatisation (USEPA, 2009; Nikolaou 2013; Madikizela et al. 2017; Points 2018; Madikizela et al. 2020).

The importance of HPLC in micropollutant analysis was earlier stressed by Picó and Barceló (2015) that the importance of HPLC in the environmental analysis is centred on its ability to couple with very sensitive detectors such as MS detectors. As noted, MS analysis of mixture can be done directly for structural information in the analysis of samples containing organic compounds. However, the use of the LC/MS is much better because as target pharmaceuticals pass through the chromatographic column, they are separated before the eluted components reach the mass spectrometry (MS) for detection (Madikizela et al., 2020).
In Kenya, environmental monitoring was conducted using LC/MS/MS to determine the concentrations of some antibiotics and antiretroviral compounds in different media including wastewater samples, surface water, suspended particulate matters and river sediments (Kairigo et al., 2020). All the targeted compounds were determined and quantified in all the tested media, and a high concentration of the compounds was reported in surface water and sediments (Kairigo et al., 2020). Their finding was an eye-opener that suspended particulate matters are an important phase for reflection concerning the emission of micropollutants from WWTPs (Kairigo et al., 2020). Therefore, all these qualities have made the LC technique more prominent for the analysis of antiretroviral compounds in wastewater.

2.14.1 HPLC method

The use of HPLC and LC-MS-MS based techniques for the evaluation of antiretroviral compounds were reported to be rapid and having a high resolution. They can be tested without analyte derivatisation, which can easily be coupled to MS or any other detective instrument (USEPA (United States Environmental Protection Agency), 2009). This is very vital in identifying the unfamiliar contaminants, particularly environmental contaminants, transformation products (TPs), and disinfection by-products (DBPs) because of good separation that facilitates identification and reduction in matrix interferences.

Among the analytical methods for traditional Chinese medicines (TCMs), HPLC was reported as the most popular one. This is because of its easy operation, wide suitability and high accuracy for the qualitative and quantitative analyses, particularly in the field of quantification and determination of a group of constituents with similar or different structures (Jianga et al., 2010). Furthermore, investigation of 100 pharmaceutical compounds and their metabolites in different water matrices was carried out by liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/Q-TOF-MS) method (Ferrer and Thurman, 2012).

A lot of antiviral determination has been carried out with HPLC related methods (see Table 2.5) Sarkar et al. (2006) employed RP-HPLC and UV methods for the quantitative determination of d4T, NVP and 3TC. The researchers claimed that the methods are, reliable and selective, provides accurate and precise lower limits of detection (LOD) and quantification. Jung et al. (2007), as cited by Jain et al. (2013), equally used LC/MS/MS (for the analysis of some ARV compounds in human blood samples. Hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) was developed and successfully used to evaluate adefovir in human plasma. The method reported being fast, linear with a lower limit of quantification (Jianga et al., 2010). The determination of nine antiviral compounds and one metabolite was determined in France with LC/MS.

An LC/MS method was used to determine an ARV compound, RTV in one of the hospitals effluents in France (Jain et al., 2013). Also, LC-MS/MS was used for the simultaneous quantitation of 3TC, AZT, and NVP in human plasma using ABC as an internal standard (Krishna et al., 2012). Similarly, Tandem mass spectroscopy was used to determine nine antiviral drugs in the surface and wastewater in Germany (Prasse et al., 2010). The group reported that rivers and wastewater (treated and raw) contain ARV drugs, such as ACY, ABC, 3TC, NVP OC, PCV, d4T and AZT (Prasse et al., 2010). Similarly, LC-MS/MS was employed for simultaneous quantification of 12 ARV compounds, which include 3TC, ABC, AZT, d4T, ddC, ddI, EFV, IDV, LPV, NVP, RTV and TAF, in surface water (Wood et al., 2015). The scientists reported that all the 12 ARV compounds investigated were detected in the water samples analysed (Wood et al., 2015).

Similarly, Madikizela et al (2017), in an inclusive summary of ARV compounds that were detected in the wastewater and surface water in African countries were reviewed in consistence with the status of the pharmaceuticals in African water bodies. The researchers further reported that the chromatographic method was the major analytical methods used for the analysis of ARVs (Madikizela et al., 2017). This was further in agreement with the fact that LC is easily coupled with very sensitive detectors such as time-of-flight (TOF) and Orbitrap high-resolution

mass spectrometry (HRMS). This provides appropriate sensitivity, identification and quantification of a large range of micropollutants at detection limits as low as ng/L (Madikizela et al., 2020). Based on this combined qualities of HPLC in the separation of the target analytes such as ARVs in this case, most of the recent investigations conducted in the world and South Africa as discussed earlier make use of LC/MS (Aminot et al., 2018; K'oreje et al., 2018; Abafe et al., 2018; Mosekiemang et al., 2019; Mtolo et al., 2019; Mhuka et al., 2020; Mlunguza et al., 2020; Omotola and Olatunji, 2020; Kairigo et al., 2020). Table 2.5 gives a summary of some ARVs detected by the LC method.

Table 2.4: Antiretroviral drugs detected by liquid chromatography methods

S/No.	Antiretroviral drug	Method of detection	Sample matrix	Reference
1	ABC	Liquid chromatography/tandem mass spectrometry	Surface water and wastewater	(Clark et al., 2006; Abafe et al., 2018)
		High-performance liquid chromatography	Biological samples	(Ferrer et al., 2004; Savaser et al., 2007)
		Liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Water, fish and wastewater	(Swanepoel et al 2015)
2	Atazanavir (ATV)	Liquid chromatography	Biological samples	(Loregian et al., 2006, Verbesselt et al., 2007)
		Liquid chromatography/tandem mass spectrometry	Surface water and wastewater	(Abafe et al., 2018)
		Liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Water, fish and wastewater	(Swanepoel et al 2015)
		UHPLC /High-resolution Orbitrap mass spectrometry	Surface water and wastewater	(K'oreje et al. (2018)
3	AZT	Liquid chromatography/tandem mass spectrometry	Surface water and wastewater	(Abafe et al., 2018; Mosekiemang et al., 2019)
		Liquid chromatography/tandem mass spectrometry	Surface water and wastewater	(Abafe et al., 2018; Kairigo et al., 2020; Muriuki et al., 2020)
		Hydrophilic interaction chromatography coupled with electrospray ionization tandem mass spectrometry	Biological samples	(Yan et al., 2009)
4	d4T	Reverse phase high-liquid chromatography	Pharmaceutical dose	(Anbazhagan et al., 2005)
5	Darunavir (DRV)	Liquid chromatographic–tandem mass spectrometry	Biological samples	(Huang et al., 2004)
6	ddI	Reversed-phase high-performance liquid chromatography	Biological samples	(Verweij van Wissen et al., 2005)
		Liquid chromatography-tandem mass spectrometry	Biological samples	(Clark et al., 2006)
		Liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Water, fish and wastewater	(Swanepoel et al 2015)
		High-performance liquid chromatography	Biological samples	Lakshmi Sailaja et al., 2007
		Liquid chromatography/coupled with high-resolution mass spectrometry	Surface water and wastewater	(Mhuka et al., 2020)
7	EFV	Liquid Chromatography-Mass spectrometry	Surface water and wastewater	(Wood et al., 2015)
		Liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Surface water, aquatic organisms and wastewater	(Swanepoel et al 2015; (Mlunguza et al., 2020)
		UHPLC /High-resolution Orbitrap mass spectrometry	Surface water and wastewater	(K'oreje et al. (2018)
		Liquid chromatography/tandem mass spectrometry	Surface water and wastewater	(Abafe et al., 2018; Mosekiemang et al., 2019; Mtolo et al., 2019

Table 2.4 continued.

S/No.	Antiretroviral drug	Method of detection	Sample matrix	Reference
8	FTC	Liquid chromatography-tandem mass spectrometry	Surface water and wastewater	(Mosekiemang et al., 2019; Mhuka et al., 2020)
		Liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Surface water, aquatic plant and wastewater	(Mlunguza et al., 2020)
9	IDV	Liquid Chromatography-Mass Spectrometry	Biological samples	(Jayewardene et al., 2001)
		High-performance liquid chromatography	Biological samples	(Remmel et al., 2000)
		Ion pair Reversed-Phase High-Performance Liquid Chromatography	Biological samples	(Li et al., 1999)
		Solid-phase extraction and LC-MS	Biological samples	(Rose et al., 2000)
		Liquid chromatography/tandem mass spectrometry	Surface water and wastewater	(Abafe et al., 2018)
10	3TC	Liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Surface water, fish and wastewater	(Swanepoel et al., 2015)
		Liquid Chromatography-tandem mass spectrometry	Biological samples	(Robbins et al., 2007)
		Reverse Phase high-performance liquid chromatography	Pharmaceutical dose, biological samples	(Anbazhagan et al., 2005; Sarkar et al., 2006)
		Liquid Chromatography-Mass Spectrometry	Surface waters and wastewater	(Ngumba et al., 2016)
		UHPLC/high-resolution Orbitrap mass spectrometry	Surface water and wastewater	K'oreje et al. (2018)
		Liquid chromatography/tandem mass spectrometry	Surface water and wastewater	(Abafe et al., 2018; Mosekiemang et al., 2019; Kairigo et al., 2020; Muriuki et al., 2020)
		Liquid chromatography/mass spectrometry	Surface waters and wastewater	(Omotola and Olatunji, 2020)
		Liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Surface water, fish and wastewater	(Swanepoel et al 2015)
		Liquid chromatography/tandem mass spectrometry	Surface water, fish and wastewater	(Abafe et al., 2018)
		Liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Surface water, fish and wastewater	(Swanepoel et al 2015)
11	Lopinavir (LPV)	Liquid chromatography/tandem mass	Surface water and wastewater	(Abafe et al., 2018)
		Liquid chromatography/tandem mass spectrometry	Surface water and wastewater	(Abafe et al., 2018)
12	Maraviroc (MVC)	High-performance liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Surface water, fish and wastewater	(Swanepoel et al., 2015)
13	Nelfinavir (NFV)	UHPLC/coupled to high-resolution Orbitrap mass spectrometry	Surface water and wastewater	(K'oreje et al., 2018)

14	NVP	Liquid chromatography/tandem mass spectrometry	Surface water and wastewater	(Abafe et al., 2018; Mosekiemang et al., 2019; Muriuki et al., 2020)
		Liquid chromatography/tandem mass spectrometry	Wastewater, effluent suspended particulate matters, surface waters and sediments	Kairigo et al., 2020)
		Liquid chromatography/mass spectrometry	Surface water and wastewater	(Bhembe et al., 2020)
15	Raltegravir (RAL)	High-performance liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Surface water, fish and wastewater	(Swanepoel et al 2015)
16	RTV	Liquid chromatography/tandem mass spectrometry	Surface water and wastewater	(Abafe et al., 2018; Mosekiemang et al., 2019)
17		High-performance liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Surface water, fish and wastewater	(Swanepoel et al., 2015)
18	Saquinavir (SQV)	Liquid chromatography/tandem mass spectrometry	Surface water and wastewater	(Abafe et al., 2018)
		High-performance liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Surface water, fish and wastewater	(Swanepoel et al., 2015)
19	TDF	Liquid chromatography Quadrupole time-of-flight – Mass spectrometry	Surface water and wastewater	(Mlunguza et al., 2020)

2.14.2 UV Spectrometry method

Though the LC related methods such as LC-MS, LC-MS/MS, LC- TOF-MS, and LC-HRMS methods, are rapid with a high degree of resolution, they are however considered expensive methods and not readily available in most African countries (Deepali and Elvis 2010; Swati et al. 2011; Jain et al. 2013; Madikizela et al., 2020). As such, they may not be easily met for routine analysis in smaller WWTPs in the rural and smaller towns in African countries due to poverty and under-development because most African countries lack these modern scientific equipment needed for the trace analysis of emerging pollutants like ARVs (Madikizela et al., 2017; Madikizela et al., 2020). It was also recorded that few environmental monitoring conducted from most of these African countries using LC-MS were conducted in several laboratories from the Europian countries including Denmark, Cyprus, France, Belgium (Madikizela et al., 2020). All these could account for the limited research articles in this field of study.

Therefore, despite the general use of LC methods, other researchers used other methods such as UV Spectrophotometry (Anbazhagan et al., 2005; Sarkar et al., 2006; Sharada et al., 2010; Rathod et al., 2014) in pharmaceutical formulation, GC (Schoeman et al., 2017; Damkjaer et al., 2018), the ARVs of interest were derivatised before the sample injections. Montaseri et al.(2019), synthesised a quantum dot-ligand system and used them for the fluorescence detection of acetaminophen in tap and river water samples. Swati et al. (2011) reported that UV

spectrometry suffered high detection limits, low sensitivity, complex procedures for the preparation of samples or standard solutions and tedious experimental conditions. Notwithstanding, the method has been successfully used many times in the determination of ARVs in drug pharmaceutical formulation in dosage forms and reported to be simple, precise, accurate, rapid, selective and linear within some concentration limit (Anbazhagan et al., 2005; Sarkar et al., 2006; Rathod et al. 2014; Kamangu et al., 2017). The solubility of NVP in the water at neutral pH was reported to be relatively low (approximately 1 mg/ml). It is, however, more soluble in methanol and readily soluble in dilute (0.01M) HCl with pH below 3 (Anbazhagan et al., 2005; Stieger et al., 2009). Also, when NVP is dissolved in either water or methanol, it shows maximum absorbance at 283 nm but, it exhibits a shift of absorbance maximum to 315 nm in dilute HCl (Anbazhagan et al., 2005; Sarkar et al., 2006; Stieger et al., 2009).

Relative to the above illustrations, UV Visible Spectrophotometer was used to evaluate the solubility and dissolution rates of some ARV compounds at different pH, the study revealed that 3TC and AZT exhibit greater and better solubility of 276.08 and 28.90 mg/mL, respectively in 0.01 M HCl (Prakash et al., 2008). This peak-shift and better solubility as a result of solvent medium were found to be very useful. Also, they are of great importance in the identification and quantification of ARVs using UV Spectrophotometry. Most especially, in the absence of more sophisticated equipment (Stieger et al., 2009). Several types of research have employed this approach to analyse ARVs, particularly, in the tablet (Anbazhagan et al., 2005). Simultaneous quantification of d4T, 3TC and NVP in a drug formulation was carried out with UV spectroscopy. It was reported that the excipients present in various brands of the tablets did not interfere with the method. And the method can be used for routine simultaneous quantitative estimation (Anbazhagan et al., 2005). TAF, an antiviral compound was also determined using UV spectrometry (Swati et al., 2011).

Similarly, a UV spectrophotometric method was used for the quantitative determination of three antiretroviral drugs 3TC, d4T, and NVP (Sarkar et al., 2006). The work showed that the calibration curves for the entire analytes were linear at the working concentration range with a very good $R^2 > 0.999$ (Sarkar et al., 2006). The method was described as simple, selective, consistent, accurate, and precise with lower limits of detection and quantification. Also, it saves time and appropriates for regular quantitative evaluation of pharmaceuticals in dosage forms (Sarkar et al., 2006). Also, the solubility and dissolution rate of three ARVs- 3TC, d4T and NVP in four different pH media using UV Visible spectrophotometer, the result showed that all the drugs are soluble (Prakash et al., 2008). Recently, Kamangu et al. (2017) reported the application of a UV-visible spectrophotometer to evaluate the quality of six ARV drugs, including ABC, EFV, LPV boosted by RTV, d4T, NVP and AZT employed in the Kinshasa region of the Democratic Republic of Congo (DRC) (Kamangu et al. 2017). The researchers reported that the protocol demonstrated a good correlation between the introduced/induced concentration and the analytical response signified by the absorbance readings. Also, the calibration lines were validated, and the R^2 values for all the compounds were found to be approximately 0.99. Concurrently, the calibration lines were accurately used to determine the concentrations of the compounds in the samples (Kamangu et al. 2017).

A recent investigation reported the use of the UV method for the determination of naphthalene and phenanthrene concentrations in aquatic media (Wu et al., 2020) and for the quantification of sulfonamides, antibacterial agents that is mostly used in both veterinary and human medicine in seawater sample (Errayess et al., 2017). Also recently, the UV method was used to determine the concentrations of phenanthrene and its monohydroxy derivatives in both simulated seawater and ultrapure water samples (Bao et al., 2020). The UV visible spectrometry method was reported to be simple, sensitive, accurate, precise, cost-effective, and without any need for drastic experimental conditions and also effective for the routine quantification of sulfonamides in drinking water, seawater and pharmaceutical and veterinary formulations (Errayess et al., 2017). The application of the UV method was based on its availability, low cost and ability to quantitatively determine an individual compound (Errayess et al., 2017; Bao et al., 2020).

To the best of our knowledge very limited studies have been done to determine the residues of ARV compounds in wastewater samples from Cape Town metropole; most especially, to reduce their cost implications in the analysis by using UV-visible spectrophotometry. Since UV method has been used to determine different ARVs compounds (including 3TC, AZT and NVP) in pharmaceutical formulations and described to be simple, precise, accurate, and cost-effective. The need for the use of UV spectrophotometry for routine analysis of emerging pollutants such as pharmaceuticals has been considered very important because of its great benefits including simple implementation, speed, accuracy, cost-effectiveness, availability and low reagent usage (Errayess et al., 2017). Moreover, different research groups have reported that comparatively, the results obtained from the UV and the HPLC methods were similar, which means that the UV method is vigorous and promising (Errayess et al., 2017; Bao et al., 2020).

In that respect, this study aims at adopting the UV-visible spectrophotometric protocol for the determination of the residues of 3TC, AZT and NVP residues in the effluent from two WWTPs in Cape Town, South Africa. This could probably be an eye-opener especially to the African countries where more sophisticated equipment is not available. Therefore, the implementation of a simple and accurate UV-vis spectroscopic method that can be used to monitor ARVs in wastewater samples and particularly, in the areas where HIV/AIDs pandemic is predominant.

Table 2.5: Antiretroviral drugs detected by UV visible spectrophotometer methods

S/No.	Anti-retroviral drug	Method of detection	Sample matrix	References
1.		UV Spectrophotometry	Pharmaceutical dosage form	(Kamangu et al., 2017)
2.	Adefovir dipivoxil (ADV)	UV Spectrophotometry	Pharmaceutical dosage form	(Ahmed et al., 2008)
3.	ddI	UV Spectrophotometer	Pharmaceutical dosage form	(Sangshetti et al., 2007)
4.	EFV	UV Spectrophotometry	Pharmaceutical dosage form	(Kamangu et al., 2017)
		UV Spectrophotometry	Pharmaceutical dosage form	(Sharada et al., 2010)
5.	IDV	UV Spectrophotometry	Pharmaceutical dosage form	(Sarma and Sastri, 2002; Erk, 2004)
6.	3TC	UV Spectrophotometry	Pharmaceutical dosage form	(Sarkar et al., 2006)
		UV Spectrophotometry	Pharmaceutical dosage form	(Anbazhagan et al., 2005)
		UV Spectrophotometry	Pharmaceutical dosage form	(Prakash et al., 2008)
7.	NVP	UV Spectrophotometry	Pharmaceutical dosage form	(Anbazhagan et al., 2005)
		UV Spectrophotometry	Pharmaceutical dosage form	(Sharada et al., 2010)
		UV Spectrophotometry by difference	Pharmaceutical dosage form	(Sahoo et al., 2013
		UV Spectrophotometry	Pharmaceutical dosage form	(Kamangu et al., 2017)
8.	d4T`	UV Spectrophotometry	Pharmaceutical dosage form	(Anbazhagan et al., 2005)
		UV Spectrophotometry	Pharmaceutical dosage form	(Basaviah et al., 2007)
		UV Spectrophotometry	Pharmaceutical dosage form	(Prakash et al., 2008)
9.	AZT	UV Spectrophotometry	Pharmaceutical dosage form	(Prakash et al., 2008)
		UV Spectrophotometry	Pharmaceutical dosage form	(Sharada et al., 2010)
		UV Spectrophotometry	Pharmaceutical dosage form	(Kamangu et al., 2017)
10.	Indinavir sulphate	UV Spectrophotometry	Pharmaceutical dosage form	(Rathod et al., 2014)

2.15 Water treatment technologies

There are diverse treatment technologies developed to remove contaminants in water and wastewater. This process depends on different factors which include the inherent physicochemical nature of the contaminants, nature of the treatment process involved, level of purification desired, intended use, and so on (Herrmann, 2010; Chander et al., 2016). There exist about a hundred (100) differing approaches that have been applied at one time or another to ensure different levels of purified water (Oputu, 2016). These methods include among other basic and simple physical treatment procedures such as flocculation, sedimentation, filtration, and clarifiers to more specialised procedures such as the American Petroleum Institute's (API) oil-water separator, chemical methods (Fenton process), and membrane processes (membrane bioreactors and membrane distillation). Others include techniques based on advanced oxidation using UV (AOP) and those based on nanotechnology (Oputu, 2016).

As a result of the multiplicity in water treatment technologies, there exist various opinions and approaches to the classification of treatment technologies. Some authors classified the techniques based on the principles of operation, which includes physical, chemical, biological, and so on. Others prefer to classify them based on the level of efficiency or the class of water

they treat (Oputu et al., 2015). Dore (2015) classified treatment technologies into six classes based on the nature of contaminant removed by the process as presented in Table 2.6. The approach categorises water treatments technologies, but not the final water quality or water source.

Class 1 treatment technologies are a minimum treatment technology that primarily involves disinfection with chlorine to remove pathogens. This may be appropriate for some underground water that may not contain suspended solid particles.

Class 2 involves physical processes which include flocculation, sedimentation and filtration to remove the suspended solid particles, then follow by disinfection by the use of chlorine for the removal of pathogens. This method is limited because it does not remove some protozoa for instance Cryptosporidium family and Giardia lamblia. Also, it results in the formation of DBPs like the trihalomethanes (THMs), nitrosamines, chloramines, and halo acetic acids (HAAs) that cause dermatitis (Postigo and Richardson, 2014; Dore, 2015).

On the other hand, class 3 makes use of ultraviolet light for photodegradation to eliminate chlorinated phenols, chlorinated aromatic, hydrocarbons, aromatic hydrocarbons, and many pesticides through photolysis. In the photolysis system, a photosensitizer substance absorbs the light wavelength and transmits the energy to the contaminants at the light wavelength of the solar energy wavelength, where contaminants will not naturally absorb radiation. The commonest photosensitisers in water include nitrate and a generic compound, humic acids which can eliminate protozoa in water (Oller et al., 2011; Dore, 2015).

Also, Class 4 uses ozone oxidation with greater oxidising power (2.1 eV) than chlorine to selectively oxidise many recalcitrant organics (e.g. phenols, pesticides and some detergents) and inorganic species in an aqueous solution. It removes pathogens, suspended solids, and protozoa; and it was first used in Nice, France in 1906. Ozone has been extensively applied in water treatment long ago because it prevents the formation of DBPs in water. However, its application is prevented by such limitations as production cost and lack of residual disinfection, as found in chlorine disinfection. Nevertheless, there are increasing interests in ozonation technology, because of the health and environmental concern for DBPs in water (Belgiorno et al., 2007; Herrmann, 2010; Vergeynst et al., 2015; Dore, 2015).

Table 2.6: Classification of water treatment technologies (Dore, 2015).

Class	Typical treatment technology	Contaminants removed
Class 1	Chlorination	Water disinfection; removal of most pathogens
Class 2	High rate clarification and filtration	Disinfection plus suspended solid removal
Class 3	Ultraviolet	Class 2 plus removal of Protozoa
Class 4	Ozonation	Class 3 plus removal of dissolved organic matter
Class 5a	Activated carbon powered/granular carbon	Class 3 plus removal of geosmin and other taste and odour compounds, DBPs, Volatile Organic Compounds, Endocrine Disruptors, micro-pollutants, pesticides, pharmaceuticals, and personal care products
Class 5b	Advanced oxidation process	Class 5a plus higher efficacy of the removal of chemicals and other micro-pollutants (e.g. pesticides, pharmaceuticals, taste and odour concerns
Class 6	Reverse Osmosis or distillation	Class 5 plus removal of salinity

According to Dore (2015) classification, class 5 treatment technology is subdivided into two, which are listed below as:

- The use of activated carbon, powder or granular, and
- Advanced oxidation processes (AOPs).

These two forms of treatment technologies can efficiently remove odour, taste, volatile and recalcitrant organic compounds including pesticides, PPCPs and DBPs.

In class 5a, the use of granular activated carbon (GAC) has been applied in a countless large number of WWTPs in the World. It is efficient and versatile for the removal of all sorts of contaminants from water and wastewater. Moreover, it is limited since it only conveys contaminants from one water to the carbon adsorbent. Also, the comparatively high cost of GAC has limited its wide application, mostly, in resource-limited countries (Herrmann, 2010; Llorca et al., 2015; Dore, 2015).

In class 5b, an advanced oxidation process (AOP), which can be referred to as photocatalysis, is improved classical ozonation. In a broad sense, AOP is the aqueous stage oxidation approaches that mostly depend on the intermediate compound of highly reactive species (hydroxyl radicals). And in the process, the target pollutant was destroyed. Meanwhile, along the process, a metal oxide semiconductor is immersed in water and irradiated by near UV light ($\lambda < 385$ nm) to form free hydroxyl (•OH) radicals. The most commonly and widely used catalyst is TiO2 as a result of its photo-stability, harmless, relatively cheap and, water insolubility under most environmental conditions. It was reported that oxidation by TiO2 photocatalysis is relatively unaffected at a proximate neutral pH (Jiang et al., 2010). But the cost of using microfiltration membranes for the separation and recycling of the photocatalytic products, from the slurry suspension, however, increases as the concentration of the catalyst increases (Jiang et al., 2010). Correspondingly, other different catalysts like Fe II and Fe III are used together with ozone with or without UV–vis radiation (Belgiorno et al., 2007; Herrmann, 2010; Fatta-kassinos et al., 2011; Gros et al., 2013).

During the system, aqueous ozone is transformed into a radical such as the hydroxyl radical (HO•), with an oxidising power (2.8 eV) greater than the power exhibited by the ozone molecule. At times, the transformation is enhanced by other materials which include activated alumina, carbon, zeolites or carbon nanotubes, (Llorca et al., 2015). The cost of using UV-vis radiation makes the method an expensive treatment technology (Braham and Harris, 2009; Prieto-rodriguez et al., 2012). The use of solar photocatalytic was employed as a cheaper approach economically to eliminate the cost of UV lamps. It was established that the catalytic and photocatalytic ozonation methods involving ROS that destroys organic compounds are considered as efficient approaches to eliminate emerging contaminants from water (Sun, et al., 2015; Yang et al., 2016).

Though, different photochemical systems including UV/O_3, UV/H_2O_2, UV/TiO_2, UV/Cl_2, UV/$CKMnO_4$ and several chemical oxidation processes such as O_3, O_3/H_2O_2, H_2O_2/Fe^{2+}, and H_2O_2/Fe^{2+}, the production of HO• which is highly reactive and non-selective for indiscriminate degradation of contaminants and persistent organic compounds in water and wastewater is common to them. This makes AOPs preferred over single ozonation for water purification and to avoid the accumulation of refractory intermediates (Herrmann, 2010; Chávez et al., 2016).

Also, other research works have identified other radicals, such as sulphate and azide radical for the removal of organic pollutants in wastewater radical (Sun, et al., 2015; Yang et al., 2016).

The class 6 techniques, which include reverse osmosis, nano-filtration and distillation, remove all target compounds of Class 5 techniques and salinity from water. Regardless of the method's perfection, there is a great drawback when applying the method at a large scale in term of operational cost (Dore, 2015).

2.16 Advances in water purification by oxidation

The early use of first-generation chemical oxidants as disinfectants in water treatment has been in existence for a century. Chemical oxidants that had been applied at one time or the other during water or wastewater treatment to lessen organic and inorganic contaminants present in the water include chlorine, ozone, chlorine dioxide, chloramines, ferrate (VI), Photo- Fenton, permanganate, bisulphate activated permanganate (Hu et al., 2011; Sun et al., 2015; Ling et al., 2016; Von Gunten, 2018). The addition of free chlorine in the form of chlorine gas (Cl2) or sodium hypochlorite (NaOCl) to water forms hypochlorous acid (HOCl) and hypochlorite (OCl^-). Chlorine reacts with double bonds, neutral amines and activated aromatic systems (Lee and von Gunten, 2010b; Von Gunten, 2018). The use of "chlorine" as free available chlorine (FAC) has several benefits over other disinfectants; because it is cheaper, it is effective against many waterborne pathogens and offers residual disinfectant during the delivery system (Remucal and Manley, 2016; Von Gunten, 2018).

A few years later, there were reports on the formation of DBPs like chloroform and other halogenated (trihalomethanes (THMs)) DBPs during chlorination. These DBPs are produced when the chemical oxidants react with water or wastewater matrices including the dissolved organic matter (DOM), iodide and bromide (Sun et al., 2015; Richardson and Kimura, 2016; Li and Mitch, 2018). And cases of an epidemic of bladder cancer and miscarriages were detected. However, the causal factors of these cases were traced to the use of chlorine in water treatment. Inexplicably, this effect brought a shift from the full-scale use of chlorine as a disinfectant by some countries (Deborde and Gunten, 2008). In some nations, a compromise was made between disinfection and the DBP problem in water treatment (Von Gunten, 2018; Li and Mitch, 2018). In most cases, disinfestation takes the lead (Von Gunten, 2018; Li and Mitch, 2018).

In other to circumvent the DBPs problems, other disinfectants like chloramine and chlorine dioxide gained popularity. This resulted in a substantial reduction in THM formation. Besides, other DBPs such as N-nitrosodimethylamine (NDMA) or chlorite/chlorate were formed. This arouses great interest in how to eliminate pollutants and other natural organic matter (NOM) in water and wastewater (Gordon et al., 1990; Mitch, 2003; Richardson et al., 2007). This paves way for the oxidation process for the transformation of hazardous pollutants into non-hazardous or less toxic ones (Lee and von Gunten, 2010; Hübner et al., 2015).

Out of these oxidants, the use of ozone was more common, it was originally applied as a disinfectant for the production of drinking water. Observably, the result of using ozone in removing the taste, odour, colour and many micropollutants was successfully (Lee and von Gunten, 2010; Sun, et al., 2015). Ozone is not stable in water; it decomposes to form O3 and OH radicals (HO•), which are considered as strong oxidants in water. Disinfection occurs

mainly by ozone, but oxidation processes may occur through both ozone and HO• radicals. Ozone is a selective oxidant; it is widely applied in water treatment, because of its excessive oxidising power (2.1 eV), which enables it to selectively oxidise many organic and inorganic species in an aqueous solution. It specifically attacks the activated aromatic systems, the double bonds, and the neutral amines at a much greater rate than chlorine. On the other hand, HO• reacts randomly with many dissolved compounds in the water matrix and faster (Von Gunten, 2003; Huber et al., 2005). Ozone as an oxidant enhances the coagulation and biodegradation of dominant organic matters (DOM). During ozonation process, ozone reacted with bromine to form DBPs such as bromate, with a potential worse health effect than their chlorinated counterparts and oxygen-rich compounds such as carboxylic acids, aldehydes and ketones (Von Gunten, 2003; Hammes et al., 2007; Herrmann, 2010; Ramseier et al., 2011).

The use of chlorine dioxide came into existence, chlorine dioxide on its part reacts with tertiary amines and activated aromatic compounds. Its reactivity is higher than chlorine but lower than ozone. The application of chlorine dioxide is usually applied at optimal or regulated doses to avoid the build-up of oxidation by-products chlorite and chlorate (Guan et al., 2010). Another oxidant used in the oxidation process is ferrate. Ferrate (VI) is effective for the disinfection of microorganisms, it partially degrades and/or oxidise the organic and inorganic contaminants and get rid of the suspended and colloidal materials because it can as well produce ferric coagulating species (Ramseier et al., 2011; Odabasi, 2016).

Another oxidation process made use of was photocatalysis. This is a chemical oxidation process, where a metal oxide semiconductor submerges in water and irradiated with near UV light ($\lambda <$ 385 nm) results in the production of HO•. TiO_2 is the most widely used catalyst (Belgiorno, et al., 2007; Rodríguez et al., 2012). Also, other different catalysts such as Fe II and Fe III are used, together with ozone in the presence of UV–vis radiation (Herrmann, 2010). However, this method is an expensive treatment technology due to the cost of UV-visible radiation, a large amount of catalyst required at the optimum operating performance, and the cost of removing and recycling the photocatalytic materials from the slurry suspension using microfiltration membranes (Braham and Harris, 2009; Jiang et al., 2010; Oller et al., 2011). Solar detoxification by photo-Fenton was described as a more efficient process than TiO2 photocatalysis (Belgiorno et al., 2007; Oller et al., 2011; Kanakaraju et al., 2018). It functions at acidic pH below 4 to prevent iron oxide precipitation. Also, its limitations include:

- High cost involved in the adjustment of pH of a large quantity of water from near neutral to acidic and back to neutral (Prieto-rodriguez et al., 2012; Barbosa et al., 2016), and
- The elevation of water salinity due to the use of a large number of chemicals (Prieto-rodriguez et al., 2012; Barbosa et al., 2016).

2.17 Removal of antiviral compounds

The main route of pharmaceutical compounds, including antiviral compounds, into the environment is primarily through an aquatic medium, while other common means include food-chain because of their low volatility (Nikolaou, 2013; Gogoi et al., 2018).

Moreover, antiviral compounds were expected to react or behave in a similar way to the antibiotics. This presumes that the removal processes used for antibiotics could be successfully employed for antiviral drugs. Although, several methods including adsorption, anaerobic

biological processes, oxidation using chlorination, H_2O_2-UV, ozonation, membrane separation techniques and electrochemical methods are proposed and obtainable in the literature for the elimination of antibiotics from wastewater. Their effectiveness, however, depends on other factors which include the nature of matrices in the influent, prevailing weather conditions, design and operation of WWTPs, the concentration of the pharmaceuticals in the sewage and the physicochemical nature of the compounds (Jain et al., 2013; Chander et al., 2016).

Biological removal of antiviral compounds is considered to be economical. Nonetheless, the survey conducted in many previous types of research on the elimination of antiviral compounds in wastewater pointed out that, only a limited number of ARVs could be eliminated with biodegradation without any guarantee of total mineralisation. In many instances, the TPs could be more persistent than the parent compound. Typical examples are carboxy-ACY and PCVTP 251, the TPss of ACY and PCV, respectively which could cause major deviations in the normal metabolism of the living organism were reported to be persistent with biological degradation (Prasse et al., 2011; Jain et al., 2013).

2.18 Proposed methods for the removal/degradation of antiviral

As earlier reported, most pharmaceutical compounds such as antiviral compounds escaped conventional wastewater or drinking water treatment technologies (Jain et al., 2013; Wood et al., 2015). Similarly, some of them are still not effectively removed with unconventional methods such as UV photolysis and oxidation by chlorine, ozone and adsorption by GAC (Jain et al., 2013; Chávez et al., 2016; Guo et al., 2017; Chan et al., 2018).

In essence, this indicates that a single process is not sufficient for degradation, including the total mineralisation of recalcitrant chemicals such as ARVs compounds. This demonstrates the need for an improved removal process. Explicably, according to previous studies, it was projected that the integration of two or more processes is more beneficial in achieving better efficiency, as well as minimising the development of TPs (Xiong et al., 2010; Jain et al., 2013; Guo et al., 2017).

Different methods have been proposed and considerably studied, which can be applied individually or used in combination with other processes for the removal/degradation of antiviral compounds. These methods include:

i. Advanced oxidation process (AOPs),
ii. Membrane separation processes: ultrafiltration, electrodialysis, reverse osmosis, Low-pressure membrane filtration, such as microfiltration (MF) and ultrafiltration (UF) (Hu et al., 2011; Sun et al., 2015; Boix et al., 2016; Chan et al., 2018), and
iii. Electrochemical methods such as electrooxidation, electrocoagulation, and electroflotation (Jain et al., 2013; Moreira et al., 2017).

2.18.1 Advanced oxidation processes (AOPs)

AOPs are efficient technologies for the elimination of refractory pollutant such as pharmaceutical compounds (Jain et al., 2013; Gadipelly et al., 2014; Rana et al., 2017). It operates in two stages, which are:

i. formation of strong oxidants, and

ii. the reaction of these oxidants with organic contaminants in water.

The generated HO• promotes organic matter oxidation at high reaction rates. This is important because non-selective radical reacts indiscriminately with several organic compounds, which includes organic pollutants and biomolecules such as proteins and nucleic acids for their removal from water and wastewater (Jiang et al., 2010; Peng et al., 2014; Vergeynst et al., 2015).

The conventional AOPs, which include UV/O_3, H_2O_2/O_3, UV/H_2O_2 and Fenton-based methods largely rely on the production of HO•. The generated HO• ensures high reactivity, which promotes its wider application in the elimination of several types of persistent compounds (Jain et al., 2013; Remucal and Manley, 2016). Many previous works have implemented these methods to eliminate different compounds including pharmaceutical compounds such as antibiotics, carbamazepine, ibuprofen, clenbuterol, caffeine, gemfibrozil, metoprolol, naproxen propranolol, bisphenol A, primidone and so on (Sun et al., 2015; Remucal and Manley, 2016; Chan et al., 2018).

The setback encountered in the major dependence on high reactivity of HO• revealed that HO• is reactive with other water matrices such as DOM, alkalinity (bicarbonate), etc. These compounds scavenge HO• and thereby limiting the HO• effectiveness toward the target compounds (Rosario-ortiz et al., 2010; Lee and von Gunten, 2010; Hübner et al., 2015; Sun et al., 2016). Moreover, the process requires an enlarged physical size of the plant, which is considered to can be highly capital intensive in operation due to high energy demand and high amount of chemical-cost to quench the residual oxidants (Remucal and Manley, 2016; Guo et al., 2017).

2.18.1.1 UV/chlorine process

The UV/chlorine method is an evolving AOP for the elimination of micropollutants (Guo et al., 2017; Wang et al., 2019). Reports have shown that the process operates within pH 4- 8 typical of natural water, and without amines and ammonia present in the water, the aqueous chlorine exists as an equilibrium mixture of hypochlorite (OCl^-) and its conjugate hypochlorous acid (HOCl) at pKa 7.5 (Chan et al., 2012; Chávez et al., 2016). During this process, numerous mechanisms and reactions occur that are divided into three stages are illustrated in Equations 2.1 – 2.6 (Sun et al., 2015; Guo et al., 2017; Wang et al., 2019).

i. direct reaction of HOCl and OCl^-,
ii. production of primary oxidants (HO• and Cl •) by UV irradiation photolysis

$HOCl/OCl^- \xrightarrow{h\nu} HO^{\cdot}/O^{\cdot} + Cl^{\cdot}$ ..……...Equation 2.1

iii. production of secondary reactive species $Cl_2^{\bullet-}$, ClO•

$Cl^{\bullet} + Cl^- \underset{\leftrightarrow k2}{\overset{h\nu\ k1}{}} Cl_2^{\bullet-}$ ……Equation 0.1

($k_1 = 6.5\times 10^9\ M^{-1}s^{-1}$), ($k_2 = 1.1\times 10^5\ M^{-1}s^{-1}$)

$HO^{\bullet} + HOCl \rightarrow ClO^{\bullet} + H_2O$ ……..Equation 0.2

($k= 2.0\times10^9\ M^{-1}s^{-1}$)

$HO^{\bullet} + OCl^- \rightarrow ClO^{\bullet} + OH^-$ ……Equation 0.3

($k= 8.8\times10^9\ M^{-1}s^{-1}$)

$Cl\bullet + HOCl \rightarrow ClO^{\bullet} + H^+ + Cl^-$ ……Equation 0.4

($k= 3.0\times10^9\ M^{-1}s^{-1}$)

$Cl^{\bullet} + OCl- \rightarrow ClO^{\bullet} + Cl^-$ ..……Equation 0.5

($k= 8.2\times10^9\ M^{-1}s^{-1}$) (Guo et al., 2017)

HO• oxidises a wide range of compounds, but other reactive species called reactive chlorine species (RCS), (Cl•, $Cl_2^{\bullet-}$ and ClO•) are selective in their reactions, and it enhances better performance (Wang et al., 2015; Fang et al., 2017). Also, Cl• possesses advanced reactivity than HO• toward some specific organic compounds, such as phenol, benzoic acid and chlorobenzene (Mártire et al., 2001); $Cl_2^{\bullet-}$ targets hydroxy and methoxy substituents of aromatics and olefinic compounds attached to amino groups, whereas ClO• attacks aromatics having methoxy groups (Chan et al., 2012). Degradation of trimethoprim, ibuprofen and carbamazepine were accomplished with RCS, (HO•) and both (HO• and Cl•), respectively (Guo et al., 2017). Since most of these functional groups exist in pharmaceuticals (ARVs) and considering the reactivities of other RCS formed in chlorine photolysis; then, there is a potential for the elimination of our target compounds in wastewater.

2.18.1.2 Benefits of chlorine photolysis

Chlorine photolysis exhibited some key advantages such as its application in both disinfestation and degradation of persistent organic contaminants (ARVs) (Chan et al., 2012; Hübner et al., 2015). Other key advantages of chlorine photolysis include its use in reducing concentrations of DBPs, provision of the residual effect of chlorine for disinfection process (Chuang et al., 2017), and its great potential to transforming the existing chlorine-based water treatment facilities (such as wastewater treatment plants, swimming pools, and so on) into AOPs for enhanced degradation of micropollutants (Wang et al., 2015).

Many studies utilised low pressure-UV (LP UV) monochromatic radiation at 254 nm or medium pressure-UV (MP UV) polychromatic radiation having wavelengths ranging from 200–400 nm. Few investigations made used solar radiation to initiate the UV-based AOPs (Chan et al., 2012; Hübner et al., 2015; Chan et al., 2018; Remucal and Manley, 2016). The studies reported that sunlight can successfully initiate the UV/chlorine AOP in place of UV lamps, which could be high cost demanding (Chan et al., 2012; Hübner et al., 2015; Chan et al., 2018; Remucal and Manley, 2016). The method is reported to be effective for the removal of the diversity of micropollutants. These include trichloroethylene, atrazine, benzoic acid, taste and odour compounds such as 2-methylisoborneol and geosmin (Wang et al., 2015; Fang et al., 2017). Some pharmaceutical compounds and hormones, including nitroimidazoles, trimethoprim, sulfamethoxazole, ibuprofen, carbamazepine, diclofenac, metoprolol and 17a-

ethinylestradiol have been degraded by UV/Cl_2 methods; and carbamazepine, sulfamethoxazole, and trimethoprim are better degraded by UV/chlorine than UV/H_2O_2 (Guo et al., 2017).

Likewise, in aqueous media with minimal concentrations of dissolved organic carbon (DOC) at very low acidic pH values (i.e., pH ≤ 6.5), UV/chlorine is more effective at producing HO• for the degradation of target contaminants than UV/H_2O_2 (Hübner et al., 2015; Wang et al., 2015). Substantially, the UV/chlorine method has been described as more effective than the popular UV/H_2O_2 process in real water treatment. Chlorine generates higher HO• with more efficient radical production. Chlorine species (HOCl and OCl^-) absorb a larger amount of light; it generates higher quantum yields than H_2O_2, and lower chemical and energy cost are required (Hübner et al., 2015; Chan et al., 2018). Recently, many municipal WWTPs using AOPs are changing from the UV/H_2O_2 to the UV/chlorine process (Guo et al., 2017; Chuang et al., 2017).

2.18.1.3 Sunlight/$KMnO_4$ process

Permanganate was reported to react with double bonds by donating oxygen, it can as well remove hydride ions electrons, or hydrogen atoms (Tratnyek, 2006; Guan et al., 2010; Hu et al., 2011). Permanganate enhances coagulation and removal of micropollutants. A small amount of permanganate was demonstrated to enhance the exclusion of tannic acid more than coagulation by aluminium chloride alone. It has been used as an oxidising agent to remove different organic and inorganic compound such as phenol, Mn^{2+}, tannin, As (III), and some heavy metal ions, such as Co (II), Cr (III) and Pb^{2+} (Farré et al., 2008; Jiang et al., 2010; Prieto-rodriguez et al., 2012; Rubirola et al., 2014; Sun et al., 2015).

Some antibiotics including lincomycin, ciprofloxacin and trimethoprim are oxidised by potassium permanganate ($KMnO_4$, Mn(VII) in a water treatment operation, but without tangible mineralisation (Hu et al., 2011). Notwithstanding, it has been observed that for target pharmaceutical removal, higher permanganate quantities and/or longer reactor times will be needed. This signifies that it could be very useful for the elimination of pharmaceutical particularly, in WWTPs operations that contains higher organic matrix and operate longer retention times. Understandably, the longer retention time will help in consuming any residual permanganate (Hu et al., 2011). Therefore, combining permanganate with radiation from the sun as AOP could yield better mineralisation.

2.18.1.4 Benefits of $KMnO_4$ photolysis

Relative to other oxidants such as chlorine, chlorine dioxide, potassium ferrate ozone and ozone, permanganate is sometimes the favourite due to its reduced cost, easy manipulation, applicability over a wide pH range, and its relative stability at the subsurface level (Guan et al., 2010; Chan et al., 2018). Oxidation with permanganate produces a non-hazardous insoluble product that can be easily removed by filtration or sedimentation. More importantly, permanganate is an environmentally pleasant oxidant, it does not enhance the development of chlorinated or brominated by-products, which are extremely hazardous. All this enhances the application of permanganate as an oxidant in water and wastewater treatment (Hu et al., 2011; Hübner et al., 2015).

Also, the possibility of enhancing the oxidation of micropollutants by permanganate has been predicted (Hu et al., 2010). Thus, integrating permanganate with radiation from the sun as AOP would yield better mineralisation.

2.18.1.5 Reasons for the oxidation of target compounds

Enumerated below are the determinable reasons for the oxidation of the target compounds.

- South Africa consumes more ARV drugs than other parts of the world (Wood et al., 2015; Abafe et al., 2018; Stanley and Kaplan, 2018).
- ARV compounds are found in significant quantities in WWTPs in South Africa (Boulle et al., 2010; Wood et al., 2015; Schoeman et al., 2015; Schoeman et al., 2017).
- Conventional wastewater treatments cannot adequately remove many ARVs because they are highly bioactive and resistant to biological degradation. (Mascolo et al., 2010; Schoeman et al., 2015; Wood et al., 2015; Hübner et al., 2015).
- ARVs have been reported to cause bacterial and viral resistance in aquatic organisms such as dabbling ducks, sterility, feminisation, and interference with the endocrine system in the higher animal. This may cause antiviral resistance in humans after a long time of exposure (Broder, 2010; Jain et al., 2013; Madikizela et al., 2017).

The increase in the consumption of ARV compounds in South Africa can lead to the accumulation of ARVs and their TPs in WWTPs, with greater potential to contaminate the water bodies (Levy et al., 2005; Boulle et al., 2010; Stinson et al., 2014; Stinson et al., 2017).

2.19 Summary

In summary, this chapter presented a review of literature that covers, emerging contaminants, pharmaceutical and personal care products. A particular focus was on antiretroviral compounds and their toxic effects in the aquatic environment which necessitates the need to remove them.

As a case study, the literature was narrowed down to the investigation of specific ARV compounds which are 3TC, AZT and NVP in Bellville and Khayelitsha in Cape Town metropole, South Africa.

The common analytical techniques for the analysis of the compounds in the aquatic environment and the several advancements in water treatment technologies from past decades to the present time were also discussed. The chapter emphasised on AOPs which show improvement in the removal of emerging contaminants and antiretroviral compounds.

In the concluding section of this chapter, the potential and benefits of the two proposed AOPs sunlight (UV)/Cl_2 and sunlight (UV)/$KMnO_4$ treatment were discussed. These include the production of RCS which enhances selectivity and better performance, ease of application in both disinfestation and degradation of persistent organic contaminants, cost-effectiveness and $KMnO_4$ for being environmentally friendly.

CHAPTER 3

EXPERIMENTAL DESIGN

3.1 Introduction

This chapter is both field and laboratory-based orientated. It presents fieldwork which deals with the collection of wastewater samples from both Bellville and Khayelitsha WWTPs identified as WWTP 1 and WWTP 2, respectively. Samples were collected for twelve months using standard methods as contained in EPA (2009) protocols and the research conducted by Prasse et al. (2010). Also, it highlights samples treatment and preservation using standard methods described by EPA (2009) protocols and Prasse et al. (2010).

Moreover, in this chapter, the evaluation of ten physicochemical parameters, the determination of the three pharmaceutical formulations (3TC, AZT and NVP) from the wastewater samples using standard methods were discussed. As part of the discussions present in this chapter, the development of two new advanced oxidation processes (AOPs) which are sunlight (UV)/$Ca(ClO)_2$ and sunlight(UV)/$KMnO_4$ methods are also discussed. The optimised conditions for the developed AOPs methods were used to degrade the three pharmaceutical formulations of interest in standard samples, as well as in the real wastewater samples. The degradation was monitored with both UV Spectrophotometry and HPLC techniques.

3.2 Graphical abstract

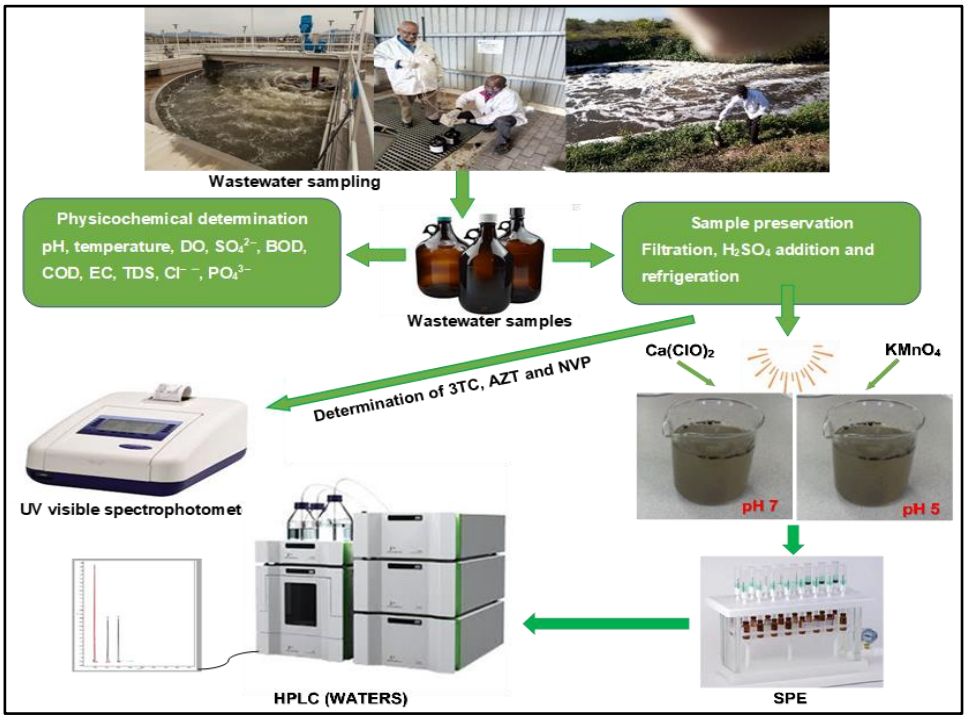

Figure 3.2: Flow diagram representation of the graphical abstract of the study.

3.3 Sampling Methodology

3.3.1 Sampling material and sample containers

The sampling material was a 1 L plastic container attached to a rope and sinker while the sample containers for influents and effluent were 2.5 L Winchester bottles. In the laboratory, before sample collection, both sample containers and sampling material were thoroughly washed with liquid soap. They were rinsed several times with tap water, three times with high purity milli-Q water. The containers were air-dried and labelled appropriately with the intended content by a waterproof label. Each bottle was put in a bubble wrap bag sheeting plastic bag (EPA, 2009; Schoeman et al., 2017).

3.3.2 Description of the WWTPs

The first WWTP is located in the Bellville South Industrial Area (Figure 3.2). The total capacity of WWTP 1 is 70 Mega litre (ML). It received influent from domestic and industrial run-off water. Raw influent passes through coarse and fine screening, respectively, for the removal of solid particles. It then undergoes anaerobic and aerobic digestion with activated sludge and moving bed bioreactors (MBBR) digestion for organics removal (by converting organic matter into methane and carbon dioxide) nitrification, denitrification and detoxification. Disinfection is attained by chlorination. Lastly, the final effluent is passed through UV radiation as the tertiary treatment before its discharge (Olujimi et al., 2010; Veolia Water Technologies, 2019).

The second WWTP is located outside the Khayelitsha Informal Settlement (Figure 3.2). In contrast, the total capacity of WWTP 2 is only 40 ML, and the influent came mainly from domestic and agricultural wastes. The plant utilizes the same treatment process except for the tertiary treatment that uses UV radiation (Olujimi et al., 2010; Veolia Water Technologies, 2019).

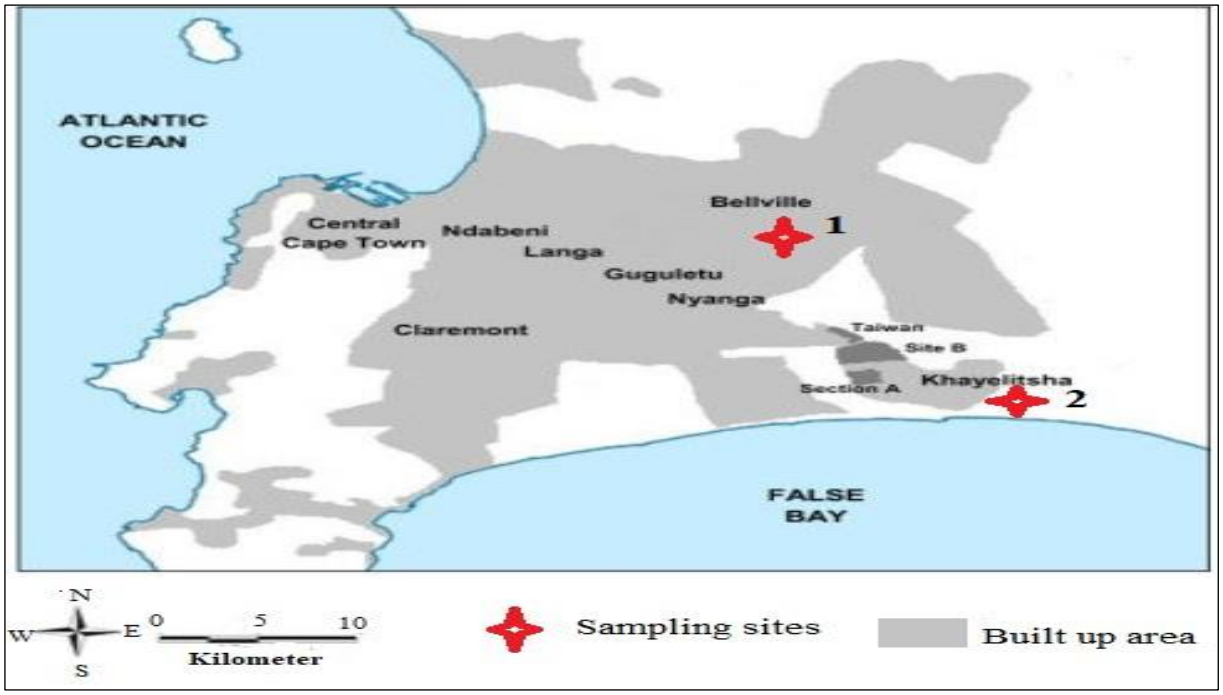

Figure 3.2: Map showing sampling sites employed in this study.

3.3.3 Sampling procedure

Monthly grab samples were collected from both WWTPs in Western Cape, South Africa as shown in the sampling sites in Figure 3.2. Sampling was done from April 2018 to April 2019 following the standard protocols described by USEPA (2009) and Prasse et al. (2010). The WWTPs were mentioned earlier in this study as WWTP 1 and WWTP 2. Raw influent (RI) and two types of final effluent (before UV treatment (BUVT) and after UV treatment (AUVT)) samples were collected from WWTP 1 located near the CPUT campus in Bellville. Final effluent was collected in WWTP 2 located near Khayelitsha. These samples were collected in triplicate into pre-washed 2.5 L Winchester dark-brown bottles, after rinsing thrice with the wastewater (WW).

More so, the dates of samples collection are as presented in Table 3.1. A total of one hundred and eight samples were collected from WWTP 1, while thirty-six samples were collected from WWTP 2 within the sampling period. The samples were arranged in a strong plastic box and immediately transported to the laboratory within 30 minutes.

Table 3.1: Dates of sample collection.

Wastewater Treatment Plant (WWTP) 1	Wastewater Treatment Plant (WWTP) 2
9/ 4/2018	10/ 4/2018
10/5/2018	10/5/2018
17/6/2018	17/6/2018
31/7/2018	22/7/2018
18/8/2018	18/8/2018
15/9/2018	15/9/2018
26/10/2018	21/10/2018
13/11/2018	11/11/2018
6/12/2018	2/12/2018
13/2/2019	13/2/2019
26/3/2019	24/3/2019
21/4/2019	21/4/2019

3.3.4 Sample pre-treatment

Subsequently, an aliquot of the samples was taken immediately in the laboratory for the determination of some physicochemical parameters. This includes pH, temperature, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), BOD_5 and COD, SO_4^{2-}. Cl^-, PO_4^{3-}. The pH of the remaining samples was simultaneously adjusted to pH 2.5, with 3.5 M sulphuric acid to inhibit microbial activity, and then stored in the refrigerator at 4 ^oC for further analysis within 4 days of sample collection (Prasse et al., 2010).

3.4 Determination of physicochemical parameters

Physicochemical parameters, temperature, pH, electrical conductivity (EC) and total dissolved solids (TDS) were measured with Lovibond SensoDirect 150 multimeter. Dissolved oxygen was measured with a Hanna multi-parameter probe HI 9828 after proper calibration according to the manufacturer's manual. All determinations were performed in triplicate.

3.4.1 Determination of temperature

The temperature of the wastewater samples was determined once the sample gets to the laboratory, by dipping the temperature probe of Lovibond SensoDirect 150 multimeter into the aliquots of the samples in a 100 mL beaker.

3.4.2 Determination of pH

The Lovibond SensoDirect 150 multimeter was calibrated according to the manufacturer manual, with three standards buffers pH 7, pH 4 and pH 9. Thereafter, the pH of the wastewater samples was determined by dipping the pH probe the multi-meter into the sample, while the pH was read on stability.

3.4.3 Determination of electrical conductivity (EC)

The electrical conductivity of the wastewater samples was measured with Lovibond SensoDirect 150 multimeter by the manufacturer manual.

3.4.4 Determination of total dissolved solids (TDS)

The total dissolved solids of the wastewater were measured by multiplying the EC values with a 0.64 factor in line with the method of Rusydi (2018).

3.4.5 Determination of dissolved oxygen (DO)

The DO in the wastewater samples were determined with Hanna multi-parameter probe according to the manufacturer manual after calibrating the meter with zero (0) and one hundred (100) solutions.

3.4.6 Determination of sulphate

Sulphate content was determined according to the method by Mussa et al. (2009). A 1000 ppm stock solution of sulphate standard was prepared by dissolving 1.5098 g of analytical reagent (AR) Na_2SO_4 salt in a 1000 mL of Milli-Q-water. Subsequent working standards of 0, 10, 20, 40, 80 and 100 ppm were prepared by making up 0 mL, 1 mL, 2 mL, 4 mL, 8 mL, 10 mL of the stock solution into 100 mL in a standard flask with Milli-Q water.

The calibration curve was prepared by mixing 10 mL of working solution standards, 10 mL of Milli-Q water and 6 mL of 2 M acetate buffer pH 6. And the resulting solution was gently stirred on an orbits shaker for 5 minutes. Thereafter, about 0.15 g of $BaCl_2$ salt was added and stirred for another 2 minutes. The standards were removed from the shaker and allowed to rest for five minutes. Meanwhile, the Jenway 7305 Spectrophotometer was zero with the 0 ppm blank solution at 420 nm, and the absorbance of different concentrations of the standards was measured. A standard calibration curve (Appendix A) was constructed from the plot of absorbance against concentration. A similar procedure was applied for the wastewater samples, and the sulphate content was determined from the regression coefficient of the standard calibration curve

3.4.7 Determination of chemical oxygen demand (COD)

Determination of chemical oxygen demand (COD) was carried out with open reflux colourimetric method for 20 to 1500 mg/L range as described by the United State Environmental Protection Agency (USEPA, 1980). A 1000 mg/L stock solution of standard potassium hydrogen phthalate (KHP) was prepared by dissolving 850 mg of previously oven-dried KHP in a 1000 mL standard flask. From this, a series of KHP calibration standards covering 20 to 1500 mg/L COD were prepared.

A 2.5 mL of digesting solution (12.259g $K_2Cr_2O_7$ +544 mL H_2SO_4 +5.5 g Ag_2SO_4) was added to 2.5 mL of the standards, wastewater and dilution water (blank vial) accordingly. The flasks were mixed well and digested simultaneously for two hours under reflux at 150 ^oC. After digestion, the flasks were allowed to cool at room temperature, and the absorbances were read from Jenway 7305 Spectrophotometer at 600 nm.

A standard calibration curve (Appendix B) for the different concentrations of KHP standard was plotted from the series of absorbance values of the standards from the Jenway 7305 Spectrophotometer at 600 nm. Subsequent COD concentrations of the samples and blank were determined from the calibration curve (USEPA, 1980).

3.4.8 Determination of biochemical oxygen demand (BOD)

The biochemical oxygen demand (BOD) was determined by the Standard Methods for the Examination of Water and Wastewater by American Public Health Association (APHA/AWWA/WEF, 2012). To the wastewater, 0.5 mL of sodium thiosulphate was added to remove any present chlorine which, can hinder proper biological activity during the BOD test. The pH of the sample was measured and adjusted to pH 7 by the addition of either 0.1 M NaOH or 0.1 M H_2SO_4. The sample was maintained at pH 7 by the addition of 0.1 M phosphate buffer 7.

Serial dilution of the wastewater was done to obtain 0.100%, 0.010%, and 0.001% of the original wastewater concentration. This was done by accurately pipetting 50 mL of the wastewater into a 500 mL standard flask and makeup to prepare 0.100% of the original wastewater. The process was repeated with 0.100% and 0.010% to prepare 0.01% and 0.001%, respectively. The 300 mL BOD bottles were filled with different concentrations of the wastewater samples. Three bottles were filled with dilution water as control and treated like the wastewater except for the addition of wastewater samples.

The initial dissolved oxygen (DO_1) in both dilution water and sample bottles were measured with a DO meter. The bottles were incubated in the dark at 20 °C for five days. The final dissolved oxygen (DO_5) concentration (mg/L) was measured on the fifth day (± 3 hours). The BOD concentration (mg/L) was then calculated according to Equation 3.7 (APHA/AWWA/WEF, 2012).

BOD concentration (mg/L) = $(DO_{1s} - DO_{5s})$ - $(DO_{1c} - DO_{5c})$ x FD ……Equation 0.6

Where:

DO_{1s} = Initial dissolved oxygen of the sample on day 1;
DO_{5s} = Final dissolved oxygen of the sample on day 5;
DO_{1c} = Initial dissolved oxygen of the control on 1;
DO_{5c} = Final dissolved oxygen of the control on day 5;
FD = Dilution factor.

3.4.9 Determination of chloride

A modified method of Mesqita et al. (2002) was used for the determination of chloride in the wastewater. A 1000 mg/L standard chloride stock solution was prepared by dissolving 0.5 g of previously dried NaCl in 500 mL of water. Different ranges of chloride working standards were appropriately prepared in the order of 2–10 mg/L, 10–50 mg/L and 50–400 mg/L. A stock solution of 0.1 M silver nitrate was prepared by dissolving 1.7 g $AgNO_3$ in 100 mL of water and standardise with a standard NaCl solution. A 2 M HNO_3 solution was prepared by the dilution of 13.95 mL of concentrated HNO_3 to 100 mL.

The precipitating reagent (0.005 M AgNO3) solution was prepared as follows. A 0.0125 g polyvinyl alcohol (PVA) was dissolved in boiling water and allowed to cool. After cooling, 9.4 mL of concentrated nitric acid (d ~ 1.40; 65%) and 5 mL of 0.1 M $AgNO_3$ stock solution were added in a 100 mL standard flask. The solution was prepared up to volume with Milli-Q water in a 500 mL standard flask. And the solutions were prepared in the dark, while the standard flasks were wrapped with aluminium foil.

Based on the procedure applied, chloride was determined by a little modification of turbidimetric determination by Mesquita et al. (2002). The solutions were added in the sequential order of 2.5 mL precipitating reagent, 0.8 mL of 2 M nitric acid solution and 1.80 mL standard chloride solution and shacked together. The absorbance of the turbidity formation of different concentrations of the standard was measured in Jenway 7305 Spectrophotometer at 410 nm. The calibration curve (Appendix C) for the standard was obtained by plotting the absorbance against concentration. The same procedure was used for the wastewater samples, and the concentration was determined from the equation of the calibration curve of the standards.

3.4.10 Determination of phosphate

Phosphate was determined by the modified method of Pradhan and Pokhrel (2013). Phosphate stock solution (100 ppm) was prepared by 0.4393 g of potassium dihydrogen phosphate (KH_2PO_4) in Milli Q water and made up to 100 mL in a volumetric flask. The working phosphate solutions were prepared by its further dilution. Sodium molybdate (2.50% working solution) was prepared by dissolving 6.25 g in Milli-Q water in a 250 mL standard flask and made to mark with the same Milli Q water. A 5 M sulphuric acid solution was prepared by diluting 28 mL of concentrated sulphuric acid solution to mark in a 100 mL volumetric flask. A 0.15 g of hydrazine sulphate was dissolved in 100 mL Milli-Q water to prepare a 0.5 M solution of hydrazine sulphate.

3.4.10.1 Preparation of calibration curve for phosphate standard

From 100 ppm phosphate solution, series of working solutions of 12 mg/L ppm 10 ppm, 8 ppm 6 ppm, 4 ppm, 2 ppm and 1 ppm by pipetting 3.00 mL, 2.5 mL, 2 mL, 1.5 mL, 1 mL, 0.5 mL and 0. 25 mL, respectively, into 25 mL standard flask.

The calibration curve was prepared in the sequential order of 25 mL standard flask, a 2 mL solution of sodium molybdate (2.50%) and 0.5 mL of 5 M sulphuric acid (0.10 M) solutions

were mixed. Then, 1 mL of 0.5 M hydrazine sulphate solution was added, and the volume was made up to mark with Milli-Q water. The solution was left for 45 minutes at 30^{o}C for maximum colour development, while absorbance was read at 840 nm. The calibration curve was derived by plotting the absorbance against the concentration of the standard solutions. However, the same procedure was repeated for the filtered wastewater sample solutions. The amount of phosphate in ppm (mg/L) was measured with the equation of the standard calibration curve (Appendix D).

3.5 Spectroscopic determination of selected anti-retroviral compounds in wastewater samples

3.5.1 Preparation of stock and working solutions

All chemicals used in the preparation of standard and working solutions were of analytical grade and purchased from local chemical suppliers in Cape Town (South Africa). All solutions were prepared with water obtained from a Milli-Q (Millipore, 18 MΩ/cm) water purification system. The instrument used were UV-1800 Shimadzu Spectrophotometer (Japan), Jenway 7305 Spectrophotometer and Crison pH meter GLP 21+.

The ARV tablets used in this study were obtained for research purposes from a local pharmacy in Bellville, Cape Town. This route was followed for the pharmaceutical grade of ARVs, partly to track the fate of the ARVs dispensed at local community hospitals, pharmacies and day clinics in the Western Cape. Also to alleviate the issues experienced with the approval from the Department of Health (Pretoria) and the high cost involved. Each of the tablets contained a single active ingredient which is 3TC, AZT and NVP and their brand names are Epivir®, Retrovir® and Viramune®, respectively. The active percentage of 3TC (Epivir), AZT (Retrovir) and NVP (Viramune) in the tablets according to the label claims are 44.35%, 77.44% and 25.14%, respectively.

Each tablet was accurately weighed on an analytical balance before crushing and later pulverized into powdery form. An individual stock solution of 1000 mg/L (1000 ppm) was prepared for each of the 3TC, AZT and NVP formulations, by weighing the appropriate quantity of the powdered drug calculated according to the reported information on the label as shown in Table 3.2. The active ingredient was thereafter extracted according to the protocol reported by Sarkar et al. (2006). Concisely, the pulverized tablets were extracted thrice by sonication in 0.01 N HCl for 30 min. Each extract was filtered with doubled Starlab Scientific filter paper (medium speed filtration) into 100 ml standard flasks. The flasks were made up with 0.01 M HCl to give a stock of 1,000 mg/L. Appropriate dilutions of the stock solutions were made to prepare seven mixed standards solutions with different concentration ranging from 2,000-24,000 µg/L for 3TC and AZT and 4,000-40,000 µg/L for NVP (Anbazhagan et al. 2005; Sarkar et al. 2006).

Table 3.2: Preparation of stock solutions of 3TC, AZT and NVP.

Tablet (trade name)	Average weight (mg)	Label claim/tablet (mg)	Amount of drug containing 1 mg active ingredient (mg)	Amount dissolved in 100 mL (mg)	Concentration (mg/L)
3TC (Epivir)	338.2	150	2.260	226.0	1000
AZT (Retrovir)	387.4	300	1.291	129.1	1000
NVP (Viramine)	795.7	200	3.978	397.8	1000

The filler materials in the tablet did not affect the stock solutions because the responses from different concentrations of the working solutions used for calibration curves were linear with the concentrations measured and the R^2 for all the 3 drugs were > 0.997.

3.5.2 Determination of maximum absorbance of 3TC, AZT and NVP

The determination of absorbance readings for prepared standard solutions was done using a UV-1800 Shimadzu Spectrophotometer (Japan). This was done by scanning the solution of each compound entirely in the UV region (200 – 400 nm) on the UV-vis spectrometer. The λ max was found to be 280 nm, 267 nm and 313 nm for 3TC, AZT and NVP, respectively. And the maximum absorbance readings were consequently used for the analytical protocol of the standard calibration curves construction, followed by the analysis of the wastewater samples.

3.5.3 Preparation of calibration curves

The calibration curves (Appendix E, F and G) were prepared by measuring the absorbance of different concentrations of seven mixed standard solutions at 280 nm, 267 nm and 313 nm for 3TC, AZT and NVP, respectively. The values obtained were then used to plot the calibration curves for each compound (Anbazhagan et al., 2005; Sarkar et al., 2006).

3.5.4 Spectroscopic determination of 3TC, AZT and NVP in wastewater

The wastewater samples were analysed according to the protocol of Anbazhagan et al. (2005), which was modified for this study. Approximately 49 µL of concentrated HCl was added to a 30 mL aliquot of the wastewater sample in a 50 mL standard volumetric flask. The flask was filled to the calibrated mark with the same wastewater sample to prepare a 0.01 M hydrochloric acid solution. The sample solution was mixed and filtered twice with a Starlab Scientific filter paper (medium speed filtration). A blank sample was prepared in the same way with Milli-Q water but without the wastewater sample. The absorbance of the blank sample and wastewater sample was measured at the same wavelength as the standard solution. And the concentration of each ARV in the wastewater sample was determined from the correlation coefficient of the standard calibration curves for the corresponding ARV drug investigated.

3.5.5 Method validation

The method was validated by the International Conference on Harmonization (ICH) guidelines for the validation of analytical procedures reported by Rathod et al. (2014). The system precision, linearity, method precision (Intra-day and inter-day precision) and accuracy were determined by applying the method of Rathod et al. (2014).

3.5.5.1 System precision

The absorbance readings of seven replicate samples of 120 µg/L, 120 µg/L and 240 µg/L for 3TC, AZT and NVP were measured at 280 nm, 267 nm and 313 nm, respectively according to the protocol described by Rathod et al. (2014).

3.5.5.2 Linearity

The linearity of the method was determined from the absorbance readings of six different concentrations of the standards between 10 – 240 µg/L for 3TC and AZT and 20 – 400 µg/mL for NVP. For each standard, the absorbance readings of the three replicated samples were measured, and the average absorbance reading was used to construct standard calibration curves by plotting the average absorbance reading against the concentrations.

3.5.5.3 Method precision (intra-day and inter-day precision) evaluation

The precision of the method was determined by performing the following:

i. intra-day (repeatability) measurements of the absorbance readings, and
ii. inter-day (intermediate) precision measurements.

3.5.5.3.1 Intra-day precision (repeatability) measurements

For repeatability, seven consecutive readings of the three replicated samples of 3TC, AZT and NVP were measured at different times in one day.

3.5.5.3.1 Inter-day precision (intermediate) measurements

The inter-day (intermediate) precisions of the three pharmaceutical formulations were measured on three consecutive days. This was performed by measuring the absorbance readings of three different concentrations (20 µg/L, 120 µg/L, and 200 µg/L) for 3TC and AZT, whereas the absorbance readings of 40 µg/mL, 240 µg/mL and 400 µg/L were measured for NVP.

3.5.5.4 Accuracy measurements

The accuracy of the method was evaluated through the recovery experiments, and the percentage recovery of the three replicate samples of 3TC and AZT at 2,000 µg/L, 12,000 µg/L, and 20,000 µg/L and NVP at 4,000 µg/L, 24,000 µg/L and 40,000 µg/L were measured.

3.5.5.5 Limit of detection (LOD) and limit of quantification (LOQ) for 3TC, AZT and NVP pharmaceutical formulations

Limit of detection (LOD) for the compounds was determined by the method of Thomsen et al. (2003). The absorbances of ten replicate blank solutions of each compound were recorded with UV visible spectrophotometer. The LOD was determined as thrice the standard deviation of the mean of 10 repeated measurements of the blank divided by the slope of the calibration curve of the standard.

$$LOD = \frac{3.3\sigma}{S} \quad \text{.....Equation 3.8}$$

While, the limit of quantification (LOQ) is the lowest concentration on the standard curve which can be determined or measured with accuracy, precision and variability (Sarkar et al. 2006). It was determined as ten times the standard deviation of the mean of 10 repeated measurements of the blank divided by the slope of the calibration of the standard.

$$LOQ = \frac{10\sigma}{s} \quad \text{.....Equation 3.9}$$

Where:

σ = Standard deviation of the absorbance of 10 readings of the blank solution.
S = Slope of the regression line.

3.6 Degradation of 3TC, AZT and NVP with sunlight (UV)/Chlorine and sunlight (UV)/$KMnO_4$ Advanced Oxidation Processes

3.6.1 Preparation of solution

3.6.1.1 Preparation of $Ca(ClO)_2$ and $KMnO_4$ oxidants

The oxidants were prepared by the method of Gray (2014). Different 20,000 µM stock solutions of $Ca(ClO)_2$ and $KMnO_4$ were prepared by dissolving 426.81 mg and 316.07 mg, respectively, in separate 100 mL of Milli-Q water and stored in amber bottles. The high concentration of the oxidants was chosen to prevent the dilution of the samples for analysis. To prevent the decay of the oxidants, they are prepared fresh and used within 24 hours.

3.6.1.2 Preparation of $Na_2S_2O_3$ solution

A 40,000 µM solution of sodium thiosulphate was prepared by dissolving 632.44 mg of sodium thiosulphate in 100 mL water and stored in an amber bottle. The solution was prepared fresh and used within 24 hours.

3.6.1.3 Preparation of phosphate buffer solutions

Different Phosphate Buffer Solutions used in the degradation experiment were prepared according to European Pharmacopoeia Chapter 4, section 4.1.3 (EP) (European, 2005). Phosphate buffer solution of pH 4 This was prepared by dissolving 6.80 g of potassium dihydrogen phosphate in 800 mL of Milli-Q water. The pH was adjusted to 4.0 with potassium hydroxide and made up to calibration mark with Milli-Q water in a 1000 mL standard flask. Other phosphate buffers of different pHs were prepared as indicated in Table 3.3

Table 3.3: Preparation of phosphate buffer solutions

Phosphate buffer solution	Quantity of potassium dihydrogen (g)	Adjusted to the required pH with
pH 5	2.72	1.0 M potassium hydroxide solution
pH 6	6.80	2.0 M sodium hydroxide solution
pH 7	5 + 11 (dipotassium hydrogen phosphate)	1.0 M sodium hydroxide solution
pH 8	0.523 + 16.73 (dipotassium hydrogen phosphate)	–
pH 9	17.4	1.0 M potassium hydroxide solution

3.7.1 Determination of suitable experimental conditions

Literature established that the rate of contaminant elimination depends on some factors such as pH, irradiation intensity and contaminant-oxidant ratio (Remucal and Manley, 2016; Wu et al., 2017). During this process, each of the experimental condition was first optimised systematically.

3.7.2.1 Determination of solar irradiation intensity

Before commencing these experiments, the weather forecast was monitored to facilitate the selection of a suitable day for the degradation experiment. Generally, the appropriate days chosen are those with favourable weather condition for our experiment viz. The average temperature considered was 23 ± 2.0 ^{o}C and the average solar irradiance 250 ± 25 mW/m^2 (when the solar radiation was very high) (Downs et al., 2016; Gies et al., 2018). The actual weather conditions (temperature and solar irradiance) on the days and times (within 12 noon - 2 p.m.) of the experiments were measured by spectroradiometer from the Solar Energy Unit, Department of Mechanical Engineering, Cape Peninsula University of Technology, Bellville, South Africa. All outdoor experiments were conducted between 12 noon and 2 pm when the weather was clear and sunny.

3.7.2.2 Selection of appropriate volume of buffer solution for the degradation processes

In an attempt to determine the appropriate (optimum) pH for this work, different 50 mL of mixed standard solutions containing 5,000 µg/L (20 µM) of 3TC, AZT and NVP at pH 4-9 were

chosen for the experiment. The solution was prepared from 1000 ppm stock solutions of 3TC, AZT and NVP by adding 1.25 mL of each stock solution into a 250 mL standard flask containing 75 mL of varying phosphate buffer solutions (pH 4 – 9). The flasks were made up to the calibration mark with Milli-Q water. From each of these solutions, a 50 mL (~20 µM 3TC, AZT and NVP) aliquot was measured in duplicate into 100 mL Pyrex beakers and spiked with 140 µM (OAR 7:1) Ca $(ClO)_2$ or $KMnO_4$.

The initial concentration (5,000 µg/L) of the analyte and the oxidant ratio added was following the ratio reported in the earlier literature (Chan et al., 2012) and the concentrations that also fall within the concentration earlier determined in section 3.6.4. The pH of the solutions was tested with a pH meter, but a sharp rise in the pH was observed when the buffered mixed standard solutions were spiked with the $(Ca(ClO)_2$. To work at the required pH (4 – 9), different ratios of mixed standard solution and buffers solution that could hold the solution at the required pH was initially optimised. This was attained by checking the initial and final pH of different ratio of ARV-buffer solution before and after adding the oxidants. The ratio of ARVs solution - buffer solution found to be optimal was 7:3 (Appendix H). This ratio was used to prepare an ARV standard solution in all the subsequent experiments. The addition of KMnO4 did not show a similar rise in the pH; although, for consistency and accurate comparison, the same ARVs - buffer solution ratio used for $Ca(ClO)_2$ was used in all the subsequent preparations.

3.7.2.3 Determination of optimum time for the degradation processes

To optimise the effect of time, other parameters (pH 4 and oxidant dose) were kept constant. Because the samples had been preserved at the acid condition, this optimisation was conducted at pH 4. Likewise, the initial OAR 7:1 chosen was based on past literature (Different 5,000 µg/L (~20 µM) mixed standard solutions of 3TC, AZT and NVP were prepared from individual 1000 ppm stock solutions of 3TC, AZT and NVP. This was done by pipetting 1.25 mL of each stock solution into a 250 mL standard flask containing 75 mL of phosphate buffer pH 4 (7:3 for ARVs–buffer solution). The flasks were made up to the calibration mark with Milli-Q water and swirled thoroughly. A duplicate portion of 50 mL was measured into 100 mL Pyrex beakers and spiked to 140 µM to make OAR 7:1 for Ca $(ClO)_2$ or $KMnO_4$. These were arranged in another flat tray containing water (1 cm height) to minimise the effect of temperature changes in the outdoor environment. The set up was shaken in an orbital shaker for 15 minutes to ensure equilibrium before it was put outdoors under the sunlight at Bellville campus, Cape Peninsula University of Technology, Cape Town, South Africa. Aliquots were collected periodically at 0, 30, 60, 90, 120, 15, 180 and 210 minutes for sunlight/Cl_2 and 0, 15, 30, 45, 60, 75, 90 and 105 minutes for Sunlight/$KMnO_4$ based on the previous literature (Hu et al., 2010; Chan et al, 2012). The residual oxidants were quenched with excess sodium thiosulphate (280 µM), and the reaction progress was monitored by measuring the remaining concentrations of 3TC, AZT and NVP with Jenway 7305 Spectrophotometer (Hu et al., 2011; Chan et al, 2012). The percentage removal was calculated according to Equation 3.10.

$$\frac{C_o - C_t}{C_o} \times 100 \quad \text{……..……..Equation 0.7}$$

Where:

C_o = initial concentration (100%) at time 0 minute;

C_t = final concentration (remaining %) at time t.

3.7.2.4 Determination of optimum pH for the degradation experiment

The set up for the optimisation of pH was conducted as explained in section 3.7.2.3 at the optimised time, 90 minutes but at different pH (pH- 9).

3.7.2.5 Determination of optimum oxidant-analyte ratio (OAR)

After the optimisation of time and pH for both process $Ca(ClO)_2$ and $KMnO_4$, the optimum OAR was investigated by subjecting a duplicate portion of 50 mL of mixed standards (5 µg/L 3TC, AZT and NVP) in either phosphate buffer pH 7 for UV/Cl_2 process or pH 5 for UV/$KMnO_4$ process. The samples were spiked to different OARs of 3:1; 5:1;7:1; 9:1 and 11:1. These were arranged in another flat tray containing water (1 cm height) to minimise the effect of temperature changes in the outdoor environment. The set up was shaken in an orbital shaker for 15 minutes to ensure equilibrium before it was put outdoors under the sunlight for 90 minutes (sunlight (UV)/Cl_2 process) and 45 mins (sunlight(UV)/$KMnO_2$ process) at CPUT, Bellville Campus, South Africa. The residual oxidants were quenched with excess sodium thiosulphate (280 µM), and the reaction progress was monitored by measuring the remaining concentrations of 3TC, AZT and NVP with Jenway 7305 Spectrophotometer (Hu et al., 2011; Chan et al, 2012).

3.7.2.6 Control Experiment: Dark control experiment (treatment with oxidant alone) and treatment with UV radiation alone

At the established optimised conditions which are 90 mins., pH 7 and OAR 7:1 for sunlight (UV)/Cl_2 process and at 45 mins, pH 5 and OAR 7:1 for sunlight(UV)/$KMnO_4$ process, two sets of control experiment were carried out for each treatment process which involved

a) Treatment with $Ca(ClO)_2$ alone and b)treatment with UV radiation alone

Likewise, for sunlight(UV)/$KMnO_4$ process involved

a) Treatment with $KMnO_4$ alone and b) treatment with UV radiation alone.

These were carried out concurrently to compare the combined factors with an individual factor as enumerated below

i. The dark control experiment was done in a similar way and under the same environmental conditions of 90 mins, pH 7 and OAR 7:1 established for sunlight(UV)/$Ca(ClO)_2$ process and at 45 mins, pH 5 and OAR 7:1 for sunlight(UV)/$KMnO_4$ process as described in section 3.7.2.6 but the beakers containing the samples were covered with aluminium foil to shield them from the sunlight radiation.

ii. The second control experiment was carried out similarly and exposed to sunlight (UV) radiation, but without the addition of $Ca(ClO)_2$ or $KMnO_4$ as the case may be (Hu et al., 2011; Chan et al., 2012).

The percentage of removal was calculated according to Equation 3.10.

3.7.2.7 Degradation of different concentrations of analytes at the optimised conditions

Once the degradation conditions viz a vis; average solar irradiance of 250 ± 25 mW/m^2, pH (pH 7 for UV/Cl_2 and pH 5 for $UV/KMnO_4$), OAR 7:1 and reaction time (90 minutes for UV/Cl_2 and 45 minutes for $UV/KMnO_4$) have been established. Degradation of different concentrations of our target analytes was performed with the optimised reaction conditions as follows in order to evaluate the effect of the initial concentration of the ARVs on their removal by the two processes. For each concentration (1,000 µg/L, 5,000 µg/L, 10,000 µg/L, and 20,000 µg/L), two sets of quadruplicate portions of 50 mL mixed standards were prepared. The first set was prepared in phosphate buffer pH 5 and spiked with $KMnO_4$ to OAR 7:1 (20 µM ARVs: 140 µM $KMnO_4$, and the second set prepared in phosphate buffer pH 7 was spiked with $Ca(ClO)_2$ to 7:1 OAR. These were arranged in a flat tray containing water (1 cm height) to minimise the effect of temperature changes in the outdoor environment. The set up was shaken in an orbital shaker for 15 minutes to ensure equilibrium. Thereafter, duplicate portions from each set to determine the initial concentrations at time zero were spiked with excess sodium thiosulphate solutions to 280 µM (14:1 of $Na_2S_2O_3$: ARV). The remaining duplicate portions were set under sunlight in CPUT, Bellville Campus, South Africa. The residual oxidants were quenched with excess sodium thiosulphate (280 µM) solutions at the end of the reaction time of 45 minutes and 90 minutes for UV/KMnO4 and UV/Cl_2 experiments, respectively. The reaction progress was monitored by measuring the remaining concentrations of 3TC, AZT and NVP with Jenway 7305 Spectrophotometer using Equation 3.10 (Hu et al., 2011; Chan et al, 2012).

3.8 HPLC analysis

A Waters high-performance liquid chromatography (HPLC) furnished with Waters 1525 binary pump, Waters 2707 auto-sampler, Waters 2487 dual λ Absorbance detector and operated on Breeze software.

3.8.1 Chromatographic conditions

The method was set according to Ngumba and Gachanja (2015). Column: Zorbax Eclipse Plus C18 (4.6 * 100mm, 3.5 microns); Mobile phase: aqueous phase A– 99.9% + 0.1% (v/v) formic acid and organic phase B– 99.9% acetonitrile + 0.1% (v/v) formic acid; Flow rate:1.0 mL/min; Sample injection volume: 10 µL; Gradient elution: B was held at 20.0% for the first 2 min, then linearly increased to 100.0% in 3 min. B was then lowered to 20.0% in 5 min and held there for 2 min. The column was then equilibrated for 7 min before the next injection. The total run time for each injection was 19 minutes. All the chromatograms were first analysed at 260 nm and

265nm based on the previous studies (Joshi and Adeyeye, 2012; Shamili et al., 2018). The chromatogram was later analysed at 265, which produces the best peaks and retention time.

3.8.2 Preparation of stock solutions for HPLC analysis

Different 1000 µg/mL (1,000,000 µg/L) stock solutions (1) of 3TC, AZT and NVP were prepared by dissolving 100 mg of each compound in 60.0% methanol in separate 100 mL standard flasks. The second stock solution 10 µg/mL (10,000 µg/L) was prepared by diluting 1 mL of each stock solution 1 to 100 mL.

3.8.3 Preparation of calibration standards and quality control sample

Ten different mixed standards with concentrations ranging from 1.00–300.00 µg/L were prepared for calibration by appropriate dilution of stock solutions 2 with Milli-Q water as shown in Table 3.4. Three different concentrations (5 µg/L, 25 µg/L and 100 µg/L) for the quality control sample were also prepared separately. In addition, both calibration standards and quality control samples were prepared in triplicate (Ngumba and Gachanja, 2015).

Table 3.4: Table showing the volume of the stock solution measured for the preparation of calibration standards for analysis of 3TC, AZT and NVP.

Conc.(µg/L)	mL
1	0.025
5	0.125
10	0.250
25	0.625
50	1.250
100	2.500
150	3.750
200	5.000
250	6.250
300	7.500

3.8.4 Sample extraction and clean up

The calibration standards, quality control sample and wastewater samples were filtered with a sterile filter (CA 0.45 µm 25 mm). Thereafter, their pH was adjusted to pH 9 with dilute NH_4OH solution to boost the recovery of the analytes.

In the meantime, the Phenomenex SPE cartridges (Strata X, 33 µM Polymeric Reversed Phase, 500 mg, 6 mL tube) were sequentially conditioned with 6 mL methanol and 6 mL ultrapure Milli-Q water adjusted to pH 9. Replicate 100 mL samples ($n = 3$) were then loaded, hold for 60 seconds and flow using a vacuum manifold at a flow rate of 10 mL per minute. Then, the cartridges were dried in a vacuum for 5 min, washed with 5 mL of 2% methanol solution in 5% aqueous NH_4OH and dried for 10 min.

The analytes were eluted twice with 3 mL of acetonitrile/methanol/acetic acid (50:50:2 v/v) and the solvent evaporated in a stream of nitrogen at 40 °C. And the concentrates were reconstituted to 1 mL with acetonitrile/water (20:80 v/v), filtered through PTFE 0.2 µm cellulose acetate syringe filter into 2 mL chromatographic vial (Ngumba and Gachanja, 2015).

3.8.5 Analyte identification and preparation of calibration curve for HPLC analysis

Firstly, an individual ARV standard was identified through its retention time, this was done by injecting triplicate portions of each ARV standard (25 µg/L) into the HPLC system. For the calibration curve, ten different concentrations of mixed standards (Table 3.3) were pass-through SPE for sample extraction and clean up as described in subsection 3.8.4. Thereafter, they were injected in triplicate into the HPLC system and the chromatograms were recorded. Responses of triplicate measurements of the calibration standards were recorded. The plot of average area (µV Sec) against concentration was used to prepare a 7-point calibration curve (Figures 6.21 – 6.23) for 3TC, AZT and NVP, respectively (Ngumba and Gachanja, 2015).

3.8.6 Method validation (HPLC)

Validation of an analytical method is a laboratory process that ascertained that the performance characteristics of the method used to meet the requirements for the intended analytical application (Bhardwaj et al., 2015). Validation is required for a new or amended method to ensure reproducible and reliable results when used by different operators employing the same equipment in the same or different laboratories (Bhardwaj et al., 2015). The method was validated for the three analytes according to the ICH guidelines. The following parameters were tested for the validation:

i. linearity,
ii. repeatability,
iii. % recovery,
iv. matrix match effect, and
v. limit of detection (LOD) and limit of quantitation (LOQ).

3.8.6.1 Linearity

The linearity of the calibration was determined from the correlation coefficient (R^2) of the calibration curve recorded when seven different concentrations between 2 - 300 µg/L were injected in triplicate into the HPLC system.

3.8.6.2 Repeatability

The instrumental intra-day and inter-day repeatability of the instrument was assessed by three different concentrations (20 µg/L, 60 µg/L and 200 µg/L) of mixed standards. The samples were injected six times ($n = 6$) at a different time of the day for intra-day repeatability and in three consecutive days for inter-day repeatability. The percentage relative standard deviation (RSD) of the responses were determined.

3.8.6.3 Percentage recovery

The SPE recovery was evaluated with three different concentrations of 20 µg/L, 60 µg/L and 200 µg/L, correspondingly. For each concentration, triplicates samples of 1000 mL Milli-Q water were divided into two of 500 mL portions A and B, respectively. Triplicate samples of portion A for each concentration was spiked with the stock mixed standard solution to the concentrations of 20 µg/L, 60 µg/L and 200 µg/L, respectively. The second part (B) was not spiked. Both A and B were passed through the SPE process. B was later spiked with the stock solution to 20 µg/L, 60 µg/L and 200 µg/L, respectively, after the SPE process. The concentrates of both A and B were reconstituted into 1mL with acetonitrile/water (20:80 v/v) in a 2 mL chromatographic bottle and injected into the HPLC (Ngumba and Gachanja, 2015 modified).

$$\% \text{ recovery} = \frac{A}{B} \times 100 \quad \text{.....Equation 0.8}$$

Where:

A = peak area of A (peak area of analyte added before the SPE process);
B= peak area of B (peak area of analyte added after the SPE process).

3.8.6.4 Matrix effect

It has been reported that in many cases, exact matrix blanks are difficult to find especially for environmental samples taken from different locations (Gosetti et al., 2010; Wood et al., 2015). Where applicable, matrix effect can be accounted for by using the isotopically labelled standard for each target compound which is also very costly (Gosetti et al., 2010; Wood et al., 2015). Due to the unavailability of exact matrix blanks for all the wastewater sample, and the high cost of obtaining isotopically labelled standard for the three compounds, the BUVT sample in WWTP 1 that has not been treated with UV radiation was used for the matrix match blank sample. The reason behind this is that since WWTP 2 does not make use of UV in their treatment and to ensure good comparison, the use of BUVT sample in WWTP 1 will fairly represent different samples and different WWTPs.

The BUVT sample for this test was passed through the SPE several times to remove the ARVs present. The three ARVs content were monitored with Jenway 7305 Spectrophotometer at each time (six times) until the absorbance of the ARV could not be detected (0.000 absorbances) from the spectrophotometer. This treated BUVT effluent sample was used to evaluate the matrix effect as follows. Triplicate samples of both Milli-Q water and treated BUVT samples were spiked with a standard solution to 10 µg/L before and after the SPE process, respectively. Then, the peak areas of the samples were measured in the HPLC, and the ME was evaluated using Equation 3.12 as cited by Ngumba and Gachanja (2015).

$$\text{ME } (\%) = \frac{X}{Y} \times 100 \quad \text{.....Equation 0.9}$$

Where:

X = the peak area of the ARV standard recorded for the in Milli-Q water.
Y = the peak area of the ARV standard recorded for the extracted BUVT effluent sample spiked with ARV standard after SPE.

3.8.6.5 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for each compound were determined based on the standard deviation (SD) of the response of the blank solution and the slope. The SD (σ) of the blank solution was obtained by measuring the response of blank solution of each compound in HPLC seven times, and the slope (S) was derived from the calibration curve of each compound earlier determined (Bhardwaj et al., 2015).

The detection limit (DL) is determined as:

$$LOD = \frac{3.3\sigma}{S} \quad \text{.....Equation 3.13}$$

$$LOQ = \frac{10\sigma}{s} \quad \text{.....Equation 3.14}$$

Where:

σ = the standard deviation of the blank sample response;
S = the slope of the calibration curve.

3.8.7 Degradation of standard solution sample with sunlight (UV)/Cl and sunlight (UV)$KMnO_4$

Two sets of four replicate samples of 50 mL mixed standards (5,000 µg/L 3TC, AZT and NVP) were prepared. The first set was prepared in phosphate buffer pH 5 and spiked with $KMnO_4$ to 7:1 OAR. The second set was prepared in phosphate buffer pH 7 and spiked with $Ca(ClO)_2$ to 7:1 OAR. Both sets were arranged in a flat tray containing water (1 cm height) to minimise the effect of temperature changes in the outdoor environment. The set up was shaken in an orbital shaker for 15 minutes to ensure equilibrium. Thereafter, the duplicate portion from each set (to determine the initial concentrations) was spiked with excess sodium thiosulphate solutions to 280 µM. The remaining duplicate portions (to determine final concentration) were set outdoors under sunlight on the campus of the Cape Peninsula University of Technology, Bellville Campus, South Africa. The residual oxidants were quenched with excess sodium thiosulphate (280 µM) solutions at the end of the reaction time of 45 minutes and 90 minutes for UV/$KMnO_4$ and UV/Cl_2 experiments, respectively. Thereafter all the eight samples for the determination of both initial and final concentrations were treated for sample extraction as explained in subsection 3.8.4 and injected in triplicate into the HPLC system as described in subsection 3.8.5. Percentage removal was calculated according to Equation 3.15.

$$\% \text{ removal} = \frac{x-y}{x} \times 100 \quad \text{.....Equation 0.105}$$

Where

x = initial concentration of the ARV obtained from the regression equation of the standard calibration curve;
y = final concentration of the ARV obtained from the regression equation of the standard calibration curve.

3.8.8 Degradation of ARVs from the real wastewater samples

3.8.8.1 Selection of wastewater samples

The optimises degradation conditions viz a vis; average temperature: 23 ± 2.0 ^{o}C and average solar irradiance: 250 ± 25 mW/m^2 pH (5 for UV/$KMnO_4$ and 7 for UV/Cl_2), OAR and time were applied for the degradation of the real wastewater samples from the two plants. As reported in subsection 3.4.2.6, samples were collected for 12 months over the four seasons (autumn, winter, spring and summer) in Cape Town. For the selection of a particular month within the season, some criteria were put into consideration. These include: The months that contained the highest, middle and lowest concentrations of ARVs were selected, this was done to evaluate the effect of concentration variation on the degradation efficiency. The collective existence of the ARVs at different concentrations in the wastewater samples was also considered and the months in which the concentrations of 3TC, AZT and NVP are closer were selected. In summary, a representative month was selected from each of the seasons making a total of four months. From each month, every category of the sample collected (RI, BUVT, AUVT and FE) were degraded in triplicate. This makes a total of a forty-eight sample comprising thirty-six from WWWP 1 and twelve from WWTP 2 were degraded and analysed. The representative samples chosen are shown in Table 3.5.

Table 3.5: Table showing months selected per season for wastewater samples degraded.

Month	Season	Sample and Average concentration of 3TC, AZT and NVP (µg/L)			
		1 RI	1 BUVT	1 AUVT	2 FE
Jul. 2018	Winter	9,721	4,363	4,482	4,181
Sept. 2018	Spring	14,479	4,802	4,962	4,089
Nov. 2018	Summer	8,680	2,900	2,832	2,852
Mar. 2019	Autumn	34,447	9,977	9,828	8,897

(1 = WWTP 1, 2 = WWTP 2, RI = raw influent sample, BUVT = before UV treatment effluent sample, AUVT = after UV treatment effluent sample and FE = final effluent)

3.8.8.2 Degradation of wastewater sample with UV/Cl_2

Approximately 75 mL of phosphate buffer pH 7 solution was added to 170 mL of raw wastewater to ensure a ratio of 7:3 for sample: buffer solution. The solution was thoroughly mixed and a 4 × 50 mL portion was spiked with $Ca(ClO)_2$ to OAR 7:1 based on their concentrations as indicated in Appendix I. Thereafter, the procedure described in subsection 3.8.7 for the standard sample was followed accordingly for the degradation of the ARVs.

3.8.8.3 Degradation of wastewater sample with UV/$KMnO_4$

To 170 mL of raw wastewater was added 75 mL of phosphate buffer pH 5 solution to ensure ratio 7:3 for sample: buffer solution. The solution was thoroughly mixed and a 4 × 50 mL portion was spiked with $KMnO_4$ to OAR 7:1 based on their concentrations as indicated in Appendix J. Thereafter, the procedure described in subsection 3.8.7 for the standard sample was followed accordingly.

3.9 Statistical analysis

The data obtained in this research were analysed by graph pad prism software. For significant F value, the least significance difference (LSD) was utilised for mean comparison at 5% level. Statistical analysis was used to indicate significant differences ($P < 0.05$). The difference is detected as greatly significant when the P-value is lower than 0.001, statistically significant if P-value is lower than 0.05 and non-significant if the P-value is greater than 0.05. Microsoft Excel software was also used to execute the descriptive statistics.

3.10 Summary

This chapter focused on the scientific methods employed to successfully carry out this research study. It started by highlighting the theories and principles of the techniques used. This was followed by the sampling methodology, sampling location details and sample preparation.

Furthermore, monthly wastewater sampling from the two WWTPs between the autumn of 2018 and autumn of 2019 and the essential sample pretreatment was discussed. Thereafter, the prevailing physicochemical conditions in the two wastewater were evaluated through the determination of ten different physicochemical parameters in the wastewater samples using standard methods. The chapter also discussed the development of an accurate and cost-effective UV visible spectroscopic method for the effective determination of our three targeted ARVs compounds.

Likewise, the chapter showed how the two new AOPs which are sunlight (UV)/Cl_2 and sunlight(UV)/$KMnO_4$ were developed and optimised. The methods were used for the removal of the three pharmaceutical compounds of interest in Milli-Q water and the % removal was monitored with UV spectrophotometry. Finally, the established optimised conditions were used for the removal of the target compounds from the real wastewater samples and measured with the HPLC technique.

CHAPTER 4

PHYSICOCHEMICAL PROPERTIES OF WASTEWATER EFFLUENT FROM TWO SELECTED WASTEWATER TREATMENT PLANTS (CAPE TOWN) FOR WATER QUALITY IMPROVEMENT[β]

4.1 Introduction

Wastewater is complex matrices that contain numerous physical and chemical components that can influence the activities or reactions in the wastewater ecosystem. The 3rd objective of this work was to determine the level of specific physicochemical parameters in the wastewater samples collected from the two plants between autumn 2018 and autumn 2019. and to investigate their co-influence on the target ARVs. The procedure for this investigation was contained in section 3.5 in chapter 3.

This chapter discusses the results of ten physicochemical parameters evaluated. The parameter discussed here includes temperature, pH, EC, TDS, DO, SO_4^{2-}, COD, BOD_5, Cl^-, and PO_4^{3-}. The chapter also discussed the impact of these physicochemical parameters on the target ARVs within the sampling period.

4.2 Physicochemical properties to wastewater

The result of monthly variation of physicochemical properties of the final effluents from the WWTP 1 and 2 includes temperature, pH, EC, TDS, DO, SO_4^{2-}, COD, BOD_5, Cl^-, and PO_4^{3-} are presented below.

4.2.1 Monthly variation of temperature in the WWTPs 1 and 2

Figure 4.1 presents the monthly variation of temperature in WWTPs 1 and 2. The temperature ranged between 16.9 – 25.3 ^oC in both plants. The temperatures recorded in the two plants were lower compared to the values (19.0-36.0 ^oC) reported in the literature for wastewater effluents (Agoro et al., 2018). All the temperature range in both plants conformed with the regulatory limits for discharged effluent into water bodies by the Department of water affairs and forestry (DWAF), South Africa and the United State Environmental Protection Agency (USEPA) as shown in Table 4.1. The lowest temperature (16.9 ^oC) was obtained from the WWTP 2 while the highest temperature (25.3 ^oC) was obtained in plant 1. The variation in temperature range recorded between the two plants might have occurred because plant 1 received a lot of industrial

[β] G. S. Olabode, O. F. Olorundare, and V. S. Somerset (**2020**). Physicochemical properties of wastewater effluent from two selected wastewater treatment plants (Cape Town) for water quality improvement. *International Journal of Environmental Science and Technology*, Published. doi.org/10.1007/s13762-020-02788-9.

effluent in addition to the domestic effluent while plant 2 received mainly domestic and agricultural effluent. It has reported that effluent generated from industrial cooling processes is one of the principal sources of thermal pollution that result in high temperature (Lokhande et al., 2011).

Table 4.1: Regulatory guidelines by DWAF and USEPA (*Department of water affairs and forestry (DWAF), South Africa. **United State Environmental Protection Agency; Drinking Water Regulations and Health Advisories, EPA 822-R-94-001, May 1994).

Parameters	Units	*DWAF	** USEPA	Average concentration	
				WWTP 1	WWTP 2
T	oC	35	Below 35	21.06	20.77
pH	-	5.5-9.5	6.5–8.5	7.20	7.32
EC	(µS/cm)	750	2500	1133.04	1200.97
TDS	(mg/L)	450	500	725.15	768.67
DO	(mg/L)	7.5	6.0-9.5	4.21	3.97
SO42-	(mg/L)	200	250	60.50	55.88
COD	(mg/L)	75	below 1000	85.97	348.58
BOD	(mg/L)	3.0-6.0	below 500	40.20	107.18
Cl-	(mg/L)	100	250	176.06	115.61
PO43-	(mg/L)	10	4	3.57	5.52

The lowest temperature was recorded in July 2018 which happened to be the peak of winter in the southern hemisphere where the pilot study was conducted. The highest temperature value (25.3 ^{o}C) was recorded in March 2019, the peak of Autumn in the study area. As observed within the first five months (April – August 2018) of samples collection, the temperatures recorded in both plants except April and May in WWTP 2 are lower and fairly stable. They did not show any significant difference ($P > 0.05$). From September however, the temperature in the two WWTPs started rising and peak in February/March 2019. The temperature variation might have resulted mainly due to prevailing environmental factors viz a viz, climate change and seasonal variation in the Cape region, South Africa. The result observed in both plants is consistent with the findings for temperature variation of dairy wastewater (Tikariha and Sahu, 2014). They recorded a lower temperature during the winter while a higher temperature was recorded during the summer months (Tikariha and Sahu, 2014). A similar observation of temperature variation of 18.1 and 28.5°C was observed in the three different sampling periods which was also attributed to seasonal variations (Iram et al., 2013).

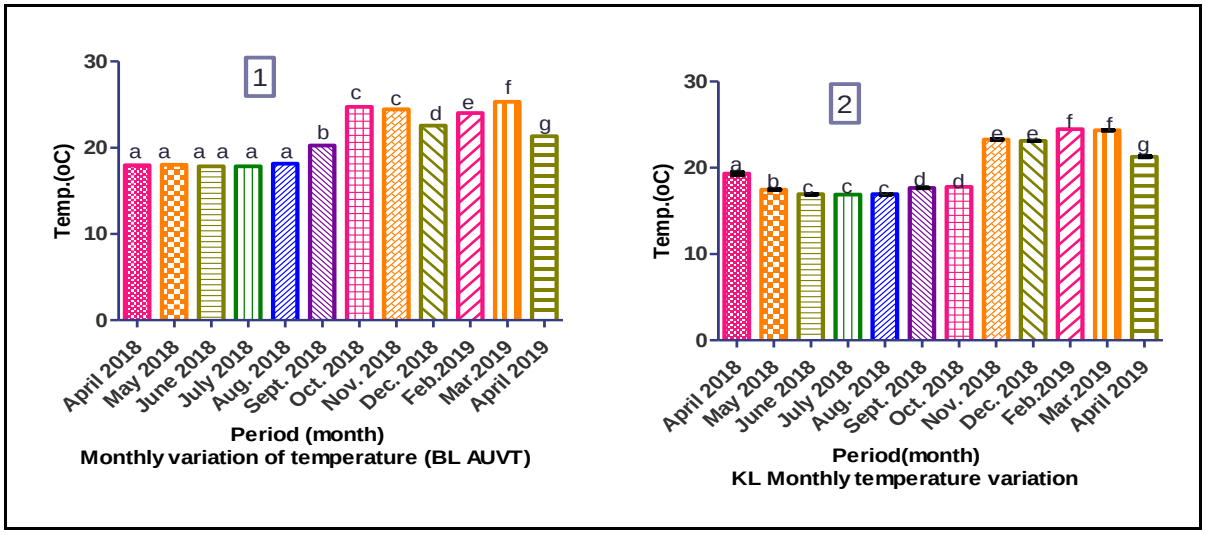

Figure 4.1: Results for monthly variation of temperature in discharged final effluents from the WWTP 1 and WWTP 2 samples.

4.2.2 Monthly variation of pH in the WWTPs 1 and 2

The results of the monthly variation of pH within the sampling period (April 2018-April 2019) in WWTP 1 and 2 are presented in Figure 4.2. The pH for the two WWTPs ranged between 5.85 in August 2018 and 7.85 in February 2019 which both occurred in WWTP 2. The pH range (5.85–7.85) recorded in WWTP 1 study shows a similar trend of observation to the pH range (6.1–7.7) reported in India for wastewater effluent from a dairy farm (Tikariha and Sahu, 2014). Most of the pH values recorded within the sampling period in WWTP 1 are around neutral (pH 7), but they are significantly different ($P < 0.05$) from one another. In WWTP 2, however, the pH values recorded in the majority of the months in WWTP 2 are fairly stable around pH 7.5 except August (5.85) and October (6.56) which were significantly different from one another and the rest of the months. The result obtained in WWTP 2 is close to the pH values (7.22, 7.78) as reported in other research work (Shrestha et al., 2017). The stability observed for these results could be because there was a steadiness in the pattern of the domestic influent discharge received by the plant.

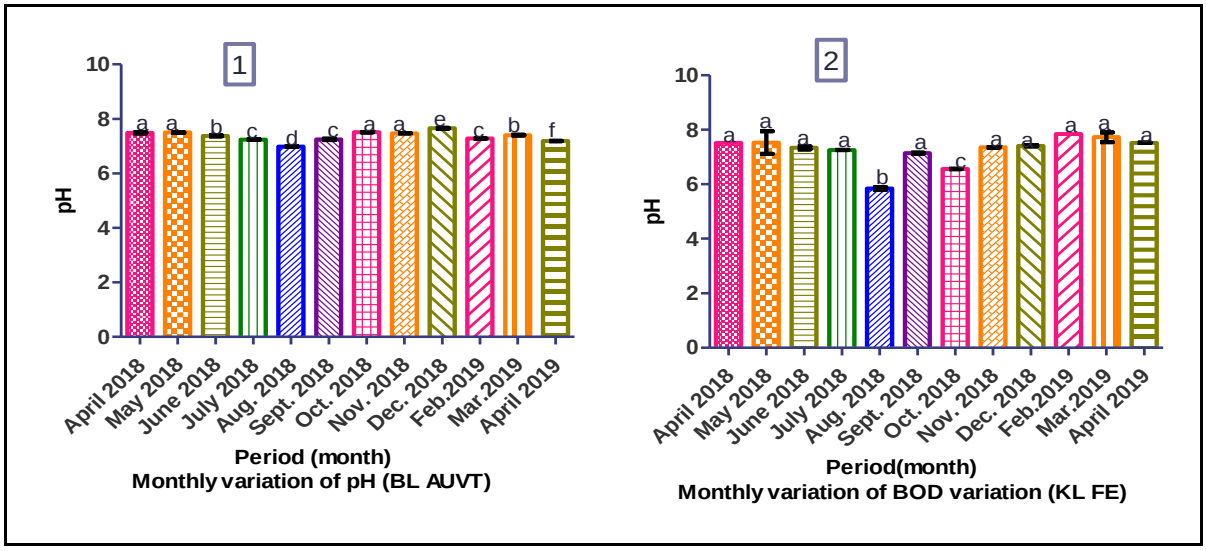

Figure 4.2: Results for monthly variation of pH in discharged final effluents from the WWTP 1 and WWTP 2 samples.

However, the stable pH values tending towards alkalinity could be a result of a high level of carbonate use in the treatment method. This was evident because the sample always produces foam during sampling. A similar observation was Rawalpindi and Islamabad in Pakistan (Iram et al., 2013). In their study, they reported that a high level of pH and organic matter content could render the majority of trace elements immobilized which invariably could lead to an increase in pH value (Iram et al., 2013). The pH range in the two plants complies with the regulatory limit for wastewater discharge effluent to water bodies by both DWAF and USEPA (Table 4.1).

4.2.3 Monthly variation of EC the WWTPs 1 and 2

Results of monthly variation of EC in WWTPs 1 and 2 between April 2018 and April 2019 are presented in Figure 4.3 The EC in WWTP 1 varied between 923.00 in April 2019 and 1,294.17 µS/cm in October 2018, respectively. The lowest (923.00 µS/cm) and highest (1,294.17 µS/cm) EC values occurred in WWTP 1 and WWTP 2, respectively. The lowest EC value (923.33 µS/cm) in April 2019 varies significantly ($P < 0.05$). from all other ECs values obtained during the sampling months and it occurred in Autumn. Likewise, the highest EC value was recorded in October which was the Summer period. This point to the fact that seasonal variation could affect EC value This was clearly shown in August 2018 when the EC value dropped to 1055.50 µS/cm which could be attributed to the very cold weather in the winter. However, a steady and gradual increase in EC sets in from September in both WWTPs which marks the beginning of spring and continues through the summer period. In both WWTPs, (except July 2018) lower values of EC experienced during winter/autumn and higher EC values recorded in the spring/summer could be the effect of variation in temperature within the sampling period. This observed trend is in agreement with the report from another research work that electrical conductivity is usually higher in the summer and decreases in the winter because temperature variation affects electrical conductivity (Kriegel and Palmour III, 2012). A similar trend was also observed at the Parkside sampling site area during the winter period in the determination of physicochemical variation of Buffalo River estuary, in Eastern Cape Province of South

Africa. The EC value recorded was significantly lower than the values observed for the other seasons (Chigor et al., 2013). An earlier study conducted using five different types of water with different salinity and chemical composition confirmed that, at the real environmental monitoring range of 0-30 ^{o}C, the relationship between temperature and EC is directly proportional and linear (Hayashi, 2004; Pal et al., 2015). It was also reported that the EC of Rudrasagar lake in India had maximum EC concentration during the summer than in the winter (Pal et al., 2015). All these show that in an aquatic ecosystem, an increase in temperature could enhance the performance of EC as the flow of dissolved ions that make up EC could be more active, this was consistent with our finding.

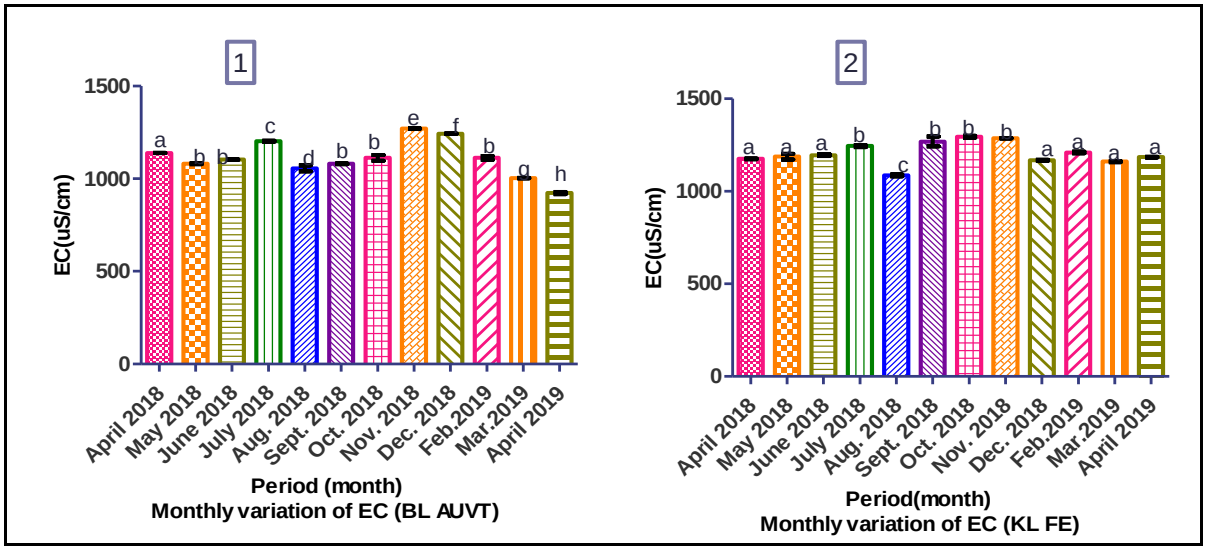

Figure 4.3: Results for monthly variation of EC in discharged final effluents from the WWTP 1 and WWTP 2 samples.

The range of EC in WWTP 2 (1085.67 – 1294.17 µS/cm) was observed to be a little higher than that observed in WWTP 1 (923.00–1270.33 µS/cm). This could be the effect of the higher altitude in WWTP 1 compared to WWTP 2. A similar result was reported by Oyem et al. (2014) that lower ECs (8.23 – 14.46 µS/cm) and (8.24 – 8.25 µS/cm) were recorded in Owa Alero and Alihame in Nigeria, respectively, with lower altitude whereas an average of 12.22 µS/cm and 12.23 µS/cm was reported respectively for Agbor and Owa that have higher altitude (Oyem et al., 2014). The relationship between EC and altitude could be attributed to the effect that WWTP 1 with higher altitude could have a lower water table and hence experience a higher net leaching effect than WWTP 2 (Oyem et al., 2014). The EC values in both plants did not comply with the DWAF standard (750 µS/cm) limit but they all comply with the USEPA discharge limit of 2500 µS/cm (Table 4.1).

4.2.4 Monthly variation of TDS in the WWTPs 1 and 2

Figure 4.4 presents the monthly variation of TDS recorded for wastewater effluent from WWTP 1 and 2. The TDS values in both WWTPs ranged between 590.72 in April 2019 and 828.27 mg/L in October 2018, respectively. The lowest and highest concentrations were obtained from WWTP 1 and WWTP 2, respectively. The TDS range (590.72 – 828.27 mg/L) in both plants

are fell within the range (20.3 – 23,350 mg/L) reported for Buffalo River water in Eastern Cape, South Africa (Chigor et al., 2013). All the TDS values in both WWTPs are higher than the recommended standards for wastewater discharge by both DWAF (450 mg/L) and 500 mg/L by USEPA. The result within the sampling period shows varying significant differences ($P < 0.05$). The lowest TDS value in WWTP 1 (590.72 mg/L) in April 2019 and the lowest value in WWTP 2 (694.83 mg/L) in August 2018 are significantly different ($P < 0.05$) from the TDS concentration in all the other sampling periods. The highest concentration (813.01 mg/L) in WWTP 1 differs significantly ($P < 0.05$) from all other TDS values. The highest TDS (828.27 mg/L) in WWTP 2, on the other hand, shows no significant ($P > 0.05$) differences between 796.05 mg/L in July, 811.84 mg/L in September and 823.60 mg/L in November 2018. This point to the fact that the TDS values in WWTP 2 are generally higher than what is obtainable in WWTP 1.

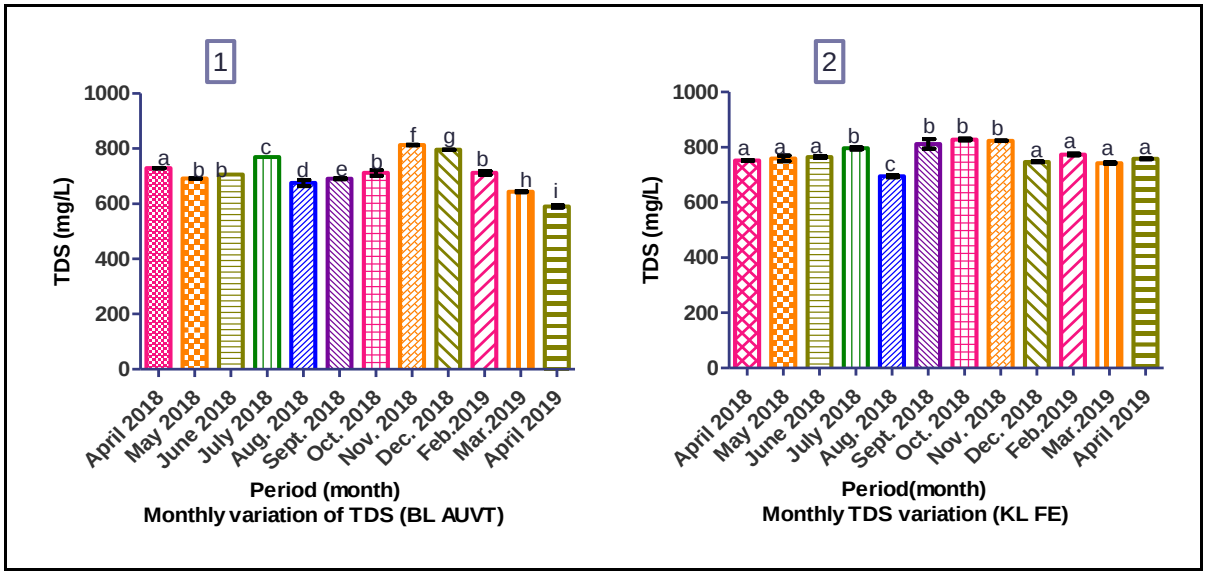

Figure 4.4: Results for monthly variation of TDS in discharged final effluents from the WWTP 1 and WWTP 2 samples.

The concentrations of TDS (742–774 mg/L) in about 60% of the sampling periods in WWTP 2 are fairly stable and they did not show any significant differences ($P > 0.05$). This could probably point to a level of stability in the quantity of salts content that flow to the plant which as a result of stability in the lifestyle of people around the WWTP 2. It could also suggest a level of precision, stability or efficiency of the treatment plant in removing dissolved solids. Comparing the two plants, we could say that WWTP 1 is more efficient than WWTP 2 in removing TDS.

The lowest concentrations of TDS recorded in both plants (WWTP 1: 590.72 mg/L; WWTP 2: 694. 83 mg/L) within the sampling period is close to the average TDS (644.5 mg/L) obtained from effluents from engineering industries from Taloja Industrial Area of Mumbai, India (Lokhande et al., 2011).

TDS is a measure of water salinity and it determines the concentration of salts in a water body. . These salts largely include chlorides, carbonates, bicarbonates, sulphates, phosphates, and nitrates of calcium, magnesium, sodium, potassium, iron, and manganese (Rusydi, 2018). The

density of water can be greatly affected by the quantity of dissolved solid elements in it. This can consequently, influences osmoregulation in aquatic organisms, reduces the solubility of gases such as oxygen and this can eventually determine the application/uses of such water (Lokhande et al., 2011; Sun et al., 2015).

4.2.5 Monthly variation of DO in the WWTPs 1 and 2

Figure 4.5 illustrates the monthly variation of DO in WWTP 1 and 2 that ranged between 1.30 mg/L and 5.50 mg/L. The lowest and highest DO were both recorded at WWTP 2. The lowest DO concentration in September (1.30 mg/L) in WWTP 2 was significantly different from the DO content in any other months ($P < 0.05$). This could likely be due to the dredging activities that took place at the sampling site in September 2018. The dredging was done to remove the sediments to allow easy flow of the wastewater. The samples collected during that month were very cloudy, dirty and foul odour.

The relatively high concentration of DO recorded from October 2018 to April 2019 in WWTP 2 could probably be due to the longer duration of bright sunlight during the long day in spring/summer/autumn. This is consistent with the report of Patil et al. (2012) that the longer day and the extended bright sunlight duration experienced during summer enhances photosynthesis by phytoplankton which make use of carbon dioxide and eventually releases more oxygen into the water body. On the other hand, lower DO values (3.41–3.67 mg/L) was recorded around the same period (December 2018 to April 2019) in WWTP 2 compared to what was obtained in the earlier part of the sampling period. This could be attributed to the presence of some inorganic compounds present in the effluent from the surrounding industries that compete with the available oxygen in the effluent. The previous study reported that inorganic compounds such as ammonium nitrates, hydrogen sulphates, and ferrous ions present in water could compete for oxygen in water thereby lowering its DO content (Agoro et al., 2018).

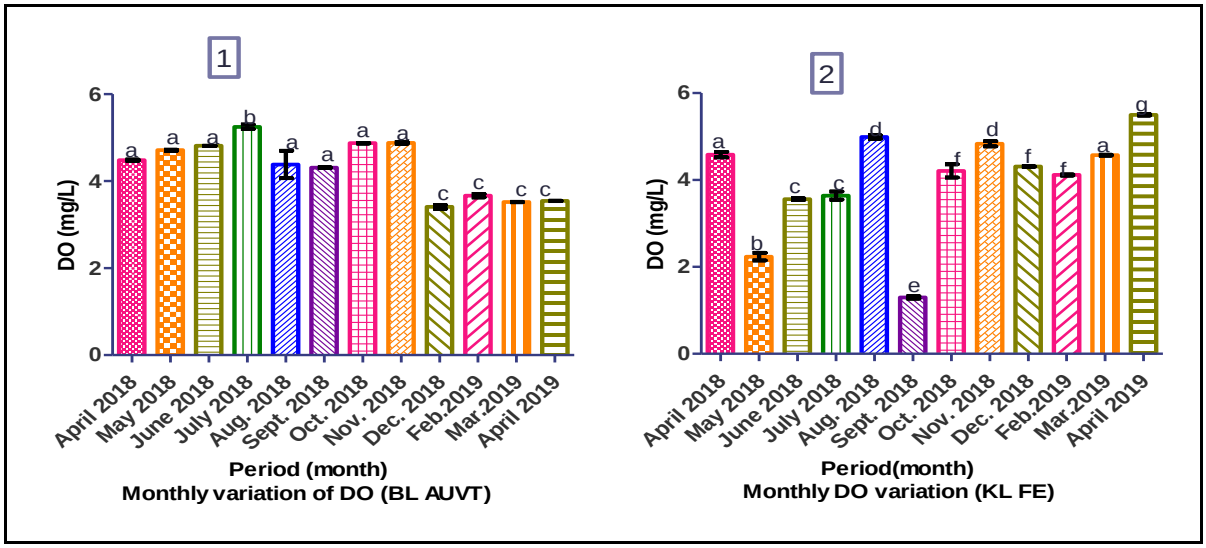

Figure 4.5: Results for monthly variation of DO in discharged final effluents from the WWTP 1 and WWTP 2 samples.

Some previous findings also reported that DO is a parameter for measuring the level of pollution by organic matter, it guides the physical and biological activities in water such as the rate of decomposition of organic substance (Osuolale and Okoh, 2015; Agoro et al., 2018). The discharge effluents with low dissolved oxygen, high BOD and turbidity is an indicator of potential danger to the receiving surface water. Low concentrations of dissolved oxygen, when combined with the presence of toxic substances, could constitute a stress to aquatic ecosystems because the toxicity of some elements increases with low concentrations of DO (Osuolale and Okoh, 2015). The standard DO value acceptable for aquatic organisms is 4–5 mg/L (Agoro et al., 2018), the result from the two WWTPs indicates that about 67% of the recorded DO values (4–5 mg/L) is good for aquatic life. Nevertheless, the DO values recorded in both plants (1.3–5.50 mg/L) ranged below the recommended standards for both DWAF (7.5 mg/L) and USEPA (6-9 mg/L).

4.2.6 Monthly variation of SO_4^{2-} in the WWTPs 1 and 2

Figure 4.6 presents the monthly variation of SO_4^{2-} in WWTPs 1 and 2. The SO_4^{2-} concentrations in the two WWTP s ranged between 3.23 and 163.18 mg/L in June 2018 and November 2018 respectively within the sampling period. Both the lowest and highest SO_4^{2-} levels were recorded in WWTP 1. The values were generated from the standard calibration curve of sulphate in Appendix A. The result shows a level of variation within the sampling period. The concentrations of SO_4^{2-} in April 2018, June 2018, July 2018, September 2018 and April 2019 were very low (3.23–11.21 mg/L). Similar results were also obtained in June 2018 (19.18 mg/L), July 2018 (4.62 mg/L) and August 2018 (7.93 mg/L) in WWTP 2. This could be ascribed to a high level of organic matter in the wastewater leading to a low level of dissolved oxygen in the wastewater. An earlier report showed that if an aquatic environment is deprived of sufficient oxygen, sulphate being an electron acceptor could be used to break down the organic matter present in water to produce hydrogen sulphide with a rotten egg smell (Kavitha et al., 2012).

The concentrations of sulphate in other months in both plants were higher within the sampling period. This is likely to be associated with excessive use of detergent and soap in WWTP 1. Likewise, cartridge wastes containing ink and barium sulphate used as an additive in plastic industries could be responsible for the high level in WWTP 2 (Shrestha et al., 2017).

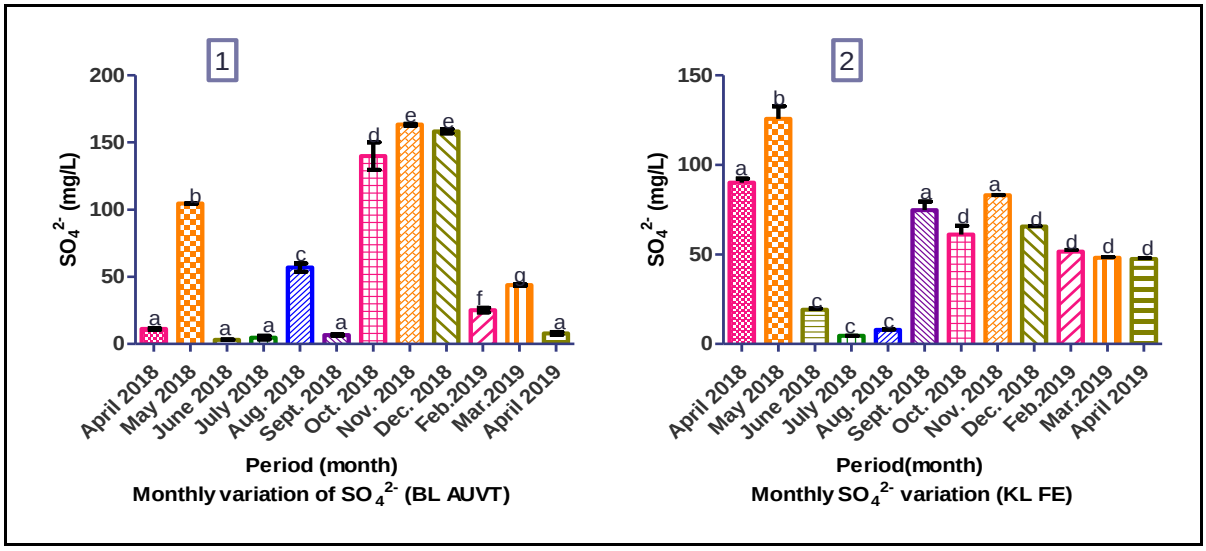

Figure 4.6: Result for monthly variation of SO_4^{2-} in discharged final effluents from the WWTP 1 and WWTP 2 samples.

The values of SO_4^{2-} in both plants within the sampling period are lower than 300 mg/L reported for effluents from a pharmaceutical industry in Bangalore, India (Kavitha et al., 2012). It was, however, higher than the 15.97 – 87.38 mg/L reported for wastewater effluent in Nullah Lai and Koh-e-Noor textile in Pakistan (Iram et al., 2013). The normal level of sulphate in the water had been reported to be optimal for the plant need, a low level of SO_4^{2-} could cause laxative in human and excessive sulphate in water could lead to diarrhoea and other gastrointestinal diseases (Kavitha et al., 2012; Agoro et al., 2018). The level of sulphate in both WWTPs fell within the recommended limits by both DWAF (200 mg/L) and 250 mg/L by USEPA (see Table 4.1).

4.2.7 Monthly variation of COD in the WWTPs 1 and 2

Figure 4.7 presents the monthly variation of COD in WWTP 1 and 2 within the sampling period which was produced from the standard calibration curve of the KHP standard (Appendix B). The result from the two WWTPs showed that the COD concentration ranged from 23.70 – 898.58 mg/L in WWTP 1 and 2, respectively. The result shows that the concentration of COD in WWTP 1 were generally lower compared with that in WWTP 2. The value of COD obtained in 50% of the sampling period in WWTP 1 in April 2018 (24.75 mg/L), July 2018 (23.70 mg/L), September 2018 (54.35 mg/L), October 2018 (35.49 mg/L), November 2018 (66.33 mg/L) and February 2019 (33.33 mg/L) falls within the recommended values for DWAF (75 mg/L) for wastewater discharge but all the levels in WWTP 2 are higher than the permissible limit of 75 mg/L by DWAF. As per the USEPA recommended limit, the COD level in both plants comply with the limit (< 1000 mg/L) set by USEPA.

This indicates that WWTP 1 was a little effective in removing COD pollution than WWTP 2. The higher level of COD in WWTP 2 could be associated with high population density and illegal dumping of organic materials due to the high level of informal settlement around WWTP 2. The concentrations of COD (23.70–898.58 mg/L) in the two plants within the sampling period was lower compared to 352–927 mg/L reported for the final effluents from Waluj industrial area in Indian (Kale and Bandela, 2016). The values were however higher than 46.80–93.60 mg/L reported for Boji Owa and Owa-Alero in Nigeria (Oyem et al., 2014).

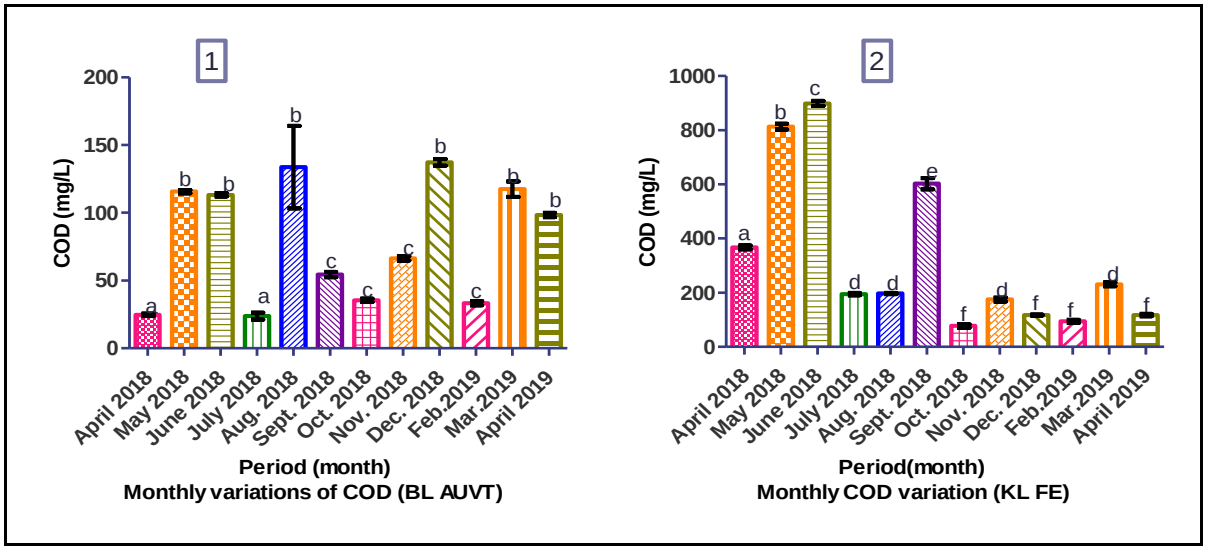

Figure 4.7: Results for monthly variation of COD in discharged final effluents from the WWTP 1 and WWTP 2 samples.

COD values indicate the level of domestic and industrial pollution in water and wastewater. It measures the amount of oxygen required for the oxidation of organic matters in water under specified conditions of oxidizing agent usually potassium dichromate and temperature (150 ^{o}C) for 2 hours (Kale and Bandela, 2016). A high quantity of COD is an indication that the effluent could contain hazardous materials that are resistant to microbial degradation and these are dangerous to the receiving water bodies (Paltahe et al., 2018).

4.2.8 Monthly variation of BOD in the WWTPs 1 and 2

The monthly variation of BOD in WWTPs 1 and 2 is presented in Figure 4.8. BOD levels ranged between 9.17 and 252.44 mg/L in July 2018 and March 2019, respectively. The lower level of BOD could arise from the effectiveness of WWTP 1 efficiency in removing organic matter (Osuolale and Okoh, 2015). The high level of BOD, particularly in WWTP 2 could be the result of direct discharge of plants, animals and other domestic waste into the sewage system by the residents which dwell mostly in an informal setting around WWTP 1 (Paltahe et al., 2018). The concentration range (9.17 – 252.44 mg/L) in both plants is higher than 3.7 – 14 mg/L reported for effluent from municipal wastewater from Eastern Cape, South Africa (Adewumi et al., 2010; Agoro et al., 2018). The values in both plants were however lower compared to 535.80 mg/L and 604.80 mg/L reported for wastewater effluents from paint and engineering industries, respectively (Lokhande et al., 2011) These values were also lower than 535.80 mg/L and 776.20 mg/L reported for effluents from paint and dyes industries, respectively (Paltahe et al., 2018).

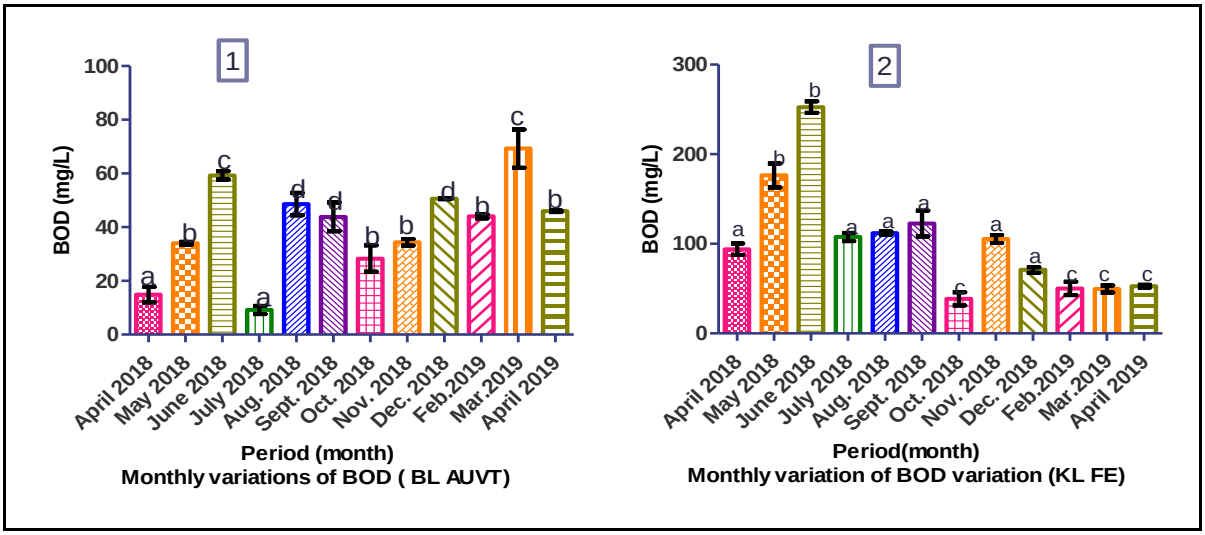

Figure 4.8: Results for monthly variation of BOD in discharged final effluents from the WWTP 1 and WWTP 2 samples.

The BOD in both WWTPs are far above the recommended limit of 3–6 mg/L for the discharged effluent by DWAF but all of them fell within the 500 mg/L limits set by USEPA (Table 1). Based on the result obtained in the two plants, the levels of BOD in WWTP 2 (38.54 – 252.44 mg/L) were generally higher than the values in WWTP 1 (9.17 to 69.25 mg/L). Low BOD content in water suggests good and qualitative water. High BOD concentration indicates a high level of organic matter content and this signifies potential danger to the receiving water bodies. This reduces the quantity of dissolved oxygen available in water and constitutes a potential risk to aquatic organisms in the water body (Osuolale and Okoh, 2015; Paltahe et al., 2018).

4.2.9 Monthly variation of Cl^- in the WWTPs 1 and 2

Figure 4.9 illustrates the monthly variation of chloride ion in WWTP 1 and 2 within the sampling period of April 2018 and April 2019. The values were generated from the calibration curve of chloride standards Appendix C. The chloride concentration in both WWTPs varied between 48.17 mg/L in WWTP 2 and 378.48 mg/L in WWTP 1 respectively. The lowest concentration (48.17 mg/L) recorded in WWTP 2 was not significantly different ($P < 0.05$) from 50.60 mg/L and 59.19 mg/L in June 2018 and December 2018, respectively but differed significantly from what was obtained in other months within the sampling period in WWTP 1.

The highest concentration recorded in May in WWTP 1 was significantly different from the concentrations obtained in other months. The variation in the chloride concentrations in the two plants showed that the concentration of chloride in WWTP 2 conforms to both DWAF and USEPA better than what was obtainable in WWTP 1. About 20% of chloride concentration complies with the recommended limit (100 mg/L) by DWAF and 75% conforms with the USEPA limit of 250 mg/L in WWTP 1, In WWTP 2, however, 80% of chloride concentrations were within the 100 mg/L recommended by DWAF and all (100%) of the chloride concentrations conforms with USEPA (250 mg/L) limits. The results obtained in the two plants were higher than 3.25 – 224 mg/L reported in the literature (Agoro et al., 2018) but lower than

(127.72 – 396.16 mg/L) reported for Nullah Lai and Koh-e-Noor textile industries located in Rawalpindi and Islamabad, Pakistan (Iram et al., 2013).

The human diet is one of the major sources of sodium chloride found in WWTPs, they are readily soluble in wastewater and not substantially affected by wastewater treatment processes (Chigor et al., 2013). When the concentration of sodium chloride in water is above 250 mg/L, the water becomes unpleasant due to the salty taste and also unfit for irrigation purpose (Chigor et al., 2013). Similarly, in an aquatic ecosystem, a high chloride content can threaten the sustainability of ecological food sources, thereby constituting dangers to the survival of some species because their growth and reproduction are seriously affected (Aniyikaiye et al., 2019).

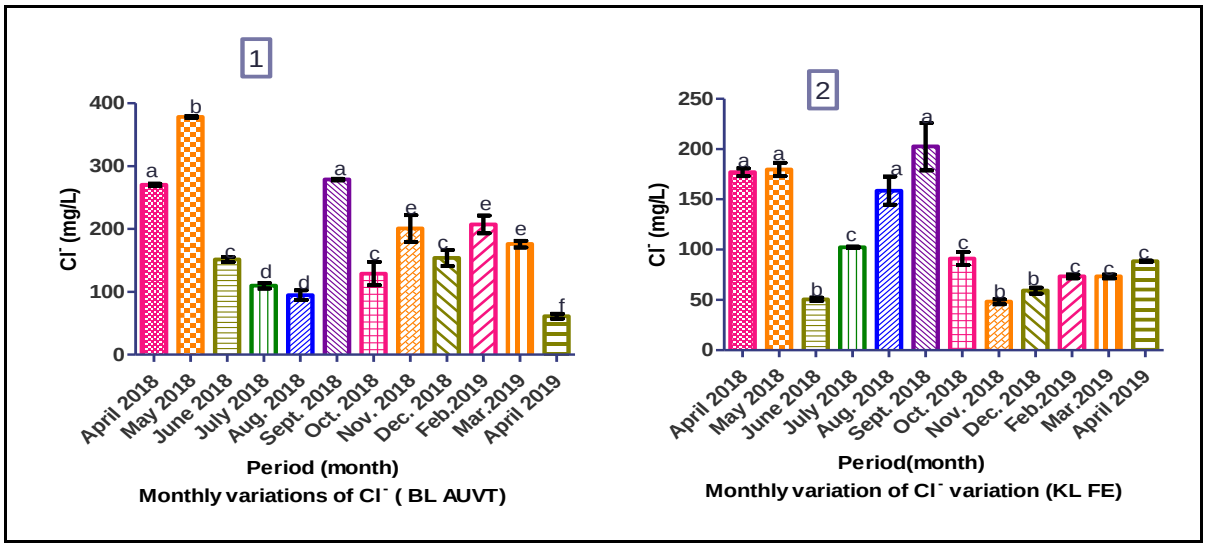

Figure 4.9: Results for monthly variation of Cl^- in discharged final effluents from the WWTP 1 and WWTP 2 samples.

4.2.10 Monthly variation of PO_4^{3-} in the WWTPs 1 and 2

Figure 4.10 presents the monthly variation of phosphate in WWTPs 1 and 2. The concentrations varied from 0.10 mg/L in September 2018 to 11.32 mg/L in November 2018. The values were generated from the standard calibration curve of phosphate (Appendix D). The lowest and highest concentrations were observed in WWTP 1. This demonstrates the extent of variation in the phosphate concentration in WWTP 1 than WWTP 2. The phosphate concentrations range obtained in plant 2 was more consistent within the sampling period because there was no significant difference ($P > 0.05$) between the concentrations recorded in about 70% of the sampling period. The concentrations (0.1 – 0.51 mg/L) in the first six months of the sampling period (except May 2018) were very low. An abrupt rise in the concentration of PO_4^{3-} occurred in October 2018 and continued for the rest of the sampling period. A similar trend was also observed in the last four months in WWTP 2. This can be attributed to different factors which include uncontrolled use of fertilizers in agricultural practices, the influx of effluent from food and meat processing industries and undue detergent waste disposal from households. Similar high phosphate pollution was observed in all the effluent samples investigated in Nepal by Shrestha et al. (2017).

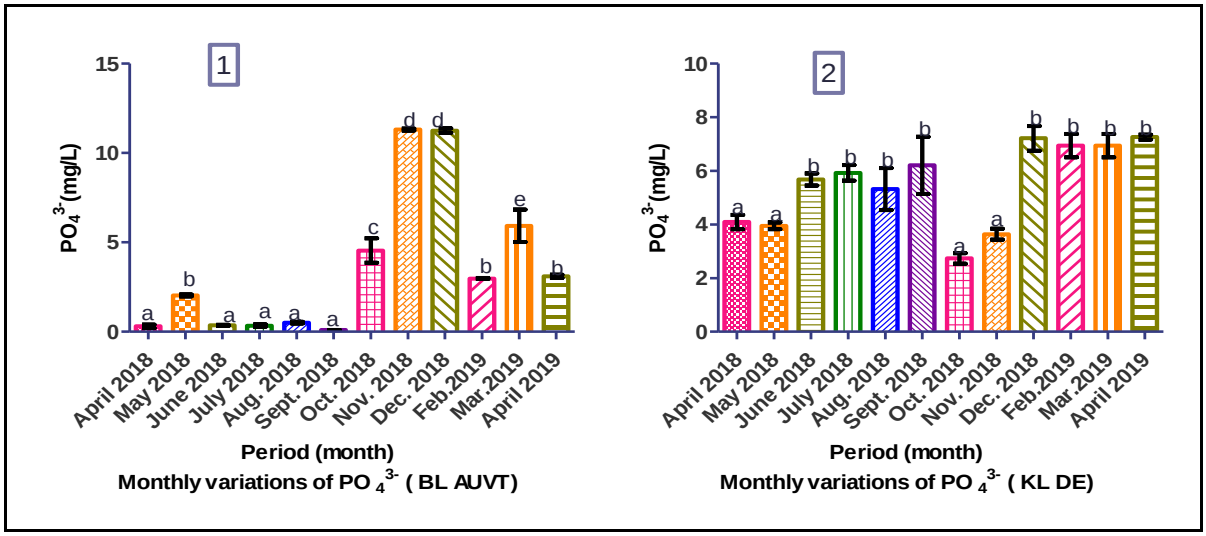

Figure 4.30: Results for monthly variation of PO_4^{2-} in discharged final effluents from the WWTP 1 and WWTP 2 samples.

The concentration range recorded in the major parts of the sampling period in the two plants (10 out of 12 months in WWTP 1 and 8 out of 12 months in WWTP 2) were similar to 0.04–5.00 mg/L reported for wastewater effluent in Eastern Cape Province, South Africa (Agoro et al., 2018). More than 90% of the phosphate concentrations in the two plants comply with the DWAF set limit of 10 mg/L. Based on the USEPA standard, 8 months (about 70%) conforms in WWTP 1 while only 3 months complies in WWTP 2 (Table 4.1). The range of the PO_4^{3-} in the entire sampling period was close to (1.21 to 9.92 mg/L) reported for five wastewater effluents from an industrial area in Nepal (Shrestha et al., 2017). Reports have shown that a high concentration of phosphate and nitrates promote eutrophication in the aquatic ecosystem which can increase oxygen demand which and hence cause several objectionable ecological problems in the affected ecosystem (Agoro et al., 2018; Paltahe et al., 2018).

4.3 Evaluation of impact physicochemical parameters on target ARVs

Wastewater is a complex environmental matrix with numerous physicochemical parameters that determine the complex reactions that are taking place there (Metcalfe et al., 2010; Fatta-kassinos et al., 2011; Ferrer and Thurman, 2012; Pereira et al., 2015; Antonin et al., 2015; Schoeman et al., 2015; Aminot et al., 2016; Boix et al., 2016). It has been established that whatever happen to one component in an aquatic ecosystem like wastewater is influenced by other physicochemical parameters present therein (Iram et al., 2013; Hübner et al., 2015; Miklos et al., 2018; Aniyikaiye et al., 2019). Upon this understanding, we considered the impacts of the determined physicochemical parameters on our target pharmaceutical formulations.

4.3.1 Effect of temperature changes on the monthly variation of 3TC, AZT AND NVP

The effect of temperature changes on the monthly variation of 3TC, AZT and NVP in WWTP 1 and 2 within the sampling period is illustrated in Figure 4.11. The temperatures in both plants ranged between 16.9 – 25.3 ºC, 3TC ranged between 1,178.20 – 5,289.16 µg/L, AZT between 86.79 – 6,698.20 µg/L and NVP between 5,469.00 – 20,770.35 µg/L. In WWTP 1, between April and August 2018, the temperature slightly dropped from 18 – 17.87 ºC, and simultaneously increased the concentrations of 3TC from 3,400 – 4,700 µg/L, AZT from 539 – 683 µg/L and NVP from 8,215–9,707 µg/L.

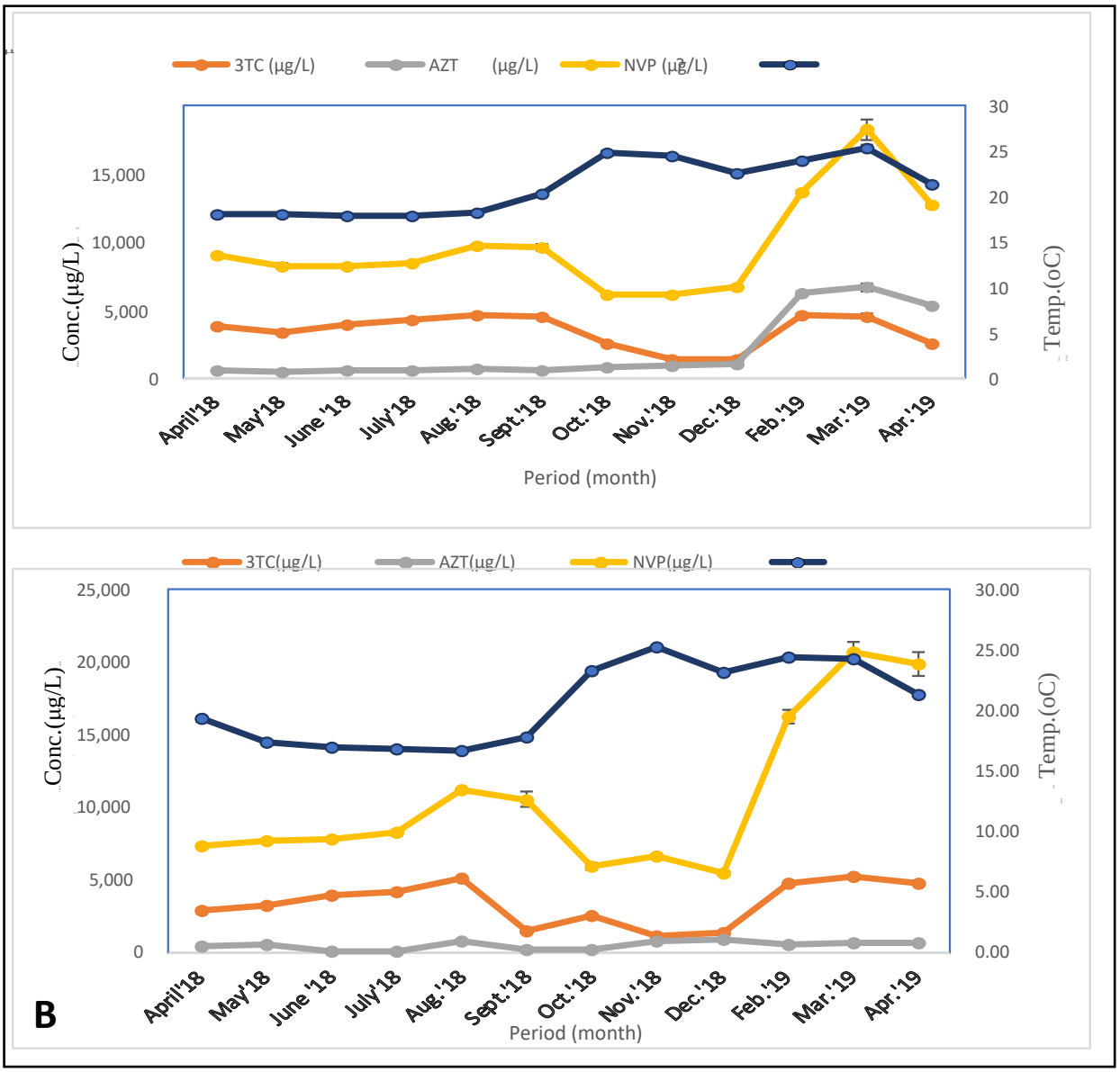

Figure 4.41: Effect of temperature on the monthly variation of 3TC, AZT and NVP in the final effluents from the WWTP 1 and WWTP 2.

Similarly, in the WWTP 2, the temperature reduces from 19.33 – 16.67 ºC and concurrently increased the concentrations of 3TC (2,933 – 5,096 µg/L), AZT (430 – 773 µg/L) and NVP (7,375 – 11,204.55 µg/L). Observably, the increase in the concentrations of the three ARVs that

were recorded between April and September 2018 in both plants could be attributed to the low temperature recorded around Autumn/winter.

Between October and December 2018 in both plants, the temperature rose to an average of 24^{o}C. Within this period of temperature increase, a greater level of reduction in the concentrations of 3TC (4,567 – 1,407 µg/L) and NVP (9,661 – 6,135 µg/L) for WWTP1 and 3TC (5,096 – 1,178 µg/L) and NVP (11,204 – 5,469 µg/L) for the WWTP 2 were recorded. This effect could be attributed to an increase in temperature and higher solar radiation in the summer.

Finding derived validates some previous results that claimed that the removal of pharmaceutical products is enhanced during the warm season (Matamoros et al., 2015). Also, this is in agreement with other scientific reports that asserted that high temperature and elevated radiation are abiotic environmental factors that enhance the removal of pharmaceutical compounds in wastewater (Fatta-kassinos et al., 2011; Al Qarni et al., 2016). In essence, this is because an increase in temperature increases the rate of degradation of the compounds due to the enhanced physical and chemical reactions (Iram et al., 2013)

4.3.2 Effect of pH changes on the monthly variation of 3TC, AZT and NVP

The effect of pH changes on the monthly variation of 3TC, AZT and NVP in wastewater samples from the WWTP 1 and 2 is depicted in Figure 4.12. The pH ranged between 6.50 – 8.50, while 3TC ranged between 1,178.20 – 5,289.16 µg/L, AZT between 86.79 – 6,698.20 µg/L and NVP between 5,469.00 – 20770.35 µg/L. In both plants, the concentrations of the three ARVs increase as the pH decreases and vice versa in most of the months within the sampling period. Noticeably, as pH decreases from 7.50 – 6.99 between May and August 2018 a gradual increase in the concentration of the three ARVs was recorded in the two plants. In WWTP 1, , 3TC increases from 3,400 – 4567 µg/L, AZT from 539.40 – 683.16 µg/L and NVP from 8,215.42 – 9707.51 µg/L. Whereas in WWTP 2, 3TC increases from 4,180.03 – 5,096.06 µg/L, AZT 119.05 – 773 µg/L and NVP from 8,245.06 – 11,204.55 µg/L as a result of decrease in pH from 7.53 – 5.85.

Similarly, an increase in pH of 6.99 – 7.66 in WWTP 1 and 7.15 – 7.85 in WWTP 2 from August – December 2018 except October 2018 in WWTP 2, a decrease in the concentrations of the ARVs were observed. 3TC reduces from 4,698.47 – 1,458.58 µg/L and NVP 9,707.51 – 6,161.54 µg/L in WWTP 1, while 3TC decreases from 5,096.06 – 1,178.20 µg/L, AZT from 773.78 – 234.78 and NVP from 11,204.55 – 5,469.00 µg/L in WWTP 2.

This variations in concentration could be a result of a change in the pH. In this case, our findings substantiate the report that specified that the changes in the pH coupled with some physicochemical parameters like salinity and organic matter can determine the fate of pharmaceutical residues in an aquatic ecosystem (Gaw et al., 2014). This is also consistent with the earlier reports that the nature of consumed food can affect the absorption of ARVs compounds (Beach, 1998; Cimino et al., 2018). For instance, protein can bind about 40% of

3TC and food rich in fat could delay AZT absorption (Beach, 1998; Cimino et al., 2018). This can occur by altering the electrostatic interactions of charged compounds (Kyu et al., 2015).

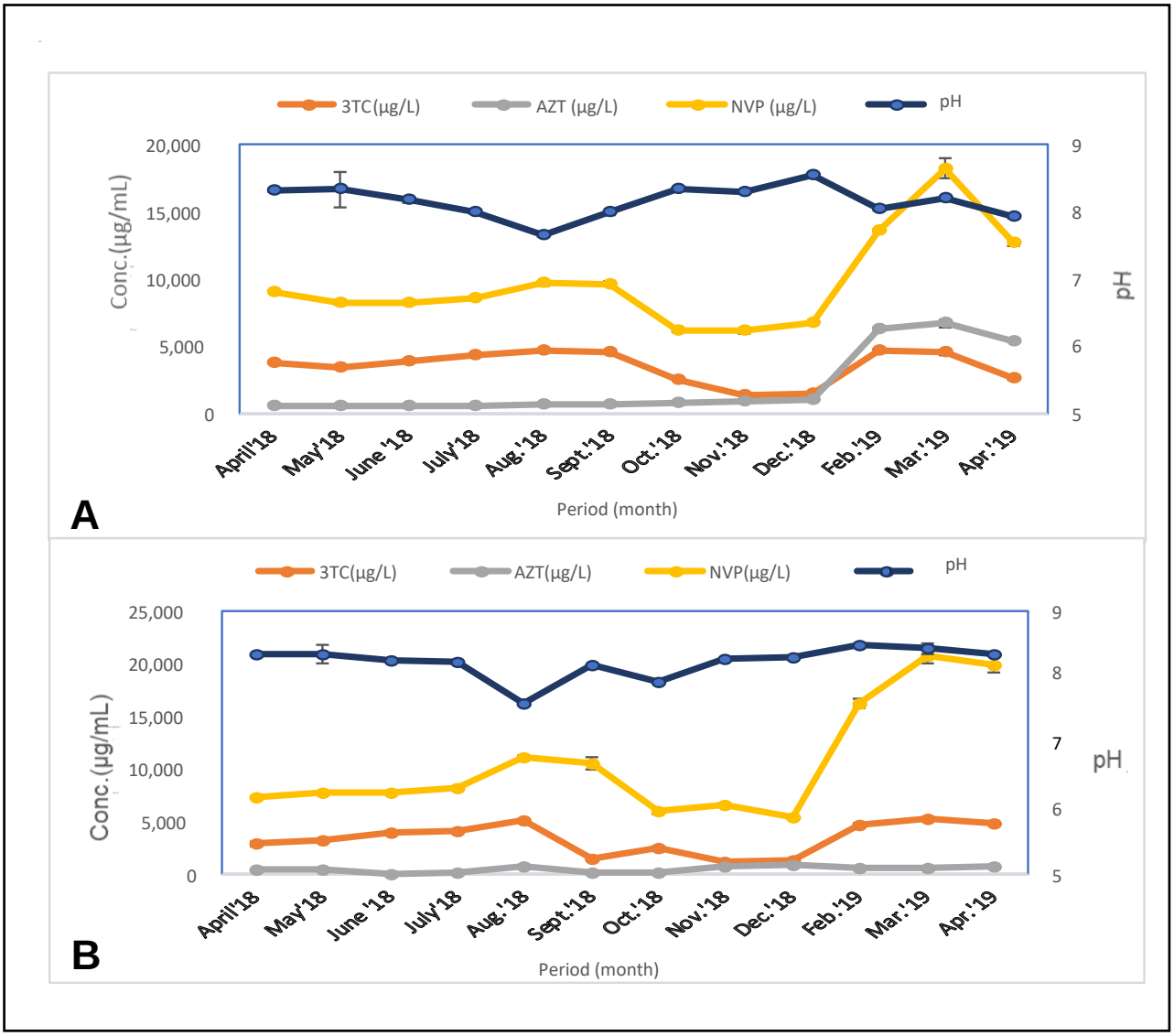

Figure 4.52: Effect of pH on the monthly variation of 3TC, AZT and NVP in the final effluents of WWTP 1 and WWTP 2 wastewater samples.

Research has reported that pH is one of the most important biotic factors that control many chemical reactions in the environment because of their sensitivity to changes in the pH (Kale and Bandela, 2016). The pH of wastewater during the treatment process is very vital for the elimination of organic compounds and heavy metals. Basic pH enhances the precipitation of some metals (Aniyikaiye et al., 2019).

4.3.3 Effect of changes in EC monthly variation of 3TC, AZT and NVP

Graphical illustration of the effect of changes in EC on the monthly variation of 3TC, AZT and NVP in the final effluent samples from the WWTP 1 and 2 is presented in Figure 4.13. The EC ranged between 923.00 – 1,270.33 µS/cm in the two WWTPs while 3TC, AZT and NVP ranged

from 1,178.20 – 5,289.16 µg/L, 86.79 – 6,698.20 µg/L and 5,469.00 – 20,770.35 µg/L, respectively. Between May and July 2018, the EC steadily increased from 1,081.00 – 1,203.00 µS/cm in WWTP 1. Similarly, in WWTP 2, the EC also gradually increased from 1,175.17–1,243.83 µS/cm. The ARVs concentrations were found to slightly increase along with the same trend within this period, except AZT in June 2018 in WWTP 1, and June and July 2018 in WWTP 2. In August 2018, the EC concentrations dropped and the concentration of the three ARVs increased. The increase in the ARV concentration could be associated with the low temperature during the peak of winter in August which could lower the effect of EC on the ARVs removal. This was consistent with a report that the EC of Rudrasagar lake in Indian had higher EC during the summer than the winter season (Pal et al., 2015). These show that in an aquatic ecosystem, an increase in temperature could enhance the concentration, the flow and the reactivities of dissolved ions that constitute EC to promote the removal of our compounds and EC and vice versa.

From September 2018, the EC began to rise until it reaches the peak of 1,270.33/1,243.67 µS/cm in November/December 2018 in the WWTP 1 and 1,294.17/1,268.00 µS/cm in October/November in WWTP 2. Around this period, a reduction in the concentration of 3TC (4,567.01 – 1,407.54 µg/L) and NVP (9,661.40–6135.90 µg/L) was recorded in WWTP 1. In addition, a reduction from 5,096.06–1,178.20 µg/L, 773.40–234.24 µg/L and 11,204.55 – 5,469.00 was recorded for 3TC, AZT and NVP, respectively in the WWTP 2. The decrease in concentrations observed in the two plants from October–December 2018 can probably be attributed to the synergy effect of the increase in ECs coupled with the rise in temperatures. This was evident as the temperatures in October, November and December 2018 rose to an average of 24 ^{o}C in both WWTPs. On the other hand, the increase in EC in July 2018 to 1,203.00 µS/cm in WWTP 1 and 1,243.83 µS/cm in WWTP 2 did not demonstrate any corresponding decrease in the three ARVs concentrations due to the effect of low temperatures of 17.86 ^{o}C in WWTP 1 and 16.98 ^{o}C in WWTP 2 in July 2018.

Studies indicated a positive correlation between an increase in temperature and EC (Lackey, 2012; Oyem et al., 2014). Similarly, the synergy effect between temperature and EC in wastewater at high temperature was ascribed to the decrease in viscosity, which is caused by an increase in temperature (Lackey, 2012). Conductivity is a critical parameter for health consideration, EC is of health concern when it exceeds 370 µS/cm (Kale and Bandela, 2016). Electrical conductivity is the water ability to transmit electric current. Therefore, this is subject to the presence of ions, their concentration, valence, mobility, and temperature of measurement. EC is a parameter to assess water quality (Qureshimatva et al., 2015). Conductivity measurements are employed in many industries and environmental application as a means to measure ionic content in solution (Tikariha and Sahu, 2014).

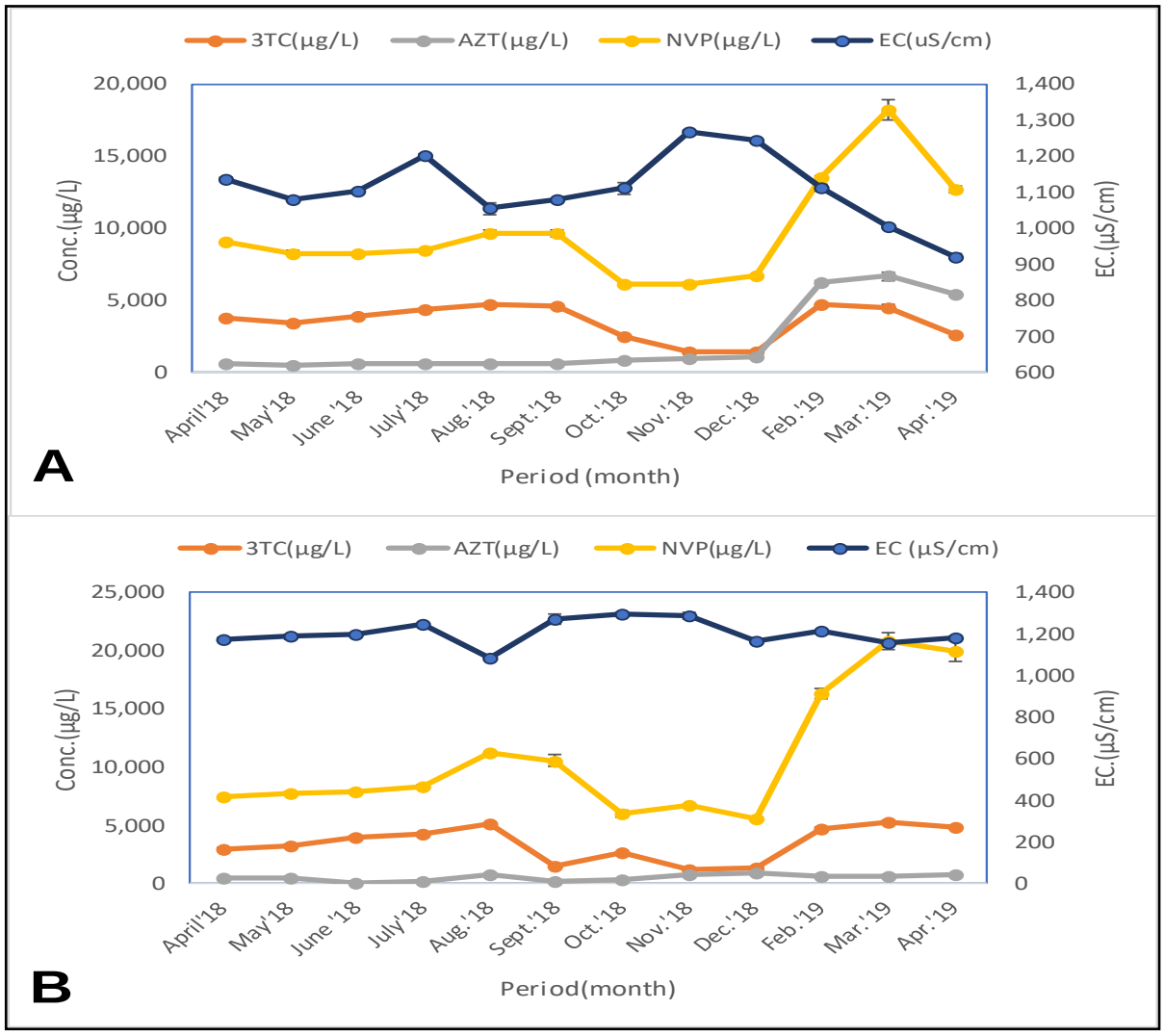

Figure 4.63: Effect of EC on the monthly variation of 3TC, AZT and NVP in the effluent samples at WWTP 1 and WWTP 2.

4.3.4 Effect of changes in TDS on the monthly variation of 3TC, AZT and NVP

The effect of changes in TDS on the monthly variation of 3TC, AZT and NVP in the final effluent samples at the WWTP 1 and 2 is graphically illustrated in Figure 4.14. TDS varied from 590.72 mg/L in WWTP 1 in April 2018 to 828.7 mg/L in WWTP 2 in November 2018. 3TC ranged between 1,178.20 – 5,289.16 µg/L, AZT between 86.79 – 6,698.20 µg/L and NVP between 5,470.00 – 20,770.35 µg/L.

Between April and May 2018, a slight decrease in the concentration of TDS was observed with a corresponding reduction in the concentrations of the three ARVs. From May–July 2018 in WWTP 1, there was an increase in the concentration of TDS (691.84–769.92 mg/L) and all the compounds of interest (except NVP in June 2018) increased, such as 3TC (3,400.76–4,329.52 µg/L), AZT (539.04 – 596.72 µg/L) and NVP (8,284.59–8,521.74 µg/L), accordingly. A similar trend was also observed between April-July 2018 in WWTP 2. The concentrations of the ARVs (except AZT in June and July 2018) increased with an increase in TDS concentrations**.** A relatively conspicuous decrease in TDS concentration was recorded in August 2018 but, the concentration of the three ARVs increases against the trend between April and July 2018.

Figure 4.74: Effect of TDS on the monthly variation of 3TC, AZT and NVP in the final effluent samples at WWTP 1 and WWTP 2.

From the two plants, we observed that the impact of TDS on the ARVs also depends on seasonal changes and some other environmental condition in the wastewater. For instance, from April to July 2018 with relatively lower temperature, a relative increase in the concentration of most of the ARVs occurred despite the increase in TDS. However, between October and December 2018 in the summer when the TDS and temperature were relatively higher, (except TDS in October 2018 in the WWTP 1), the concentration of the three ARV compounds were comparatively abated. The trend observed is consistent with the finding that affirmed that there is a positive relationship between TDS and temperature (Oyem et al., 2014). The group reported that increased temperature can cause an increase in TDS due to an enhanced solubility of ions at a higher temperature. Also, it was reported that high TDS points to the presence of a high quantity of ions such as chlorine (Agoro et al., 2018). In that case, this could be responsible for the reduction in ARVs concentration that occurred between October and December 2018, when TDS and temperature were relatively higher. The increase in the concentrations of TDS recorded between February and April 2019 could be a result of lower concentrations of TDS and shift in the season from summer to Autumn (Chigor et al., 2013; Agoro et al., 2018).

4.3.5 Effect of changes in DO on the monthly variation of 3TC, AZT and NVP

The effect of DO on the concentration of 3TC, AZT and NVP in final effluent from the WWTPs 1 and 2 is illustrated in Figure 4.15. The concentrations of DO in the WWTPs 1 and 2 varied from 1.30 – 5.50 mg/L within the sampling period while 3TC, AZT and NVP ranged between 1,178.20 – 5,289.16 µg/L, 86.79 – 6,698.20 µg/L and 5,469.00 – 20,770.35, respectively. The months of May-August 2018 were characterised by the increase in the concentrations of DO (4.71–5.25 mg/L) in WWTP 1 and (2.23 – 4.99 mg/L) in WWTP 2. This finding was consistent with the earlier study that clarified that a decrease in the water temperature increases dissolved oxygen concentration because the solubility of oxygen and some gases increase with decreases in temperature (Oyem et al., 2014). But concerning the ARVs, a positive correlation was observed in the concentration of the three ARVs. 3TC increases from 3,400 – 4,698 µg/L, AZT from 539 – 683 µg/L and NVP from 8,284 – 9,707 µg/L (except NVP in June 2018) in the WWTP 1. In the WWTP 2, 3TC increases from 3,225 – 5,096 µg/L, AZT from 519 – 773 µg/L and NVP from 7,721 – 11,204 µg/L.

Between October and December 2018, the concentrations of 3TC and NVP were lowered when the concentrations of DO were increased. This can be attributed to an enhanced microbial activity on the ARVs at an appropriate temperature for the anaerobic process. This result corroborates the report in the United States of America that identified higher DO level in the aquatic environment as one of the conditions that favour the degradation of pharmaceutical products by microorganisms (Stadler et al., 2012; Stadler, 2016). This happens because according to the finding, high DO increase the number of available microorganisms species, and at the same enhances their performances (Liu and Wang, 2015; Stadler et al., 2016).

The variation observed on the impact of DO on ARVs in May – August 2018 (higher ARVs values) and October – December 2018 (lower ARV values) could be attributed to the synergetic effect between DO and other factors such as temperature that enhances microbial activities. The reduction in the level of ARVs between October and December 2018 (summer) could mean that the temperature (24 ^{o}C) around this season favours the microbial activities than 17 – 19 ^{o}C between May and August 2018. This was consistent with the report of Al Qarni et al. (2016).

Figure 4.85: Result showing the effect of DO on the monthly variation of 3TC, AZT and NVP in BL AUVT and BL BUVT wastewater samples.

A sharp decrease in the concentration of DO was recorded in September 2018, which was more conspicuous in the WWTP 2 than WWTP 1. This could be attributed to the heavy pollution of the wastewater with organic matter from the residents or pollution from industries.

4.3.6 Effect of changes in SO_4^{2-} on the monthly variation of 3TC, AZT and NVP

With a similar approach, the effect of changes in SO_4^{2-} on the monthly variation of 3TC, AZT and NVP in the final effluent from WWTP 1 and WWTP 2 is illustrated in Figure 4.16. The concentrations of SO_4^{2-} varied between 3.28 mg/L in June 2018 to 163.18 mg/L in November 2018, respectively. 3TC ranged from 1,178.20 – 5,289.16 µg/L, AZT from 86.79 – 6,698.20 µg/L and NVP from 5,469.00 – 20,770.35 µg/L.

The concentrations of SO_4^{2-} are comparably low between April and September 2018, except in May 2018 in the WWTP 1, the same trend was observed between June and August 2018. Within this period, it was observed that the variation in the concentration of SO_4^{2-} did not show much impact on the concentrations of the ARVs, because the three compounds maintained a gradual increased in concentration. This may be ascribed to the low solubility of SO_4^{2-} in the wastewater

during winter as a result of low temperature. Sulphate, as a component of TDS, is described as one of the dissolved inorganic anions in water, which can easily combine with inorganic cations such as sodium, potassium, magnesium and to form salts. Sulphate is generally distributed in nature; it is commonly found in natural waters at concentrations ranging from a few to several hundred milligrams per litre (Lowa Department of Natural Resources, 2009).

A sharp increase in the concentration of SO_4^{2-} and reduction in the concentrations of 3TC, AZT and NVP (except AZT in the WWTP 1) were recorded between October and December 2018 in the two plants and in September of the year in the WWTP 1. Similarly, when a reduction in the concentrations of SO_4^{2-} was noted between February and April 2019, the concentrations of the three ARVs increases and was more noticeable on NVP. This is consistent with scientific findings that high sulphate dose has been reported to enhance the removal of pharmaceutical residue when other conditions as pH and temperature are favourable (Gao et al., 2012; Antonin et al., 2015).

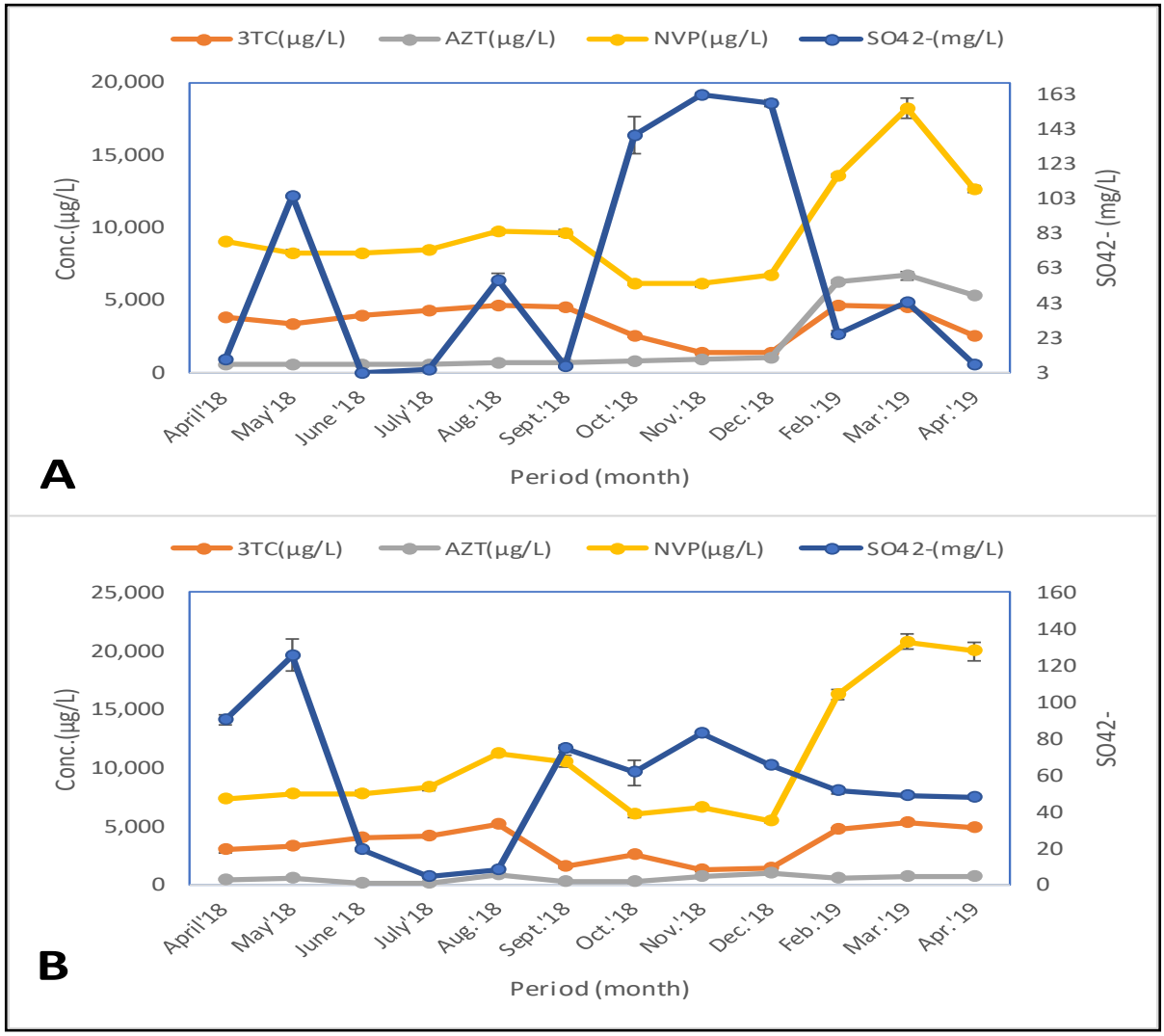

Figure 4.96: Effect of SO_4^{2-} on the monthly variation of 3TC, AZT and NVP in BL AUVT and BL BUVT wastewater samples.

In both plants, the impact of SO_4^{2-} on the ARVs observed between September 2018 and April 2019, when the temperature is warm and with better average daily solar radiation compared to

May and August (winter) suggests that seasonal or environmental variation is a contributory role. This observation is consistent with the report of Gao et al. (2012) on the degradation of sulfamethazine in water including a study carried out by Vincent-akpu et al. (2015) on the evaluation of physicochemical properties and metal contents of water and sediments of Bodo Creek, Niger Delta, Nigeria.

4.3.7 Effect of changes in COD on the monthly variation of 3TC, AZT and NVP

The graphical illustration presented in Figure 4.17 depicts the effect of COD on the monthly variation of 3TC, AZT and NVP in the final effluent samples from the WWTPs 1 and 2. Within the sampling period, the concentrations of COD varied between 23.70 and 898.58 mg/L in July and June 2018, respectively. 3TC, AZT and NVP ranged from 1,178.20 – 5,289.16 µg/L, 86.79 – 6,698.20 µg/L and 5,469.00 – 20,770.35, respectively. Between April and September 2018 in both WWTPs, the concentrations of COD to the concentration of our target compounds did not follow a specific trend. An interwoven low and high concentrations of COD was recorded within the sampling period. Therefore, a clear picture of the effect of COD on our target compounds was not evident. For instance, in May and June 2018, when the concentrations of COD were high, the concentrations of the ARVs were relatively low.

On the other hand, when the concentrations of COD were high in August in WWTP 1 and September in WWTP 2, the concentrations of the ARVS (except 3TC in September) were high. This can be explained in relations to some other prevailing conditions in the wastewater. The previous investigation pointed out that the elimination of pharmaceutical in WWTPs is subject to the properties of the substance, treatment process and prevailing temperature (Radjenovic et al., 2007; Gros et al., 2013; Richardson and Ternes, 2014).

Around the summer period, low concentrations of the ARVs were recorded between October and December 2018, when the concentrations of COD were minimal (except in December 2018 in the WWTP 1). This is consistent with reports from the previous studies that the reduction in the level of COD in wastewater is one of the indicators of a better removal rate of pharmaceuticals (Radjenovic et al., 2007; Gadipelly et al., 2014). Also, it pointed out that their elimination in the WWTPs is subject to the properties of the substance, treatment process and the prevailing temperature (Radjenovic et al., 2007; Richardson and Ternes, 2014). Our finding is in agreement with these reports because the average temperature of the two WWTPs between April and September 2018 was 18 ^{o}C, while the average temperature in October to December 2018 where lower concentrations of the ARVs were obtained was 23 ^{o}C. Improved ARV's removal around this period could be attributed to the favourable temperature that ensures high concentration and activities of organisms in the long retention time MBR process applied by the two plants; thus, enhance better performance (Radjenovic et al., 2007; Gadipelly et al., 2014).

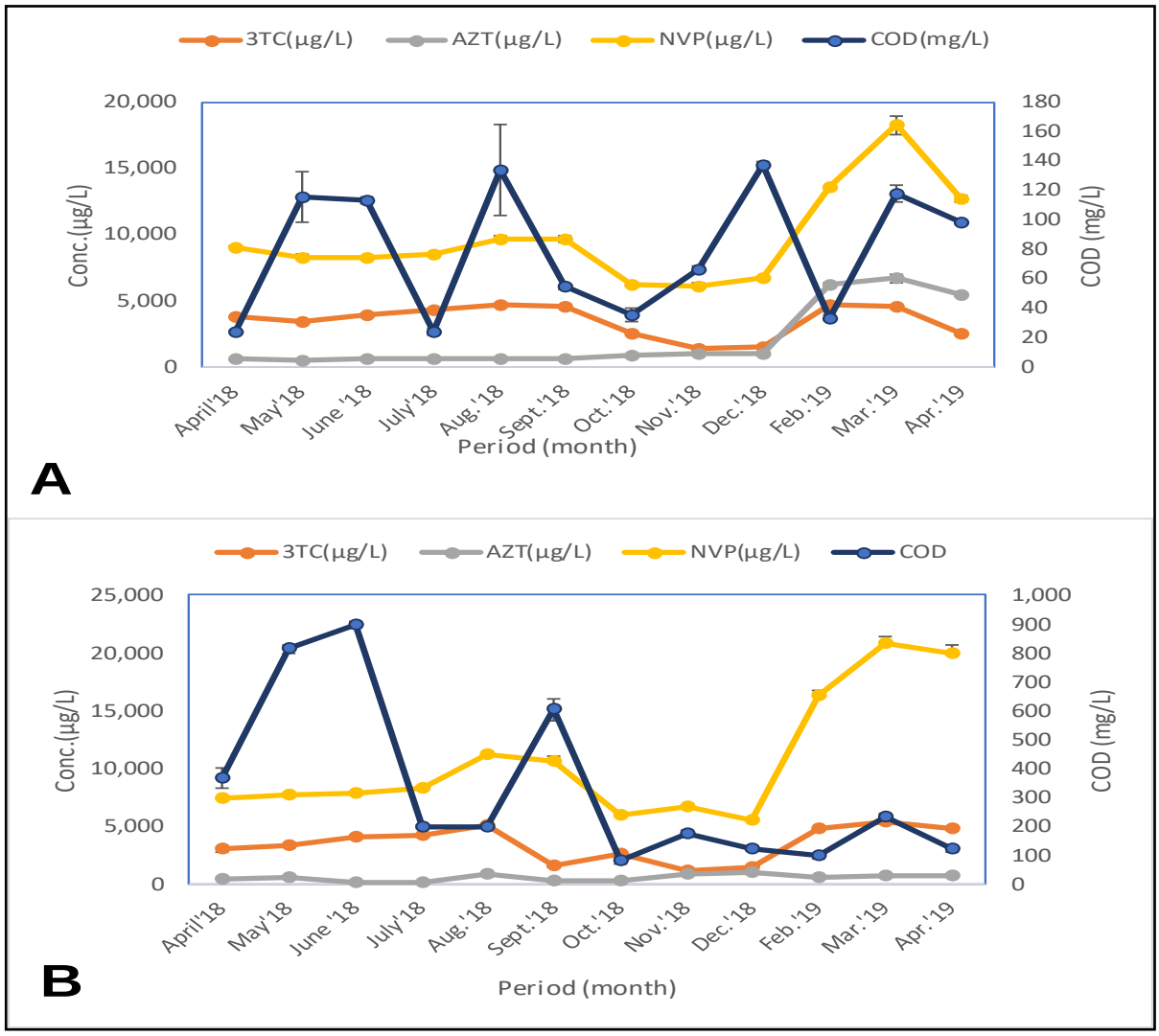

Figure 4.107: Effect of COD on the monthly variation of 3TC, AZT and NVP in BL AUVT and BL BUVT wastewater.

4.3.8 Effect of changes in BOD on the monthly variation of 3TC, AZT and NVP

The effect of BOD on the monthly variation of 3TC, AZT and NVP in the final effluent from the WWTPs 1 and 2 is presented in Figure 4.18. Within the sampling period, the concentrations of BOD varied between 9.17 and 252 .67 mg/L in July 2018 and June 2018, respectively. 3TC, AZT and NVP ranged from 178.20 – 5,289.16 µg/L, 86.79 – 6,698.20 µg/L and 5,469.00 ─ 20,770.35 µg/L, respectively.

Between April and August 2018 (except April in WWTP 1 and June/July 2018 for AZT in WWTP 2), a gradual increase in the concentrations of the three ARVs was observed in the two plants. In the summer period, between October and December 2018, the concentration for the three compounds reduce from what was operational in the first five months of the sampling period. For the remaining part of the sampling period (February to April 2019), the concentrations of the three ARVs rise again. The elimination rates of PPCPs in different types of WWTPs vary. But generally, systems with efficient removal of conventional wastewater constituents (BOD_5 and NH_4^+) similarly demonstrate an enhance elimination of many PPCPs (Matamoros et al., 2009).

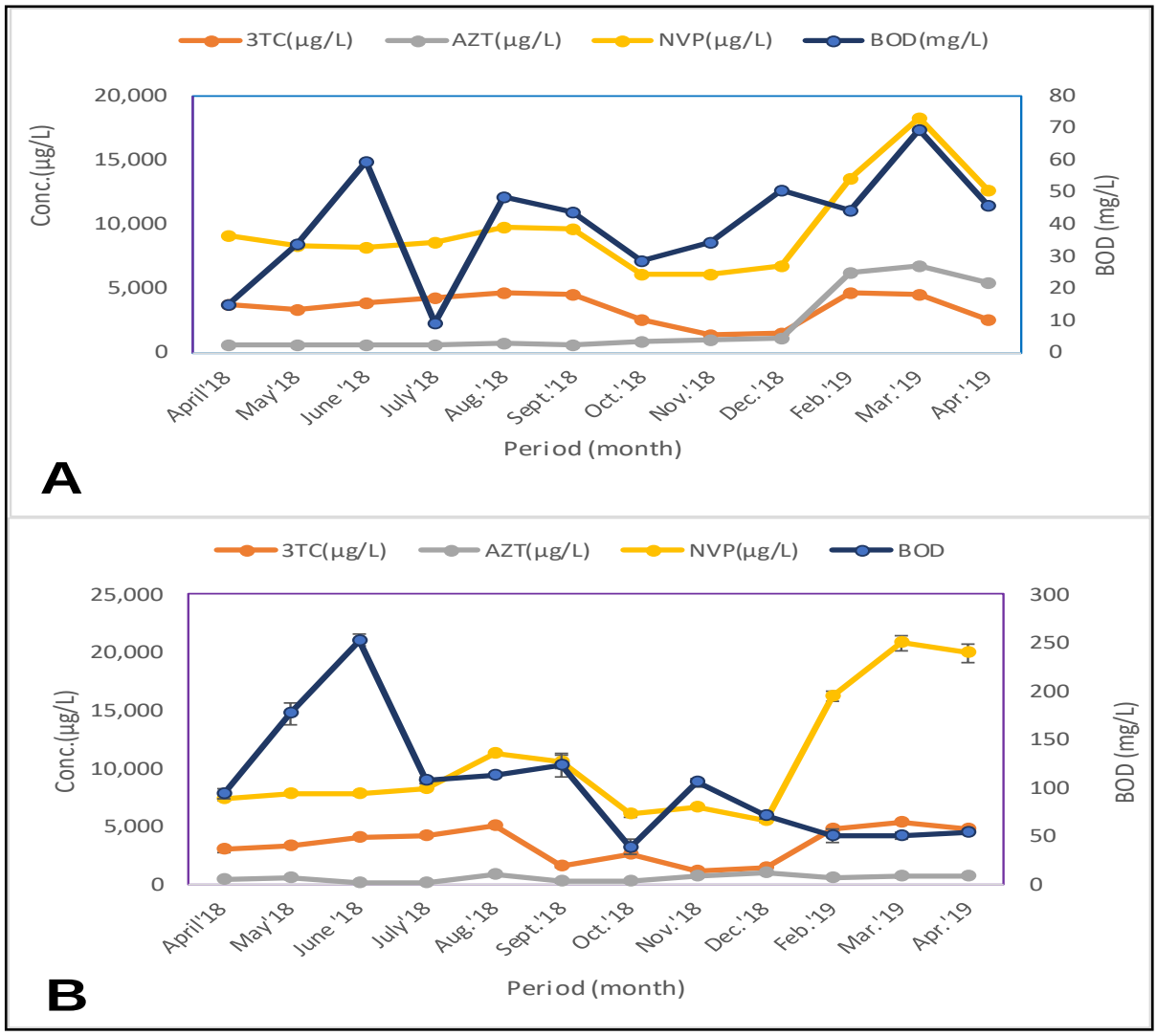

Figure 4.18: Effect of BOD on the monthly variation of 3TC, AZT and NVP in BLAUVT and BLBUVT wastewater.

4.3.9 Effect of changes in Cl^- on the monthly variation of 3TC, AZT and NVP

Figure 4.19 illustrates the effect of Cl^- on the monthly variation of 3TC, AZT and NVP in the final effluent from the WWTPs 1 and 2. The concentrations of Cl^- varies between 48.17 mg/L in April 2019 and 278.75 mg/L in September 2018. 3TC, AZT and NVP ranged from 178.20 – 5,289.16 µg/L, 86.79 – 6,698.20 µg/L and 5,469.00 – 20,770.35 µg/L, respectively. For most parts of the sampling period, the trend observed was the inverse variation between the concentration of chlorine and the target compounds; for instance, a steady reduction in the concentration of Cl^- (278.48 – 94.85 mg/L) was observed between May and August 2018. Within this period, the three ARVs experienced increase in concentration of 3TC (3,400.76 – 4,698.47 µg/L), AZT (539.04 – 683.16 µg/L) and NVP (8,284.59 – 9701.51 µg/L). This finding is consistent with the scientific reports that the degradation of some groups of antibiotics is minimal at lower concentrations of chloride ions (Rivera-Utrilla et al., 2013; Serna-Galvis et al., 2017). The reverse was the case in WWTP 2. For instance, the concentrations of the three ARVs increased between June and August in this pattern 3TC (3,986.01 – 5,096.06 µg/L), AZT

(86.75 – 773.78 µg/L) and NVP (102.35 – 1,104.55 µg/L) despite the sharp increase in the concentrations of chlorine (50.60 – 158.59 µg/L). This observation could be as a result of other components of the wastewater matrix present in the wastewater during this period that is more reactive to chlorine. This is consistent with the literature that the component of the water matrix is very crucial to the reaction that takes place there (Miklos et al., 2019).

Between August and September 2018, in both plants, the concentration of chlorine increased sharply (94.85 – 278.75 mg/L) and a reduction in the concentrations of the three ARVs was recorded. In WWTP 1 3TC (4,698.47 – 4,567.01 µg/L), AZT (683.16 – 656.13 µg/L) and NVP (9,707.51 – 9,661.40 µg/L); WWTP 2 (5,096.06 – 1493.43 µg/L), AZT (773.78 – 234.76 µg/L) and NVP (11,204.55 – 10,537.55 µg/L) accordingly. As observed in both plants, the concentrations of the ARVs (particularly 3TC and NVP) decreased between October and December 2018 despite the variation in the concentrations of chloride in both plants. This shows that other environmental factors, including other matrixes such as pH and temperature, can contribute to the changes in ARV concentrations. This finding affirmed early report that claimed that the efficiency of chlorine for pharmaceutical degradation is pH-dependent (Sirés and Brillas 2012; Serna-galvis et al., 2017). Also, due to its pH-dependent, various species of chlorine (HOCl, ClO^-, Cl_2, etc.) may be available in solution (Deborde and Gunten, 2008). And these forms of chlorine indicate significant differences in their reactivity with micropollutants, which eventually shows the variation in removal efficiency (Deborde and Gunten, 2008).

The reduction in the concentrations of the three ARVs experienced majorly around September and December in both WWTPs could be the combined effect of chlorine content, high temperature and UV radiation, which usually have high index during the Spring/Summer period (Fatta-kassinos et al., 2011; Al Qarni et al., 2016). Some exceptional cases as observed in AZT in November and December can probably be the level of the reactivities of the compounds with chlorine. Explicably, finding from the previous study mentioned that the selective reactivity of Cl^- with the target compounds could be positive or negative effect Deng et al. (2013), as experienced in different seasons and a different chemical ration of our target compounds (3TC, AZT and NVP) within the sampling period. Also, this observation substantiates the study carried out by Kwon et al. (2015) on the effects of three anions (Cl^-, $NO3^-$, and $SO4^{2-}$) on ibuprofen removal from wastewater samples. The group reported that the influence of Cl^- on the target pharmaceutical compounds depends on the relative reactivity of the target compounds with Cl^- (Kwon et al., 2015).

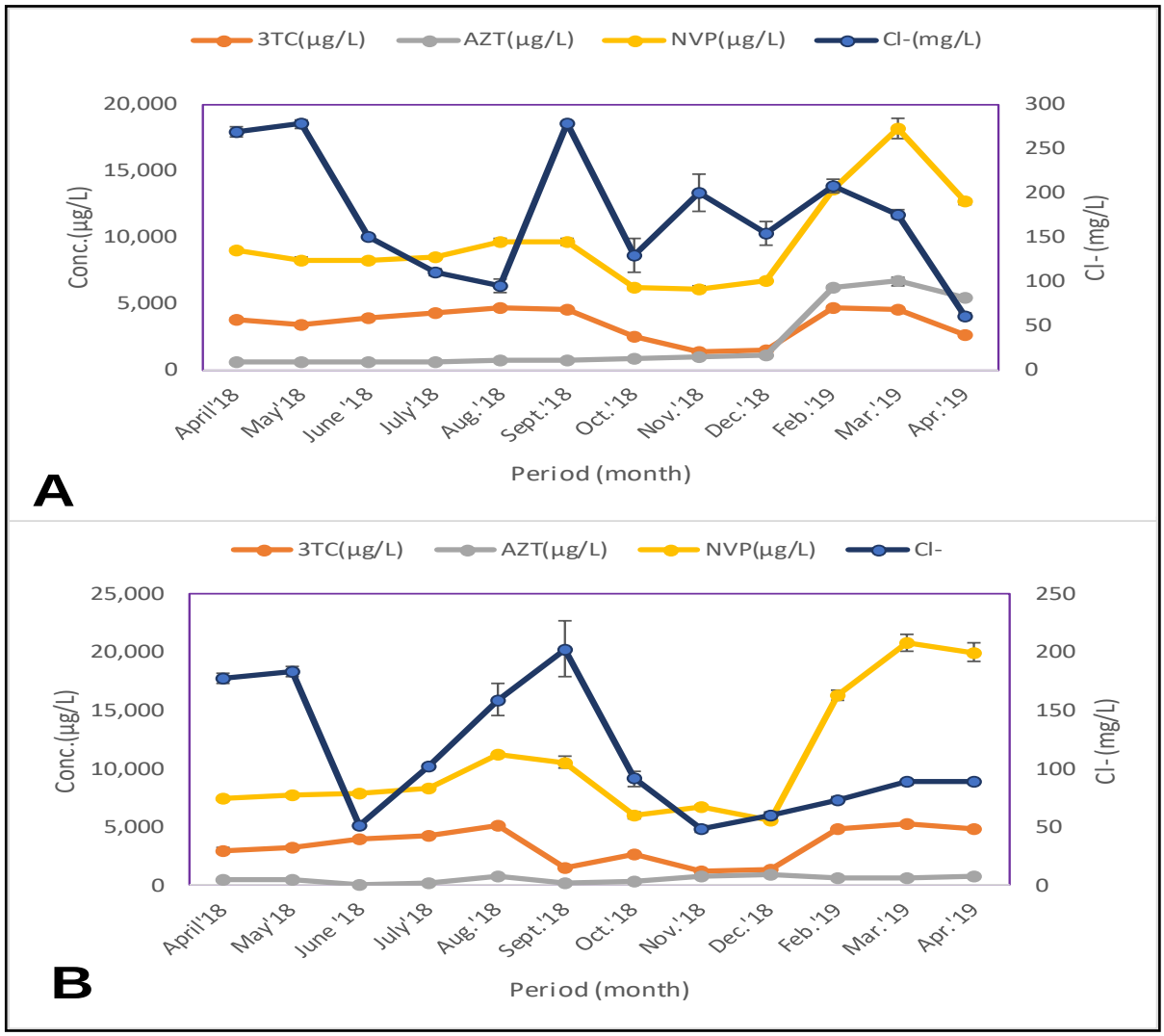

Figure 4.19: Effect of Cl^- on the monthly variation of 3TC, AZT and NVP in the final effluent samples from WWTP 1 and WWTP 2.

4.3.10 Effect of changes in PO_4^{3-} on the monthly variation of 3TC, AZT and NVP

The effect of PO_4^{3-} on the monthly variation of 3TC, AZT and NVP in the WWTPs 1 and 2 is presented in Table 4.20. The phosphate content varied between 0.10 mg/L in September 2018 and 11.32 mg/L in November 2018, while 3TC, AZT and NVP ranged from 178.20 – 5,289.16 µg/L, 86.79 – 6,698.20 µg/L and 5,469.00 – 20,770.35 µg/L, respectively.

As the concentrations of $PO4^{3-}$ within the two-plant varied, it was noted that between April – September 2018 in the WWTP 1 and April – November in the WWTP 2, the concentrations of $PO4^{3-}$ were lower compared to the latter part of the sampling period. For most of this period, the concentration of the three ARVs (except July in NVP in the WWTP 1 and June and July 2018 for AZT in the WWTP 2) gradually increased. In November and December in the WWTP 1 and December in the WWTP 2, the concentrations of phosphate were very high and the concentrations of the ARVs decreased.

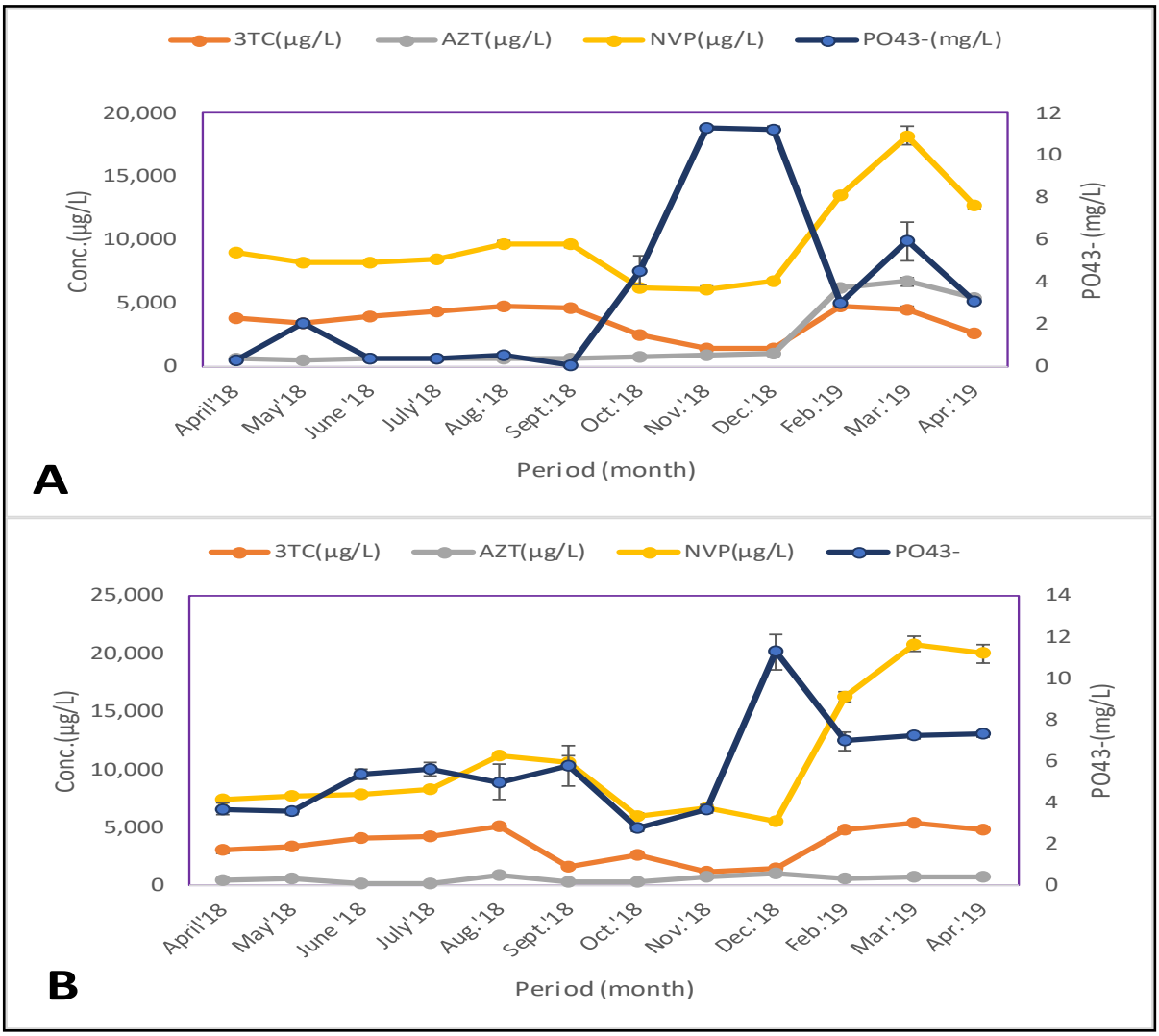

Figure 4.110: Effect of PO_4^{3-} on the monthly variation of 3TC, AZT, NVP in the final effluent sample from WWTP 1 and WWTP 2.

Besides, it is noteworthy that both the lowest and highest concentrations of PO_4^{3-} exist between October and December 2018 in WWTP 2, respectively. Notwithstanding, the concentrations of the three ARVs reduced around this same period within the sampling period. This shows that PO_4^{3-} has little impact on our target pharmaceutical compounds. This little impact could be a result of the effect of other physicochemical parameters such as temperature and other matrices in the wastewater.

Our finding agrees with the report that temperature is one of the main factors that affect many physicochemical equilibria, most reactions such as degradation in the aquatic environment are enhanced by the increase in temperature (Delpla et al., 2009). Similarly, correlations between temperature and phosphates, nitrates and Total Phosphorus (TP) have been established in the Mekong River (Prathumratana et al., 2008).

4.4 Summary

The results of monthly variation of ten physicochemical parameters investigated within April 2018 and April 2019 showed that only three parameters (temperature, pH and SO_4^{2-}) out of the ten complied with the recommended limits by both regulatory bodies. EC, COD and BOD values in both plants and chloride (in WWTP 2) conformed with the USEPA limits while phosphate levels in WWTP 2 complied with the DWAF limit of 10 mg/L. This shows that effluents from these plants are sources of pollution to the receiving water bodies and the environment at large. Therefore, some of the defined objectives of national guideline values, including access to clean and safe water, prevention of health-related waterborne diseases and eradication of gross pollution to surface water sources were not fully met. This is not good for a semi-arid country like South Africa where water is scarce and wastewater effluent is used in agriculture, landscaping and industrial purposes.

The chapter also showed how different physicochemical parameters affect the concentration of the ARVs investigated within the sampling period.

CHAPTER 5

SPECTROSCOPIC DETERMINATION OF SELECTED ANTI-RETROVIRAL COMPOUNDS IN WASTEWATER SAMPLES[χ]

5.1 Introduction

This chapter discusses the results obtained from the UV-visible spectrophotometric determination of three pharmaceutical compounds (3TC, AZT and NVP) in wastewater samples collected for 12 months from the two WWTPs. The level of removal of our target ARV compounds during treatment processes at WWTP 1 and how the treatment of the final effluent (BUVT) with UV radiation in WWTP 1 affects the removal of the ARVs was also enumerated. In the case of WWTP 2, only the results obtained for the ARVs in this study will be discussed, investigating the efficiency of the plant to remove the target ARVs. The chapter further presents the results obtained for the validation of the simple UV-visible spectroscopic analysis, using the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures.

5.2 Method validation (UV)

The spectroscopic method used for the determination of the three pharmaceutical formulations was validated according to the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures reported by Rathod et al. (2014). The system precision, linearity, method precision (Intra-day and inter-day precision) and accuracy were determined according to the method of Rathod et al. (2014). The seven points calibration curves for the 3TC, AZT and NVP were prepared as described in section 3.6.3 and they are presented in Appendix E, F and G, respectively.

5.2.1 System precision

The absorbance readings of seven replicate samples of 120 µg/L, 120 µg/L and 240 µg/L for 3TC, AZT and NVP measured at 280 nm, 267 nm and 313 nm, respectively is presented in Table 5.1. The relative standard deviation (RSD) of the absorbance readings obtained for seven replicates of each compound was less than 2% as depicted in Table 5.1. This indicates that the system has high precision and reproducible (Rathod et al., 2014).

[χ] **Olabode, G.S**. & Somerset, V.S. (**2020**). UV-visible spectroscopic determination of antiretroviral compounds in wastewater samples. *Water, Air, and Soil Pollution*. (**In Review**).

Table 5.1: Results for the system precision for 3TC, AZT and NVP formulations.

n	3TC (µg/L)	Abs.(3TC)	3TC (µg/L)	Abs. (AZT)	3TC (µg/L)	Abs. (NVP)
1	120	0.453	120	0.183	240	0.34
2	120	0.453	120	0.184	240	0.34
3	120	0.454	120	0.184	240	0.342
4	120	0.454	120	0.185	240	0.343
5	120	0.454	120	0.185	240	0.343
6	120	0.455	120	0.185	240	0.344
7	120	0.455	120	0.185	240	0.344
Average		**0.454**		**0.184**		**0.342**
SD		**0.001**		**0.001**		**0.002**
%RSD		**0.180**		**0.427**		**0.498**

5.2.2 Linearity

The linearity of the method was determined from the seven-point calibration curve for different concentrations of the standards between 10 – 240 µg/L for 3TC and AZT, whereas it was from 20 – 400 µg/L for NVP, respectively. The results obtained for each pharmaceutical formulation exhibited a good relationship between the absorbance readings and different concentrations within the concentration range of the standards. The correlation coefficients (R^2) for the three pharmaceutical formulations within the calibration ranges are higher than 0.998 as presented in Table 5.2 which showed that the method is linear (Chaudhari and Bhalerao, 2012).

Table 5.2: Results for the linearity of the calibration curves.

Validation parameters	3TC	AZT	NVP
Calibration Range (µg/L)	10-240	10-240	20-400
Regression equation	y = 0.1171 x + 0.0188	y = 0.1112 x + 0.0054	y = 0.026 x + 0.0058
Correlation coefficient (R^2)	0.9984	0.9985	0.9983

5.2.3 Method precision (intra-day and inter-day precision)

The precision of the method was determined by:

i. intra-day (repeatability) of the measured value and
ii. inter-day (intermediate) precision.

5.2.3.1 Intra-day precision (repeatability)

The result of intra-day precision is presented in Table 5.3 and the %RSD for the three pharmaceutical formulations is less than 2% assay. This indicates that the method developed is precisely based on the repeatability test, and it offers consistent reproducible results.

Table 5.3: Results for the intra-day precision measurements for 3TC, AZT and NVP pharmaceutical formulations.

	3TC			AZT			NVP		
n	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
	Assay 1	**Assay 2**	**Assay 3**	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 1**	**Assay 2**	**Assay 3**
1	102.55	102.09	102.32	98.89	99.44	100.56	100.33	99.73	100.03
2	102.55	102.09	102.32	99.44	100.00	100.56	100.33	99.73	100.03
3	102.79	102.32	102.55	99.44	100.00	101.11	100.92	100.03	100.03
4	102.79	102.32	102.55	100.00	100.56	101.11	101.22	100.03	100.33
5	102.79	102.32	102.55	100.00	100.56	101.67	101.22	100.33	100.33
6	103.02	102.55	102.79	100.00	100.56	101.67	101.52	100.33	100.33
7	103.02	102.55	102.79	100.00	100.56	101.67	101.52	100.33	100.33
Avg.	**102.79**	**102.32**	**102.55**	**99.68**	**100.24**	**101.19**	**101.01**	**100.07**	**100.20**
SD	**0.19**	**0.19**	**0.19**	**0.44**	**0.44**	**0.50**	**0.51**	**0.27**	**0.16**
%RSD	**0.18**	**0.19**	**0.19**	**0.44**	**0.44**	**0.49**	**0.50**	**0.27**	**0.16**

5.2.3.2 Inter-day precision (intermediate)

Tables 5.4. ─ 5.6 present inter-day method precisions for 3TC, AZT and NVP, respectively. The % RSD for the three pharmaceutical formulations were within the acceptable limits of less than 2% assay within and between the three days (Chaudhari and Bhalerao, 2012; Rathod et al., 2014). This indicates that the method applied is inter-day precise.

Table 5.4: Results for the inter-day method precision measurements of the 3TC pharmaceutical formulation.

	Day 1			Day 2			Day 3		
n	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
	Assay 1	**Assay 2**	**Assay 3**	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 1**	**Assay 2**	**Assay 3**
1	97.92	101.50	98.85	101.39	101.39	99.83	100.00	100.70	99.69
2	97.92	101.50	98.85	101.39	101.39	99.83	100.00	100.70	99.69
3	97.92	101.73	98.99	102.79	101.39	99.92	100.00	100.70	99.69
4	97.92	101.73	98.99	102.79	101.63	99.92	101.39	100.70	99.83
5	99.31	101.73	99.13	102.79	101.63	100.11	101.39	100.70	99.83
6	99.31	101.96	99.13	102.79	101.63	100.11	101.39	100.93	99.97
7	97.92	101.96	99.13	104.18	101.63	100.11	101.39	100.93	99.97
Ave.	**98.32**	**101.73**	**99.01**	**102.59**	**101.53**	**99.92**	**100.79**	**100.79**	**99.81**
SD	**0.68**	**0.19**	**0.13**	**0.96**	**0.12**	**0.13**	**0.74**	**0.124**	**0.13**
%RSD	**0.69**	**0.19**	**0.13**	**0.94**	**0.12**	**0.13**	**0.74**	**0.12**	**0.13**

Table 5.5: Results for the inter-day method precision measurements of the AZT pharmaceutical formulation.

	Day 1			Day 2			Day 3		
n	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
	Assay 1	**Assay 2**	**Assay 3**	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 1**	**Assay 2**	**Assay 3**
1	96.67	98.89	97.67	100.00	99.44	97.33	103.33	98.33	97.33
2	96.67	99.44	97.67	103.33	99.44	97.67	103.33	98.33	97.33
3	100.00	99.44	97.67	103.33	99.44	97.67	106.67	98.89	97.33
4	100.00	100.00	98.00	103.33	100.00	97.67	106.67	98.89	97.00
5	100.00	100.00	98.00	103.33	100.00	97.67	106.67	98.89	97.00
6	100.00	100.00	98.00	103.33	100.56	97.67	106.67	99.44	97.00
7	100.00	100.00	98.00	103.33	100.56	97.67	106.67	99.44	97.00
Ave.	**99.05**	**99.68**	**97.86**	**102.86**	**99.92**	**97.62**	**105.71**	**98.89**	**97.14**
SD	**1.63**	**0.44**	**0.18**	**1.26**	**0.50**	**0.13**	**1.63**	**0.45**	**0.18**
%RSD	**1.64**	**0.44**	**0.18**	**1.23**	**0.50**	**0.13**	**1.54**	**0.46**	**0.18**

Table 5.6: Results for the inter-day method precision measurements of the NVP pharmaceutical formulation.

	Day 1			Day 2			Day 3		
n	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
	Assay 1	**Assay 2**	**Assay 3**	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 1**	**Assay 2**	**Assay 3**
1	101.96	100.33	99.48	100.18	100.03	99.48	101.96	99.73	99.13
2	101.96	100.33	99.48	100.18	100.03	99.48	101.96	99.73	99.30
3	101.96	100.92	99.48	100.18	100.63	99.66	101.96	100.03	99.30
4	103.75	101.22	99.84	101.96	100.92	99.66	103.75	100.03	99.48
5	103.75	101.22	100.02	101.96	101.22	99.66	103.75	100.33	99.48
6	103.75	101.52	100.02	101.96	101.22	99.84	103.75	100.33	99.66
7	103.75	101.52	100.02	101.96	101.22	99.84	103.75	100.33	99.66
Ave.	**102.99**	**101.01**	**99.76**	**101.20**	**100.75**	**99.66**	**102.98**	**100.07**	**99.43**
SD	**0.96**	**0.51**	**0.27**	**0.96**	**0.54**	**0.15**	**0.96**	**0.27**	**0.20**
%RSD	**0.93**	**0.50**	**0.27**	**0.94**	**0.54**	**0.15**	**0.93**	**0.27**	**0.20**

5.2.4 Accuracy measurements

Data obtained for the accuracy of the three pharmaceutical formulations are presented in Table 5.7. The accepted limits of recovery for accuracy ranged between 98% - 102% (Rathod et al., 2014). All observed data are within this range, in essence, this demonstrates good recovery values and hence the accuracy of the method developed**.**

Table 5.7: Results for the accuracy of the method for the three pharmaceutical formulations

	3TC			AZT			NVP		
Actual Conc.(µg/L)	20	120	200	20	120	200	40	240	400
Mean (Abs.)	0.083	0.454	0.724	0.035	0.184	0.303	0.060	0.342	0.562
Mean Conc.(µg/L)	20.1	123.2	198.5	20.2	119.0	198.4	40.8	242.0	399.1
SD	**0.02**	**0.02**	**0.02**	**0.05**	**0.05**	**0.34**	**0.06**	**0.12**	**0.38**
%RSD	**1.09**	**0.12**	**0.115**	**2.50**	**0.44**	**1.71**	**1.42**	**0.50**	**0.94**
% recovery	**100.00**	**102.32**	**99.28**	**100.95**	**99.68**	**99.19**	**101.98**	**101.01**	**99.76**

5.2.5 Limit of detection (LOD) and limit of quantification (LQD)

The LOD was determined as thrice the standard deviation of the mean of 10 repeated measurements of the blank values divided by the slope of the calibration curve of the standard. The LOD are 0.68 µg/L, 1.86 µg/L and 7.59 µg/L for 3TC, AZT and NVP, respectively. As indicated in Table 5.8

$$LOD = \frac{3.3\sigma}{S}$$ ……...……Equation 5.1

While the Limit of quantification (LOQ) is the lowest concentration obtained from the standard calibration curve that can be determined or (measured) with precision, accuracy, and variability (Sarkar et al., 2006). It was determined as ten times the standard deviation of the mean of 10 repeated measurements of the blank divided by the slope of the calibration curve of the standard. The LOQ are 2.06 µg/L, 5.63 µg/L and 22.99 µg/L for 3TC, AZT and NVP, respectively shown in Table 5.8

$$LOQ = \frac{10\sigma}{S}$$ ……...Equation 5.2

Where:

σ = Standard deviation of the absorbance of 10 readings of the blank solution;
S = Slope of the regression line.

Table 5.8: Results for the Limit of detection (LOD) and limit of quantification (LOQ) (µg/L).

n	3TC	AZT	NVP
Blank 1	0.002	0.000	0.000
Blank 2	0.002	0.000	0.000
Blank 3	0.001	0.001	0.001
Blank 4	0.001	0.001	0.002
Blank 5	0.000	0.002	0.001
Blank 6	0.000	0.002	0.001
Blank 7	0.000	0.001	0.001
Blank 8	0.001	0.001	0.001
Blank 9	0.001	0.000	0.001
Blank 10	0.001	0.000	0.002
Ave.	**0.001**	**0.001**	**0.001**
Std	**0.001**	**0.001**	**0.001**
LOD (3.3 σ/S)	**68**	**186**	**759**
LOQ (10 σ/S)	**206**	**563**	**2,299**

5.3 Monthly variation of 3TC in final effluent from WWTP 1 and WWTP 2

The monthly variation of 3TC in WWTP 1 and 2 samples is presented in Figure 5.1. Overall concentration ranged between 11.78 µg/L in November 2018 to 52.89 µg/L in March 2019. In WWTP 1, the concentration of 3TC varied between 14.10 µg/L in November 2018 to 47.02 µg/L in February 2019. The lowest concentration (14.10 µg/L) recorded in November 2018 was close to 14.58 µg/L recorded in December 2018 but differed significantly ($P < 0.05$) from other concentrations obtained in subsequence months within the sampling period. In WWTP 2, however, the lowest value of 11.78 µg/L in November 2018 was not different from the 14.94 µg/L and 13.63 µg/L obtained in September 2018 and December 2018 respectively. The value was significantly different ($P < 0.05$) from the values obtained in other months within the sampling period.

More so, the lower concentrations recorded between October and December in WWTP 1, and between September and December in WWTP 2 could be as a result of biodegradation and photodegradation. This could have been caused by changes in weather condition, such as a rise in temperature and intense sunlight radiation in September, which marks the beginning of spring/summer. A similar observation was reported in another study by Aminot et al. (2016). The researchers provided clarity on the pharmaceutical concentration in the estuarine of the Garonne River. They discovered that the concentration decreases along the estuary during the summer period. This was attributed to the rise in the temperature and particulate matter during the summer that impacted the stability of pharmaceuticals (Aminot et al., 2016). The highest concentration of 47.02 µg/L in February 2019 in WWTP 1 was not different from 46.98 µg/L, 45.67 µg/L, and 45.51 µg/L reported in August 2018, September 2018, and March 2019, respectively.

On the other hand, the result obtained for WWTP 2 revealed the highest concentration (52.89 µg/L) value obtained. This was significantly different ($P < 0.05$) from the values obtained in the subsequence months except in August 2018 (5,096 µg/L). The maximum concentrations of 47.02 µg/L and 52.89 µg/L for WWTP 1 and 2, respectively were higher than 3.99 µg/L and 5.43 µg/L reported for wastewater effluent and river water in Kenya (Ngumba and Gachanja 2015). Also, it is greater than 0.0026 µg/L reported for Bordeaux (WWTP) sampling point in France (Aminot et al., 2016). The concentration range of 11.78–52.89 µg/L within the sampling period was higher than 19.90–31.007 µg/L reported for wastewater effluent in Kenya (K'oreje et al., 2016). The reason for the higher concentration reported could be due to the high rate of consumption level of ARVs compounds in South Africa as the highest user of ARV drugs in the World (Wood et al., 2015; Cornell et al., 2017; Abafe et al., 2018). Besides, the difference in the sampling period and location could be another reason as similarly reported in Kenya (Ngumba and Gachanja 2015). For this study, the sampling period was between April 2018 to April 2019. And from literature surveyed, there is a continuous rise in the number of people on ARV therapy, it was reported that the number of infected people in South Africa in 2016 was more than double of 2.35 million infections reported in 2012 (Cornell et al., 2017; Avert, 2018).

The higher concentrations recorded between February 2019 and April 2019 could be a result of the concentration of the sample due to lack of rain and reduction in the volume of wastewater around this period (Wood et al., 2015). Conversely, the slightly higher concentrations observed from June to August/September in both WWTP could be as a result of low biodegradability of

the compound characterized by low temperature during the winter period (Matamoros et al., 2015; Al Qarni et al., 2016). In South Africa, 3TC is a component of the first line regime for the treatment of HIV in children, pregnant women, and adults (Schoeman et al., 2017).

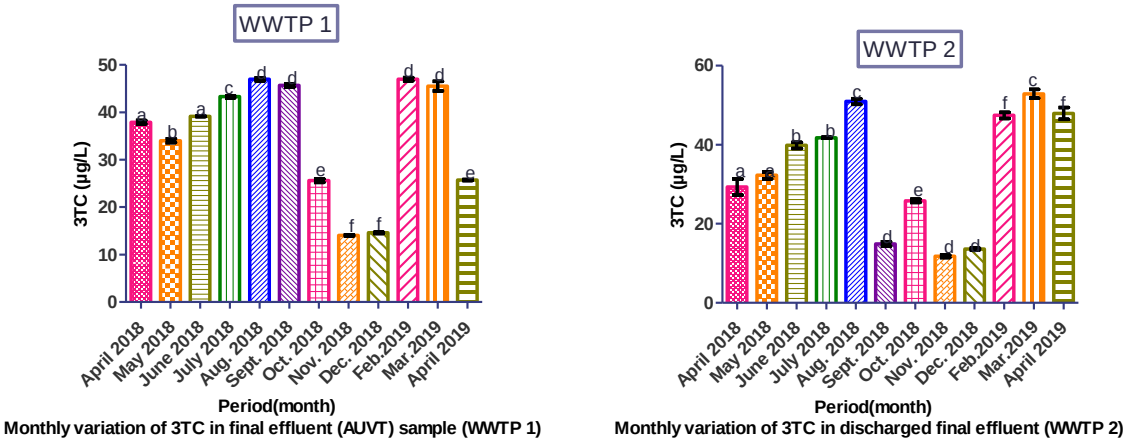

Figure 5.1: Results for monthly variation of 3TC in discharged final effluents of WWTP 1 and WWTP 2.

5.4 Monthly variation of AZT in final effluent from WWTP 1 and WWTP 2

Figure 5.2 showed the level of AZT between April 2018 and April 2019 in WWTP 1 and 2. The concentrations of AZT within the sampling period ranged between 0.90 µg/L in June 2018 and 66.98 µg/L in March 2019. The concentration varied between 5.39 µg/L in May 2018 to 66.98 µg/L in WWTP 1 and it ranged from 0.90 µg/L to 9.17 µg/L in June to December 2018 in WWTP 2. The lowest concentration, 9.0 µg/L obtained in June 2018 was not different from 1.20 µg/L obtained in July 2018, but significantly different ($P < 0.05$) from the result obtained in other months. In that case, this can be ascribed to the reduction in the level of the compound in the influent around this period. The highest concentration of 66.98 µg/L observed in March could probably come from excessive pollution from direct disposal of expired or unused AZT from household or hospital into the sewage system (Jain et al., 2013). In WWTP 1, the concentration of AZT in the first seven months (April-October 2018) of the sampling period were close to one another. This could be attributed to a level of stability in plant efficiency.

The effluent concentration range of 0.90–67.40 µg/L in both plants was higher than 0.51 µg/L and 0.11 µg/L that was reported for wastewater effluent by Ngumba and Gachanja (2015) and K'oreje et al. (2016), respectively. It was also greater than 0.97 µg/L, 7.70 µg/L and 17.40 µg/L recorded in surface water (Wood et al., 2015; Ngumba and Gachanja, 2015; K'oreje et al., 2016). The values were exceedingly higher than 0.0033 µg/L reported for the Bordeaux sampling point of estuarine Garonne River in France (Aminot et al., 2016). Notwithstanding, the concentration recorded in our study in the major part of the sampling period (April to December 2018) was comparable to 0.13 µg/L for wastewater effluent (Abafe et al., 2018).

AZT has been reported in surface water and WWTPs (influent and effluent) (Prasse et al., 2010; K'oreje et al., 2016; Abafe et al., 2018). It is used with 3TC in combination with one NNRTI

such as EFV or NVP as the first-line regimen for the treatment of HIV in South Africa (Marconi et al., 2008; Boulle et al., 2010; Idele et al., 2014).

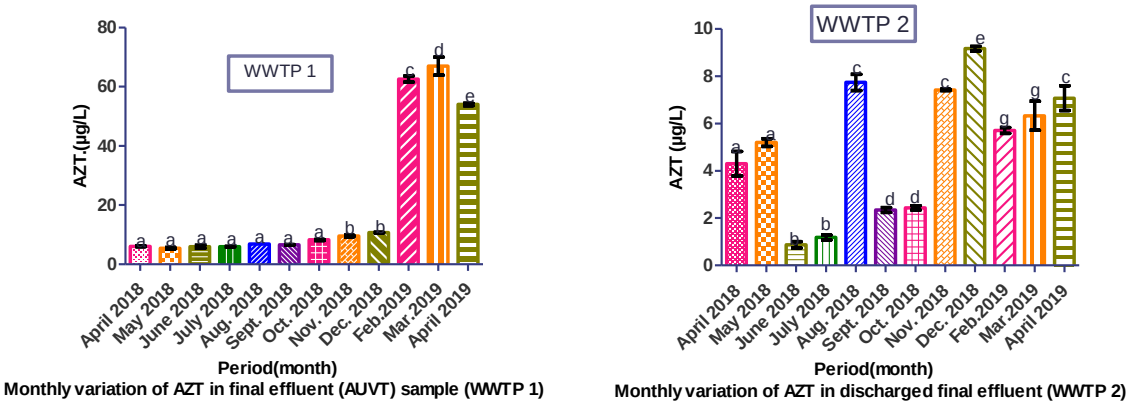

Figure 5.12: Results for monthly variation of AZT in discharged final effluents of WWTP 1 and WWTP 2.

5.5 Monthly variation of NVP in final effluent from WWTP 1 and WWTP 2

Figure 5.3 depicts the monthly variation of the concentration of NVP in WWTP 1 and 2. In the two plants, NVP ranged from 54.70 µg/L in December 2018 to 207.70 µg/L in March 2019. The lowest and highest concentrations were recorded in WWTP 2. In WWTP 1, the concentration of NVP ranged between 61.35 µg/L in November 2018 to 182.33 µg/L in March 2019. In WWTP 2, the concentration ranged from 54.70 µg/L in December 2018 to 207.70 µg/L in March 2019. The lowest concentration (61.35 µg/L) in WWTP 1 was not different from 59.95 µg/L and 67.00 µg/L in October and December 2018 respectively, but significantly different ($P < 0.05$) from the concentration recorded in other months within the sampling period. Similarly, the lowest concentration (54.70 µg/L) in WWTP 2 was not different from 59.78 µg/L recorded in October 2018 but demonstrated a level of difference from the result obtained in other months within the sampling period. The lowest concentrations range recorded between October and December in both WWTPs could be attributed to the effect of weather around the summer period (Al Qarni et al., 2016).

It has been reported in China and Saudi Arabia that the level of elimination of pharmaceutical compounds in WWTPs depends on different factors (Chen et al., 2014; Al Qarni et al., 2016). These include the prevailing weather conditions (rainfall/dilution effect, temperature, the intensity of solar radiation), physicochemical properties of the analyte, treatment technology applicable in the WWTP such as hydraulic retention time (HRT), and solids retention time (SRT) (An et al., 2011; Z. Chen et al., 2014; Al Qarni et al., 2016). These factors affect microbial activities and growth, which consequently cause variation in the effluent quality (An et al., 2011; Chen et al., 2014; Al Qarni et al., 2016). The highest concentration of 182.33 µg/L recorded in WWTP 1 was significantly different ($P < 0.05$) from the concentrations in other months.

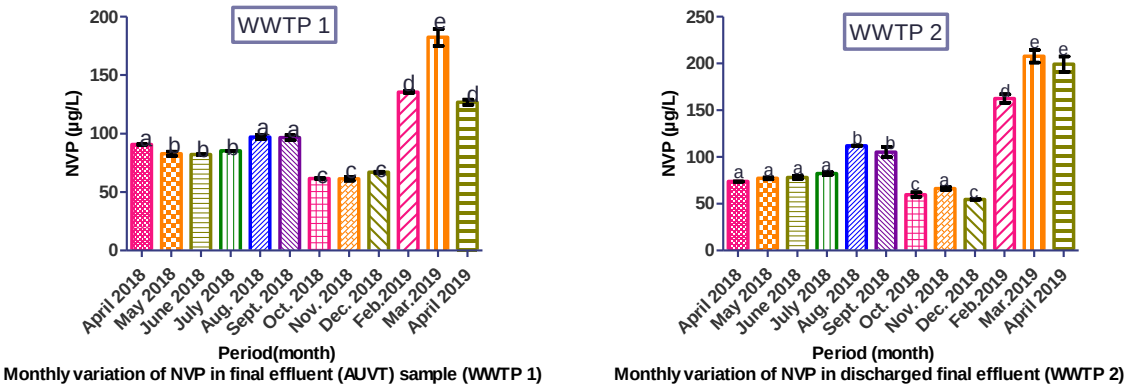

Figure 5.3: Results for monthly variation of NVP in discharged final effluents of WWTP 1 and WWTP 2.

Additionally, the highest concentration (207.70 µg/L) in WWTP 2, on the contrary, was not different from 199.33 µg/L obtained in April 2019, but significantly different ($P < 0.05$) from the concentrations of NVP in other months. Therefore, this can be ascribed to excessive use of the drug within this period. NVP is used extensively in South Africa to prevent mother to child infection of HIV (Boulle et al., 2010; Stinson et al., 2017). Within the sampling period, despite the concentration differences in both plants, the trend of variation in the concentrations of NVP follow a similar pattern. The concentration was almost the same between April and July. The concentration increased slightly in August and September, and the lowest concentration range was recorded between October and December 2018.

The concentrations of NVP reported here was higher than 0.00043 µg/L reported for effluent from WWTP in South Africa (Swanepoel et al., 2015) and 0.0005 µg/L reported for Garonne River in France (Aminot et al., 2016).

5.6 Relative comparison of occurrence of 3TC, AZT and NVP in the two plants

Figure 5.4 compares the concentration of AZT with that of 3TC and NVP in the effluent samples. Except for February to April 2019 in WWTP 1, the concentrations of AZT were found to be generally low (less than 10 µg/L) in both plants. This result concurred with the report of Swanepoel et al. (2015) in the investigation of the presence, concentrations, and potential implications of HIV in some designated water resources in South Africa. The group reported that AZT (0.0003 µg/L) was the lowest in natural water investigated (Swanepoel et al., 2015). The low concentration of the compound could be attributed to its high photo degradability, transformation into triphosphate, and its rapid absorption in the gastrointestinal tract (GIT). Thus, this suggests that little amount is being excreted as parent AZT through urine or faeces (Vaňková, 2010; Jain et al., 2013). This result, however, contradicts the report that claimed that AZT occurred in high concentration in South Africa surface water alongside with d4Tand NVP (Wood et al., 2015).

The rise in the concentration of AZT observed between February and April 2019 in the WWTP 1 sample could be attributed to the increase in the population elevated the influent load. Another possible reason could be improper sanitation by the residents of some informal

settlement/locations around WWTP 1. In essence, this can result in the direct release of waste into the sewage system (Ngumba and Gachanja 2015; K'oreje et al. 2016). Similarly, the higher concentration recorded could be a result of glucuronide conjugates excretion in humans (Abafe et al., 2018).

On the other hand, the concentration of NVP was always on the high side. The lowest concentrations of NVP which are 61.35 µg/L and 54.70 µg/L in WWTP 1 and 2 samples, respectively were higher than 47.02 µg/L and 52.89 µg/L, which are the highest concentration values for 3TC in WWTP 1 and WWTP 2 samples, respectively. Similarly, apart from the exceptionally high concentration of AZT (62.59 and 66.98 µg/L) observed in February and March 2019 in WWTP 1 samples, the lowest concentration of NVP (61.35 µg/L) was higher than the concentrations of AZT in the remaining part of the sampling period. Also, in WWTP 2, the lowest concentration of NVP (54.70 µg/L) was higher than 9.17 µg/L, which is the highest concentration of AZT. The result obtained in this study concurred with other reports from the literature that stated that NVP had the highest concentration in Nairobi River Basin water (K'oreje et al., 2012). Similarly, the concentration of NVP was reported to be about twice the concentration of AZT in the Nairobi river (K'oreje et al., 2012). From the general investigation executed across South African surface water, Wood et al. (2015) reported that NVP had the highest average concentration (apart from caffeine) and was the second pharmaceutical that occurred most frequently in the nation-wide survey of pharmaceuticals in surface water in South Africa.

Similarly, our finding was consistent with the report of Swanepoel et al. (2015) on different water sources in South Africa. In natural water NVP (0.0068 µg/L) had the highest concentration. In drinking water samples, NVP occurred in five samples, AZT was only identified in two drinking water samples, while 3TC was below the limit of detection or limit of quantification. In groundwater samples also, NVP was reported to have the highest concentration of 0.0003 – 0.0054 µg/L and found in eight samples, while 3TC with the concentration of 0.00003 – 0.00009 µg/L occurred in 2 samples only (Swanepoel et al., 2015).

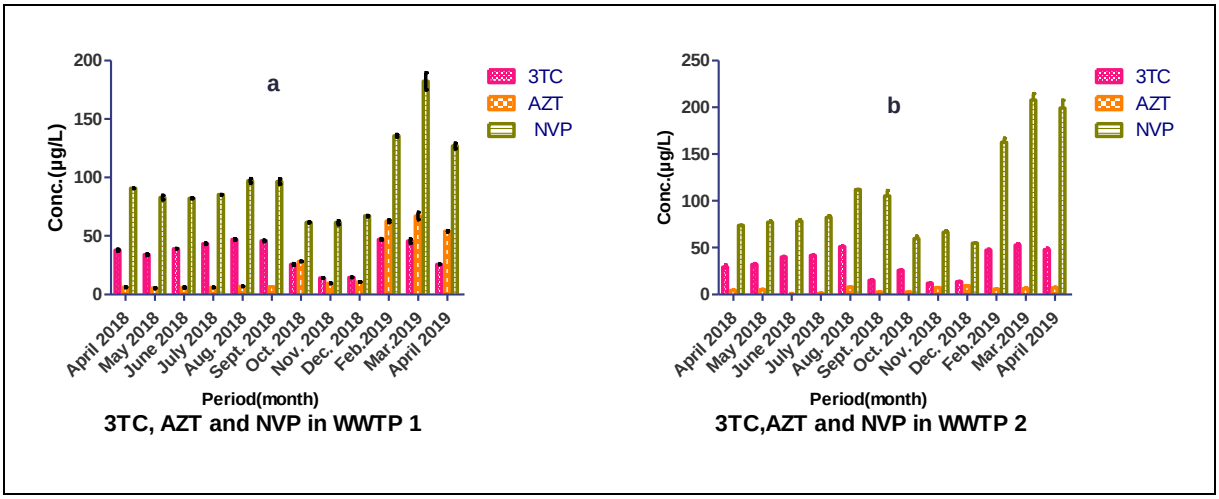

Figure 5.4: Comparison of occurrence of 3TC, AZT and NVP pharmaceutical formulations (a=WWTP 1, b=WWTP 2).

This finding was inconsistent with the study reported on the Kenyan river water where NVP had the lowest concentration compared to 3TC and AZT, which is, 167 µg/L, 17 µg/L, and 6 µg/L for 3TC, AZT, and NVP, respectively (Ngumba and Gachanja, 2015; K'oreje et al., 2016). Essentially, our finding also differs from the findings in Germany that reported a higher concentration (0.170 µg/L) for AZT and a lower concentration (0.017 µg/L) for NVP (Prasse et al., 2010). Essentially, their findings could be location-specific based on the type and quantity of ARV administered in those countries. For instance, the ARV compounds, ACY, ganciclovir and RBV were reported in Chinese WWTPs and some river water samples, whereas the commonly reported ARVs such as d4T and AZT in African WWTPs and river water samples, were not detected (Peng et al., 2014; Mzukisi et al., 2017).

NVP has been detected in the low ng/L range in German surface water (Prasse et al., 2010). It occurred at a higher level in river water and WWTP samples (influent and effluent) in Kenya (Ngumba and Gachanja 2015; K'oreje et al., 2016; Abafe et al., 2018). It is expected to be present at much higher concentrations in South Africa due to the level of its prominent therapeutic use for the prevention of mother to children transmission in South Africa (Schoeman et al., 2015; Wood et al., 2015). The highest concentration of NVP compared to 3TC and AZT pharmaceutical formulations could be attributed to factors such as i) its low removal ability at the WWTPs– during the treatment processes due to the de-conjugation of its hydroxylated metabolites, and ii) its hydrophilic nature which limits its removal from wastewater through sorption to sludge. All these factors point to the recalcitrant nature of the NVP (Wood et al. 2015; Madikizela et al., 2017; Schoeman et al., 2017; Abafe et al., 2018).

Reports from the literature surveyed categorized NVP as one of the persistent and bioactive ARV compounds in the environment (Prasse et al., 2010; Jain et al., 2013; Wood et al., 2015). Its persistent nature was due to its long half-life and photo-stability, and it has been reported as being toxic to larger animals such as rat (Jain et al., 2013). In South Africa, NVP is an NNRTI ARV compound that is mostly used in combination with two NRTI to treat HIV infection and massively used for the prevention of HIV transfer from mother to child (Boulle et al., 2010; Stinson et al., 2017). This kind of high concentration of pharmaceutical compounds, as observed in NVP, have been reported to have a consequential effect of reducing WWTPs efficiency (Slater et al., 2010; Abafe et al., 2018).

5.7 The percentage removal of 3TC, AZT and NVP in WWTP 1 and WWTP 2

Figure 5.5 presents the monthly percentage removal of 3TC, AZT, and NVP pharmaceutical formulations in WWTP 1 wastewater samples within the sampling period between April 2018 and April 2019. The percentage removal (PR) was obtained by comparing the concentration of pharmaceutical formulations in the RI with concentrations in the AUVT samples. As illustrated in graph a of Figure 5.5, the plant efficiency in removing 3TC during the sampling period ranged between 50.55% in October 2018 to 79.76% in April 2019. The lowest percentage removal (50.55%) in October 2018 was not different ($P > 0.05$) from 51.13% in April 2018 but significantly different ($P<0.05$) from the results in other months. In April 2019, the highest percentage estimate of 79.76% concentration of compounds was removed, which was significantly ($P < 0.05$) different from the values in other months. Other higher % of concentrations of compounds removed were recorded in August 2018 (73.26%), September 2018 (73.81%), and December 2018 (71.88%). The removal efficiency (RE) obtained in our study was higher than (24.01–59.40%) reported by K'oreje et al. (2016). This finding was comparable to the report of the previous study done by Prasse et al. (2010), in which case 3TC was one of the ARV compounds with a higher removal rate above 80.00%.

Graph b of Figure 5.5 shows the percentage monthly removal of the AZT in WWTP 1 samples. The degradation level varies from 52.98% in October 2018 to 81.18% in August 2018. The highest level of degradation in August 2018 did not show much different from that obtained in September 2018, December 2018, March 2019, April 2019, December 2018, and March 2019 which are 78.72%, 75.57%, 73.54%, 77.17%, 75.57%, and 73.54%, respectively. In October 2018, the lowest percentage removal of 52.98% was not significantly different ($P > 0.05$) from 58.48% obtained in April 2018. In addition, percentage removal of 67.29%, 72.19%, 71.05%, 69.73%, and 64.97% were recorded in May 2018, June 2018, July 2018, November 2018, and February 2019, correspondingly. These were not different from one another within the sampling period, but significantly different ($P < 0.05$) from the percentage removal in other months within the sampling period.

The average percentage removal of AZT within the sampling period was above 70.24%. This average estimate is close to the RE of 76.00% in WWTP 1, but lower than RE of 93% in WWTP 2 reported in Germany (Prasse et al., 2010). In addition, the average estimate is lower than the removal efficiency (RE) of 95% in WWTP 2 and 3 (K'oreje et al., 2016). More so, a trend of RE above 90% was reported for AZT in all the three WWTPs investigated in KwaZulu-Natal, South Africa (Abafe et al., 2018).

Similarly, graph c of Figure 5.5 presents the percentage of NVP removed in WWTP 1 samples evaluated within the sampling period of this study. The compound was removed at varying degrees within the period of investigation, and percentage estimates removed range from 25.86% in April 2018 and 76.89% in April 2019. The highest percentage of NVP removed in April 2019 was significantly ($P < 0.05$) different from the degradation recorded in all other months within the sampling period. Conversely, the least estimate of the concentration of compound was removed in April 2018 which was significantly different ($P < 0.05$) from the values obtained in other months.

In summary, the results obtained showed that the percentage removal of our target compounds in WWTP 1 samples occurred at varying levels. This can be attributed to the nature of the compounds, the type or composition of the wastewater, and the treatment operation in the WWTP (Schoeman, 2015). Also, the differences in the results of the percentage removed are related to other factors, such as the plant operational residence time, temperature, bacterial communities, sunshine, etc. (Matamoros et al., 2014; Aminot et al., 2016).

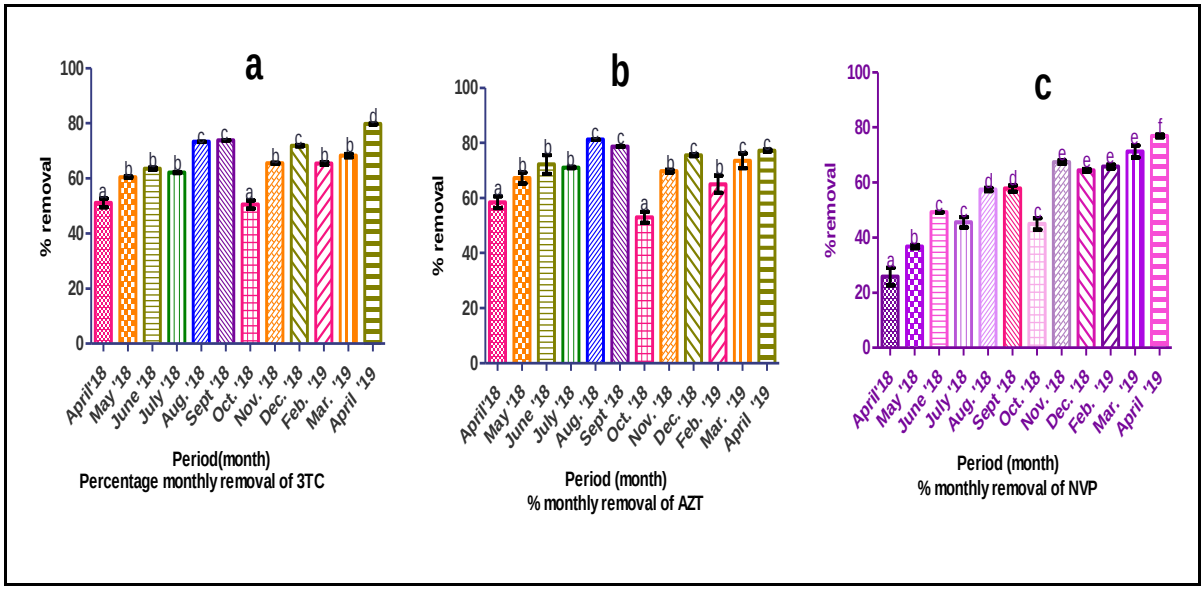

Figure 5.5: Graphical illustration of the monthly percentage removal of 3TC, AZT and NVP pharmaceutical formulations in WWTP 1 samples.

On average, the percentage removed for AZT, 3TC, and NVP are 70.20%, 65.41% and 55.34%, respectively. With a simple perspective, the order of efficiency of percentage removed as attained in this study is AZT>3TC>NVP. The high level of susceptibility to photolysis, hydrolysis, sorption and biodegradability might be the responsible factors for the greater level of percentage removal achieved for AZT (K'oreje et al., 2016). On the other hand, the low level of removal results of NVP could be attributed to its persistent nature (Wood et al., 2015). The "in-vitro" investigation of the removal rate of NVP in the bottled experiment reported that NVP is non-biodegradable and that it could be persistence in the environment (Vaňková, 2010). Other investigations elsewhere corroborate this finding, with a report from Europe indicating that NVP was not removed in the two WWTPs investigated (Prasse et al., 2010). A study from South Africa identified the persistent nature of NVP in the WWTPs evaluated as high levels of NVP that was reported in wastewater effluent samples (Schoeman et al., 2015).

5.8 Effect of UV treatment

Figure 5.6 shows the effect of UV treatment on the concentration of 3TC, AZT and NVP pharmaceutical formulations in WWTP 1 samples. The 2-way ANOVA was used to compare the concentrations of the three ARVs in the BUVT and AUVT samples within the sampling period of this study. As shown in graph (a) of Figure. 5.6, only the results of June 2018 and February 2019 demonstrated significant differences of ($P < 0.001$) and ($P < 0.01$), respectively

between the concentrations of 3TC in BUVT and AUVT samples. These were indicated as letters a and b on the BUVT and AUVT bars in graph a of Figure 5.6. All other months within the sampling period did not show any level of significant difference in the concentrations of 3TC between BUVT and AUVT samples. Similarly, in both graph b and c of Figure 5.6, there was no significant difference ($P > 0.05$) in the concentrations of AZT and NVP in any of the months within the sampling period as indicated on the bars. The result shows that the final tertiary treatment with UV radiation before the wastewater is finally discharged has a negligible effect on the elimination of our target compounds.

In that case, this could be ascribed to insufficient UV radiation that results from inadequate contact time between the UV radiation and wastewater. This could occur if the wastewater flows across the UV radiation at a much faster rate, and how the wastewater flows perpendicular to the flow direction of the UV radiation (EPA, 1999; Olaolu et al., 2014; Wu, et al., 2016). Similarly, it was reported that the success of UV radiation in the disinfestation and removal of micropollutants from water and wastewater depends on some factors such as the intensity or dose of UV radiation, the exposure duration, the type of reactor, and the presence of refractory dissolved organic matter (DOM) that could absorb UV light; thus, reducing the impact or effective treatment (EPA, 1999; Wu et al., 2017; Chan et al., 2018).

Another likelihood causal factor may be the volume or depth of the wastewater as it passes through the UV radiation. Earlier reports showed that a UV-based method in the removal of micro-pollutants is more effective with a thin layer or shallow water, but the effectiveness decreases as the volume and depth of the water increases (Hübner et al., 2015; Von Gunten, 2018). A reduced impact of UV radiation for the removal of contaminants in coastal water which is deeper compared to shallow surface water has been reported (Gaw et al., 2014). The researchers explained the limitation, among other factors, based on the effect of light attenuation as the water body becomes deeper (Gaw et al., 2014).

Similarly, since the plant uses chlorination as part of the secondary treatment process before the wastewater gets into the UV radiation treatment section; therefore, the inability of the UV radiation to show significant difference or reduction in the concentration of the ARVs compounds in BUVT and AUVT samples may be due to a low level of the chlorine content of the wastewater reaching UV radiation section (Ye et al., 2017; Huang, et al., 2017; Chan et al., 2018). Reports have shown that for the success of a UV/chlorine process, an optimised dose of chlorine is required to ensure the success of the process for effective contaminants removal (Ye et al., 2017; Huang, et al., 2017; Chan et al., 2018).

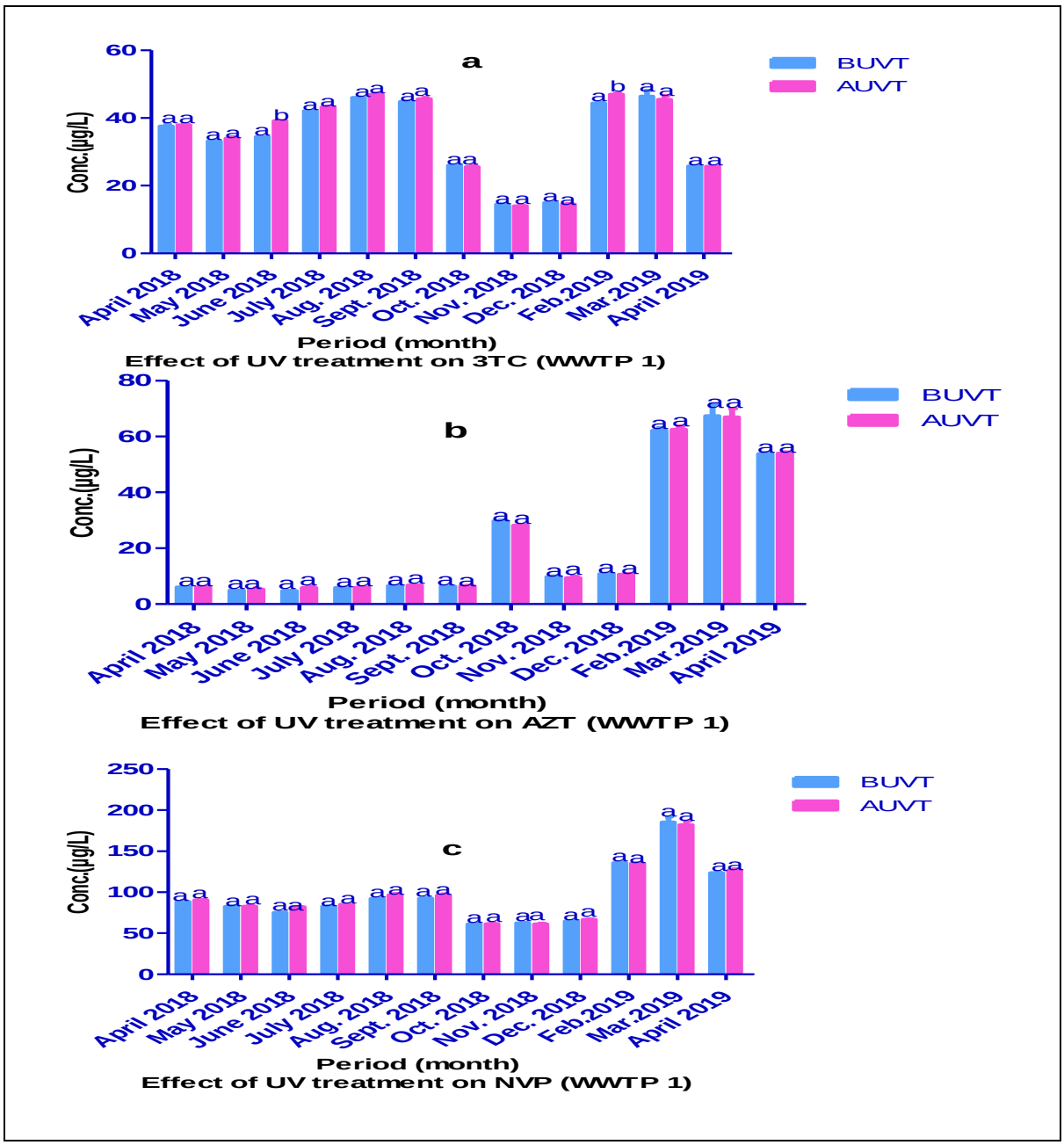

Figure 5.6: Graphical illustration of the effect of UV treatment on (a) 3TC, (b) AZT and (c) NVP pharmaceutical formulation in WWTP 1 samples.

This suggests that the flow rate, the depth of the BUVT wastewater flowing through the UV radiation and the chlorine content during treatment can be revisited. Previous studies that compared direct photolysis with the combined UV/chlorine process found that chlorination alone or UV radiation alone was not effective for the removal of the majority of pharmaceuticals

as compared to the combined UV/chlorine process (Guo et al., 2017; Wang, et al., 2017; Chan et al., 2018).

5.9 Summary

All the three investigated ARV compounds (3TC, AZT, NVP) were detected and quantified by UV-visible spectrophotometry technique. Within the sampling period, their concentrations ranged ranged between 1,410 – 4,702 µg/L, 539 – 6,698 µg/L, and 6,135 – 18,233 µg/L for 3TC, AZT, and NVP, respectively for WWTP 1. It was 1,178 - 5,289 µg/L, 90 – 917 µg/L, and 5,470 – 20,770 µg/L for 3TC, AZT, and NVP, respectively in WWTP 2 sample. The validation of the analytical method according to ICH guidelines produced good linearity within 2 – 24 µg/mL for 3TC and AZT and 4 – 40 µg/mL for NVP; the precision based on RSD was < 2% and the percentage mean recovery complied with accepted range (98 – 102%) for accuracy studies. The limit of detection (LOD) and limit of quantification (LOQ) were 0.068 µg/L and 0.206 µg/L for 3TC, 0.186 µg/L and 0.563 µg/L for AZT, and 0.759 µg/L and 2.299 µg/L for NVP, respectively. This indicates that the method is simple, accurate, precise, linear, fast, and cost effective for the determination of ARVs in WWTPs, and most especially small WWTPs.
Our findings also showed that WWTP 1 was able to remove the major part of the three compounds investigated. However, NVP appeared to be recalcitrant and persistent because it contained the highest concentrations in the two plants.

CHAPTER 6

DEGRADATION STUDIES OF SELECTED ANTI-RETROVIRAL COMPOUNDS IN WASTEWATER SUBJECTED TO SUNLIGHT(UV)/CL_2 AND SUNLIGHT(UV)/$KMNO_4$ TREATMENT

6.1 Introduction

The result of UV visible spectroscopy in chapter five established the presence of our target ARVs in wastewater samples from both WWTPs. This is consistent with several scientific reports that ARVs are not completely removed in WWTPs operations and effluent from WWTPs constitute a source of pollution to the aquatic environment (Prasse et al., 2010; Ngumba and Gachanja, 2015; Wood et al., 2015). This chapter focuses on the development and optimisation of two AOPs treatments which are sunlight (UV)/Cl_2 and sunlight (UV)/$KMnO_4$ processes for the removal of the ARV compounds, to eradicate or minimize the level of environmental pollution by the ARV compounds in the aquatic ecosystem.

6.2 Optimisation of degradation parameters

According to the literature, some parameters and conditions which include irradiation intensity, pH, the oxidant to analyte ratio (OAR) and contact time are essential for the degradation of micropollutants (Remucal and Manley, 2016; Guo et al., 2017). This section investigates and optimises each of the parameters to ensure the good performance of the novel methods.

6.2.1 Selection of solar irradiance

Since the objective of this work was to use open solar radiation which may not be altered or controlled. The weather forecast was monitored before the days of the experiments to facilitate the selection of a suitable day for the degradation experiment. The appropriate days selected for the experiments were clear and sunny days with an average solar irradiance between 250 ± 25 mW/m^2 when the UV index was very high and an average temperature of 23 ± 2.0 ^oC (Downs et al., 2016; Gies et al., 2018). The actual weather conditions (solar irradiance and temperature) on the days and times of the experiments were measured by a spectroradiometer (ASD FieldSpec 4 Hi-Res: High-Resolution Spectroradiometer) from the Solar Energy Unit, Department of Mechanical Engineering, Cape Peninsula University of Technology, Bellville, South Africa. All degradation experiments were conducted in open-air between 12 noon and 2 pm when the weather was clear and sunny following the protocol of Chan et al. (2012).

6.2.2 Influence of time on pharmaceutical degradation using sunlight (UV)/Cl_2

This section investigates and optimises the influence of time on the degradation of our target ARVs. The method for the determination of the optimum time for the degradation of 5 µg/mL (≈20 µM) 3TC, AZT and NVP was conducted as described in section 3.7.2.2. The results showing the effect of different time on the removal of 3TC, AZT and NVP with sunlight (UV)/$Ca(ClO)_2$ are shown in Figures 6.1 – 6.3. The Figures show the % removal of 3TC, AZT and NVP at different contact times (t). From Figures 6.1– 6.3. the greatest percentage reduction in the concentration of the analyte occurred at 90 minutes. The percentage removal between 0 – 210 mins were 0.00 –11.05 %, 0.00 –20.46 %, and 0.00 –17.40 % respectively. It was 0.00 %, 16.61 %, for 3TC, AZ and NVP, respectively. In the three compounds, the highest % removal occurred at 90 minutes and the values were 11.05 %, 20.46 %, and 17.40 % for 3TC, AZT and NVP, respectively. Therefore, the subsequent experiments were carried out at 90 minutes.

Reaction time has been described as an important parameter usually measured in degradation experiment (Pan et al., 2017; García-Espinoza et al., 2018). In electrochemical oxidation of CBZ with NaCl as supporting electrolyte, the optimal time for the degradation of CBZ was 12.45 mins (García-Espinoza et al., 2018). In the degradation of NVP with FL-BP@Nb2O5, the reaction mixture was illuminated for 3 h but the optimised time was 180 mins when the % removal was 56.2% at the initial concentration of 5ppm. Before and after this optimised time, the percentage removal recorded were lower (Bhembe et al., 2020). In an investigation to determine factors responsible for the degradation of 53 pharmaceuticals (including 8 ARV compounds) discharged into Garonne River in Bordeaux, France, the investigation was time-bound and was conducted for 4 weeks (Aminot et al., 2018)

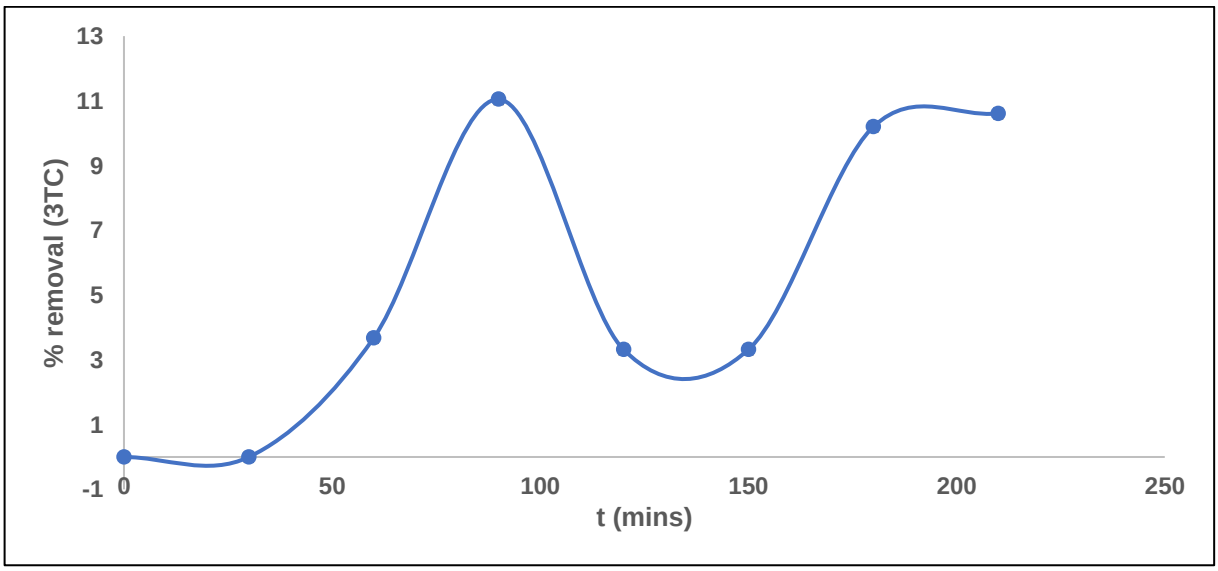

Figure 6. 1: Effect of contact time on the removal of 3TC.

(Reaction conditions: T_0 = 25 ± 0.2 °C, pH = 4, solar irradiance 250± 25 mW/m^2, $[A]_0$ = (20 µM of 3TC), $[O]_0$ = 140 µM, O = $Ca(ClO)_2$).

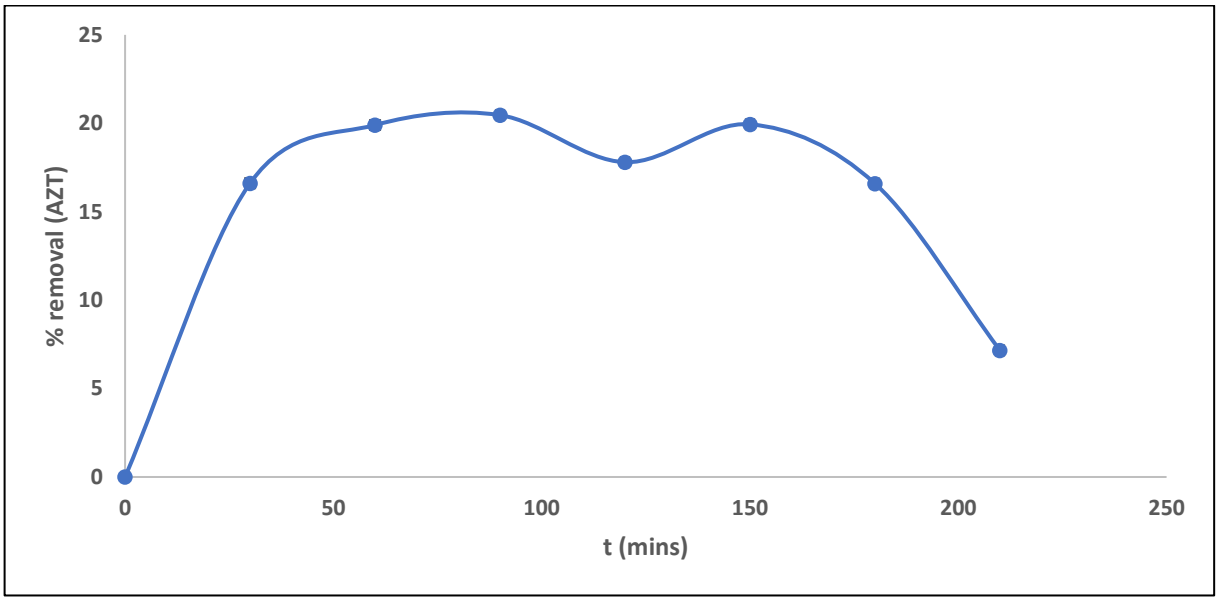

Figure 6. 2: Effect of contact time on the removal of AZT.

(Reaction conditions: T_0 = 25 ± 0.2 °C, pH = 4, solar irradiance 250± 25 mW/m^2, $[A]_0$ = (20 µM of AZT), $[O]_0$ = 140 µM, O = $Ca(ClO)_2$).

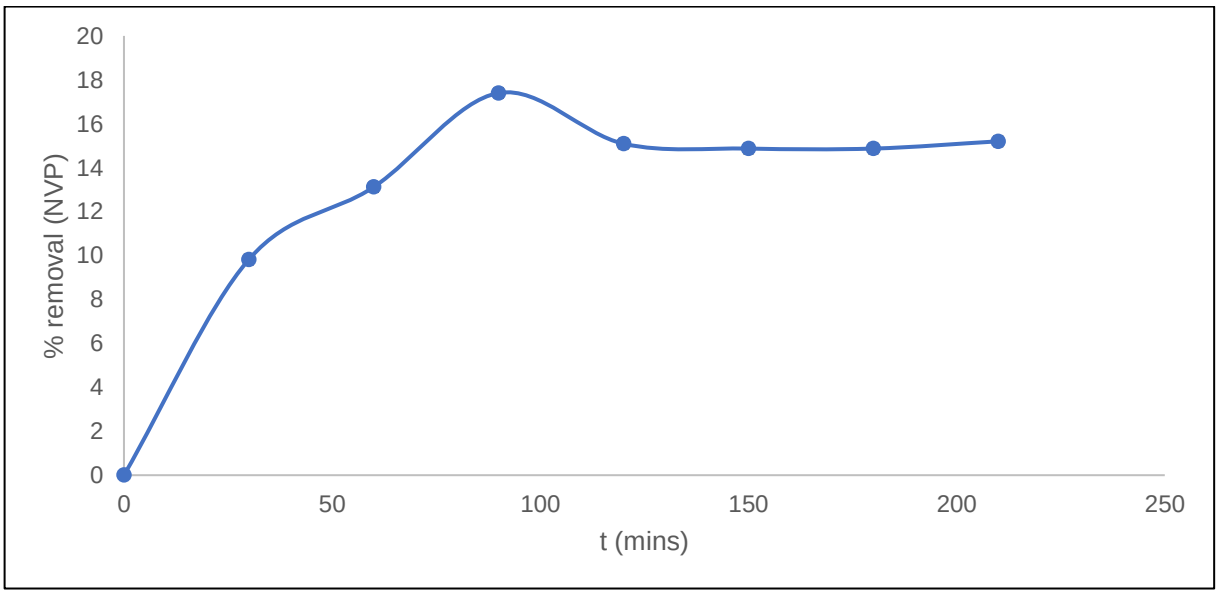

Figure 6. 3: Effect of contact time on the removal of NVP.

(Reaction conditions: T_0 = 25 ± 0.2 °C, pH = 4, solar irradiance 250± 25 mW/m^2, $[A]_0$ = (20 µM of NVP), $[O]_0$ = 140 µM, O = $Ca(ClO)_2$).

6.2.3 Influence of time on pharmaceutical degradation using sunlight(UV)/$KMnO_4$

The impact of time on the removal of the target compounds was investigated by optimising the contact time. The procedure for the optimisation of contact time was described in section 3.7.2.2. The result showing the impact of contact time on the removal of the three pharmaceutical compounds was presented in Figures 6.4 – 6.6. From Figure 6.4, the % removal recorded for 3TC varied between 0.11 – 4.34 %, it was 43.22 – 56.63 % for AZT (Figure 6.5) while it ranged between 39.00 – 50.04 % for NVP (Figure 6.6). The findings showed that the highest level of % removal for 3TC and AZT occurred at 45 mins. The highest % reduction for NVP (50.05 %) was recorded in both 45 and 60 mins. For consistency with the two other compounds, and since the standards were prepared as a cocktail to reflect the real practical environmental situation in which the compounds co-exist together in wastewater, 45 minutes which correspond with the other compounds was picked as the optimum time for the subsequent studies. Research has shown that time is a major factor in degradation processes, the degradation of AZT was conducted in different conditions (acidic, basic, and neutral) in reflux for 72 h while the oxidative degradation with hydrogen peroxide was conducted at a room temperature for 10 h (Devrukhakar et al., 2017).

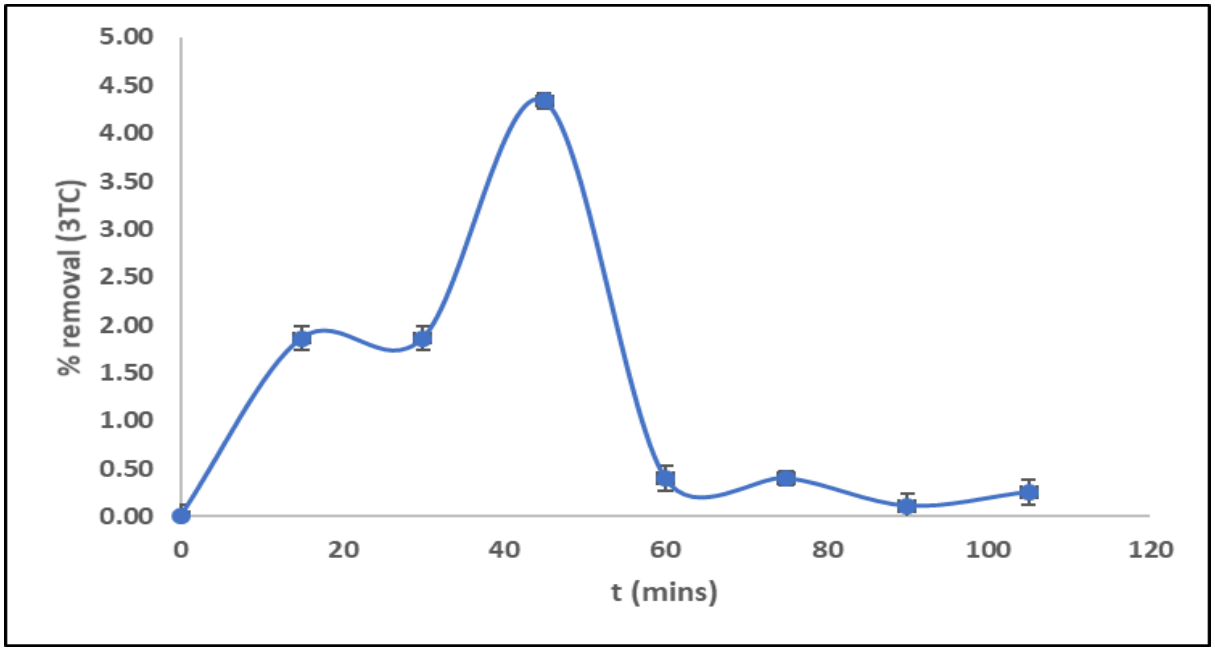

Figure 6. 4: Effect of contact time on the removal of 3TC.

(Reaction conditions: T_0 = 25 ± 0.2 °C, pH = 4, solar irradiance 250± 25 mW/m^2, $[A]_0$ = (20 µM of 3TC), $[O]_0$ = 140 µM, O = $Ca(ClO)_2$).

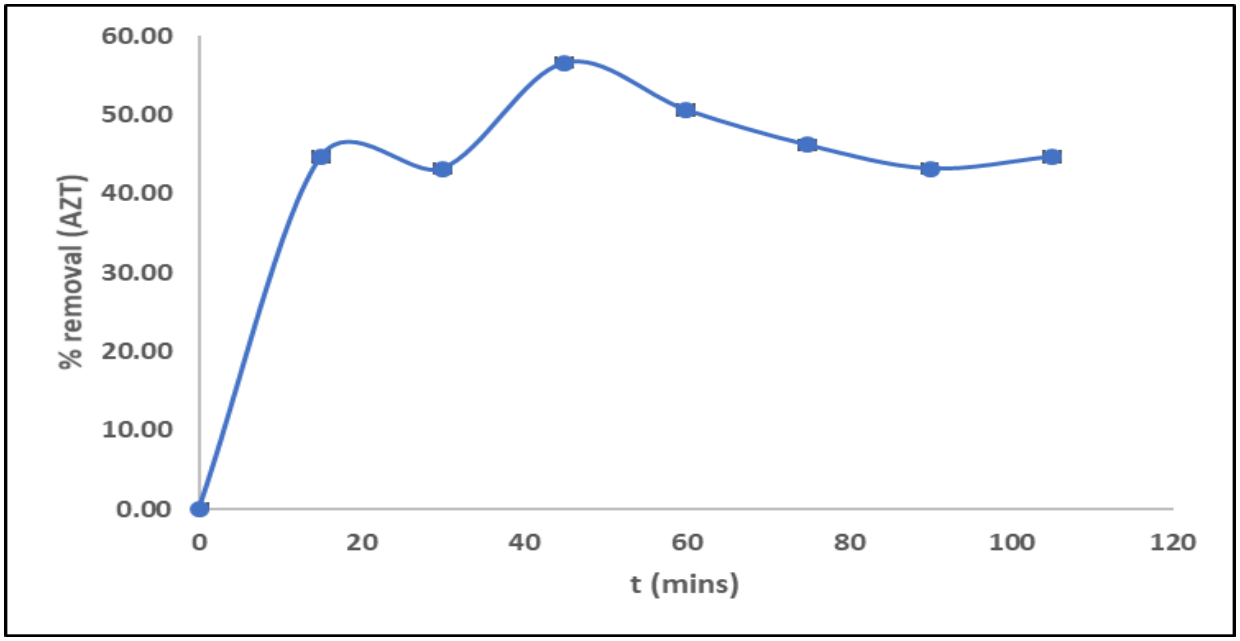

Figure 6. 5: Effect of contact time on the removal of AZT.

(Reaction conditions: T_0 = 25 ± 0.2 °C, $_pH$ = 4, solar irradiance 250± 25 mW/m^2, $[A]_0$ = (20 µM of AZT), $[O]_0$ = 140 µM, O = $Ca(ClO)_2$).

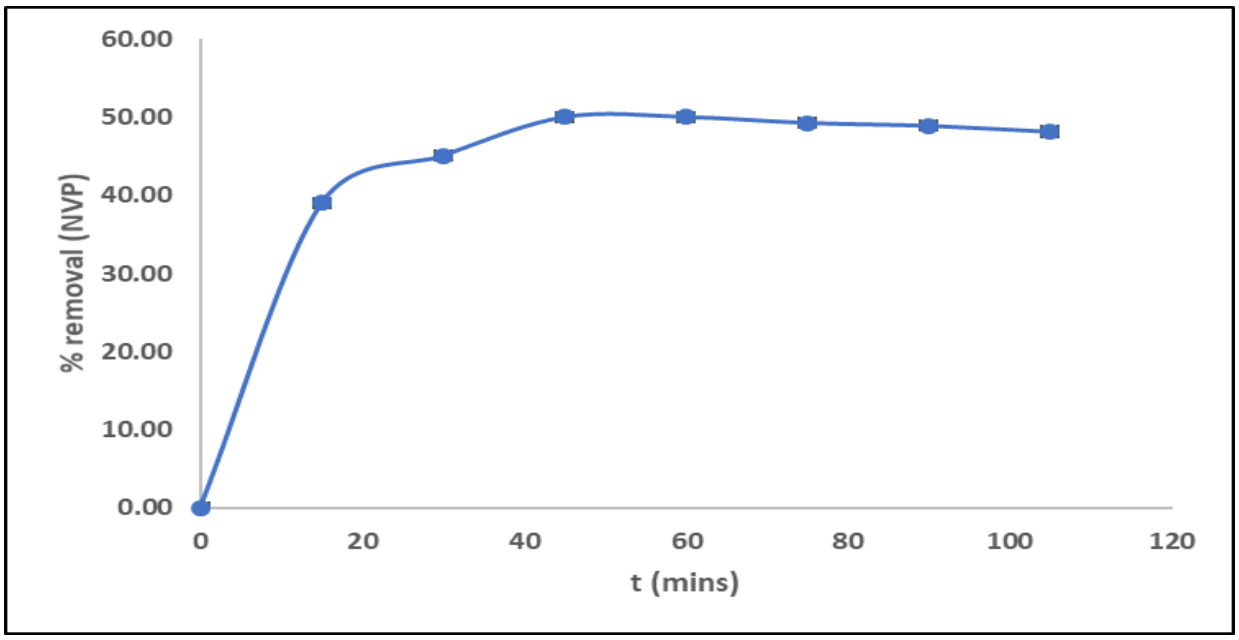

Figure 6. 6: Effect of contact time on the removal of NVP.

(Reaction conditions: T_0 = 25 ± 0.2 °C, $_pH$ = 4, solar irradiance 250± 25 mW/m^2, $[A]_0$ = (20 µM of NVP), $[O]_0$ = 140 µM, O = $Ca(ClO)_2$).

The time (45 minutes) corroborates the report that three antibiotics (ciprofloxacin, lincomycin, and trimethoprim) were efficiently removed below 60 minutes which is practically crucial in the water treatment process (Hu et al., 2010). This time was however higher than that reported in some other scientific works (Sun, et al., 2015; Chow and Sze-Yin Leung, 2019). This could however be due to the enhancement of manganese oxidants with bisulphite in their work. Time

has been described as one of the operational conditions put into consideration as a performance index of WWTPs towards the removal of pollutants (K'oreje et al., 2018).

6.2.4 Influence of pH on pharmaceutical degradation using sunlight (UV)/Cl_2

This section investigates and optimises the influence of different pH on the degradation of our target ARVs. The method for the determination of the optimum pH for the degradation of 5 µg/mL (≈20 µM) 3TC, AZT and NVP was conducted as described in section 3.7.2.4. The results showing the effect of different pH on the % removal of 3TC, AZT and NVP are shown in Figures 6.7 – 6.9.

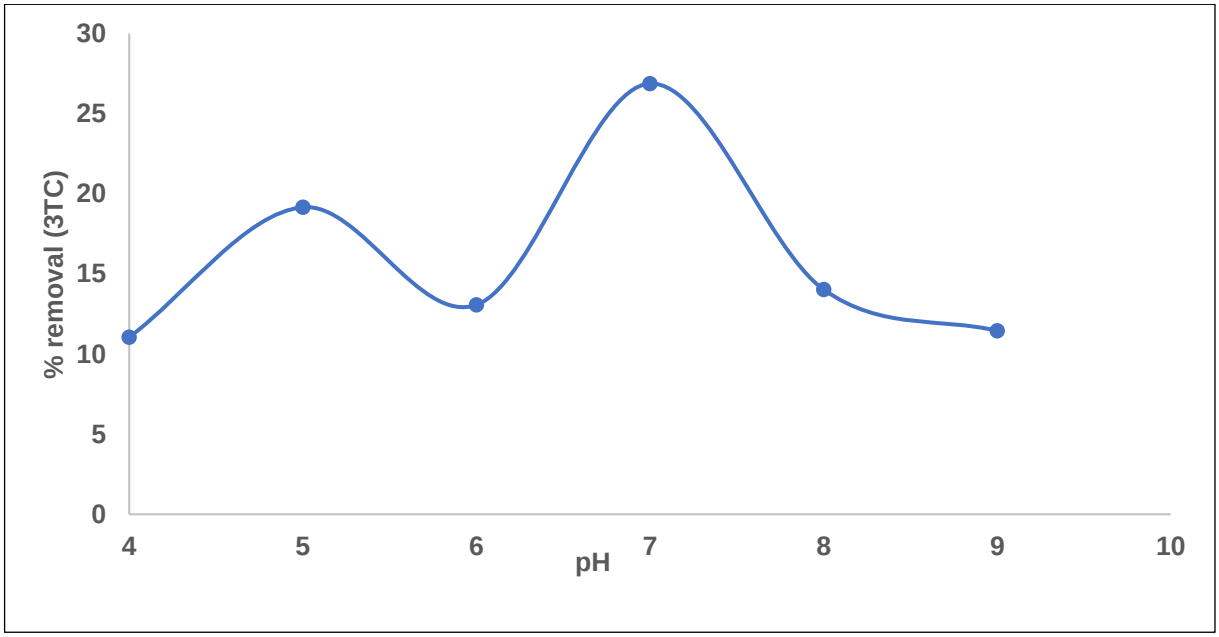

Figure 6. 7: Effect of pH on the removal of 3TC (sunlight (UV)/Cl_2 method).

(Reaction conditions: T_0 = 25 ± 0.2 °C, pH = 4 – 9, solar irradiance 250± 25 mW/m^2, t = 90 mins, $[A]_0$ = (20 µM of 3TC), $[O]_0$ = 140 µM, O = $Ca(ClO)_2$).

The percentage removal recorded between pH 4 – 9 for 3TC, AZT and NVP varied from 11.05 – 26.88%, 0.00 – 26.96% and 18.24 – 24.55%, respectively. In all the 3 compounds, pH 7 showed the greatest percentage reduction of 26.88% (Figure 6.7), 26.96% (Figure 6.8)and 24.55% (Figure 6.9) for 3TC, AZT and NVP, respectively. Therefore, the subsequent experiments were carried out at pH 7 for 90 minutes.

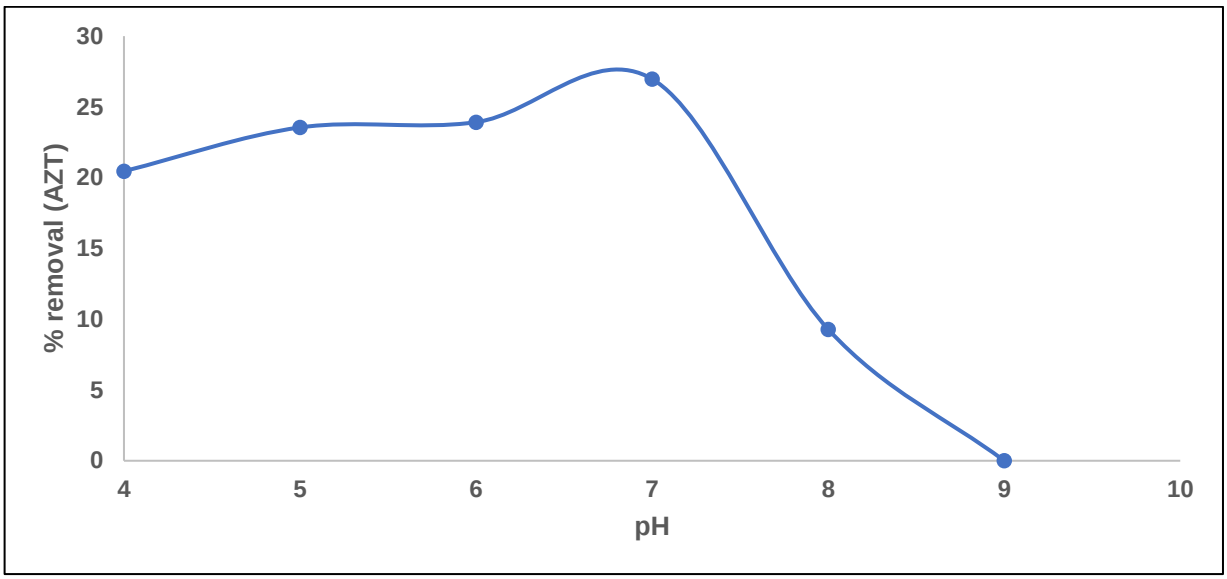

Figure 6. 8: Effect of pH on the removal of AZT (sunlight (UV)/Cl_2 method).

(Reaction conditions: T_0 = 25 ± 0.2 °C, pH = 4 – 9, solar irradiance 250± 25 mW/m^2, t = 90 mins, $[A]_0$ = (20 µM of AZT), $[O]_0$ = 140 µM, O = $Ca(ClO)_2$).

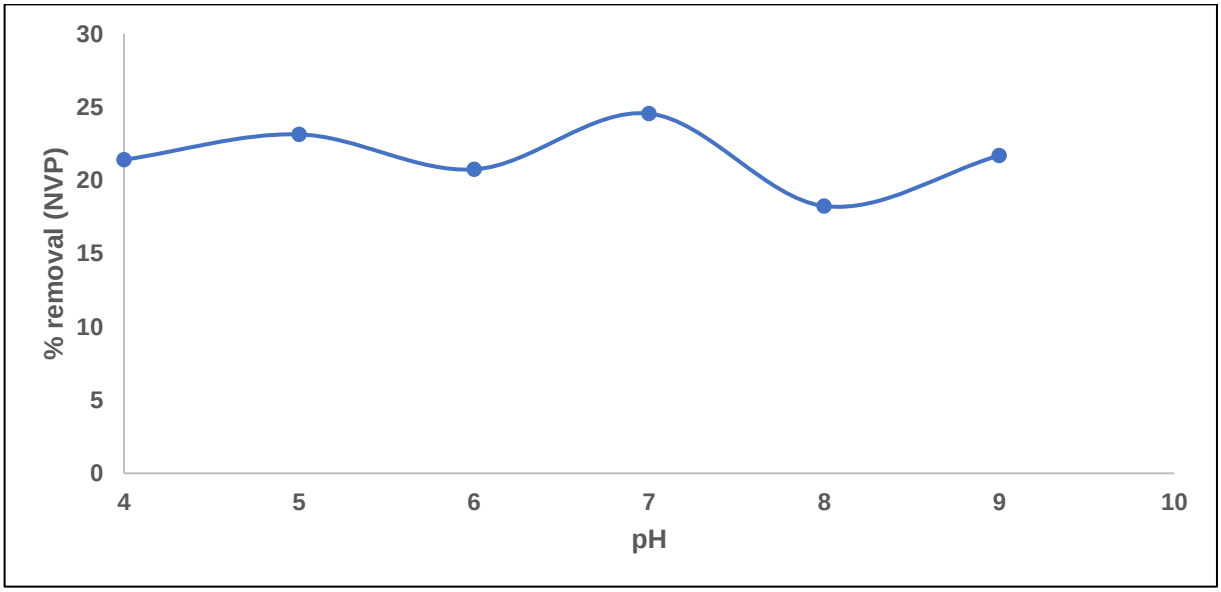

Figure 6. 9: Effect of pH on the removal of NVP by (sunlight(UV)/Cl_2 method).

(Reaction conditions: T_0 = 25 ± 0.2 °C, pH = 4 – 9, solar irradiance 250± 25 mW/m^2, t = 90 mins, $[A]_0$ = (20 µM of NVP), $[O]_0$ = 140 µM, O = $Ca(ClO)_2$).

It has been reported that the physicochemical properties of pharmaceuticals have a major impact on the degradation of pharmaceuticals, for instance, non-ionic pharmaceuticals were reported to be recalcitrant while the weak acidic pharmaceuticals demonstrated fast degradation rates (Grossberger et al., 2014). However, reports on the effect of pH on ARV compounds are very scarce in the literature. The majority of past and recent journals on ARVs were on monitoring rather than degradation (Abafe et al., 2018; K'oreje et al., 2012; Mosekiemang et al., 2019; Mlunguza et al., 2020; Omotola and Olatunji, 2020). Consequently, the effect of pH on our compounds removal was discussed with the recent reports with other pharmaceuticals found in the literature that have similar physicochemical properties and where a similar degradation

method is used as in this work (Akenga et al., 2021). Some pharmaceuticals such as CBZ, lamotrigine, caffeine, sildenafil, sulfapyridine and metoprolol were reported to be similar being non-ionic (Grossberger et al., 2014), some also have similar log Dow, for instance, CBZ and NVP (log Dow 2.5) (Chuang et al., 2019) therefore the basis for using them.

Concerning the structure of our ARV compounds, at pH 7, when both OCl^- and HOCl exist at equilibrium and in the presence of UV radiation there will be a kind of synergy reaction occasioned by the OCl^- and H^+ from the HOCl. For, 3TC, the ether group is opened up for reaction, so, the H^+ attacks the oxygen atom of the ether group that caused protonation of the oxygen atom of the ether group. Then, the OCl^- attacks the carbon atom of the ether group which thereby caused the degradation of the compound. This was consistent with the proposed degradation of 3TC to protonated cytosine when the oxathiol group in 3TC was replaced by a hydrogen atom. This later result in the formation of other metabolites due to subsequent loss of NH_3, H_2O, and HNCO (Bedse et al., 2009). Though this report showed that 3TC is stable at a neutral pH during hydrolysis (Bedse et al., 2009), the difference in their report could emanate from the condition of degradation which was just by the heating the compound in water without the addition of any other compound such as buffer solution, chlorine dose and radiation.

For AZT, the azide group is the most reactive part, there is a protonation caused by the attack of H^+ on the negatively charged nitrogen at the azide group, then, the OCl^- attacks the positively charged nitrogen atom which increases the degradation of the AZT.

In the case of NVP, the H^+ from HOCl attack the lone pair on the amine functional groups and the carbonyl group is attacked by the OCl^-. The result of degradation studies conducted separately on NVP using 3-chloroperoxybenzoic acid (MCPBA) and H_2O_2 at different pH showed that both compounds degraded NVP at neutral and pH but the oxidation did not occur at the acidic pH (Qiu et al., 2009). The group explained that the result obtained could arise from the decrease in the electron density at the double bonds because of the protonation of the N_2 in the quinoline moiety. Similarly, it was reported that the total degradation of NVP occurred most rapidly in the 90s in an unbuffered HPLC grade water (apparently around pH 7) than at pH 8. Whereas in an acidic pH, about 80% of NVP remained undegraded even at 110 s when the reaction stops. Thus, the increased reactivities and degradation of NVP could be due to the existing species NVP at pH 7 (Wood et al., 2016). Similarly, the increased degradation at pH 7 could be attributed to the contribution of ClO• that could be better produced at pH 7. This is consistent with the report that the production of ClO• slightly increased at pH 7 that slightly enhances the removal of gemfibrozil and bezafibrate (Kong et al., 2018). The group also reported that at pH 7, the dissociation constant value for the two compounds increased correspondingly with chlorine dose.

Our report is consistent with many findings for better removal of pharmaceuticals at pH 7 (Wu et al., 2016; Guo et al., 2017; Guo et al., 2018). The degradation of some pharmaceuticals such as naproxen, diclofenac, gemfibrozil, trimethoprim and propranolol are promoted at around pH 7 (Wu et al., 2016; Guo et al., 2017). The result from a similar study also showed that the optimum degradation of phenacetine with UV/Cl_2 occurred at pH 7.2 which is very close to this work (Zhu et al., 2018). Similarly, Guo et al. (2018) reported that UV/Cl_2 was able to better degrade 16 pharmaceuticals than UV/H_2O_2 at pH 7. Previous research attributed the better removal of pharmaceutical compound trimethoprim (TMP) in the water around neutral pH to the dissociation of TMP that is pH-dependent (Wu et al., 2016).

There are diverse reports on the influence of pH on the degradation of pharmaceuticals under the UV/Cl_2 process (Wu et al., 2016; Guo et al., 2017; Yin et al., 2018). Yin et al. (2018) reported that pH does not influence the degradation of prednisolone. However, it was well reported in the literature that pH value is very important for the degradation of pharmaceutical residues in the aquatic system by the radiation – relation processes (Wang and Chu, 2016; Guo et al., 2017; Wu, et al., 2018a; Cai et al., 2019). Several scientific findings documented that the degradation efficiency of pharmaceuticals residues with UV/Cl_2 process at alkaline condition (generally pH > 10) is generally lower compared to what operates at weakly acid to neutral condition (Wang and Chu, 2016; Guo et al., 2017; Wu, et al., 2018a; Cai et al., 2019). The justification provided for these variations was that the generation of HO• and RCS at weakly acid cum neutral pH is higher and this eventually determine their effectiveness. This is because, at this pH, HOCl which is the dominant species at acidic cum neutral pH generates more HO• and RCS that favour contaminants removal than ClO^- (Guo et al., 2017; Wang et al., 2019). On the other hand, In a WWTP in South Africa where chlorination is mainly used for disinfection, a reduced removal of NVP was reported at the acidic pH at which the plant operates (Wood et al., 2015). This clearly shows that other experimental or real-life situations such as the target analyte, added reagent and catalyst could also affect the optimal pH (Bhembe et al., 2020).

6.2.5 Influence of pH on pharmaceutical degradation using sunlight (UV)/$KMnO_4$

One of the advantages of oxidation of micropollutant with $KMnO_4$ is that it can work at different pH ranges (Von Gunten, 2018), however, most oxidation that is $KMnO_4$ related are conducted at one pH or the other (Hu et al., 2011; Sun et al., 2015). The optimisation is important and very necessary because it makes the work more scientific (Hu et al., 2011; Sun et al., 2015). This section presents the result of the influence of pH on the removal of our target compounds by sunlight(UV)/KMnO4 in Milli -Q water. The experimental procedure for the determination of the optimum pH as described in section 3.7.2.4. Figures 6.10 ─ 6.12 depict the effect of different pH (4-9) on the removal of 3TC, AZT and NVP treated with sunlight(UV)/$KMnO_4$. The % removal between pH 4 – 9 ranged between 4.34 – 27.21%, 4.42 – 80.22% and 6.08 – 54.81% for 3TC, AZT and NVP, respectively. From the list of % removal, the highest values for 3TC, AZT and NVP 27.21%, 80.22% and 54.81%, respectively

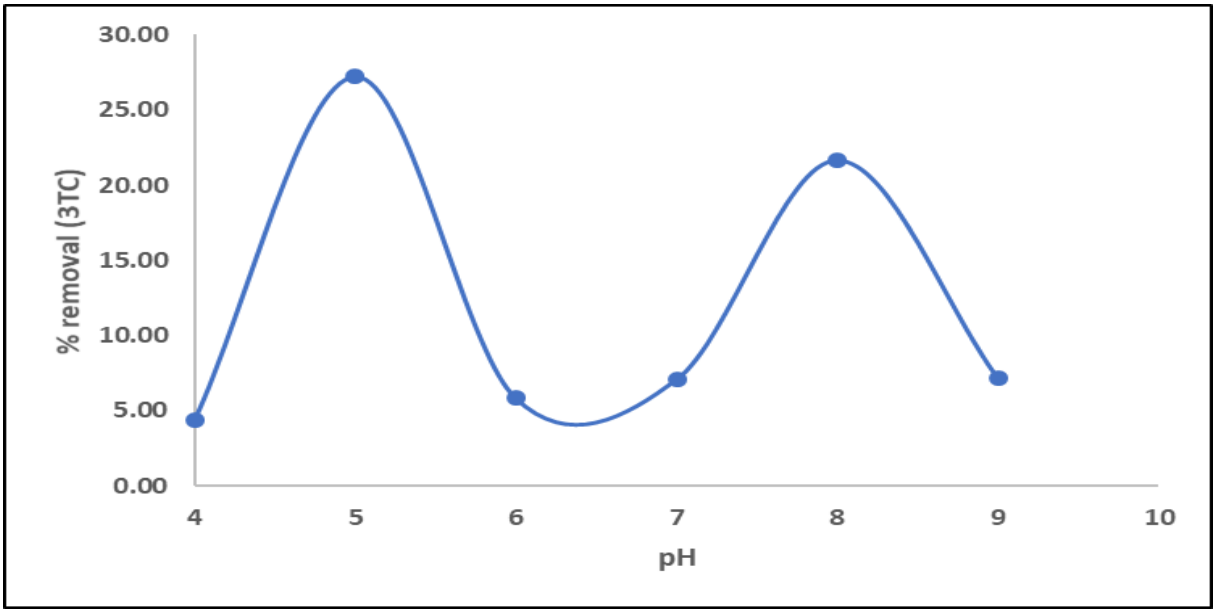

Figure 6. 10: Effect of pH on the removal of 3TC (sunlight(UV)/$KMnO_4$ method).

(Reaction conditions: T_0 = 25 ± 0.2 °C, pH = 4 – 9, solar irradiance 250± 25 mW/m^2, t = 45 mins, $[A]_0$ = (20 µM of 3TC), $[O]_0$ = 140 µM, O = $KMnO_4$).

The result shows the varying percentage removal of the three compounds at different pH (4-9). Figures 6.10 ─ 6.12 show that for all the pH (4-9), the highest reduction in the concentrations of the 3 ARVs occurred at pH 5.

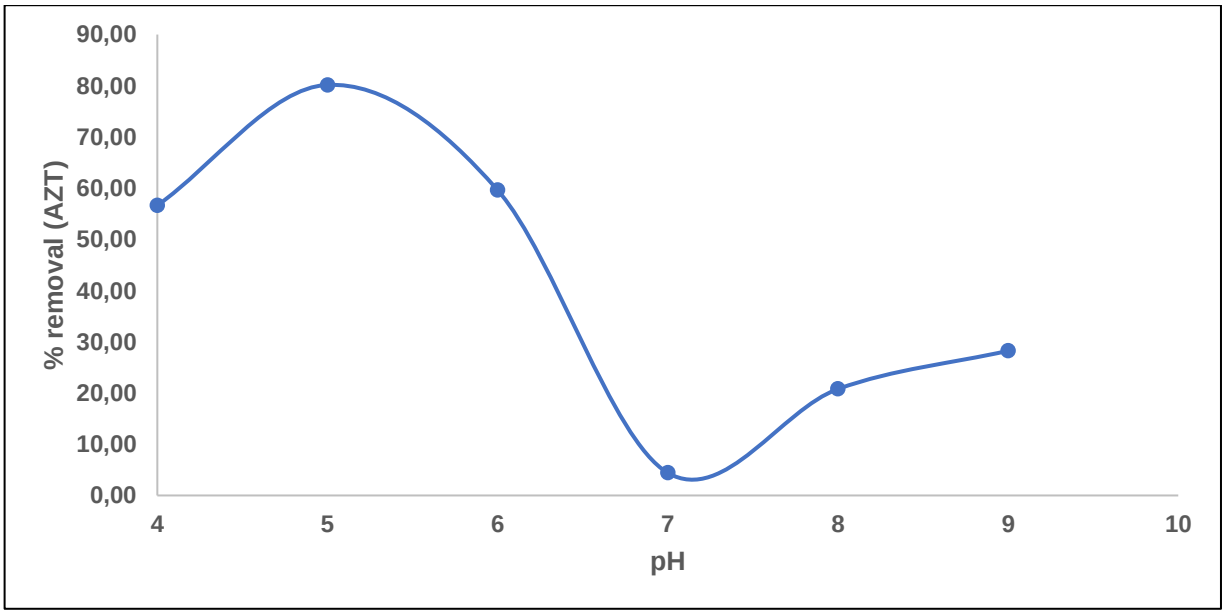

Figure 6. 11: Effect of pH on the removal of AZT (sunlight(UV)/KMnO4 method).

(Reaction conditions: T_0 = 25 ± 0.2 °C, pH = 4 – 9, solar irradiance 250± 25 mW/m^2, t = 45 mins, $[A]_0$ = (20 µM of AZT), $[O]_0$ = 140 µM, O = $KMnO_4$).

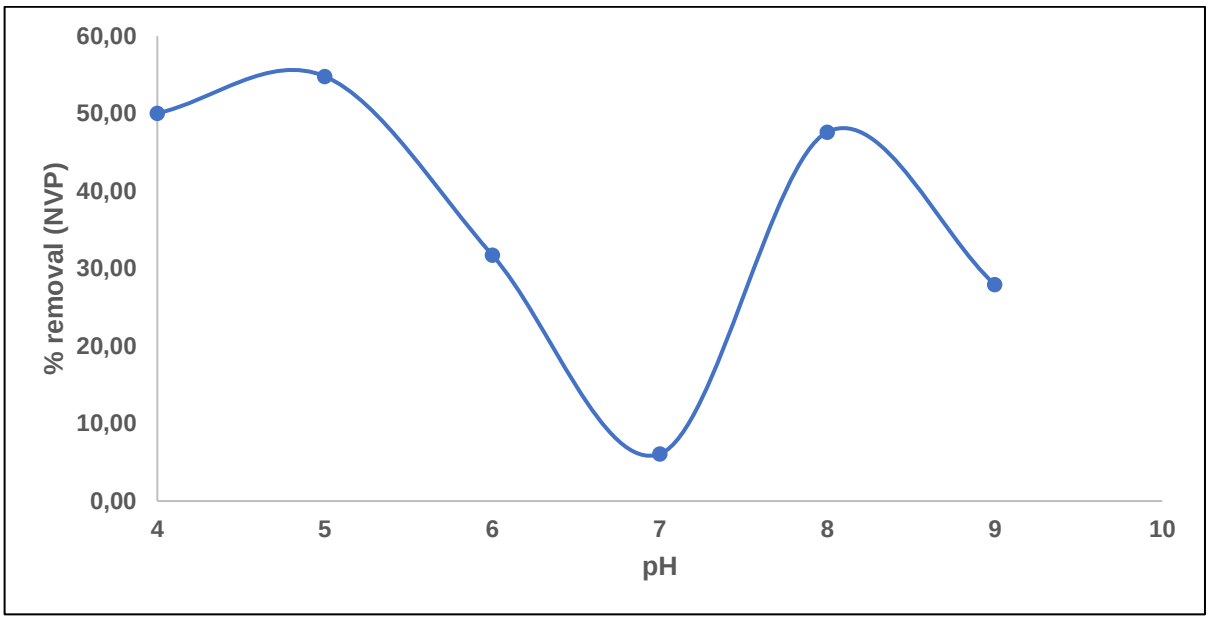

Figure 6. 12: Effect of pH on the removal of NVP (sunlight(UV)/KMnO4 method).

(Reaction conditions: T_0 = 25 ± 0.2 °C, pH = 4 – 9, solar irradiance 250± 25 mW/m^2, t = 45 mins, $[A]_0$ = (20 µM of NVP), $[O]_0$ = 140 µM, O = $KMnO_4$).

For 3TC, at pH 5, the H^+ attacks the oxygen atom in the ether group that has a lone pair of an electron which caused protonation of the oxygen atom. Then, the manganate ion which is negatively charged attacks the carbon atom of the ether group which result in the opening of the ether group. Bedse et al. (2009) reported the degradation of 3TC in an acidic condition with 0.1N HCl when heated.

In the case of AZT, the H^+ from the acidic pH attacks the negatively charged nitrogen atom of the azide group. Subsequently, the manganate opens up the ether ring as it attacks the neutral nitrogen ion at the azide group which caused the electron to shift to the positively charged nitrogen atom of the azide group which then caused the compound to degrade. This was consistent with the report in the literature (Devrukhakar et al., 2017). The group reported the formation of two product ions m/z 227 and m/z 127 by the protonation of AZT and 5-methyl pyrimidine-2,4(1H,3H)-dione) respectively. The degradation of AZT, when subjected to a forced degradation in an acidic condition under reflux for 72 h, was reported by Devrukhakar et al. (2017). The same compound was reported to be stable in alkaline and neutral conditions.

For NVP, there was an earlier report that the removal of NVP is hindered at the acidic condition where chlorine is used as a disinfectant (Wood et al., 2016). However, the degradation of NVP could be caused by the $KMnO_4$ being a powerful oxidising agent. At pH 5 where the % removal was the highest, H^+ from the acidic pH attacks the nitrogen atom of the amine functional group that has a lone pair of electron and the manganate ion attacks the carbonyl group in the NVP to cause the removal of the compound. The findings of photocatalytic degradation of NVP reported high degradation of NVP at the acidic condition in which the degradation efficiency was 46.6%, 42.1% and 33.2% for pH 3, pH 5, and pH 1, respectively. The effect could be related to the pKa of NVP which is 2.8. This shows that the compound is weakly basic with the ability to be protonated in an acid medium (Bhembe et al., 2020). This disparity in the effect of pH could also be caused by some other factors such as the other reagent and conditions attached.

For example, the inability of the compound to degrade at the acidic condition as reported by Wood et al. (2016) was based on the use of chlorine for disinfection. The degradation recorded in our case and the report of Bhembe et al. (2020) at acidic condition could also be related to the reagents involved which were $KMnO_4$ and black phosphorus /niobium (V) oxide. This is consistent with the literature that the degradation of NVP at acidic pH could be attributed to the surface of the photocatalysts which was positive relative to NVP which is negatively charged. Thus, NVP adsorbed on the surface of the catalysts due to electrostatic attraction which enhances the adsorption of NVP hence promoted the degradation efficiencies (Bhembe et al., 2020).

The result here is in agreement with the findings from another scientist that investigated the impact of manganese oxidant activated by with bisulphite on the removal of organic pollutant (Sun et al., 2015). The study reported an enhanced removal of ciprofloxacin, methyl blue and phenol at pH 5.0 with rates ($k_{obs} \approx 60-150$/s) that were $5-6$ orders of magnitude faster than those recorded for treatment with permanganate alone, and $\sim 5-7$ orders of magnitude faster than conventional AOPs for water treatment (Sun et al., 2015).

6.2.6 Determination of optimum oxidant to analyte ratio (OAR) for Sunlight (UV)/Cl_2

Another major determining factor for the successful degradation of micropollutants in AOPs is the oxidant to analyte ratio (OAR) in terms of their mole ratios. To establish the OAR for the optimum performance of our novel AOPs, a different ratio of $Ca(ClO)_2$ to the ARVs was tested at 90 minutes and pH 7 already established for the UV/Cl_2 process. This section presents the results % removal obtained for different OARs (3:1 5:1, 7:1, 9:1 and 11:1) investigated. The experiment that determines the optimum OAR for the oxidation of 5 µg/mL (20 µM) pharmaceutical compounds was investigated according to the protocol described in section 3.7.2.5.

Figures 6.13 – 6.15 describe the % removals for the three ARVs at various OARs. In Figure 6.13, at the optimised time (90 mins) and pH (pH 7) the % removal recorded for 3TC at 3:1; 5:1;7:1; 9:1 and 11:1OAR are 33.35 %, 37.35 %, 53.26 %, 43.27 % and 12.28 %, respectively. The 53.26 % produced at OAR 7:1 was the highest compared to 33.35 %, 37.34 %, 43.27 and 12.28 % removals obtained at OARs 3:1, 1:5, 9:1 and 11:1, respectively.

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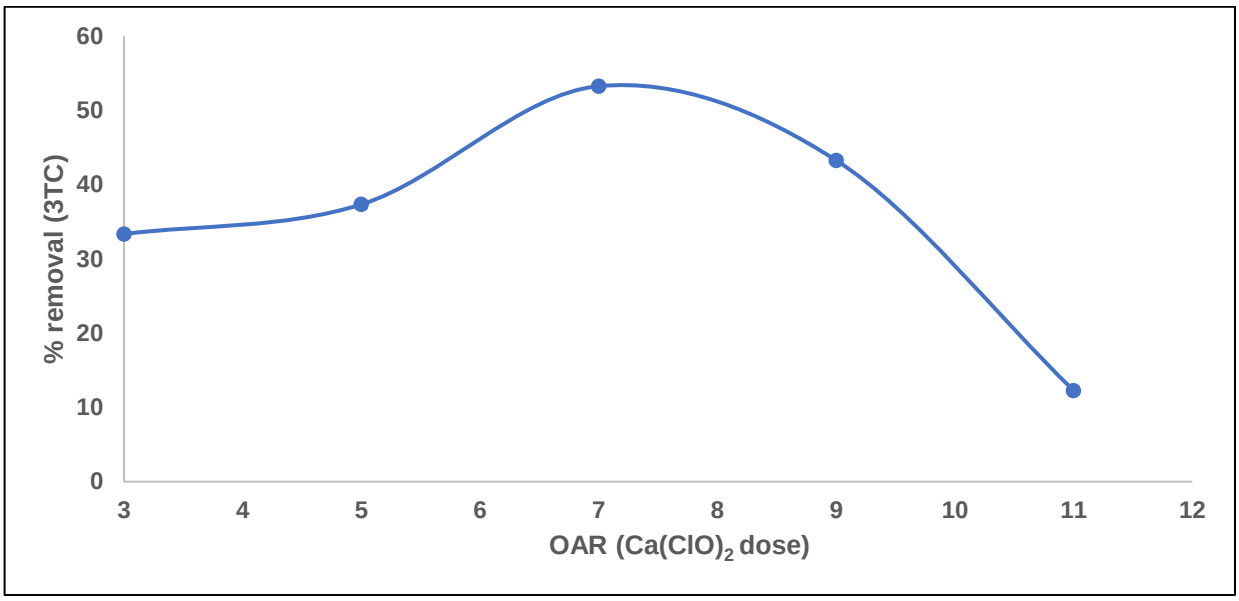

Figure 6. 13: Effect of different doses of $Ca(ClO)_2$ oxidant on the removal of 3TC

(Reaction conditions: OAR = 3:1, 5:1, 7:1, 9:1, 11:1, T_0 = 25 ± 0.2 °C, pH = 7, solar irradiance 250± 25 mW/m^2, t = 90 mins, [A]$_0$ = (20 µM 3TC), O = $Ca(ClO)_2$, OAR means oxidant to analyte ratio).

Similarly, in Figure 6.14, the treatment of AZT with OAR 7:1 resulted in 55.41 % removal. This was the greatest % removal relative to 26.68%, 33.16%, 13.87% and 13.87% for treatment with OAR 3:1, 5:1, 9:1 and 11:1, respectively.

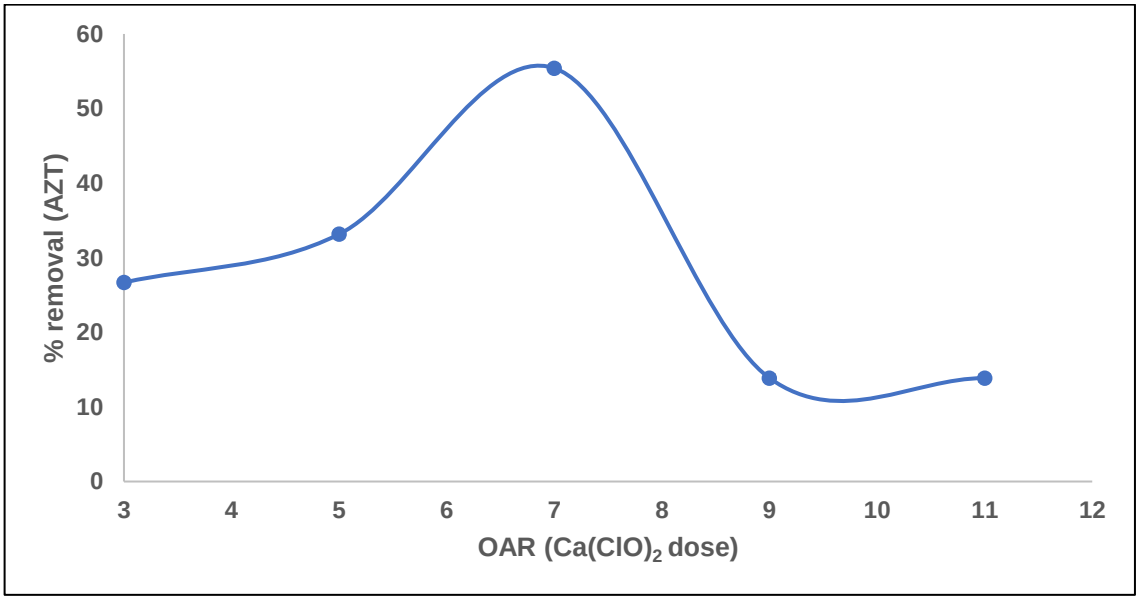

Figure 6. 14: Effect of different doses of $Ca(ClO)_2$ oxidant on the removal of AZT

(Reaction conditions: OAR = 3:1, 5:1, 7:1, 9:1, 11:1, T_0 = 25 ± 0.2 °C, pH = 7, solar irradiance 250± 25 mW/m^2, t = 90 mins, [A]$_0$ = (20 µM AZT), O = $Ca(ClO)_2$, OAR means oxidant to analyte ratio).

In Figure 6.15, the % removal recorded for NVP at OARs 3:1; 5:1;7:1; 9:1 and 11:1are 33.35 %, 37.35%, 50.72%, 43.27% and 12.28%, respectively. Out of these, the highest % removal was 50.72 % recorded at OAR 7:1.

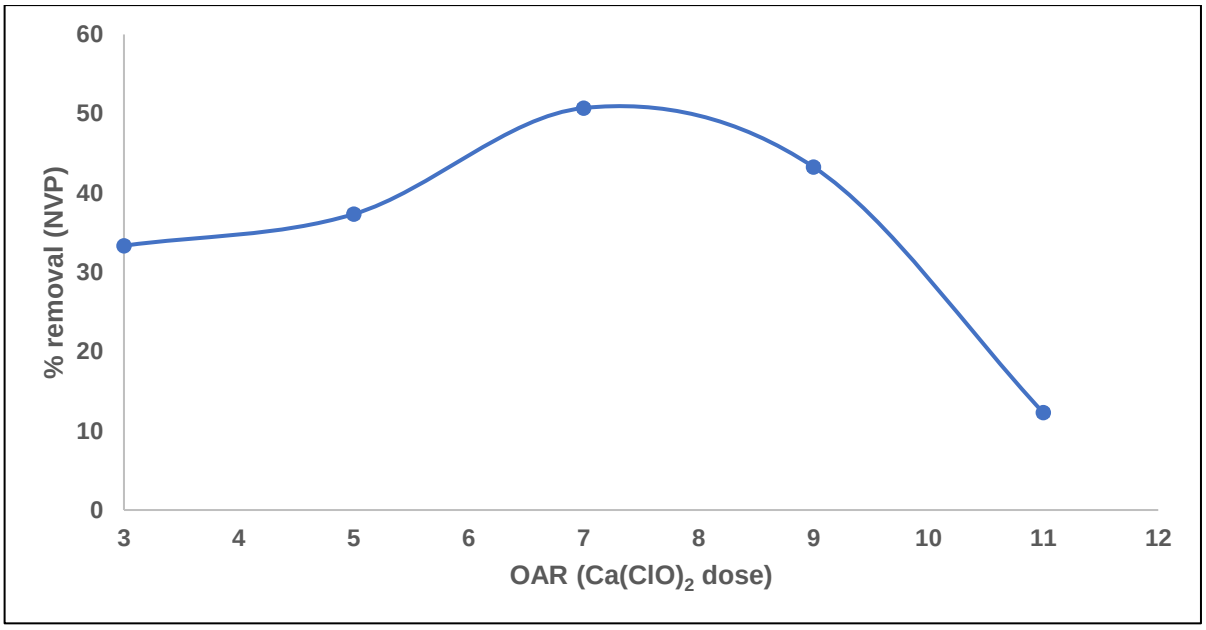

Figure 6. 15: Effect of different doses of $Ca(ClO)_2$ oxidant on the removal of NVP

(Reaction conditions: OAR = 3:1, 5:1, 7:1, 9:1, 11:1, T_0 = 25 ± 0.2 °C, pH = 7, solar irradiance 250± 25 mW/m^2, t = 90 mins, [A]$_0$ = (20 µM NVP), O = $Ca(ClO)_2$, OAR means oxidant to analyte ratio).

The general trend observed for the % removal of the three ARVs is that the % removal increases as the doses of $Ca(ClO)_2$ increases from 3:1 to 7:1 as follows, 3TC (33.38%, 37.35%, 53.26%); AZT (26.72%, 33.18%, 55.41%); NVP (33.38%, 37.35%, 50.72%) for OARs 3:1; 5:1 and 7:1, respectively. It however decreases for OARs 9:1 and 11:1 in this order 3TC (43.27% and 12.28%), AZT (13.87% and 13.87%) and NVP (43.27% and 12.28%). This could be attributed to the quantum variation as the oxidant doses change. Our result agrees with the submissions of other researchers (Remucal and Manley, 2016; Dong et al., 2017). They reported that during UV/Cl_2 processes, the quantum yield of active chlorine that generates the radicals increases as the concentration of active chlorine increases, but it begins to reduce as the chlorine concentration increased beyond a certain level when the excess chlorine act as OH radical scavenger (Remucal and Manley, 2016; Dong et al., 2017). This could be the case when in this investigation the OAR was increased to 9:1 and 11:1, when the percentage of degradation for 3TC was lowered to 17.78 and 33.69%, AZT reduced to 13.87% for both 9:1 and 11:1 OAR while NVP reduced to 43.27 and 12.08%, respectively.

The increase in the % removal experienced in the three compounds as the chlorine dose increases up OAR 7:1 could also be explained by the production of more HO• and Cl• as the chlorine dose increases. This trend was reported in the degradation of CBZ with the UV/Chlorine process, the degradation rate constants (K_{obs}) increases as the chlorine dose increases up to 20 mg/L when it reaches the peak before it began to fall. This was explained that more HO• and Cl• were generated which caused the corresponding increase in the removal of the compounds increases as the chlorine dose increases (Pan et al., 2017). Bhembe et al.

(2020) reported an increase in the degradation of NVP from 56.2% to 68.5% when the dose of few-layer black phosphorus combined with niobium (V) oxide (FL-BP@Nb_2O_5) composite catalyst dose increased from 5 mg to 15 mg, respectively. The degradation however reduced to 63.8% upon the addition of 20 mg. The increase in the degradation experienced was because there was an increase in the number of active sites for the catalyst and more reactive species. On the other hand, the reduction in the degradation performance when the excess catalyst was added was attributed to the aggregation of the catalyst that eventually blocks the active sites.

6.2.6.1 Treatment with sunlight(UV)/Cl_2 compared with the treatment with chlorine alone and UV radiation alone

Oxidation of micropollutant with chlorine has been in existence for decades because of the ability of chlorine to oxidize many contaminants but with some setback which includes the formation of DBPs (Li and Mitch, 2018; Von Gunten, 2018). Previous research, however, demonstrated that UV/Cl_2 is an evolving AOP method that ensures an enhanced removal of micropollutants and also lowers the level of DBPs (Chan et al., 2018; Wang et al., 2019). The efficiency of sunlight(UV/Cl_2) in removing our target analytes was compared with two control experiments which involved the removal of the three ARVs compounds with

i. chlorine alone (without UV radiation) and
ii. UV alone (without chlorine dose)

These experiments were carried out as control experiments I and II, respectively in section 3.7.2.6.

Table 6.1compares the removal efficiency of sunlight(UV/Cl_2), chlorine alone (absent of UV radiation) and UV radiation alone (no addition of $Ca(ClO)_2$) on the three pharmaceutical compounds at the optimised conditions (irradiation intensity of 250 ± 25 mW/m^2, pH 7, 90 minutes and OAR 7:1). From Table 6.1, it could be seen that the % of removal recorded when the 3 ARVs were treated with UV/Cl_2 (the real experiment) were better than those of the control experiments (treatment with Cl_2 alone or treatment with UV alone).

The % removal recorded for the treatment of 3TC with sunlight(UV/Cl_2), chlorine alone and UV radiation alone was 53.26%, 24.79% and 14.09% respectively. The % removal for UV/Cl_2 (53.26%) was greater than chlorine alone (24.79%) and UV radiation alone (14.09%).

Table 6. 1 The efficiency of sunlight(UV)/C l_2 compared with Cl_2 alone and sunlight(UV) alone.

(Reaction conditions: OAR = 7:1, T_0 = 25 ± 0.2 °C, pH = 7, solar irradiance 250± 25 mW/m^2, [A] $_0$ = (20 µM 3TC), t = 90 mins, O = $Ca(ClO)_2$, OAR means oxidant to analyte ratio).

	% removal		
Treatment	3TC	AZT	NVP
UV/Cl_2	53.26	55.41	50.73
Cl_2 alone	24.79	33.86	28.57
UV alone	14.09	9.30	3.19

Similarly for AZT, the % removal for chlorine alone, UV alone and combined UV/Cl_2 were 33.86%, 9.30% and 55.41%, respectively. This result showed that the % removal with UV/Cl_2 (55.41%) was the greatest compared to 33.86% and 9.30% obtained for chlorine alone and UV radiation alone, respectively. For NVP, 50.72% removal was recorded for the combined UV/Cl_2, this was the highest compared with 28.54% and 3.19% for treatment with chlorine alone and UV alone, respectively.

In the three compounds, the combined UV/Cl_2 steadily removed ARVs better than a single parameter. This is consistent with other findings that photolysis of chlorine during combined UV/Cl_2 treatment better destroys organic pollutants in the aquatic system than treatment with Cl_2 or UV radiation alone (Nowell and Hoigné, 1992; Deng et al., 2013; Wu et al., 2017; Pan et al., 2017). This result is also in agreement with other studies that direct photolysis (UV radiation alone) was not as effective as the combined UV/chlorine process for the removal of the majority of pharmaceutical residues in water (Guo et al., 2017). Wang et al. (2016) also reported that UV/free chlorine is a fast-growing AOP due to its effectiveness in contaminants removal than chlorination or UV photolysis. The better performance of UV/Cl_2 in removing ARVs could be attributed to the better formation of OH radicals and RCS in the combined UV/Cl_2 process (Guo et al., 2017; Wang et al., 2019). For the 3 ARV compounds, the efficiency of their removal follow the order sunlight/(UV)/Cl_2 > Cl_2 alone > UV alone. This is consistent with previous findings (Wang et al., 2019).

6.2.7 Determination of optimum oxidant to analyte ratio (OAR) for (Sunlight (UV)/$KMnO_4$)

As done for the UV/Cl_2 process, in this section, we also optimised the dose of $KMnO_4$ oxidant required for the optimum degradation of the target ARVs. Different ratios of $KMnO_4$ to a fixed ratio (20 μM) of ARVs were investigated at the optimised pH (pH 5) for the UV/$KMnO_4$ process. The experiment that determined the optimum OAR for the oxidation of 5 μg/mL (20 μM) of our pharmaceutical compounds was investigated according to the method described in section 3.7.2.6. This section presents the results of different % removal obtained for the three ARVs at different OARs (3:1 5:1,7:1,9:1 and 11:1) examined.

Figures 6.16, 6.17 and 6.18 present the effect of treating a mixed standard containing a 5 μg/mL (20 μM) of 3TC, AZT and NVP with different $KMnO_4$ doses (OAR 3:1 5:1, 7:1, 9:1, 11:1). As indicated in Figure 6.16, different percentages of removal were observed at different OARs. For the OARs 3:1 5:1, 7:1, 9:1 and 11:1 the % removal recorded were 22.22%, 23.39%, 69.92%, 52.67% and 63.91%, respectively. Out of these, 69.92% for OAR 7:1 was the highest (Figure 6.16).

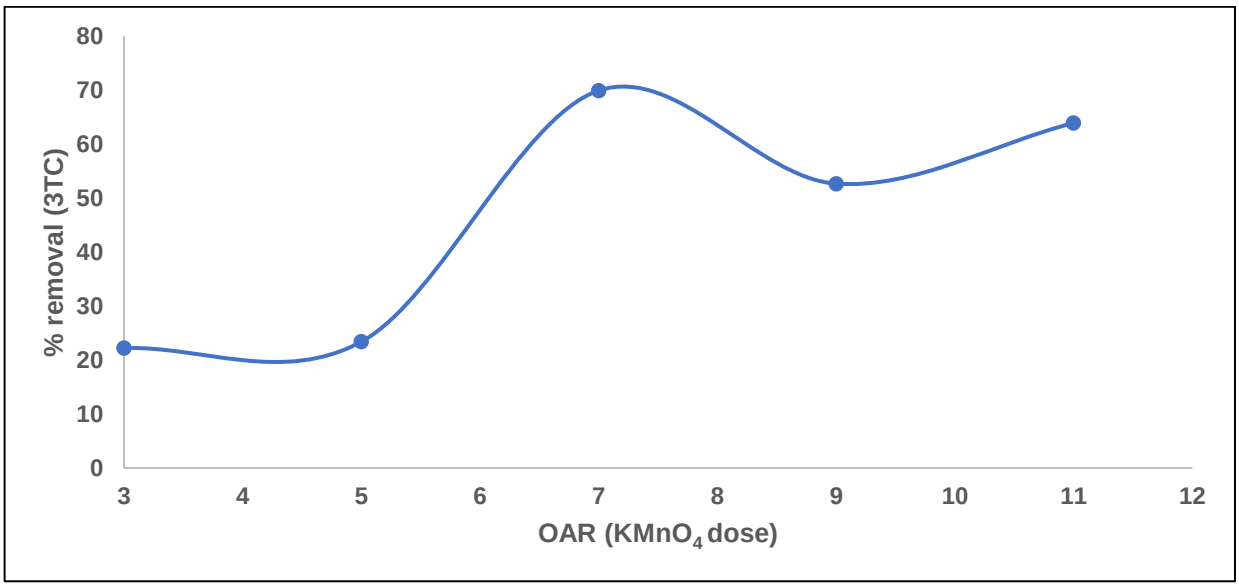

Figure 6. 16: Effect of different doses of $KMnO_4$ oxidant on the removal of 3TC

(Reaction conditions: OAR = 3:1, 5:1, 7:1, 9:1, 11:1, T_0 = 25 ± 0.2 °C, pH = 5, solar irradiance 250± 25 mW/m^2, t = 45 mins, [A]$_0$ = (20 µM 3TC), O = $KMnO_4$, OAR means oxidant to analyte ratio).

In Figure 6.17 the % removal of AZT at different OARs of $KMnO_4$ were 27.79%, 37.32%, 74.20%, 66.82% and 60.25% for the OARs 3:1 5:1, 7:1, 9:1 and 11:1, respectively. The highest % removal of AZT as shown in Figure 6.17 was 74.20% at OAR 7:1.

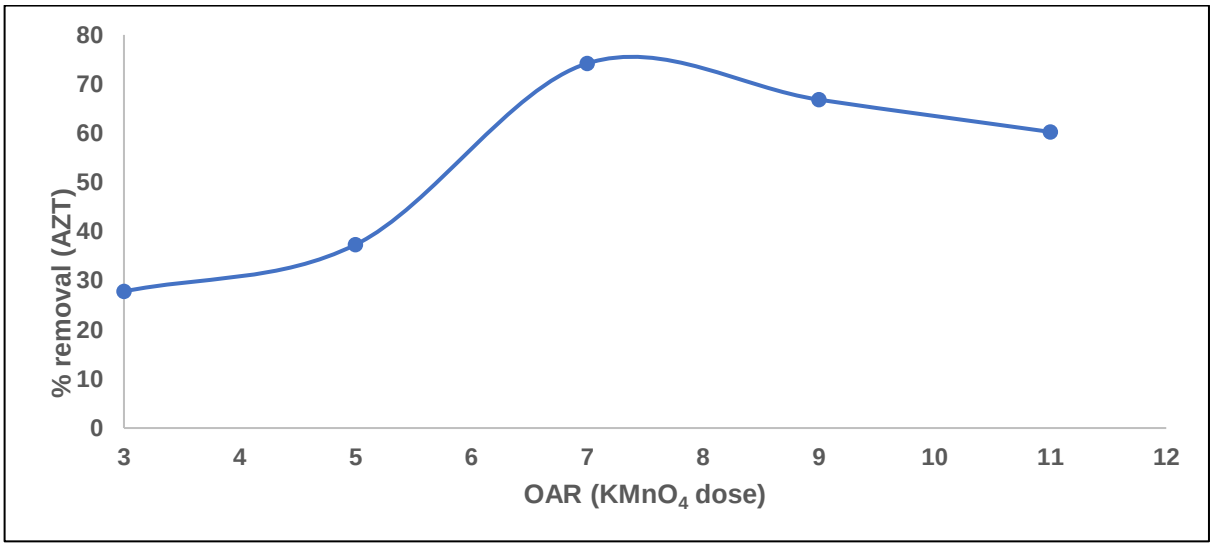

Figure 6. 17: Effect of different doses of $KMnO_4$ oxidant on the removal of AZT.

(Reaction conditions: OAR = 3:1, 5:1, 7:1, 9:1, 11:1, T_0 = 25 ± 0.2 °C, pH = 5, solar irradiance 250± 25 mW/m^2, t = 45 mins, [A]$_0$ = (20 µM AZT), O = $KMnO_4$, OAR means oxidant to analyte ratio).

In Figure 6.18, the % removal recorded for NVP at OARs 3:1 5:1, 7:1, 9:1 and 11:1, were 33.82%, 39.13%, 48.83%, 30.77% and 28.00%, respectively. Likewise, the 47.83% recorded at OAR 7:1 was the highest % removal.

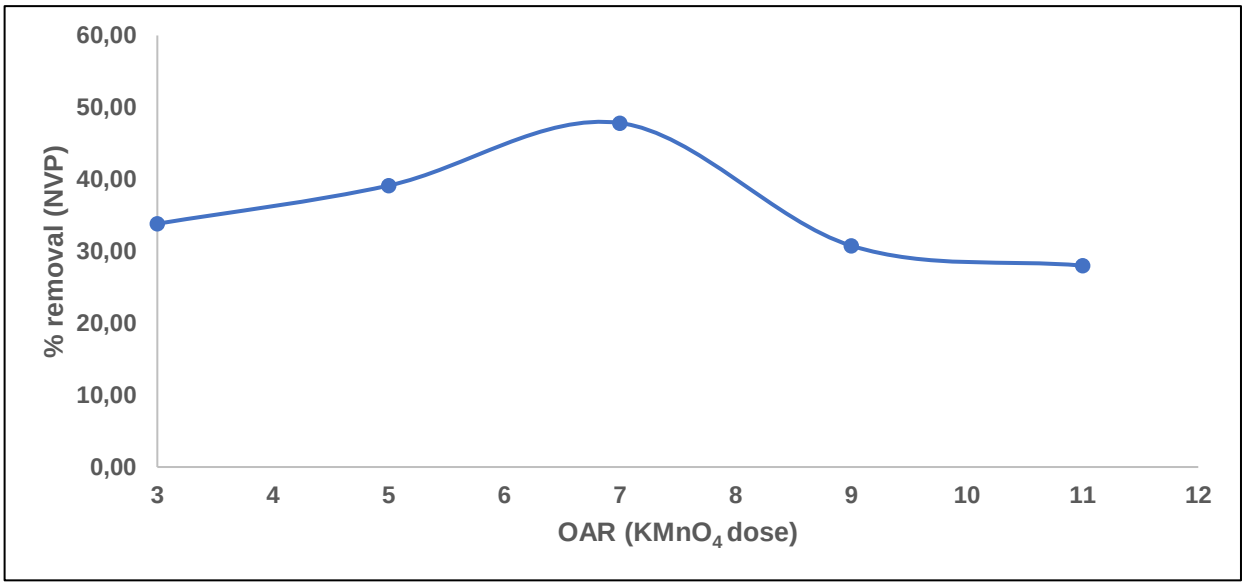

Figure 6. 18: Effect of different doses of $KMnO_4$ oxidant on the removal of NVP.

(Reaction conditions: OAR = 3:1, 5:1, 7:1, 9:1, 11:1, T_0 = 25 ± 0.2 °C, pH = 5, solar irradiance 250± 25 mW/m^2, t = 45 mins, [A]$_0$ = (20 µM NVP), O = $KMnO_4$, OAR means oxidant to analyte ratio).

The general trend observed for the three ARV compounds was that the % removal increases as the dose of $KMnO_4$ increases up to OAR 7:1, beyond this ratio, the % reduction began to reduce. This is consistent with the findings of Bhembe et al. (2020) that the degradation of NVP increases from 20.9% to 30.3% when the quantity of Nb_2O_5 increase from 5 mg to 15 mg but decreased when 20 mg was added. The reduction in the degradation efficiency was attributed to the aggregation of the catalyst that hampers the active sites for the degradation process (Bhembe et al., 2020).

The highest % removal recorded at OAR of 7:1 in this work for the three pharmaceutical compounds was close to the 1:10 OAR used for the oxidation of triclosan (Jiang et al., 2009). It was, however, less than OAR 20:1 used by Guo et al. (2018). The use of a very high ratio of 20:1 for their investigated compounds and micropollutants could be because UV/$KMnO_4$ was used for the first time (Guo et al., 2018).

Likewise, the optimum OAR obtained in this work was closer to the OAR of 5:1 used by other researchers for the oxidation of three antibiotics; ciprofloxacin, lincomycin, and trimethoprim (Hu et al., 2011). The lower $KMnO_4$ dose used by Hu and his group for the degradation of selected antibiotics and micropollutants could be because the main objective of the study was to identify the metabolites and pathways of the reaction.

6.2.7.1 Treatment with sunlight(UV)/$KMnO_4$ compared to treatment with $KMnO_4$ and UV radiation alone

As done with the UV/Cl_2 process, the effect of treating the target analytes with UV/$KMnO_4$ was compared with the two-control experiments that utilized $KMnO_4$ alone and UV alone. The results are presented in Table 6.2.

Table 6. 2: The efficiency of sunlight(UV)/$KMnO_4$ compared with $KMnO_4$ alone and sunlight(UV) alone. (Reaction conditions: OAR = 7:1, T_0 = 25 ± 0.2 °C, pH = 5, solar irradiance 250± 25 mW/m^2, [A] $_0$ = (20 µM 3TC), t = 90 mins, O = $Ca(ClO)_2$, OAR means oxidant to analyte ratio).

	% removal		
Treatment	**3TC**	**AZT**	**NVP**
UV/KMnO4	69.9248	74.198	47.8261
KMnO4 alone	58.804	69.5092	36.76
UV alone	12.1265	19.9262	10.3448

The treatment with UV/KMnO4 was able to remove 69.92% of 3TC compared to 58.80% and 12.13% for treatment with $KMnO_4$ alone and treatment with UV alone, respectively. Similarly, 74.20% of AZT was removed for the treatment of AZT with UV/$KMnO_4$. This was higher than 69.51% and 19.93% produced for the treatment with $KMnO_4$ and UV radiation alone, respectively. In the same way, as shown in the table, 47.85% removal recorded for treating NVP with UV/$KMnO_4$ was greater than 32.35% for the treatment with $KMnO_4$ alone and 10.35% for UV alone, respectively.

The proficient removal of the 3 ARVs as by the UV/$KMnO_4$ than either $KMnO_4$ alone or UV radiation alone could be attributed to the synergetic reaction between UV radiation and $KMnO_4$. This enhances the production of some reactive manganese species (RMnS) during the combined UV/$KMnO_4$ as the case with other UV based AOPs such as UV/Cl_2 or UV/H_2O_2 (Guo et al., 2018). This RMnS could likely be Mn(V) which is very reactive and likely to be produced better in an enhanced $KMnO_4$ process at the acidic medium (Sun et al., 2015). This is consonant with the recent report that permanganate alone did not produce Mn(V) but the combination of ultraviolet radiation and potassium permanganate does (Guo et al., 2018) recently reported.

Similarly, according to some earlier reports that during UV/$KMnO_4$ processes, an excited form of Mn(V) peroxide, $[MnO_2(\eta 2\text{-}O_2)^-]^*$ can be produced (Thornley and Bitterwolf, 2015; Hu et al., 2016). From there, the normal Mn(V) peroxide $[MnO_2(\eta 2\text{-}O_2)^-]$ that is a more reactive oxidizing agent than permanganate could be formed (through O−O bond resulting from two Mn=O bonds of permanganate) for the enhanced degradation of micropollutants (Thornley and Bitterwolf, 2015; Crandell et al., 2017). The mechanism for the UV photolysis of permanganate is comparable to that of UV photolysis of H_2O_2. The mechanism produces Mn(V) peroxide which subsequently produced HO• (a powerful oxidizing species) from the UV photolysis of the O−O bond of Mn(V) peroxide (Thornley and Bitterwolf, 2015; Crandell et al., 2017; Guo et al., 2018).

6.3 Degradation of different concentrations of the analytes with sunlight(UV)/Cl_2

The optimised experimental conditions obtained in the earlier section which are: average solar irradiance of 250 ± 25 mW/m^2, pH 7, OAR 7:1 and reaction time at 90 minutes were applied to degrade different concentrations (10 µg/L, 50 µg/L, 100 µg/L and 200 µg/L) of the ARVs. The concentrations of the ARVs selected for this experiment were based on the concentration ranges earlier determined in the wastewater sample as discussed in sections 5.2 – 5.4 of chapter five. The experimental procedure for this section was described in section 3.7.2.5. The purpose of

this was to apply the optimised conditions for the removal of different concentrations of the ARVs to investigate the effect of initial concentrations of the analytes on the removal level.

Figure 6.19 shows the % removal of different concentrations of the three pharmaceutical compounds when treated with $Ca(ClO)_2$. For all the compounds, the % removal decreased as the initial concentrations of the analytes increased. The % removal of 3TC decreased from 57.33 – 18.49% when the initial concentration increased from 10 µg/L to 200 µg/L, AZT decreased from 60.02 – 22.96% and NVP decreased from 38.99 –16.84%.

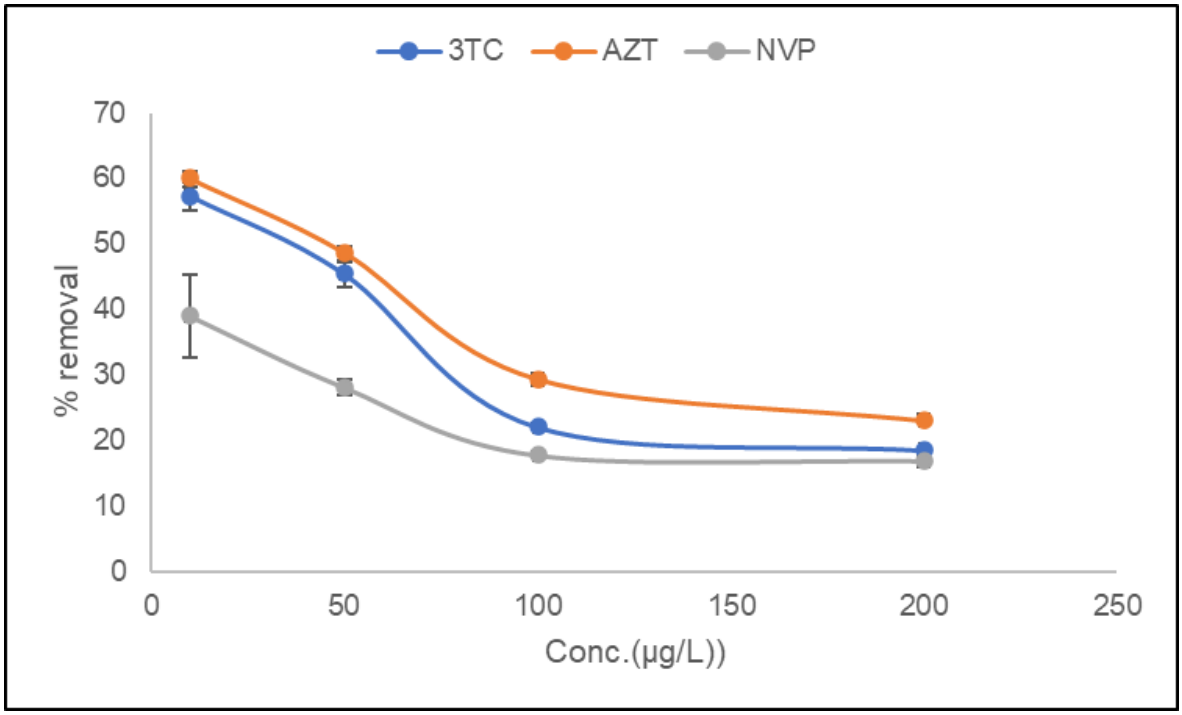

Figure 6. 19: The % removal of different concentrations of 3TC, AZT and NVP with sunlight (UV)/Cl_2 at the optimised condition.

(Reaction conditions: OAR = 7:1, T_0 =25 ± 0.2 °C, PH= 7, solar irradiance 250± 25 mW/m^2, $[A]_0$= (20 µM 3TC, AZT, NVP), O = $Ca(ClO)_2$, OAR means oxidant to analyte ratio).

This finding is consistent with previous reports that the removal of contaminants decreases as the initial concentrations increases (Yang et al., 2010; Shah et al., 2014; Wang and Chu, 2016). This can be attributed to several factors, Yang et al. (2010) associated this to the fact that at higher initial concentrations of contaminants, the contaminants compete with the oxidants and absorb more radiation so the remaining limited available radiation may not be enough to activate the oxidant for effective degradation of the target compounds. Also, the presence of higher concentrations of the analytes (substrates) could occupy more of the active sites of the oxidant, thus, inhibits the generation of the much-needed radicals and reactive species for the degradation of the target compounds (Yang et al., 2010).

Shah et al. (2014) explained the lower degradation at the higher initial concentration on the basis that, at higher concentrations, the number of degradation intermediates and degradation product(s) formed from photodegradation related processes could increase as the initial concentration increases. This invariably can lead to a higher level of competition between the degradation intermediate formed and the target contaminants for the reactive species (Equation

6.1 and 6.2). This is possible because most degradation intermediates are reactive and not stable, therefore could lead to an increased probability of the reactions (Shah et al., 2014).

Reactive species + Contaminants → Degradation intermediates ……...Equation 0.11

Reactive species + Degradation intermediates → Degradation products ……Equation 0.12

6.4 Degradation of different concentrations of the analytes with sunlight(UV)/$KMnO_4$

Similarly as done with UV/Cl_2, the optimised experimental conditions obtained for UV/$KMnO_4$: average solar irradiance of 250 ± 25 mW/m^2, pH 5, OAR 7:1 and reaction time of 45 minutes were applied to degrade different concentrations (10 µg/L, 50 µg/L, 100 µg/L and 200 µg/L) of the analyte. This helps to elucidate the effect of initial concentrations of the ARV on the performance of the UV/$KMnO_4$ process. The concentrations of the ARVs chosen for this experiment were based on the concentration ranges earlier determined in the wastewater sample as discussed in chapter five (section 5.2 – 5.4). The procedure of the experiment that described this was described in section 3.7.2.5.

Figure 6.20 presents the result of % removal of different concentrations of the three pharmaceutical formulations with sunlight (UV)/$KMnO_4$ at the optimised reaction conditions. As indicated, the % removal of the three compounds decreases with the increase in the initial concentrations of the three analytes. The Figure also shows that when the initial concentrations of the compounds increased from 10 ─ 200 µg/L, the % removals decreased from 58.69 ─ 35.09%, 66.03 ─ 35.94%, 50.53 ─ 27.64% for 3TC, AZT and NVP, respectively. This result agrees with the report of other researchers that the % degradation of antineoplastic cytarabine and that of endosulfan in aqueous solution decrease with an increase in the initial concentrations of cytarabine and endosulfan (Ocampo-Péreza et al., 2011; Rivera-Utrilla et al., 2013). It also conforms to a scientific finding that the % removal of theophylline at 30 minutes were 99.00%, 68.00%, and 11.00% for the initial concentrations of 3, 30, and 300 ppm, respectively (Liang et al., 2013). This could be attributed to the reduction in the penetration potential of the light radiation entering the drug solution due to an increase in concentration, thus decreasing the absorption of the photon by the oxidants at higher drug concentration (Kaur et al., 2015).

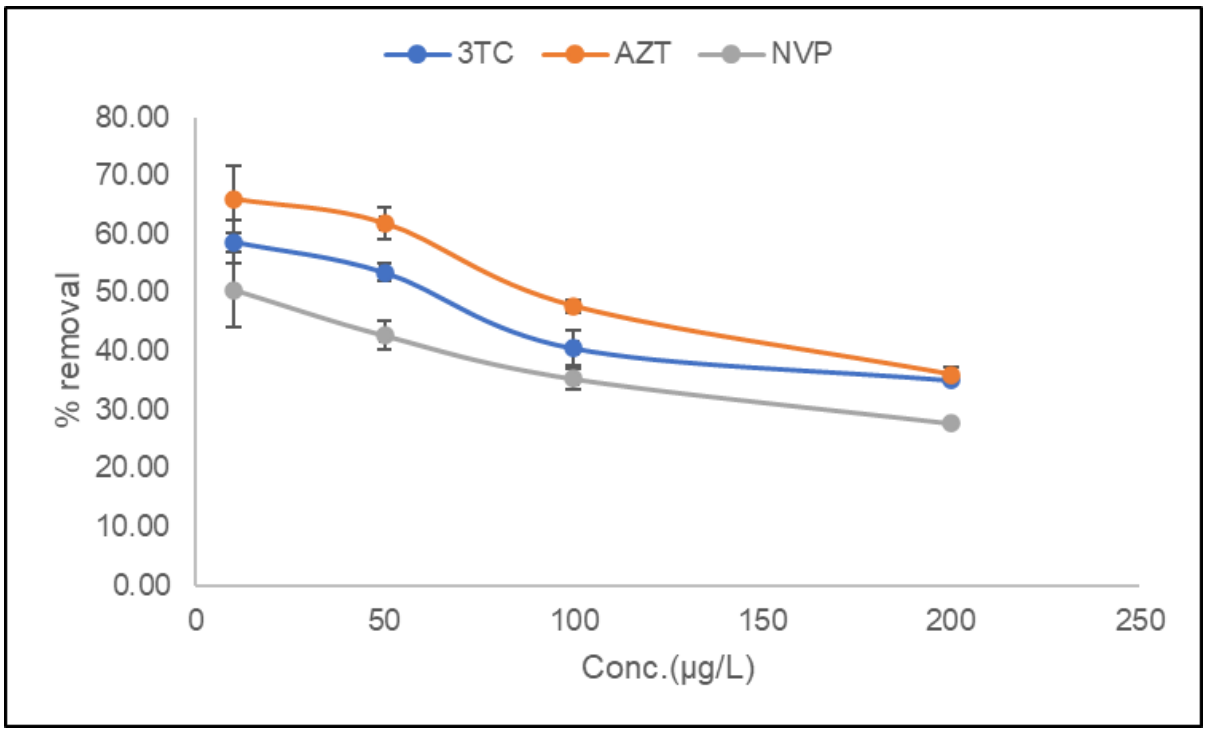

Figure 6. 20: The % removal of different concentrations of 3TC, AZT and NVP with sunlight (UV)/$KMnO_4$ at the optimised condition.

(Reaction conditions: OAR = 7:1, T_0 =25 ± 0.2 °C, PH= 5 $[A]_0$= (20 µM 3TC, AZT, NVP), O = $KMnO_4$, OAR means oxidant to analyte ratio).

6.5 HPLC Method

This research work deals with the spectroscopic and chromatographic analysis of target ARV compounds in wastewater samples. Section 6.1 deals with the optimisation of the degradation conditions. Section 6.2 and 6.3 used the spectroscopic method which is less expensive compared to HPLC to investigate the degradation of our ARVs in Milli-Q water. This section (6.4) discusses the use of HPLC to evaluate the degradation of our target analytes in both Milli-Q water and real wastewater samples. This is because wastewater is a complex matrix that contains other numerous compounds that require separation. The use of HPLC was necessary to separate our target compounds from other complex matrices in the wastewater which UV-Visible spectrophotometer cannot do.

A high-performance liquid chromatography (HPLC) furnished with Waters 1525 binary pump, Waters 2707 auto-sampler, Waters 2487 dual λ absorbance detector and operated on Breeze software was used for this process. The optimised chromatographic condition used was as described in section 3.8.1. Thereafter, the individual compound in the sample vial was injected into the HPLC for the identification test to determine the retention time of each compound.

6.5.1 Calibration curve

Standard calibration curves for the three analytes were prepared as described in section 3.8.5. Triplicate measurements of peak areas of seven different concentrations (2 – 300 µg/L) for 3TC,

AZT, and NNP were determined using the HPLC. The plot of area (µV Sec) against concentration was used to prepare the 7-points calibration curves for each pharmaceutical compound as shown in Figures 6. 21, 6.22 and 6.23 for 3TC, AZT and NVP, respectively. Also, typical chromatograms for the calibration standard are presented in Figure 6.24 and 6.25.

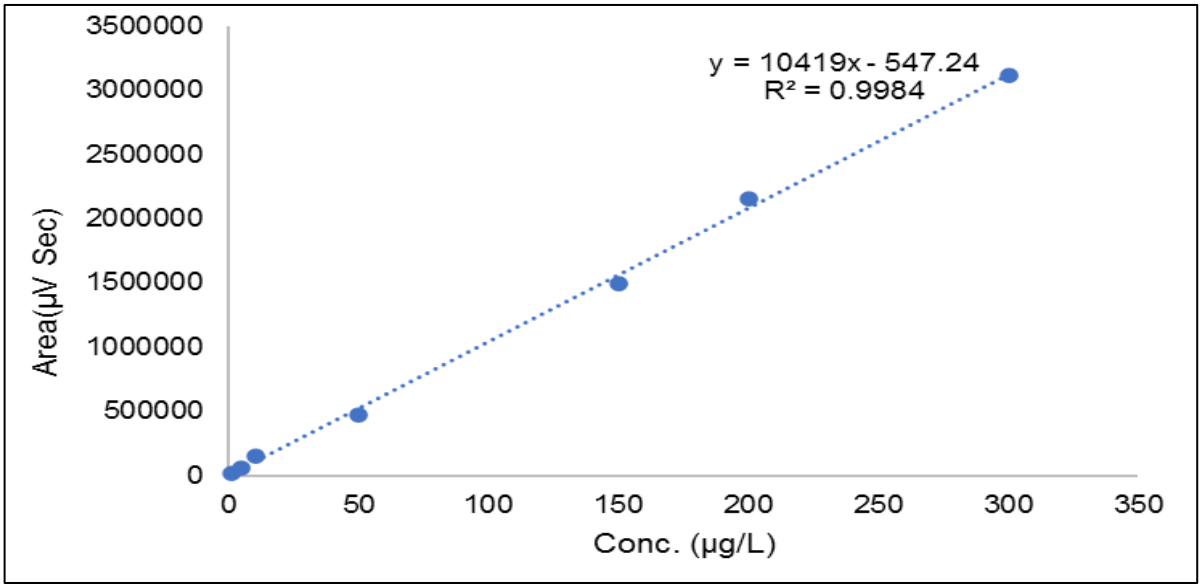

Figure 6. 21: Calibration curve for 3TC standard.

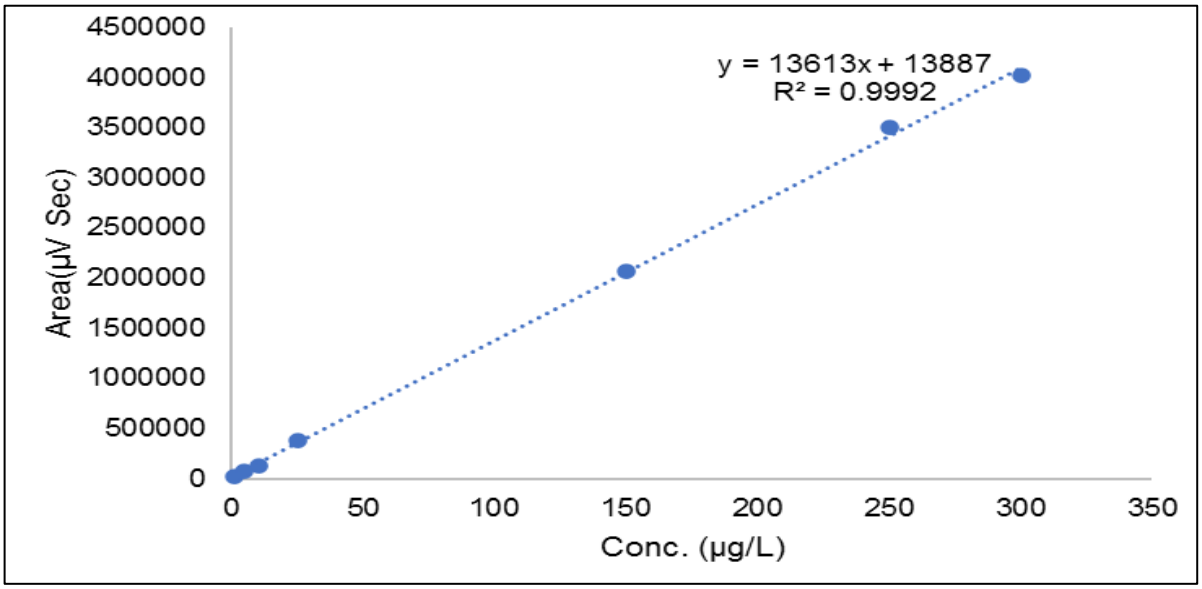

Figure 6. 22: Calibration curve for AZT standard

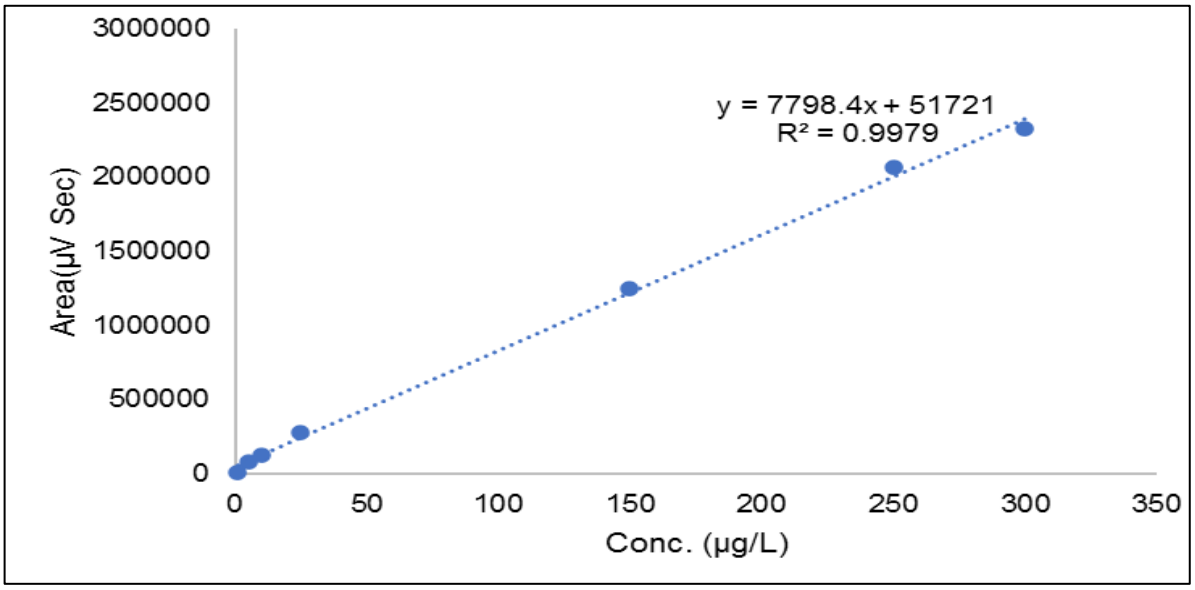

Figure 6. 23: Calibration curve for NVP standard.

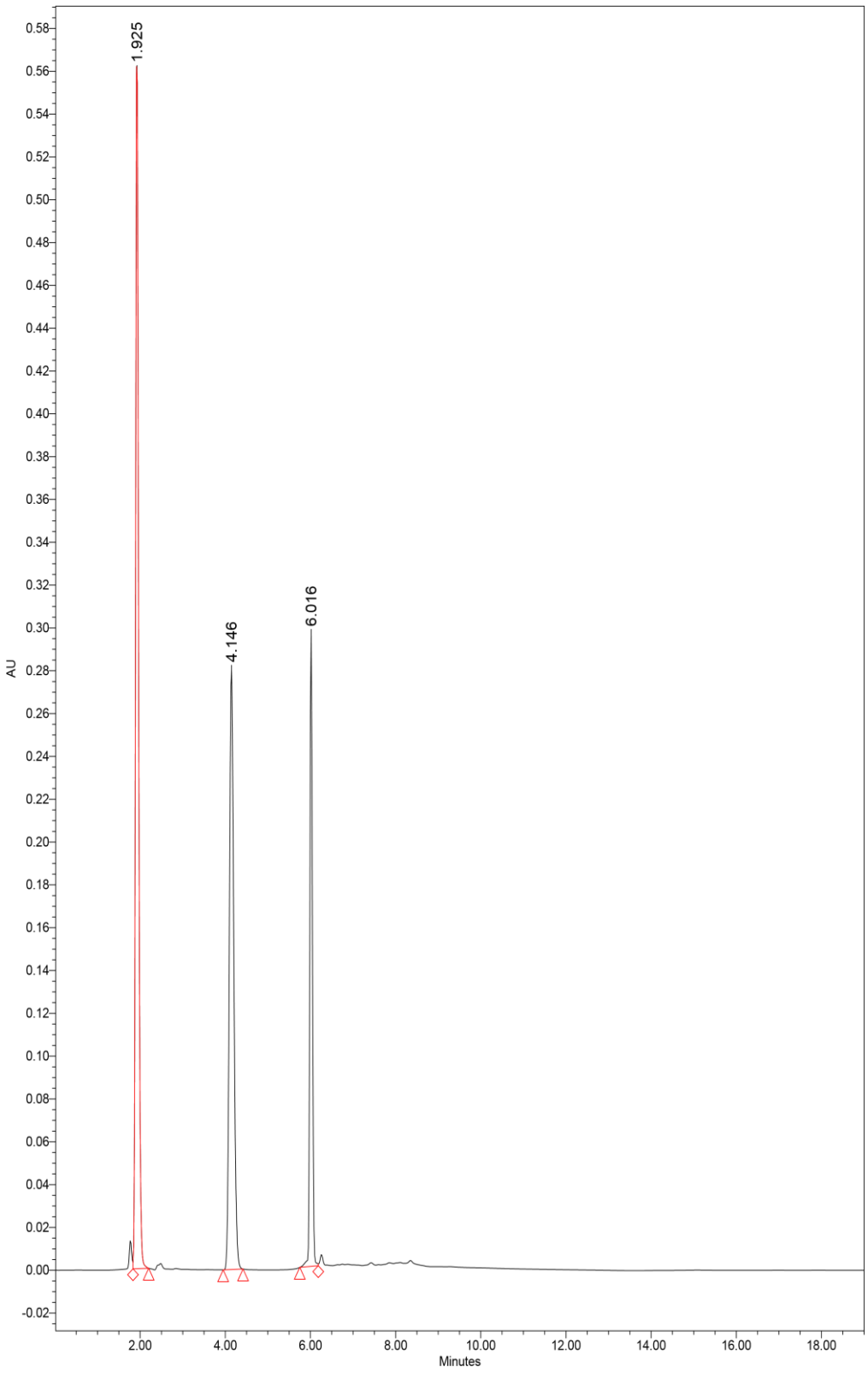

Figure 6. 24: Sample chromatogram showing the resolution of 3TC, AZT, and NVP, respectively (150 µg/L).

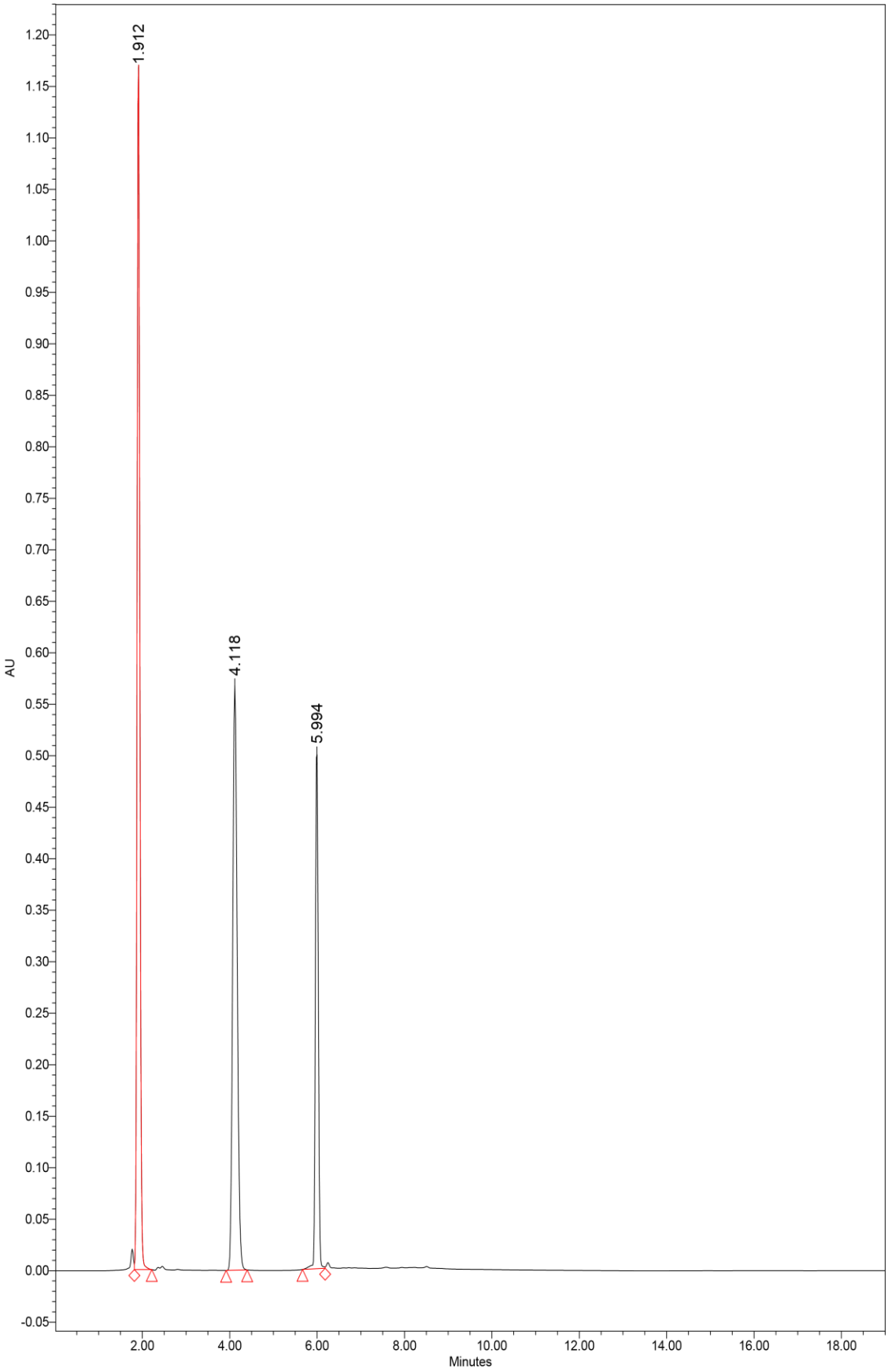

Figure 6. 25: Sample chromatogram showing the resolution of 3TC, AZT, and NVP, respectively (300 µg/L).

6.5.2 Validation of analytical method (HPLC)

Validation of an analytical method is the laboratory practise that established the quality, reliability and consistency of analytical results or procedure. It authenticates the performance characteristics of a method and shows that it meets the necessities for the envisioned analytical claim (Bhardwaj et al., 2015). Validation is essential for a novel or modified method to ensure that it can produce similar and reliable results when used by other operators using the same equipment in the same or different laboratories (Bhardwaj et al., 2015). The analytical validation performed include linearity, percentage recovery, repeatability, matrix effect, the limit of detection and limit of quantification.

6.5.2.1 Linearity

The result of the linearity of the analytical method is presented in Table 6.1. and it was determined as described in section 3.8.6.1. The response area (µVSec) of the three analytes were linear to the measured concentrations of the standards. The R^2 values for the three compounds were 0.9984, 0.9992 and 0.9979 for 3TC, AZT and NVP, respectively. The result showed that a good correlation was obtained between the peak areas and concentrations.

6.5.2.2 Repeatability

The results of intra-day and inter-day evaluated are presented in Table 6.1. These were estimated based on relative standard deviation (RSD) at the same experimental conditions as described in the experimental procedure contained in section 3.8.6.2. The values for the three concentrations examined ranged between 0.039 – 1.31 and 1.02 – 3.42 for intra-day and inter-day, respectively. The RSDs for the compounds are within the acceptable limit (less than 20%) for repeatability test for the HPLC method (United Nations, 2009; Bhardwaj et al., 2015). This shows that the method was repeatable and reliable.

6.5.2.3 Percentage recovery

Percentage recoveries for the three compounds are presented in Table 6.1, this was determined by the experimental procedure described in section 3.8.6.3. The value ranged between 68.09 and 93.74%. The recoveries for all the pharmaceutical formulations (except 3TC at 60 µg/L) were higher than 70%. Among the three compounds, 3TC had the least recovery compared to the other compounds. This could be attributed to its high solubility in aqueous media that restricted its retention on SPE (Ngumba and Gachanja, 2015). On the other hand, NVP maintained the highest recoveries at the three levels of determinations and this can be attributed to its low polarity and low solubility in water (Sarkar et al., 2006; Jain et al., 2013).

6.5.2.4 Matrix effect

The result of the matrix effect is as shown in Table 6.1. The experimental procedure for the matrix effect was described in section 3.8.6.4. All three compounds were prone to matrix effects. The matrix effects ranged between 67.58 and 74.03% which increased in the following order: 3TC > NVP > AZT. Matrix effect has been reported as one of the difficulties encountered in the analysis of environmental samples. This may be suppression or enhancement of signal or response from the target analyte thus affecting the accuracy of analytical methods (Cimetiere et al., 2013). Analytical inaccuracy could result from different sources which include sample composition, compounds released during sample pre-treatment or extraction, mobile phase additives, sample to matrix ratios, matrix type and extraction methodology (Wood et al., 2015). To avoid the effect, samples may either be analysed by external calibration using matrix-matched standards or by standard addition (Cimetiere et al., 2013; Wood et al., 2015).

6.5.2.5 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) of an analytical technique is the lowest concentration of an analyte that can be detected or distinguished (though not quantified) from the noise of an analytical procedure or of an instrument. It is usually stated as a concentration at a signal to noise ratio of 3:1 (Bhardwaj et al., 2015; Vidushi et al., 2017).

The limit of quantitation (LOQ) on the other hand is the least concentration of an analyte that can be successfully quantified in a sample with satisfactory precision and accuracy under specified working conditions (Bhardwaj et al., 2015). It is usually calculated as a signal: noise ratio of 10:1 as recommended by ICH (Bhardwaj et al., 2015; Vidushi et al., 2017). Both LOD and LOQ were determined as described in section 3.8.6.5. Table 6.1 presents the LOD for the three pharmaceutical formulations and it varied from 0.25 – 0.66 µg/L. LOQ ranged between 0.79 – 1.99 µg/L.

Table 6. 3: Linearity, % recoveries, matrix effect, LOD, LOQ, Intra-day repeatability and inter-day repeatability for ARV compounds.

Analyte	Linearity (R^2)	% Recovery (SD)			% ME (SD)	LOD (µg/L)	LOQ (µg/L)	Intra-day repeatability (RSD)			Inter-day repeatability (RSD)		
		20 µg/L	60 µg/L	300 µg/L				20 µg/L	60 µg/L	300 µg/L	20 µg/L	60 µg/L	300 µg/L
3TC	0.9984	86.49 (3.55)	68.09 (8.21)	72.46 (5.33)	74.03 (1.06)	0.46	1.38	0.97	0.99	1.13	1.30	1.61	2.08
AZT	0.9992	92.24 (7.54)	84.50 (3.48)	87.32 (3.91)	67.58 (3.95)	0.25	0.79	0.54	0.97	0.99	1.02	2.27	1.91
NVP	0.9979	93.74 (2.56)	86.94 (5.57)	89.94 (8.28)	70.53 (2.16)	0.66	1.99	0.39	0.81	1.31	1.84	3.42	1.98

6.6 Comparison of the result of ARVs measurement from the two processes

The result of ARV monitoring with the UV method was compared with the result of the HPLC method. The result of the concentrations of wastewater samples determined by the UV method in section 3.5.4 was compared with the result gotten with the HPLC method for the same set of samples that were used for the degradation on the real wastewater samples. The samples chosen were July 2018, September 2018, November 2018 and March 2019 which are the winter, the spring, the summer and the autumn, respectively (Table 3.5). Figures 6.26 – 6.28 present the results of the ARV concentrations in the two methods.

From Figures 6.26 – 6.28, the general trend was that the result obtained for the HPLC method was higher than that for the HPLC method. But at the same time, there was a correlation in the proportion of the concentrations measured in both methods. In Figure 6.26 for the concentrations of 3TC for instance, where the concentration of the RI samples recorded for the HPLC method was higher, a similar trend (though at a low concentration) was observed in the concentrations measured with the UV. Also in the November samples when the concentrations of the 3TC were generally low, it was similarly observed that the concentrations recorded for the UV method were lower in all the four sample matrices compared to the value recorded with the HPLC. The decreasing trend observed for 3TC in both UV and HPLC methods in November 2018 samples were UV (40.77 µg/L, 14.53 µg/L, 14.07 µg/L, and 11.78 µg/L), HPLC method (67.88 µg/L, 62.00 µg/L, 51.88 µg/L and 44.60 µg/L) for the RI, BUVT, AUVT and FE sample, respectively. This showed a level of proportionality in their concentrations.

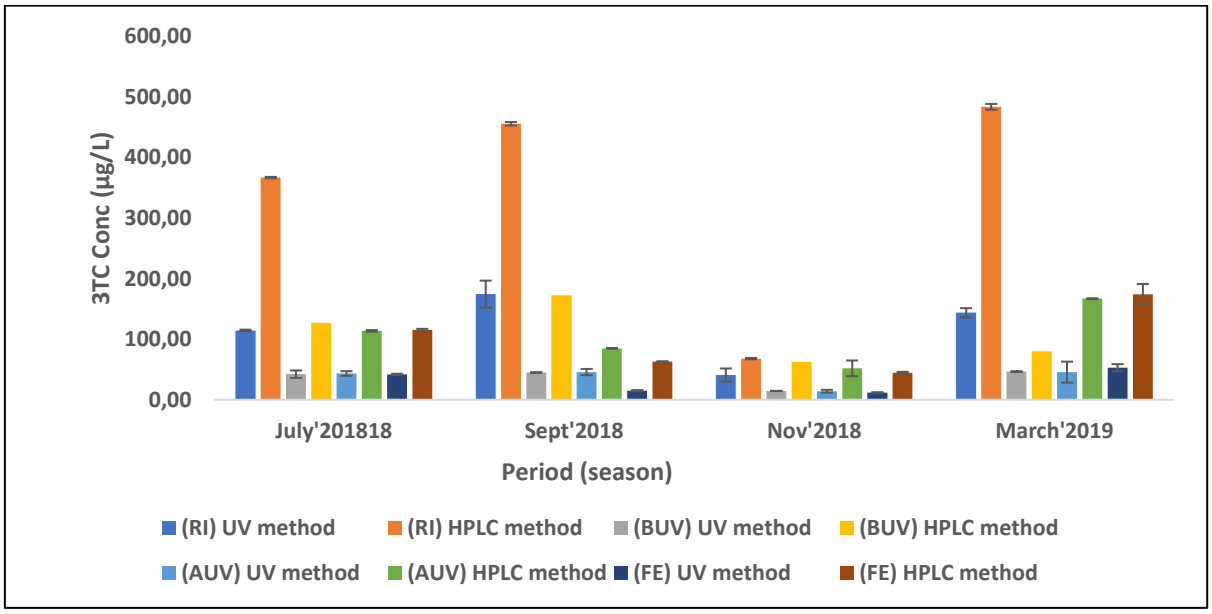

Figure 6. 26: Concentrations of the 3TC determined by UV visible spectroscopic and HPLC method.

In Figure 6.27 for AZT as well, in July, September and November when the concentrations of AZT measured with the HPLC method were low, the majority of concentrations measured with

the UV were much lower. The value obtained for the UV methods were seen majorly in March 2019 sample when the concentrations recorded for the HPLC method were correspondingly higher compared to other months. The highest concentration of AZT for the HPLC method (200.28 µg/L) was recorded in the RI sample in March 2019 and the corresponding highest concentration for the UV method was 154.03 µg/L.

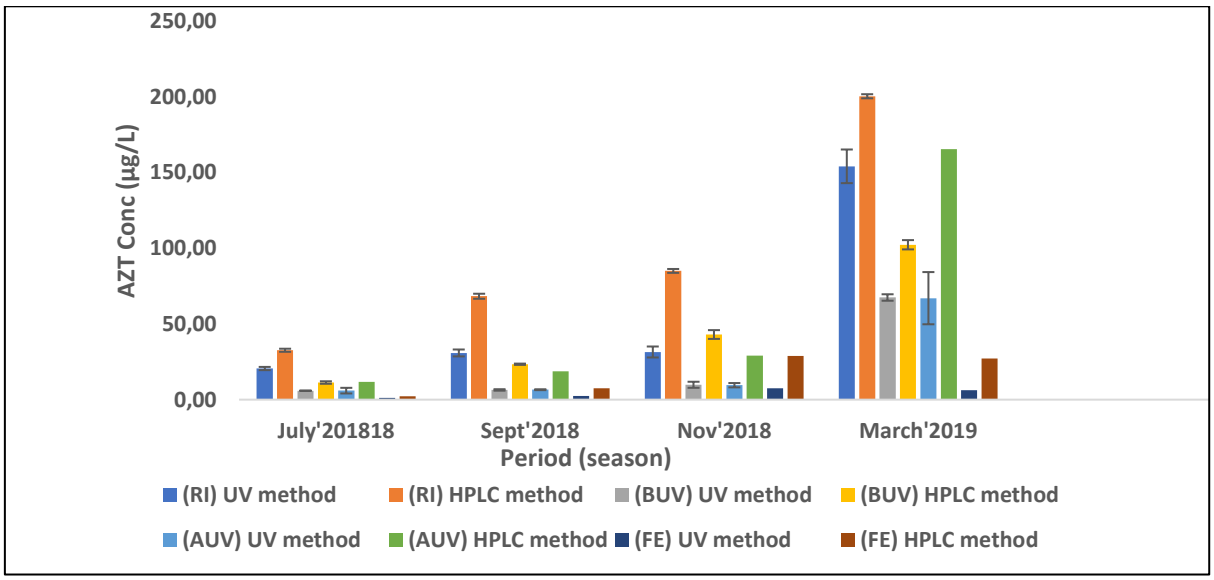

Figure 6. 27: Concentrations of the AZT determined by UV visible spectroscopic and HPLC method.

A similar trend was recorded for the NVP in all the months and all the sample matrices, concentrations recorded with the HPLC mehod were higher than that for the UV method (Figure 6.28).

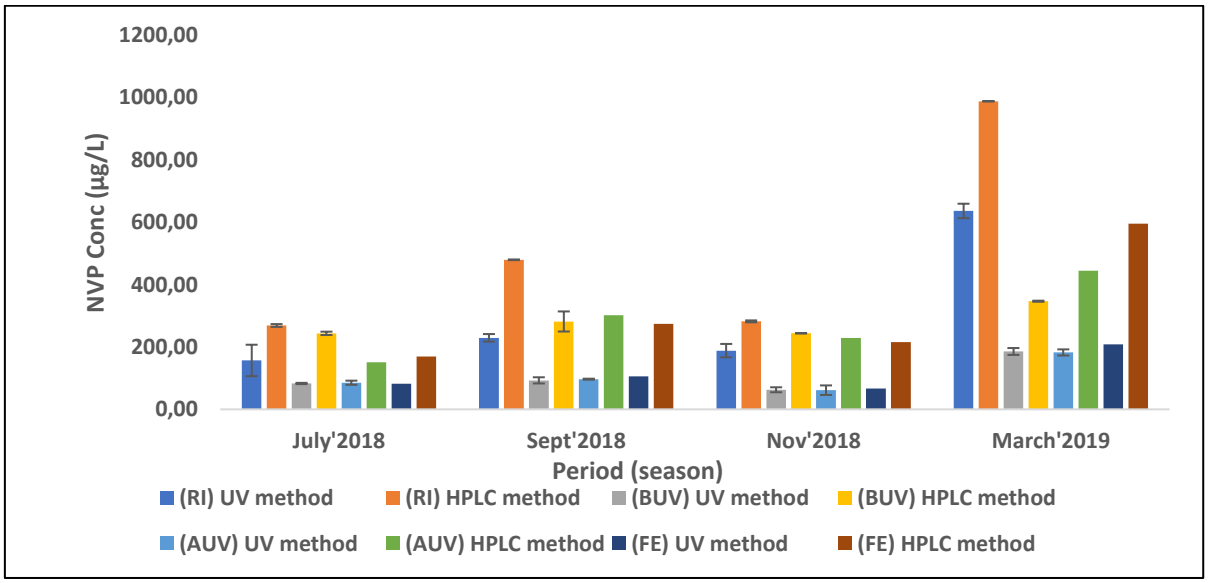

Figure 6. 28: Concentrations of the NVP determined by UV visible spectroscopic and HPLC method.

From the result obtained so far, in all the months and the sample matrices, the concentrations of the three ARVs obtained with the HPLC method were generally higher compared to the results obtained for the UV method. The high value recorded in the HPLC method could have come from the high sensitivity of the HPLC method. This is in agreement with previous (Anbazhagan et al., 2005; Sarkar et al., 2006). The approaches had been successfully used to determine our target compounds though in different matrices (pharmaceutical dose) and reported to be most suitable (Anbazhagan et al., 2005; Sarkar et al., 2006). This shows that for the wastewater sample the HPLC method is more sensitive and accurate than the UV method. This is also in consonance with earlier report that the chromatographic method has been the major analytical methods used for the analysis of ARVs in environmental samples (Madikizela et al., 2017).This was evident in the method validation for the two methods. For UV method LOD (0.68 – 7.59 µg/L), LOQ (2.06 – 22.99 µg/L) while for HPLC, LOD (0.25 – 0.66 µg/L), LOQ (0.79 – 1.99 µg/L).

Nonetheless, the detection and determination of the three compounds by the two methods, (though at different concentrations) were possible. Also, there was a direct correlation in the values obtained from the two methods because where a high value was obtained in the HPLC method, the values in the UV method follow the same trend and vice versa though not the same values. Lastly, based on the objective of this research which is the use of low-cost analytical equipment in an African setting and particularly in the WWTPs in the rural areas where they may not be able to afford sophisticated equipment (Madikizela et al., 2020) This means that this low-cost UV method could be useful for semi-quantitative measurement of the ARVs. Another important factor for consideration that can enhance the performance of this UV method is the application of the same sample treatment which involved the use of SPE for sample extraction and concentration. This was consistent with the literature that the use of SPE in environmenal sample for cleansing and concentration was an added advantage to achieve a low detection range (Errayess et al., 2017). This would limit matrix effect thus improving the sensitivity and applicabiliy of UV method. This could give better opportunities for the monitoring of ARVs and other pharmaceutical residues in an aquatic environment.

6.7 Degradation of ARV samples with sunlight(UV)/Cl_2 and sunlight (UV)$KMnO_4$

This section describes the implementation of the established reaction conditions (UV/Cl_2 process: solar irradiance 250 ± 25 mW/m^2, pH 7, OAR 7:1, reaction time 90 minutes. UV/$KMnO_4$ process: solar irradiance 250 ± 25 mW/m^2, pH 5, OAR 7:1 reaction time at 45 minutes) for the elimination of the three ARVs in Milli-Q water. Their % removal was monitored by the HPLC method. The experimental description of this section is contained in section 3.8.7. Three different concentrations, 20 µg/L, 35 µg/L and 150 µg/L within the range of the calibration curve in section 6.3.1 were chosen for the experiment. The purpose of using different concentrations was to evaluate the impact of the initial concentrations of the analyte on the % removal efficiency of UV/Cl_2 and UV/$KMnO_4$ methods.

The result showing the % removal of different concentrations of ARVs standards is shown in Figure 6. 29. In section (a) of Figure 6. 29, for the three concentrations, observed, the sunlight (UV)/Cl_2 was able to remove all three compounds to varying percentages. It was observed in all three compounds that the level of removal decreases as the concentration increases. The average % removal by UV/Cl_2 method for 20 µg/L, 35 µg/L and 150 µg/L are as follows: 3TC (51.69%, 49.42% and 45.08%), AZT (80.40% , 61.59% and 54.44%) and NVP (33.42% , 25.91% and 23.54%), respectively.

Similarly, for the UV/$KMnO_4$ process, there were variations in the % removal for different concentrations of the ARVs. A similar trend was recorded in the average % removal which decreased with an increase in the concentrations of the three analytes: For 3TC the % removals were 39.32%, 31.77% and 26.62% for 20 µg/L, 35 µg/L and 150 µg/L, respectively. For AZT, they were 80.75% and 77.88% and 62.30% for 20 µg/L, 35 µg/L and 150 µg/L, respectively and for NVP, the % removal were 30.44% , 20.39% and 18.43% for 20 µg/L, 35 µg/L and 150 µg/L, respectively.

In the two processes, the highest and the lowest % removal were recorded in 20 µg/L and 150 µg/L respectively. The highest % removal recorded in 20 µg/L could be associated with the availability of enough reaction sites because of the low concentration of the analyte molecules. Likewise, the lowest % removal recorded at 150 µg/L can be attributed to the effect of blockage and reduction in the reactive sites of the oxidants as the concentration of the target compounds increases (Yang et al., 2010). Also, at increased concentration, means more particles of the contaminants could absorb part of the available photons (radiation) available to the oxidant thereby reducing the quantity of radiation needed by the oxidants for oxidation during the photodegradation process (Yang et al., 2010; Shah et al., 2014; Wang and Chu, 2016). This finding agrees with the other scientific findings that benzotriazole was not effectively removed at a high initial concentration (Hübner et al., 2015). It is also consistent with the report of Shah et al. (2014) that the removal efficiency of endosulfan decreased correspondingly with an increase in the initial concentration of endosulfan.

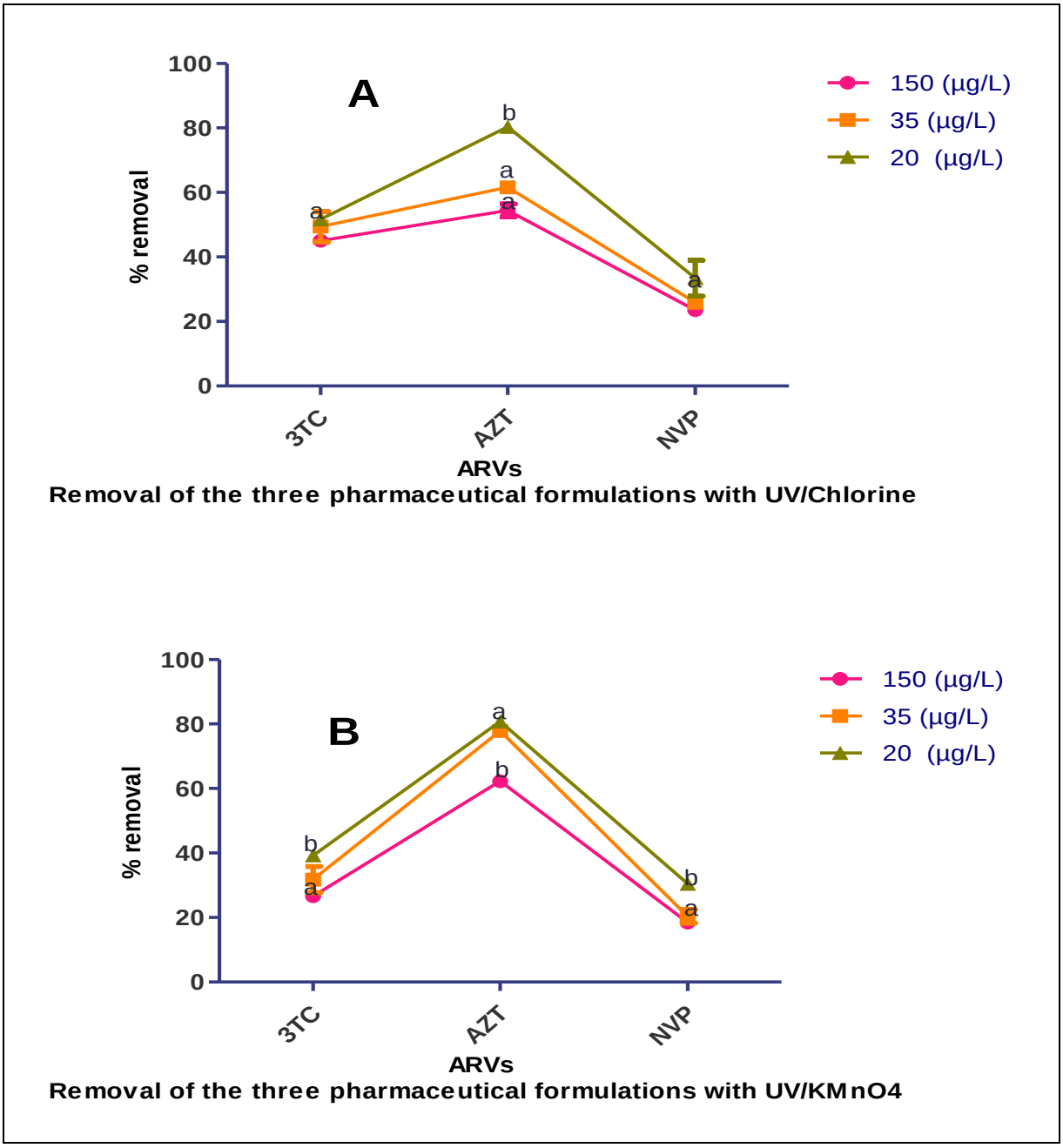

Figure 6. 29: Result of degradation of a standard solution of three pharmaceutical formulations with sunlight (UV)Cl_2 and sunlight (UV)MnO_4.

(Values with the same letter means no significant difference, values having different letters indicate significant difference).

In both treatments, the highest level of degradation was recorded in AZT. This could be attributed to the high degradability of AZT due to its low half-life and glucuronidation (Purvin et al., 2014; Joshy et al., 2018). On the other hand, NVP had the lowest % removal and this is consistent with its environmental persistence and high half-life (Sabo et al., 2002; Wood et al., 2016).

Statistical analysis with two-way ANOVA as shown in section A of Figure 6.29 indicates that the % removal of 3TC and NVP for the three concentrations studied were not significantly different ($P > 0.05$). However, the % removal of AZT at 20 µg/L was significantly different ($P < 0.01$) from those of 35 µg/L and 150 µg/L. In section B of Figure 6.26, the % removal

recorded for 3TC and NVP at 20 µg/L was significantly different ($P < 0.01$) from those of 35 µg/L and 150 µg/L. This can be attributed to the higher penetrating power of the UV radiation at the lowest concentration of analytes thus enhancing the photolysis of the oxidant. For AZT however, the % removal at 150 µg/L was significantly different ($P < 0.01$) from those recorded in 20 µg/L and 35 µg/L which showed no significant difference between one another. This can be ascribed to blockage in the reactive sites of the oxidant and reduction in the available photons required by the oxidant at a higher concentration of the ARVs.

6.8 Removal of ARV compounds in real wastewater samples by sunlight(UV)/Cl_2 method

As enumerated earlier, the aim of this research was to use sunlight(UV)/Cl_2 and sunlight(UV)/$KMnO_4$ as novel AOPs for the removal of 3TC, AZT and NVP contaminants from wastewater samples. This section presents and discusses the results obtained when the optimised methods were applied for the removal of the target analytes from the representative wastewater samples collected for 12 months in both plants. The initial concentrations of the ARVs in the samples before the degradation experiment and the final concentrations after the experiment were determined using Waters HPLC. The detailed description of the experimental procedure for the result discussed here can be found in section 3.8.8.

6.8.1 Removal of 3TC with sunlight (UV)/Cl_2

The % removal of 3TC from different wastewater samples matrices (RI, BUVT, AUVT and FE) after treatment with sunlight (UV)/Cl_2 is presented in Figure 6.30. The % removal for the four-season varied from 7.31 to 70.79% and they both occurred in RI in WWTP 1. The lowest % removal (7.31%) in the RI sample in September (autumn) 2018 was not significantly different ($P > 0.05$) from the RI sample in (July and November 2018), BUVT samples in (July, September and November 2018), AUVT samples in (September 2018) and FE sample (November 2018). The % removal (7.31%) is comparable to 5% reported for the removal of NVP in wastewater sample (Wood et al., 2015) but lower compared to 80.89% recorded for cytarabine (Ocampo-Péreza et al., 2011) and 99% for photodegradation of ketorolac tromethamine drug with sunlight(UV)/TiO_2 quantum dots (Kaur et al., 2015).

The low percentage removal could be attributed to the high concentration of natural organic matter (NOM), bicarbonate and high alkaline content present in the wastewater matrices. This agrees with the report of Wu et al. (2016) that at pH 7.1 the presence of NOM and bicarbonate reduced the extent of degradation of TMP by the UV/chlorine process. Similarly, differences in natural water matrices were given as a reason for variation in % degradation of some pharmaceuticals compounds in China (Yang et al., 2016). It has been reported severally in the literature that the presence of NOM could react in a different way such as radical scavenger, UV filter and as chlorine consumer that hinders the effective degradation of pharmaceutical in the UV/chlorine process (Lee and von Gunten, 2010; Wu et al, 2016; Guo et al., 2017). Substantial scavenging of ClO• which caused a great decrease in the degradation of PPCPs was attributed to the presence of high content of NOM in water and wastewater (Guo et al., 2017).

Also, excessive bicarbonate in natural water could scavenge both HO• and Cl• which are essential species for the degradation of micropollutants in UV/Cl_2 to form CO_3^{-} according to Equations 6.3 and 6.4 thereby causing a low degradation process (Wu et al., 2016; Zhu et al., 2018).

$HCO_3^- + HO\bullet \rightarrow H_2O + CO_3^{-}$ $\left(K=8.5\times10^{6}\ M^{-1}S^{-1}\right)$ …….…… Equation 0.13

$HCO_3^- + Cl\bullet \rightarrow H^+ + Cl^- + CO_3^{-}$ $\left(K=2.2\times10^{8}M^{-1}S^{-1}\right)$ ….……Equation 0.14

The highest % removal (70.99%) recorded was not significantly different ($P > 0.05$) from 60.01% recorded for the BUVT sample in the same month (autumn) but differed significantly from all other % removal recorded in other seasons and wastewater matrices. This finding is comparable to 87% removal reported for sulfadiazine (Wang and Chu, 2016) and 87.9% recorded for TMP at pH 7.1 (Wu et al., 2016). It also falls within the range of 59 ─ 99% and 27 ─ 92% reported for pharmaceutical residues in natural water (Yang et al., 2016). Our finding is however lower compared to the 95% removal efficiency reported for chloramphenicol degraded by UV/chlorine (Dong et al., 2017). The relatively high % removal recorded in the RI sample in March 2019 could be associated with the effective performance of HO• and multiple RCS (Cl•, $Cl_2\bullet^-$ and ClO•) and a low level of NOM in the March 2019 sample. This is because of a higher level of elimination of the ARVs in most of the water matrices in this month compared to other months. This is consistent with a report that high degradation of TMP was enhanced by RCS (Wu et al., 2016). It has been documented also that HO• is a non-selective oxidant that reacts with a diversity of contaminants at nearly diffusion-controlled rates. On the other hand, Cl• is more selective in its reactivities particularly with compounds containing an aromatic ring and electron-rich moieties (Wu et al., 2016; Fang et al., 2017; Guo et al., 2017).

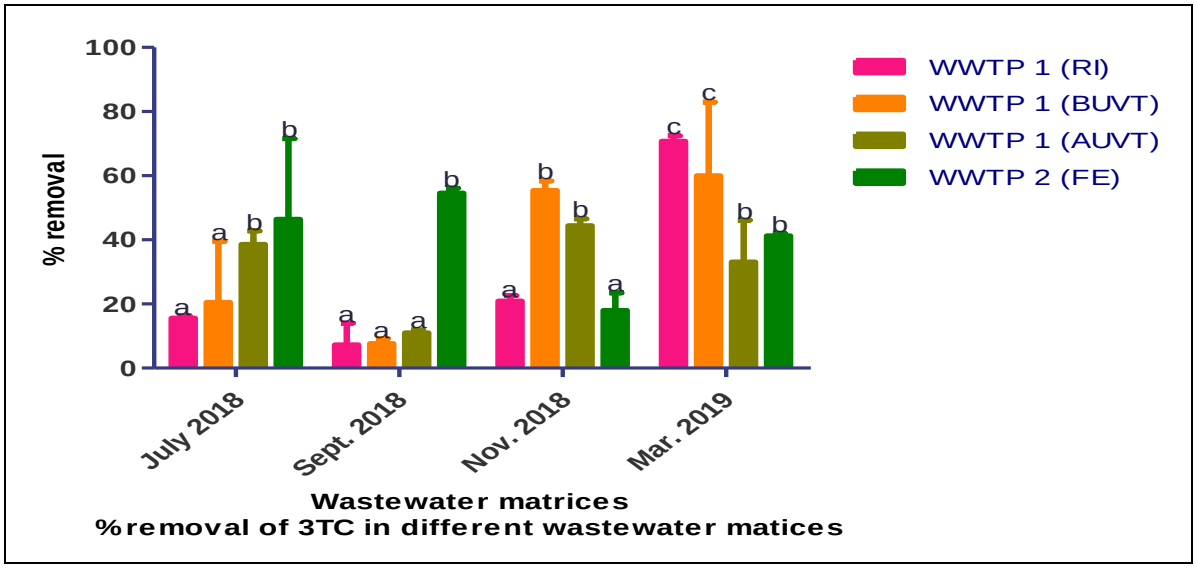

Figure 6. 30: Result of percentage removal of 3TC from different wastewater matrices using sunlight (UV)/Cl_2.

The previous study carried out in China on the degradation of atrazine (ATZ) reported that the degradation efficiencies of UV/chlorine at neutral pH indicate that UV/Cl_2 is a good AOPs that can be practically applied to real water because of its dual role particularly in the areas of high degradation of pollutants and disinfection purpose (Kong et al., 2016). Wu et al. (2016) also pointed out that the UV/chlorine effectively degraded carbamazepine, diclofenac and sulfamethoxazole than the UV/H_2O_2 process. Concerning the compound structure, the presence of pyrimidine group in 3TC is another criteria for the removal of 3TC by UV/Cl_2 because this group can be readily attacked by chlorine (Deborde and Gunten, 2008; Miklos et al., 2019).

6.8.2 Removal of AZT with sunlight (UV)/Cl_2

Figure 6.31 shows the % removal of AZT, in the four matrices (RI, BUVT, and AUVT in WWTP1 and FE in WWTP 2) when treated with sunlight(UV)Cl_2. The % removal ranged between 29.64 ─ 91.69% in the BUVT sample in September 2018 and the FE sample in March 2019. It is worthy to note that under the same experimental conditions of radiation, pH, OAR and time, the compounds were removed to varying degrees in different wastewater matrices. This could suggest the effect of different wastewater matrices on the extent of degradation. This is consistent with earlier findings by Bolton and Stefan (2002). The group reported that water quality affects the UV/H_2O_2 process in the area of UV-transmittance and radical scavenging which result in a higher level of electrical energy per order (E_{EO}) (Bolton and Stefan, 2002).

The lowest % removal (29.64%) recorded in the BUVT sample in September 2018 was close to the results obtained in RI and AUVT samples in the same month. This result is comparable to 29.7% and 31.1% reported for the degradation of estrone (E1) and 17α-ethinylestradiol (EE2), respectively (Deng et al., 2015). The result can be attributed to differences in the four wastewater matrices. Many reports assert the fact that water quality can negatively affect the efficiency of the UV/Cl_2 process toward the removal of contaminants in water (Wang et al., 2015; Wang et al., 2016; Wu et al., 2017; Guo et al., 2017; Miklos et al., 2019). Another finding

also corroborates our result that at the same chlorine dose, in the UV/chlorine process, the % degradation recorded for CBZ in WWTPs effluent decreased by approximately 30% compared to the result obtained for the same chlorine dose in ultrapure water (Wang et al., 2016). From another result, however, a recent report on the impact of different qualities of water (pure water, drinking water, groundwater and wastewater) on the E_{EO} of the UV/H_2O_2 process. The result showed that there was no significant difference in the E_{EO} values of this water (Miklos et al., 2018). The group reported that pure water expected to have the lowest E_{EO} had the highest median E_{E}o values (Miklos et al., 2018). However, the possible explanation given for the result was the predominant use of pure water in lab-scale experiments against the real wastewater samples used in this case.

The highest % removal (91.69%) indicates that UV/Chlorine can degrade AZT in wastewater samples. This is consistent with many reports in the literature (Wang et al., 2015; Richardson and Kimura, 2016; Wang et al., 2017; Aghdam et al., 2017; Chan et al., 2018; Guo et al., 2018b). Several other reports point out that different types of micropollutants which include pesticides, pharmaceuticals, personal care products and natural organic matter have been successfully degraded by the UV/free chlorine process (Xiang et al., 2016; Sun et al., 2016; Dong et al., 2017; Wang, Wu, et al., 2017; Fang et al., 2017; Xiang et al., 2018). This effect was attributed to the better yield of hydroxyl radicals (HO•) and reactive chlorine species (RCS) which include Cl•, $Cl_2^{\bullet-}$ and ClO•. This occurs when free chlorine (HOCl/ OCl^-) is simultaneously exposed to UV radiation according to the mechanism of UV/Chlorine as shown in Equations 6.5 to 6.11 thereby leading to more effective removal of contaminants (Huang et al., 2017; Wang et al., 2017; Zhu et al., 2018; Zhang et al., 2019).

$HOCl \leftrightarrow OCl^- + H^+$ …… Equation 0.15

$HOCl/OCl^- + hv \rightarrow HO^{\cdot}/O^{\cdot -} + Cl^{\cdot}$ ……Equation 0.16

$Cl^{\cdot} + Cl^- \underset{\leftarrow(K_2)}{\overset{\rightarrow(k_1)}{\rightleftharpoons}} Cl_2^{\cdot -}$ ($K_1 = 6.5\times10^9 M^{-1}S^{-1}$, $K_2 = 1.1\times10^5 M^{-1}S^{-1}$) ……Equation 0.17

$HOCl + HO^{\cdot} \rightarrow ClO^{\cdot} + H_2O$ $\left(K = 2.0\times10^9 M^{-1}S^{-1}\right)$ ……Equation 0.18

$OCl^- + HO^{\cdot} \rightarrow ClO^{\cdot} + OH^-$ $\left(8.8\times10^9 M^{-1}S^{-1}\right)$ ……...Equation 0.19

$HOCl + Cl^{\cdot} \rightarrow ClO^{\cdot} + H^+ + Cl^-$ $\left(K = 3.0\times10^9 M^{-1}S^{-1}\right)$ ……Equation 0.20

$ClO^- + Cl^{\cdot} \rightarrow ClO^{\cdot} + Cl^-$ ($K = 8.2\times10^9 M^{-1}S^{-1}$) ……Equation 0.21

(Wu et al., 2016; Guo et al., 2017)

During UV/chlorine oxidation, RCS attack double bonds, aromatic groups and phenolic groups which are electron-rich with high electron-donating capacity (Fang et al., 2017; Guo et al., 2017; Chan et al., 2018; Zhu et al., 2018a). In this case, the presence of pyrimidine and azide groups enhance the removal of AZT in the combined UV/Cl_2 process. This is consistent with reports that pyrimidine and azide being electron donor groups are readily reactive to chlorine (Deborde and Gunten, 2008; Guo et al., 2017; Miklos et al., 2019).

Similarly, in scientific research, the degradation kinetics of different molecular weight (MW) fractions showed that UV/chlorine oxidation was able to degrade high MW fractions into low MW fractions, with a high degradation rate of (>3000 Da) (Wang, et al., 2017). Also, the UV/chlorine process as an AOP, have been reported to be more effective for the degradation of emerging contaminants such as ibuprofen, carbamazepine, caffeine and other pharmaceuticals than UV irradiation alone or dark chlorination alone (Yang et al., 2016; Pan et al., 2017).

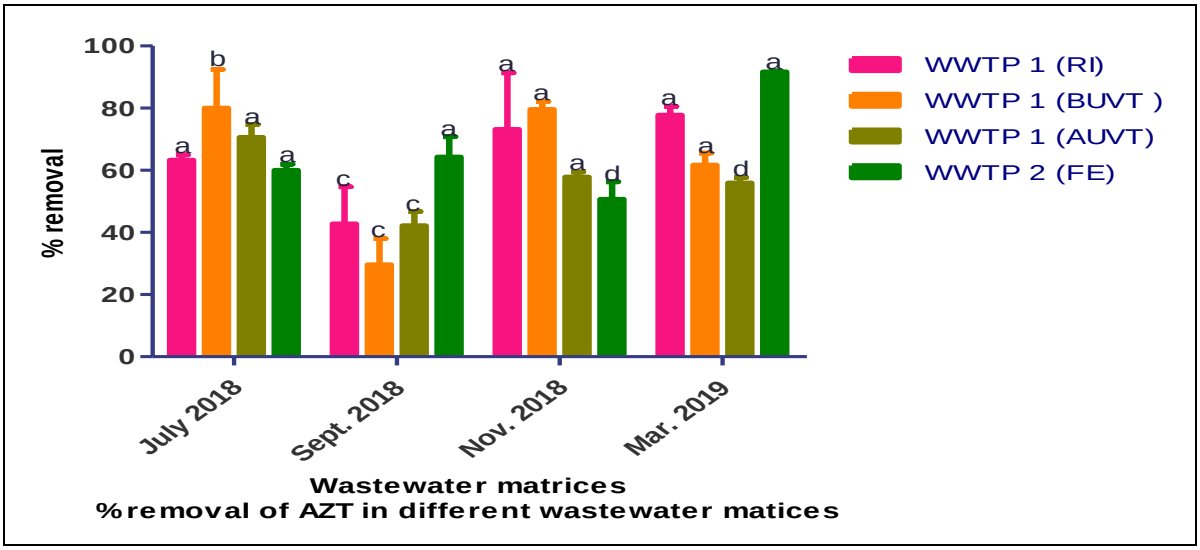

Figure 6. 31: Result of percentage removal of AZT from different wastewater matrices using sunlight (UV)/Cl_2.

Similarly in another study, Kong et al. (2016) reported that a low level of HCO_3^- /CO_3^{2-} which commonly occurs in natural water and used to represent inorganic carbon and radical scavenger showed negligible effect on the atrazine degradation at pH 7. Since this experiment was conducted at pH 7, the high % removal recorded could mean that the level of inorganic and dissolved organic matter (DOM) in the samples were low.

6.8.3 Removal of NVP with sunlight(UV)/Cl_2

Figure 6.32 shows the % removal of NVP in different wastewater matrices (RI, BUVT, AUVT in WWTP 1 and FE in WWTP 2) in the four, seasons in Cape Town, South Africa. The % removal varied from 15.60 to 67.77% in the BUVT sample in September 2018 (spring) and the AUVT sample in March 2019 (autumn), respectively. The lowest % removal (15.60%) was not significantly different ($P > 0.05$) from those recorded in RI samples in July 2018, (winter), September 2018, November 2018; BUVT sample in July 2018, and FE samples in September and November 2018. The lowest % removal (15.60%) was higher than 5% reported for the removal of NVP in the wastewater sample (Wood et al., 2015). The low % reduction might be associated with the possibility of a high level of NOM, bicarbonate, chloride, and ammonia in the water matrices. This trend has been reported in the literature that the presence of NOM, bicarbonate and chloride ions in surface water and wastewater can retard the degradation of micropollutants (Ocampo-Péreza et al., 2011; Rivera-Utrilla et al., 2013; Wu et al., 2017).

Wood et al. (2016) similarly reported the low degradation of nevirapine as a result of the excessive chlorine demand by the NOM, nitrites and ammonia in the wastewater sample. All these can significantly affect the reaction of chlorine resulting in the low % removal recorded (Heeb et al., 2014; Wu et al., 2017).

Kong et al. (2018) also showed how NOM (1 mg/L) and ammonia (50 mM) in water decreased the degradation of gemfibrozil (GFRZ) by 50.3% and 66.3%, respectively and that of bezafibrate (BZF) by 36.2%, and 7.1%, respectively. NOM is an optically active compound that plays significant roles in freshwater ecosystems; it can be used to estimate the level of water quality, it can also convey nutrients, heavy metals and other pollutants from the terrestrial to the aquatic ecosystems (Zhang et al., 2011). Therefore, the occurrence of NOM and bicarbonate in water and wastewater is a great challenge because it seriously affects the qualities of water such as colour, turbidity and odour (Zhang et al., 2011; Wang et al., 2019). It constitutes problems in water and wastewater treatment plant as it negatively affects the effectiveness of water treatment processes such as granular activated carbon filtration and membrane filtration. Additionally, it reduces the attenuation effect of ultraviolet radiation on micropollutants, it can also decrease the efficiency of oxidants and disinfectants and leads to the production of undesirable disinfection by-products during oxidation processes (Baghoth et al., 2010; Varanasi et al., 2018; Wang et al., 2019). Similarly, the presence of other ions such as bromide and ammonium ions which are generally common in wastewater could also be responsible for the low % removal (Heeb et al., 2013; Wood et al.,2015; Wu et al., 2017).

The highest % removal (67.77%) in the RI sample in March 2019 (autumn) was not significantly different ($P > 0.05$) from the % removal recorded for some samples which include, all AUVT samples in July 2018 to March 2019, BUVT samples in November 2018 as well as FE samples in July 2018 and March 2019. The result suggests the low level of chlorine oxidant scavenger and UV absorbance and the possibility of an effect of seasonal variation that could have lowered the concentrations of scavenger and UV absorbance component in the water matrices. This % removal is comparable to the 70% removal of sotalol by direct chlorination (Miklos et al., 2019).

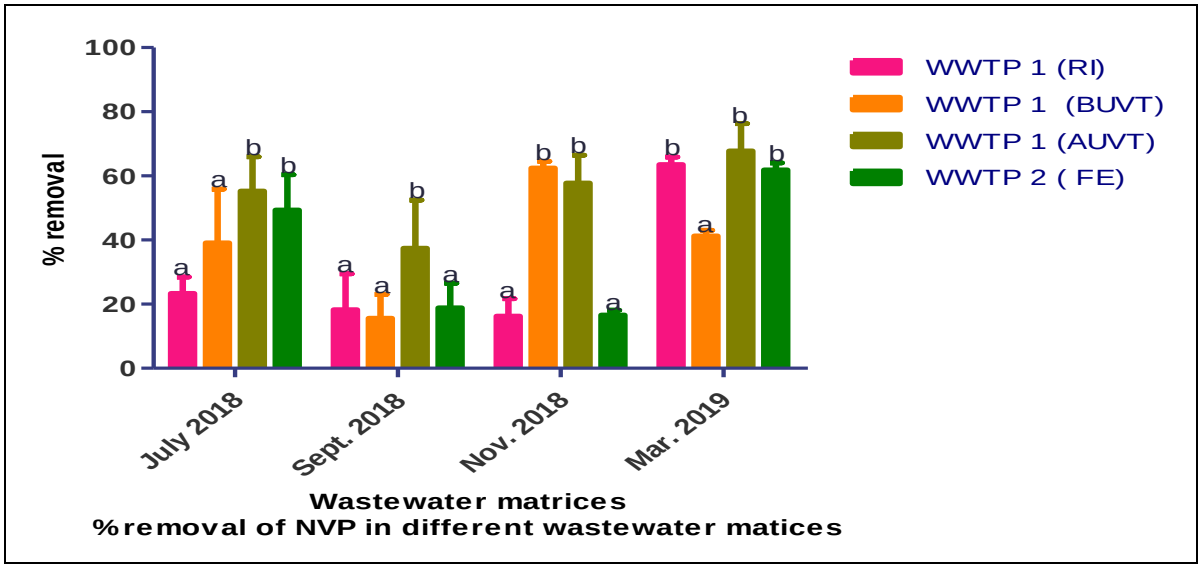

Figure 6. 32:Result of percentage removal of NVP from different wastewater matrices using sunlight (UV)/Cl_2.

The relatively high degradation can be attributed to the high photolysis rate constants of $HOCl/OCl^-$, high quantum yield, high molar absorption coefficient and production of HO• radicals and RCS which enhance the removal of our target pharmaceutical compounds. Previous studies carried out showed that the high degradation of contaminants can be attributed to the high photolysis rate constants of $HOCl/OCl^-$ quantum yields of 1.0 and 0.9 mol/E and molar absorption coefficients of 59 and 66 M^{-1} cm^{-1}, respectively. Also, the production of primary radical which are HO• and Cl• and the secondary reactive chlorine species (RCS), such as $Cl_2^{\bullet-}$ and ClO• which are copious during the UV/chlorine process also enhances the effective removal of organic contaminants (Zhang et al., 2009; Wu et al., 2017; Miklos et al., 2019). Other factors could be the differences in the water matrices and the low level of NOM within the period. This trend was also reported by Varanasi et al. (2018) concerning the application of UV/Chlorine for wastewater treatment in Los Angelis and they found out that the variation in degradation was ascribed to the variation in the water matrices and NOM concentrations.

6.9 Removal of ARV compounds in real wastewater samples by sunlight(UV)/$KMnO_4$ method

6.9.1 Removal of 3TC with sunlight(UV)/$KMnO_4$

Figure 6.33 shows the result of % removal of 3TC from the four wastewater matrices (RI, BUVT, AUVT in WWTP 1 and FE in WWTP 2). The % removal varied from 2.70% in July 2018 FE sample in WWTP 2 to 100.00% in March 2019 samples. The lowest % removal recorded was not significantly different ($P > 0.05$) from the values recorded in RI and BUVT samples in July 2018. This % removal is lower compared to 34% to 37% and 29.70% to 37.10% recorded for the degradation of three natural estrogens: estrone (E1), 17β-estradiol (E2), and synthetic 17α-ethinylestradiol (EE2), with $KMnO_4$ alone and ultrasound radiation alone, respectively (Deng et al., 2015). This could be associated with the reactions of unstable reactive manganese intermediates Mn(III), manganese oxide, Mn(V) and Mn(VI) formed during

permanganate oxidation that transformed to MnO_2 which is less reactive and also inhibits the removal of our ARV compounds. This is consistent with a report that at weakly acidic conditions, Mn (V) and Mn(VI) are not stable, they disproportionate at a very faster rate to form MnO_2 (Gao et al., 2019). Likewise, in the absence of ligands, Mn(III) and Mn(V) can equally be oxidized and reduced at the same time to form MnO_2 and Mn(II) which could retard micropollutant oxidation (Kenneth Klewicki and Morgan, 1998; Yang et al., 2013; Gao et al., 2019). This also conforms with earlier reports that manganese ion, inhibits the oxidation of CMZ (Hu et al., 2009; Jiang et al., 2010).

The highest % removal (100%) was recorded in March 2019 sample for the four matrices. This occurred because the concentrations of the final sample were not detected (ND) by the HPLC whereas the initial concentration was quantifiable. The 100% removal was comparable to > 95% removal recorded for sulfamethoxazole and methyl blue, respectively but higher than 84% and 75% reported for ACE and CBZ by permanganate/bisulphite (PM/BS) processes (Chow and Leung, 2019). The high removal can be associated with a high level of some factors such as humic acid, a component of the dissolved organic matter (DOM) in the wastewater samples in March 2019. This finding is in agreement with the report that water matrices can both accelerate or retard the removal of micropollutants (Guan et al., 2010). Similarly, a higher removal efficacy of levofloxacin, phenol, triclosan and 17 b-estradiol during permanganate related process reported was ascribed to the creation of secondary oxidants including Mn^{3+}, $O_2^{\cdot -}$, in the presence of humic acid (Jiang et al., 2009; Yang et al., 2013; Chow and Leung, 2019). It can also be attributed to the enhanced production of RMnS as a result of sunlight(UV)/MnO_4 treatment. This agrees with scientific reports that UV irradiation is an effective means of activating permanganate to enhance the production of RMnS: Mn(III), MnO_2^{-}, Mn(V), MnO_4–, and MnO_4^{2-} (Guo et al., 2018). Other factors such as the influence of other environmental factors such as temperature and pH of wastewater samples in March 2019 can also contribute to the high removal efficiency of the compound as seen in summer/ autumn in this case. Our finding corroborates the reports from the USA and Portugal that higher removal efficiency of endocrine-disrupting compounds, pharmaceuticals and personal care products were recorded in summer than winter (Yu et al., 2013; Pereira et al., 2015).

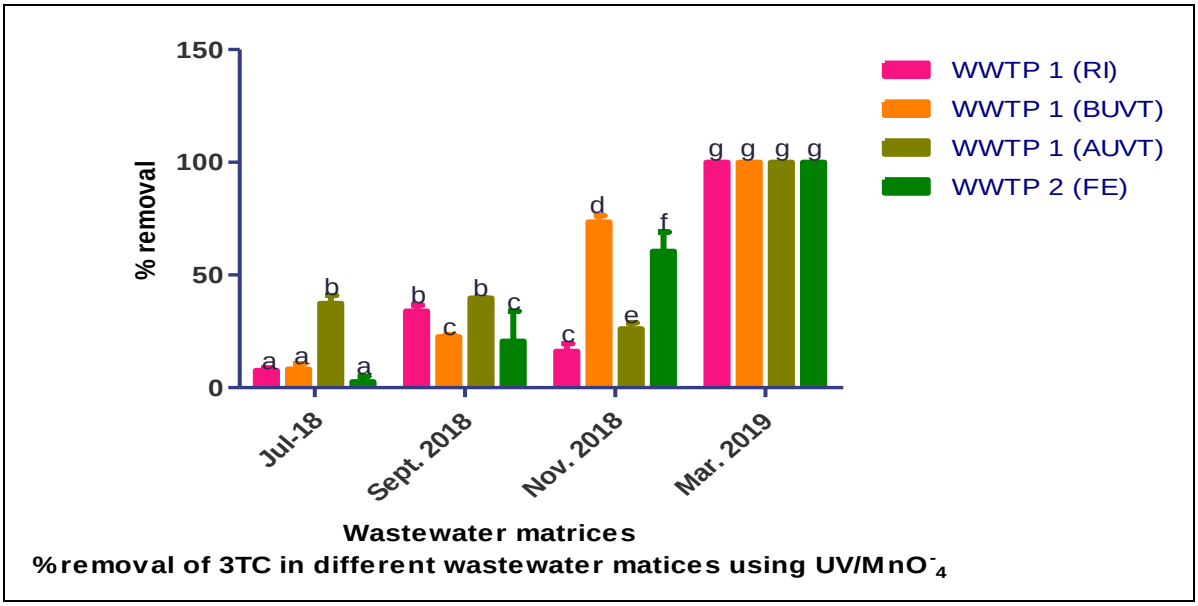

Figure 6. 33: Result of percentage removal of 3TC from different wastewater matrices using sunlight(UV)/$KMnO_4$

6.9.2 Removal of AZT with sunlight(UV)/$KMnO_4$

The % removal of AZT from the four wastewater matrices which are RI, BUVT, AUVT from WWTP 1 and FE samples from WWTP 2 is presented in Figure 6.34. The removal ranged between 6.48 – 100.00% in the RI sample in September 2018 (spring) and all samples in March 2019, respectively. The lowest concentration recorded was not significantly different ($P > 0.05$) from the values obtained in RI samples in July 2018 and November 2018. This could be associated with the presence of some compounds and ions in the RI samples in those months that consumes the $KMnO_4$ or UV radiation resulting in reduced removal efficiency of the UV/$KMnO_4$. This is consistent with the other findings that consumption of permanganate by water matrices is a critical factor that inhibits the removal of micropollutant from water (Yang et al., 2013; Wu et al., 2017).

On the other hand, the highest % removal (100%) was recorded in all the March 2019 samples and they did not exhibit any significant difference ($P > 0.05$) from one another. This can be attributed to the environmental impact and some compounds present in NOM that can induce indirect photolysis attenuation of organic contaminants (Grebel et al., 2012). It can also be attributed to the coupling effect of UV/$KMnO_4$ that ensures better production of permanganate reactive species in the March samples. This is consistent with the scientific finding that the effective removal of certain micropollutants by the UV/permanganate system showed that some reactive species are better produced during the simultaneous treatment with UV and permanganate (Guo, et al., 2018). More importantly, it has been shown that permanganate can be reduced to several RMnS; (Mn(VI), Mn(V), Mn(IV), Mn(III) and Mn(II). Out of all these species, Mn(VI) is reported to be less reactive and can be produced in alkaline condition, similarly, Mn(IV) and Mn(II) exist mostly as stable manganese reduction products which make them less reactive to most micropollutant (Lee and von Gunten, 2010a; Guan et al., 2010; Sun et al., 2015). Therefore, of all the RMnS, Mn(V) and Mn(III) could be majorly responsible for the degradation recorded. This is consistent with the finding that the Mn(V) is a selective oxidant that was responsible for the removal of benzoic acid, terephthalic acid, p-chlorobenzoic

acid, and nalidixic acid during the UV/permanganate process because it can oxidize the α-C atom of primary and secondary alcohols to aldehydes through hydrogen transfer (Guo, et al., 2018). The reason provided for this is that when $KMnO_4$ is exposed to UV radiation, excited state manganese(V) peroxo complex $[MnO_2(\eta^{2}\text{-}O_2)^{-}]$ is formed which is more reactive than MnO_4^{-} to form O─O bond from two Mn=O bonds of permanganate (Crandell et al., 2017; Guo, et al., 2018).

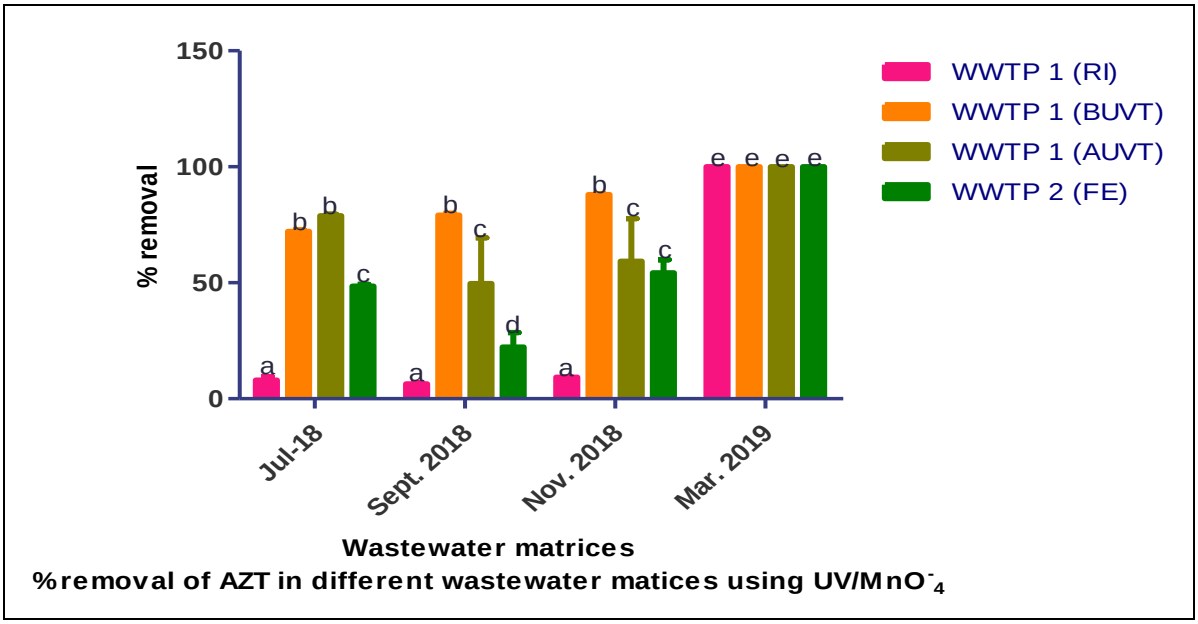

Figure 6. 34: Result of percentage removal of AZT from different wastewater matrices using sunlight(UV)/$KMnO_4$

Also considering the structure of the compound, AZT contains pyrimidine that is a possible reactive site for Mn(VII) oxidation. This site has been projected as a likely target site for ozone and chlorine reactions with TMP (Dodd and Gunten, 2006; Dodd and Huang, 2007; Chávez et al., 2016). Hu et al. (2010) also proposed pyrimidine as the reactive site for lincomycin in the degradation of the compound with $KMnO_4$, they further confirmed this by using 3,4,5-trimethoxytoluene and 1,4-diaminopyrimidine which are two structural analogues of pyrimidine for kinetic studies and it was reported that the site is the possible reacting site for Mn(VII) attack for the degradation (Hu et al., 2011). This is also in agreement with the finding that oxidation of nucleic acid with Mn(VII) occurred at 5,6 double bond of pyrimidine ring (Hu et al., 2010).

6.9.3 Removal of NVP with sunlight(UV)/$KMnO_4$

The result of % removal of NVP in four wastewater matrices (RI, BUVT, AUVT in WWTP 1 and FE samples in WWTP 2 is presented in Figure 6.35. The % removal ranged between 15.41% in the BUVT sample in March 2019 ─ 100% in the AUVT sample of WWTP 1 in March 2019. The lowest % removal did not differ significantly ($P > 0.05$) from the % removal recorded in July 2018 (RI and FE samples), September 2018 (RI, AUVT and FE samples), November 2018 (FE sample) and March 2019 (RI sample). The lowest values which existed in about 50% of the water sample could be associated with the recalcitrant nature of NVP. Previous research findings documented the recalcitrant nature of NVP in a bottled experiment using laboratory

water (Vaňková, 2010) and in wastewater (Prasse et al., 2010; Wood et al., 2015). This can also be attributed to the weakly acidic condition (pH 5) in which the research was conducted. The recent report associated the low removal of NVP in wastewater with its reduced degradation in acidic pH (Wood et al., 2016; Abafe et al., 2018).

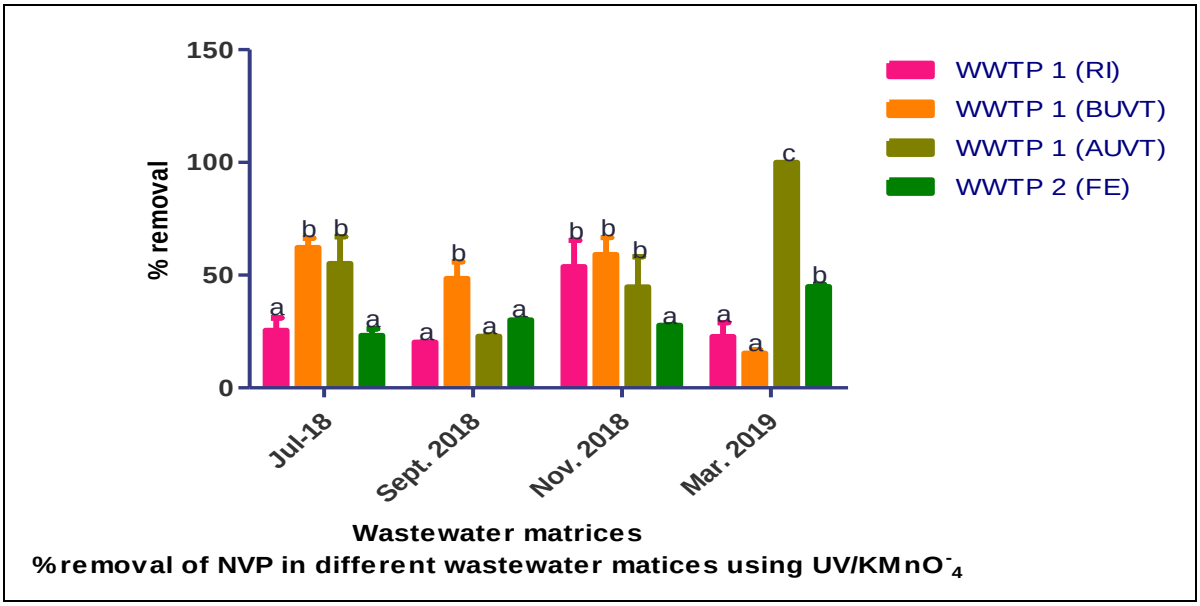

Figure 6. 35: Result of percentage removal of NVP from different wastewater matrices using sunlight(UV)/$KMnO_4$.

The highest % removal recorded in the AUVT sample in March 2019 can be related to the low level of NOM in the AUVT sample and the high molar absorption coefficient of permanganate that promotes the generation of HO• (Shen and Schäfer, 2015). Guo et al. (2018) recently reported that the effectiveness of $KMnO_4$ related AOP towards the degradation of micropollutants can be attributed to the high molar absorption coefficient of permanganate (637 M^{-1} cm^{-1}) and the enhanced generation of HO• and the generation of RMnS in the weak acidic medium used in this investigation. This has been reported in the literature that the degradation of micropollutant by permanganate depends on the RMnS generated at weakly acidic conditions (Sun et al., 2015).

The group explained that when permanganate was activated with bisulfite at pH 5, phenol, ciprofloxacin and methylene blue were better degraded to about three to six orders of magnitude under the same experimental conditions than the result obtained at alkaline condition (Sun et al., 2015). Similarly, they reported that the degradation was highly enhanced at the same pH 5 when $KMnO_4$ was activated with $NaHSO_3$ than using $KMnO_4$ or $NaHSO_3$ alone and thereby reporting that activated $KMnO_4$ at pH can degrade contaminants that are supposedly recalcitrant to oxidation by ordinary $KMnO_4$ (Sun et al., 2015).

6.10 Comparison between sunlight(UV)/Cl_2 and sunlight(UV)/$KMnO_4$ methods

By comparing the two methods, it is understood that the sunlight(UV)/Cl_2 can be easily applied because most of the WWTPs already have/included chlorination stage in their treatment

processes. More so, it will only include the incorporation of sunlight radiation unit operation at the optimised condition at a minimal operational cost. Also, the pH 7 at which the sunlight(UV/Cl_2) was optimised is another advantage for its environmental relevance because most operations are carried out around this pH. Another advantage is the use of the method to promote the inactivation of chlorine resistant microbial pathogen and reduces the formation of DBPs (Kong et al., 2019; Wang et al., 2019).

The use of UV/$KMnO_4$ demonstrated an improvement on the advantages of $KMnO_4$ which include being an environmentally friendly material, moderate cost, easy and safe storage and delivery, no probability of forming chlorinated or brominated by-products. Also, the optimised processes took only 45 minutes for the elimination of the compounds investigated. As established in all March 2019 samples where the removal efficiency were 100%, points out that UV/$KMnO_4$ AOP method if well implemented could be an excellent AOP to eliminate contaminants in water/wastewater.

In the two methods, the highest % removal was consistent in March 2019 samples. This could be attributed to the seasonal effect on sample removal. This could be further explored to achieve optimal removal of ARVs.

6.11 Summary

In summary, this chapter discussed the development and optimisation of the two AOPs techniques which are sunlight(UV/)Cl_2 and sunlight(UV)/$KMnO_4$ in Milli-Q water for the removal of 3 ARV compounds. The optimised reaction conditions for the sunlight(UV)/Cl_2 method are pH 7, 90 minutes, OAR 7:1 and solar irradiance 250 ±25 mW/m^2. For the sunlight(UV)/$KMnO_4$ process, it was average solar irradiance of 250 ± 25 mW/m^2, pH 5, OAR 7:1 and reaction time 45 minutes. The two methods at the optimised conditions degraded the three ARVs in Milli-Q water to different percentages which were measured by both UV spectroscopic and HPLC methods. The HPLC method was able to distinctively resolve the three compounds in the Milli Q water and wastewater sample and as well accurately measured the percentage removal of the 3 compounds in different wastewater samples. The method validation for the HPLC by the International Conference on Harmonization (ICH) showed that the method is accurate, repeatable and reliable, having a good recovery low LOD and LOQ.

CHAPTER 7

CONCLUSION, RECOMMENDATIONS AND FUTURE WORK

7.1 Introduction

This chapter concludes the results presented in the study and makes recommendations for future work.

7.2 Conclusion

The main aim of this study was to investigate whether specific anti-retroviral compounds of lamivudine (3TC), zidovudine (AZT) and nevirapine (NVP) are present in the wastewater of two selected wastewater treatment plants in the Cape Town Metropole.

The following conclusions are made from each specific objective:

The **first objective** focussed on the determination of the monthly and seasonal levels of 3TC, AZT and NVP contaminants in wastewater samples from the Bellville and Khayelitsha WWTPs. The results obtained towards this objective with the use of UV-Visible spectrophotometry technique have shown that the three ARV compounds (3TC, AZT, NVP) investigated occurred in the wastewater samples from the two plants at different concentrations within the sampling period. From our findings, NVP consistently occurred at a high concentration in both WWTP 2 throughout sampling while AZT occurred at low concentrations. The validation of the method produced good linearity, precision, mean recovery, LOD and LOQ for the three compounds within the acceptable range. This indicates that the method is simple, accurate, precise, linear, faster and cost-effective for the determination of ARVs in WWTPs, and most especially in small WWTPs where the use of sophisticated equipment may not be available. Thus the first objective was successfully met. Though the value got from this method was higher compared to the values from the HPLC method, however, this finding indicates that the method is simple, linear, faster, and cost-effective which could be used for a fast semi-quantitative determination of ARVs in WWTPs, and most especially in small WWTPs where the use of sophisticated equipment may not be available.

The **second** objective was to evaluate the efficiency of WWTP 1 in eliminating 3TC, AZT and NVP contaminants through the qualitative determination of the ARVs residues in influent (RI) and effluent (AUVT) wastewater samples using UV/visible spectrophotometer analysis. The result obtained from this study showed that WWTP 1 partially removed the three compounds investigated to different degrees. AZT exhibited the highest % removal while NVP had the

least % removal. The effect of treating the BUVT sample with UV radiation in WWTP 1 demonstrated a negligible effect of UV treatment on the reduction of the ARV compounds. This indicates the achievement of the second objective.

The **third** objective was to determine the level of specific physicochemical parameters in the wastewater samples in compliance with the national and international regulatory bodies' limits and to investigate their co-influence on the target ARVs. The results obtained from this study showed that only three (temperature, pH and SO_4^{2-}) out of the ten parameters studied fully complied with the recommended limits by both regulatory bodies. This indicates that effluents from these plants are probable sources of pollution to the receiving water bodies and the environment at large. This is not good for a semi-arid country like South Africa where water is scarce and wastewater effluent may be used in agriculture, landscaping and industrial purposes.

The result of the impact of physicochemical parameters on the removal of target ARVs indicates variation in their effects. However, temperature and pH appeared to be the principal factors because most of the positive or negative effects demonstrated by other physicochemical parameters are connected with the prevailing temperature and pH values.

The **fourth** objective was to develop novel advanced oxidation treatment technologies, including exposure to:

- Sunlight(UV)/Cl_2 treatment;
- Sunlight(UV)/$KMnO_4$ treatment.

for the degradation or removal of 3TC, AZT and NVP from wastewater samples.

The optimisation of different degradation conditions including time, pH, contact time, and OARs showed that each of the parameters influenced the removal of our target compounds. At the respective optimised conditions, both methods were able to remove the three pharmaceutical compounds in Milli-Q water and different wastewater matrices (RI, BUVT, AUVT in WWTP 1 and FE in WWTP 2) to different degrees.

The results of the control experiments demonstrated an enhanced removal of the ARVs with the combined AOPs treatment processes than the control experiments that utilise a single treatment process. This showed the effectiveness of our developed methods over single treatment processes. This is good and will go a long way to stop or reduce environmental pollution arising from ARVs pollution, thereby improving and ensuring a cleaner environment.

At the established optimised conditions, the investigation of the effect of the initial concentration of ARVs on the removal efficiency demonstrated that the removal efficiency of the two methods decreased when the initial concentrations increased. The chromatographic separation of the 3 ARVs produced highly resolved chromatographic peaks. The validation of the HPLC method was accurate with good linearity, percentage recovery and repeatability.

The ARVs contaminants were removed in real wastewater samples by the two methods to varying degrees and UV/$KMnO_4$ removed our target compounds more than the UV/Cl_2 method. Thus, the fourth objective of this investigation was achieved.

7.3 Recommendations

Due to the partial removal of the ARVs by the two WWTPs, and non-compliance of the majority of physicochemical parameters to both national and international limits, this study, recommends an urgent need to upgrade the two WWTPs and any other existing WWTPs in Cape Town, South Africa. Also, the regulatory organizations in South Africa should have routine pollution monitoring and enforce strict compliance with the international and national set standards for effluent discharge. It should be reported that both the WWTPs have in 2020 undergone major renovations that will be investigated in future studies.

Similarly, to ensure optimum performance of the UV radiation treatment process in WWTPs, the existing operation situations should be revisited at the municipal level. The conditions that enhance the performance of UV radiation in the elimination of contaminants which include the flow rate, the depth of wastewater that passes through the UV system as well as chlorine dose must be revisited and optimised. These will go a long way to stop or minimize the risk of pollution to the receiving water bodies by both regulated physicochemical parameters and the yet to be regulated ARV compounds. Thus, ensuring a safe and healthy environment for residents of Cape Town and the entire South Africans.

Since the degradation methods developed were able to remove the target ARVs, the application of the optimised methods will be of great benefit in ensuring a safe aquatic environment. Also, the consistent highest % removal recorded in March 2019 samples in the majority of the samples, could be further explored to achieve optimal removal of ARVs.

7.4 Suggested Future work

This present work investigated the presence and removal of target ARVs in wastewater. Future work should:

(i) Examine the likely transformation products/metabolites generated by the two AOPs.

(ii) Investigate the toxicity of the compounds on some aquatic species.

(iii) Study the possibility of the ARV compounds in biosolids generated during wastewater treatment processes. This will go along way to avoid the consumption of these compounds and their metabolites in farm products due to the use of the biosolids as farm manure.

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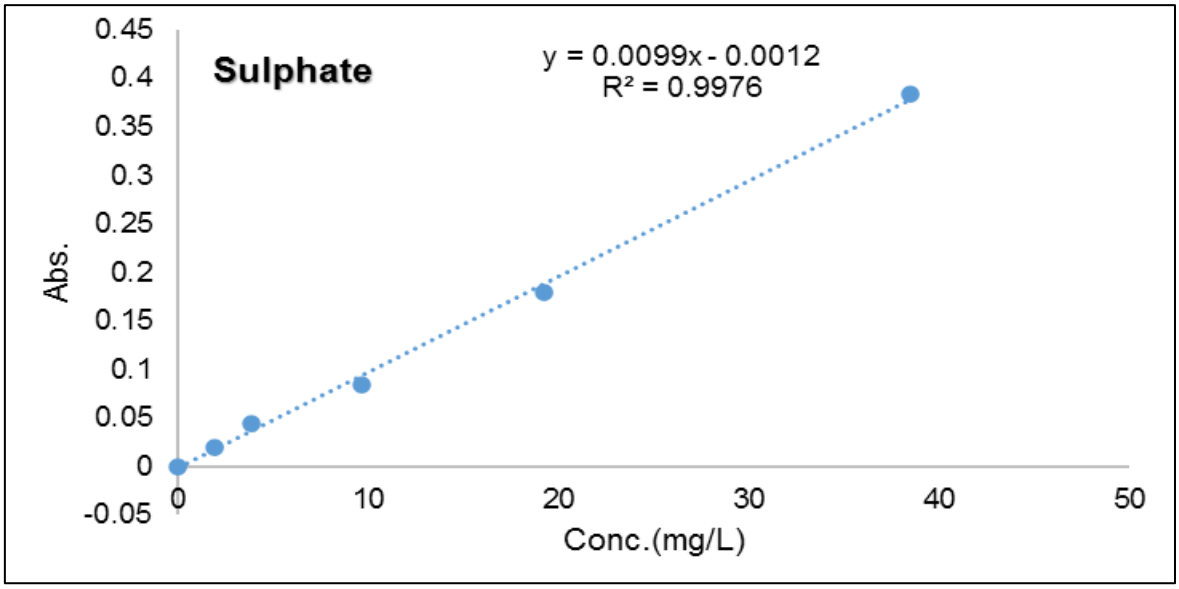
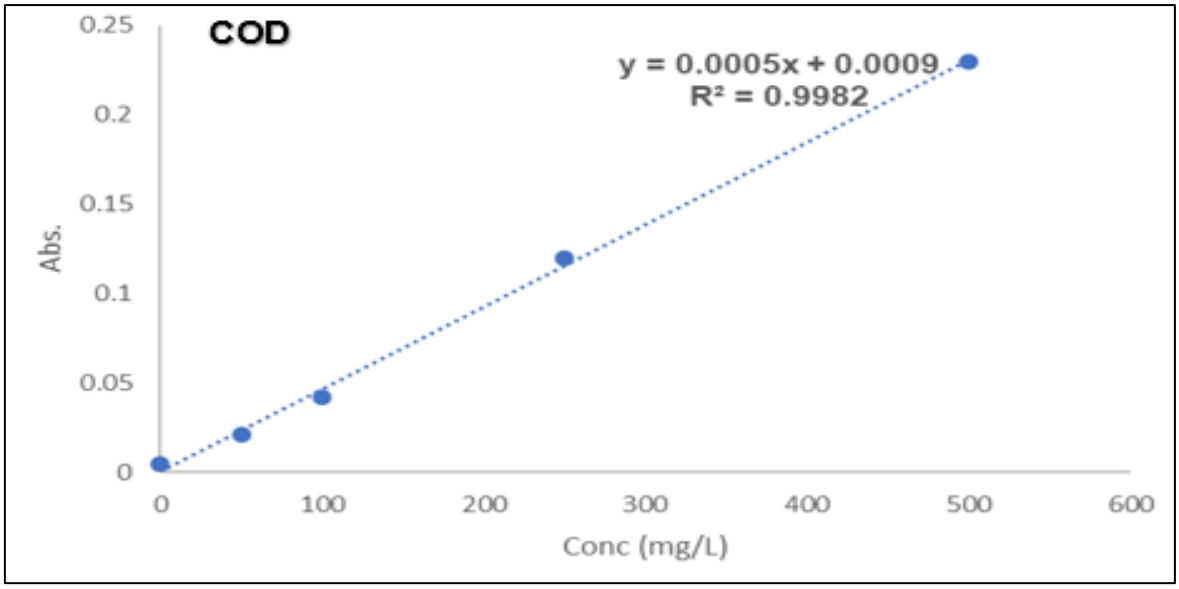
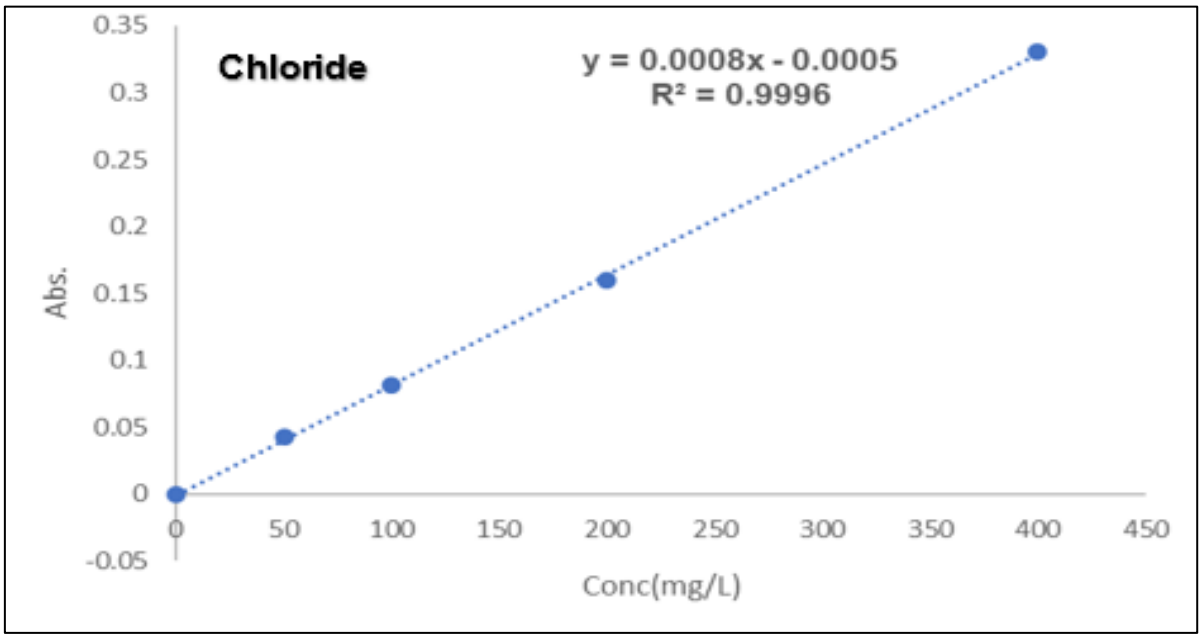
List of Appendices



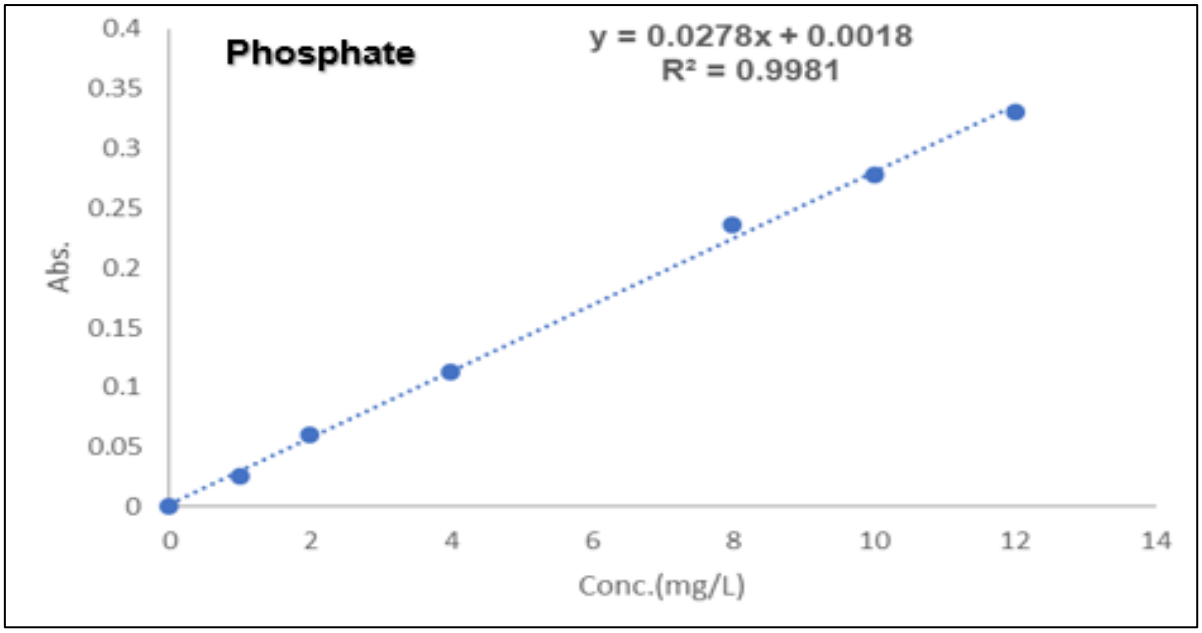
Appendix A: Standard calibration curve for sulphate.



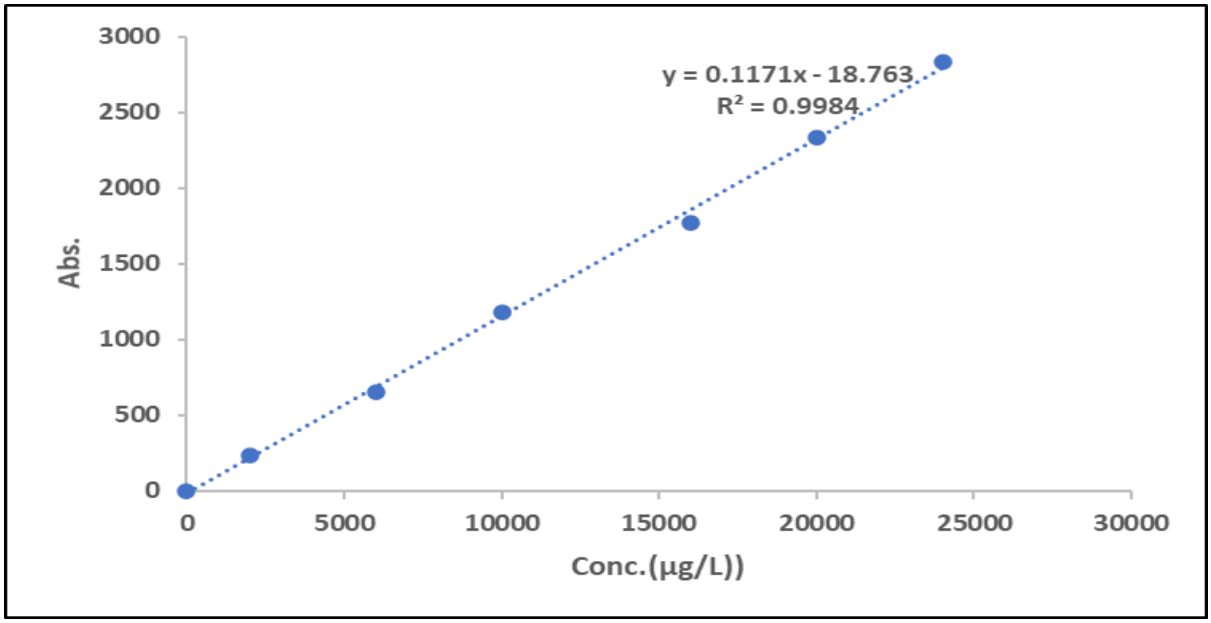
Appendix B: Standard calibration curve for COD.



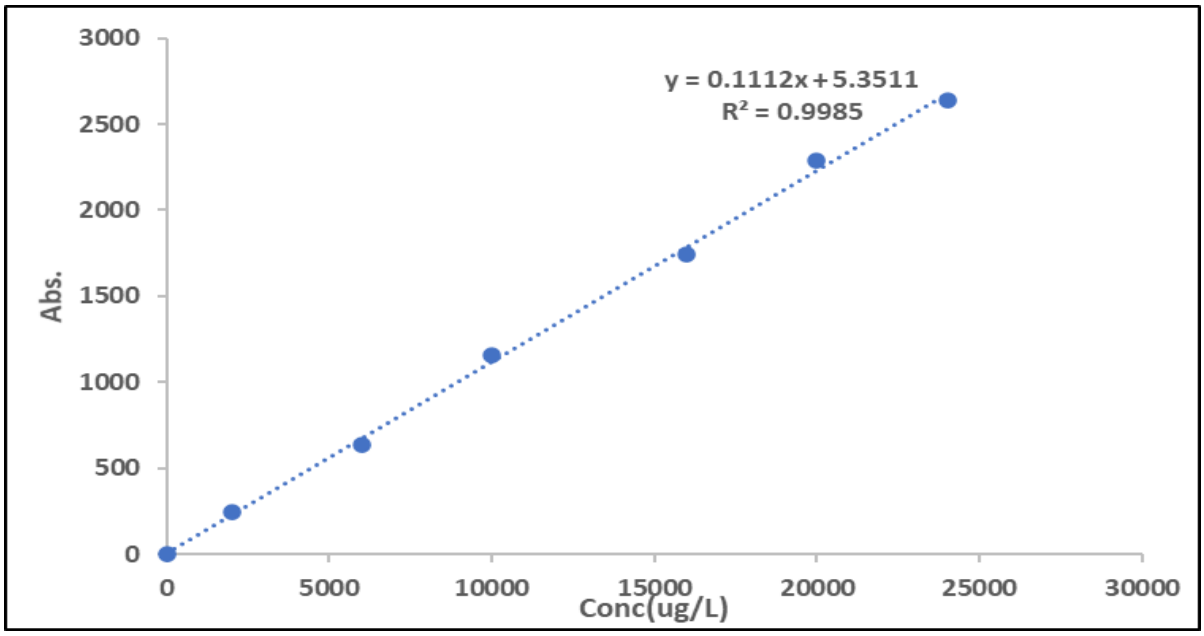
Appendix C: Standard calibration curve for chloride.



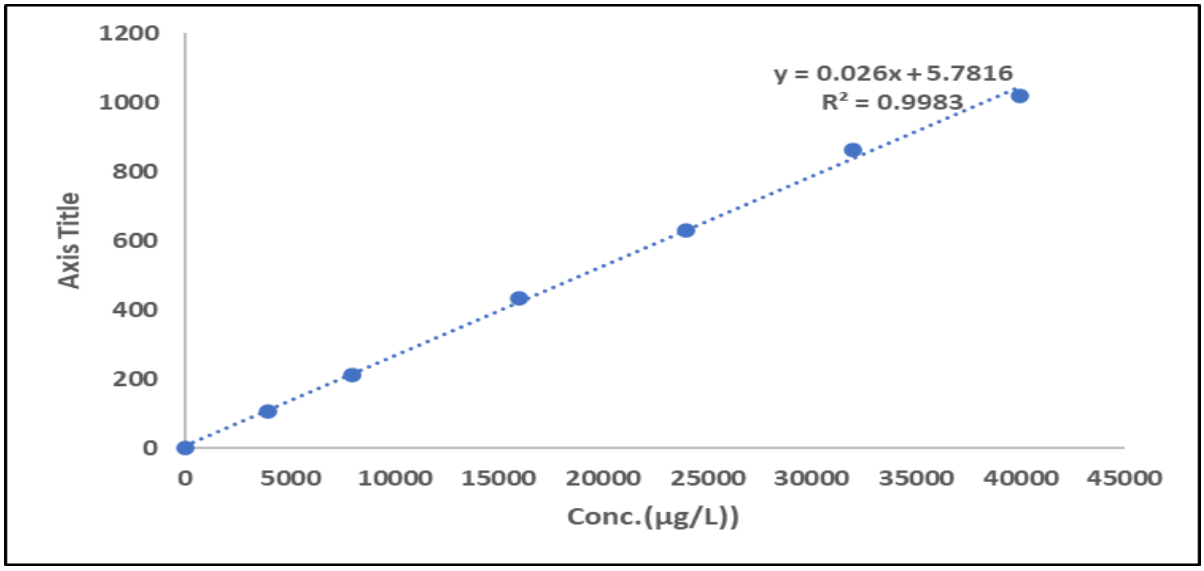
Appendix D: Standard calibration curve for phosphate.



Appendix E: Standard calibration curve for the 3TC (UV spectroscopic Method).



Appendix F: Standard calibration curve for the AZT (UV spectroscopic Method).



Appendix G: Standard calibration curve for NVP (UV spectroscopic Method).

Volume of ARV solution (mL)	Volume of buffer (mL)	Initial pH	Volume of $(Ca(ClO)_2)$ (mL)	Final pH
9.90	0.10	7.23	0.05	10.98
9.90	0.10	7.22	0.05	10.99
9.00	1.00	7.14	0.05	9.66
9.00	1.00	7.15	0.05	9.64
8.50	1.50	7.11	0.05	8.99
8.50	1.50	7.12	0.05	9.00
8.00	2.00	7.10	0.05	8.50
8.00	2.00	7.10	0.05	8.50
7.50	2.50	7.10	0.05	8.21
7.50	2.50	7.10	0.05	8.19
7.00	3.00	7.01	0.05	7.01
7.00	3.00	7.01	0.05	7.00
6.50	3.50	7.02	0.05	7.01
6.50	3.50	7.02	0.05	7.01
6.00	4.00	7.02	0.05	7.01
6.00	4.00	7.02	0.05	7.02
5.00	5.00	7.01	0.05	7.01
5.00	5.00	7.01	0.05	7.01

A. **Appendix H: Determination of optimum ARV – buffer solution ratio required to maintain specific pH for the degradation of three pharmaceutical formulations.**

B. **(The optimised ratio (7:3) is coloured red).**

Sample	Sample conc.(µg/L)		No of mole	$Ca(ClO)_2$ (20,000 µg/L)		$Na_2S_2SO_4$ (40,000 µg/L)	
	Before	After	in buffered	Volume added	No of mole	Volume added	No of mole
	Buffer (µg/L)	Buffer (µg/L)	Sample (µM)	(µL)	(µM)	(µL)	(µM)
1JR-Cl	9.72	6.80	27.22	476.00	190.51	476.00	381.02
1JB-Cl	14.48	10.14	40.54	710.00	283.78	710.00	567.56
1JA-Cl	8.68	6.08	24.30	425.00	170.13	425.00	340.26
2JE-Cl	34.45	24.11	96.45	1688.00	675.16	1688.00	1350.33
1SR-Cl	4.36	3.05	12.22	214.00	85.51	214.00	171.02
1SB-Cl	4.80	3.36	13.45	235.00	94.14	235.00	188.27
1SA-Cl	2.90	2.03	8.12	143.00	56.84	143.00	113.68
2SE-Cl	9.98	6.98	27.94	489.00	195.55	489.00	391.10
1NR-Cl	4.48	3.14	12.55	220.00	87.84	220.00	175.67
1NB-Cl	4.96	3.47	13.89	243.00	97.24	243.00	194.49
1NA-Cl	2.83	1.98	7.93	139.00	55.50	139.00	110.99
2NE-Cl	9.83	6.88	27.52	482.00	192.61	482.00	385.22
1MR-Cl	4.18	2.93	11.71	205.00	81.96	205.00	163.91
1MB-Cl	4.09	2.86	11.45	200.00	80.14	200.00	160.27
1MA-Cl	2.85	2.00	7.98	140.00	55.89	140.00	111.78
2ME-Cl	8.90	6.23	24.91	436.00	174.38	436.00	348.77

Appendix I: Quantity of $Ca(ClO)_2$ spiked into different concentrations of wastewater samples for degradation. (1= WWTP 1, 2 = WWTP 2, J = July 2018, S = September 2018, N = November 2018, M = March 2019, R= RI sample , B = BUVT sample, A = AUVT sample, E= FE sample).

Sample	Sample conc. (µg/L)		No of mole	$KMnO_4$ (20,000 µg/L)		$Na_2S_2SO_4$ (40,000 µg/L)	
	Before	After	in buffered	Volume added	No of mole	Volume added	No of mole
	Buffer (µg/L)	Buffer (µg/L)	Sample (µM)	(µL)	(µM)	(µL)	(µM)
1JR-K	9.72	6.80	27.22	476.00	190.51	476.00	381.02
1JB-K	14.48	10.14	40.54	710.00	283.78	710.00	567.56
1JA-K	8.68	6.08	24.30	425.00	170.13	425.00	340.26
2JE-K	34.45	24.11	96.45	1688.00	675.16	1688.00	1350.33
1SR-K	4.36	3.05	12.22	214.00	85.51	214.00	171.02
1SB-K	4.80	3.36	13.45	235.00	94.14	235.00	188.27
1SA-K	2.90	2.03	8.12	143.00	56.84	143.00	113.68
2SE-K	9.98	6.98	27.94	489.00	195.55	489.00	391.10
1NR-K	4.48	3.14	12.55	220.00	87.84	220.00	175.67
1NB-K	4.96	3.47	13.89	243.00	97.24	243.00	194.49
1NA-K	2.83	1.98	7.93	139.00	55.50	139.00	110.99
2NE-K	9.83	6.88	27.52	482.00	192.61	482.00	385.22
1MR-K	4.18	2.93	11.71	205.00	81.96	205.00	163.91
1MB-K	4.09	2.86	11.45	200.00	80.14	200.00	160.27
1MA-K	2.85	2.00	7.98	140.00	55.89	140.00	111.78
2ME-K	8.90	6.23	24.91	436.00	174.38	436.00	348.77

Appendix J: Quantity of $KMnO_4$ spiked into different concentrations of wastewater samples for degradation. . (1= WWTP 1, 2 = WWTP 2, J = July 2018, S = September 2018, N = November 2018, M = March 2019, R= RI sample , B = BUVT sample, A = AUVT sample, E= FE sample).

The percentage removal of three pharmaceutical products from wastewater samples by sunlight (UV)/Cl_2												
Dates	**WWTP 1 (RI)**			**WWTP 1 (BUVT)**			**WWTP 1 (AUVT)**			**WWTP 2 (FE)**		
	3TC (sd)	**AZT (sd)**	**NVP (sd)**	**3TC (sd)**	**AZT (sd)**	**NVP (sd)**	**3TC (sd)**	**AZT (sd)**	**NVP (sd)**	**3TC (sd)**	**AZT (sd)**	**NVP (**
Jul. 2018	15.66 (0.80)	63.27 (1.67)	23.37 (5.09)	20.51 (19.01)	80.08 (12.38)	39.08 (28.58)	38.59 (4.22)	70.64 (4.06)	55.27 (16.06)	46.48 (2.5)	60.08 (4.95)	49.39 (2
Sep. 2018	7.32 (6.75)	42.77 (11.05)	18.31 (11.17)	7.67 (1.55)	29.64 (8.36)	15.60 (7.42)	11.01 (0.84)	42.18 (4.46)	37.43 (15.01)	54.59 (1.54)	64.25 (10.87)	18.85 (7
Nov. 2018	20.98 (1.67)	73.23 (18.17)	16.30 (5.44)	55.50 (2.86)	79.59 (2.55)	62.46 (0.68)	44.42 (4.55)	57.87 (1.62)	57.81 (2.4)	18.00 (5.47)	50.68 (4.73)	16.60 (1
Mar. 2019	70.79 (0.77)	77.75 (2.79)	63.55 (0.57)	60.01 (22.95)	61.71 (3.85)	41.28 (1.77)	33.16 (12.96)	55.96 (1.78)	67.77 (0.77)	41.30 (0.74)	91.69 (2.93)	61.88 (2

Appendix K: Result of % removal of three pharmaceutical products from wastewater samples by sunlight (UV)/Cl_2.

The percentage removal of three pharmaceutical products from wastewater samples by sunlight (UV)/$KMnO_4$												
Dates	**WWTP 1 (RI)**			**WWTP 1 (BUVT)**			**WWTP 1 (AUVT)**			**WWTP 2 (FE)**		
	3TC (sd)	**AZT (sd)**	**NVP (sd)**	**3TC (sd)**	**AZT (sd)**	**NVP (sd)**	**3TC (sd)**	**AZT (sd)**	**NVP (sd)**	**3TC (sd)**	**AZT (sd)**	**NVP (sd)**
Jul. 2018	7.72 (0.96)	8.08(1.51)	25.52(5.53)	8.35(2.23)	72.16(0.68)	62.37(4.11)	37.44(3.45)	78.99(0.56)	55.15(11.96)	2.70(2.41)	48.62(0.69)	23.34(2.83)
Sep. 2018	34.06(2.61)	6.48(0.46)	20.35(0.18)	22.63(0.94)	79.21(0.75)	48.55(7.41)	39.84(0.14)	49.66(19.83)	22.97(0.43)	20.69(13.27)	22.29(6.33)	30.18(0.57)
Nov. 2018	16.20(3.32)	9.25(0.12)	53.76(11.73)	73.60(2.75)	88.21(0.14)	59.18(7.57)	26.17(2.64)	59.18(10.63)	44.76(13.40)	60.62(8.50)	54.35(5.56)	27.84(0.13)
Mar. 2019	100.00(0.00)	100(0.00)	22.81(6.17)	100.00(0.00)	100.00(0.00)	15.41(1.52)	100.00(0.00)	100.00(0.00)	100.00(0.00)	100.00(00)	100.00(0.00)	44.95(1.02)

Appendix L: Result of % removal of three pharmaceutical products from wastewater samples by sunlight (UV)/$KMnO_4$.

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ORIGINAL PAPER

Physicochemical properties of wastewater effluent from two selected wastewater treatment plants (Cape Town) for water quality improvement

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Abstract

Effluent from wastewater treatment plants (WWTPs) is one of the main sources of pollution that seriously affect the physicochemical parameters in water bodies. This occurs particularly when partially treated or untreated effluent is released to various aquatic ecosystems. This constitutes a potential danger to the general environment. To safeguard the lives of human, aquatic and other living organisms, regular monitoring of wastewater effluent to ascertain their compliance with both national and international regulatory limits for water quality improvement is very crucial. This research work was carried out using standard methods to evaluate ten physicochemical parameters from the effluent of two WWTPs in Cape Town, South Africa. Our findings were: temperatures (16.90–25.33 °C), pH (5.85–7.85), EC (923.00–1294.17 µS/cm), TDS (590.72–828.27 mg/L), DO (1.30–5.50 mg/L), SO_4^{2-} (3.23–163.18 mg/L), COD (23.70–898.58 mg/L), BOD (9.17–252.44 mg/L), Cl^- (48.17–378.48 mg/L) and PO_4^{3-} (0.10–11.32 mg/L). Temperature, pH and SO_4^{2-} levels in the two WWTPs were within the recommended limit for wastewater discharge by the Department of Water Affairs and Forestry (DWAF), South Africa and the United State Environmental Protection Agency (USEPA). However, other parameters did not comply fully with both DWAF and USEPA standards. The result showed that effluents from these WWTPs are possible sources of pollution to the receiving water bodies and calls for enhancement of the WWTPs operations to ensure safe effluent discharge.

Keywords Physicochemical analysis · Wastewater treatment plants · Wastewater effluent · Permissible limit

Introduction

Human activities are not static, and the contaminations of the environment (water, air and soil) globally are the results of increased population and various anthropogenic activities such as agriculture, industrialization, urbanization, change in the pattern of life and climate change (Olujimi et al. 2012; Ullah et al. 2018). Majority of the results or products of these contamination go to the wastewater treatment plants (WWTPs) and consequently have a great impact on the physicochemical properties of wastewater (Paltahe et al. 2018). Wastewater treatment plants (WWTPs) have therefore been identified as one of the main sources of variation in the physicochemical parameters of the aquatic ecosystem due to the discharge of partially treated effluent containing different hazardous materials into the receiving water bodies (Fatta-Kassinos et al. 2011; Paltahe et al. 2018).

Wastewater is generated via different sources including homes, industries, farmland and other usage points (Koopaei and Abdollahi 2017). Therefore, it encompasses various organic and inorganic compounds as well as pathogenic microorganisms (Lokhande et al. 2011; Rana et al. 2017). These constitute potential dangers to the natural water system and hence becomes hazardous to life (Lokhande et al. 2011; Koopaei and Abdollahi 2017). The continuous pollution of the aquatic environment with wastewater has a detrimental impact on health and the environment (Lokhande et al. 2011; Rana et al. 2017; Koopaei and Abdollahi 2017).

The challenge of water scarcity arising as a result of reduction in the quantity and quality of freshwater resources available to man as well as the ecosystems is a global phenomenon (Abbott et al. 2019). Globally, 2.1 billion people

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Appendix M: Published article 1.

3

Advances in Chromatographic Determination of Selected Anti-Retrovirals in Wastewater

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Abstract

Rising market and technological society using a continuously growing number of man-made chemicals have expanded the coast of pollutants beyond industrial or agricultural chemicals. Emerging pollutants (EPs) are defined as substances that have been detected, which is not included in the routine monitoring programs at the EU level, and whose fate, behavior, and ecotoxicity effects are not well understood. EPs cover a wide range of man-made chemicals which are in use worldwide and 'indispensable' for modern society such as pharmaceuticals used in human and veterinary medicines. Eps occur in the different part of the world such as Europe, United nation, France, China, and Africa. Common detection methods from the literature include SPE-LC-TOF-MS, HR-MS, LC–MS/MS, and GC-HR-MS. EPs scattered into the environment through various anthropogenic activities, dispersed throughout environmental matrices resulting in constituting an array of contaminants into the aquatic ecosystem and finally detrimental to human health because life is interdependent.

Keywords: Anti-retrovirals, emerging pollutant extraction, chromatography, wastewater

3.1 Introduction

In the past, industrial and agricultural chemicals are considered as major sources of pollutants in the environment. However, advances in environmental

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Appendix N: Published book chapter.