Analysis of Targeted Pharmaceuticals and Metabolites at a Wastewater Treatment Plant in KwaZulu-Natal, South Africa

by

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PREFACE

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

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

_____

Signed: Professor Brenda Moodley

Date: November 2022

DECLARATION 1: PLAGIARISM

I, Nikitha Inarmal, declare that:

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

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

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

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

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

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

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

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

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

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CONFERENCES AND OTHER PARTICIPATIONS

(a) Oral presentation at the School of Chemistry and Physics postgraduate seminar session, 17 September 2021.

(b) Flash presentation at UKZN's College of Agriculture, Engineering and Science Postgraduate Research Innovation Symposium, 2nd place in the School of Chemistry and Physics, 10 December 2021.

(c) Oral presentation to Umgeni Water, 31 March 2022.

(d) Oral presentation at the School of Chemistry and Physics postgraduate seminar session, 12 October 2022.

(e) Oral presentation at the South African Chemical Institute's Postgraduate Symposium, placed 1st within the masters category, 10 November 2022.

(f) Flash presentation at UKZN's College of Agriculture, Engineering and Science Postgraduate Research Innovation Symposium, 3rd place in the School of Chemistry and Physics, 9 December 2022.

(g) Manuscript titled "Selected Pharmaceutical Analysis in a Wastewater Treatment Plant during COVID-19 Infection Waves in South Africa" accepted for publication in the Royal Society of Chemistry's "Environmental Science: Water Research & Technology" journal, 27th March 2023.

Signed: Nikitha Inarmal

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ABSTRACT

Globally, surface water bodies are shown to be severely contaminated by chemical compounds as a result of human activity. Some of these compounds are inclusive of pharmaceuticals and their metabolites which are classified as emerging contaminants (ECs). A major contributor to this pollution event is treated wastewater effluent. This is an area of growing concern, particularly for emerging economies, due to an increase in demand for clean and potable water arising from rapid urbanisation and significant population growth. In South Africa, there is limited information outlining concentrations of various pharmaceuticals in treated wastewater effluent and surface water bodies and there is currently no legislation indicating maximum concentrations to be allowed in wastewater effluent. This research study aimed to identify and quantify concentrations of selected pharmaceuticals and metabolites present at various stages within the Wastewater Treatment Plant (WWTP). Additionally, the study aimed to determine seasonal pharmaceutical trends in relation to SARS-CoV-2 (COVID-19) and influenza infections, removal efficiencies and undertake an environmental risk assessment (ERA). The pharmaceuticals that were analysed included metformin, caffeine, sulfamethoxazole hydroxylamine, sulfamethoxazole, nevirapine, prednisolone, valsartan, rifampicin, 17α-ethinylestradiol (EE2) and ivermectin, all of which being pharmaceuticals that are commonly prescribed for the treatment of medical conditions that are prevalent in South Africa and for the treatment and prevention of COVID-19. Analyte concentrations were assessed in wastewater samples taken from the inlet, balancing tank, secondary effluent, and maturation river over three sampling seasons. Analytes were extracted using a solid phase extraction (SPE) method and extracts were analysed using liquid chromatography – mass spectrometry (LC – MS). Method percentage recoveries ranged from 73.53 – 100.70% while limit of detection (LOD) and limit of quantification (LOQ) overall ranged from 0.0330 – 0.886 mg L^{-1} and 0.0990 – 2.68 mg L^{-1}, respectively. Analyte concentrations quantified in inlet samples ranged from 0.007587 – 1.243 mg L^{-1} while balancing tank concentrations ranged from 0.007599 – 1.042 mg L^{-1}. Secondary effluent and maturation river concentrations ranged from 0.0005244 – 0.9483 mg L^{-1} and 0.0002507 – 0.9286 mg L^{-1}, respectively. Non-detection of certain analytes in some samples could imply the possibility that the pharmaceutical converted into transformation products and thus evaded detection. Influent pharmaceutical concentrations showed distinct seasonal variation, and this was statistically shown using a one-way ANOVA analysis. P-values were recorded as < 0.05 for caffeine, sulfamethoxazole hydroxylamine, sulfamethoxazole, EE2 and ivermectin, thus implying that significant differences were observed between seasonal levels of

pharmaceutical compounds and in relation to the COVID-19 and influenza infection peaks. Analyte removal efficiencies based on the plant treatment processes ranged from 7.70 – > 99.99 %. The ERA performed indicated ratios within the range of 0.0863 and 10.5x 10^{8}, which is of significant concern as values greater than 1 would severely impact the health of aquatic organisms and ecosystems within the receiving surface water body.

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To my parents, mom and dad – words will never do justice to the love and appreciation I hold for you in my heart. Thank you for being my pillars in life. Thank you for your support, guidance, countless sacrifices, for your endless love and patience. Thank you for giving me the greatest gift of all, my education. Dad, although you never got to read my thesis, you are in every page. To my sister, I love and miss you dearly. Thank you for being my guardian angel through it all. My fiancé, Ashael Ramiah, you are one of my greatest blessings. Thank you for always pushing me to do my best, for having unwavering faith and believing in me when I could not believe in myself.

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To the analytical technical staff, Mrs Thiloshini (Anita) Naidoo and Ms Unathi Bongoza, I am eternally grateful for all your help, motivation and advice.

Thank you to Umgeni Water for their financial assistance for this research project. I would also like to extend my immense gratitude to the staff at the Wastewater Treatment Plant for their kind assistance during my sampling trips.

DEDICATION

To my father, the brightest star in the sky.

Sunil Lakhichand Inarmal
1963 – 2021

All that I am, I owe to you.

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ARBs	Angiotensin II receptor blockers
ARV	Antiretroviral
CAF	Caffeine
COVID-19	SARS-CoV-2
DWS	Department of Water and Sanitation
ECs	Emerging contaminants
EDCs	Endocrine disrupting compounds
EE2	17α-ethinylestradiol
EFF	Effluent
ERA	Environmental risk assessment
FR	Flow rate
HIV	Human immunodeficiency virus
HPLC	High pressure liquid chromatography
INF	Influent
IS	Internal standard
IVE	Ivermectin
LC	Liquid chromatography
LC – MS	Liquid chromatography – mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MEC	Measured environmental concentration
MET	Metformin
ML	Mass load
MS	Mass spectrometry
NEV	Nevirapine
OTC	Over the counter
PNEC	Predicted no effect concentration
PRED	Prednisolone
RdRp	Ribonucleic acid dependent ribonucleic acid polymerase

RIF	Rifampicin
RNA	Ribonucleic acid
RQ	Risk quotient
RSD	Relative standard deviation
SAHPRA	South African Health Products Regulatory Authority
SMX	Sulfamethoxazole
SMX N-OH	Sulfamethoxazole hydroxylamine
SPE	Solid phase extraction
TB	Tuberculosis
UK	United Kingdom
UV	Ultraviolet
VAL	Valsartan
WHO	World Health Organisation
WWTPs	Wastewater treatment plants

CHAPTER 1: INTRODUCTION

1.1 General Overview

South Africa has a world class and diverse economy that encompasses first world infrastructure prevailing alongside widespread poverty (Peltzer *et al*., 2010). South Africa has by far the biggest market for pharmaceuticals within the region of Africa (Peltzer *et al*., 2010, Department of Trade and Industry, 2017). Pharmaceuticals are chemical compounds that are synthesised for the prevention, treatment, or cure of various diseases or for the restoration, correction, or modification of organic functions within the human body (Bavumiragira *et al.,* 2022). They are an integral part of everyday life for many South Africans. They are the most frequently used medical treatment and their potential for both aid and harm is immense (Institute of Medicine, 2007). Globally, well over 3 000 chemical compounds have been approved by several regulatory bodies as constituents of pharmaceutical products (Löffler and Ternes, 2003).

In recent times, there has been growing concern in lieu of the occurrence of emerging contaminants (ECs) in the environment. ECs refer to synthetic compounds that are detected in the environment and have harmful effects on living organisms due to prolonged exposure over long periods of time (Moodley *et al*., 2016). These compounds are inclusive of pharmaceuticals and personal care products (Li *et al*., 2016). Pharmaceuticals are not completely metabolised by the human body and are excreted in sewage either unchanged or alternatively as metabolites. Some metabolites have potent psychoactive properties that have unknown effects on the aquatic environment due to limited studies on them (Cosenza *et al.,* 2018). Sewage containing pharmaceuticals and their metabolites, amongst other contaminants, is then transported to wastewater treatment plants (WWTPs) for further treatment *via* a network of municipal sewage systems. Wastewater treatment refers to the process of removing organic, inorganic, and biological matter from a waste stream thus making it suitable for release back into the environment. There are two basic stages in wastewater treatment namely, primary, and secondary treatments. During the primary stage of treatment, solids potentially settle and are filtered out of the wastewater (EPA, 1998). Primary treatment alone is unable to meet communities' requirements for better water quality therefore, secondary treatment is employed. Conventional WWTPs utilise biological processes as a secondary treatment to further purify

wastewater. This stage removes approximately 85% of nutrients and organic matter from wastewater using bacteria (EPA, 1998).

The interest displayed in the amount of pharmaceuticals discharged into the environment is resultant of two factors: i) the increased use of these compounds and ii) the potential environmental impact that they may have (Cosenza *et al.*, 2018). Prior studies have detected numerous pharmaceuticals and their metabolites present in wastewater effluent because of the inability of conventional WWTP processes to remove them (Evgenidou *et al.,* 2015). A favourable characteristic of pharmaceuticals is their bioactive nature in the human body to aid with the healing of various ailments. However, they resist degradation in the environment, and this ultimately leads to their persistence (Mastrup *et al*., 2005).

According to Gracia-Lor *et al*., (2012), wastewater from urban areas is presently deemed the largest source of pharmaceutical compounds found in the aquatic environment. WWTPs were originally designed to remove organic pollutants in the form of organic matter and nutrients, to name a few, but not complex pharmaceutical compounds (Gracia-Lor *et al*., 2012). Additionally, the SARS-CoV-2 (COVID-19) outbreak impacted the globe in 2020. The outbreak was classified as a global pandemic by the World Health Organisation (WHO). COVID-19 is an extremely contagious respiratory virus that has led to the loss of millions of lives since the outbreak started to present. The incidence of the virus resulted in a significant increase in the use and administration of pharmaceuticals with the aim of preventing and treating infection. Ultimately, if not removed, these compounds can reach surface water, groundwater and in due course, drinking water (Petrovic *et al.,* 2009). One of the contributing factors to the inefficient removal is the rapid increase in the population, leading to more effluent being sent to WWTPs which forces these facilities to operate beyond their working capacity (Archer, 2018). Another proposed explanation is insufficient human resources for the operation and maintenance of the treatment plants. Monitoring the concentrations of pharmaceuticals and their metabolites in WWTPs enables: i) an increase in knowledge of the amount of these compounds discharged into the environment; ii) an estimation of the effects they may have in aquatic environments and iii) an educated assessment of the removal efficiency of these compounds by the processes currently employed in the treatment plant (Cosenza *et al*., 2018).

In 2008, the South African Department of Water and Sanitation (DWS) launched a Green Drop Programme to assess the performance of WWTPs around the country. The Green Drop

Programme is an incentive-based mechanism that intends on providing rewards for compliance with regards to the treatment of wastewater, thus refining the quality of wastewater management in South Africa (Ntombela *et al*., 2016). An operating WWTP receives Green Drop status if they comply with specific criteria for effective wastewater treatment, as set out by the DWS. The criteria included: having professionally trained and skilled workers in operations, maintenance and management positions, wastewater sample analysis and submission of these results and effluent quality compliance, just to name a few. According to DWS, in 2013 there were 824 wastewater treatment plants located throughout the country. However, only 60 of those plants received Green Drop awards (Ntombela *et al.,* 2016). The importance of effectively functioning WWTPs is paramount to the health of humans, the environment and water security in a water scarce country like South Africa.

Based on the statistics of the Green Drop Programme, more than 49% of WWTPs are functioning at a poor and critical state. This can be enhanced by the periodic testing of water samples from the WWTP to establish effluent quality compliance. Analytes present in wastewater that are of concern are pharmaceuticals due to their persistence and their potential risk to the environment. Pharmaceuticals that will be focused on in this research study includes metformin, caffeine, sulfamethoxazole hydroxylamine, sulfamethoxazole, nevirapine, prednisolone, valsartan, rifampicin, 17α-ethinylestradiol (EE2) and ivermectin. These compounds were selected due to their high number of prescriptions, known abuse and threat to the environment.

1.2 Rationale

Wastewater treatment from urban areas has become an increasingly prioritised segment of water and sanitation to reduce the input of pharmaceutical compounds into the aquatic environment. The implications of poor-quality wastewater being discharged back into the environment are dire as it affects the health of aquatic ecosystems and human health, alike. Research on the identification and quantification of pharmaceuticals and their concentrations in wastewater is therefore of significant importance in order to assess removal efficiencies of constituent analytes.

1.3 Aim

This research study aims to identify and quantify concentrations of pharmaceuticals; metformin, caffeine, sulfamethoxazole hydroxylamine, sulfamethoxazole, nevirapine, prednisolone, valsartan, rifampicin, 17α-ethinylestradiol (EE2) and ivermectin, present in a wastewater treatment plant situated in KwaZulu-Natal, South Africa.

1.4 Objectives

1.4.1. Develop and validate a method for extraction, clean-up, qualitative and quantitative analysis of pharmaceuticals and their respective metabolites present in wastewater samples.
1.4.2. Analyse for selected analytes *via* the use of Liquid Chromatography – Mass Spectrometry (LC – MS).
1.4.3. Investigate seasonal trends of pharmaceutical usage.
1.4.4. Establish the overall removal efficiency of analytes based on the current removal process within the WWTP, and
1.4.5. Estimate potential environmental effects that the effluent may have on aquatic ecosystems through environmental risk assessment (ERA).

1.5 Hypothesis

Analytes of interest should have highest concentrations within inlet samples and a significant reduction should be observed in effluent samples, ensuring that processes employed within the treatment plant are effective at removing these contaminants. Furthermore, concentrations of antibiotics and immune support medication should be lowering at the end of KwaZulu-Natal's projected fourth wave SARS-CoV-2 (COVID-19) peak in February 2022 and highest at the start of the projected fifth wave peak in April 2022. In addition, concentrations quantified in the rainy season should be lower than those found in the dry season/s.

1.6 Research Scope

The analysis of pharmaceutical and selected metabolites is centred around the Pietermaritzburg area in KwaZulu-Natal, South Africa, which is the second most densely populated province

found in South Africa. The WWTP discharges effluent into a nearby river. This river acts as a major source of water for domestic, agricultural, and industrial uses within the province.

Wastewater samples were collected from the inlet, balancing tank, secondary effluent, and maturation river. Studies that comprised the analysis of pharmaceuticals have previously been conducted in South Africa, but there are no present publications concerning concentrations of ivermectin and sulfamethoxazole hydroxylamine in wastewater. The analyses presented in this work will provide vital information for Umgeni Water as well as further research in this field of study.

1.7 Outline of Dissertation

Each chapter focuses on the discussion of wastewater treatment, pharmaceuticals, and respective metabolites. The dissertation is presented in a manuscript approach. The details of each chapter is outlined below.

Chapter 1 outlines a general overview to pharmaceuticals, selected metabolites, and wastewater treatment. Additionally, the rationale and aim and objectives for the research are also provided.

Chapter 2 provides a literature review on pharmaceuticals and metabolites found in wastewater, wastewater treatment, as well as a discussion of the extraction method and instrumentation used in this study.

Chapter 3 is centred around the materials and methods used for the extraction and analysis of targeted analytes. This includes method development and validation parameters.

Chapter 4 details results obtained during the fourth and fifth COVID-19 waves observed in KwaZulu-Natal, simultaneously occurring during our wet and dry seasons, respectively.

Chapter 5 outlines results detailing removal efficiencies and an environmental risk assessment.

Chapter 6 provides conclusions, challenges, and future work.

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CHAPTER 2: LITERATURE REVIEW

Water is an indispensable resource that plays a vital role in the human body. Living beings can survive several weeks without food, but merely a few days without water. South Africa is classified as a semi-arid country that receives low volumes of rainfall, averaged at 500 mm per annum and high evaporation which is averaged at 1700 mm per annum (Eberhard and Robinson, 2003). These factors therefore give rise to water scarcity that is experienced within the country.

Water is utilised by small- and large-scale farmers, industrial users, municipal water users and individuals that utilise water resources for leisure or tourism purposes (Berger *et al*., 2007). Water resources are therefore allocated to irrigation, potable water, industrial production, environmental facilities, and recreation (Berger *et al*., 2007). Within KwaZulu-Natal, Umgeni Water provides bulk potable water to the municipality. Through social development and the radical increase in the global population, water consumption has increased far beyond sustainable levels in countries around the world (Chen *et al*., 2013). Droughts, depletion of groundwater, the deterioration of water quality and climate change makes the present water crisis even worse. Herein lies the need for alternative water sources such as the recycling of wastewater and desalination of sea water.

Many countries do not have separate sewage collection pipes which result in greywater (wastewater from households, excluding toilets), blackwater (wastewater from toilets), industrial, medical, and commercial wastewaters being discharged into the same sewage system. Consequently, municipal wastewater contains an extensive spectrum of chemical pollutants such as pharmaceuticals, as well as organic matter, inorganic particles, and pathogens – each of which are of probable risk to the environment and subsequently human health (Chen *et al*., 2013).

Pharmaceuticals and their metabolites are becoming of scientific and public concern as a new class of environmental pollutants due to their unknown environmental fate and toxicological effects on organisms. These ECs are of interest due to their frequent use, persistence and potential risk to aquatic organisms and humans alike. Previous studies are indicative of mentioned ECs persisting through wastewater treatment processes and ultimately being discharged into the environment (Evgenidou *et al.*, 2015).

2.1 Pharmaceuticals

Pharmaceuticals are an integral part of everyday life for many South Africans. They are the most frequently used medical treatment and their potential for both aid and harm is immense (Institute of Medicine, 2007). Pharmaceutical consumption is increasing rapidly throughout the world (Gracia-Lor *et al*., 2012). The South African pharmaceutical sector is the largest in Africa (Department of Trade and Industry, 2017). An estimated R 48.6 billion was the output for the pharmaceutical industry in 2017, which encompassed both the public and private sectors (Antonie *et al*., 2018). In South Africa, over the counter (OTC) medication can be obtained from pharmacies, government clinics, informal convenience stores in townships and general stores in malls and shopping centres (Bennin and Rother, 2015). OTC medication obtained from these stores often provide limited information pertaining to side effects and overdose (Bennin and Rother, 2015). Endocrine disrupting compounds (EDCs) can interfere with the normal functioning of the endocrine system in humans and wildlife (Aris *et al*., 2014). EDCs can affect the endocrine system by mimicking the effects of specific hormones or disrupting the synthesis and metabolism of those hormones (Aris *et al*., 2014). Pharmaceuticals are not completely metabolised by the human body and enter sewage either unchanged or as metabolites (Li *et al.*, 2016). Wastewater treatment plants were initially designed to remove inorganic and organic pollutants such as dissolved organic matter, solids, and nutrients, but not pharmaceuticals or their metabolites (Gracia-Lor *et al*., 2012).

The physical and chemical properties of the pharmaceuticals and their respective metabolites selected for this study are shown in Table 2.1. Solubility is expressed at 25 ºC.

Table 2.1: Physical and chemical properties of selected pharmaceuticals and their metabolites focused on in this study

Class	Compound	CAS number	Molecular formula[a]	Molar mass[a]/ g mol^{-1}	Melting point[a]/^{o}C	Boiling point[b]/^{o}C	Solubility[a]/mg mL^{-1}
Endocrine disruptors	17α-ethinylestradiol	57-63-6	$C_{20}H_{24}O_2$	296.40	143.0	457.2	0.0113
	Prednisolone	53-03-2	$C_{21}H_{28}O_5$	360.44	235.0	570.6	0.223
Diabetes	Metformin	657-24-9	$C_4H_{11}N_5$	129.16	224.5	172.5	1.38
Hypertension	Valsartan	137862-53-4	$C_{24}H_{29}N_5O_3$	435.52	116.5	85.5	0.00141
Antiretroviral	Nevirapine	129618-40-2	$C_{15}H_{14}N_4O$	266.30	248.0	415.4	0.00071
Tuberculosis	Rifampicin	13292-46-1	$C_{43}H_{58}N_4O_{12}$	822.94	185.5	937.0	1.30
Stimulant	Caffeine	58-08-2	$C_8H_{10}N_4O_2$	194.19	236.5	178.0	16
Antibiotic	Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253.28	169.0	482.1	0.50
	Sulfamethoxazole hydroxylamine	114438-33-4	$C_{10}H_{11}N_3O_4S$	269.28	130.0	502.2	-
Anthelmintic	Ivermectin	70288-86-7	$C_{47}H_{72}O14$	875.10	155.0	940.4	0.004

[a](PubChem), [b](ChemSpider), -: No data available.

A key facilitator in the freedom of women over the past few decades was the development of the contraceptive pill (Mastrup *et al*., 2005). Currently, a widely used contraceptive is 17α-ethynylestradiol, also known as EE2 (Mastrup *et al*., 2005). EE2, shown in Figure 2.1, is a synthetic estrogen derived compound that is used in all modern contraceptive pills and as a result, is one of the most frequently used medications (Mastrup *et al*., 2005, Aris *et al*., 2014). Due to its recurrent use, it has become a problem in aquatic environments because of its persistence and its ability to accumulate and concentrate in biota. In the environment, EE2 has the potential to alter sex determination and delay sexual maturity of exposed biota, even at ng L^{-1} levels (Aris *et al*., 2014). Incomplete absorption of these contraceptives in the human body leads to a significant proportion of these compounds being excreted (Mastrup *et al*., 2005). A major metabolite of EE2 is 2-hydroxyethinylestradiol. Previous studies carried out by Barr *et al*., 2012, indicated that the hydroxylation of the estrogen EE2 resulted in a decrease in estrogenic activity. This implies that their effect on the environment and organisms as an endocrine disruptor would not be as detrimental as those with EE2. For this reason, only the parent compound will be focused on in this study.

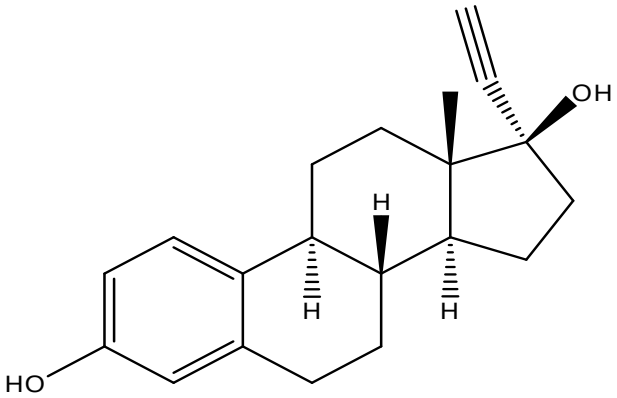

Figure 2.1: Chemical structure of EE2

Prednisone, as shown in Figure 2.2(a), is a prescription glucocorticoid drug that is used as an anti-inflammatory and as an immunosuppressant (Chang *et al*., 2007). Prednisone is a prodrug to its metabolite, prednisolone. A prodrug is one that must undergo complete hepatic metabolism to become pharmaceutically active (Puckett *et al*., 2019). The chemical structure of prednisolone is shown in Figure 2.2(b). Side effects as a result of the use of prednisone may include acne, insomnia, hypertension and increased risk of infections (Puckett *et al*., 2019).

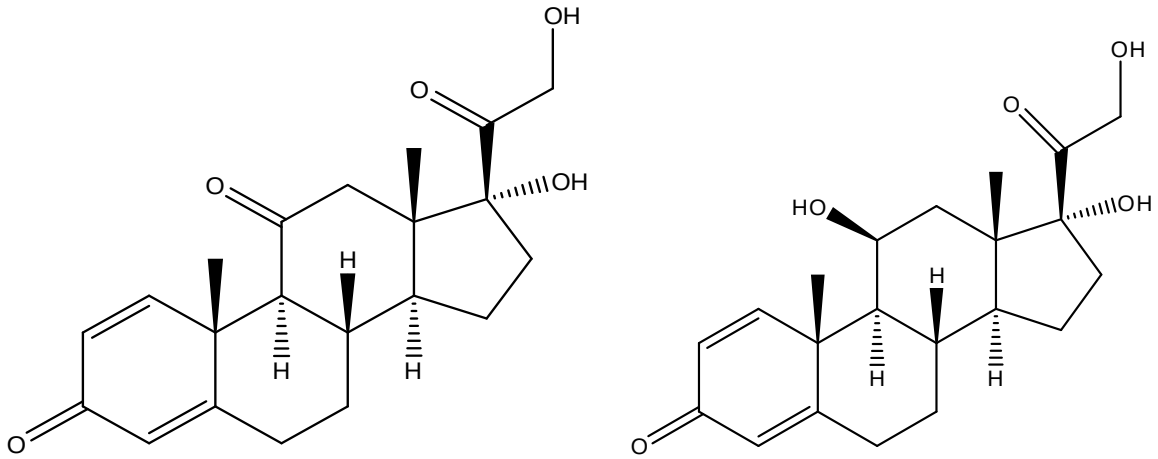

Figure 2.2: Chemical structure of (a) prednisone and (b) prednisolone

Diabetes mellitus is a metabolic disease arising from defects in insulin secretion or action (Kharroubi, 2015). There are two types of diabetes, Type 1 and Type 2. More than 90% of diabetes patients are diagnosed with Type 2 (Kharroubi, 2015). Metformin, Figure 2.3, is the first-line treatment for Type 2 diabetes. It works by decreasing the amount of sugar that is produced by the liver and the amount that is absorbed by the stomach or intestines (Nasri and Rafieian-Kopaei, 2014). Metformin has been observed to reduce diabetes related mortality and complications by 30% when compared to insulin (Nasri and Rafieian-Kopaei, 2014). Metformin is not metabolised within the human body and is excreted unchanged in urine (Gong *et al*., 2012).

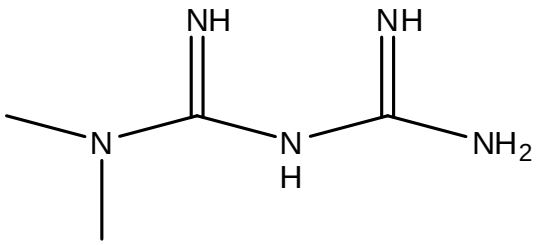

Figure 2.3: Chemical structure of metformin

Presently, hypertension affects roughly 1.13 billion people worldwide (WHO, 2019). It is the most seen condition in South Africa (Schellack and Malan, 2014). Hypertension is a key risk factor for the development of cardiovascular diseases and stroke. Valsartan, shown in Figure 2.4, belongs to the angiotensin II receptor blockers (ARBs) class of antihypertensive drugs. It works by constricting blood vessels to reduce blood pressure (Nixon *et al*., 2009). ARBs are

one of the first-line antihypertensive drugs available for treatment of hypertension. A study undertaken on one pharmaceutical group in Gauteng indicated that over a five-year period, 413 578 tablets containing valsartan were sold – the highest volume compared to other ARBs (Pillay, 2019). Common side effects include fatigue, dizziness, and joint pain (Nixon *et al*., 2009). Less than 10% of an administered dose of valsartan is metabolised in the liver. Excretion occurs mainly in bile and, to a lesser extent, via the kidneys largely as the unchanged drug (Dina and Jafari, 2000).

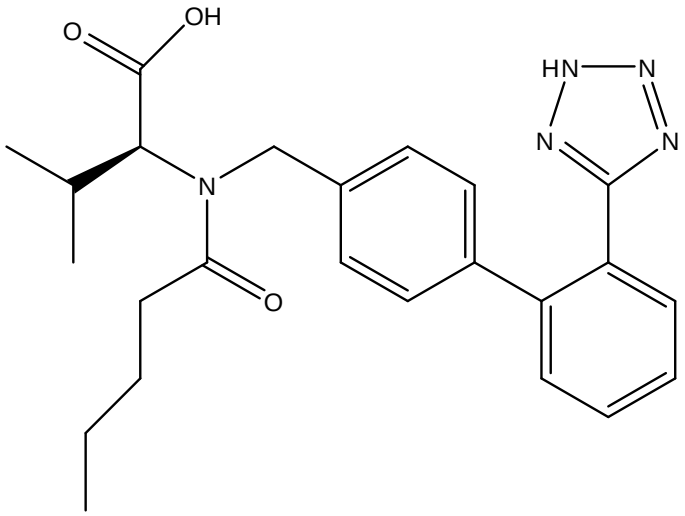

Figure 2.4: Chemical structure of valsartan

South Africa has the largest incidence of human immunodeficiency virus (HIV) in the world. The introduction of antiretroviral (ARV) treatment has brought a substantial decrease in the number of acquired immunodeficiency syndrome (AIDS) related morbidity and mortality (Montessori *et al*., 2004). As a result, South Africa has the largest ARV treatment programme globally (UNAIDS, 2021). In South Africa, an estimated 6.3 million people were living with HIV/AIDS in 2013 and around 200 000 people were estimated to have died from the dreaded disease the same year (Swanepoel *et al*., 2015). In the year 2020, an estimated 7.8 million adults and children were living with HIV in South Africa (UNAIDS, 2022). ARVs are used in combination to exhibit synergistic effects on patients (Swanepoel *et al*., 2015).

Nevirapine, shown in Figure 2.5, is one of the most common types of ARV medication that is used in the management of HIV/AIDS in South Africa (Swanepoel *et al*., 2015). It is prescribed as a first-line regimen for the treatment of HIV (Meintjies *et al*., 2017). In a study undertaken by Swanepoel, 2015, on ARVs in different waterbodies in South Africa, nevirapine was found in natural waters, WWTP effluents, drinking water and groundwater. Side effects from the use

of ARVs include nausea, fatigue, and the development of autoimmune disorders (Montessori *et al*., 2004).

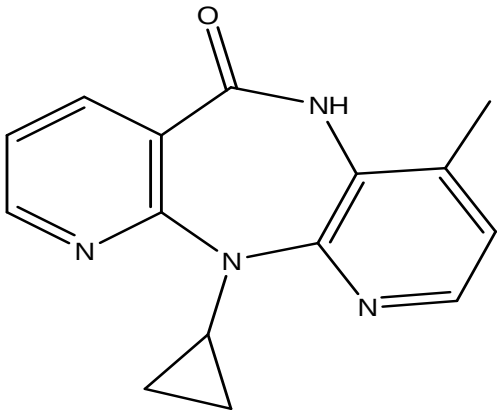

Figure 2.5: Chemical structure of nevirapine

Tuberculosis (TB) is an ancient infection that has been around for over 4 000 years (Zaman, 2010). It is a long-lasting infection that is caused by a bacterium called *Mycobacterium tuberculosis*, and is spread between humans by air (Zaman, 2010). TB often affects the lungs of patients which leads to severe coughing, chest pains and fever (Fogel, 2015). There are two main types of TB infections: latent and active TB. Latent TB is a state where humans are infected with *Mycobacterium tuberculosis* but do not show any clinical symptoms – the infection causing bacteria is in a dormant state (Lee, 2016). Active TB is a state whereby the patient shows symptoms, and the infection becomes contagious. Latent TB can develop into active TB (Lee, 2016). While being administered as treatment for latent TB, rifampicin is also used for the treatment of active TB. South Africa is burdened with one of the highest numbers of TB cases in the world, with an estimated 500 000 cases of TB being reported in 2011 and roughly 330 000 of those infected were also HIV positive (South African Government, 2017). The introduction of resistant strains of TB has led to combinations of anti-TB drugs being used to combat the infection (Klein *et al*., 2016). Rifampicin, Figure 2.6, is a potent anti-TB drug (Schonell *et al*., 1972).

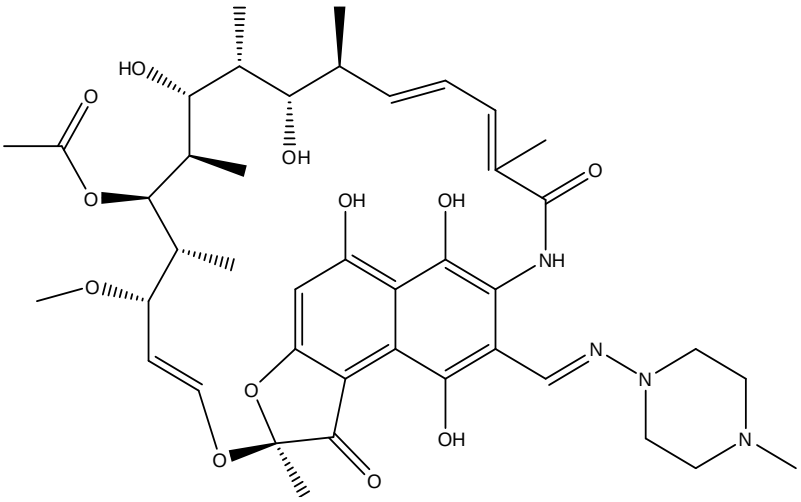

Figure 2.6: Chemical structure of rifampicin

An antibiotic is a chemical substance that inhibits the growth and/or metabolic activities of bacteria as well as other micro-organisms that cause illness and disease (Waksman, 1947). Sulfamethoxazole, Figure 2.7(a), is an antibacterial sulfonamide that is metabolised to sulfamethoxazole hydroxylamine (Figure 2.7(b)) in the liver (Cribb and Spielberg, 1992). The presence of antibiotics in the environment gives rise to the development of antibiotic resistant bacteria (Gleckman *et al*., 1981). This phenomenon leads to commonly used antibiotics not being effective against bacteria strains any longer. Due to the development of antibiotic resistance, combinations with sulfamethoxazole has become more common. A combination of antibiotics with sulfamethoxazole leads to enhanced therapeutic efficacy, improved antibacterial activity and a decrease in the emergence of antibiotic resistance (Gleckman *et al*., 1981).

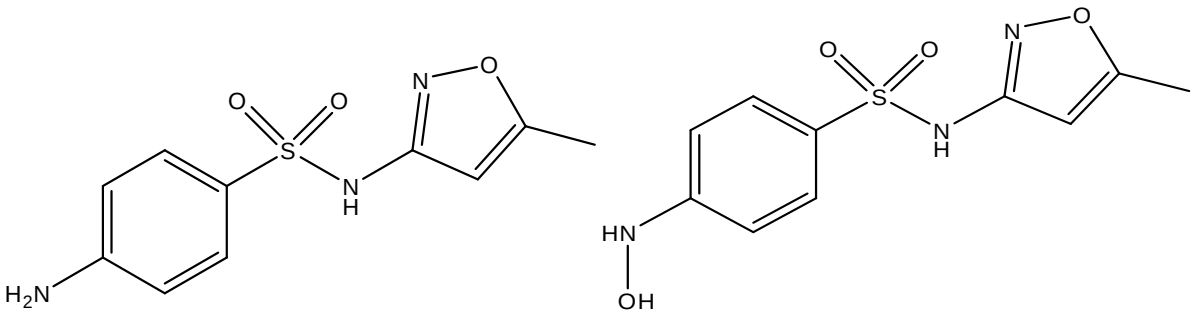

Figure 2.7: Chemical structure of (a) sulfamethoxazole (parent) and (b) sulfamethoxazole hydroxylamine (metabolite)

Pollution markers are essential in detecting and locating sources of water pollution (Buerge *et al*., 2003). The monitoring of pollution markers is applied to evaluate environmental pollution caused by humans (Gonçalves *et al*., 2017). A good chemical marker for domestic wastewater contamination is caffeine, illustrated in Figure 2.8, due to its regular and constant consumption (Beurge *et al*., 2003). Caffeine is found naturally in many foods and beverages such as chocolate, energy bars, soda, tea, and coffee. Caffeine is likely to persist in water largely due to its high solubility and negligible volatility. Its undoubtable anthropogenic origin makes it a marker of choice for domestic contaminated wastewater (Gonçalves *et al*., 2017).

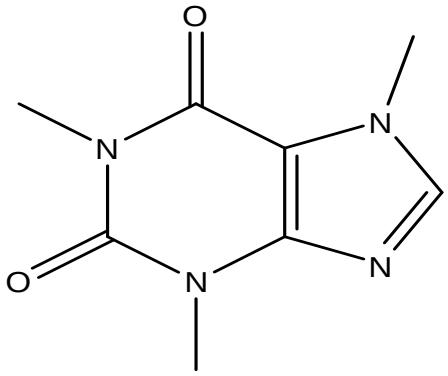

Figure 2.8: Chemical structure of caffeine

Ivermectin, shown in Figure 2.9, is an anthelmintic class of drug that is currently used in veterinary medicine. It is an adaptable drug with significantly unique characteristics (Rizzo, 2020). Ivermectin shows antibacterial, antiviral, and anticancer activity (Rizzo, 2020). Since the start of the COVID-19 pandemic, small scale observational studies have shown ivermectin as a possible treatment for the COVID-19 infection (Bryant *et al*., 2021). A study undertaken by Swargiary, 2020, demonstrated that ivermectin binds to an active site on the SARS-CoV-2 ribonucleic acid (RNA) – dependent RNA polymerase (RdRp), which is suggestive of possible inhibition of viral replication. Due to the cost-effectiveness and therapeutic efficacy of this drug, its use has been significant during the global pandemic (Bryant *et al*., 2021).

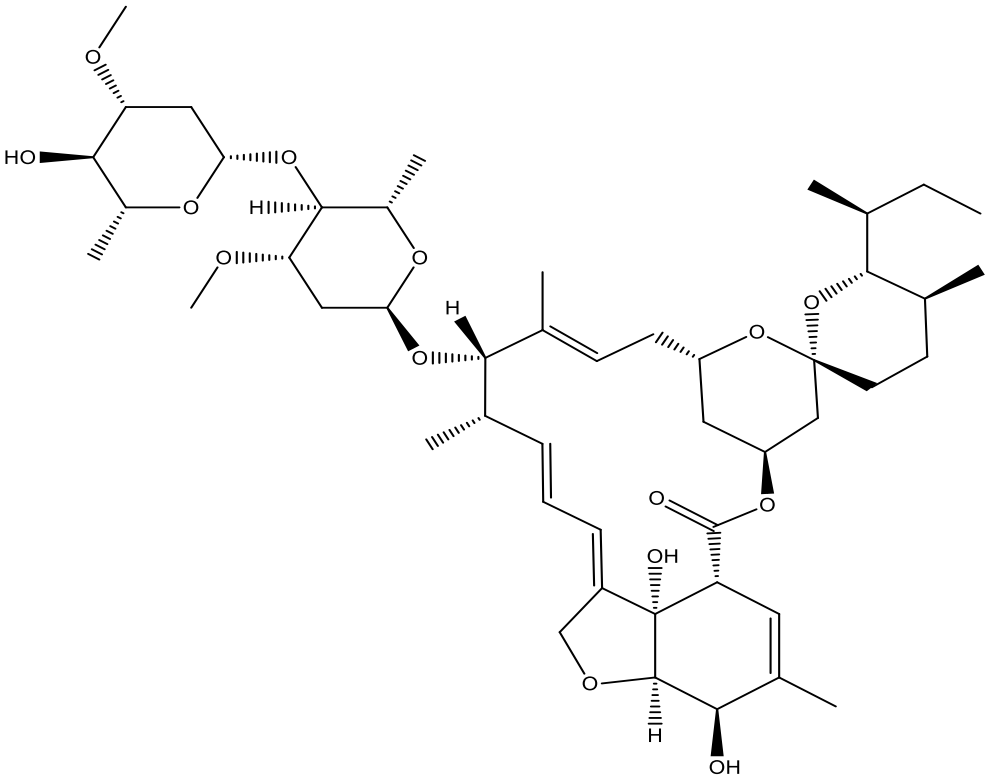

Figure 2.9: Chemical structure of ivermectin

2.1.1 Previous Research Conducted on Pharmaceuticals in Wastewater

Comparative studies focusing on the concentrations of pharmaceuticals and metabolites from several WWTPs were evaluated in lieu of those selected for this study. The studies reviewed spanned a period from 2007 up to and including 2018 across a local and international geographic footprint that comprised KwaZulu-Natal, Gauteng, Western Cape, Spain, United Kingdom (UK), Germany, Netherlands, and Bavaria. The comparative analysis revealed that there are no strong correlations between concentrations of pharmaceuticals and geographical areas from the selected studies. This data is outlined in Table 2.2.

Table 2.2: Comparison of concentrations of pharmaceuticals and metabolites from various WWTPs

Class	Pharmaceutical	Concentration/µg L^{-1}	Location	Source	Reference
Endocrine disruptor	EE2	0.0100 – 0.0950	KwaZulu-Natal	WWTP influent	Manickum and John, 2014
		0.001000 – 0.00800	KwaZulu-Natal	WWTP effluent	Manickum and John, 2014
Diabetes	Metformin	129	Germany	WWTP influent	Scheurer *et al*., 2009
		11.0	Germany	WWTP effluent	Scheurer *et al*., 2009
		101	Germany	WWTP influent	Scheurer *et al*., 2009
		2.20	Germany	WWTP effluent	Scheurer *et al*., 2009
		73.7	Netherlands	WWTP influent	Oosterhuis *et al*., 2013
		1.82	Netherlands	WWTP effluent	Oosterhuis *et al*., 2013
Hypertension	Valsartan	8.10	Bavaria	WWTP influent	Bayer *et al*., 2014
		1.10	Bavaria	WWTP effluent	Bayer *et al*., 2014

Table 2.2 continued…

Class	Pharmaceutical	Concentration/µg L^{-1}	Location	Source	Reference
		1.93	Netherlands	WWTP influent	Oosterhuis *et al*., 2013
		0.21	Netherlands	WWTP effluent	Oosterhuis *et al*., 2013
		0.342	South Wales (UK)	WWTP influent	Kasprzyk-Horden *et al*., 2009
		0.192	South Wales (UK)	WWTP effluent	Kasprzyk-Horden *et al*., 2009
Antiretroviral	Nevirapine	0.240 – 1.48	Gauteng	Surface water	Wood *et al*., 2015
		2.61 – 2.99	KwaZulu-Natal (Phoenix)	WWTP influent	Abafe *et al*., 2018
		1.34 – 1.46	KwaZulu-Natal (Phoenix)	WWTP effluent	Abafe *et al*., 2018
		1.95 – 2.25	KwaZulu-Natal (KwaMashu)	WWTP influent	Abafe *et al*., 2018
		1.83 – 1.97	KwaZulu-Natal (KwaMashu)	WWTP effluent	Abafe *et al*., 2018
		0.650 – 0.690	KwaZulu-Natal (Northern WWTP)	WWTP influent	Abafe *et al*., 2018

Table 2.2 continued…

Class	Pharmaceutical	Concentration/µg L^{-1}	Location	Source	Reference
		0.510 – 0.570	KwaZulu-Natal (Northern WWTP)	WWTP effluent	Abafe *et al*., 2018
Antibiotic	Sulfamethoxazole	0.100 – 0.200	Western Cape	WWTP influent	Hendricks and Pool, 2012
		0.0800 – 0.100	Western Cape	WWTP effluent	Hendricks and Pool, 2012
		0.450	Valencia (Spain)	WWTP influent	Gracia-Lor *et al*., 2012
		0.0500	Valencia (Spain)	WWTP effluent	Gracia-Lor *et al*., 2012
		59.3	KwaZulu-Natal (Northern WWTP)	WWTP influent	Matongo *et al*., 2015
		0.0290	South Wales (UK)	WWTP influent	Kasprzyk-Horden *et al*., 2009
		0.0100	South Wales (UK)	WWTP effluent	Kasprzyk-Horden *et al*., 2009

Table 2.2 continued…

Class	Pharmaceutical	Concentration/μg L^{-1}	Location	Source	Reference
		0.03100	South Africa	WWTP influent	Swartz *et al.*, 2018

There is a limited amount of local information linking these pharmaceutical contaminants to the health of humans, wildlife, and the environment. Literature shows that it is far too simplistic to assume that the non-detection of certain compounds parallels to complete drug removal and the alleviation of related risks. Research has shown some pharmaceutical compounds may elude detection by converting into transformation products (Yadav *et al*., 2017)

An increase in the usage of pharmaceuticals may have undesirable impacts on the future of human and environmental health as a result of the constant discharge of wastewater-borne compounds into the environment (Yadav *et al*., 2017). A study undertaken by Jendrzejewska and Karwowska, 2018, on a lab-scale simulation of wastewater treatment showed the influence antibiotics have on wastewater. During wastewater treatment, the concentrations of antibiotics are generally reduced however, antibiotics and their metabolites may remain in surface waters (Jendrzejewska and Karwowska, 2018). A significant increase in the number of antibiotic resistant bacteria was observed. After the treatment process, multi-resistant strains were isolated from the sludge used for the removal of organic and biological matter. This study is indicative of a possibility that resistant strains for diseases, such as TB, could have been formed as a result of large concentrations of antibiotics present in the environment. Consequently, there is a need to conduct monitoring studies on South African wastewater and surface water to determine the fate of pharmaceuticals and their metabolites.

2.2 Environmental Risk Assessment

Environmental risk assessment (ERA) is a scientific process that identifies and evaluates the likelihood of a compound to threaten living organisms, natural habitats, and ecosystems. It is determined by considering the predicted no effect concentration (PNEC) and the measured environmental concentration (MEC). PNEC refers to the maximum concentration of a compound that will exhibit no adverse effects on an organism or ecosystem. ERA is calculated by Equation 2.1.

$$\frac{MEC}{PNEC} = \text{Risk quotient (RQ)} \qquad \text{Equation 2.1}$$

An RQ value greater than or equal to 1 implies that the compound is of environmental concern.

2.3 Wastewater Treatment Plants (WWTPs)

A wastewater treatment plant facilitates the removal of organic and inorganic matter from the wastewater stream thus making it suitable for releasing back into the environment. During wastewater treatment, the removal of biological and chemical pollutants is achieved by physical (filtration and settling), chemical (adsorption and precipitation), and biological (microbial transformation) processes (Gerba, 2005). The basic operating model of a WWTP occurs in primary and secondary treatment steps, as illustrated in Figure 2.10.

Inlet works is inclusive of screening and de-gritting (Brouckaert *et al*., 2016). The purpose of the screen is to remove large, floating objects such as branches and litter, amongst other large solids (EPA, 1998). The raw sewage then enters three primary settlers – each of which has rotating bridges that aids in the removal of oil and scum from the surface of the water, which is then disposed. The now settled sewage is discharged to the balancing tank prior to being pumped into the activated sludge plant. During the treatment of wastewater, solids are separated from the liquids. The resulting solids are then treated to yield a nutrient-rich, semi-solid product referred to as sludge. The activated sludge plant comprises an anaerobic tank immediately followed by a three-lane aeration tank (Brouckaert *et al*., 2016). The sludge is loaded with bacteria which serves to break down organic matter in the sewage into nontoxic by-products. The sludge, which is now enriched with additional bacteria as well as other tiny organisms, can be reused within the aeration tank (EPA, 1998). The addition of alum facilitates the chemical precipitation of particles (Brouckaert *et al*., 2016). Thereafter, discharge into the secondary settlers occurs. During this step excess sludge and bacteria is removed and recycled back into the treatment process. The effluent is then transferred to a maturation river (not shown) where it is chlorinated preceding discharge into a nearby river (Brouckaert *et al*., 2016). Within KwaZulu-Natal, this river is a key source of water for domestic, agricultural, and industrial use.

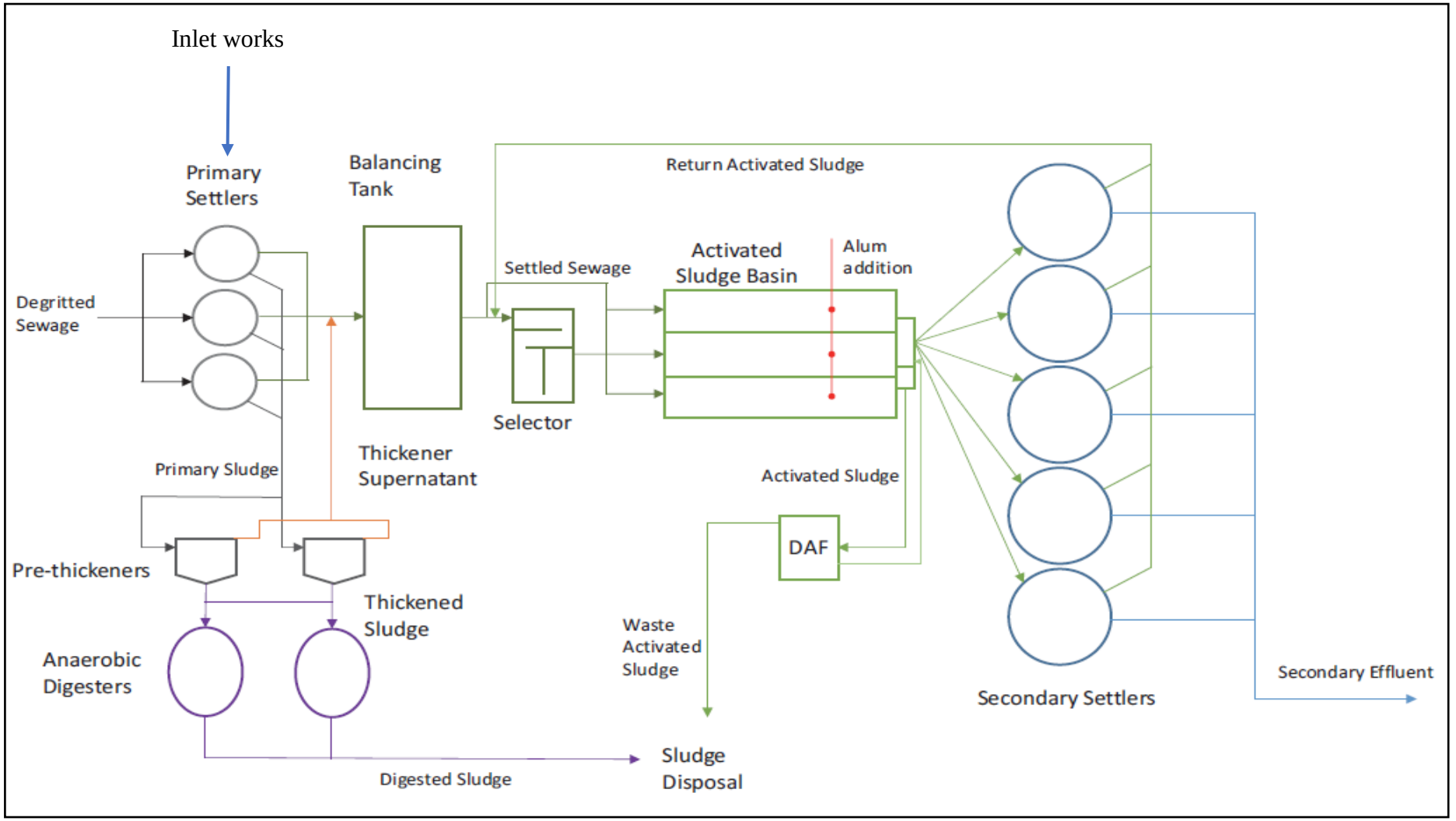

Figure 2.10: Simple schematic diagram showing the process undertaken at a WWTP (Brouckaert *et al*., 2016).

2.4 Methods used for the determination of pharmaceuticals

Methods of extraction of organic compounds from water samples include liquid/liquid extraction and solid phase extraction (SPE). Liquid liquid extraction aims to transfer water soluble impurities present within the sample to the aqueous phase leaving organic analytes of interest in the organic phase. Disadvantages with this extraction method includes the use of large volumes of solvents and long extraction times. SPE however, is the most commonly used extraction technique used for this scope of research. This method uses smaller solvent volumes, and extraction times span over a shorter period of time. There are various quantification methods used in the analysis of organics present in wastewater. Some of these include Gas Chromatography – Mass Spectrometry (GC-MS), High Pressure Liquid Chromatography (HPLC) with a UV detector, Liquid Chromatography – Mass Spectrometry (LC-MS) and lastly Tandem Mass Spectrometry (MS/MS), which is not easily available. GC-MS results in the analysis and quantification of organic compounds however, this method is not suitable for compounds that are thermally unstable.

2.4.1 Solid Phase Extraction

Solid phase extraction (SPE) is an analytical technique that is useful in the isolation and concentration of an analyte present in a matrix (Poole, 2002). Analytes are separated in accordance with their chemical and physical properties. SPE can be performed under reverse phase conditions, normal phase conditions, ion exchange or mixed mode. Reverse phase SPE makes use of a non-polar stationary phase and a polar mobile phase. Retention of organic analytes from the sample matrix onto the SPE stationary phase is resultant of non-polar analyte – non-polar packing material interactions, also known as Van der Waal forces (Poole, 2002). To elute an adsorbed analyte from the stationary phase, a polar solvent is used. Mixed mode SPE, as explored in this study, can retain analyte/s based on two modes of interactions. This technique is beneficial when the sample matrix has numerous analytes, each with different properties (Williams *et al.*, 2006). The SPE stationary phase is an octadecyl group bonded type silica gel or other reverse phase packing material with an ion exchange group bonded onto it. Cation exchange phases most often have a negatively charged sulfonate group that is bonded to the reverse phase packing material (Supelco, 1998). This analytical technique allows the pH

of the sample to be adjusted to make analyte/s positive or negative to be retained by the cation or anion phase (Williams *et al.*, 2006).

2.4.2 Liquid Chromatography – Mass Spectrometry

Liquid chromatography (LC) is an analytical technique that is used to separate individual components or analytes of a liquid sample (Gratz *et al.*, 2000). Separation is based on the individual component's affinity towards the mobile and stationary phases (Gratz *et al.*, 2000). There are different phases of LC, normal phase, reverse phase, and ion exchange. The nature of the sample, solvent and stationary phase selected will determine the retention time of the individual components of interest (Petrova and Sauer, 2017). As the individual components pass through the column, those that have strong interactions with the stationary phase will have longer retention times (Petrova and Sauer, 2017). Upon exiting the column, the individual components are transported to the detector. The detector can be an ultraviolet (UV) detector, refractive-index detector, or mass spectrometer, just to name a few. Figure 2.11 shows the schematic layout of a typical LC – MS instrument.

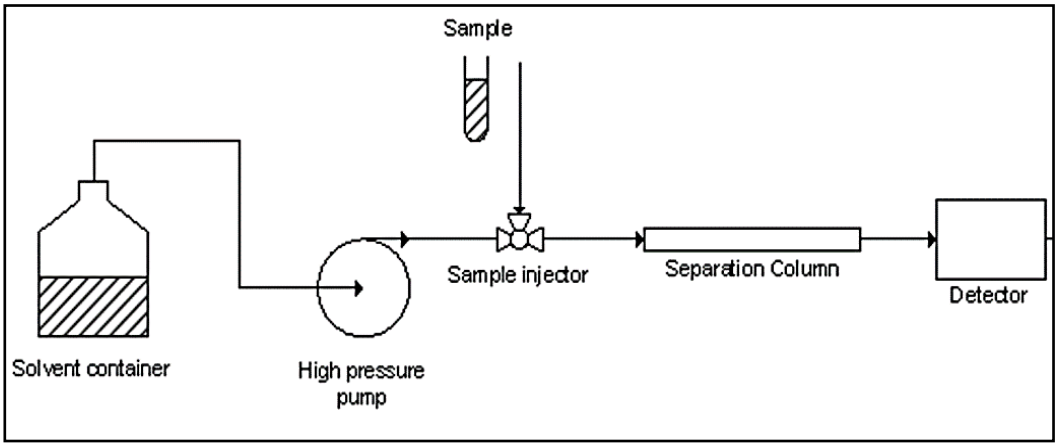

Figure 2.11: Typical layout of a LC instrument (de Corral and Pfister, 2005).

The coupling of mass spectrometry (MS) to chromatographic techniques is advantageous due to the high sensitivity of MS. The characteristics of MS have ensured unsurpassed sensitivity, detection limits and variety of applications (de Hoffmann and Stroobant, 2007). While LC enables the separation of individual components, MS allows for the structural identification of the individual components respective to their retention time. A mass spectrometer consists of four major components; ion source, mass analyser, ion detector and a data processing system, as illustrated in Figure 2.12 (Premier Biosoft, 2020).

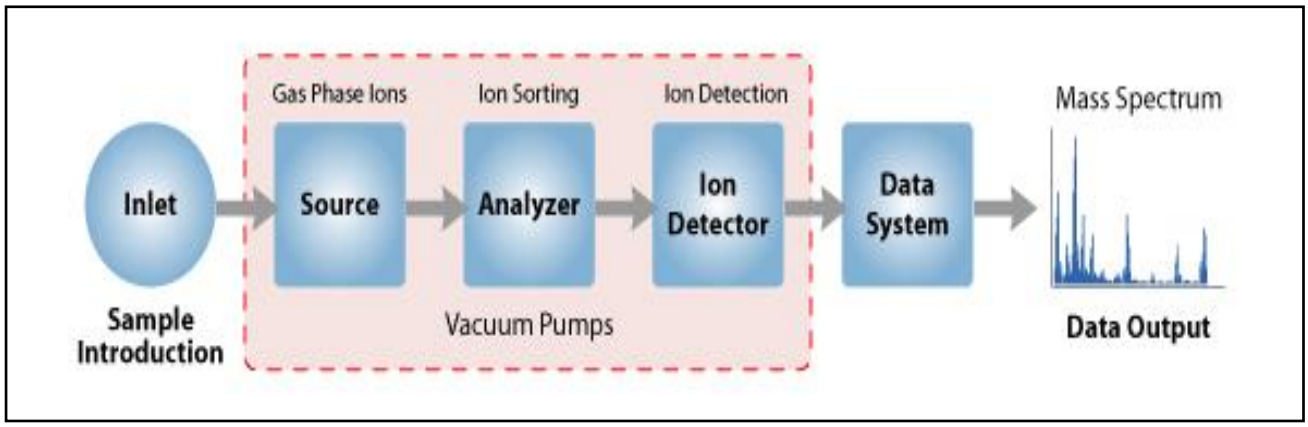

Figure 2.12: Typical layout of a mass spectrometer (Premier Biosoft, 2020).

Individual components separated from the LC column enter the ionisation source chamber, where multiple negative and positive ions are generated (de Hoffmann and Stroobant, 2007). The method used to generate ions in this scope of research is electrospray ionisation (ESI). ESI is a technique that produces an aerosol spray using a high voltage. The ions formed from the ESI process move to the mass analyser which separates the generated ions according to their mass-to-charge ratios (*m/z)* (de Hoffmann and Stroobant, 2007). The mass analyser used for this study is a single quadrupole. The now separated ions that are passed from the mass analyser enter the detector where they are detected and transformed into a usable signal while the data processing system produces the mass spectrum (de Hoffmann and Stroobant, 2007). The detector used for this study is comprised of a conversion dynode and an electron multiplier. The signal is generated from an electric current that is proportional to the ions abundance (de Hoffmann and Stroobant, 2007).

2.5 Summary

This chapter addressed pharmaceuticals, their metabolites, and their negative effects on the environment. Additionally, the wastewater treatment process, extraction procedures and instrumentation principles that were followed was outlined in this chapter. This study will also investigate whether the selected emerging organic contaminants were adequately removed from wastewater during the treatment process used at the WWTP. The inadequate removal of organic pollutants poses a great risk to aquatic ecosystems as these contaminants have the potential to adversely affect the environment, as described in this chapter. To the best of my knowledge, analysis of sulfamethoxazole hydroxylamine and ivermectin in wastewater has not

yet been investigated in this area of interest. A detailed methodology outlining the identification and quantification of targeted analytes is covered in Chapter 3.

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CHAPTER 3: METHODS AND MATERIALS

3.1 Sampling Sites

The area of study chosen for this research project is a WWTP situated in Pietermaritzburg, KwaZulu-Natal, South Africa. The WWTP, under Umgeni Water management, services the greater Pietermaritzburg community. This treatment plant manages a flow of approximately 100 ML day^{-1} of wastewater and covers an area of approximately 46 acres of land.

Water samples were collected once daily for a period of seven consecutive days. Mentioned samples were collected over three sampling periods, February 2022, April, and June 2022. These specific sampling periods were chosen based on their season and in relation to the COVID-19 predicted waves in South Africa. February 2022 samples correlated to the wet season and the period after the COVID-19 forth wave peak, while April 2022 samples represented the period approaching the COVID-19 fifth wave. June 2022 samples corresponded to the dry season and the period after the COVID-19 fifth wave peak.

Sampling points, listed in Table 3.1, included the inlet (after degritting), balancing tank, secondary effluent, and maturation river. The WWTP is situated alongside the western bank of a nearby river into which treated wastewater effluent is discharged. The river is a key source of water for domestic, agricultural, and industrial uses within the surrounding communities of Msahweni and Sobantu. An aerial view of the treatment plant is depicted in Figure 3.1.

Table 3.1: Sampling sites and a basic site description

Sampling site	Site description
Inlet	Raw wastewater flows through the inlet
Balancing tank	Once influent has been filtered, the water sits in a balancing tank to further settle contaminants
Secondary effluent	Point where effluent from all 5 secondary settlers flows
Maturation river	Immediately prior discharge into a nearby river

Figure 3.1: Aerial view of the WWTP including maturation river (Google Earth Pro)

3.2 Storage and Preservation

The collected water samples were stored in 2.5 L amber glass bottles to prevent UV degradation of analytes. These bottles were washed threes time with liquid soap (DynaChem), tap water and deionised water prior to sampling. Water samples were stored on ice during transportation to the laboratory and acidified with three drops of acetic acid for preservation. In the laboratory, samples were stored in the fridge at 4 ℃.

3.3 Chemicals

Chemical solvents used for the analysis included high performance liquid chromatography (HPLC) grade methanol (MeOH), acetic acid and ammonium acetate, all purchased from Sigma Aldrich, South Africa. Ultra-pure type 1 Millipore water (resistivity of 18.2 MΩ.cm at 25°C) used for the analysis was obtained with a Synergy ® UV water purification system. Certified reference standards of each analyte of interest were utilised. This included metformin, nevirapine, prednisolone, valsartan, rifampicin, ivermectin and internal standard primidone, which were purchased from Sigma Aldrich, South Africa. Caffeine, hydroxylamine sulfamethoxazole, sulfamethoxazole and EE2 were purchased from DLD Scientific. Filter discs used were manufactured by Filter-Bio and had a pore size of 0.45 µm. Oasis® HLB cartridges (60 mg, 3 cc) were purchased from Microsep (Pty) Ltd.

3.4 Analysis of Pharmaceuticals and Metabolites

The methods used for the analysis of targeted analytes were adapted from studies performed by Castiglioni *et al.*, 2006, and Archer, 2018.

3.4.1 Filtration

Samples were initially filtered using gravity filtration to remove larger particles. Whatman Filter Paper, No. 40 (15 cm diameter) was used for this purpose. A volume of 50 mL of the filtered wastewater sample was further filtered through a 0.45 µm filter disc. The filtration and sample preparation of all water samples were conducted within two weeks of sampling.

3.4.2 SPE using Oasis HLB Cartridges

Filtered wastewater samples were adjusted to pH 7 prior to SPE (Archer *et al.*, 2017). The SPE cartridges were conditioned with 2 mL of methanol and 2 mL of ultra-pure Millipore water. Samples were then passed through cartridges using a vacuum manifold, at approximately 2 mL min^{-1} followed by vacuum drying for 15 minutes. The extracts were then eluted with two 2 mL portions of methanol. The eluate was thereafter evaporated to dryness under vacuum. Dried samples were then reconstituted in 1 mL using the 6 mg L^{-1} multi-analyte standard mixture (spiking solution). The resulting sample was then ready for LC – MS analysis.

3.4.3 Preparation of Standard Solutions

A 100 mg L^{-1} multi-analyte stock solution was prepared by diluting 1 mg of pure analyte in 10 mL of HPLC grade methanol. Thereafter, successive dilutions were made using the dilution factor method shown in Equation 3.1, where C and V represents concentration and volume, respectively. A set of standards with concentrations ranging from 2 mg L^{-1} to 10 mg L^{-1} were prepared in HPLC grade methanol.

Sample calculation: $$C_1V_1 = C_2V_2 \quad \text{Equation 3.1}$$

$$(100 \text{ mg L}^{-1})V_1 = (2 \text{ mg L}^{-1})(0.01 \text{ L})$$

$$= \frac{(2 \text{ mg L}^{-1})(0.01 \text{ L})}{(100 \text{ mg L}^{-1})}$$

$$V_1 = 0.002 \text{ L} = 200 \text{ µL}$$

Table 3.2 outlines the volume of the working standard used to prepare the set of standards. Volumes were calculated using the dilution formula, as shown in Equation 3.1.

Table 3.2: Volume of 100 mg L^{-1} stock solution used to prepare required set of standards

Concentration/mg L^{-1}	**Volume of working standard/µL**
2	200
4	400
6	600
8	800
10	1000

3.4.4 Analysis of Samples with LC – MS

A Shimadzu LC – MS – 2020 liquid chromatography – mass spectrometer was used for pharmaceutical analysis. Table 3.3 shows the LC-MS instrument parameters that were used.

Table 3.3: LC-MS instrument parameters for the analysis of pharmaceuticals and their respective metabolites

Parameter	
Column	Shimadzu, Shim-pack GIST Reverse phase C18 column, 2.1 x 100 mm, 3 µm
Flow rate	0.22 mL min^{-1}
Injection volume	5.00 µL
Solvents	A: Ultra-pure Millipore water + 5 mM ammonium acetate + 3 mM acetic acid B: 100% MeOH
Gradient	0 – 1 min (20% MeOH) 1 – 18.5 mins (20% - 90% MeOH) 18.5 – 22.5 mins (90% MeOH) 22.5 – 25 mins (90% - 20% MeOH) 25 – 27.5 mins (20% MeOH)
Mass spectrometer	Single quadrupole, electrospray ionisation (ESI) Ionisation chamber voltage: -3.50 kV Ion source temperature: 200 ℃ Detector voltage: -1.45 kV

3.5 Quantification of Pharmaceuticals and Metabolites

Individual concentrations of pharmaceuticals and their metabolites present in the sample extract were calculated from the equation from the standard calibration curve, as shown in the sample calculation below. Further calculations involved analyte concentrations in the sample, mass loads, and removal efficiency.

3.5.1 Individual Analyte Concentrations

The peak area of nevirapine in the chromatogram of a sample was found to be 546 517 mAU. The equation from the standard calibration curve for nevirapine (Appendix A5) is y = 76 202x + 17 370.

$$Y = 76\,202x + 17\,370$$

$$546\,517 = 76\,202x + 17\,370$$

$$x = 6.944 \text{ mg } L^{-1}$$

Samples were spiked with 6 mg L^{-1} standard mixture. The purpose of spiking samples is further explained in Section 3.6.1. The concentration of nevirapine from the sample extract is:

$$6.944 \text{ mg } L^{-1} - 6 \text{ mg } L^{-1} = 0.944 \text{ mg } L^{-1}$$

Analyte concentrations present in each sample were determined using Equation 3.2 below. Where C_s corresponds to the concentration of analyte in the sample, V_s refers to the volume of wastewater sample used, C_e corresponds to the concentration of analyte found in the sample extract and V_e refers to the volume of the sample extract.

$$C_sV_s = C_eV_e \qquad \text{Equation 3.2}$$

$$C_s = \frac{CeVe}{Vs}$$

$$= \frac{(0.944 \text{ mg } L^{-1})(1 \text{ mL})}{(50 \text{ mL})}$$

$$= 0.0189 \text{ mg } L^{-1}$$

3.5.2 Mass Loads

Mass loads are calculated to compensate for the varying flow rates at the treatment plants. Influent and effluent streams are expressed as g day^{-1}. For this calculation, the influent (Inf) or effluent (Eff) concentration of analyte is used and is expressed as $\frac{\text{mg}}{\text{L}}$. Flow rate (FR) refers to the daily flow rate of 'fresh' wastewater into the treatment plant as well as the daily flow rate of treated wastewater out of the plant and is expressed as ML day^{-1}.

$$\text{Mass loads} = \text{Inf or Eff } \left(\frac{\text{mg}}{\text{L}}\right) \text{ x FR } \left(\frac{\text{ML}}{\text{day}}\right) \text{ x } \underbrace{\frac{1\text{ g}}{1000\text{ mg}} \text{ x } \frac{1\,000\,000\text{ L}}{1\text{ ML}}}_{1000} \qquad \text{Equation 3.3}$$

Sample calculation: The daily average flow rate obtained for Monday's effluent stream was recorded as 86 ML day^{-1} and using the concentration of nevirapine calculated above.

$$\text{Mass load}_{(\text{effluent})} = 0.0189 \text{ mg } L^{-1} \text{ x } 86 \text{ ML } day^{-1} \text{ x } 1000$$
$$= 1\,625.4 \text{ g } day^{-1}$$

3.5.3 Removal Efficiency

Removal efficiency indicates the amount of analyte removed during the treatment process, expressed as a percentage. The abbreviations ML(influent) and ML(effluent) refers to the calculated mass load for an analyte in the influent and effluent, respectively.

$$\text{Removal efficiency} = \frac{\text{ML(influent)} - \text{ML(effluent)}}{\text{ML(influent)}} \text{ x } 100 \qquad \text{Equation 3.4}$$

Sample calculation: The ML(influent) is calculated as 2 392.2 g day^{-1} and the ML(effluent) is 1 625.4 g day^{-1}.

$$\text{Removal efficiency} = \frac{2\,392.2 \text{ g day}^{-1} - 1625.4 \text{ g day}^{-1}}{2\,392.2 \text{ g day}^{-1}} \text{ x } 100$$
$$= 32.05\ \%$$

3.6 Method Development and Validation

The extraction methods discussed in Section 3.4 were validated through means of recovery studies, the determination of limits of detection (LOD) and limits of quantification (LOQ) and through inter-day and intra-day analysis.

3.6.1 Recovery Studies

Recovery studies forms part of an investigation determining whether the experimental procedure applied to the water samples are efficient in the extraction of targeted analytes. This was carried out by spiking and extracting analytes from a tap water sample using the method described in Section 3.4. An additional tap water sample, not spiked with any standard was extracted through the same methods shown in Section 3.4. Percentage recovery is calculated using Equation 3.5. Acceptable recoveries fall between the range of 60 to 120 % recovered.

$$\% \text{ recovery} = \frac{\text{spiked sample concentration} - \text{unspiked sample concentration}}{\text{concentration used to spike}} \text{ x } 100 \qquad \text{Equation 3.5}$$

Sample calculation showing percentage recovery for metformin:

$$\% \text{ recovery} = \frac{\text{spiked sample concentration} - \text{unspiked sample concentration}}{\text{concentration used to spike}} \text{ x } 100$$

$$= \frac{4.99 \text{ mg L}^{-1} - 0 \text{ mg L}^{-1}}{6 \text{ mg L}^{-1}} \text{ x } 100$$

$$= 83.17 \%$$

3.6.2 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD refers to the lowest concentration of an analyte that can be detected *via* an analytical method. While LOQ refers to the lowest concentration at which the performance of an analytical method is acceptable for usage.

Equation 3.6 and Equation 3.7 that will be used to calculate these parameters are as follows:

$$\text{LOD} = 3.3 \text{ x } \frac{\text{standard deviation}}{\text{gradient}} \qquad \text{Equation 3.6}$$

$$\text{LOQ} = 10 \text{ x } \frac{\text{standard deviation}}{\text{gradient}} \qquad \text{Equation 3.7}$$

Analysis of standards were done in triplicate. Calibration curves were generated for each replicate. These curves were drawn using the peak area from the resulting chromatograms in relation to the standard concentrations. The standard deviation that exists between the y-intercept values of each equation is used as well as the average gradient.

Sample calculation: Calibration curves from triplicate analysis obtained for metformin had the following equations: y = 96828x + 89878; y = 101097x + 88278; y = 98473x + 109454.

The average gradient from the following equations is 98799.33 and the standard deviation observed in the y-intercept values is 9627.52. Inserting these values in Equations 3.6 and 3.7 gives rise to the following data:

$$\text{LOD} = 3.3 \times \frac{\text{standard deviation}}{\text{gradient}} \qquad \text{Equation 3.6}$$

$$= 3.3 \times \frac{9627.54}{98799.33}$$

$$= 0.322 \text{ mg } L^{-1}$$

$$\text{LOQ} = 10 \times \frac{\text{standard deviation}}{\text{gradient}} \qquad \text{Equation 3.7}$$

$$= 10 \times \frac{9627.54}{98799.33}$$

$$= 0.974 \text{ mg } L^{-1}$$

3.6.3 Inter-day and Intra-day Studies

To ensure that the analysis of each sample is reliable, inter-day and intra-day analyses were carried out. Intra-day analysis refers to analysing samples at various time intervals within the same day. Whilst inter-day analysis refers to analysing the same samples over consecutive days. From the analyses, the percentage relative standard deviation will be determined. This would give an indication as to how consistent the LC – MS instrument is.

3.7 Quality Assurance

In order to ensure that the analysis of samples was done consistently, a 6 mg L^{-1} standard mixture was analysed at intervals during the analysis on the LC – MS. Additionally, a methanol blank was analysed after each triplicate sample analysis to ensure that there was no carryover between sample runs. Quality assurance was also confirmed using an internal standard – primidone. A constant response of the internal standard during each sample run ensured that instrumental sensitivity was consistent throughout the analysis.

3.8 References

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CHAPTER 4: SELECTED PHARMACEUTICAL ANALYSIS IN A WASTEWATER TREATMENT PLANT DURING COVID-19 INFECTION WAVES IN SOUTH AFRICA

4.1 Abstract

Globally, an extensive range of pharmaceuticals are consumed daily to treat a variety of illnesses and diseases. Since the occurrence of the SARS-CoV-2 virus (COVID-19) outbreak, the use of pharmaceuticals has increased drastically in order to treat and prevent infection. Studies have shown that pharmaceutical usage is largely dependent on seasonal colds and influenza, brought upon by changes in seasonal temperatures. This was explored in the present study and was verified by the results we obtained. Versatile solid phase extraction (SPE) and liquid chromatography – mass spectrometry (LC – MS) methods were developed and validated for the accurate detection of target pharmaceuticals. Resulting concentrations of pharmaceuticals used to treat chronic ailments such as diabetes, hypertension, tuberculosis, and HIV/AIDS showed consistent daily usage while pharmaceuticals used for the treatment of COVID-19 and influenza showed distinct seasonal trends. Trends also directly correlated with the total number of active COVID-19 cases experienced in South Africa during sampling periods.

4.2 Introduction

Over the past century, the field of medicine has made significant progress in the lengthening of life expectancy and reduction of morbidity (Chen and Lin, 2009). Presently, more than 3000 chemical compounds have been approved by various regulatory bodies as constituents of pharmaceutical products (Löffler and Ternes, 2003). Pharmaceuticals are not entirely metabolised by the human body and are excreted either unchanged or as metabolites (Li et al., 2016).

Wastewater refers to used water that encompasses any combination of industrial, commercial, medical, domestic, and agricultural activities, stormwater, and sewer inflow (Kanama, 2018). Pharmaceutical contaminants are not sufficiently removed during conventional processes operated at most wastewater treatment plants (WWTPs). Therefore, their occurrence in environmental media, such as water bodies, implies possible danger to overall public health,

biota, and the environment (Kanama, 2018). There is increasing indication that a wide variety of pharmaceuticals persist in natural water resources (Archer, 2018). These organic pollutants are classed within the emerging contaminants (EC) group and enter various water systems through sewage, leeching, industrial waste, and improper disposal. The detection of pharmaceuticals in South African water systems has increased sporadically over the last few years. Adverse effects associated with the occurrence of pharmaceuticals in the environment includes bacterial resistance to numerous antibiotics, feminisation and infertility amongst aquatic animals and unnecessary exposure of these animals to pharmaceuticals that they simply do not need. Medication has the tendency to persist within the human body to aid in the healing of various ailments however, this often leads to the same persistence observed within the environment (Mastrup, 2005).

The SARS-CoV-2 (COVID-19) outbreak affected the world in 2020. The World Health Organisation (WHO) classified this outbreak as a global pandemic. COVID-19 is a highly contagious respiratory virus that has resulted in the loss of millions of lives globally (Ciotti *et al.,* 2020). The occurrence of the global pandemic gave rise to a substantial increase in the use of pharmaceuticals to prevent and treat infections. Pharmacies and medical facilities reported an increased demand for pharmaceuticals such as antibiotics, antivirals and immunosuppressants during this period (Ciotti *et al.,* 2020). Although interactions between humans play a vital role in transmission of the virus, temperature and humidity were found to affect virus survival as well as human immune functioning and response (Byun *et al.,* 2021). During the period of time in which COVID-19 became classified as a global pandemic, geographical regions that experienced higher ambient temperatures (being > 20 ℃), were found to have reported lower number of COVID-19 cases compared to regions that experienced lower temperatures. Apart from other meteorological conditions such as precipitation, wind speed and solar radiation that were also found to have had effects on transmission (Byun *et al.,* 2021). The consumption of certain pharmaceuticals, inclusive of antimicrobials and antibiotics, has a distinct seasonal trend of increased usage during the winter months and decreased usage in the summer months, both of which ran parallel with influenza and COVID-19 cases (O' Flynn *et al.,* 2021). Epidemiologists in South Africa predicted the end of the fourth COVID-19 infection wave to occur at the end of January 2022. The start of the potential fifth wave was forecasted for April 2022. The peak of the fifth wave was experienced around June 2022, during the cold, winter month. Using this information, sampling trips were centred around these periods of time in order to compare pharmaceutical concentrations during the high and low

infection periods. In South Africa, ivermectin was used as a treatment for COVID-19 infection. Initially, the use of ivermectin to treat the virus was prohibited as there were limited available studies on the effectiveness of this drug in the treatment of COVID-19. In January 2021, the South African Health Products Regulatory Authority (SAHPRA) approved the use of ivermectin in the treatment of COVID-19, but only under controlled, compassionate usage under Section 21 of the Medicines and Related Substances Act (SAHPRA, 2021). Despite the initial prohibition of widespread use of ivermectin, the drug was largely available on the black market. In May 2022 SAHPRA had terminated the compassionate usage of ivermectin programme on the basis that additional large scale clinical studies had shown little to no improvement in COVID-19 cases with the use of ivermectin. Additionally, the regulatory body had expressed a rapid decline in the number of patients and medical facilities applying for usage and dispensing of the drug (SAHPRA, 2022).

This research study aims to identify and quantify the concentrations of selected pharmaceuticals and metabolites present in the influent stream at a Wastewater Treatment plant, in KwaZulu-Natal. Pharmaceuticals of interest were selected due to them being used for medical conditions that are prevalent in South Africa as well as being commonly prescribed to treat and prevent COVID-19 and influenza. These analytes of interest are outlined in Table 4.1. Additionally, the study aimed to show trends that exist between pharmaceutical usage and seasonal changes.

Table 4.1: Basic description of the ten pharmaceuticals evaluated in this study

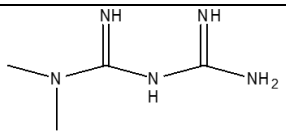
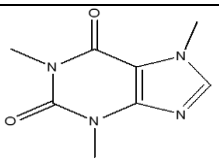
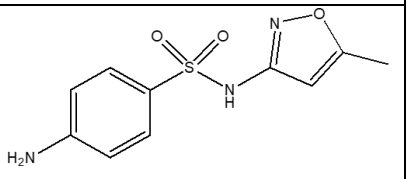
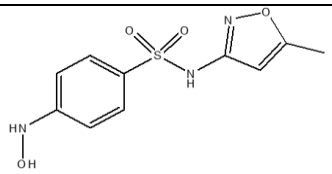
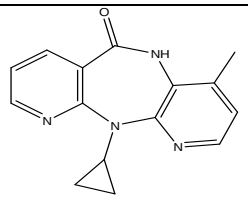
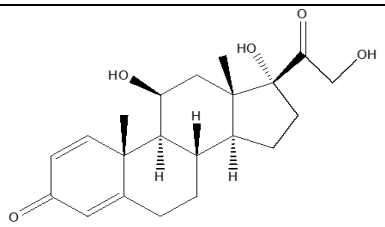
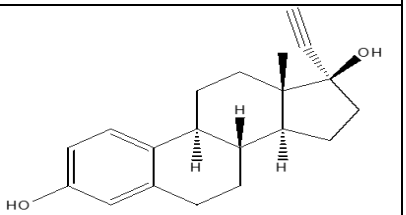
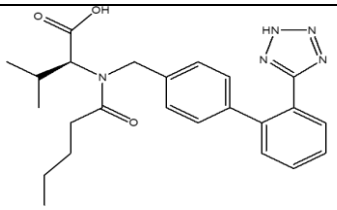
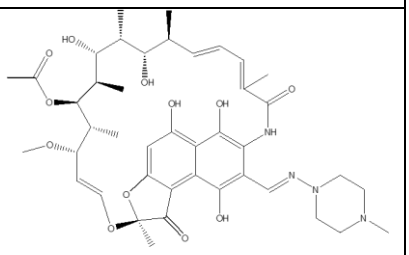
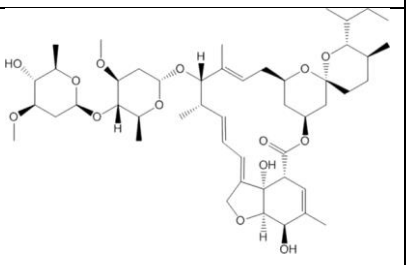
Class	Pharmaceutical	Chemical structure
Diabetes	Metformin	
Stimulant	Caffeine	
Antibiotic	Sulfamethoxazole	

Table 4.1 continued…

Class	Pharmaceutical	Chemical structure
	Sulfamethoxazole hydroxylamine	
Antiretroviral	Nevirapine	
Endocrine Disruptors	Prednisolone	
	17α-ethinylestradiol (EE2)	
Hypertension	Valsartan	
Tuberculosis	Rifampicin	
Anthelmintic	Ivermectin	

4.3 Materials and Methods

4.3.1 Chemicals and Reagents

This study involved the analysis of ten pharmaceutical standards and one internal standard (IS). Certified reference standards used for analysis included metformin, caffeine, sulfamethoxazole hydroxylamine, sulfamethoxazole, primidone (IS), nevirapine, prednisolone, valsartan, rifampicin, EE2 and ivermectin, all of which were purchased from Sigma Aldrich (South Africa) and DLD Scientific. Ammonium acetate, HPLC grade acetic acid and HPLC grade methanol was purchased from Sigma Aldrich, South Africa. Oasis® HLB cartridges (60 mg, 3 cc) was purchased from Microsep (Pty). Ultra-pure type 1 Millipore water (resistivity of 18.2 MΩ.cm at 25°C) used for experimental analysis was obtained from a Synergy ® ultraviolet (UV) water purification system.

4.3.2 Study Site and Sampling Procedure

The study site for this research project was a WWTP, situated in Pietermaritzburg, KwaZulu-Natal. Figure 4.1(a) depicts a map of South Africa with the province KwaZulu-Natal highlighted. An expansion of KwaZulu-Natal, shown in Figure 4.1(b), highlights the location of Pietermaritzburg in relation to the province. Wastewater from stormwater pipes, hospitals, residential and industrial areas flow into the WWTP. Treated wastewater effluent from the WWTP flows directly into a nearby river which serves as a source of water for domestic and agricultural use.

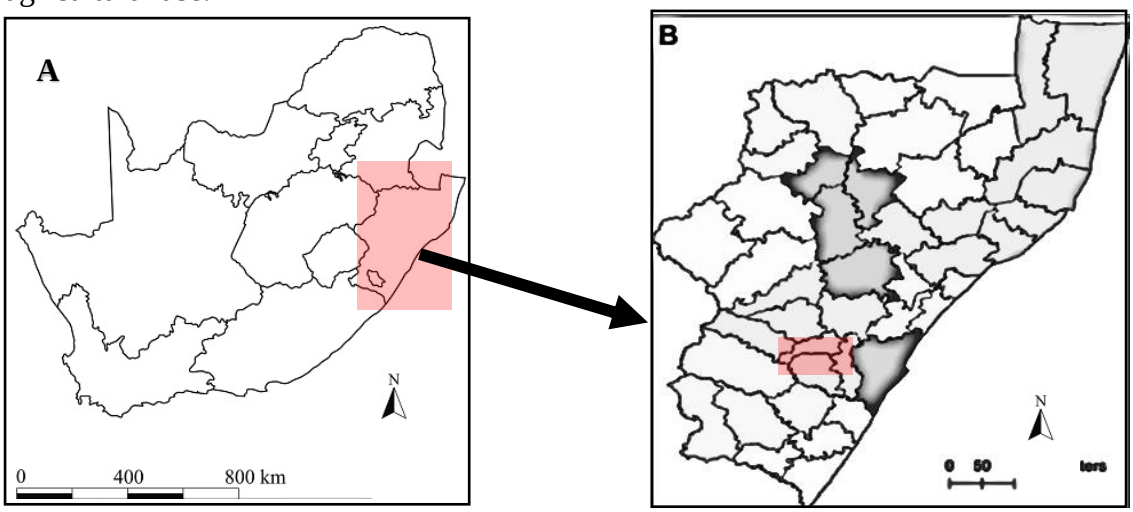


Figure 4.1: Geographical location of A) KwaZulu-Natal within South Africa as a country and B) Pietermaritzburg, the study area within KwaZulu-Natal (about South Africa, 2004)

Wastewater samples were collected from four sampling sites within the WWTP, which services the Pietermaritzburg population and has a mean wastewater inflow of 100 ML day^{-1}. Sampling points include the inlet, balancing tank, secondary effluent, and maturation river. Samples were collected for seven consecutive days (Monday to Sunday) over three sampling periods, February 2022, April 2022, and June 2022. Wastewater samples were collected in glass amber bottles and sample pH was recorded immediately after collection, prior to being acidified with acetic acid for preservation. These pH values are included in Appendix B1. The samples were kept on ice during transportation to the laboratory. At the laboratory, samples were stored in the fridge at 4 ℃. Filtration and extraction of all samples were performed within two weeks of collection.

4.3.3 Solid Phase Extraction of Pharmaceuticals and Metabolites

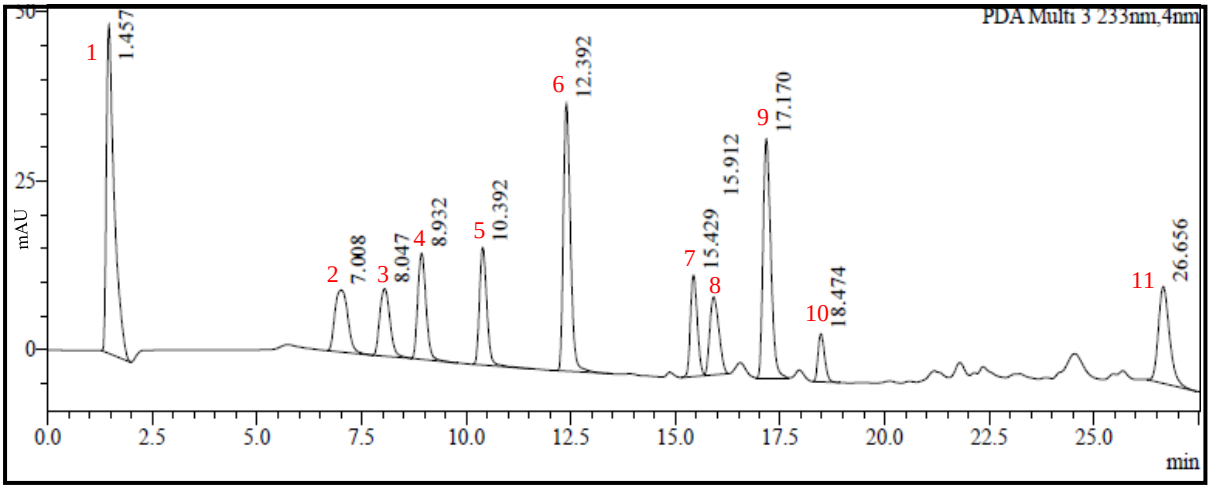
Wastewater samples were filtered through gravity filtration. A volume of 50 mL was further filtered using a 0.45 µm filter disc. The pH of the filtered sample was adjusted to 7 (Archer *et al.,* 2017). An Oasis® HLB cartridge was conditioned with 2 mL of methanol followed by 2 mL of ultra-pure Millipore water. The water sample was then loaded onto the cartridge and passed through at a rate of approximately 2 mL min^{-1}. The cartridges were then dried under vacuum for a period of 15 minutes. Extracts were eluted with 4 mL of methanol into amber vials. The 4 mL of methanol was added in two batches on 2 mL each. Upon the first 2 mL elution, the methanol was evaporated under vacuum and the final 2 mL was then added. The eluate was then evaporated to dryness under vacuum and thereafter reconstituted in 1 mL of 6 mg L^{-1} standard mixture.

4.3.4 LC-MS Analysis of Pharmaceuticals and Metabolites

A Shimadzu LC – MS – 2020 Liquid Chromatography – Mass Spectrometer was used for pharmaceutical analysis following a method adapted from Archer, 2018. The chromatographic method used for the quantification of analytes is described in Table 4.2. Method blanks containing methanol were run after each sample to ensure quality control for the analysis. Figure 4.2 illustrates chromatographic separation of pharmaceuticals, at a wavelength of 233 nm, obtained through the use of the method outlined below.

Table 4.2: Instrument parameters for the analysis of targeted analytes

Parameter		
Column	Shimadzu, Shim-pack GIST Reversed phase C18 column, 2.1 x 100 mm, 3 µm	
Flow rate	0.22 mL/min	
Injection volume	5.00 µL	
Mobile phases	A: ultra-pure Millipore water + 5 mM ammonium acetate + 3 mM acetic acid B: 100% MeOH	
Gradient	Time/min	%B
	0.01	20
	1.00	20
	18.50	90
	22.50	90
	25.00	20
	27.50	20
Mass spectrometer	Single quadrupole, electrospray ionisation (ESI) Ionisation chamber/ interface bias voltage: -3.50 kV Ion source temperature: 200 ℃ Detector voltage: -1.45 kV	



1 – metformin, 2 – caffeine, 3 – sulfamethoxazole hydroxylamine, 4 – sulfamethoxazole, 5 – primidone (IS), 6 – nevirapine, 7 – prednisolone, 8 – valsartan, 9 – rifampicin, 10 – ee2, 11 – ivermectin.

Figure 4.2: LC-MS PDA chromatogram showing analyte separation observed at 233 nm for 6 mg L^{-1} analytical standard

4.3.5 Statistical Analysis

Statistical analysis for this study was performed using Microsoft Excel Analysis ToolPak. The analysis is expressed by means of a one-way ANOVA. The concentrations of selected pharmaceuticals were compared against the various sampling campaigns to determine whether there were statistically significant differences between sampling campaigns. Significant differences are recorded as a factor of $p < 0.05$.

4.4 Results and Discussion

4.4.1 Method Development and Validation

Parameters that were investigated to validate the extraction and quantification method includes linearity, limit of detection (LOD), limit of quantification (LOQ), percentage recovery, interday and intraday analyses – all of which are described in Table 4.3.

Table 4.3: Method validation data obtained for analytes of interest

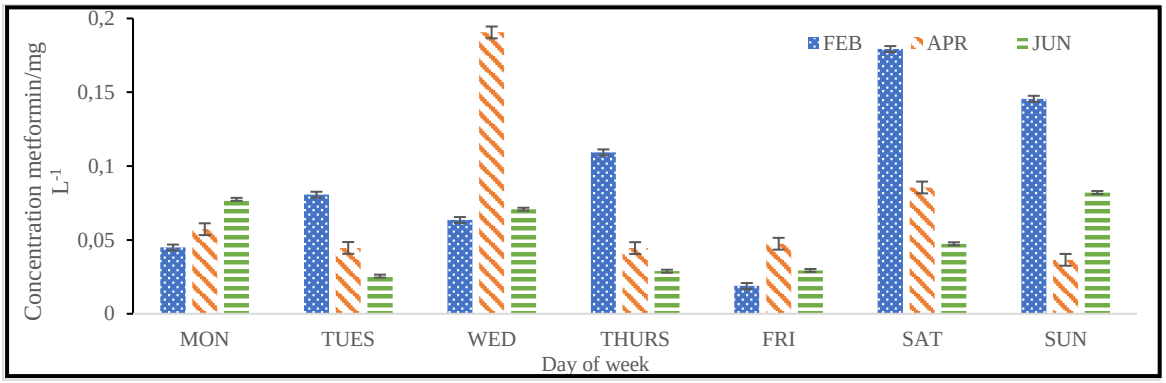
Pharmaceutical	LOD/ mg L^{-1}	LOQ/ mg L^{-1}	Recovery/ %	Interday/ %RSD	Intraday/ %RSD	R^2
Metformin	0.322	0.974	83.17	3.75	2.63	0.9909
Caffeine	0.033	0.099	86.42	2.32	1.47	0.9936
Sulfamethoxazole hydroxylamine	0.071	0.214	79.65	4.52	3.22	0.9957
Sulfamethoxazole	0.072	0.219	73.53	0.96	1.58	0.9942
Nevirapine	0.066	0.200	100.70	1.36	1.61	0.9971
Prednisolone	0.886	2.684	95.56	1.05	1.89	0.9970
Valsartan	0.220	0.667	89.61	1.84	1.91	0.9950
Rifampicin	0.062	0.187	82.33	0.56	1.31	0.9933
EE2	0.215	0.653	105.80	1.21	1.07	0.9938
Ivermectin	0.589	1.784	88.35	1.54	1.63	0.9952

The determination of LOD and LOQ values is necessary to identify the lowest analyte concentrations that can be detected and quantified by the instrument, respectively. In this study, LOD values ranged from 0.033 to 0.886 mg L^{-1}, while LOQ values ranged from 0.099 to 2.684 mg L^{-1}. The purpose of conducting recovery tests is to ensure the efficiency of the methods used for the extraction of selected analytes. Recoveries of analytes at pH 7 ranged from 73.53 to 105.8%. The acceptable recovery range outlined by ICH ranges from 80 – 120% (ICH, 2005). All analytes fell within this range with the exception of sulfamethoxazole and sulfamethoxazole hydroxylamine. EPA guidelines, however, requires analytes to be recovered within the range of 70 – 120%. Analytes from this study fell within the EPA accepted recovery range of 70 to 120%. The importance of interday and intraday analyses relates to the ability of an instrument to reproduce consistent data output and is expressed as percentage relative standard deviation (%RSD). To ensure reliable reproducibility, %RSD values for the interday and intraday analyses should be below 10% (UNODC, 2009). The results obtained are all below 5% which is indicative of consistency in the instrument's ability for the analysis performed in this study. R^2 values corresponding to linearity of calibration curves for each analyte were all 0.9909 and above. A value of 1.000 for linearity is ideal. The method validation

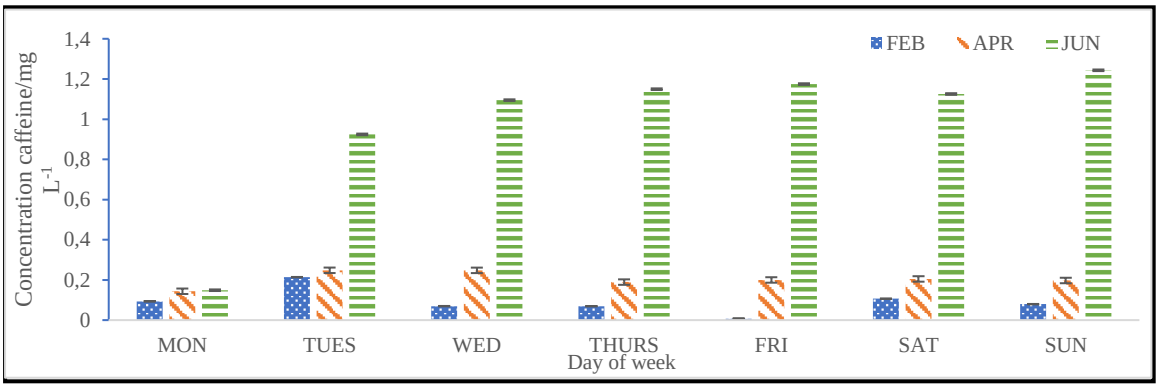
parameters shown in Table 4.3 are suggestive that the methods used for the extraction and analysis of analytes were suitable for the study.

4.4.2 Concentrations of Pharmaceuticals and Metabolites

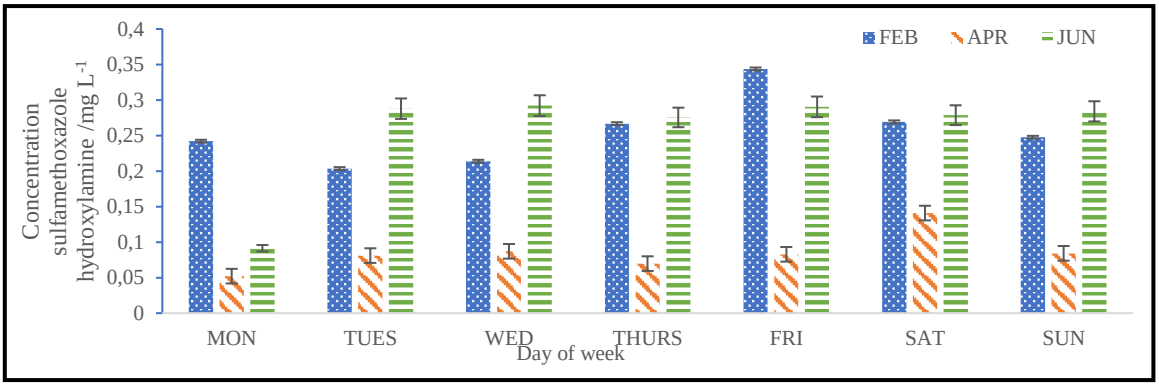
For this study, a total of ten pharmaceuticals were quantified in wastewater influent samples which represented eight different pharmaceutical classes. The results obtained are shown in Figures 4.3(a) to 4.3(j), which outlines the various influent concentrations quantified. Detailed results outlining tables of concentrations are included in Appendix B2. Major variations observed between inlet concentrations can be linked to numerous factors, some of which includes daily variations in the human consumption of these pharmaceuticals or pollution events between the three sampling periods.



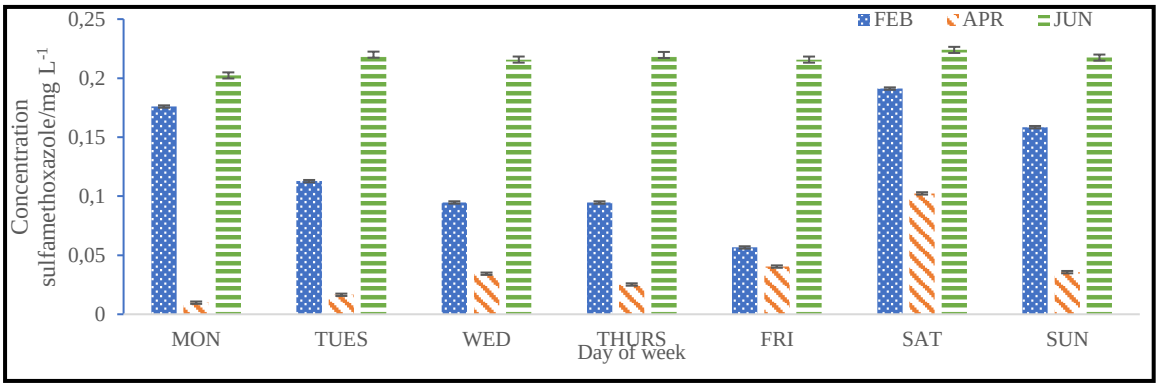
(a) Metformin



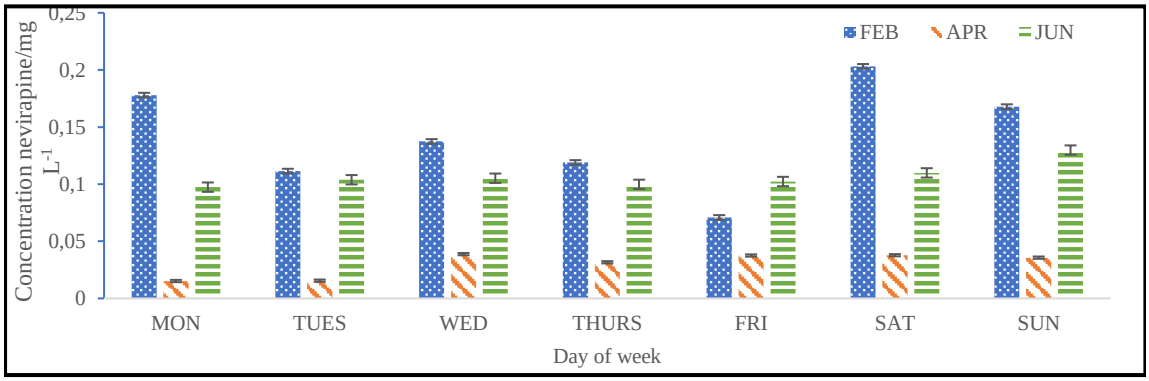
(b) Caffeine



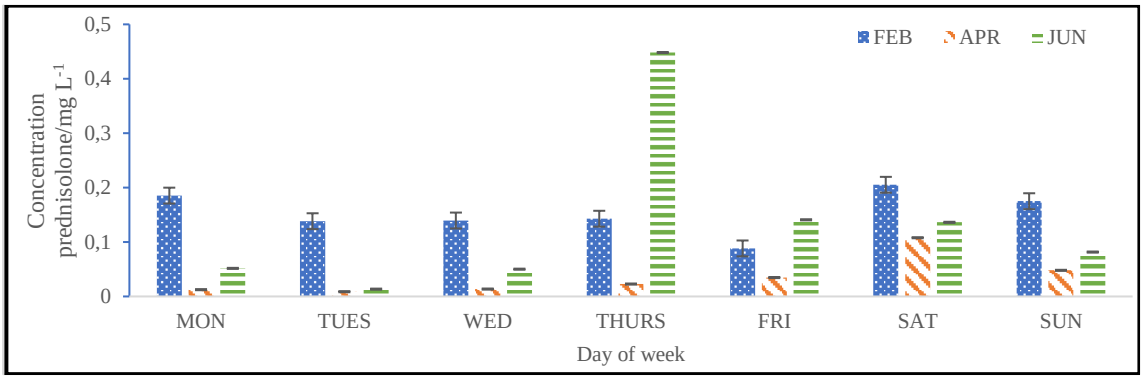
(c) Sulfamethoxazole hydroxylamine



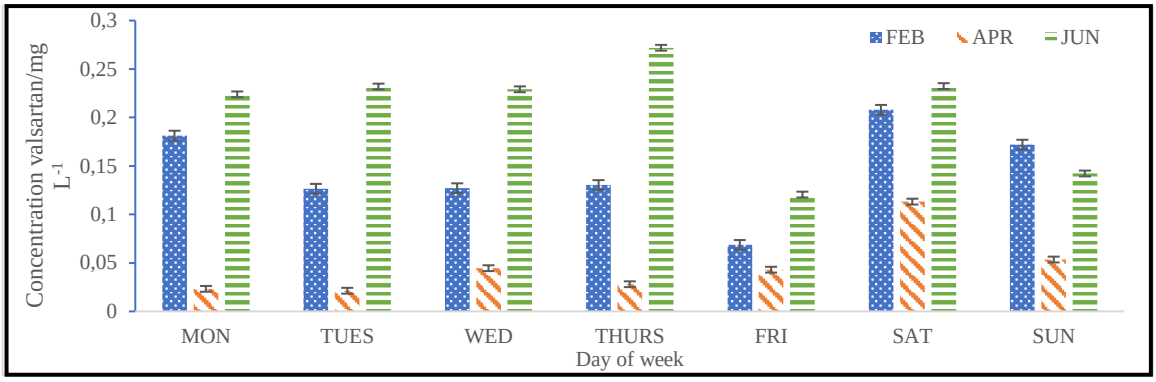
(d) Sulfamethoxazole



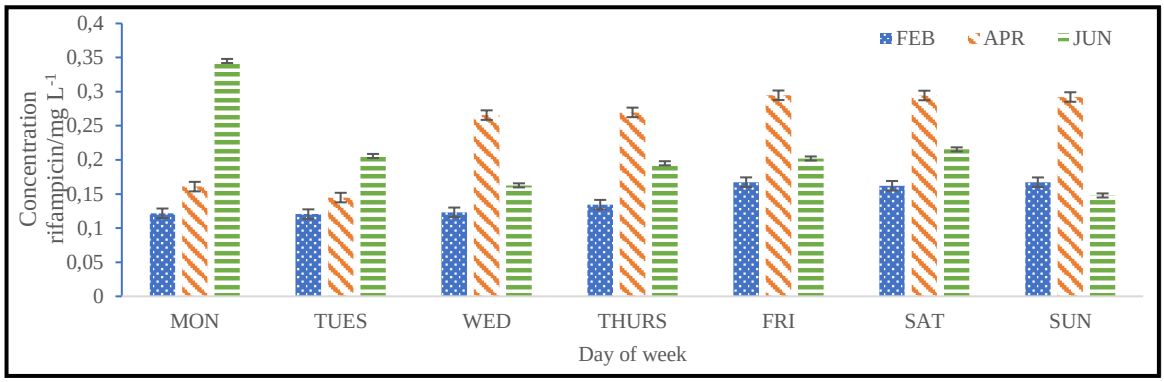
(e) Nevirapine



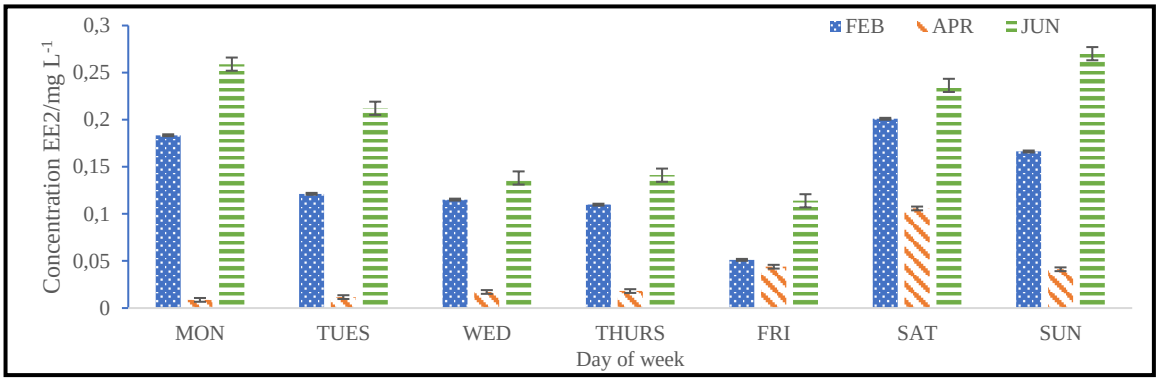
(f) Prednisolone



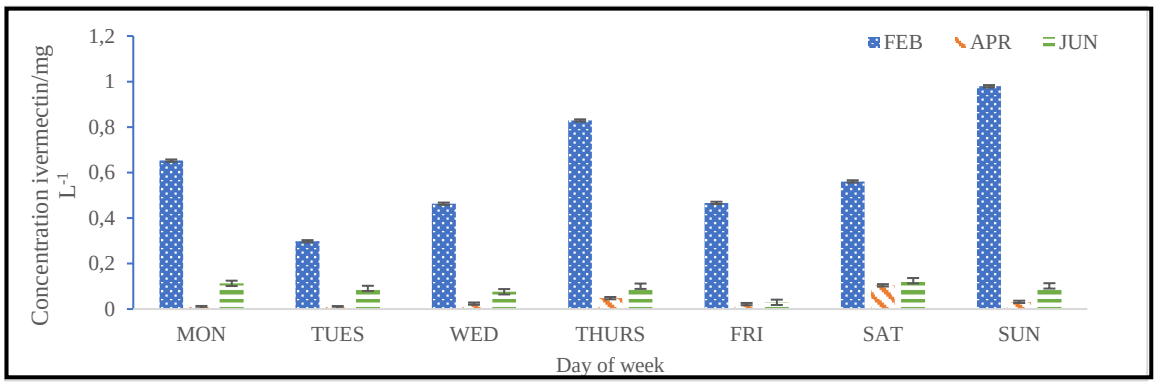
(g) Valsartan



(h) Rifampicin



(i) EE2



(j) Ivermectin

Figure 4.3: Concentrations of analytes quantified daily shown over three sampling seasons

4.4.2.1 Pharmaceuticals Prescribed for Chronic Illnesses

Pharmaceuticals such as metformin, nevirapine, valsartan and rifampicin are prescribed for chronic illnesses ranging from diabetes, HIV/AIDS, hypertension, and tuberculosis, respectively. Data observed in Figures 4.3(a), (e), (g) and (h), indicate quantified concentrations of each compound for each sampling season. Pharmaceutical concentrations throughout the week were relatively consistent, which is indicative of daily consumption. Overall, April 2022 samples showed lower pharmaceutical concentrations as compared to February and June 2022. This could be resultant of the April 2022 flood that was experienced in KwaZulu-Natal. Usual rainfall experienced in Pietermaritzburg during the month of April is approximately 40 mm however, an average of 200 mm of rainfall was recorded during the flooding period. Lower pharmaceutical concentrations could be explained by WWTP influent being saturated with rainwater and surface runoff as opposed to majority of the influent comprising household, hospital, and industrial waste. Over the three sampling periods, concentrations of metformin in wastewater influent ranged from 0.01883 to 0.1905 mg L^{-1}. The concentration spike observed for metformin, 0.1905 mg L^{-1}, in the Wednesday sample from the April 2022 sampling period could possibly be a consequence of incorrect or illegal disposal of the pharmaceutical. This is due to the irregularly high concentration that is observed in this sample when comparing to concentrations observed for other days within the April 2022 sampling period. Concentrations of rifampicin ranged from 0.1205 to 0.1674 mg L^{-1} in February, 0.1448 to 0.2947 mg L^{-1} in April and 0.1479 to 0.3449 mg L^{-1} in June. Additionally, the high concentrations of rifampicin observed daily during the April 2022 sampling period, illustrated in Figure 4.3(h), could be attributed to incorrect usage or improper disposal of expired or unused medication. This could be explained by the metabolism of rifampicin. Rifampicin is largely metabolised by the human body and a relatively low percentage is excreted as the unchanged drug. Concentrations of rifampicin quantified during February and June 2022 sampling trips were significantly lower in comparison to April 2022, immediately following the KwaZulu-Natal flooding. Concentrations of all other pharmaceuticals were found to be lower during this time and this was due to the substantially higher influx of storm and surface water runoff. Therefore, improper disposal potentially combined with significantly improper usage could have potentially led to the noticeable difference in concentrations between April samples compared to February and June samples. Additionally, many homes and buildings were washed away as a result of the severe flooding that was experienced. This could have led to unused medication belonging to patients being washed away in the process.

The prescribed term for the treatment of tuberculosis is a period of six months. An overall increase in concentrations observed between February and June samples would be indicative of more people being diagnosed with tuberculosis and thus being on rifampicin treatment. Therefore, the sudden increase observed in April and then an immediate drop in concentration following in June would unlikely be a result of human usage as the recommended treatment period for the disease is six months.

4.4.2.2 Stimulants and Self-Prescribed Pharmaceuticals

Caffeine is found in beverages such as tea, coffee, energy drinks and soft drinks, as well as chocolates and other snacks. The concentrations of caffeine, outlined in Figure 4.3(b), shows a steady increase in caffeine consumption as seasons transition from warm (February) to cold (June). Concentrations ranged from 0.00759 to 0.2138 mg L^{-1} in February and 0.1488 to 1.2432 mg L^{-1} in June. A decrease in temperature usually leads to an increase in consumption of hot beverages such as tea and coffee, intending to warm the body and provide a sense of comfort. EE2, is a synthetic estrogen that is used in almost every contraceptive pill (Mastrup et al., 2005). Concentrations obtained for EE2, depicted in Figure 4.3(i), shows consistent daily usage, which is prescribed within this class of pharmaceutical. The highest concentrations of EE2 can be observed in the month of June 2022, which falls within the winter season in South Africa. Concentrations ranged from 0.1139 to 0.2701 mg L^{-1}. This could be explained by referring to data published by Stats SA which indicates that 876 435 babies were born and registered for the year 2016 (Stats SA, 2017). From the data set, it was found that the months of March and September had the highest births compared to other months of the year. This implies that increased conception was found in June, which is winter, and December, which is the festive season (Stats SA, 2017). The data obtained in this study verifies the information given by Stats SA, indicating that more babies are conceived in June. This would explain the significant increase in the use of contraceptives observed, as there would be a rise in sexual activity during this time of the year. Many females would begin to take contraceptive medication during this time however, contraceptive medications are only effective if taken on a daily basis over a long period of time. The results from an ANOVA test performed for caffeine and EE2 indicated that each pharmaceutical showed significant seasonal differences in concentrations, by means of $p < 0.05$. Caffeine had a p-value of 9.65×10^{-7}, while EE2 had a p-value of 5.98×10^{-5}.

4.4.2.3 Pharmaceuticals Used for the Treatment of Influenza and COVID-19

Figure 4.4 outlines the total active number of COVID-19 cases experienced in South Africa since the start of the global pandemic (World-O-Meter, 2022). In South Africa, there were a total of five COVID-19 infection waves since the start of the pandemic up to July 2022. Table 4.4 shows the total number of active cases that were recorded during the sampling trips. It is important to note that during the February and June 2022 sampling periods there was an observed decrease in COVID-19 cases as the week progresses (i.e., from Monday to Sunday) which corresponds to a decline in the wave, while in April 2022 an increase in COVID-19 cases is observed as the week progresses, which corresponds to an incline observed in the wave.

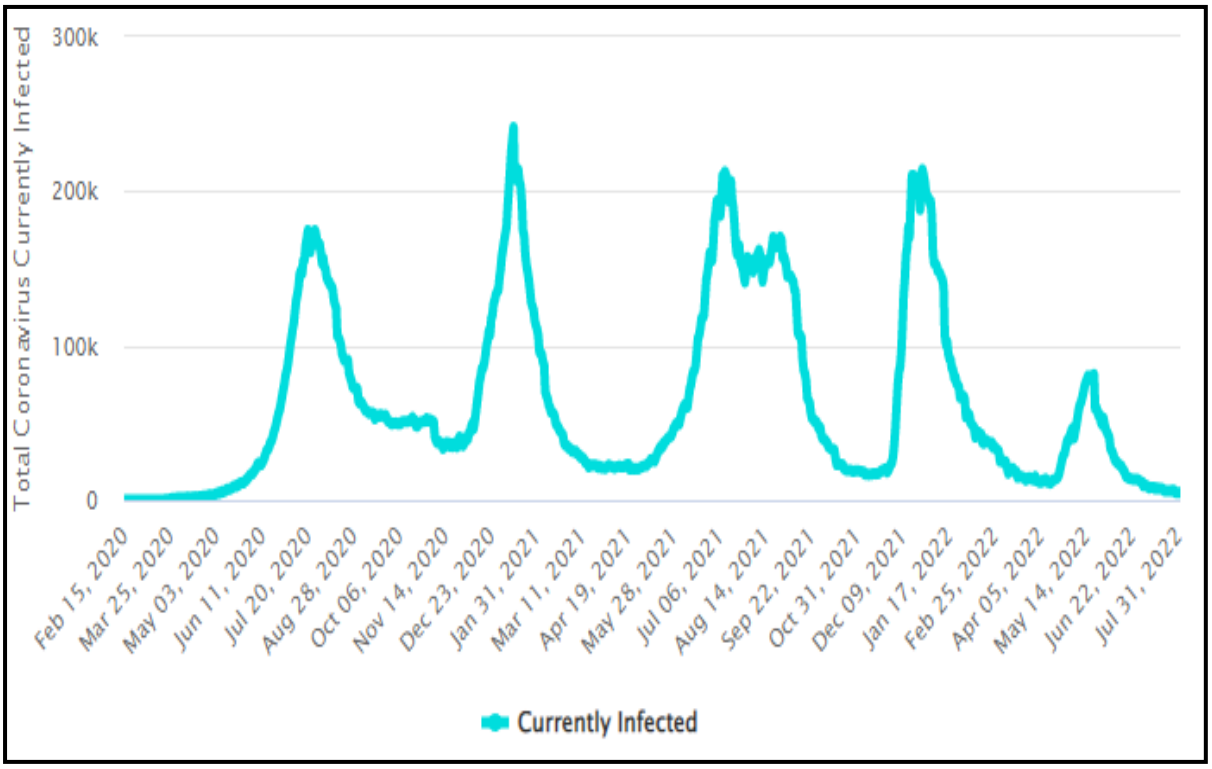


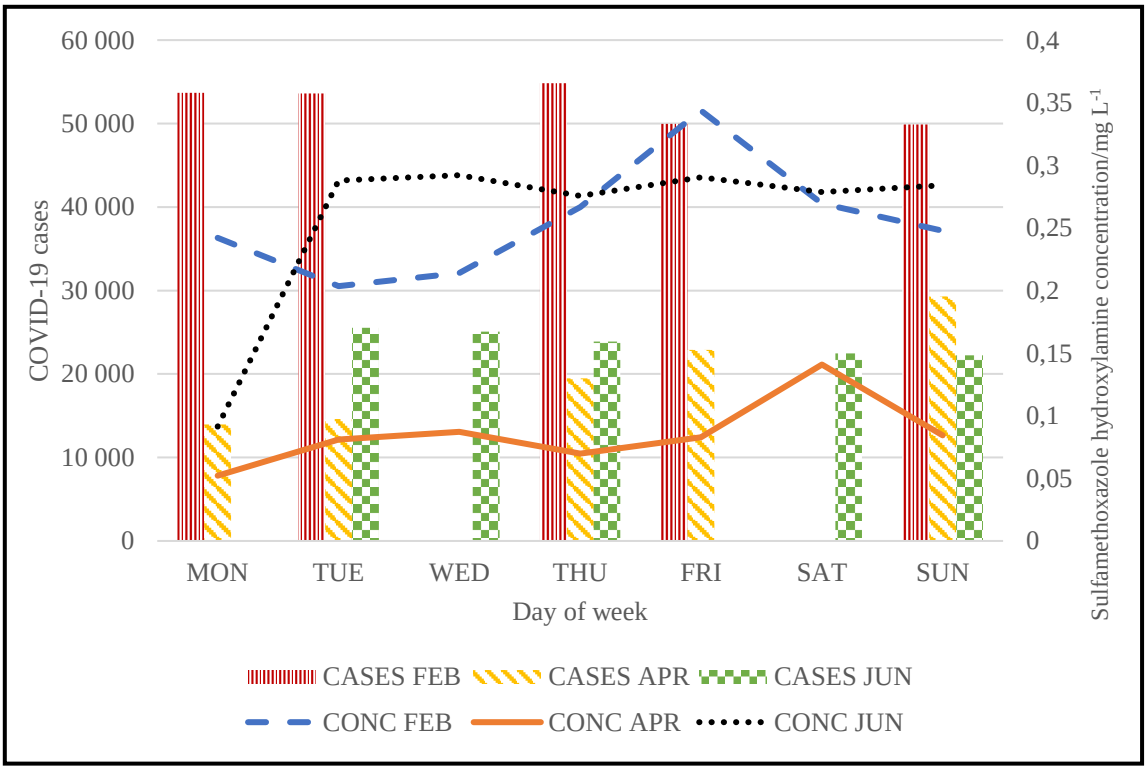
Figure 4.4: Graphical representation of total active COVID-19 cases in South Africa (World-o-Meter, 2022)

Table 4.4: Total number of active COVID-19 cases in South Africa during sampling periods (World-o-Meter, 2022)

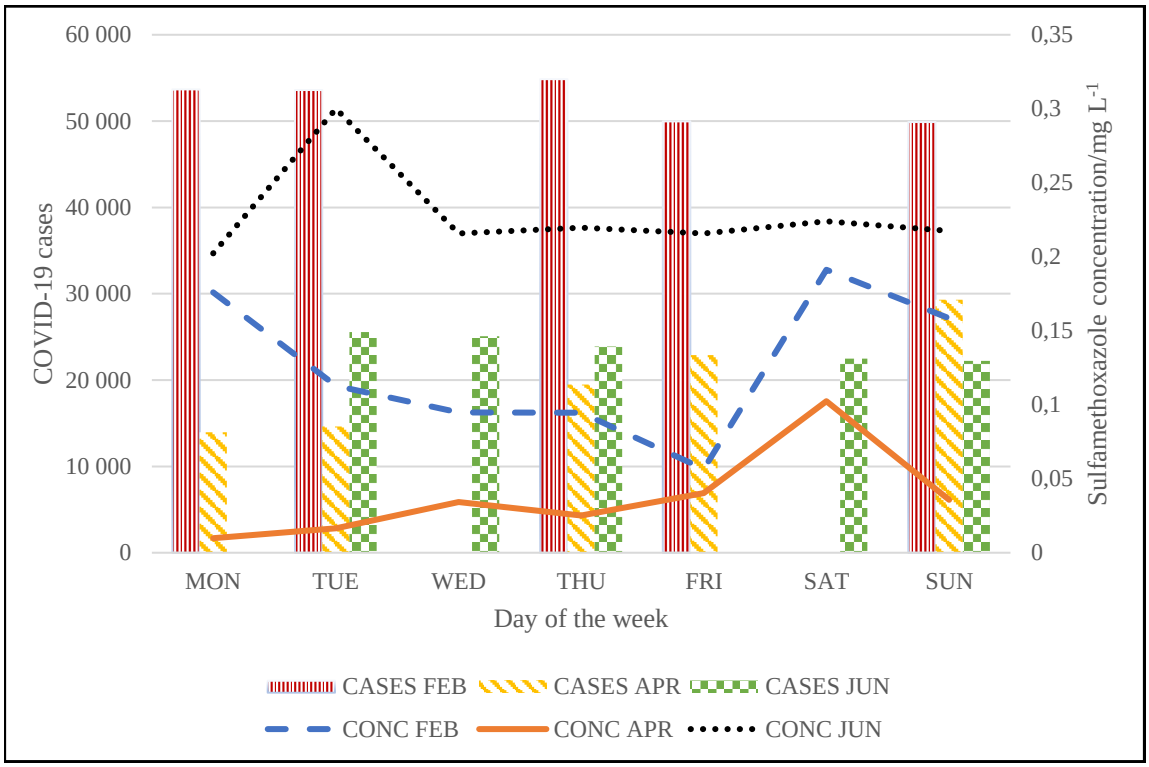
Day of week	Total number of active cases recorded		
	31 January – 6 February 2022	18 – 24 April 2022	6 – 12 June 2022
Monday	53 668	13 957	–
Tuesday	53 601	14 611	25 556
Wednesday	–	–	25 065
Thursday	54 849	19 472	23 897
Friday	49 967	22 891	–
Saturday	–	–	22 482
Sunday	49 878	29 311	22 233

–: No data available.

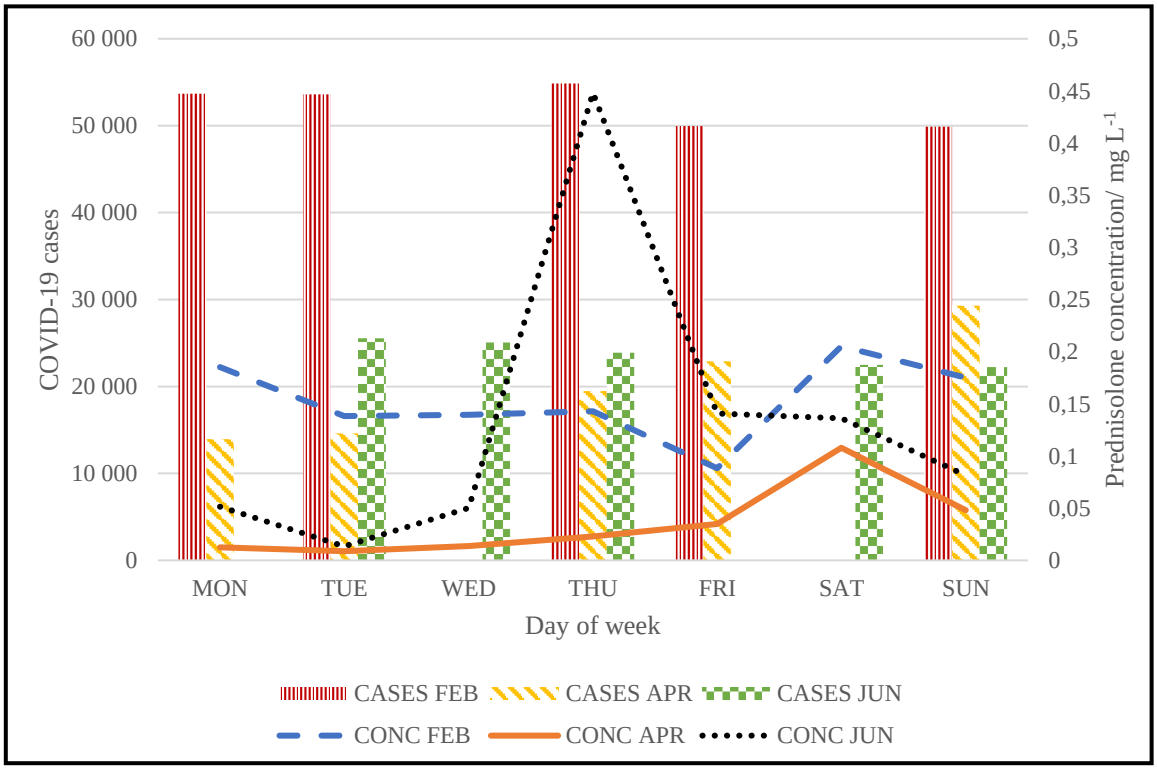
Collating the data obtained from Figures 4.3(c), (d), (f) and (j) with the data shown in Table 4.4, Figure 4.5 depicts the relationship that exists between pharmaceutical concentration and COVID-19 cases experienced during sampling periods.



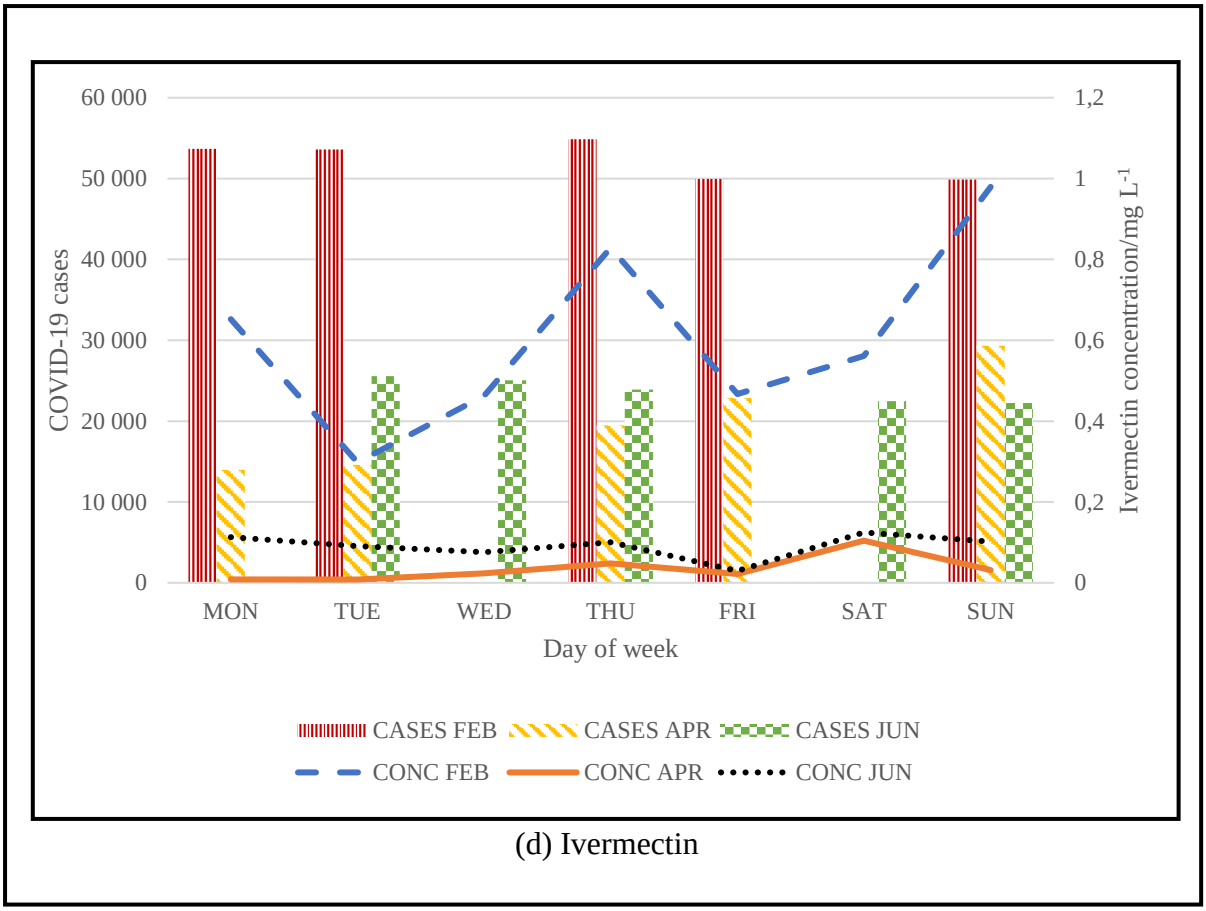
(a) Sulfamethoxazole hydroxylamine



(b) Sulfamethoxazole



(c) Prednisolone



(d) Ivermectin

Figure 4.5: Relationship between pharmaceutical concentration and COVID-19 cases

From the data illustrated in Figure 4.3(a) and (b), the samples taken in February 2022 were found to have had substantially high concentrations of antibiotics – sulfamethoxazole hydroxylamine and sulfamethoxazole, which are metabolite and parent compound, respectively. Concentrations during this period of time for sulfamethoxazole hydroxylamine and sulfamethoxazole ranged from 0.2035 to 0.3438 mg L^{-1} and 0.05661 to 0.1912 mg L^{-1}, respectively. This correlates to the data shown in Table 4.4, which indicates that the higher number of active COVID-19 cases were during February, when compared to the other two sampling months. The concentrations of hydroxylamine sulfamethoxazole and sulfamethoxazole quantified in June 2022 were comparable to that found in February 2022, as shown in Figures 4.5(a) and (b), albeit the total number of active COVID-19 cases were substantially lower. Concentrations found for the parent compound ranged from 0.2023 to 0.3061 mg L^{-1} and metabolite concentrations were between 0.09136 and 0.2921 mg L^{-1}. This can be attributed to June being a colder month and the start of the influenza season experienced in South Africa. An increase in the prescription of sulfamethoxazole containing antibiotics used to treat influenza would have contributed to the high concentrations found in wastewater

samples. Prednisolone concentrations, depicted in Figure 4.3(f), follows a similar usage trend when comparing results to the curve shown in Figure 4.4. Prednisone was predominantly used for the treatment of COVID-19, this was confirmed as the highest concentrations were found in February 2022 (0.0883 to 0.2052 mg L^{-1}), corresponding to the highest number of active cases recorded. A rapid decline in usage is observed in April 2022, which directly corresponds to the lowest number of active cases recorded for this period of time. Thereafter, a slight rise in concentration is observed as the COVID-19 cases start to pick up again in June 2022 (0.01343 to 0.4482 mg L^{-1}). This is illustrated in Figure 4.5(c). The 0.4482 mg L^{-1} prednisolone outlier observed within Figure 4.3(f) could be resultant of irregular usage, as prednisone tablets will have to be internally metabolised by the human body in order to become prednisolone, which was detected. Concentrations of ivermectin found during sampling trips, illustrated in Figure 4.3(j), shows direct correlation to the data shown in Figure 4.4 and Table 4.4. As shown in Figure 4.5(d), the highest observed concentrations were found in February 2022, ranging from 0.2979 to 0.9794 mg L^{-1}. A major decrease in ivermectin usage is observed as the COVID-19 fifth wave approached. Concentrations quantified for April ranged from 0.00818 to 0.1043 mg L^{-1}, while June concentrations ranged from 0.07600 to 0.1246 mg L^{-1}. This could be due to the fewer number of active cases recorded for this period as well as the prohibition of the use of ivermectin as per SAHPRA. Furthermore, the low concentrations observed could be due to the fact that most people were now vaccinated against COVID-19 as well as milder COVID-19 cases were being experienced as the virus continued to mutate. The ANOVA test was performed for each pharmaceutical within this category. Sulfamethoxazole hydroxylamine, sulfamethoxazole and ivermectin all showed significant differences between seasons as $p < 0.05$. P-values obtained were as follows, sulfamethoxazole hydroxylamine, $p = 6.85 \times 10^{-6}$; sulfamethoxazole, $p = 1.47 \times 10^{-7}$ and ivermectin, $p = 4.37 \times 10^{-7}$. Prednisolone showed no significant difference as $p = 0.05579$, which is greater than the significance level of 0.05. This is resultant of the single outlier obtained during the Thursday sample from the June sampling campaign. The value shown on the graph in Figure 4.3(f) shows concentrations in agreement with each other, with the exception of the June Thursday sample, which classified it as an outlier. If the Thursday outlier was instead a result within the concentration range for the sampling campaign, then the ANOVA test would indicate a significant difference between the different sampling campaigns, i.e., $p < 0.05$.

4.5 Conclusions

This study aimed to quantify targeted pharmaceuticals and metabolites present in wastewater influent and to provide a link between pharmaceutical usage, COVID-19 infections, and the change in seasonal weather temperatures. Results obtained showed direct correlation between the consumption of pharmaceuticals and the total number of COVID-19 cases reported. Pharmaceuticals prescribed to treat chronic ailments were found to have consistent daily usage, as required with this class of compounds.

4.6 References

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CHAPTER 5: REMOVAL EFFICIENCIES AND ENVIRONMENTAL RISK ASSESSSMENT OF SELECTED PHARMACEUTICALS AND METABOLITES AT A WASTEWATER TREATMENT PLANT IN PIETERMARITZBURG, SOUTH AFRICA.

5.1 Abstract

This research study critically evaluates the concentrations of selected pharmaceuticals found within wastewater and at various treatment stages within the wastewater treatment plant. Generally, the analyte concentrations quantified within influent samples were higher than effluent samples which is indicative of the treatment process being able to partially remove some of the analytes. As expected, analyte concentrations were observed to decrease as wastewater went through the treatment process with the greatest reduction in analyte concentrations observed between secondary effluent and maturation samples, which could be indicative of photodegradation of compounds. Influent concentrations of analytes ranged from 0.008184 to 1.243 mg L^{-1} while maturation river concentrations ranged from not detected to 0.9286 mg L^{-1}. Removal efficiencies ranged from 7.70% to >99.99% of analyte removed. Environmental risk assessment ratios ranged from 0.0863 to 10.5 x 10^8. Ratios for metformin and valsartan were well below 1, indicating that they would not be of environmental risk. While ratios for ivermectin were substantially high, ranging from 25.4 x 10^7 to 10.5 x 10^8 indicating that it is of severe environmental risk and is of significant concern.

5.2 Introduction

Emerging contaminants (ECs) are described as chemical compounds that have adverse effects on the health of humans, animals, and the environment. Despite their negative impact, these compounds are still essentially unregulated, and their fate in the environment is still not sufficiently understood (O' Flynn *et al*., 2021). ECs are inclusive of pharmaceuticals, hormone regulating medications and their relative metabolites. The effects of human influence in combination with these EC's, have detrimental consequences on surface water as stated in previous research (Archer, 2018, Aris *et al.,* 2014).

Pharmaceuticals are synthesised to be stable by nature and exhibit their required biological effects at relatively low concentrations – characteristics of which consequently leads to

environmental persistence. A major source of pharmaceutical contamination in surface water is from effluent discharged from wastewater treatment plants (WWTPs). The rate at which these pharmaceuticals enter the surface water, is much higher than the rate of degradation and this leads to a pseudo-persistence (Archer, 2018). This phenomenon is the result of rapid urbanisation and exponential population growth which has caused wastewater treatment plants to operate beyond their original design capacity. Globally, municipal WWTPs primarily deal with wastewater arising from domestic, medical, and industrial sources however, it is common for stormwater runoff to also be treated at WWTPs.

The most common type of WWTPs situated in urban areas make use of the activated sludge treatment method for the removal of compounds such as nutrients and inorganics. This method is efficient as it ensures high removal of nutrients, organic matter as well as suspended solids at relatively low operational costs (O 'Flynn *et al.*, 2021). However, this treatment process is not specifically tailored to remove pharmaceuticals and other ECs from wastewater, resulting in their presence in the treated effluent from WWTPs. Removal efficiencies observed at various WWTPs depend on numerous factors, some of which includes the physio-chemical properties of the compound, climate conditions (such as temperature, sunlight intensity and rainfall), operational conditions of the treatment process (relating to temperature, redox conditions, and retention times) in addition to the age and condition of the activated sludge (Gracia-Lor, 2012). Prior studies undertaken by Mines *et al.*, 2007, showed strong correlations between rainfall intensity and WWTP flow rates thus, indicating the extent to which meteorological conditions influence the treatment processes conducted at WWTPs. As a result of these factors, removal efficiencies are observed to vary significantly among WWTPs, as well as within a specific plant at different time intervals (Gracia-Lor, 2012).

Although the vast majority of research done globally shows various ECs to be present in wastewater and receiving water bodies, the fate and impact that they have within treatment plants and environmental water bodies are still inadequately described. This study aims to identify and quantify analyte concentrations within various stages of the wastewater treatment process and hence determine relative removal efficiencies. Additionally, the study aimed to undertake an environmental risk assessment on the selected analytes and to determine whether they are of environmental concern. Analytes selected for this study included pharmaceuticals used to treat various medical conditions that are prevalent in South Africa. These pharmaceuticals and their chemical properties are outlined in Table 5.1.

Table 5.1: Chemical properties of pharmaceuticals selected for this study

Class	**Compound**	**Molecular formula[a]**	**Molar mass[a]/ g mol^{-1}**	**Melting point[a]/^{o}C**	**Boiling point[b]/^{o}C**	**Solubility[a]/mg mL^{-1}**
Diabetes	Metformin	$C_4H_{11}N_5$	129.16	224.5	172.5	1.38
Stimulant	Caffeine	$C_8H_{10}N_4O_2$	194.19	236.5	178.0	16
Antibiotic	Sulfamethoxazole hydroxylamine	$C_{10}H_{11}N_3O_4S$	269.28	130.0	502.2	-
	Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.28	169.0	482.1	0.50
Antiretroviral	Nevirapine	$C_{15}H_{14}N_4O$	266.30	248.0	415.4	0.00071
Endocrine disruptors	Prednisolone	$C_{21}H_{28}O_5$	360.44	235.0	570.6	0.223
	17α-ethinylestradiol	$C_{20}H_{24}O_2$	296.40	143.0	457.2	0.0113
Hypertension	Valsartan	$C_{24}H_{29}N_5O_3$	435.52	116.5	85.5	0.00141
Tuberculosis	Rifampicin	$C_{43}H_{58}N_4O_{12}$	822.94	185.5	937.0	1.30
Anthelmintic	Ivermectin	$C_{47}H_{72}O14$	875.10	155.0	940.4	0.004

[a](PubChem), [b](ChemSpider), -: No data available.

5.3 Materials and Methods

5.3.1 Chemicals and Reagents

Analytical standards purchased from Sigma Aldrich, South Africa and DLD Scientific, included metformin, caffeine, sulfamethoxazole hydroxylamine, sulfamethoxazole, primidone (internal standard), nevirapine, prednisolone, valsartan, rifampicin, 17α-ethynylestradiol (EE2) and ivermectin. Ultra-pure type 1 Millipore water (resistivity of 18.2 MΩ.cm at 25°C) was acquired from a Synergy ® UV water purification system. HPLC grade methanol, ammonium acetate and acetic acid was purchased from Sigma Aldrich, South Africa. Oasis® HLB cartridges (60 mg, 3 cc) were purchased from Microsep (Pty) Ltd.

5.3.2 Study Site and Sample Collection

The study site for this research endeavour was a Wastewater Treatment Plant, situated in Pietermaritzburg, KwaZulu-Natal, South Africa. This WWTP has a mean influent flow rate of 100 ML day^{-1} and services the immediate Pietermaritzburg population. Wastewater samples were collected once daily from four sampling sites within the plant, over a period of seven consecutive days. Samples were taken over three sampling campaigns: February 2022, April 2022, and June 2022. Sampling points included the inlet, balancing tank, secondary effluent, and maturation river. A simple schematic diagram illustrating the basic stages of wastewater treatment followed by the WWTP is shown in Figure 5.1. Upon collection, wastewater samples were acidified with three drops of acetic acid and were kept on ice during transportation to the laboratory. Once at the laboratory, samples were stored in the fridge at 4 ℃. Filtration and extraction of all samples were done within two weeks of sample collection.

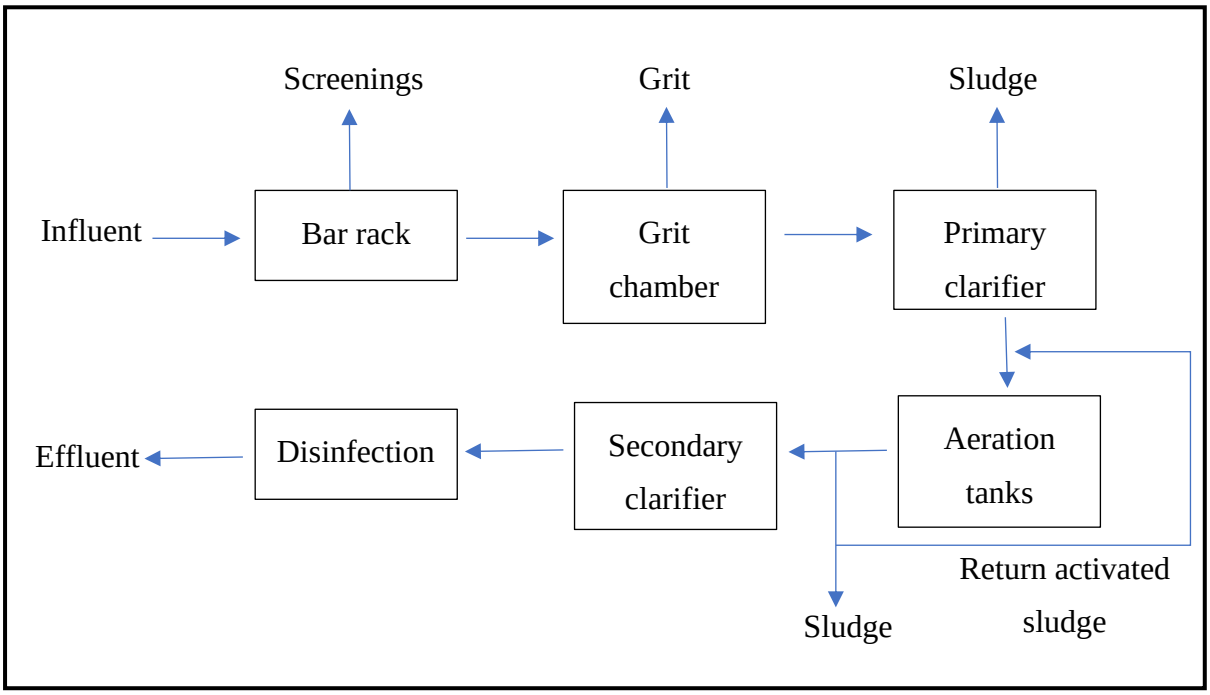


Figure 5.1: Simple schematic showing basic stages of wastewater treatment

5.3.3 Solid Phase Extraction (SPE) of Analytes of Interest

Wastewater samples were initially gravity filtered using Whatman No. 40 filter paper to remove larger particles, such as scum. Thereafter, a volume of 50 mL was further filtered using a 0.45 µm filter disc.The pH of the resulting sample was adjusted to 7. A pH of 7 was chosen for extraction as prior studies have shown it to be an optimum pH for the extraction of these analytes (Archer *et al.*, 2017). An Oasis® HLB cartridge was used to conduct SPE. Cartridges were conditioned with 2 mL of methanol and followed by 2 mL of ultra-pure Millipore water. The double filtered water sample was then loaded onto the cartridge and was allowed to pass through at a flow rate of approximately 2 mL/min. SPE cartridges were then dried under vacuum for a period of approximately 15 minutes to ensure that the eluate was allowed sufficient time to elute. Extracts were then eluted with 4 mL of methanol into amber glass vials. The 4 mL of methanol was added in two batches on 2 mL each. Upon the first 2 mL elution, the methanol was evaporated under vacuum and the final 2 mL was then added. The eluate was evaporated to dryness under vacuum and thereafter reconstituted in 1 mL using a 6 mg L^{-1} standard solution, which was used to spike the samples.

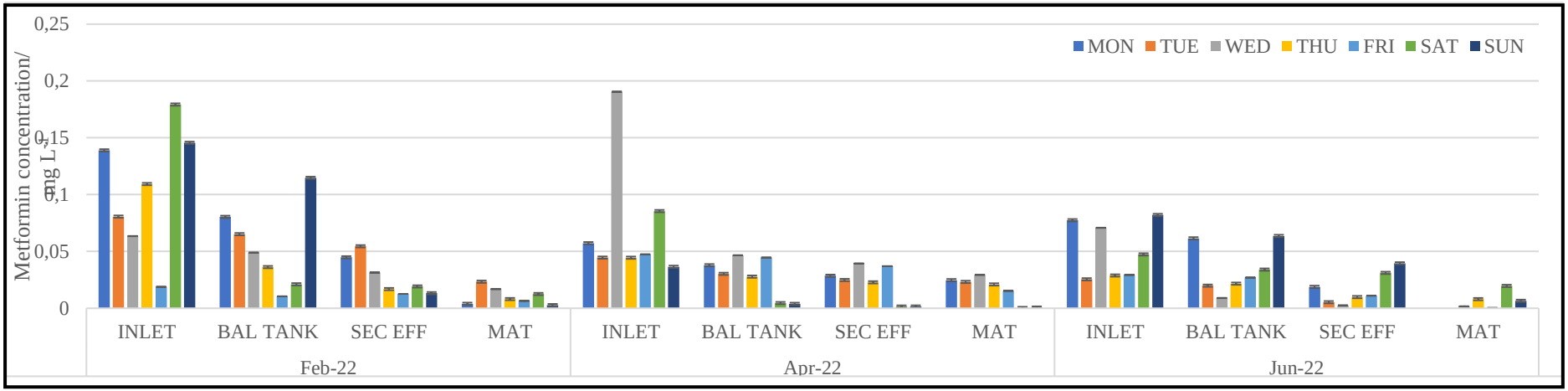
5.3.4 Liquid Chromatography – Mass Spectrometry (LC -MS) Analysis of Analytes of Interest

A Shimadzu LC – MS – 2020 system was used for the analysis of targeted analytes following a method adapted from Archer, 2018. A Shimadzu Shim-pack GIST reversed phase C18 column was used, with 2.1 x 100 mm dimensions and 3 µm particle size. Mobile phases used for the analysis were (A) ultra-pure Millipore water consisting of 5 mM ammonium acetate and 3 mM acetic acid and (B) 100% high pressure liquid chromatography (HPLC) grade methanol. The mobile phase flow rates and injection volumes were kept consistent at 0.22 mL min^{-1} and 5 µL, respectively. Method blanks containing methanol were inserted after each sample run. Gradient elution was performed by the following method: 0 – 1 minute: 20% B isocratic hold; 1 – 18.50 minutes: from 20% B to 90% B; 18.50 – 22.50 minutes: 90% B isocratic hold; 22.50 – 25 minutes: 90% B – 20% B; 25 – 27.50 minutes: 20% isocratic hold to recondition the column to initial conditions. The mass spectrometer had a single quadrupole mass analyser and electrospray ionisation. The ionisation chamber and detector voltages were -3.50 kV and -1.45 kV, respectively, with an ion source temperature of 200 ℃.

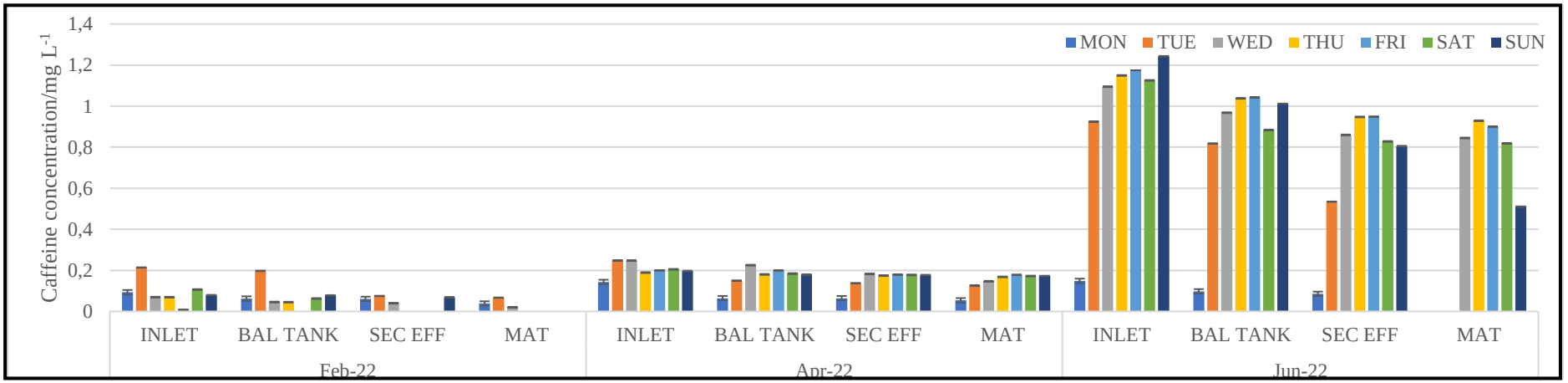
5.4 Results and Discussion

5.4.1 Concentrations of Analytes Within the Various Stages of the Treatment Process

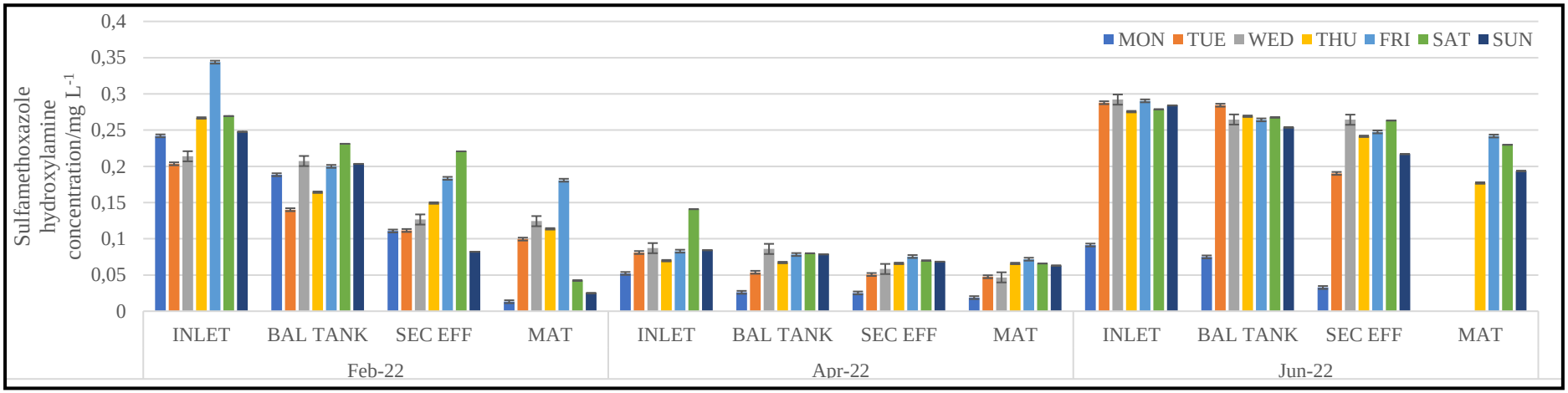
Wastewater samples were collected once a day over a period of seven consecutive days. Figure 5.2(a) to (j) illustrates analyte concentrations at stages of the treatment process that were sampled. Concentrations are expressed in mg L^{-1}. Detailed tables outlining concentrations of analytes are included in Appendix B2.



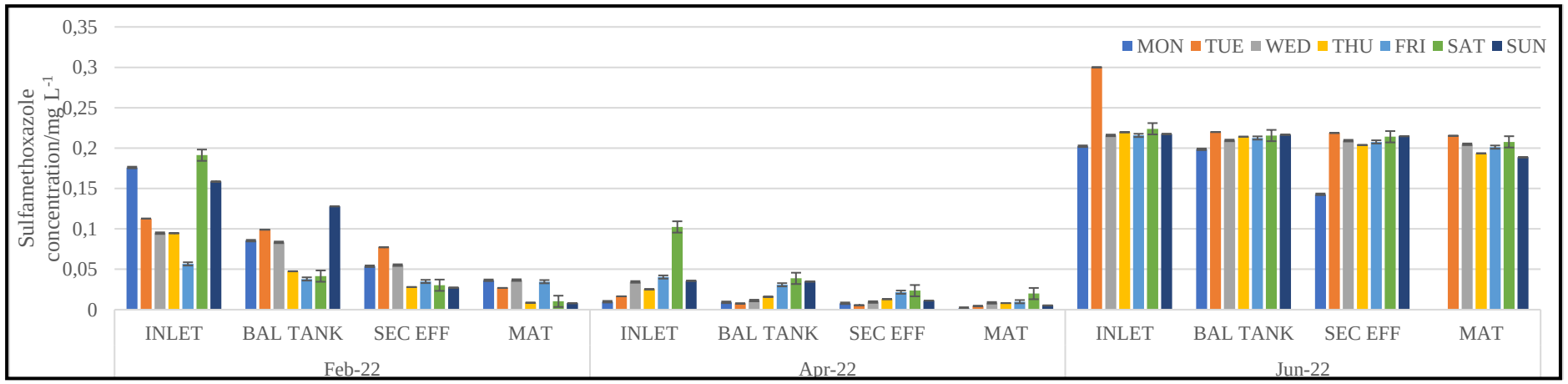
(a) Metformin



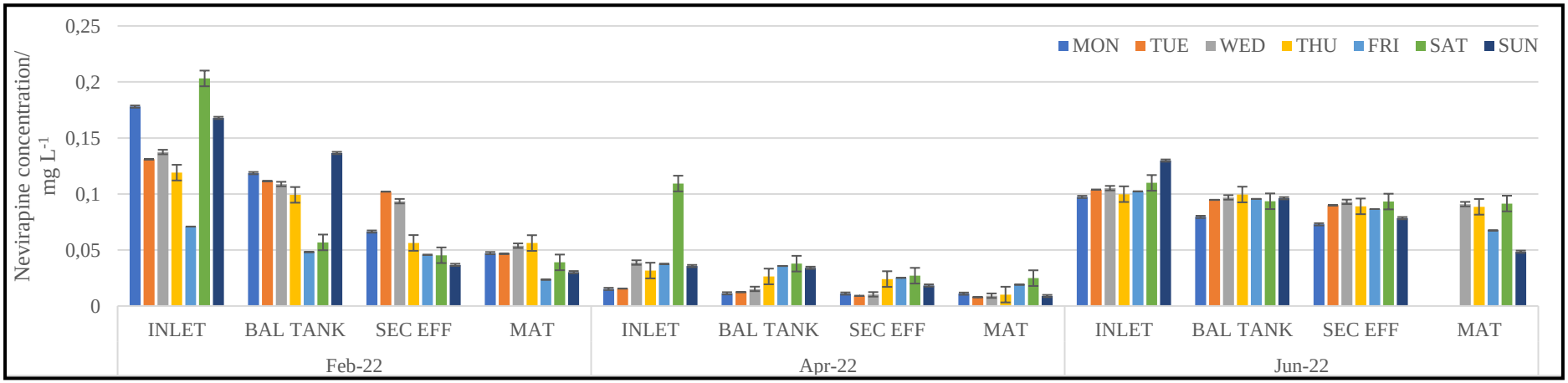
(b) Caffeine



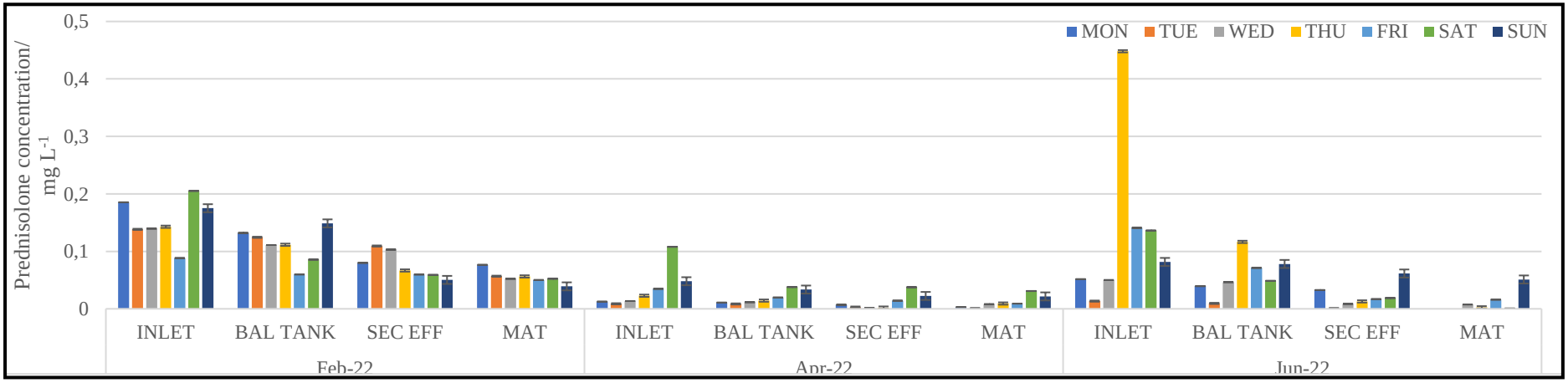
(c) Sulfamethoxazole hydroxylamine



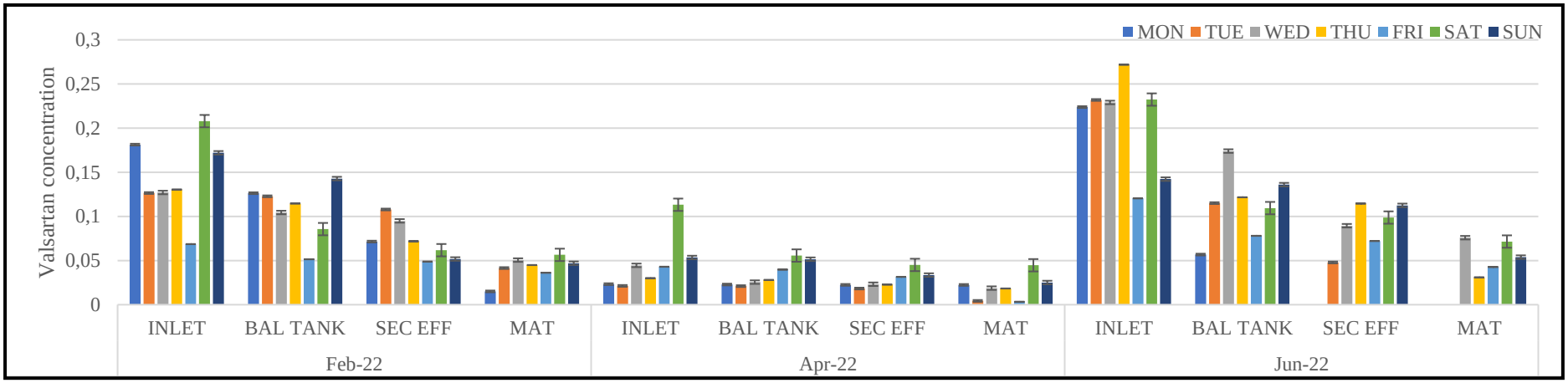
(d) Sulfamethoxazole



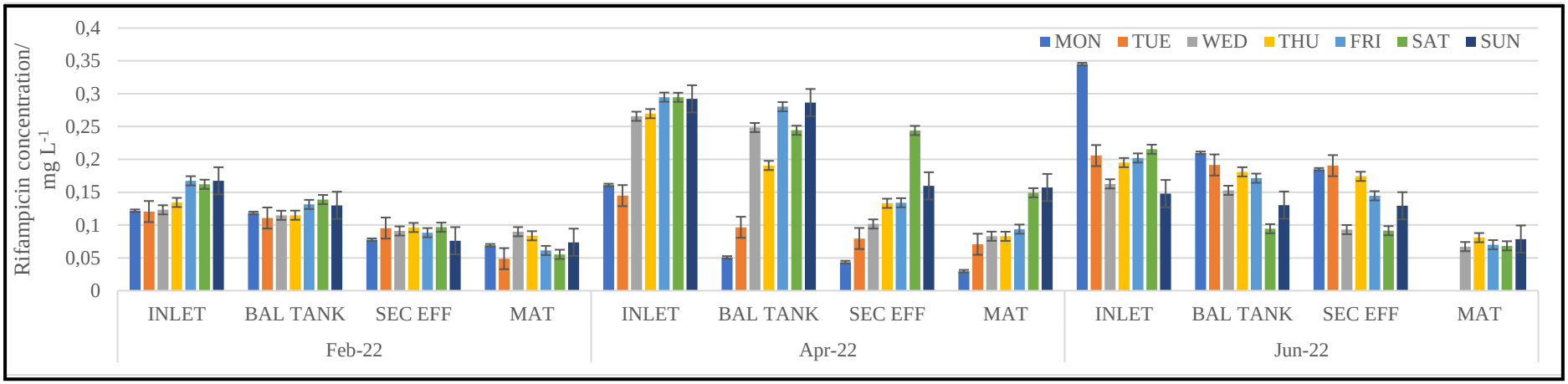
(e) Nevirapine



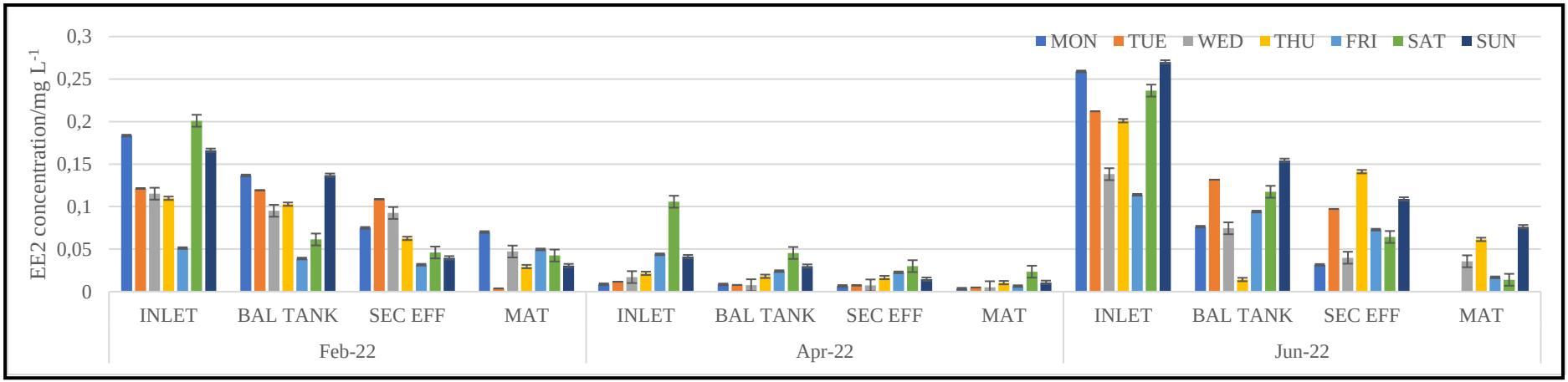
(f) Prednisolone



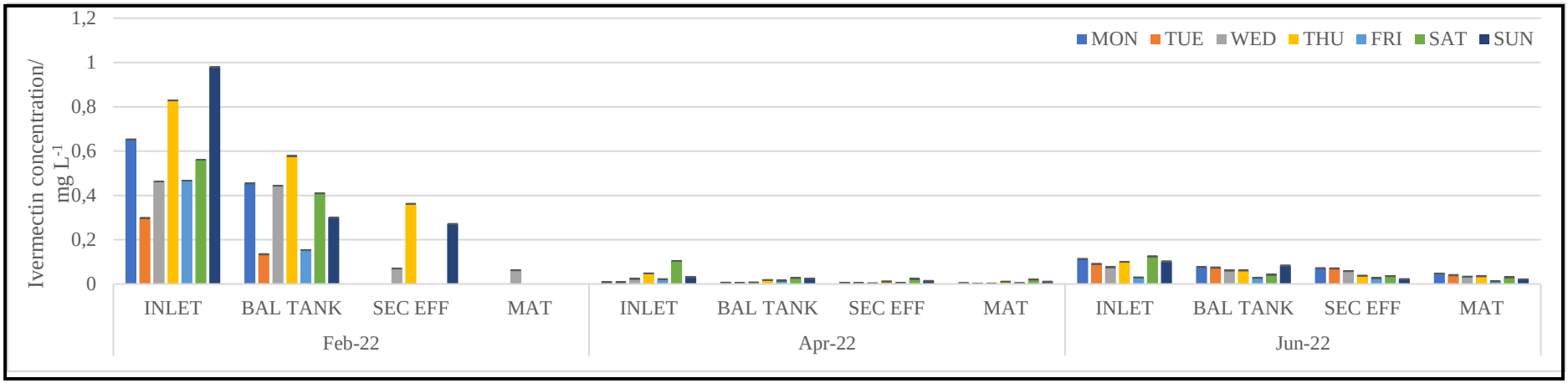
(g) Valsartan



(h) Rifampicin



(i) EE2



(j) Ivermectin

Figure 5.2: Trends observed for pharmaceutical concentrations within the plant process during sampling trips

There was an observable change in the concentration of the analytes between samples collected from the influent and effluent of the wastewater treatment plant. The highest concentrations of analytes were detected in the inlet samples, with only a slight decrease in the analyte concentrations of the wastewater samples collected from the balancing tank. The wastewater sampled from the secondary effluent showed a minimal reduction in concentration compared to that found in the balancing tank despite the wastewater being treated with activated sludge. This could be due to the sludge mainly being able to target the digestion of nutrients and the breakdown of organics such as proteins, fats, and carbohydrates, rather than complex chemical compounds such as pharmaceuticals (Navalon *et al.,* 2011). In most cases, a significant reduction in pharmaceutical concentration can be observed in the maturation river samples. This could be attributed to the treated wastewater being exposed to the elements of nature during its flow from the maturation river into the river to which it is discharged. As the maturation river flows, it is exposed to ultraviolet (UV) rays from the sun. This phenomenon could ultimately lead to photodegradation of many pharmaceuticals which is confirmed from the significantly lower concentrations found from this sampling site. A study undertaken by Kawabata *et al., (*2013), investigated the effects that sunlight has on a range of pharmaceuticals found within aquatic media. The results concluded that sunlight (predominantly UV-B radiation) was responsible for the partial degradation of several pharmaceutical compounds. The complete photodegradation of a few pharmaceuticals did occur however, the variation is majorly due to the chemical structure of the compounds and the presence of certain functional groups (Kawabata *et al.,*2013). At the WWTP, the maturation river flows for a few kilometres within a man-made cement, or equivalent, channel. This therefore creates an increased surface area for the photodegradation of pharmaceuticals. Adsorption or leaching into the soil and sediment surrounding the channel is a possibility however, if adsorption or leaching into sediment were to occur along the maturation river channel, it could happen at any point within the treatment process and not exclusively at this point as all the channels and pipes within the plant are made from similar materials. Further studies analysing soil and sediment from within the WWTP is an area for investigation.

Analyte concentrations quantified within the maturation river were significantly lower than that from the inlet however, the mere presence of certain compounds such as antibiotics and antituberculosis pharmaceuticals allows for the development of drug-resistant bacteria. Concentrations of sulfamethoxazole hydroxylamine (metabolite) and sulfamethoxazole (parent) detected in the maturation river samples ranged from not detected – 0.2298 mg L^{-1} and

not detected – 0.2152 mg L^{-1}, respectively. Whilst there are no published South African guidelines for these analytes in wastewater, prior studies have shown that antibiotics should ideally be present in concentrations below 1.0000 x 10^{-5} mg L^{-1} to avoid the development of resistant bacterial strains (Le Page *et al.,* 2017). Caffeine concentrations detected in the inlet samples ranged from 0.007587 – 1.243 mg L^{-1} and between not detected – 0.9286 mg L^{-1} in the maturation river. Caffeine at high concentrations has been found to have the potential to alter physiochemical features within soil and water organisms (Al-Janabi, 2011). Selected bacterial species use caffeine as a significant source of carbon to meet their nutritional requirements however, other species experience repressed growth rates and inhibited metabolic processes when in the presence of caffeine (Al-Janabi, 2011).

Concentrations of EE2 quantified within the inlet samples ranged from 0.008684 – 0.2701 mg L^{-1} compared to the maturation river sample concentrations of not detected – 0.07638 mg L^{-1}. Despite a significant decline in the concentration levels, the presence of EE2 in aquatic environments could potentially lead to the feminization of male aquatic organisms and infertility amongst the female population due to its ability to bioaccumulate (Aris *et al.,* 2014). This is also possible at concentration levels as low as ng L^{-1} and would ultimately lead to the extinction of various aquatic species over time as a result of the inability to reproduce. Concentrations of metformin, nevirapine, prednisolone and valsartan within the inlet samples ranged from 0.02550 – 0.1905 mg L^{-1}, 0.01522 – 0.2031 mg L^{-1}, 0.008775 – 0.4482 mg L^{-1} and 0.02129 – 0.2719 mg L^{-1}, respectively. While maturation river concentrations ranged from not detected – 0.02939 mg L^{-1} for metformin, not detected – 0.09092 mg L^{-1} for nevirapine, not detected – 0.07650 mg L^{-1} for prednisolone and not detected – 0.07589 mg L^{-1} for valsartan. The concentration of ivermectin quantified in the inlet samples ranged from 0.008184 – 0.9793 mg L^{-1} and for the maturation river samples from not detected – 0.06237 mg L^{-1}. Prior studies have shown that the bioaccumulation of ivermectin in the tissue of aquatic organisms can reduce motor activity, affect the rate of reproduction and growth (Ding *et al.,* 2001).

5.4.2 Mass Loads

Wastewater influent and effluent flow rates differ among treatment plants as well as within a single treatment plant at different times of the day. The flow rate is dependent on various factors such as, rainfall, variation in the population serviced by the WWTP and water usage by the population within the area. Mass loads are therefore calculated to compensate for the varying

flow rates at the treatment plant influent and effluent streams. It is calculated using equation 5.1 and represented as g day^{-1}. Calculated mass loads are outlined in Appendix C1.

Mass loads = calculated influent or effluent concentration x flow rate x 1000 Equation 5.1

The flow rate refers to the rate at which raw wastewater enters the treatment plant as well as the rate treated wastewater exits the plant on a daily basis, this is expressed as ML day^{-1}. Table 5.2 outlines the average daily influent and effluent flow rates recorded at the WWTP. Additionally, the daily average rainfall experienced in the area serviced by the WWTP, Pietermaritzburg, is also shown.

Table 5.2 Average daily influent and effluent flow rates, and rainfall data for sampling period

Date	Inflow/ML day^{-1} [a]	Outflow/ML day^{-1} [a]	Average rainfall [b]/mm
31/01/2022	90	86	0.0
1/02/2022	75	71	0.8
2/02/2022	86	82	2.8
3/02/2022	76	72	1.7
4/02/2022	83	79	15.4
5/02/2022	93	89	5.8
6/02/2022	103	99	4.9
18/04/2022	112	107	0.6
19/04/2022	112	107	0.0
20/04/2022	110	105	0.0
21/04/2022	105	101	0.0
22/04/2022	114	109	0.0
23/04/2022	90	85	0.2
24/04/2022	116	110	1.1
6/06/2022	66	63	0.1
7/06/2022	70	67	0.0
8/06/2022	67	64	0.0
9/06/2022	67	64	0.0
10/06/2022	76	72	0.0
11/06/2022	71	68	0.0
12/06/2022	67	64	0.0

[a]: Average daily inflow and outflow flow rates were provided by the Wastewater Treatment Plant.

[b]: (World Weather Online, 2022).

According to Table 5.2, rainfall potentially influences daily flow rate of wastewater into the WWTP. During June 2022, the lowest overall flow rates were observed with average weekly rainfall of 0.1 mm recorded. The highest flow rates were recorded for April 2022, ranging from 85 to 116 ML day^{-1}. However, a total of 1.9 mm of rainfall was recorded for this week. This could have resulted from the April 2022 flood experienced in KwaZulu-Natal, that occurred a week before samples were collected. An estimated average of around 200 mm of rainfall was

experienced in the province during this time and as a result, WWTPs would have experienced significantly higher inflow, mainly comprising of stormwater and surface water runoff. The sampling period during February 2022 was recorded to have had intermediate flow rates, when compared to that of April and June.

5.4.3 Removal Efficiencies

Removal efficiency, equation 5.2, indicates the amount of analyte that is removed during the treatment process and is expressed as a percentage. The abbreviations ML(influent) and ML(effluent) refers to the calculated mass load for an analyte in the influent and effluent stream, respectively.

$$\text{Removal efficiency} = \frac{\text{ML(influent)} - \text{ML(effluent)}}{\text{ML(influent)}} \times 100 \qquad \text{Equation 5.2}$$

Removal efficiencies calculated for each sampling period are outlined in Table 5.3. The weekly averages are also shown for each analyte to provide an overall representation of the removal efficiency of the treatment process.

Table 5.3: Removal efficiencies obtained for sampling periods

Date	Removal efficiency/%									
	MET	**CAF**	**SMX N-OH**	**SMX**	**NEV**	**PRED**	**VAL**	**RIF**	**EE2**	**IVE**
31/01/2022	97.28	60.54	94.82	80.26	74.66	60.56	92.07	45.78	63.50	>99.99
1/02/2022	72.57	70.32	53.67	77.44	66.31	61.16	68.94	61.69	97.03	>99.99
2/02/2022	74.64	72.26	44.57	63.15	62.64	64.30	62.08	30.42	60.92	87.15
3/02/2022	92.97	>99.99	59.62	91.43	55.28	62.63	67.38	41.05	74.55	>99.99
4/02/2022	67.15	>99.99	49.95	41.80	68.41	45.91	49.79	65.17	7.70	>99.99
5/02/2022	93.30	>99.99	84.95	94.84	81.67	75.56	74.02	67.32	79.77	>99.99
6/02/2022	98.15	>99.99	90.33	95.18	82.71	78.53	73.77	57.61	82.31	>99.99
Weekly average	**85.15**	**85.59**	**68.27**	**77.73**	**70.24**	**64.09**	**69.72**	**52.72**	**66.54**	**97.31**
18/04/2022	58.72	63.96	65.56	80.83	30.96	77.13	8.08	82.39	61.65	55.04
19/04/2022	50.25	51.52	43.89	73.98	51.79	94.01	80.65	53.31	59.64	82.90
20/04/2022	85.27	43.49	48.77	76.52	77.35	43.33	59.84	70.19	70.70	89.68
21/04/2022	54.67	14.59	9.10	69.04	69.05	61.09	41.15	70.45	52.63	78.84
22/04/2022	69.12	14.69	17.14	76.63	51.49	75.01	92.62	69.59	85.91	82.83
23/04/2022	99.72	20.63	55.84	81.68	78.53	72.87	62.72	52.16	79.04	81.58
24/04/2022	98.35	16.95	29.13	86.09	76.35	57.32	55.45	49.01	74.80	68.30

Table 5.3 continued…

Date	Removal efficiency/%									
	MET	**CAF**	**SMX N-OH**	**SMX**	**NEV**	**PRED**	**VAL**	**RIF**	**EE2**	**IVE**
Weekly average	**73.72**	**32.26**	**38.49**	**77.83**	**62.22**	**68.68**	**57.22**	**63.87**	**69.20**	**77.03**
6/06/2022	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	>99.99	59.85
7/06/2022	>99.99	>99.99	>99.99	31.34	>99.99	>99.99	>99.99	>99.99	>99.99	57.51
8/06/2022	97.75	26.30	>99.99	9.35	17.45	85.61	68.36	60.57	75.31	58.25
9/06/2022	73.31	22.81	38.64	15.87	15.30	99.43	89.14	60.44	70.81	66.19
10/06/2022	99.07	27.41	21.11	11.62	37.58	89.26	66.39	67.20	86.00	59.77
11/06/2022	60.25	30.35	21.04	11.17	20.28	99.53	70.52	69.63	94.35	76.30
12/06/2022	92.35	60.77	34.92	17.14	64.35	40.20	63.81	49.21	72.99	80.02
Weekly average	**88.67**	**52.23**	**58.96**	**27.93**	**50.42**	**87.43**	**79.46**	**72.15**	**85.35**	**65.41**

MET: metformin, CAF: caffeine, SMX N-OH: sulfamethoxazole hydroxylamine, SMX: sulfamethoxazole, NEV: nevirapine, PRED: prednisolone, VAL: valsartan, RIF: rifampicin, EE2: 17α-ethynylestradiol, IVE: ivermectin.

From Table 5.3, February was determined to have the highest average removal efficiency. During this time, intermediate flow rates were recorded, as shown in Table 5.2, ranging from 75 to 103 ML day^{-1} and the percentage of analytes removed during the treatment process ranged from 7.70 to >99.99%. A percentage of >99.99% removal relates to the analyte not being detected during LC – MS analysis of the maturation river samples as this implies that the concentration of the analyte in the sample was below the limit of detection (LOD) of the instrument and it would not be statistically correct to assume that 100% of the analyte was removed. Prior studies have indicated that non-detection of an analyte does not necessarily imply that complete removal has occurred and hence the alleviation of associated risks but could simply suggest conversion of the analyte into transformation products within the sample has occurred (Yadav *et al.,* 2017). The lowest average removal efficiency was determined from the April samples, and this could be attributed to the higher flow rates observed in the treatment plant. Removal efficiencies observed for June ranged from 9.35 to >99.99% with the lowest daily average flow rate recorded, ranging from 63 to 76 ML day^{-1}. This could be an indication that slower flow rates allow for more contact time with each stage of the treatment process and possibly result in higher analyte removal. However, there are various additional factors that could affect the removal of analytes from a wastewater stream, such as the age, pH and temperature of the activated sludge used for the treatment process. Biomass concentrations, other meteorological conditions, and interruption within the plant due to maintenance or loadshedding, also affect the removal efficiency. The pharmaceutical with the highest average removal efficiency was ivermectin (97.31%), established for the February 2022 sampling period, and the lowest average was sulfamethoxazole (27.93%), quantified for the June 2022 sampling period. It is not possible for wastewater treatment processes to be able to remove all types of pharmaceutical or chemical compounds in use today as there are a substantial variety available with most having vastly different chemical structures and properties.

5.4.4 Environmental Risk Assessment

Environmental risk assessment (ERA) is a scientific process used to identify and evaluate the likelihood of a chemical compound to threaten living organisms, natural habitats, and ecosystems. It is determined by considering the predicted no effect concentration (PNEC), which is taken from literature, and the measured environmental concentration from the study (MEC). PNEC refers to the maximum concentration of a compound that will exhibit no adverse effects on an organism or ecosystem. ERA, equation 5.3, is calculated by determining the ratio

of the MEC value to PNEC. A risk quotient (RQ) value greater than or equal to 1 implies that the chemical compound is of environmental concern. Table 5.4 outlines the PNEC values obtained from various literature sources and the calculated ERA ratio for each analyte.

$$\frac{MEC}{PNEC} = \text{Risk quotient (RQ)} \qquad \text{Equation 5.3}$$

Table 5.4: ERA ratios obtained for each pharmaceutical

Pharmaceutical	**PNEC/ mg L^{-1}**	**Reference**	**ERA ratio**		
			February 2022	**April 2022**	**June 2022**
MET	0.060	Bergmann *et al.*, 2011	0.177	0.273	0.0863
CAF	0.011	Bergmann *et al.*, 2011	1.63	13.2	51.9
SMX N-OH	-	-	-	-	-
SMX	5.9×10^{-4}	Bergmann *et al.*, 2011	39.1	14.0	293
NEV	8.0×10^{-5}	Cid *et al.*, 2021	529	162	690
PRED	2.3×10^{-4}	Ren *et al.*, 2022	238	51.3	48.4
VAL	0.090	Van der Aa *et al.*, 2011	0.463	0.217	0.436
RIF	9.1×10^{-5}	Fass, 2022	756	10.4×10^{2}	572
EE2	1.0×10^{-8}	Bergmann *et al.*, 2011	3.90×10^{6}	9.30×10^{5}	2.91×10^{6}
IVE	3.0×10^{-11}	Bergmann *et al.*, 2011	29.7×10^{7}	25.4×10^{7}	10.5×10^{8}

-: No data available. MET: metformin, CAF: caffeine, SMX N-OH: sulfamethoxazole hydroxylamine, SMX: sulfamethoxazole, NEV: nevirapine, PRED: prednisolone, VAL: valsartan, RIF: rifampicin, EE2: 17α-ethynylestradiol, IVE: ivermectin.

The results in Table 5.4 were obtained by using the average weekly maturation river concentrations for each analyte. The maturation river is the final stage of the treatment process and flows directly into a nearby river. ERA ratios ranged from 0.0863 to 10.5×10^8. A RQ ratio below 1 indicates that the analyte is not of environmental concern. Ratios obtained for metformin and valsartan were below 1 for each of the three sampling occasions. The ERA ratios that are of concern are those for caffeine, sulfamethoxazole, nevirapine, prednisolone, and rifampicin. Furthermore, according to Table 5.4, pharmaceuticals that are of significant concern are EE2 and ivermectin, as these compounds could have detrimental effects on aquatic organisms as they are not adequately removed by the wastewater treatment process. EE2 is classified as an endocrine disrupting compound (EDC) and hence has the potential for detrimental impact on aquatic life within the receiving river. Some of these effects would include the feminization of male species and infertility amongst female species which could potentially lead to the extinction of certain species over time (Aris *et al.,* 2014). Prior studies done by Laurenson *et* al., 2014, on wastewater effluent from various WWTPs in the United States, found maximum EE2 RQ values of 4.6. The RQs established for this study were significantly and alarmingly higher than those found by Laurenson *et al.* 2014. Although ERA studies are becoming more frequent, there is not enough information detailing the extent of the ecological and behavioural consequences that long-term exposure to these pharmaceuticals have on wildlife. As a result of the persistence of these pharmaceuticals, further in-depth studies are required due to their difficult removal and significant risk to the environment.

5.5 Conclusion

This study aimed to identify and quantify analyte concentrations within various stages of the wastewater treatment process and determine removal efficiencies of those analytes. Additionally, an environmental risk assessment on the selected analytes and determination of whether they are of environmental concern was performed. The research study has highlighted the trend of increasing pharmaceutical usage and the relative increase in mass loads found in wastewater. The pharmaceuticals selected for this study showed varying removal patterns during the wastewater treatment process, some of which being almost completely removed and others showing effluent concentrations comparable with influent concentrations. Significant ERA ratios are also of major concern as aquatic life within the receiving river are at risk.

5.6 References

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CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

6.1 Introduction

This research study investigated the identification and quantification of selected pharmaceuticals at a wastewater treatment plant in KwaZulu-Natal. The study additionally aimed to compare seasonal trends of pharmaceutical usage in relation to COVID-19 and influenza peak periods. Further aims included determination of the removal efficiency of the current processes employed within the treatment plant and an environmental risk assessment. A solid phase extraction method was used for the extraction of analytes from wastewater samples. A liquid chromatography – mass spectrometry (LC – MS) method was developed to ensure the best detection and separation of analytes were achieved. Parameters that were optimised included, solvent compositions, flow rates, oven temperature and continuous modifications to the gradient method applied. Method validation parameters included linearity, limit of detection, limit of quantification, percentage recoveries, interday and intraday analyses.

Wastewater samples were collected from four sampling sites at the wastewater treatment plant once a day for a period of seven consecutive days, over three sampling periods. Results shown in Chapter 4 indicate pharmaceutical concentrations within influent samples having distinct seasonal trends especially when it came to sulfamethoxazole hydroxylamine, sulfamethoxazole, prednisolone and ivermectin – which are classified as pharmaceuticals prescribed for the treatment of COVID-19 and influenza. Concentrations directly correlated to COVID-19 wave peaks and lows as well as the start of the influenza season experienced in June in South Africa. Pharmaceuticals that stood out due to their negative impact on the environment and their persistence were sulfamethoxazole, sulfamethoxazole hydroxylamine, rifampicin, EE2 and ivermectin.

Results outlined in Chapter 5 show that the majority of analyte removal occurs between the secondary effluent and the maturation river and not as a result of the activated sludge treatment. This is expected as the activated sludge is known to primarily remove organics such as proteins, fats, and carbohydrates, rather than complex pharmaceutical compounds. Removal efficiencies were the best for ivermectin and the lowest for sulfamethoxazole. The environmental risk assessment showed substantially high ratios for sulfamethoxazole, nevirapine, prednisolone,

rifampicin, EE2 and most significantly, ivermectin found in wastewater effluent indicating that these compounds pose a high risk to environmental organisms within the receiving water body. Metformin and valsartan were the only two analytes that were found to not be of significant risk to aquatic life, with ratios below the RQ < 1 threshold. As a result, it is imperative to regulate the concentrations of pharmaceuticals within surface water bodies in order to minimise the negative health implications that these compounds can have on aquatic life and ultimately human life.

6.2 Challenges

Challenges faced within this study was inclusive of the COVID-19 pandemic and the national lockdown which resulted in a significant delay in project plans. Further COVID-19 restrictions including restricted working hours on campus upon the easing of national lockdown regulations also caused delays. Further challenges included a fire that broke out in a research laboratory in the Chemistry Department which damaged equipment, resources and led to further time away from laboratory work for project progress. Instrument downtime and necessary maintenance resulted in additional delays in the analysis of extracted wastewater samples.

6.3 Future Work

In order to fully understand the effects that treated wastewater effluent has on the environment, further studies are recommended to be performed with a wider range of pharmaceuticals and over longer sampling periods.

(i) In depth environmental risk assessments should be undertaken to determine the true threat that aquatic ecosystems face.

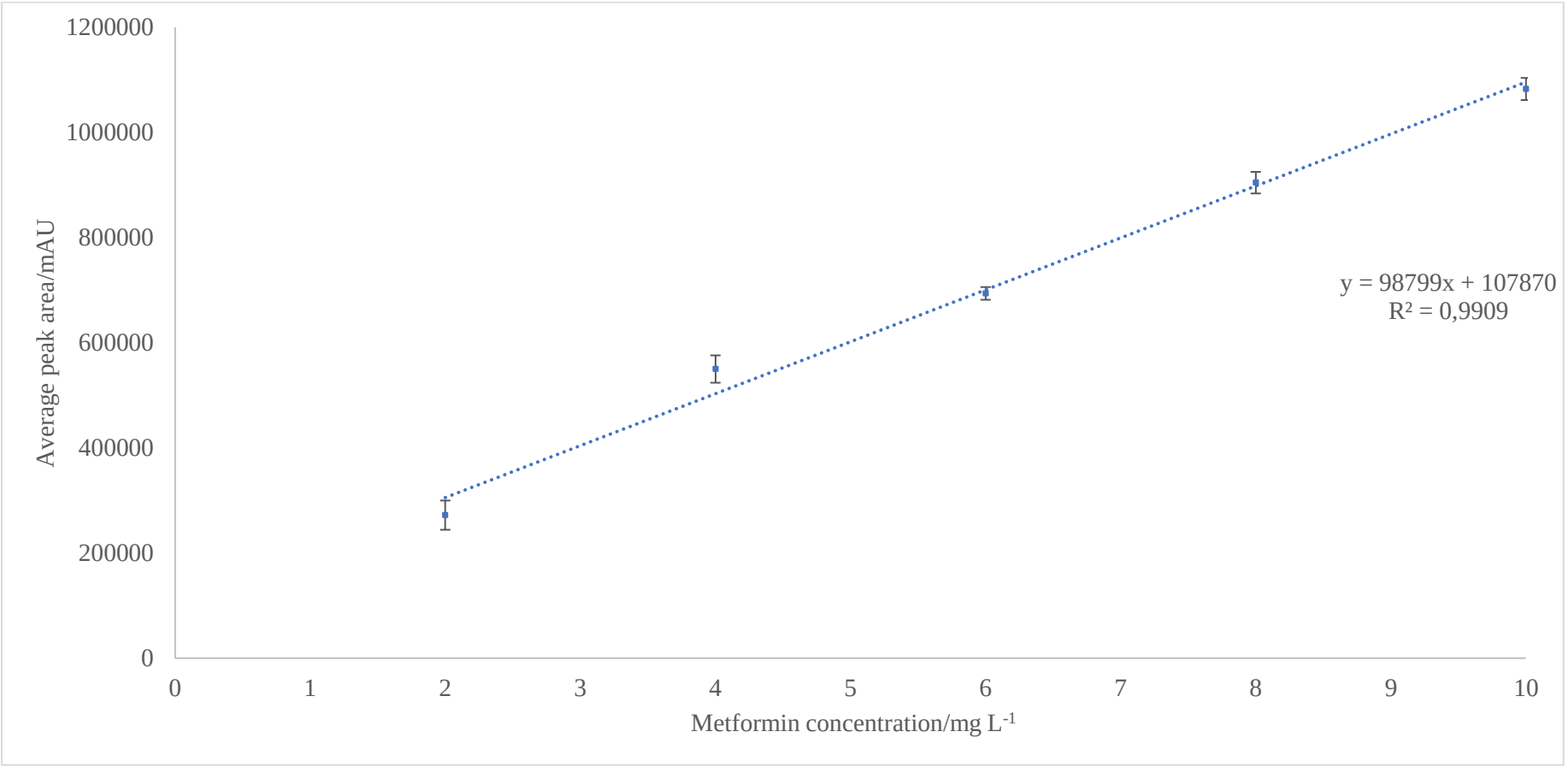
(ii) Additionally, large scale studies incorporating a substantial range of pharmaceuticals should be undertaken to assist with the publishing of legislature indicating maximum concentrations of pharmaceuticals that are allowed to be in wastewater effluent, as there are no current guidelines available.

(iii) Another area to be investigated is the analysis of photodegradation products of pharmaceuticals within wastewater effluent and the receiving river water as many of these compounds could still be of environmental risk.

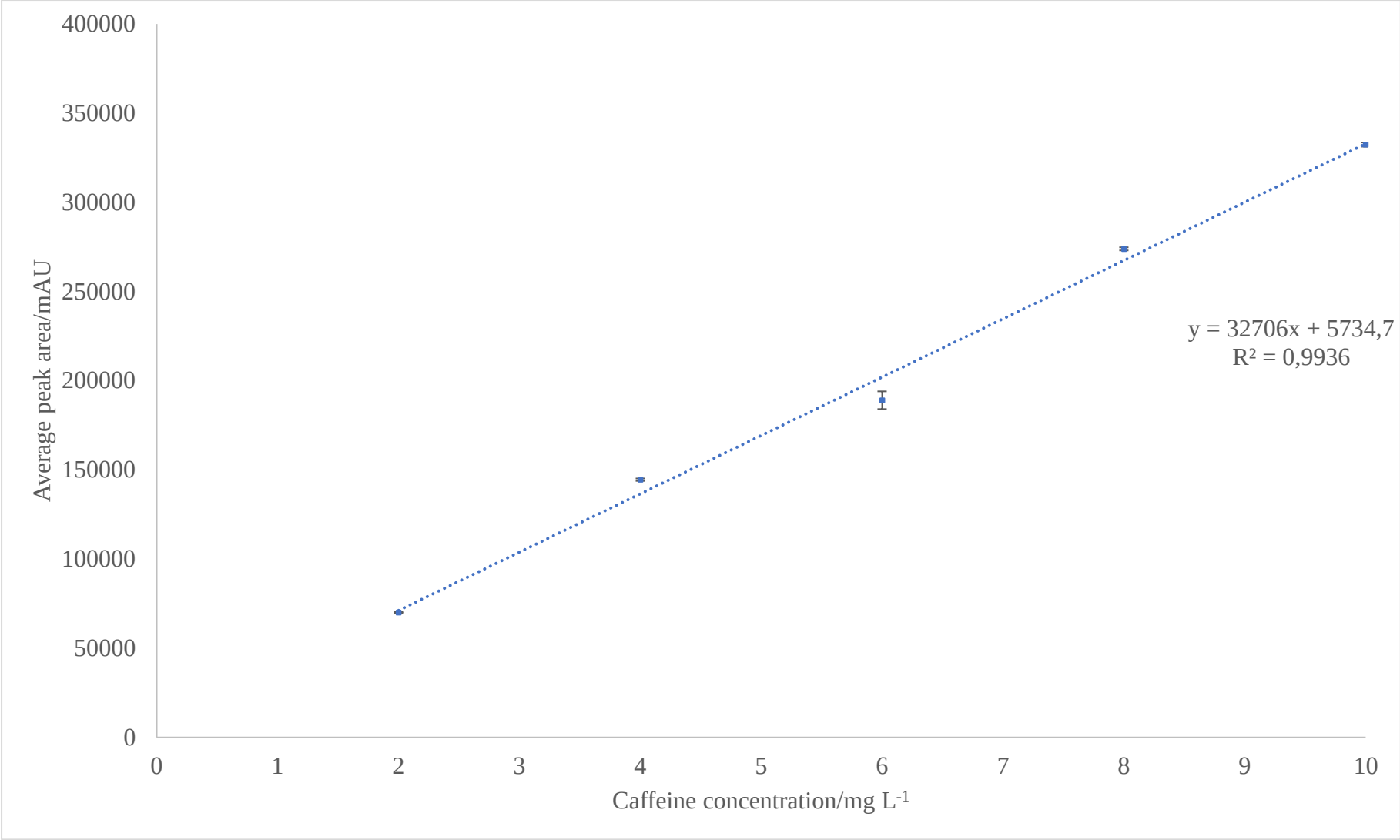
(iv) It is important to note that the receiving rivers flow towards the source of drinking water treatment plants. Studies investigating the concentrations of various pharmaceuticals in

drinking water is also of dire need as wastewater effluent contains significantly high concentrations of these analytes.

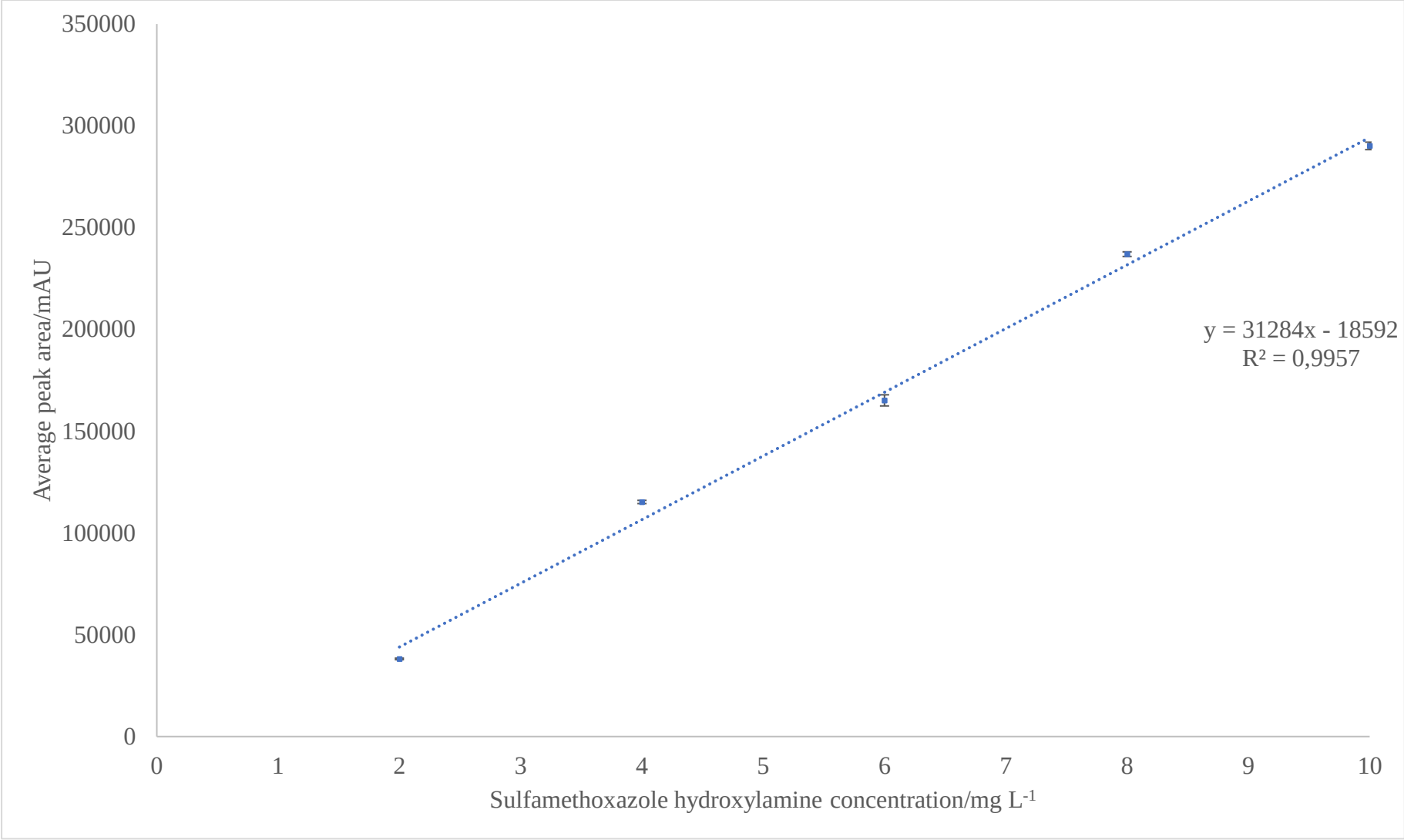
APPENDIX A: ANALYTE CALIBRATION CURVES



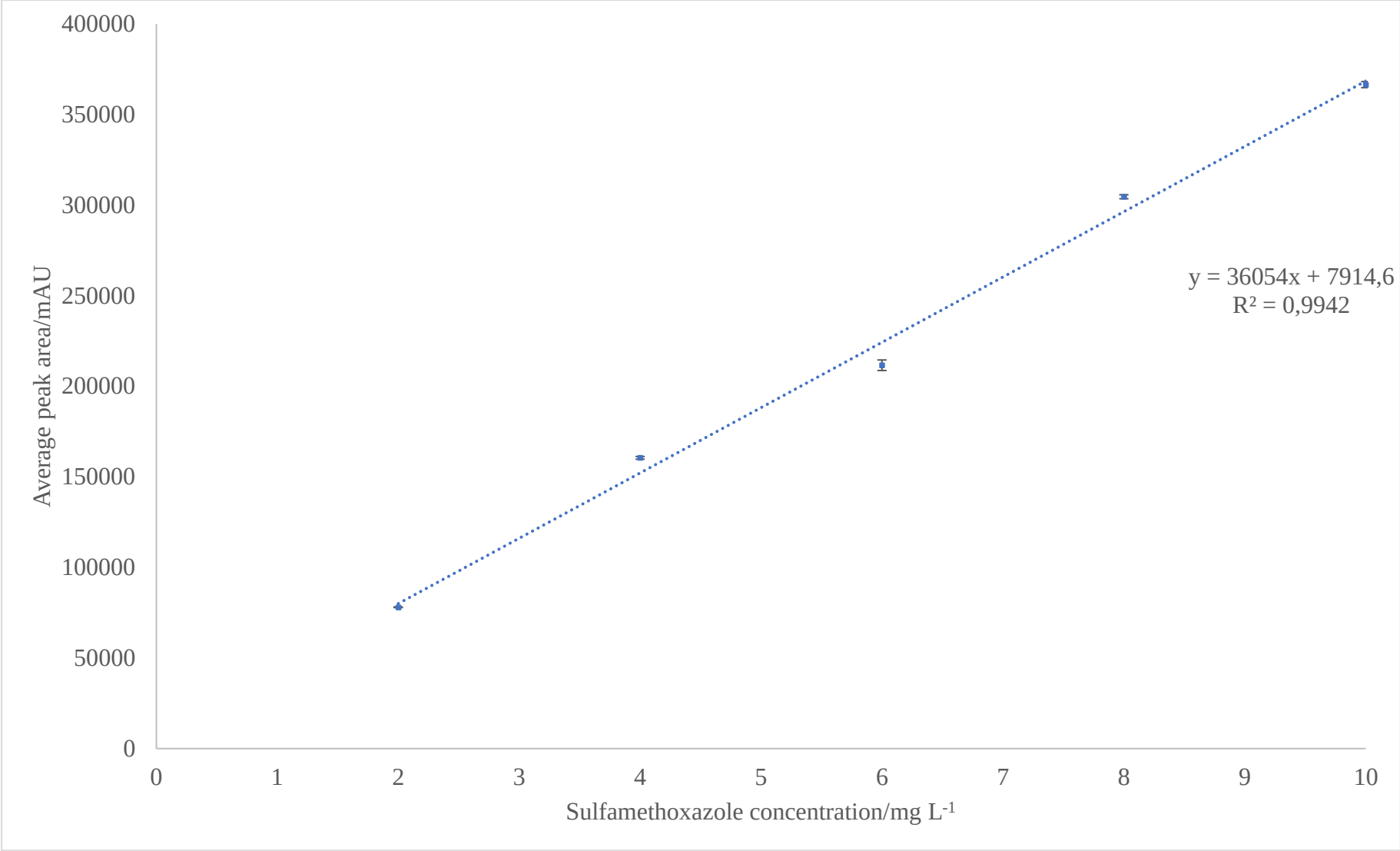
Appendix A1: Calibration curve of metformin.



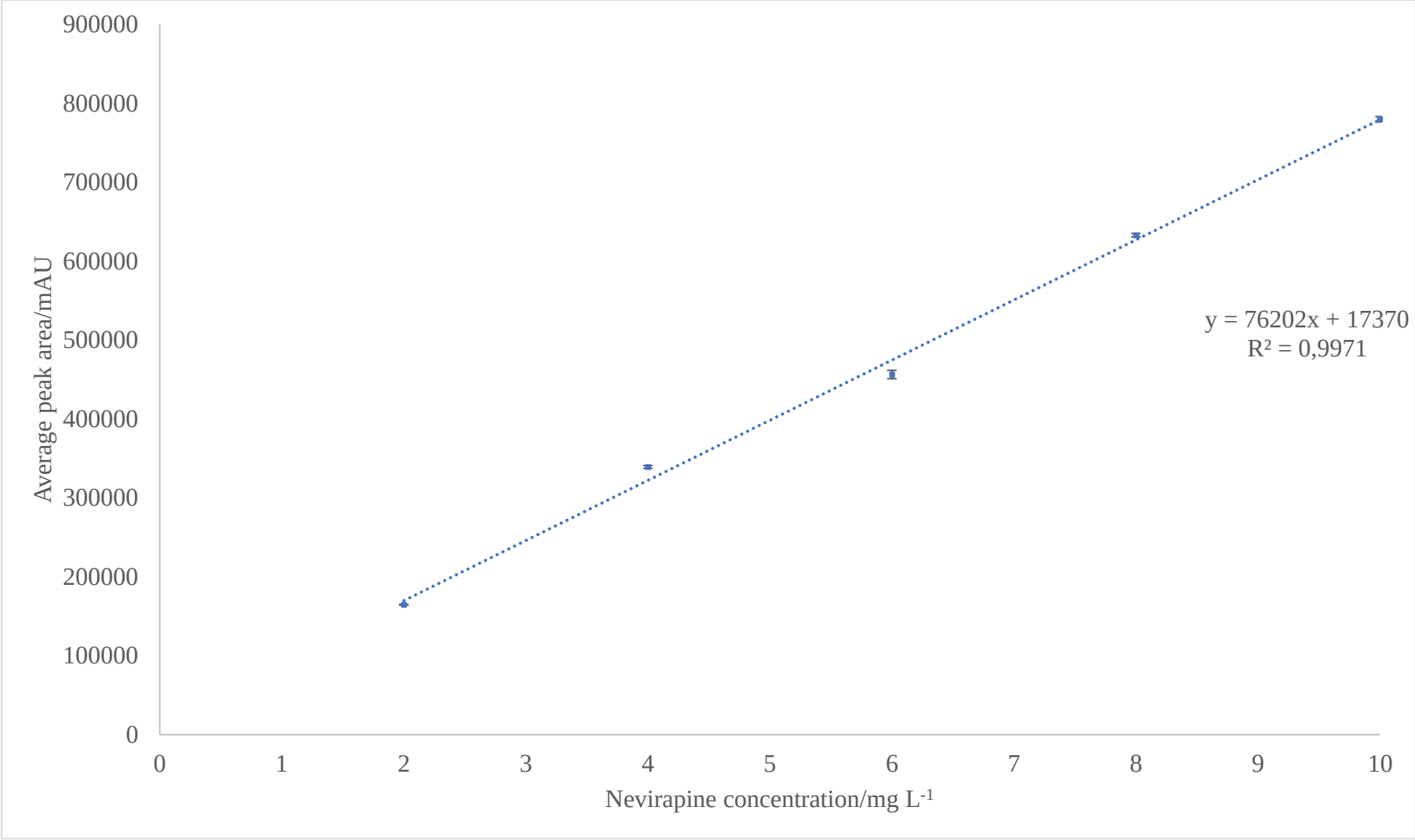
Appendix A2: Calibration curve for caffeine.



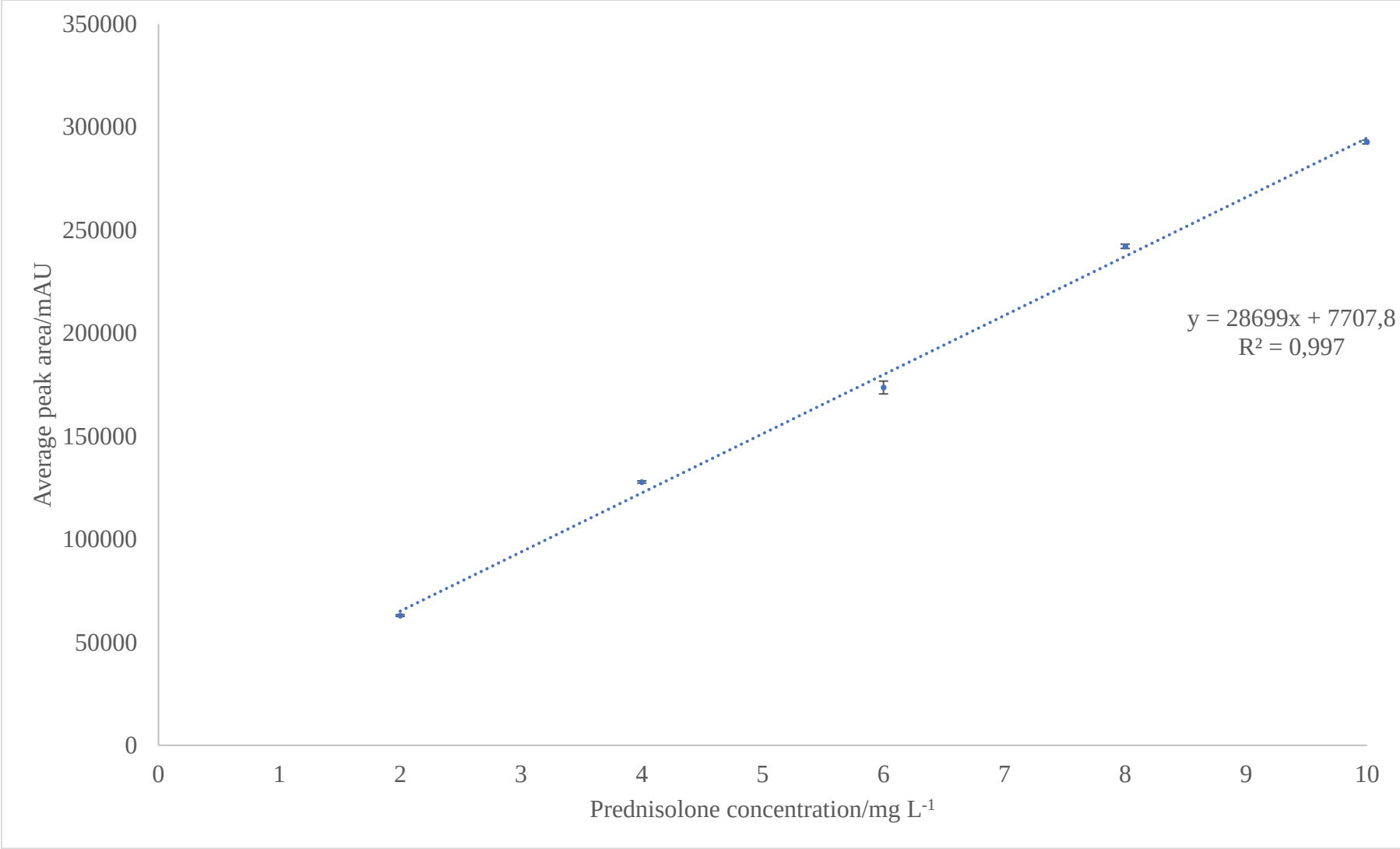
Appendix A3: Calibration curve for sulfamethoxazole hydroxylamine.



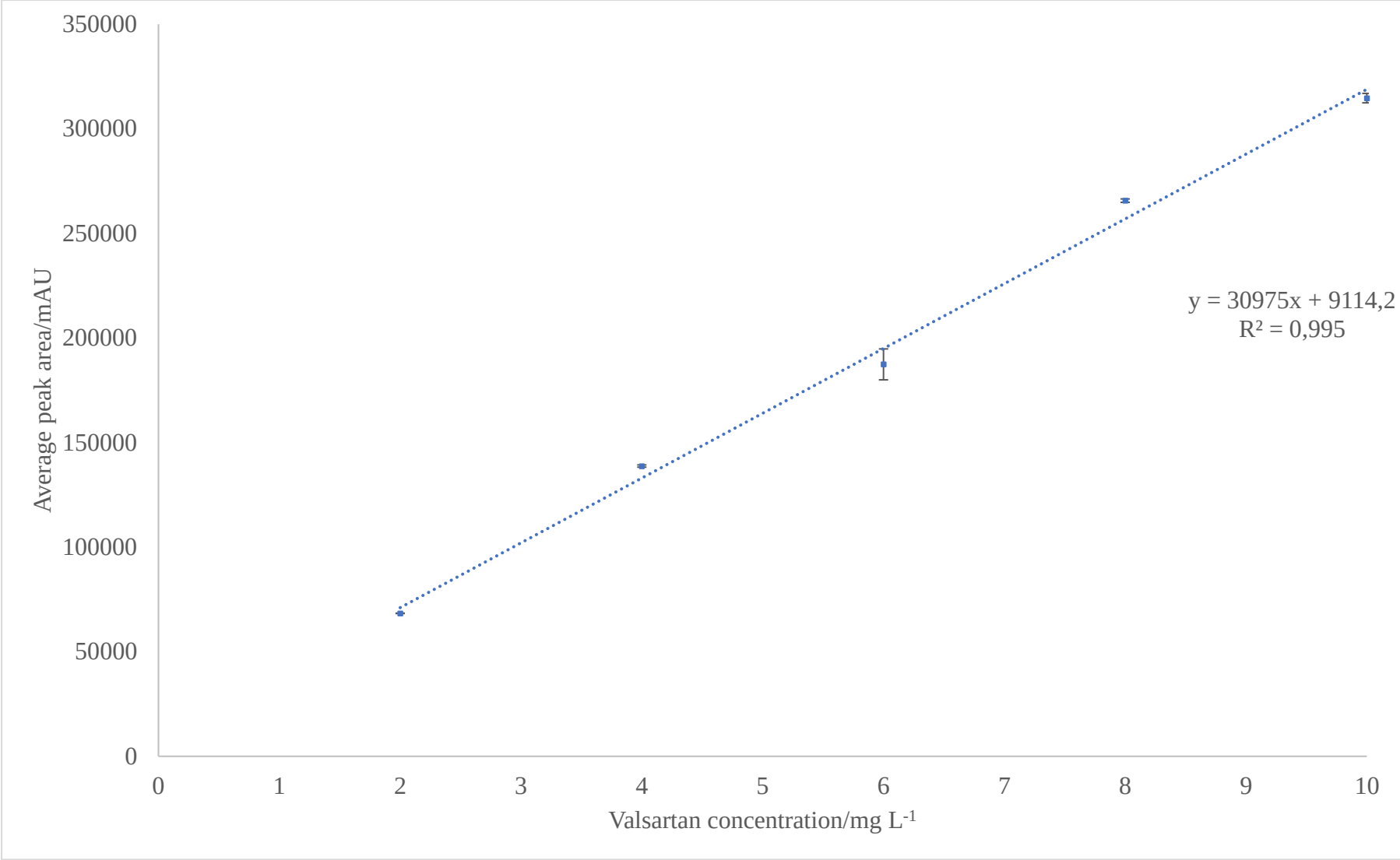
Appendix A4: Calibration curve for sulfamethoxazole.



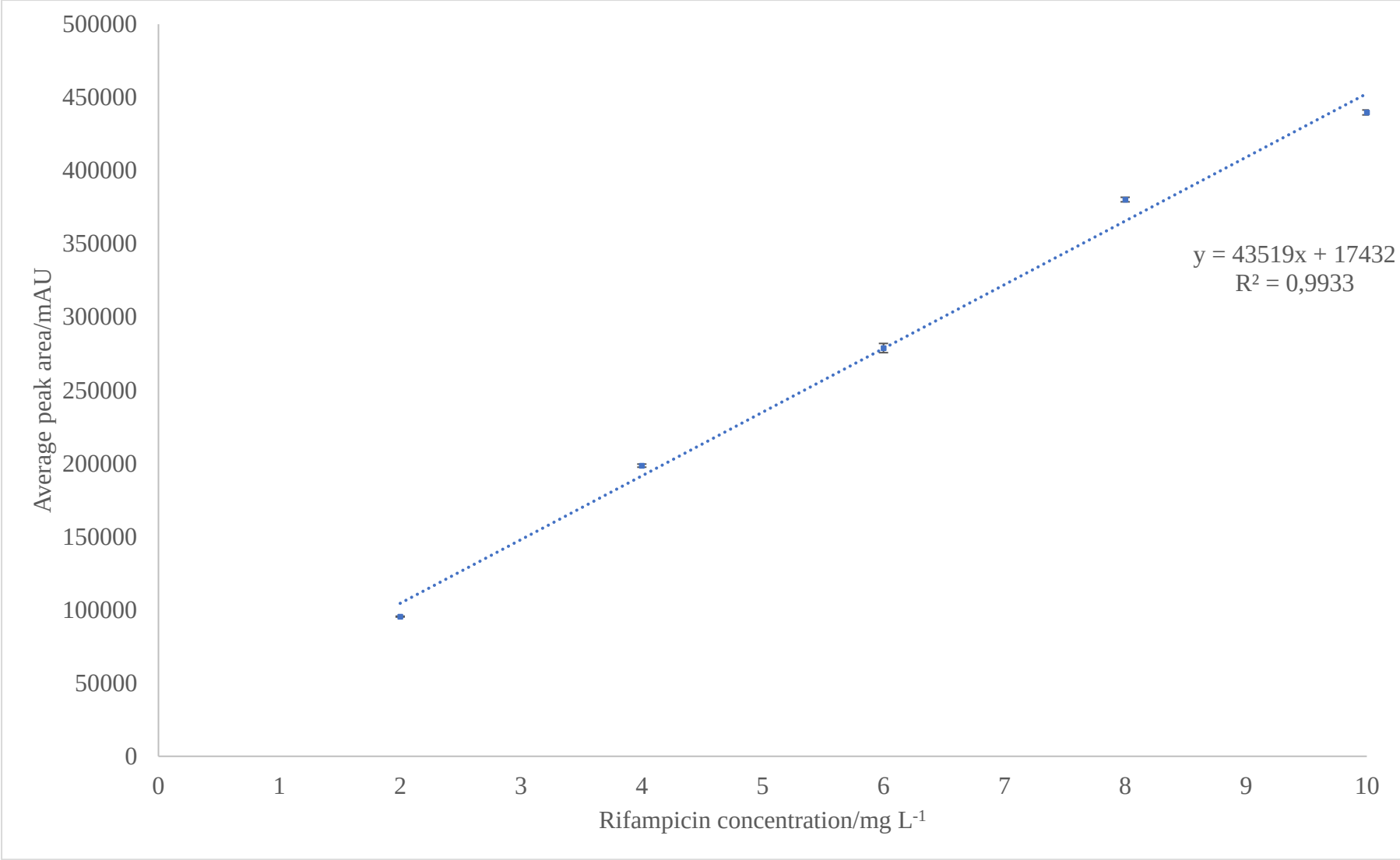
Appendix A5: Calibration curve for nevirapine.



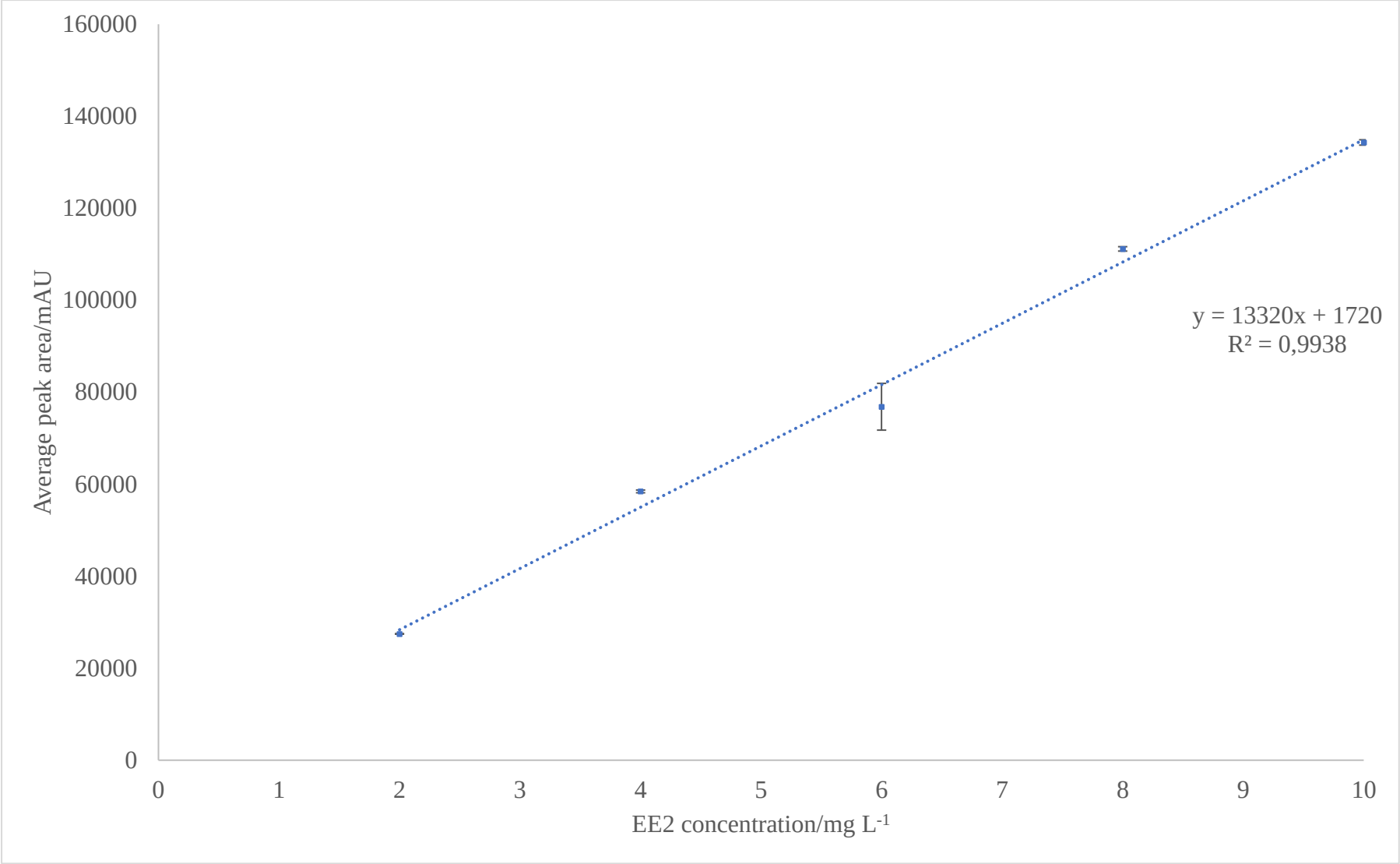
Appendix A6: Calibration curve for prednisolone.



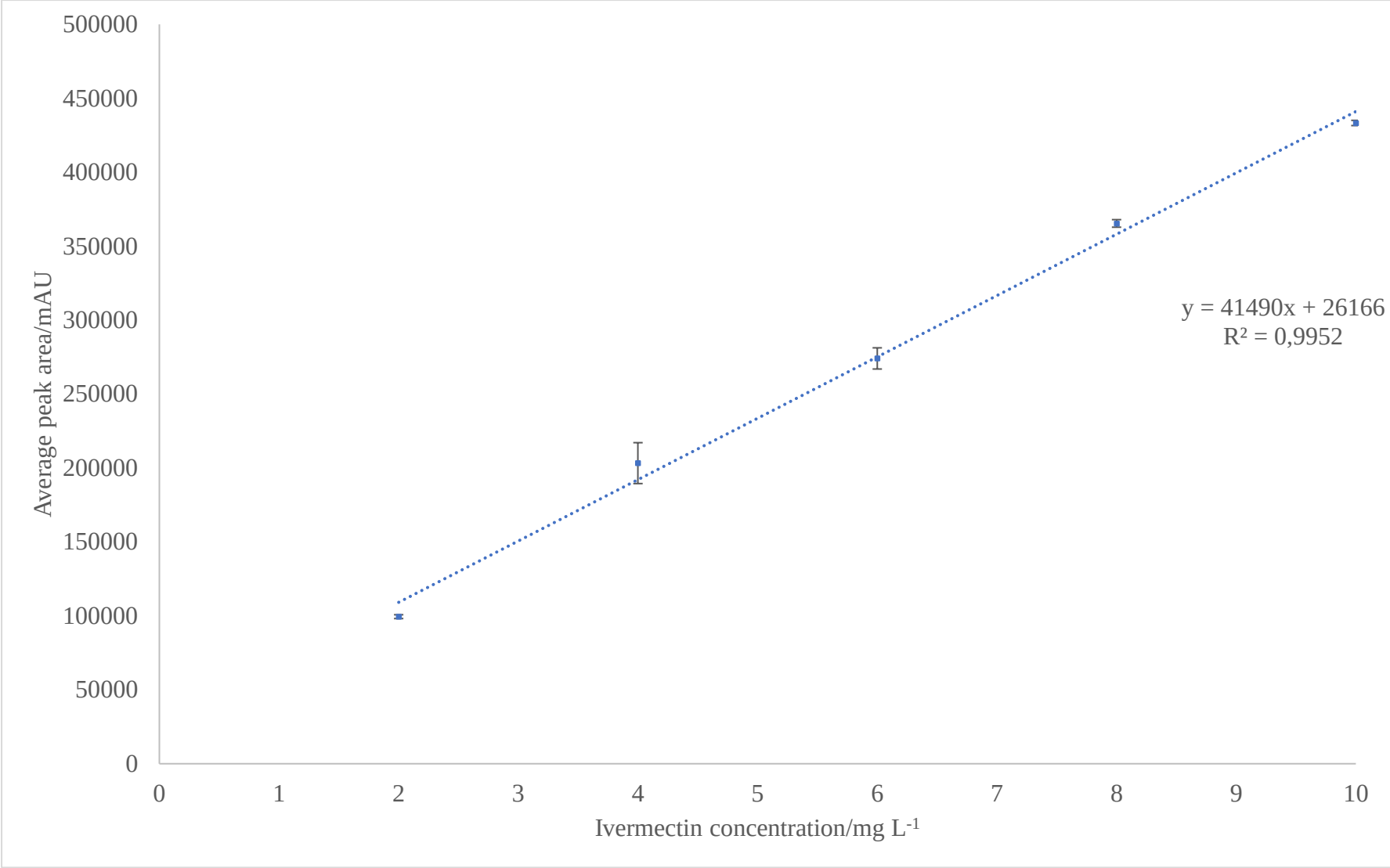
Appendix A7: Calibration curve for valsartan.



Appendix A8: Calibration curve for rifampicin.



Appendix A9: Calibration curve for EE2.



Appendix A10: Calibration curve for ivermectin.

APPENDIX B: SAMPLE pH AND ANALYTE CONCENTRATIONS

Appendix B1: pH readings of samples.

	Date	**pH at sampling points**			
		Inlet	**Balancing tank**	**Secondary effluent**	**Maturation river**
January/February 2022	31/01/2022	4.68	6.63	7.02	7.97
	1/02/2022	5.21	6.03	7.34	7.63
	2/02/2022	5.36	6.64	6.97	7.09
	3/02/2022	5.47	6.97	7.22	7.75
	4/02/2022	6.41	6.91	7.85	7.69
	5/02/2022	5.10	7.25	7.41	7.84
	6/02/2022	4.31	6.12	7.77	7.61
April 2022	18/04/2022	6.37	6.33	7.36	7.56
	19/04/2022	6.45	6.41	7.21	7.87
	20/04/2022	6.81	6.96	7.02	7.96
	21/04/2022	5.36	6.77	7.69	7.41
	22/04/2022	6.83	5.37	7.87	8.82
	23/04/2022	5.62	6.82	8.36	7.65
	24/04/2022	5.77	6.45	6.99	7.90
June 2022	6/06/2022	5.19	6.91	7.42	8.14
	7/06/2022	6.16	6.19	7.31	7.66
	8/06/2022	5.78	6.88	7.91	8.52
	9/06/2022	5.91	6.46	7.08	7.69
	10/06/2022	5.12	6.31	7.63	7.47
	11/06/2022	6.31	6.28	7.55	7.23
	12/06/2022	6.07	6.74	7.38	7.44

Appendix B2: Analyte concentrations from each sampling site over different sampling campaigns.

Table 1: Concentrations of analytes quantified during each sampling period.

Analyte	Day	Analyte concentration/mg L^{-1}											
		Feb 2022				April 2022				June 2022			
		INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT
MET	Mon	0.1390 ± 0.008815	0.08046 ± 0.01046	0.04487 ± 0.006275	0.003950 ± 0.0005361	0.05727 ± 0.0001500	0.03788 ± 0.002700	0.02859 ± 0.0005400	0.02474 ± 0.0006100	0.07748 ± 0.003010	0.06155 ± 0.004820	0.01884 ± 0.006840	ND
	Tues	0.08065 ± 0.01126	0.06520 ± 0.01211	0.05455 ± 0.009667	0.02336 ± 0.01698	0.04458 ± 0.004890	0.03031 ± 0.0006200	0.02486 ± 0.0007800	0.02321 ± 0.0002800	0.02550 ± 0.002497	0.01989 ± 0.001146	0.005336 ± 0.001430	ND
	Wed	0.06350 ± 0.007637	0.04898 ± 0.009400	0.03142 ± 0.008084	0.01688 ± 0.006726	0.1905 ± 0.0004100	0.04664 ± 0.0004200	0.03936 ± 0.0006700	0.02939 ± 0.001040	0.07081 ± 0.01697	0.009029 ± 0.007800	0.002536 ± 0.0004660	0.001669 ± 0.0001080
	Thurs	0.1093 ± 0.01427	0.03625 ± 0.03492	0.01690 ± 0.006386	0.008106 ± 0.006396	0.04449 ± 0.0005300	0.02783 ± 0.001640	0.02284 ± 0.001230	0.02096 ± 0.0003100	0.02884 ± 0.004940	0.02166 ± 0.001820	0.009839 ± 0.002120	0.008059 ± 0.0009020
	Fri	0.01883 ± 0.002544	0.01050 ± 0.001929	0.01264 ± 0.007353	0.006500 ± 0.0007893	0.04744 ± 0.0009400	0.04459 ± 0.002200	0.03704 ± 0.001020	0.01532 ± 0.001110	0.02933 ± 0.001369	0.02696 ± 0.001464	0.01108 ± 0.008210	0.0002890 ± 0.0001290
	Sat	0.1793 ± 0.007225	0.02111 ± 0.005806	0.01920 ± 0.01335	0.01255 ± 0.007074	0.08553 ± 0.0004900	0.004559 ± 0.0006600	0.001529 ± 0.0001857	0.0002510 ± 0.0001430	0.04740 ± 0.006980	0.03403 ± 0.01320	0.03113 ± 0.004890	0.01967 ± 0.008190
	Sun	0.1456 ± 0.03178	0.1148 ± 0.06360	0.01328 ± 0.001838	0.002800 ± 0.001375	0.03650 ± 0.001120	0.003889 ± 0.0001400	0.001551 ± 0.0001600	0.0006350 ± 0.0002700	0.08211 ± 0.002210	0.06363 ± 0.02260	0.03954 ± 0.0003590	0.006577 ± 0.003760
CAF	Mon	0.09350 ± 0.01617	0.06256 ± 0.005565	0.06100 ± 0.01933	0.03861 ± 0.0005600	0.1436 ± 0.002720	0.06446 ± 0.0007200	0.06438 ± 0.001380	0.05415 ± 0.003390	0.1488 ± 0.08740	0.09774 ± 0.001556	0.08567 ± 0.0001200	ND

Analyte	Day	Analyte concentration/mg L^{-1}											
		Feb 2022				April 2022				June 2022			
		INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT
	Tues	0.2138 ± 0.01033	0.1976 ± 0.01587	0.07525 ± 0.006190	0.06701 ± 0.001930	0.2479 ± 0.0007700	0.1498 ± 0.003340	0.1373 ± 0.002300	0.1258 ± 0.001060	0.9241 ± 0.06865	0.8176 ± 0.08308	0.5342 ± 0.003098	ND
	Wed	0.06947 ± 0.03386	0.04565 ± 0.003090	0.04003 ± 0.01710	0.02021 ± 0.003501	0.2476 ± 0.005030	0.2252 ± 0.002020	0.1825 ± 0.001380	0.1466 ± 0.001240	1.094 ± 0.1604	0.9678 ± 0.05381	0.8596 ± 0.05070	0.8445 ± 0.2939
	Thurs	0.06928 ± 0.02598	0.04505 ± 0.002970	ND	ND	0.1892 ± 0.001650	0.1802 ± 0.002780	0.1744 ± 0.001400	0.1680 ± 0.002400	1.149 ± 0.1306	1.037 ± 0.3816	0.9470 ± 0.2209	0.9286 ± 0.3002
	Fri	0.007587 ± 0.001230	ND	ND	ND	0.1998 ± 0.0003800	0.1997 ± 0.001800	0.1790 ± 0.003290	0.1782 ± 0.0002900	1.174 ± 0.1531	1.042 ± 0.3044	0.9483 ± 0.2051	0.8999 ± 0.2731
	Sat	0.1066 ± 0.004490	0.06271 ± 0.002190	ND	ND	0.2049 ± 0.003210	0.1842 ± 0.0006600	0.1776 ± 0.08232	0.1722 ± 0.003050	1.125 ± 0.1720	0.8836 ± 0.04438	0.8278 ± 0.02304	0.8182 ± 0.1332
	Sun	0.07947 ± 0.007450	0.07828 ± 0.01834	0.06864 ± 0.03963	ND	0.1973 ± 0.003700	0.1789 ± 0.0006300	0.1773 ± 0.0004300	0.1727 ± 0.002220	1.243 ± 0.01137	1.011 ± 0.2758	0.8052 ± 0.05741	0.5105 ± 0.05835
SMX NOH	Mon	0.2421 ± 0.0383	0.1884 ± 0.01791	0.1107 ± 0.01049	0.01312 ± 0.002890	0.05215 ± 0.001690	0.02609 ± 0.001490	0.02523 ± 0.001000	0.01880 ± 0.001450	0.09135 ± 0.006360	0.07498 ± 0.03510	0.03276 ± 0.01354	ND
	Tues	0.2035 ± 0.05850	0.1401 ± 0.03158	0.1114 ± 0.01890	0.09959 ± 0.03190	0.08105 ± 0.001610	0.05372 ± 0.0008300	0.05069 ± 0.001140	0.04760 ± 0.0001600	0.2878 ± 0.1352	0.2843 ± 0.002355	0.1902 ± 0.001223	ND
	Wed	0.2139 ± 0.02570	0.2073 ± 0.01780	0.1265 ± 0.02620	0.1243 ± 0.03540	0.08705 ± 0.0003300	0.08597 ± 0.0003400	0.05829 ± 0.0001500	0.04671 ± 0.0004500	0.2921 ± 0.005850	0.2646 ± 0.01674	0.2643 ± 0.0002200	ND

Analyte	Day	Analyte concentration/mg L^{-1}											
		Feb 2022				April 2022				June 2022			
		INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT
	Thurs	0.2667 ± 0.05030	0.1643 ± 0.03583	0.1493 ± 0.008100	0.1136 ± 0.01050	0.06976 ± 0.0004000	0.06720 ± 0.0001900	0.06600 ± 0.0006100	0.06592 ± 0.0002600	0.2754 ± 0.004490	0.2691 ± 0.04376	0.2414 ± 0.0004200	0.1769 ± 0.007960
	Fri	0.3438 ± 0.03640	0.1999 ± 0.008300	0.1834 ± 0.01040	0.1808 ± 0.01300	0.08285 ± 0.0002600	0.07825 ± 0.0002800	0.07551 ± 0.0002300	0.07180 ± 0.0005000	0.2904 ± 0.04369	0.2641 ± 0.03979	0.2475 ± 0.0001600	0.2418 ± 0.007250
	Sat	0.2693 ± 0.01140	0.2311 ± 0.02920	0.2206 ± 0.03300	0.04235 ± 0.01540	0.1409 ± 0.0002600	0.07995 ± 0.0005900	0.06990 ± 0.001377	0.06587 ± 0.0004200	0.2786 ± 0.01121	0.2675 ± 0.003400	0.2632 ± 0.003960	0.2297 ± 0.02171
	Sun	0.2476 ± 0.06660	0.2033 ± 0.01337	0.08206 ± 0.03320	0.02491 ± 0.001830	0.08423 ± 0.0002200	0.07852 ± 0.0001300	0.06835 ± 0.0002800	0.06295 ± 0.0002300	0.2840 ± 0.01198	0.2536 ± 0.003710	0.2168 ± 0.06389	0.1935 ± 0.01175
SMX	Mon	0.1760 ± 0.007700	0.08542 ± 0.001151	0.05375 ± 0.0005830	0.03635 ± 0.004700	0.009818 ± 0.0006100	0.009079 ± 0.002250	0.007900 ± 0.0003200	0.001970 ± 0.0004800	0.2022 ± 0.01646	0.1985 ± 0.009470	0.1427 ± 0.01236	ND
	Tues	0.1128 ± 0.01132	0.09900 ± 0.01000	0.07725 ± 0.01000	0.02687 ± 0.001680	0.01650 ± 0.005200	0.007599 ± 0.00005900	0.005450 ± 0.0004500	0.004494 ± 0.0001200	0.3002 ± 0.001380	0.2198 ± 0.003150	0.2187 ± 0.002520	0.2152 ± 0.002334
	Wed	0.09471 ± 0.007400	0.08335 ± 0.01600	0.05515 ± 0.01500	0.03660 ± 0.00600	0.03435 ± 0.0003700	0.01129 ± 0.0008300	0.009449 ± 0.0008630	0.008450 ± 0.0005800	0.2157 ± 0.01612	0.2094 ± 0.007270	0.2092 ± 0.005530	0.2047 ± 0.01520
	Thurs	0.09465 ± 0.01250	0.04749 ± 0.002356	0.02798 ± 0.005400	0.008565 ± 0.001560	0.02521 ± 0.0003100	0.01590 ± 0.0001200	0.01300 ± 0.0005600	0.008114 ± 0.0008000	0.2196 ± 0.01367	0.2140 ± 0.008040	0.2037 ± 0.003820	0.1934 ± 0.003830
	Fri	0.05661 ± 0.002620	0.03800 ± 0.002130	0.03491 ± 0.006600	0.03461 ± 0.007500	0.04040 ± 0.0003100	0.03080 ± 0.0002900	0.02163 ± 0.0005500	0.009873 ± 0.0003000	0.2156 ± 0.01506	0.2125 ± 0.01622	0.2076 ± 0.005690	0.2012 ± 0.005490

Analyte	Day	Analyte concentration/mg L^{-1}											
		Feb 2022				April 2022				June 2022			
		INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT
	Sat	0.1912 ± 0.006100	0.04152 ± 0.01460	0.03023 ± 0.001340	0.01030 ± 0.008700	0.1024 ± 0.0005800	0.03872 ± 0.0003200	0.02349 ± 0.001711	0.01985 ± 0.0007700	0.2239 ± 0.009250	0.2155 ± 0.005610	0.2140 ± 0.002130	0.2077 ± 0.02139
	Sun	0.1585 ± 0.03500	0.1279 ± 0.06980	0.02719 ± 0.001820	0.007950 ± 0.001360	0.03569 ± 0.001230	0.03475 ± 0.0001800	0.01121 ± 0.0003900	0.005234 ± 0.0004500	0.2174 ± 0.008810	0.2165 ± 0.01363	0.2146 ± 0.004620	0.1885 ± 0.01169
NEV	Mon	0.1780 ± 0.006200	0.1186 ± 0.009530	0.06645 ± 0.005580	0.04720 ± 0.004600	0.01522 ± 0.0003600	0.01130 ± 0.001920	0.01109 ± 0.0003700	0.01100 ± 0.0003400	0.09738 ± 0.003180	0.07955 ± 0.01457	0.07292 ± 0.009340	ND
	Tues	0.1310 ± 0.08490	0.1115 ± 0.009400	0.1021 ± 0.009500	0.04661 ± 0.01660	0.01556 ± 0.004920	0.01237 ± 0.0001800	0.009063 ± 0.0006000	0.007850 ± 0.0002500	0.1038 ± 0.002470	0.09474 ± 0.01079	0.08991 ± 0.005210	ND
	Wed	0.1375 ± 0.006100	0.1089 ± 0.007600	0.09358 ± 0.0004000	0.05385 ± 0.005300	0.03878 ± 0.0004100	0.01523 ± 0.0002000	0.01038 ± 0.0002300	0.009200 ± 0.0003700	0.1052 ± 0.008040	0.09692 ± 0.002750	0.09296 ± 0.01271	0.09092 ± 0.006430
	Thurs	0.1190 ± 0.01050	0.09920 ± 0.001567	0.05630 ± 0.005200	0.05619 ± 0.005600	0.03163 ± 0.0003600	0.02635 ± 0.0001500	0.02405 ± 0.0004000	0.01017 ± 0.0001300	0.09983 ± 0.005470	0.09951 ± 0.01204	0.08895 ± 0.007480	0.08851 ± 0.009750
	Fri	0.07090 ± 0.01930	0.04815 ± 0.01740	0.04568 ± 0.005900	0.02353 ± 0.006600	0.03755 ± 0.0001100	0.03571 ± 0.0002200	0.02516 ± 0.0004600	0.01905 ± 0.0002700	0.1023 ± 0.008520	0.09557 ± 0.01388	0.08655 ± 0.03555	0.06742 ± 0.003160
	Sat	0.2032 ± 0.005700	0.05678 ± 0.005300	0.04530 ± 0.01290	0.03892 ± 0.007500	0.1093 ± 0.0005600	0.03780 ± 0.0002200	0.02705 ± 0.0001651	0.02485 ± 0.0005300	0.1099 ± 0.007980	0.09348 ± 0.003660	0.09322 ± 0.009910	0.09146 ± 0.03105
	Sun	0.1679 ± 0.03280	0.1366 ± 0.06530	0.03672 ± 0.01730	0.03020 ± 0.001290	0.03568 ± 0.0002500	0.03409 ± 0.0001200	0.01831 ± 0.0001400	0.008900 ± 0.00003400	0.1298 ± 0.02944	0.09621 ± 0.01038	0.07842 ± 0.02721	0.04845 ± 0.01996

Analyte	Day	Analyte concentration/mg L^{-1}											
		Feb 2022				April 2022				June 2022			
		INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT
PRED	Mon	0.1854 ± 0.03210	0.1320 ± 0.09000	0.08000 ± 0.005370	0.07650 ± 0.004300	0.01249 ± 0.001200	0.01085 ± 0.001940	0.007007 ± 0.0003800	0.002990 ± 0.0004500	0.05159 ± 0.005290	0.03950 ± 0.001026	0.03263 ± 0.002873	ND
	Tues	0.1383 ± 0.08430	0.1245 ± 0.008700	0.1093 ± 0.009300	0.05674 ± 0.01570	0.008775 ± 0.0001027	0.008492 ± 0.0003800	0.003068 ± 0.0007100	0.0005500 ± 0.0003200	0.01343 ± 0.002428	0.009465 ± 0.001435	0.0005240 ± 0.0001943	ND
	Wed	0.1396 ± 0.005900	0.1110 ± 0.007500	0.1030 ± 0.005400	0.05225 ± 0.005500	0.01359 ± 0.0004700	0.01156 ± 0.0009100	0.001372 ± 0.0004700	0.008071 ± 0.0003100	0.05012 ± 0.02065	0.04645 ± 0.01958	0.008557 ± 0.002410	0.007551 ± 0.0001729
	Thurs	0.1428 ± 0.01070	0.1115 ± 0.01361	0.06671 ± 0.004800	0.05635 ± 0.005300	0.02280 ± 0.0002800	0.01433 ± 0.0002400	0.002175 ± 0.0003400	0.009224 ± 0.0005000	0.4482 ± 0.07341	0.1165 ± 0.01136	0.01286 ± 0.001133	0.002670 ± 0.0001045
	Fri	0.08830 ± 0.01780	0.05980 ± 0.01540	0.05967 ± 0.005800	0.05018 ± 0.006200	0.03478 ± 0.0006300	0.01972 ± 0.0004000	0.01415 ± 0.0005700	0.009088 ± 0.0003900	0.1408 ± 0.01185	0.07121 ± 0.03781	0.01686 ± 0.001411	0.01596 ± 0.0009713
	Sat	0.2052 ± 0.005400	0.08565 ± 0.004800	0.05905 ± 0.01220	0.05239 ± 0.007200	0.1079 ± 0.0006100	0.03795 ± 0.00008600	0.03765 ± 0.001663	0.03100 ± 0.0008400	0.1362 ± 0.02599	0.04870 ± 0.01070	0.01885 ± 0.002569	0.0006640 ± 0.00004179
	Sun	0.1751 ± 0.03040	0.1487 ± 0.06220	0.05033 ± 0.01660	0.03910 ± 0.001250	0.04799 ± 0.0001800	0.03355 ± 0.0001300	0.02240 ± 0.0005100	0.02160 ± 0.0003700	0.08158 ± 0.01165	0.07809 ± 0.01230	0.06147 ± 0.008225	0.05108 ± 0.009406
VAL	Mon	0.1813 ± 0.05940	0.1263 ± 0.09050	0.07150 ± 0.005410	0.01505 ± 0.003600	0.02325 ± 0.001200	0.02281 ± 0.002010	0.02243 ± 0.0005400	0.02237 ± 0.0007600	0.2238 ± 0.01999	0.05676 ± 0.001307	ND	ND
	Tues	0.1265 ± 0.08790	0.1228 ± 0.008800	0.1078 ± 0.009300	0.04150 ± 0.01500	0.02129 ± 0.01314	0.02103 ± 0.0006400	0.01829 ± 0.0002900	0.004312 ± 0.0002100	0.2319 ± 0.02237	0.1150 ± 0.03767	0.04788 ± 0.01054	ND

Analyte	Day	Analyte concentration/mg L^{-1}											
		Feb 2022				April 2022				June 2022			
		INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT
	Wed	0.1271 ± 0.006100	0.1044 ± 0.007500	0.09493 ± 0.006200	0.05055 ± 0.005500	0.04456 ± 0.0008300	0.02558 ± 0.0005300	0.02317 ± 0.0005400	0.01874 ± 0.0006600	0.2290 ± 0.05301	0.1740 ± 0.02576	0.08946 ± 0.004370	0.07589 ± 0.001059
	Thurs	0.1304 ± 0.01120	0.1146 ± 0.01469	0.07184 ± 0.004800	0.04490 ± 0.005100	0.02999 ± 0.0005400	0.02799 ± 0.0005800	0.02277 ± 0.0002600	0.01835 ± 0.0002600	0.2719 ± 0.08014	0.1216 ± 0.02208	0.1145 ± 0.001101	0.03091 ± 0.008479
	Fri	0.06860 ± 0.01900	0.05145 ± 0.01590	0.04883 ± 0.005900	0.03618 ± 0.006400	0.04299 ± 0.0005100	0.03978 ± 0.0005900	0.03152 ± 0.0004800	0.003318 ± 0.0002800	0.1204 ± 0.02827	0.07800 ± 0.03575	0.07218 ± 0.02439	0.04274 ± 0.01001
	Sat	0.2080 ± 0.005400	0.08562 ± 0.004700	0.06177 ± 0.01140	0.05645 ± 0.006600	0.1132 ± 0.0005900	0.05568 ± 0.0002000	0.04503 ± 0.001617	0.04468 ± 0.0003600	0.2323 ± 0.01220	0.1094 ± 0.05830	0.09872 ± 0.005128	0.07153 ± 0.002231
	Sun	0.1720 ± 0.03110	0.1429 ± 0.06100	0.05175 ± 0.01590	0.04695 ± 0.001160	0.05348 ± 0.0005200	0.05152 ± 0.00009500	0.03357 ± 0.0004000	0.02512 ± 0.0001300	0.1422 ± 0.04834	0.1359 ± 0.01457	0.1125 ± 0.03565	0.05389 ± 0.007071
RIF	Mon	0.1217 ± 0.01230	0.1182 ± 0.04890	0.07761 ± 0.003220	0.06907 ± 0.002000	0.1608 ± 0.0008100	0.05061 ± 0.0008100	0.04349 ± 0.0005200	0.02964 ± 0.004020	0.3449 ± 0.008470	0.2098 ± 0.03741	0.1848 ± 0.02504	ND
	Tues	0.1205 ± 0.02280	0.1106 ± 0.003000	0.09535 ± 0.005900	0.04878 ± 0.009700	0.1448 ± 0.0003800	0.09653 ± 0.001470	0.07947 ± 0.0001390	0.07075 ± 0.002560	0.2057 ± 0.008670	0.1914 ± 0.01934	0.1904 ± 0.006080	ND
	Wed	0.1231 ± 0.003500	0.1148 ± 0.003700	0.09094 ± 0.003300	0.08987 ± 0.002800	0.2656 ± 0.001200	0.24842 ± 0.002070	0.1015 ± 0.001160	0.08294 ± 0.001010	0.1626 ± 0.02959	0.1528 ± 0.03939	0.09297 ± 0.005090	0.06715 ± 0.02001
	Thurs	0.1344 ± 0.005300	0.1149 ± 0.07240	0.09625 ± 0.002600	0.08362 ± 0.003000	0.2696 ± 0.001630	0.1907 ± 0.001130	0.1330 ± 0.0009100	0.08281 ± 0.001160	0.1950 ± 0.09376	0.1809 ± 0.002740	0.1741 ± 0.04376	0.08079 ± 0.009220

Analyte	Day	Analyte concentration/mg L^{-1}											
		Feb 2022				April 2022				June 2022			
		INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT
	Fri	0.1674 ± 0.008400	0.1313 ± 0.002500	0.08830 ± 0.003200	0.06123 ± 0.004300	0.2947 ± 0.00005400	0.2802 ± 0.0009200	0.1338 ± 0.0002100	0.09372 ± 0.0003100	0.2020 ± 0.01627	0.1713 ± 0.09406	0.1445 ± 0.02928	0.06996 ± 0.01010
	Sat	0.1621 ± 0.003900	0.1387 ± 0.002200	0.09668 ± 0.008100	0.05535 ± 0.004300	0.2944 ± 0.0008200	0.2443 ± 0.0002000	0.2440 ± 0.001360	0.1491 ± 0.0009200	0.2153 ± 0.007170	0.09432 ± 0.001285	0.09138 ± 0.001414	0.06830 ± 0.001311
	Sun	0.1673 ± 0.01460	0.1299 ± 0.03570	0.07608 ± 0.008600	0.07377 ± 0.007800	0.2921 ± 0.0002400	0.2864 ± 0.0001200	0.1596 ± 0.00006400	0.1570 ± 0.0004400	0.1478 ± 0.01434	0.1303 ± 0.02656	0.1293 ± 0.01302	0.07862 ± 0.007851
EE2	Mon	0.1835 ± 0.01130	0.1367 ± 0.09110	0.07502 ± 0.005430	0.07010 ± 0.003700	0.008684 ± 0.0006600	0.008492 ± 0.001960	0.006698 ± 0.001390	0.003486 ± 0.0003600	0.2590 ± 0.07143	0.07634 ± 0.01410	0.03144 ± 0.001451	ND
	Tues	0.1213 ± 0.09270	0.1192 ± 0.01080	0.1086 ± 0.009200	0.003807 ± 0.001560	0.01163 ± 0.006320	0.007849 ± 0.0002800	0.007367 ± 0.0006900	0.004913 ± 0.0003500	0.2121 ± 0.07863	0.1316 ± 0.02468	0.09723 ± 0.02583	ND
	Wed	0.1151 ± 0.007300	0.09510 ± 0.008600	0.09251 ± 0.006400	0.04720 ± 0.005700	0.01709 ± 0.0005000	0.007539 ± 0.0001200	0.007326 ± 0.00009600	0.005247 ± 0.0002600	0.1381 ± 0.009710	0.07452 ± 0.01721	0.03990 ± 0.002955	0.03570 ± 0.001841
	Thurs	0.1098 ± 0.01140	0.1028 ± 0.01647	0.06260 ± 0.005000	0.02949 ± 0.005500	0.02150 ± 0.0003600	0.01805 ± 0.0003400	0.016609 ± 0.0003300	0.01058 ± 0.0003200	0.2009 ± 0.01040	0.1424 ± 0.02261	0.1411 ± 0.01695	0.06141 ± 0.02150
	Fri	0.05119 ± 0.002830	0.03894 ± 0.001640	0.03166 ± 0.005700	0.04964 ± 0.006400	0.04390 ± 0.0005400	0.02411 ± 0.0004400	0.02271 ± 0.0005400	0.006469 ± 0.0004400	0.1138 ± 0.006160	0.09418 ± 0.04284	0.07291 ± 0.002185	0.01683 ± 0.001322
	Sat	0.2010 ± 0.006300	0.06131 ± 0.005200	0.04602 ± 0.01230	0.04250 ± 0.007100	0.1058 ± 0.0008700	0.04549 ± 0.0003700	0.03000 ± 0.001680	0.02346 ± 0.0006500	0.2364 ± 0.03551	0.1174 ± 0.02937	0.06428 ± 0.007850	0.01394 ± 0.001806

Analyte	Day	Analyte concentration/mg L^{-1}											
		Feb 2022				April 2022				June 2022			
		INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT	INLET	BAL TANK	SEC EFF	MAT
	Sun	0.1663 ± 0.03220	0.1369 ± 0.06440	0.03975 ± 0.001700	0.03060 ± 0.001290	0.04119 ± 0.0004400	0.03003 ± 0.00008100	0.01470 ± 0.0004700	0.01094 ± 0.0004900	0.2701 ± 0.01845	0.1544 ± 0.01247	0.1090 ± 0.006300	0.07638 ± 0.0005425
IVE	Mon	0.6525 ± 0.07831	0.4545 ± 0.05082	ND	ND	0.008520 ± 0.002240	0.006444 ± 0.002990	0.006011 ± 0.0001100	0.004010 ± 0.0006800	0.1129 ± 0.02020	0.07703 ± 0.005380	0.07122 ± 0.003070	0.04750 ± 0.001900
	Tues	0.2979 ± 0.07156	0.1344 ± 0.0007700	ND	ND	0.008184 ± 0.006100	0.005009 ± 0.0004400	0.004666 ± 0.001320	0.001465 ± 0.0004900	0.09050 ± 0.004600	0.07420 ± 0.002700	0.07033 ± 0.004700	0.04017 ± 0.005900
	Wed	0.4630 ± 0.02426	0.4438 ± 0.1653	0.07015 ± 0.03201	0.06238 ± 0.002658	0.02382 ± 0.004410	0.006987 ± 0.001410	0.003178 ± 0.0002100	0.002575 ± 0.0007200	0.07600 ± 0.01180	0.06145 ± 0.005200	0.05843 ± 0.003300	0.03322 ± 0.003300
	Thurs	0.8289 ± 0.01025	0.5779 ± 0.09895	0.3617 ± 0.04481	ND	0.04796 ± 0.0006200	0.01792 ± 0.001940	0.01120 ± 0.0005500	0.01055 ± 0.006426	0.1002 ± 0.005800	0.06175 ± 0.001730	0.03730 ± 0.002500	0.03549 ± 0.002400
	Fri	0.4668 ± 0.1307	0.1535 ± 0.003411	ND	ND	0.02185 ± 0.009190	0.01565 ± 0.001170	0.004881 ± 0.001610	0.003923 ± 0.0008000	0.02954 ± 0.008500	0.02796 ± 0.01200	0.02710 ± 0.003300	0.01254 ± 0.002800
	Sat	0.5610 ± 0.003946	0.4094 ± 0.1081	ND	ND	0.1043 ± 0.001220	0.02812 ± 0.0008900	0.02325 ± 0.001690	0.02034 ± 0.001020	0.1245 ± 0.001800	0.04221 ± 0.002800	0.03589 ± 0.009300	0.03083 ± 0.003600
	Sun	0.9794 ± 0.2776	0.3061 ± 0.01960	0.2710 ± 0.002367	ND	0.03163 ± 0.0007000	0.02447 ± 0.0006300	0.01413 ± 0.0008200	0.01057 ± 0.0003200	0.1018 ± 0.01450	0.08303 ± 0.03700	0.02195 ± 0.009900	0.02130 ± 0.009900

MET: metformin, CAF: caffeine, SMX NOH: sulfamethoxazole hydroxylamine, SMX: sulfamethoxazole, NEV: nevirapine, PRED: prednisolone, VAL: valsartan, RIF: rifampicin, EE2: 17α-ethinylestradiol, IVE: ivermectin. ND: not detected.

APPENDIX C: MASS LOADS

Appendix C1: Calculated mass loads.

Pharmaceutical	Day of week	Mass loads/g day^{-1}					
		February 2022		April 2022		June 2022	
		INFLUENT	EFFLUENT	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Metformin	Mon	12508.18	339.70	6414.03	2647.91	5114.12	0.00
	Tues	6048.75	1659.11	4993.07	2484.12	1785.09	0.00
	Wed	5461.00	1384.73	20958.53	3086.18	4744.72	106.81
	Thurs	8307.77	583.64	4671.64	2117.81	1932.25	515.78
	Fri	1562.98	513.50	5407.55	1670.08	2229.61	20.81
	Sat	16674.90	1116.95	7697.49	21.31	3365.94	1337.98
	Sun	14997.77	277.20	4235.08	69.89	5501.91	420.90
Caffeine	Mon	8414.70	3320.36	16077.60	5794.05	9823.00	0.00
	Tues	16032.55	4757.94	27774.10	13464.12	64690.20	0.00
	Wed	5974.45	1657.27	27240.36	15393.00	73344.45	54052.81
	Thurs	5265.60	0.00	19866.00	16968.00	76994.27	59431.86
	Fri	629.73	0.00	22778.07	19432.36	89264.04	64799.15
	Sat	9909.82	0.00	18447.73	14641.25	79883.57	55641.02
	Sun	8185.86	0.00	22882.85	19004.15	83294.06	32676.53
Sulfamethoxazole hydroxylamine	Mon	21785.32	1128.39	5840.80	2011.60	6029.68	0.00
	Tues	15262.50	7070.93	9077.60	5093.20	20147.42	0.00

Pharmaceutical	Day of week	Mass loads/g day^{-1}					
		February 2022		April 2022		June 2022	
		INFLUENT	EFFLUENT	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
	Wed	18391.90	10194.12	9575.50	4905.23	19571.49	0.00
	Thurs	20272.68	8185.94	7324.374	6658.03	18457.98	11326.04
	Fri	28532.94	14282.14	9444.90	7826.20	22071.19	17411.04
	Sat	25047.34	3769.15	12681.02	5599.66	19785.89	15623.63
	Sun	25504.75	2466.74	9770.65	6924.50	19031.57	12384.93
Sulfamethoxazole	Mon	15837.14	3126.10	1099.64	210.79	13351.58	0.00
	Tues	8456.25	1907.99	1848.00	480.87	21000.00	14419.02
	Wed	8145.20	3001.20	3778.50	887.25	14453.63	13102.26
	Thurs	7193.40	616.68	2647.23	819.50	14716.14	12381.30
	Fri	4698.99	2734.68	4605.60	1076.10	16392.94	14488.41
	Sat	17781.60	916.70	9214.20	1687.63	15899.49	14123.76
	Sun	16325.65	787.05	4139.78	575.74	14566.25	12069.14
Nevirapine	Mon	16021.44	4059.20	1704.88	1177.00	6427.30	0.00
	Tues	9821.25	3309.04	1742.16	839.95	7271.16	0.00
	Wed	11820.70	4415.70	4265.69	966.00	7049.66	5819.43
	Thurs	9047.54	4046.32	3321.25	1027.85	6688.89	5665.18
	Fri	5884.70	1858.93	4280.70	2076.45	7777.20	4854.66
	Sat	18892.95	3463.47	9838.71	2112.84	7802.29	6219.92

Pharmaceutical	Day of week	Mass loads/g day^{-1}					
		February 2022		April 2022		June 2022	
		INFLUENT	EFFLUENT	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
	Sun	17295.19	2989.80	4139.07	979.00	8699.25	3101.10
Prednisolone	Mon	16682.24	6579.00	1398.65	319.93	3405.35	0.00
	Tues	10372.50	4028.96	982.74	58.89	939.88	0.00
	Wed	12001.30	4284.50	1495.45	847.41	3358.54	483.28
	Thurs	10856.37	4057.20	2394.00	931.61	30030.49	170.90
	Fri	7328.90	3964.03	3964.44	990.59	10707.56	1149.51
	Sat	19083.60	4663.32	9713.53	2635.07	9672.23	45.11
	Sun	18030.24	3870.90	5567.55	2376.00	5466.32	3269.11
Valsartan	Mon	16319.85	1294.30	2604.12	2393.59	14775.33	0.00
	Tues	9487.50	2946.64	2385.02	461.42	16234.78	0.00
	Wed	10930.60	4145.10	4902.01	1968.54	15348.96	4857.09
	Thurs	9909.38	3232.80	3149.54	1853.60	18218.17	1978.33
	Fri	5693.80	2858.65	4901.11	361.70	9156.37	3077.61
	Sat	19339.35	5024.05	10189.29	3798.38	16499.81	4864.29
	Sun	17718.81	4648.05	6203.88	2764.05	9530.90	3449.22
Rifampicin	Mon	10956.70	5940.40	18014.12	3171.94	22767.69	0.00
	Tues	9040.22	3463.66	16215.06	7571.22	14400.45	0.00
	Wed	10590.28	7369.05	29218.61	8709.12	10898.26	4297.66

Pharmaceutical	Day of week	Mass loads/g day^{-1}					
		February 2022		April 2022		June 2022	
		INFLUENT	EFFLUENT	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
	Thurs	10213.12	6020.64	28310.31	8364.64	13068.91	5170.61
	Fri	13890.50	4837.45	33599.36	10216.41	15355.61	5037.30
	Sat	15072.84	4925.78	26497.08	12675.89	15293.11	4644.57
	Sun	17231.31	7303.67	33887.52	17280.68	9907.24	5032.12
EE2	Mon	16516.38	6028.60	972.63	372.97	17094.27	0.00
	Tues	9097.50	270.29	1302.31	525.65	14850.11	0.00
	Wed	9905.05	3870.40	1880.11	550.96	9254.17	2284.88
	Thurs	8345.42	2123.67	2257.44	1069.25	13465.50	3930.24
	Fri	4248.95	3921.87	5004.76	705.10	8656.14	1211.88
	Sat	18693.00	3782.50	9519.52	1994.82	16786.99	948.23
	Sun	17127.84	3029.79	4779.06	1204.32	18097.52	4888.69
Ivermectin	Mon	58727.32	0.00	954.28	429.02	7453.86	2992.50
	Tues	22345.72	0.00	916.58	156.73	6335.00	2691.59
	Wed	39819.48	5115.09	2620.53	270.39	5092.00	2126.11
	Thurs	63001.30	0.00	5035.77	1065.71	6719.19	2271.84
	Fri	38741.61	0.00	2490.95	427.59	2245.04	903.11
	Sat	52177.39	0.00	9389.18	1729.40	8846.24	2096.76
	Sun	100876.20	0.00	3669.30	1163.12	6823.37	1363.20