Synthesis and the Application of Molecularly Imprinted Polymers as Solid-Phase Extraction and Dispersive Solid-Phase Extraction Sorbents in the Extraction of Antiretroviral Drugs in Water

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By

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A dissertation submitted to
The School of Chemistry and Physics
College of Agriculture, Engineering and Science
University of KwaZulu-Natal Pietermaritzburg (UKZN), in
fulfilment of the requirements for the degree of Master of Science in Chemistry

2022

Declaration

I, Thabiso Xolo, hereby declare that apart from the guidance and assistance from my supervisor, the research reported in this dissertation that is being submitted to the University of KwaZulu-Natal for the degree of Master of Science is my own work. It has never been published nor submitted for examination in any other university.

----------(signature of the candidate)

-----_____----------(Supervisor)

School of Chemistry and Physics, University of KwaZulu-Natal, Pietermaritzburg, 2022

Dedication

This work is dedicated to my son (Khwezi Owethu Xolo), my late mother (Tshitshi Mavis Xolo), my late father (Mandlenkosi Henson Xolo) and the rest of my supportive friends and family.

Kubamba ezingelayo.

Acknowledgements

I am thankful and appreciative of the help and contributions from the following people towards completion of this amazing piece of work:

- To my supervisor Dr. P.N Mahlambi, I am forever grateful for her guidance, support, encouragement, patience, and all the knowledge she has shared with me throughout this journey.
- To Dr J.T Khutlane, Ms. Z. Ngcingwana, Ms. C. Janse van Rensburg, Dr P. Kunene, and the rest of the Analytical Chemistry research group, I thank them for their support and assistance in handling the instruments used to conduct this work and their availability for discussions regards to what I should and should not do.
- To Ms. N.A.B Myeni, Ms S.N Zulu, Mr. T Khusi, Mr. N. Nyanda, Dr. S.S Cele, and Dr. M.M Mongaule, I thank them for their consistent support and for always reminding me the reasons why I chose to embark on this journey in the first place.
- To Mr S. Zuma, I thank him for the counselling sessions through all the times I felt like I was losing my mental health when things were not working out.
- To the National Research Foundation (NRF) of South Africa, I thank them for financially supporting this study (grant number 122598).
- To the University of KwaZulu-Natal (Pietermaritzburg Campus), School of Chemistry and Physics for resources and allowing me to conduct this research work within their premises.

Abstract

A multi-template molecularly imprinted polymer was synthesized, characterized, and applied to real water samples for the specific extraction of antiretroviral drugs (ARVDs), abacavir, nevirapine, and efavirenz. A MIP was synthesized by bulk polymerization at 60 ºC using abacavir, nevirapine and efavirenz (templates), 2-vinylpyridine (functional monomer), 1,1'-azobis-(cyclohexanecarbonitrile) (initiator), ethylene glycol dimethacrylate (cross-linker) and toluene: acetonitrile (9:1, v/v) (porogenic solvent mixture) for 16 hours. The temperature was then increased to 80 ℃ for 24 hours to ensure complete polymerization. The synthesized MIP was washed with acetic acid: acetonitrile (1:9, v/v) via soxhlet extraction until all three ARVDs were undetected in the washing solvent using high performance liquid chromatography coupled with a photo-diode array detector. A non-imprinted polymer (NIP) was synthesised using the same reagents and quantities except for the templates.

Both MIP and NIP were characterized using Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), scanning electron microscope (SEM), N_2 physisorption analysis, and elemental analysis. The FTIR results showed that the polymers were similar in structure and BET showed that they were mesoporous. The SEM showed that the MIP surface was rougher when compared to the NIP and characterization with TGA showed that they were both thermally stable.

The synthesized MIP was used to study its adsorption kinetics and isotherms. Kinetics modelling revealed that the Pseudo-second-rate order kinetics was the best fitting model with correlation coefficient of 1 compared to Pseudo-first-rate order kinetics which had a correlation efficient of 0.81-0.983 for all target analytes. The best fitting adsorption isotherm was the Freundlich model with a correlation range of 0.9451-0.986 for compared to the Langmuir model which had correlation efficient of 0.6692-0.93390.0198-0.6782 for all target analytes.

The traditional solid phase extraction and the MIP-based solid phase extraction methods were applied in distilled water samples spiked with 1 $mg{\cdot}L^{-1}$ of ARVDs and recoveries obtained were ranging from 91.68-94.59% and 97.20 to 99.68%, respectively. The MIP-based dispersive solid phase extraction method was successfully optimized with recoveries ranging from 100.28% to 102.60% for all three analytes. Selectivity studies were conducted using both the NIP and MIP with lamivudine and diclofenac as competitors. The recoveries obtained for the MIP ranged between 92% to 98% for the target analytes while they were 63% to 79% for

competitors. These results showed good selectivity and strong affinity of the polymer towards the target analytes than the competitors. This is justified by the presence of imprinted recognition sites that have the same functional groups, size, and shape as the target analytes/templates hence recoveries were low for competitors. The MIP was more selective towards analytes of interest compared to the NIP (recoveries ranged from 87.9% to 91%) for the analytes of interest which indicates successful imprinting on the MIP. Reusability studies showed that the MIP can be reused for up to 8 cycles with recoveries above 92% for all target analytes.

The developed, adopted, and validated methods were then applied to wastewater, tap water and river water samples from around KwaZulu-Natal. The concentrations obtained for abacavir, nevirapine and efavirenz were 10.65-295.90 $\mu g \cdot L^{-1}$ in wastewater, 1.95-13.15 $\mu g \cdot L^{-1}$ in river water, and 2.17-6.27 $\mu g \cdot L^{-1}$ in tap water. Efavirenz was the most dominant and consistently detected ARVD in all samples. The MIP-DSPE was the most sensitive and selective extraction technique compared to SPE and MIP-SPE. Umhlathuzana and Amanzimtoti were the most ARVD's polluted wastewater treatment plants, whilst Northern wastewater water works was the least polluted. Camps Drift was the most ARVD's polluted sampling point in Msunduzi river. Napierville and Scottsville showed the most contaminated tap water samples from suburbs around Pietermaritzburg.

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Abbreviations

% RSD	Percentage relative standard deviation
3TC	lamivudine
ABC	Abacavir
ACN	Acetonitrile
AE	Absorption efficiency
AIBN	2,2'-Azobis(2-isobutyronitrile)
APIs	Active pharmaceutical ingredients
ART	Antiretroviral therapy
ARVDs	Antiretroviral drugs
BET	Brunauer-Emmett-Teller
C_e	Equilibrium concentration
C_o	Initial concentration
CHN	Carbon, hydrogen and nitrogen analyser
CHNS	Carbon, hydrogen, nitrogen, and sulfur analyser
CNS	Carbon, nitrogen, and sulfur analyser
DAD	Diode-array detector
DIC	Diclofenac
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen.
EA	Elemental analysis
EFV	Efavirenz
EGDMA	Ethylene glycol dimethacrylate
FDC	Fixed dose combination
FTIR	Fourier transform infrared spectroscopy.
GC	Gas chromatography
H_2O	Water
HIV/AIDS	Human immuno-deficiency virus/ Acquired immuno-deficiency virus
HPLC	High performance liquid chromatography
HPLC-MS	High performance liquid chromatography- mass spectrometry
HPLC-VWD	High performance liquid chromatography-variable wave detector

IF	Imprinting factor
K	Retention behaviour
k_1	Pseudo-first-rate order constant
k_2	Pseudo-second-rate order constant
K_F	Freundlich constant
K_L	Langmuir constant
Kmip	MIP retention behaviour
Knip	NIP retention behaviour
LC	Liquid chromatography
LC-MS	Liquid chromatography- Mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MeOH	Methanol
MIP	Molecularly imprinted polymer
MIT	Molecular imprinting technology
MIP-DSPE	Molecularly imprinted dispersive solid phase extraction.
MIP-SPE	Molecularly imprinted solid phase extraction.
MRL	Maximum residue limit
MS	Mass spectrometry
n	Adsorption intensity
NIP	Non-imprinted polymer.
NNRTIs	Non-nucleoside reverse-transcriptase inhibitors
NRTIs	Nucleoside reverse-transcriptase inhibitors
NSAIDS	Non-steroidal anti-inflammatory drugs
NVP	Nevirapine
PDA	Photo diode array
PIs	Protease inhibitors
Q_e	Adsorption capacity at equilibrium
Q_m	Maximum adsorption capacity
Q_{mips}	MIP adsorption capacity
Q_{nips}	NIP adsorption capacity
Q_t	Adsorption capacity at time t
R^2	Correlation coefficient
RNA	Ribonucleic acid

SA	South Africa
SEM	Scanning electron microscopy
SPE	Solid-phase extraction
TDS	Total dissolved salts
TGA	Thermogravimetric analysis
TPs	Transformation products
TOFMS	Time of flight mass spectrometry
UV-VIS	Ultraviolet-visible detector
WHO	World health organization
WWTPs	Wastewater treatment plants.

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Chapter One: Introduction

1.1 Background

Water is one of the essentials to life for humans, animals, plants, and aquatic plants and animals. The synthesis and structure of cell constituents and transportation of nutrients into different parts of the body as well as metabolism also depends on water (Sharma *et al*., 2017). A major part of the world's economy is controlled by the availability of fresh water. Adequate water supplies are necessary for agriculture, human consumption, industrial uses as well as recreation. However, studies conducted in African countries have indicated that non-steroidal inflammatory drugs (NSAIDs), antibiotics, steroid hormones, and antiretroviral drugs (ARVDs) are amongst the most detected groups of pharmaceuticals in the aquatic environment (Madikizela *et al*., 2020, Mlunguza *et al*., 2020).

Pharmaceuticals are highly active chemical compounds designed to have specific physiological effects on target organs or tissues (Fortunak *et al*., 2014). The ARVDs are drugs used to treat the human immunodeficiency virus (HIV) infection (Nannou *et al.,* 2020). They act by reducing the viral DNA replication speed, at the same time stopping its propagation in the body (Rampersad *et al.*, 2018). However, like any other pharmaceutical compounds, the ARVDs are not completely digested in the human body and hence they are excreted (as parent compounds or metabolites) via urine and faeces and from the sewer they can end up in the wastewater treatment plants (WWTPs) (Abafe *et al.*, 2018, Adeola *et al.,* 2022). The WWTPs are not designed to remove these compounds and their residues are therefore released into the receiving rivers where they can have negative effect to aquatic animals and plants (Russo *et al*., 2010). Exposure to high levels of ARVDs can affect the central nervous system (Smurzynski *et al.,* 2011). Also, from the rivers they can end up in drinking water leading to indirect ingestion by the consumers which could result to their accumulation in the body and hence possible drug resistance towards them (Russo *et al*., 2010, Schoeman *et al*., 2015). This indicates the importance for their monitoring in the environmental matrices.

Antiretroviral drugs are present at very low concentrations in the water bodies; hence they require a very sensitive analytical technique for their possible determination and extraction in the water bodies (Adeola *et al.,* 2022). Solid phase extraction (SPE) has been used for the extraction of many organic pollutants including pharmaceutical compounds due to its high sensitivity, it is easy to use and fast (Fortunak *et al*., 2014). However, its sorbents lack selectivity hence molecularly imprinted polymers (MIPs) have been introduced as sorbents to

increase selectivity and efficiency of this technique (Mtolo *et al.*, 2019). The MIPs are easy to prepare and handle, with good thermal and chemical stability in comparison to common SPE sorbents (Bhageri *at al.*, 2022). They also can be applied to a wide range of compounds and reused with excellent reproducibility. This work therefore aims to synthesize and apply molecularly imprinted polymers as SPE sorbents for the determination of antiretroviral drugs in river water, wastewater and tap water.

Bagheri, A.R., Aramesh, N., Gong, Z., Cerda, V. and Lee, H.K., 2022. Two-dimensional materials as a platform in extraction methods: A review. *TrAC Trends in Analytical Chemistry*, p.116606.

1.2 Problem statement

South Africa has the highest antiretroviral therapy programme in the world with approximately 8.2 million people reported to be taking ARVDs in 2021 (STATSA, 2021). KwaZulu-Natal province has the second largest proportion of the population recorded to be taking ARVDs (21%). Due to incomplete digestion of ARVDs in the human body, they are excreted from the body through urine and faeces and find their way to the wastewater treatment plants where they are partially removed and therefore disposed to the receiving rivers and may reach drinking water sources. Therefore, more precautions must be taken to keep up with the level of concentrations of these drugs in water and their potential effects to the aquatic environment and humans due to indirect ingestion (Kim *et al*., 2021). The frequent detection of these compounds is mainly attributed to their poor removal from WWTPs during water purification and poor disposal of unused medicines on land which may result in transportation of these compounds into nearby rivers during rainy season (Madikizela *et al*., 2020). Therefore, highly sensitive, and selective analytical methods must be developed to selectively extract these ARVDs in water and study potential effects to both humans and the aquatic life.

Unlike pesticides and fertilizers, these new emerging water pollutants, ARVDs, are present at inconsistently varying concentrations. They have no known maximum residue limits (MRL) and their potential effects to the aquatic environment, terrestrial plants and animals and humans who use the contaminated water bodies are yet to be studied and understood. The occurrence of ARVDs in water has been studied to a limited extent compared to other pharmaceuticals such as non-steroidal anti-inflammatory drugs (NSAIDs), steroid hormones and antibiotics, yet to date, there are over 30 antiretroviral medications available for the treatment of HIV (Nannou *et al*., 2020). The number of patients using ARVDs increases exponentially on a yearly basis

worldwide (Tshamba *et al.*, 2013), which increases production and introduction of these compounds into the water sources through excretion and surface run-off of poorly disposed unused and expired drugs.

A few studies analysing the occurrence of ARVDs in water have been conducted in South Africa in Gauteng, and in KwaZulu-Natal (Schoeman *et al*., 2015, Schoeman *et al*., 2017, Mtolo *et al*., 2019, Mlunguza *et al*., 2020, Qwane *et al*., 2020). Therefore, in this study the occurrence of efavirenz, abacavir and nevirapine in wastewater from Durban and Pietermaritzburg WWTPs, tap water from suburbs in Pietermaritzburg, and river water along Msunduzi river in Pietermaritzburg was investigated to understand the extent of their pollution and determine the effectiveness of using a multi-template molecularly imprinted polymers as a sorbent in the extraction process.

Liquid chromatography equipped (LC) with photodiode array detection (PDA) was used for the detection of ARVDs. The detection limits for LC-PDA were improved by the application of appropriate sample preparation technique such as traditional SPE and MIP-SPE that are used for the preconcentration of the analytes in the sample matrix.

1.3 Aim

The aim of this research project was to synthesize and apply molecularly imprinted polymers (MIPs) as solid phase extraction sorbents and dispersive solid phase extraction sorbents for the removal of ARVDs in water samples.

1.4 Objectives

The Objectives were:

1. To optimize the LC-PDA method for separation and detection of ARVDs.
2. To synthesize and characterize molecularly imprinted polymers (MIPs).
3. To optimize the solid-phase extraction (SPE), MIP-SPE and MIP-DSPE to obtain conditions that will allow high recoveries of ARVDs from river water, tap water, and wastewater.
4. To apply the optimized conditions for the effective removal of ARVDs in river water, tap water, and wastewater.
5. To compare the performance of SPE, MIP-SPE and MIP-DSPE on the extraction of ARVDs in river water, tap water, and wastewater samples.

1.5 Research questions

- Will MIPs be successfully synthesized and characterized?
- Which extraction parameters should be optimized to obtain higher recoveries for all the analytes of interest?
- Which parameters need to be optimized to allow successful rebinding of analytes of interest to the MIPs?
- Will the selected ARVDs (abacavir, nevirapine and efavirenz) be present in the area of study and at what concentrations will they be present?
- Which of the selected ARVDs is the most dominant and which of the selected sampling points is the most polluted with the ARVDs?
- Which method is more efficient between SPE, MIP-SPE and MIP-DSPE?

1.6 Research summary

This research is made up of five chapters. Chapter one covers the background and problem statements associated with ARVDs. It also covers the aims and objectives resulting from the problem statement as well as the research questions that were answered by this project. Chapter two covers the literature review on ARVDs, their uses, impact, and fate in the environment. Sources of pharmaceutical contamination of water, exposure pathways, and physicochemical parameters, are also covered. A review of the synthesis of MIPs, extraction techniques, sample preparation, separation, and detection. A brief description of the characterization techniques used in this research was also included.

Chapter three consists of methods and materials used in this project; instrumentation used, discussion of sampling sites, sample preparations and method validation.

Chapter four reports the results and discussions from the optimization of LC-PDA to the application to real environmental samples. Synthesis and characterization of the MIP factors affecting adsorption and their optimum quantities/conditions ensuring maximum adsorption onto the MIP were discussed in this chapter. The results of the optimization of SPE, MIP-SPE and MIP-DSPE, adsorption isotherms and kinetics were also included.

The results on the application of methods such as the traditional solid phase extraction, MIP based solid phase extraction and dispersive solid phase extraction technique with MIPs as the

sorbent in the extraction of ARVDs in different environmental water sample types were reported in this chapter. Chapter five consists of the conclusion and future recommendations.

Chapter Two: Literature Review

2.1 Antiretroviral Drugs

Antiretroviral drugs (ARVDs) are pharmaceutical drugs used to prevent a retrovirus, such as HIV, from replicating and are the mainly prescribed for the treatment of HIV/AIDS worldwide (Mlunguza *et al*., 2019). World Health Organization (WHO) reported in 2021 that over 8.2 million people in South Africa were infected with human immunodeficiency virus-1 (HIV-1) (Stats SA 2021, P0302). The number of infected patients exponentially increases on a yearly basis, which significantly increases both the production and the consumption of antiretroviral drugs (Mlunguza *et al*., 2020).

The ARVDs are divided into six classes; the nucleoside reverse transcriptase inhibitors (NRTIs) which acts in the early replication viral cycle preventing reverse transcription of the viral RNA into its DNA (Fortunak et al., 2014). The non-nucleoside reverse transcriptase inhibitors (NNRTIs) acts by hooking onto reverse transcriptase inhibiting its activity (Mtolo, 2019). The entry inhibitors act by preventing the virus from attaching to a body cell (Nannou *et al*., 2020). The integrase inhibitors act by preventing the insertion of viral DNA into the DNA body cell (Maertans *et al.*, 2022). The cytochrome P45 or 3A inhibitors acts as pharmacokinetic enhancers of other ARVDs and the protease inhibitors (PIs) act by preventing the latent HIV DNA from fragmenting into components required for HIV RNA strands (Fortunak *et al*., 2014). Efavirenz, abacavir and nevirapine are some of the major active pharmaceutical ingredients (APIs) that are used in the antiretroviral therapy. Active pharmaceutical ingredients (APIs) are the molecular entities that exert the therapeutic effects of medicines and are formulated to assure their stability to enhance patient compliance and to maximize therapeutic efficacy (Fortunak *et al*., 2014). Effective chemotherapy of HIV consists of a fixed-dose combination with at least three different API, e.g., two NRTIs and one NNRTI. Efavirenz and nevirapine are two NNRTIs currently used in the first line of antiretroviral therapy (Ncube *et al*., 2018, Nannou *et al*., 2020). **Figure 2.1** shows the contribution of various pharmaceutical groups computed from the studies conducted in African countries (Madikizela *et al*., 2020).

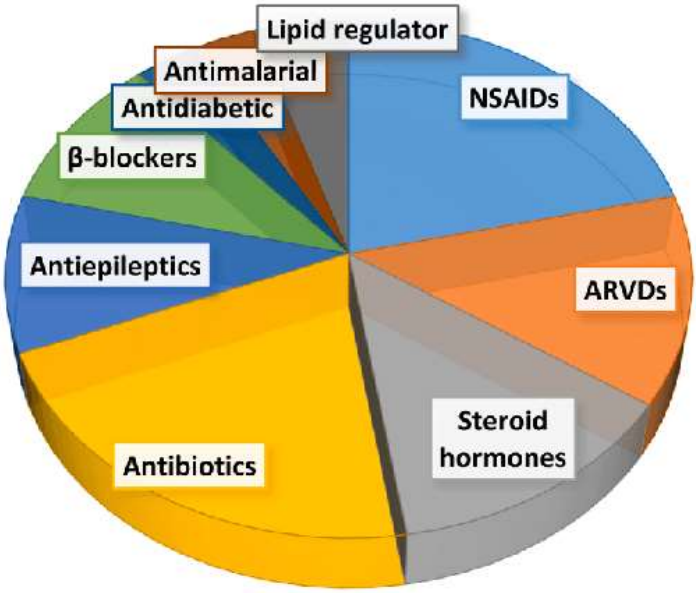


Figure 2.1 Contribution of various pharmaceutical groups in African countries (Madikizela *et al*., 2020).

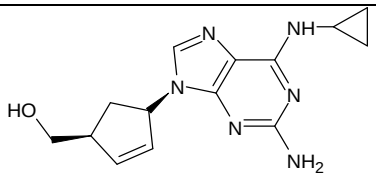
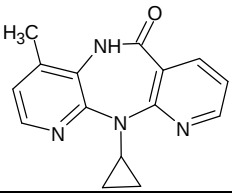
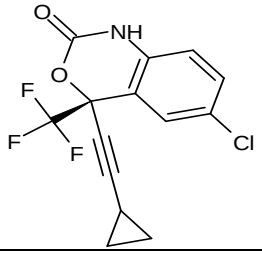
Antibiotics and NSAIDs are currently the most frequently detected groups of pharmaceuticals in the African aquatic environment (Madikizela *et al.*, 2020). The presence of antibiotics could be attributed to the global increase in antibiotic consumption through medical prescription for the treatment and prevention of bacterial infections (Madikizela *et al*., 2019). The high consumption of antibiotics observed in low- and middle-income countries is associated with approximately 80% of all antibiotics being used without proper medical prescription (Fekadu *et al*., 2019). NSAIDs are extensively used for the treatment of various diseases such as arthritis, musculo-skeletal pain, and various acute pains (Bindu *et al.*, 2020). Due to their availability over the counter without a medical prescription, they are widely used resulting to their frequent detection as derivatives or parent compounds in water sources (Mlunguza *et al*., 2019). Steroid hormones and ARVDs are amongst the new emerging water pollutants. Whilst ARVDs are associated with the world's biggest HIV pandemic, the increasing presence of steroid hormones is yet to be justified (Madikizela *et al*., 2020).

2.1.1 Properties of ARVDs

The behavior of ARVDs in the environment is mainly dependent on their physical properties such as the solubility, pKa, polarity (XLogP3), and octanol-water coefficient (log Kow) (Ngwenya, 2022). Octanol/water partition coefficient is used to measure how hydrophobic or hydrophilic a drug or chemical substance is (Nannou *et al*., 2020). Higher log Kow value indicates likeliness that a chemical substance will be found more on a solid matrix than a liquid

(Mamy *et al.,* 2015). Abacavir has the smaller log Kow value, which indicates higher water solubility and efavirenz has the higher log Kow value which indicates poor water solubility (Mtolo *et al.,* 2019, Qwane *et al.,* 2020). This indicates that efavirenz is expected to be present at very low concentrations in water samples and highly adsorb in solids compared to abacavir (Ngwenya, 2022). The pKa is an acid dissociation constant which can be related to strength and polarity of the acid or compound. High pKa values indicate strong acids (completely dissociates) and higher water solubility (polar dissolves polar), and low pKa values indicate relatively weak acids (partially dissociates) and low water solubility (polar does not dissolves non-polar (Settimo *et al*., 2014). **Table 2.1** shows the properties of some of the ARVDs.

Table 2.1. Names, structures, and chemical properties of some ARVDs (Wood *et al*., 2015)

Name	**Structure**	**Mw ($g.mol^{-1}$)**	**Kow**	**pKa**	**Solubility ($mg{\cdot}mL^{-1}$)**
Abacavir		286, 332	1.54	5.77	77.0
Nevirapine		266, 298	3.89	2.5	0.7046
Efavirenz		315, 675	4.15	-1.5	0.00855

2.1.2 How do ARVDs enter water?

Antiretroviral drugs are not completely metabolized in the human body when ingested for the promotion of health (Prasse *et al.,* 2010). The indigested parts of the ARVDs leave through urine and faecal matter into the sewage system and are transported into the wastewater treatment plants (WWTPs) (Prasse *et al.,* 2010, Rimayi *et al.,* 2018). These drugs are present at trace level concentrations, and some are highly soluble in water. The WWTPs are not designed for the removal of such drugs, as a result ARVDs easily escape during water treatment

and purification, and they end up in rivers as well as tap water (Mlunguza *et al*., 2020). The poor disposal of unused and expired drugs from household waste, pharmacies, toilet sinks, industries and hospitals may result in their presence in WWTP as well as landfills and thus end up in ground and surface waters. This results in WWTPs being the biggest contributor to water pollution with ARVDs (Madikizela *et al*., 2018). These drugs remain in water bodies and are unintentionally returned to the surface waters for human consumption, recreation, agricultural and industrial uses (Fekadu *et al*., 2019). **Figure 2.2** shows the various sources of ARVDs into water bodies.

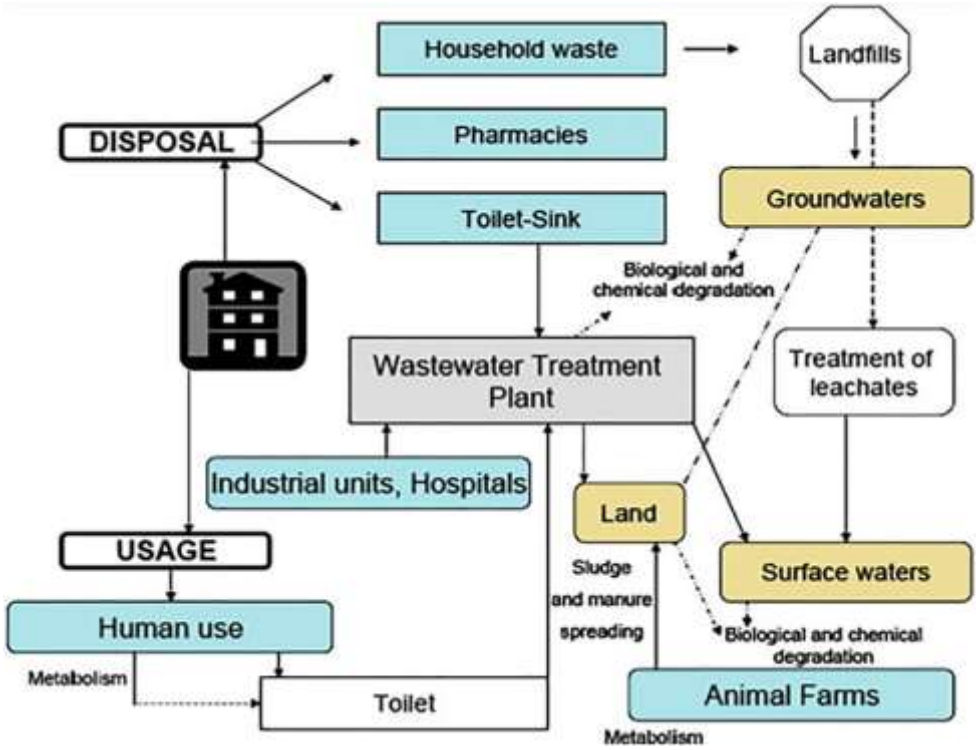


Figure 2.2. Sources of ARVDs into the water bodies (Madikizela *et al*., 2018)

2.1.3 Exposure pathways and the effects of ARVDs in humans and aquatic environment.

Exposure to ARVDs apart from self-ingestion via prescription can be through the food chain, drinking contaminated water, and eating contaminated food sources such as crops, vegetables, fruits, fish, and dairy products. The use of sludge from WWTPs by farmers as manure, and irrigation using effluent from WWTPs may contributes towards the presence of ARVDs in crops which may lead to the biomagnification of ARVDs within the food chain (Agoro *et al*., 2020). A study reported that lettuce can absorb the nevirapine, lamivudine, and efavirenz from tainted water through its roots and then transfer them to its edible leaves (Zitha et al., 2022).

The main exposure route is through WWTP to surface water and terrestrial run-off from improper disposal of expired/unused medicines (Rimayi *et al*., 2018, Mtolo *et al*., 2019). The ARVDs can potentially result in abnormalities in aquatic animals that reside in contaminated water depending on their individual toxicity. It has been reported that fish exposed to ARVDs had abnormal livers (Rimayi *et al*., 2018, Nibamureke *et al*., 2019). While effects of indirect ingestion of ARVDs by drinking the contaminated water and eating the contaminated fish are not yet thoroughly known, it has been reported that at high levels the ARVDs can cause problems in the central nervous system and could also lead to a possible drug resistance towards these drugs (Tijani *et al*., 2013, Nannou *et al*., 2020).

A study conducted by Mckinney and colleagues showed that zidovudine exposure to children can cause anaemia and lactic acidosis which may affect breathing patterns (Mckinney *et al*.,1991). Long term survivors of HIV who were through antiretroviral therapy (ART) showed several pathologies such as metabolic, lipid, cardiovascular and neurological disorders (Bijker *et al*., 2020). Another study conducted by Bertrand and colleagues showed that long term use of ART may result in cerebral vascular toxicity (Bertrand *et al*., 2019).

Vogt and co-workers conducted a study on exposure of bacteriophages and snail to ARVDs. The active pharmaceutical ingredients of ARVDs showed to have a negative impact on assemblage of viral proteins on the transmission electron microscope. This was due to that sewage treatment plants rely on the activity of bacteriophages to infect and kill most of the bacteria in the system. On the other hand, the phage depends on the metabolic activity of the bacterial host for proliferation and survival within the system. The metabolic processes of these bacteria were therefore affected by the ARVDs as they appeared to reduce the total amount of phage in the system. Since decreasing the number of phages in the system can increase the number of bacteria in the wastewater from the treatment plant. This resulted in the ARVDs effect in the productivity of wastewater treatment plants through removing bacteria from water by acting on phage in systems designed to kill and control these levels of bacteria (Vogt *et al*., 2020). Efavirenz and nevirapine also showed an overall inhibitory effect on embryonic growth of B. *tropicus*. These changes in growth rate can have a significant impact not only on interactions with other species and algae, but also on population density and demographics (Vogt *et al*., 2020). Some of the human health effects include nausea, myopathy, dyslipidemia, etc (Adeola *et al.*, 2022). Indirect ingestion may also result in liver toxicity, skin rash, formation of highly active and toxic degradation products (Nibamureke *et al*., 2019).

2.2 Extraction techniques

Different extraction techniques such as liquid-liquid extraction, solid phase micro-extraction, hollow fibre liquid phase micro-extraction and solid phase extraction have been employed for the extraction of ARVDs in water bodies, however, solid phase extraction is often used.

2.2.1 Solid Phase Extraction (SPE)

The SPE is one of the most used techniques in the extraction of pharmaceuticals in water. This is because of its high sensitivity as well as and its ability to clean-up, pre-concentrate, and extract analytes (Madikizela *et al*., 2022). The SPE consists of four steps as shown in **Figure 2.3.** Conditioning of the cartridge is the first step, and it allows for the activation of the stationary phase for consistent interaction between the analyte and sorbent. The second step is sample loading which is the introduction of the sample to the sorbent to allow maximum retention of analyte. The third step is the washing of the adsorbed impurities with an appropriate solvent that will not elute the analytes but will only remove matrix interferences. The fourth step is the elution of the adsorbed analytes with an appropriate solvent that should be strong enough to completely elute all the adsorbed analytes from the sorbent with the smallest volume possible (Vasapollo *et al*., 2011, Lu *et al*., 2019).

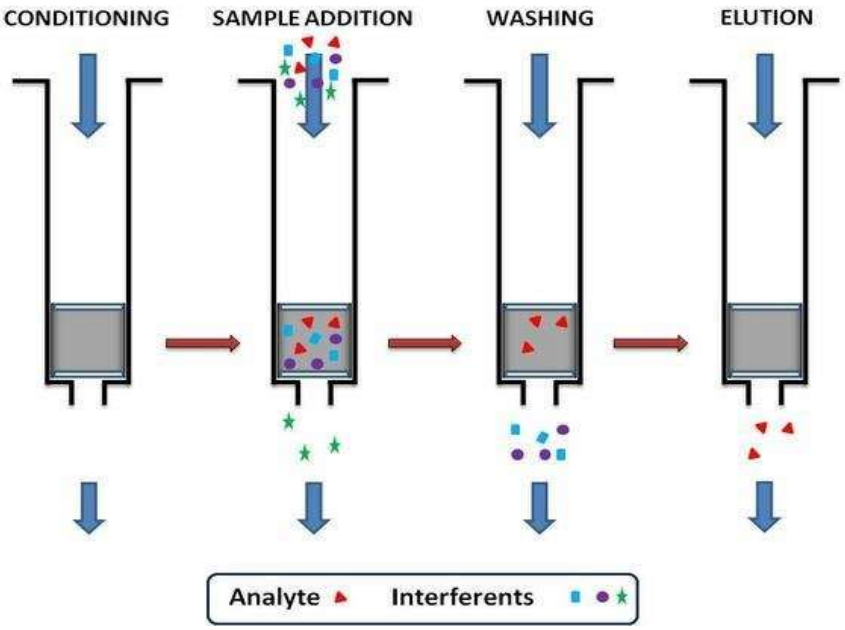


Figure 2.3. General steps for solid phase extraction (Lu *et al*., 2019).

Oasis Hydrophilic-Lipophilic-Balance is usually employed as a suitable adsorbent for many pharmaceuticals including ARVDs which is attributed to its ability to extract both hydrophilic and lipophilic-based compounds (Becskereki *et al*., 2022). Agela Cleanert PEP is another SPE

sorbent used for the extraction of pharmaceuticals from water samples (Mtolo, 2019). Even though, these SPE sorbents have good sensitivity, they have poor selectivity which often leads to co-elution of analytes with interference matrices. This major disadvantage led to the introduction of molecularly imprinted polymers (MIPs) as SPE sorbents which have a very high selectivity due to their imprinted specific recognition sites (Zwir-Ferenc *et al*., 2006, Azizi *et al*., 2019, Pichon *et al*., 2019).

2.2.2 Molecularly Imprinted Polymers (MIPs)

MIPs are synthetic polymeric materials with specific recognition sites that are complementary in shape, size, and functional groups to the analytes of interest (Lu *et al*., 2019). MIPs are cost-effective, easy to prepare and handle, they are thermally and chemically stable and applicable to wide range of compounds (Azizi *et al*., 2019). They are popular for their high selectivity hence they are combined with SPE to increase both sensitivity and selectivity of the method (Mtolo *et al.,* 2019). MIP-SPE is the conventional SPE technique which employs MIPs as the sorbent rather than Oasis HLB or Agela Cleanert PEP traditional sorbents. MIP-DSPE allows for the dispersion of MIP particles into the sample and thereafter the sorbent is collected using empty SPE cartridges (Lu *et al*., 2019). MIP-DSPE sometimes incorporates the use of MIPs coated with magnetic nanoparticles for easy collection of the sorbent with a magnet after dispersion into the sample (Chen *et al*., 2016).

Figure 2.4 shows a schematic illustration for the general preparation of MIPs. Initially, a template and functional monomer(s) are mixed in addition of a porogen which homogenizes the solution prior polymerization process. This is followed by the addition of a cross-linker and an initiator which triggers the beginning of the polymerization process. When polymerization is complete, the template is removed through multiple washing steps leaving the imprinted cavities on the surface of the polymer, complimentary in size, shape, and functional group to the functional monomer(s) (Vasapollo *et al*., 2011, Azizi *et al*., 2019).

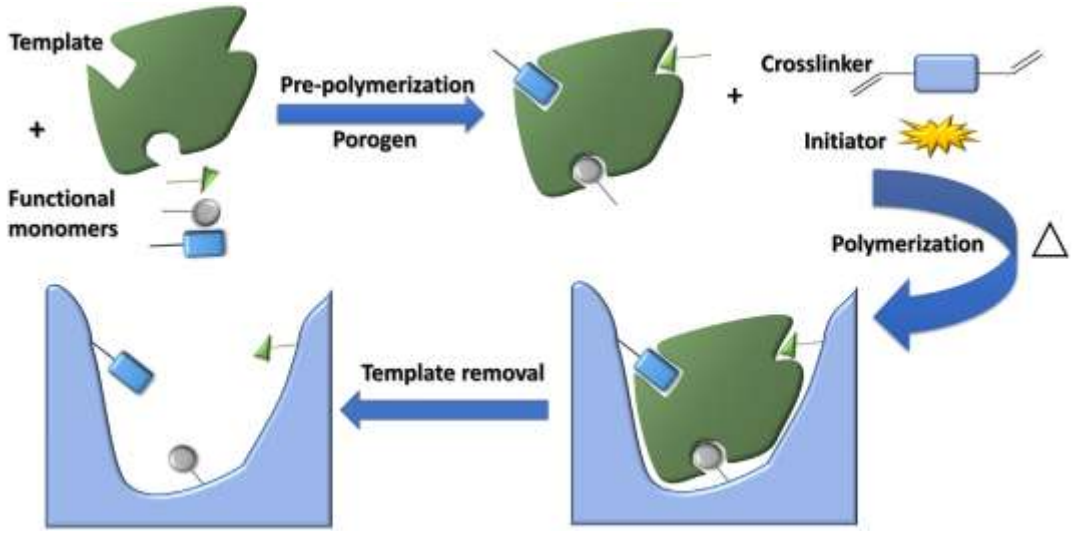


Figure 2.4. Schematic illustration for the general preparation of MIPs (Azizi *et al*., 2019)

2.2.2.1 MIP-SPE

MIP-SPE is the most common application of MIPs in sample pre-treatment. It can be coupled with an analytical instrument such as HPLC for an on-line mode. This is done by packing MIP particles in an HPLC pre-column which allows instant separation and detection of the analyte(s). An off-line mode is conducted by packing the synthesized MIP particles into empty SPE cartridges using dry or slurry packing method. Selective extraction of analytes in an off-line mode is achieved by regular steps of SPE, i.e., conditioning of the sorbent, sample loading, washing of impurities and elution of the analytes. The conditioning step of the MIP cartridge using an appropriate solvent system is done to activate the binding sites. It is then followed by loading the sample solution (Pichon *et al*., 2019). The MIP cavities then trap the analytes of interest complimentary in shape, size and functional group by adsorption and the interferences are removed during the washing step due to weak non-selective adsorption. A desorption solvent is used to disturb the interactions between the imprinted binding sites and analytes for elution (Vasapollo *et al*., 2011, Azizi *et al*., 2019). **Figure 2.5** shows the off-line mode of the MIP-SPE.

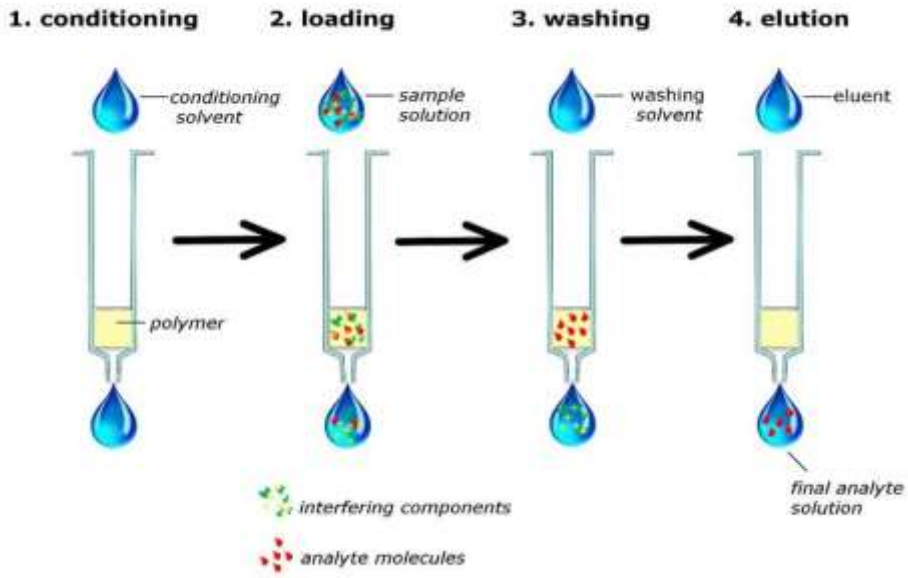


Figure 2.5. General steps for SPE using MIP as sorbent in an off-line mode (Zwir-Ferenc *et al*., 2006).

2.2.2.2 MIP-DSPE

MIP-SPE has been used as a substitute of Oasis HLB for the selective extraction of analytes from environmental water samples, however, the packing of MIPs into the SPE cartridge has disadvantages such as clogged columns and swelling of the MIPs in organic solvents (Azizi *et al.*, 2019). DSPE incorporates direct dispersion of MIP particles into the sample under stirring/shaking and/or the use of conventional beads or magnetic nanoparticles to aid the recovery of the solid phase and overcome these disadvantages (Azizi *et al*., 2019). After sufficient contact time between the sorbent and analyte(s), the particles are recovered by either centrifugation or magnetic field (if magnetic NPs are used), then introduced into an appropriate desorption solvent after a possible washing step (Lu *et al.,* 2019). **Figure 2.6** shows the preparation of MIPs and the incorporation of MIP-DSPE in the extraction of analytes.

Figure 2.6. Schematic procedures for the preparation of MIPs by precipitation polymerization (A) and MIP-DSPE (B) (Lu *et al*., 2019).

2.3 Synthesis strategies of MIPs

Different strategies can be adopted in the synthesis of MIPs to overcome application limitations such as: incompatibility of MIPs with aqueous matrices, adsorption interferences, poor imprinting effects for water soluble compounds, heterogenous and non-specific binding sites (Pichon *et al*., 2019). There are currently three strategies that can be implemented in the synthesis of MIPs, covalent imprinting, non-covalent imprinting, and semi-covalent imprinting.

2.3.1 Covalent Imprinting

The MIP synthesis is solely based on the formation of covalent bonds between the template and the functional monomer in the pre-polymerization solution (Hasanah *et al.*, 2021). MIPs synthesized with this method have the most homogeneous structures with well-defined binding sites and cavities arising from the specificity of the bond formation due to the fixed stoichiometric ratio of functional monomer: template molecules (Vasapollo *et al.*, 2011). Covalent strategies are rarely used in water analysis due to the requirement for a suitable monomer-template complex that can form readily reversible covalent bonds with the right geometry and chemistry for the extraction of target analytes from water system (Vasapollo *et al*., 2011, Pardeshi *et al*., 2016).

2.3.2 Non-Covalent Imprinting

MIPs synthesis is based on the non-covalent interactions between the template and functional monomer (Hasanah *et al.,* 2021). In this method, excess of monomer is used to enhance the formation of monomer-template complex (Vasapollo *et al.,* 2011). One of the shortcomings of this method is that it can introduce many non-specific binding sites due to excess functional monomer that leads to the reduction of the selectivity of extraction (Pardeshi *et al*., 2016). The main advantage is that the template removal does not need any cleaving of bonds since interactions between the template and functional monomer are non-covalent.

2.3.3 Semi-covalent Imprinting

MIPs are formed by a hybrid strategy that uses both the covalent and non-covalent imprinting (Azizi *et al.,* 2019). In this approach, template molecules covalently bind to the functional monomer before polymerization. The analyte rebinds through non-covalent interactions which overcomes long equilibrium time as a limitation of covalent approaches (Pardeshi *et al*., 2016). This method provides rapid uptake of analyte(s) and reduces non-selective binding (Azizi *et al.,* 2019). The main disadvantage is that preparation of MIPs through this method is complicated (Vasapollo *et al.,* 2011).

2.4 Polymerization Techniques for the Synthesis of MIPs

There are many different polymerization techniques that have been employed in the synthesis of MIPs. These include surface polymerization which uses surface imprinting technology on a thin layer of polymeric material such as Fe_3O_4, TiO_2, SiO_2 or carbon nanotubes (Duan *et al.,* 2015). Precipitation polymerization uses larger volume of porogen and Pickering emulsion precipitation which uses surfactant emulsifier or solid particles to stabilize the formation of droplets in a mixture of two immiscible liquids (Vasapollo *et al*., 2011).

However, in this project bulk polymerization technique was used. This is the greener and most used method for the preparation of MIPs for analysis of environmental contaminants such as pesticides and other pharmaceuticals in water (Azizi *et al.,* 2019). Thermal or photoinitiation is used to synthesize the polymeric material in MIP-SPE. After grinding and sieving the powdered MIP particles are collected and subjected for template removal through Soxhlet extraction. MIPs offer several advantages such as stability, resistance to a wide range of pH, temperature, and organic solvents (Vasapollo *et al*., 2011, Pichon *et al*., 2019).

2.5 Optimization of MIP Formulae

MIPs with different formulations can be prepared and used experimentally to determine the optimal composition of the MIP formulation with the highest analyte selectivity. Several factors can be optimized to improve selectivity when preparing MIP. This includes the type of template, monomer, and cross-linker as well as their ratio (Vasapollo *et al.,* 2011). The type of porogenic solvent and the volume are also considered (Azizi *et al.,* 2019). The template should pose both structural and functional group similarities to form binding affinities (Azizi *et al.,* 2019). Simulation methods can be used to select structural analogues or functional monomer which reduces cost and time effectively (Qi *et al*., 2010).

A high ratio of template: monomer (1:2) reduces the selectivity of the polymer due to excess amount of template and lower number of binding sites. The lower ratios of template: monomer (1:8) decreases the number of the template-monomer complex in pre-polymerization solution and specific cavities for adsorption (Tang *et al*., 2008, Azizi *et al*., 2019).

The ratio of monomer-crosslinking agent must be optimized based on the selectivity, efficiency, and rigidity required of MIPs which may vary depending on format and application (Vasapollo *et al.,* 2011). In a study conducted by Tang and fellow workers, 1:4 template: monomer ratio was found to be the optimal ratio for imidacloprid with the highest recoveries of 77.3%, while lower recoveries were obtained for 1:8 ratio (56.8%) and 1:2 ratio (19.4%). This indicated that the 1:4 template: monomer ratio had the highest affinity of the analyte to the cavities of the MIP for imidacloprid (Tang *et al*., 2008).

The solvent plays a crucial role in non-covalent MIPs, and it can be optimized by chromatographic evaluation. Wang *et al* (2015) studied three porogenic solvents, acetonitrile, chloroform, and toluene to imprint the phenols. The retention and imprinting factors of analytes obtained by both MIP and NIP showed that acetonitrile has a much better ability to provide a selective retention of chlorophenols compared to toluene and chloroform. The selection of the porogenic system relies on different properties of MIPs such as binding capacity, imprinting effects, the number and size of pores and specificity (Qi *et al*., 2010).

2.6 Characterisation Techniques

2.6.1 Fourier-Transform Infrared

Fourier transformation infra-red (FTIR) spectroscopy is a characterisation technique used to investigate the nature of chemical bonds in any compound or material through an absorbance or transmittance spectra (Flemban, 2017). When doing the FTIR analysis, the sample is irradiated with infrared light such that the bonds present in a material, surface, or compound

absorb certain energies when vibrating at a particular frequency. The collected absorbance data is then processed by a mathematical algorithm called the Fourier transform. FTIR has two regions associated with different vibrations, (400 – 4000 cm^{-1} is for fundamental vibrations and rotations, 4000 – 14000 cm^{-1} is for overtones and harmonic vibrations) (Kumar, 2012). The 400 – 4000 cm^{-1} region is the main region of interest in which FTIR absorbance or transmittance spectrums are typically collected. In a study conducted by Mtolo and collaborators on the MIPs synthesis of efavirenz, three characteristic functional groups were observed using FTIR analysis for the MIP. These included C-O, C=O and N-H at 1150, 1730, and 2942 cm^{-1}, respectively (Mtolo *et al*., 2019). Even though alike peaks were observed in the corresponding NIP due to similar synthetic conditions employed for MIP and NIP, the peaks were more pronounced in the unwashed MIP because of the bonds overlapping of the MIP with the template which proved successful synthesis of the MIP (Wang *et al.,* 2015). The peaks were more intense for the NIP than the washed MIP which proved successful template removal that eliminated the overlapping bonds between the template and the MIP (Mtolo *et al*., 2019).

2.6.2 Scanning electron microscope

Scanning electron microscopy (SEM) technique is used to study materials surface morphology, crystalline structure and chemical composition, and electrical behaviour. SEM electron beam is generated through heat (thermionic) or field emission gun (Flemban, 2017). The electron beam is accelerated by a large electric field following a vertical path through the column of the microscope and through the electromagnetic lens, apertures and scan coils which aims the beam at the sample as shown in **Figure 2.5**. The electrons in the beam interact with the atoms that make up the beam, backscattered with sample material to generate secondary electrons. The detector collects secondary electrons and converts them into a signal which is collected as an image that allows one to analyse the surface of the sample. The obtained image is three-dimensional and up to 1, 000, 000 times magnifications (Tare *et al*., 2009). Other properties such as composition and electrical conductivity are also obtained. A study conducted by Mtolo and colleagues on imprinting efavirenz, showed that MIP had a rough surface compared to NIP which had a relatively smooth surface, (Mtolo *et al*., 2019). The same results were obtained in another study conducted by Qwane and colleagues where abacavir was used as a template. The roughness of the MIP surface suggested more cavities which proved that the imprinting sites were present in the prepared MIPs indicating successful synthesis (Qwane *et al*., 2020).

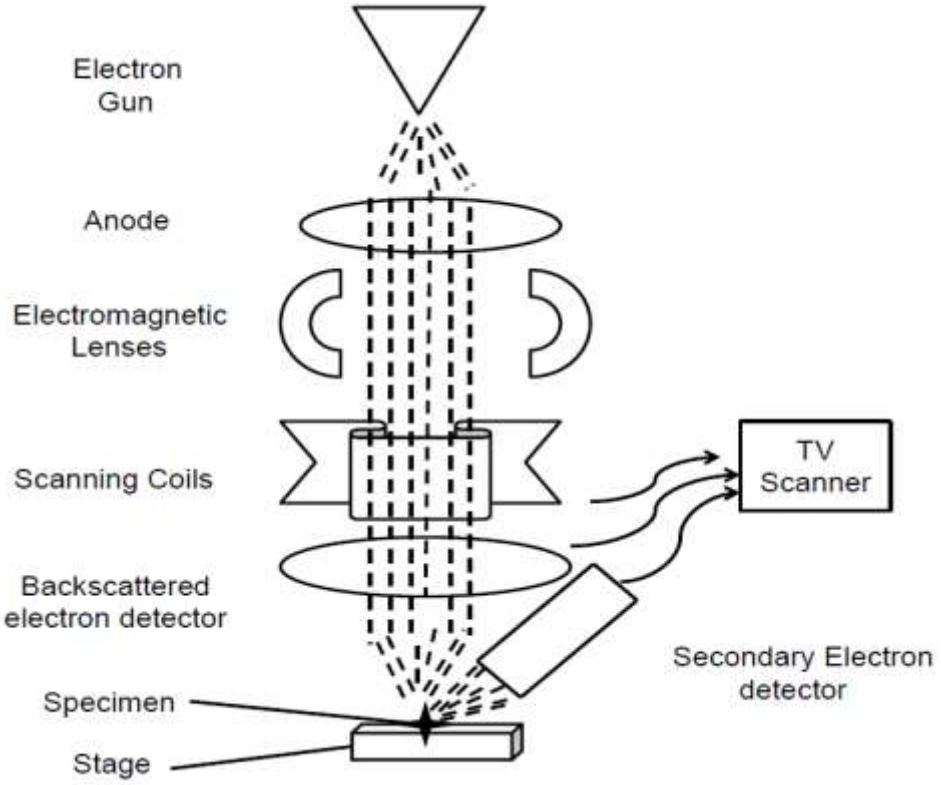


Figure 2.7. Schematic diagram of the scanning electron microscope (Tare *et al*., 2009)

2.6.3 Elemental analysis

Elemental analysis is used to determine nitrogen (N), carbon (C), hydrogen (H), sulphur (S), and oxygen (O) in a polymer. Elemental analysers can be configured in different configurations depending on the element of interest, CHN, CHNS, CNS, or N. Oxygen is measured separately by pyrolysis and sample reduction. The analysis requires a small amount of sample, which is broken down by combustion (Thompson, 2008). The elements are converted into relative combustion products from carbon-to-carbon dioxide. Additional combustion products, such as hydrogen chloride in the presence of chlorine, are removed by the adsorbent. The inactive carrier gas carries the required combustion products out of the combustion chamber and passes over the heated copper. Excess oxygen is removed, and the nitrogen oxides are converted to nitrogen gas. These gases are removed via an adsorption trap, leaving only carbon dioxide, water, nitrogen, and sulphur dioxide (Aiken *et al*., 2006). Then they are detected and quantified. If the sample is an organic/inorganic mixture, the sample should be separated, and the inorganic species analysed separately.

2.6.4 Thermogravimetric analysis

This analytical technique is used to find changes in sample weight with temperature. The sample is heated from room temperature to 800 °C or other required temperature in a nitrogen or air atmosphere to raise the temperature at a constant rate (Horvat, 2016). During the analysis, the purge gas flows through the balance, creating an inert, oxidizing, or reducing atmosphere, depending on the gas used. Then using analysis software, changes in sample weight with increasing temperature are observed and recorded on the reader (Unapumnuk *et al*., 2006). Previous studies on ARVDs (efavirenz and abacavir) imprinted MIPs showed backbone collapses at 250℃ and 355℃, respectively (Mtolo *et al*. 2019, Qwane *et al*. 2020). These results indicated that these MIPs are stable and can be applicable at temperatures up 249 °C and 354 °C, respectively.

2.6.5 Nitrogen physisorption analysis

This method is based on the adsorption of gas atoms on the surface. It provides information about the surface of the sample. Before analysing the sample, the surface of the sample is cooled with liquid nitrogen. After that, nitrogen gas is released to the sample and gas atoms are adsorbed onto the surface to form an adsorption layer. After removal from the nitrogen atmosphere, the pressure is reduced, and the adsorbed nitrogen is released and quantified (Naderi, 2015). The amount of adsorbed gas is plotted as a function of relative pressure in the form of adsorption-desorption isotherms. N_2 physisorption has the disadvantage of requiring the sample to be exposed to very low temperatures and pressures, which can cause the sample to collapse (Venkantesha, 2015). Mtolo and co-workers found a surface area of 420 m^2/g, pore volume of 0.83 cm^3/g and an average pore diameter of 8.36 nm on a MIP synthesized to extract efavirenz in water (Mtolo *et al*., 2019). Qwane and colleagues found a surface area of 372 m^2/g and an average pore diameter of 4.33 nm on a MIP synthesized to extract abacavir in water (Qwane *et al*., 2020). In both studies, the MIPs had a relatively larger surface area compared to their corresponding NIPs because of the imprinted cavities on the MIPs, which proved successful synthesis.

2.7 Separation and Detection Techniques

High performance liquid chromatography (HPLC) and gas chromatography (GC) are two techniques that have been used in the separation of ARVDs. The HPLC is an advanced

analytical technique of column chromatography used for separation, identification, and quantification of individual analytes in a mixture (Snyder *et al*., 2011). Gas chromatography is widely used for the analysis of volatile compounds or compounds that can be converted into volatile derivatives. HPLC is the preferred and mostly used technique because it can be applied for the separation of any compound that is soluble in a liquid phase. It is a relatively fast, efficient, and accurate instrument (Sah *et al*., 2021). It mainly consists of an injector that introduces sample into the column containing the stationary phase. A pump is used to move the sample and the mobile phase through the column where separation of the sample components occurs based on their interaction with the stationary phase. The separate components then exit the column where they are sensed by the detector that displays the retention time and peak area of the compounds as chromatograms (Bansal, 2010, Snyder *et al*., 2011, Sah *et al*., 2021). Analytes with higher affinity to the mobile phase will elute with shorter retention times and those with higher affinity to the stationary phase will migrate slower through the column with longer retention times). The disadvantages of HPLC are that it is expensive to purchase and maintain, it is complex, and has low sensitivity which is why it must be coupled with a very sensitive detector.

HPLC has 5 variants depending on the type of stationary phase system used. **In normal phase** a polar stationary phase and non-polar mobile phase are used, and the analyte separation is based on polarity. In r**eversed phase chromatography** the analyte separation is based on hydrophobic interaction which results from repulsive forces between non-polar stationary phase, a polar mobile phase, and the relatively non-polar analytes. In **ion exchange chromatography-** the separation is based on the attraction between solute ions and charged sites bound on the stationary phase. Ions of the same charge are excluded. In **size exclusion chromatography** the analyte separation is based on the particle size. In b**io-affinity chromatography**- analyte separation is mainly based on the specific reversible interaction of proteins with ligands.

Different detection techniques can be coupled with HPLC in the analysis and detection of ARVDs. Quadrupole time of flight-Mass spectrometry (MS), triple quadrupole time of flight MS tandem mass spectrometry, photo-diode array detector (PDA) (Mtolo *et al*., 2019). ARVDs are soluble in water and have very low concentrations, PDA detector is the most sensitive and compatible when coupled with a good sample preparation technique in the analysis of environmental samples that have analytes at low concentrations (Mtolo *et al*., 2019).

2.8 Evaluation of the Performance of MIPs prior Sample Preparation

The performance of the MIPs must be determined prior sample preparation and analysis. This is done to ensure successful extraction of analytes with high recoveries and determination of the efficiency of synthesized MIPs. Adsorption studies, chromatographic evaluation, cross-selectivity of MIPs and other methodologies are used to determine selective recognition of MIPs to ensure good performance and selectivity.

2.8.1 Adsorption studies

Adsorption properties such as equilibrium adsorption capacity of the synthesized MIP particles are considered to evaluate the performance of the MIP. The MIP particles are exposed to the analyte in aqueous matrices (Sun *et al*., 2016) or organic solvents such as acetonitrile (Sun *et al*., 2008), methanol (Yin *et al*., 2010), dichloromethane (Shaikh *et al*., 2012) or mixture of water and an organic solvent (He *et al*., 2016) for an experimentally determined interval to allow for complete equilibration. The adsorption capacity is calculated using eq. (2.1):

$$Qe = \frac{(Co-Ce)V}{W} \tag{2.1}$$

Where Co and Ce are the initial and equilibrium concentrations of the analyte in the solution. V and W are the volume of the sample and mass of the polymer used for adsorption.

The selective adsorption of properties of MIPs are commonly defined by the imprinting factor (IF) that is calculated from eq. (2.2):

$$IF = \frac{QMip}{QNip} \tag{2.2}$$

Where Q_{MIP} and Q_{NIP} are the equilibrium adsorption capacities of the analyte on MIPs and NIPs, respectively. A high IF (>1) indicates that imprinting was successfully done, with the site that selectively bind analytes in great excess of sites for non-selective uptake by the NIPs performance. The selective adsorption of target analytes is described by measuring the adsorption capacity of analytes. This is done in organic solvents or mixture of organic solvents and water to reduce strong non-specific hydrophobic interactions such as hydrogen bonding in water. The higher capacity of MIPs than NIPs show the specific interactions between the analytes and functional monomers in formed cavities of the MIPs (Azizi *et al*., 2019, Pichon *et al*., 2019). A study conducted by Mbhele and associates in 2018 showed adsorption capacity

of 38.8 mg/g for MIPs, 20.9 mg/g for NIP in the adsorption of fenoprofen in wastewater. The IF of 1.9 was obtained which demonstrated the efficiency and high interaction of fenoprofen with the imprinted cavities in the MIP (Mbhele *et al*., 2018). In another study conducted by Sun and partners in the determination of 2,4-dihydroxybenzophenone, 2,2,4,4-tetrahydroxybenzophenone, 2,2-dihydroxy-4,4-methoxybenzophenone and 2,2-dihydroxy-4-methoxybenzophenone in tap and river water, IF values greater than 34.30, 6.97, 2.02 and 1.50 were obtained respectively (Sun *et al*., 2015).

2.8.2 Chromatographic evaluation

The second measure used to characterize the selectivity of the MIPs is chromatographic evaluation. It utilizes the analyte retention factor using packed chromatographic columns with both MIP and NIP as sorbent as shown in Equation 2.3.

$$K = \frac{tr-to}{to} \qquad (2.3)$$

The selectivity is described by the IF obtained by dividing the retention factor of MIPs (K_{MIP}) by that of NIPS (K_{NIPs}). The difference between the retention factor of an analyte in MIP and NIP columns indicates the specific binding sites responsible for selective retention of analytes in MIP columns (Azizi *et al*., 2019, Pichon *et al*., 2019). A study conducted by Feng and colleagues using 2,4,6-trichlorophenol as a template in the determination of phenolic compounds in environmental water samples obtained Kmips of 1.90 and Knips of 1.13 (Feng *et al*., 2009).

2.9 Previous Analysis of ARVDs and related Pharmaceuticals

SPE method is the most published method in the extraction of ARVDs from different matrices worldwide. This is attributed to its ability to clean-up and preconcentrate samples prior analysis on the analytical instrumentation and its sensitivity (Maeterns *et al*., 2022). The ARVDs have very low vapor pressures at 25℃, which indicates that they are unlikely to be found in air and are more compatible with HPLC than GC for analysis The presence of new pollutants in surface waters is a major concern for the health and survival of ecosystems, especially fish. Also, the river water is used for irrigation, thus the ARVDs can be absorbed by the crops and affect food quality (Rimayi *et al*. 2018).

KZN has the highest detected concentration of ARVDs in the country (2-140 400 $ng{\cdot}L^{-1}$). despite being the second largest consumer of ARVDs after Gauteng (51.7-65 000 $ng{\cdot}L^{-1}$) (Mtolo et al., 2019, Horn et al., 2022). This could be due to the two provinces recording the largest numbers of people living with HIV with KZN having done more analysis of ARVDs in water. A high usage of ARVDs increases chances of possible water contamination through excretion and improper disposal of expired medicine. Kenya and South Africa have the highest detected concentrations of ARVDs in the continent ranging from 20-167 00 and 2-140 400 $ng{\cdot}L^{-1}$, respectively (Abafe *et al.,* 2018, Rimayi *et al.,* 2018, Mtolo *et al.,* 2019). This could be attributed to the world's largest ART programme being in South Africa (Mlunguza *et al*., 2019). Another reason could be the presence of advanced analytical instrumentation which has enabled South African researchers to do extensive analysis of ARVDs in water compared to other countries in the African continent (Fekadu *et al*., 2019). The concentration of ARVDs detected in water in the African countries range from 2-167 000 ng/L (K'oreje *et al.,* 2016, Abafe *et al.,* 2018), whilst the concentrations detected in the rest of the world range from 0.9-32100 ng/L (**Table 2.2**) (Prasse et al., 2010, Vergeynst *et al*., 2014). Abacavir, efavirenz, and nevirapine are the three most frequently analysed and detected ARVDs (Schoeman *et al.,* 2015, Mtolo *et al.,* 2019, Qwane *et al.,* 2020). This could be because they are the three most used drugs in the fixed dose combination (FDC) therapy of ARVDs in South Africa.

Even though not so many studies on the presence of ARVDs in the aquatic environment have been conducted in the African continent due to lack of advanced analytical instrumentation, very high concentrations are observed in this continent compared to the rest of the world. This

indicates the need for more studies to be conducted in this continent in understanding these new emerging water contaminants.

Examination of environmental samples has confirmed the presence of extremely polar pharmaceuticals in the aqueous environment. Presence of higher concentration levels of their metabolites and transformation products (TPs) indicate relevance for future surveillance campaigns (Boulard *et al*., 2018). Biodegradation of most ARVDs follows almost the same transformation reaction via oxidation of the hydroxyl group to the corresponding carboxylic acid (Mosiekemang *et al.,* 2019). Despite this reaction mechanism similarity, the conversion kinetics differ considerably, highlighting the difficulty of predicting biotransformation kinetics (Boulard *et al.,* 2018, Mosiekemang *et al.,* 2019). This could be used as a justification to the low concentrations of ARVDs reported in surface water, worldwide.

Table 2.2. Previous studies of ARVDs and related pharmaceuticals using SPE, MIP-SPE, and MIP-DSPE.

Author	Pharmaceuticals extracted	Sample type	Extraction technique	Country	Instrumentation and detection	Concentration ($ng{\cdot}L^{-1}$)	LOD ($ng{\cdot}L^{-1}$)	LOQ ($ng{\cdot}L^{-1}$)	REC (%)
Prasse *et al*., 2010	Ribavirin, acyclovir, penciclovir, abacavir, nevirapine	Wastewater and surface water	SPE	Germany	LC-MS	0.9 -1 800	0.06	0.2	83
Boulard *et al*., 2018	Emtricitbine acyclovir	groundwater, Rhine water and WWTP effluent.	SPE and freeze drying	Germany	HILIC-ESI-MS/MS	1-3.9	5-10	n.r	81
Vergeynst *et al*., 2014	Acyclovir, Lamivudine, Efavirenz, zidovudine	Wastewater	SPE	Belgium	HPLC coupled to magnetic sector HRMS	34-32 100	28	92	93
Fisher *et al*., 2016	Lamivudine, penciclovir, abacavir, acyclovir	Wastewater and groundwater	SPE	USA	HPLC coupled with triple quadrupole MS/MS	22	2.93-4.07	9.7-13.6	n.r
Peng *et al*., 2014	Ganciclovir, acyclovir, oseltamivir, stavudine, zidovudine, ribavirin	River water, reservoir, and wastewater	SPE	China	HPLC coupled with triple quadrupole MS	5.7-406	0.9-45.6	3-152	64.4-116.9
Azuma *et al*., 2015	Oseltamivir, zanamivir	River water and sewage effluent	SPE	Japan	LC-MS/MS	2-125	0.15	n.r	42-70

Ngumba *et al*., 2016	Zidovudine, lamivudine, nevirapine	River water and wastewater	SPE	Finland	LC-MS/MS	6-11.5	3-37	10-122	57.5-96.4
K'oreje *et al*., 2016	Nevirapine and zidovudine	Surface water, ground water and wastewater	SPE	Kenya	LC-MS/MS	20-167 100	n.r	n.r	n.r
Rimayi *et al*., 2018	Efavirenz, nevirapine, disoproxil, emtricitabine, lamivudine	River water	SPE	South Africa, Northwest and KZN	LC-MS/MS	0.15-354	0.06-2	0.23-0.67	73-112
Wood *et al*., 2015	Abacavir, didanosine, efavirenz, lamivudine, stavudine, etc.	Surface water	SPE	South Africa, Northwest, Gauteng, Western cape, Free State and KZN	UHPLC-MS/MS	51.7- 430	0.002-14.4	0.01-48.0	9-125
Schoeman *et al*., 2015	Efavirenz and nevirapine	wastewater	SPE	South Africa, Gauteng	GC-TOFMS	350-17 400	1.8-7.8	6.0-25.9	106-109
Schoeman *et al*., 2017	Efavirenz and nevirapine	wastewater	SPE	South Africa, Gauteng	GC-TOFMS	92-14 000	1.8-7.8	6.0-25.9	106-109
Abafe *et al*., 2018	Abacavir, atazanavir, darunavir, efavirenz, etc.	Wastewater	SPE	South Africa, KZN	HPLC coupled with triple quadrupole MS/MS	2-87 000	2-20	12-65	24-139

Mtolo *et al*., 2019	Efavirenz	River water and wastewater	SPE, MIP-SPE	South Africa, KZN	LC-PDA	2 450-140 400	410	1 390	81
Qwane *et al*., 2020	Abacavir	Wastewater and estuarine water	MIP-SPE	South Africa, KZN	LC-Uv/Vis	22 000-41 000	n.r	n.r	n.r
Horn *et al*., 2022	Nevirapine, efavirenz, ritonavir, lopinavir and zidovudine	Wastewater	SPE	South Africa, Gauteng	LC-Q-TOF/MS	40-65 000	10-580	50-1 940	55-196
Lu *et al*., 2019	Norfloxacin and enrofloxacin	Lake water, Sea water and Tap water	MIP-DSPE	China	HPLC-DAD	4 800-479 000.	220-360	670-980	91.2-106.4
***n.r= not reported**									

Chapter Three: Research methodology

3.1 Chemicals

Efavirenz, nevirapine, lamivudine, and abacavir standard salts were purchased from J & H Chemical Co. Ltd (Hangzhou Zhejiang, China). The 2-vinylpyridine (97%), high performance liquid chromatography (HPLC) grade methanol (99.8%), 1,1'-azobis(cyclohexanecarbonitrile) (98%), ethylene glycol dimethacrylate (EGDMA) (98%), diclofenac (≥96.5%) and toluene (99.7%) were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC grade acetonitrile (99.9%) and glacial acetic acid (100%) were purchased from Merck (Darmstadt, Germany).

3.2 Instrumentation

The SPE vacuum manifold used for the extraction of analytes was purchased from Sigma Aldrich (Steinheim, Germany) and connected to the vacuum pump from Edwards (Munich, Germany). Empty SPE cartridges (3 mL) and frits (0.2 µm) employed for MIP packing were obtained from Biotage (Uppsala, Sweden). The samples were shaken using a FMH SHKO 20 orbital shaker from DLD Scientific cc (Durban, South Africa). Ultrasonic bath utilised for the dispersion of MIPs was bought from Science Tech (Durban, South Africa). The working frequency, power and temperature range of the ultrasonic bath was kept at 28 kHz, 300 W, and 30℃ respectively. The coffee grinding machine mixer for to homogenizing the samples was purchased from Clicks (Pietermaritzburg, KwaZulu-Natal).

A Shimadzu high performance liquid chromatography (HPLC-2020) system equipped with a photo-diode array detector (PDA) bought from Shimadzu (Tokyo, Japan) was used for the monitoring of ARVDs in standard solutions and samples. The detection of the ARVDs was acquired at a wavelength of 254 nm. The chromatographic separation was performed on a Shim-Pack GIST C18-HP (4.6 mm× 150 mm, 3 µm) column procured from Shimadzu (Tokyo, Japan). In each case, a sample volume of 10 µL was injected into the LC system. The mobile phase employed consisted of a mixture of acetonitrile and 0.1% water at a ratio of 70:30 (v/v) flowing at 0.4 mL/min.

A Fourier transform infrared spectrometer from Perkin Elmer (Llantrisart, United Kingdom) equipped with attenuated total reflection was used to study the vibrations of the functional groups present in the synthesized polymers. The JOEL model 6700F scanning electron microscope from JOEL LTD (Tokyo, Japan) was utilised to study the polymer morphology.

Elemental composition of the polymers was studied using ThermoScientific Flash2000 organic analyser from Thermo Fisher (New York, United States). The thermal stability of the polymers was studied using Simultaneous thermal analyser 6000 Perkin Elmer (Llantrisart, United Kingdom), coupled with Pyris Software. The polymers were heated from 20-600℃ at 10℃/min under nitrogen atmosphere flowing at 20 mL/min. The surface area and porosity of the samples were characterized by N_2 physisorption measurements using Tri-Star II3020 V1.03 Brunauer–Emmett Teller from Micromeritics (Georgia, United States). The samples were degassed for 18 hours, under vacuum and the temperature was held at 200 °C

3.3 Stock solution and standard preparation

A stock solution (100 mg/L) of ARVDs was prepared by mixing 10 mg of each analyte (abacavir, nevirapine, and efavirenz) into 100 mL of acetonitrile. A series of working standard solutions (0.2 -1.0 mg/L) were then prepared from the stock solution and used to calibrate the LC-PDA instrument. A calibration curves were then drawn for each analyte to obtain the calibration equations which were used for the quantification of ARVDs. The stock solution and working standard solutions were kept in a refrigerator during the experiment.

3.4 Sampling

The samples were collected from four wastewater treatment plants (WWTP) around Durban (Amanzimtoti WWTP, Umbilo WWTP, Umhlathuzana WWTP, Northern Works WWTP) and one in Pietermaritzburg (Darvill WWTP). The river water samples were collected in three sampling points along Msunduzi River in Pietermaritzburg (Camps drift, Woodhouse and Bishopstowe). The tap water samples were collected from five suburbs around Pietermaritzburg (Scottsville, Oribi village, Lincoln Meade, Allandale, and Napierville). The Global Positioning System coordinates for the exact location of each sewage treatment plant are Darvill (S29.59979° E30.43124°), Amanzimtoti (S30.00749º E30.91720º), NorthernWorks (S29.7957º E30.9956164º), Umbilo (S29.84556º E30.89103º), Umhlathuzana (S29.8769198º E30.8817479º). The wastewater treatment plant receives wastewater for treatment from domestic and industrial water sources for treatment, and the treated wastewater is then discharged to the nearest river. Wastewater from each plant was collected at the inlet (where the treated water enters the plant) and at the exit (where the treated water is discharged into the river). Urban sewage treatment plants have been reported to unintentionally discharge pharmaceuticals into rivers because they are not designed to treat new emerging water

pollutants, including ARVDs (Tijani *et al*., 2013). Therefore, the purpose of this work was to determines the contributions of selected wastewater treatment plants to the pollution of the rivers and tap water.

The samples were collected using dark brown 2.5L glass water bottles and were stored in a cooler box with ice to maintain sample integrity until they get to the laboratory. The sample physical parameters such as pH, conductivity, total dissolved solids (TDS), dissolved oxygen (DO) and salinity were measured using Bante900P multiparameter water quality purchased from Bante instruments (Shanghai, China), and samples were then stored in a refrigerator until analysis. The measured pH for all wastewater samples ranged between 6.89 – 7.791 (**Table 3.2**) which is within the acceptable range of 5.5 - 9.5 (Weinberg and Teodosiu, 2012). The pH influences the presence and concentration levels of ARVDs in water. The presence of specific functionalities such as -OH-, -COOH-, and -NH- groups in many pharmaceuticals ensure protein binding and blood transport for pharmacological and therapeutic effects, but these functional groups make them susceptible to deprotonation under changing pH conditions (Adeola *et al*., 2022). Efavirenz becomes anionic in the pH range above the double pKa values of 10.20 and 12.52 which decreases its concentration in water. Nevirapine has a pKa of 2.8 and is predominantly anionic at pH values above its pKa which lowers its concentration in water (Adeola *et al*., 2022). Abacavir has a pKa value of 5.06 is likely to get protonated at the peripheral nitrogen (NH_2) when the pH is 2 and carry a positive charge (NH_3^+) which decreases concentration of the parent abacavir in water (Qwane *et al*., 2020).

Total dissolved solids (TDS) measured ranged between 388 - 653 mg/L and conductivity was between 538 - 1247 μS/cm which were both below the limits of < 1000 mg/L, 1700 μS/cm, respectively (Pitts, 1993, Wanda *et al*., 2016). High TDS and conductivity concentrations indicate high concentrations of pollutants (Nyoni, 2011). High conductivity and high concentrations of TDS indicates high presence of chemicals in water since pure water is a poor conductor of electricity. This has been reported to cause coronary heart disease, cancer, arteriosclerotic heart disease, cardiovascular disease, etc (Crittenden *et al*., 2012).

Salinity (psu) was measured to determine the amount of dissolved organic compounds and salts in water (Zhang *et al*., 2012). The measured salinity ranges between 0.26-0.63 psu in all samples and values were within the acceptable limit (≤ 1 psu). High TDS, salinity and conductivity decreases extraction efficiency and kinetic uptake of analytes in water. The measured dissolved oxygen (DO) ranged between 3.83 -6.54 mg/L which was found to be below the maximum limit of 8.14 mg/L as reported by South Africa water quality in most of

the samples (Munyika *et al*., 2014). High DO indicate a high population of microorganisms available in water. Low DO in water results into unsustainable aquatic life as well as the extreme algae growth caused by phosphorus and decomposition of submerged plants (Kramer, 1987). Low DO decreases microbial activity in water which in turn increases the concentration of pharmaceuticals due to slower biotransformation of pharmaceuticals in water (Stadler *et al*., 2015).

Table 3.1. Physical parameters of the collected wastewater, river water and tap water samples.

Wastewater	**pH**		**Conductivity (µS/cm)**		**TDS (mg/L)**		**DO (mg/L)**		**Salinity (psu)**	
	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl
Darvill	7.12	7.03	793	822	502	653	4.67	5.32	0.53	0.63
Amanzimtoti	7.04	6.89	1007	1247	498	620	3.83	5.50	0.48	0.61
Northern Works	7.153	7.051	778	814	388	407	5.38	5.61	0.38	0.39
Umbilo	7.191	7.058	833	538	407	272	5.52	5.87	0.39	0.26
Umhlathuzana	7.791	7.18	800	624	401	309	6.54	6.02	0.38	0.30
River water										
Camps drift	6.18		652		10		1.50		0.22	
Woodhouse	6.16		183		11		1.57		0.15	
Bishopstowe	6.16		262		4		1.56		0.20	
Tap water										
Scottsville	6.14		96		5		1.32		0.10	
Oribi Village	6.19		169		6		1.25		0.12	
Lincoln Meade	6.23		100		3		1.23		0.15	
Allandale	6.22		101		8		1.22		0.12	
Napierville	6.24		89		10		1.27		0.10	

3.4.1 Description of sampling areas

Darvill wastewater treatment plant (**Figure 3.3a**) is in Willowton, Pietermaritzburg and serves approximately 300,000 people in the Msunduzi Municipality. The treatment plant was upgraded to a new nominal capacity of 100,000 m^3/day with an optimal operating capacity of 80,000 m^3/day. Darvill discharges its treated effluent into Msunduzi river (Sikhakhane., 2002). The Amanzimtoti wastewater treatment plant (**Figure 3.3b**) is in Athlone Park near the Amanzimtoti residential area. The sewage treatment facility is in the immediate vicinity of the Umbongintwini Industrial Estate. The processing plant has a capacity of 30,000 m^3/day from two industrial areas (Prospecton and Southgate) and suburban areas (Amanzimtoti, Athlone Park, Isipingo, Kwamakhutha, and Folweni). This treatment plant discharges its treated effluent into the Mbokodweni River (Kunene *et al*., 2019, Hlengwa *et al*., 2020). The Northern Works wastewater treatment plant (**Figure 3.3c**) has a capacity of about 53,000 m^3/day. It is in an area surrounded by industries (petrochemical, construction, pharmaceuticals, detergents, textiles, and cosmetics). It receives and treat water from industrial and domestic sources and discharges its effluent into the Seekoespruit stream, which confluences with the Umgeni River (Bakare *et al*., 2022). Umbilo WWTP (**Figure 3.3d**) is in Pinetown and is divided into two sections, the old building, and the new building. The old structure has a flow rate of 13,000 m^3/day and the new structure is 10,000 m^3/day. Wastewater from this plant is discharged into Umbilo River (Hlengwa *et al*., 2020). Umhlathuzana WWTPs is formed by the Marian Ridge WWTP and Shallcross WWTP (**Figure 3.3e**). The Marian Ridge treatment plant receives approximately 8,000 m3/day of wastewater, 30% of which is for industrial use and 70% for household use. On the other hand, Shallcross WWTP accepts only about 2000 m^3/day of domestic wastewater. After both the Marian Ridge and Shallcross tributaries have been treated by the WWTP, they are combined as a single drainage and discharged into the Umhlathuzana River (Mhlanga *et al*., 2013, Hlengwa *et al*., 2020)

Figure 3.1. The satellite image of **(a)** Darvilll, **(b)** Amanzimtoti, **(c)** Northern, **(d)** Umbilo, **(e)** Umhlathuzana WWTPs.

3.5 Sample preparation

The wastewater samples were filtered with 0.45µm filter paper, and the pH was adjusted to 6. This was done to ensure that the sample was free of particles, filtration was conducted to prevent clogging of the SPE cartridge. The sample was acidified to keep the analytes in solution, to prevent formation of precipitates and to promote better interaction between ARVDs of interest and the adsorbent (Qwane *et al*., 2020).

3.6 Experimental

3.6.1 Method for Synthesis of Molecularly Imprinted Polymer (MIP)

The method for the synthesis of both the NIP and MIP was adopted from Qwane *et al* (2020) and further optimized. A 25 mg of the templates (abacavir, nevirapine and efavirenz) and 54 µL of 2-vinylpyridine were dissolved in 10 mL of acetonitrile/toluene (1:9, v/v) in a 100 mL round-bottom flask. The mixture was stirred at room temperature for 30 minutes to prepare a pre-polymerization complex. Thereafter, a 4.77 mL of ethylene glycol dimethacrylate and 100 mg of 1,1'-azobis(cyclohexanecarbonitrile) were added into the reaction flask. The reaction mixture was deoxygenated with nitrogen gas for 10 minutes, sealed, and stirred for 16 hours at 60℃. The temperature was increased to 80℃ and the mixture was further stirred for 24 hours to ensure complete polymerization. The NIP was synthesised in the same manner, except the addition of templates. The two polymers were washed with acetic acid/acetonitrile (1:9, v/v) using Soxhlet extraction until the templates could not be detected by the LC-PDA.

3.6.2 Performance of adsorption studies

The method for batch adsorption studies was adopted from Elbalkiny *et al* (2019) and Kebede *et al* (2020) and was further optimized. Parameters (solvent effects, pH, polymer mass, concentration of analytes and contact time) that could affect adsorption of ARVDs from spiked water samples were studied and optimized in batch mode. All experiments were conducted in triplicates (n=3) and average results were computed for each parameter under study. The adsorption capacity at equilibrium (Qe), adsorption capacity at time t (Qt) and adsorption efficiency (AE, %) were calculated using equations (3.1), (3.2), and equation (3.3);

$$Qe = \frac{(Co - Ce)V}{W} \quad (3.1)$$

$$Qt = \frac{(Co - Ct)V}{W} \quad (3.2)$$

$$AE\ (\%) = \frac{(Co - Ce)}{Co} \times 100 \qquad (3.3)$$

Where Co, and Ce are the initial and equilibrium concentrations respectively, Ct is the concentration at time t, V is the volume of the analytes solution used (L), and W is the amount/mass of the polymer used for the adsorption process (g).

3.6.3 Selective adsorption studies

The method used for selective adsorption studies was adopted from Qwane *et al* (2020) with further optimization. To further demonstrate the efficiency and selectivity of the polymers, 1 $mg{\cdot}L^{-1}$ of efavirenz, abacavir, nevirapine (target analytes), and competitors (lamivudine, and diclofenac) solutions were applied to 30 mg of NIP or MIP and shaken for 60 minutes at room temperature using an orbital shaker. The samples were then ultrasonicated, shaken and transferred to 3 mL SPE cartridges with the MIP or NIP as sorbent safeguarded by two frits to avoid loss during extraction. All experiments were conducted in triplicates.

3.6.4 Reusability studies

The method used for re-usability studies was adopted from Qwane *et al* (2020), and was further optimized. A 10 mg of the MIP was applied to 10 mL of distilled water samples spiked with 1 $mg{\cdot}L^{-1}$ of abacavir, nevirapine and efavirenz in a 50 mL centrifuge tube. The contents in a centrifuge tube were shaken on a FMH SHKO 20 orbital shaker for 10 minutes. The MIP loaded with ARVDs was regenerated by eluting the compounds with 2 mL of 100% acetonitrile using an empty SPE cartridge fitted with two frits and followed by washing with 2 mL of 100% methanol. The same MIP was used for repeatedly for eight consecutive cycles of adsorption/desorption. Thereafter recoveries were calculated.

3.7 Method validation

The optimized methods were validated by evaluating the accuracy which was calculated as percentage analyte recovery from fortified samples and precision which was calculated as relative standard deviation (%RSD). Calibration curves were determined by plotting the peak area against concentration of each analyte from 0.2-1.0 mg/L concentration levels. The linearity was evaluated from the calibration curve as correlation coefficients (R^2). The limits of detection

(LOD) and limit of quantification (LOQ) were determined by spiking of the blank sample with the lowest concentration achieved in the acceptable analyte recovery range (70-120 %) and precision (RSD ≤20 %). The LOD and LOQ were expressed as the analyte concentration corresponding to 3 and 10 times the standard deviation, respectively.

This was determined in accordance with the European Commission (EC) (2019) document no. SANTE/12682/2019.

3.8 Application to real samples

The SPE-LC-PDA, MIP-SPE-LC-PDA and MIP-DSPE-LC-PDA methods were then applied to real water samples (wastewater, tap water and river water) after optimization and validation.

Chapter Four: Results and Discussion

4.1 Calibration of LC-PDA

The LC-PDA method was adopted from Mtolo *et al* (2019), with minor modification of elution wavelengths to allow reasonable separation and elution of the additional ARVDs of interest (nevirapine and abacavir). The optimum conditions were isocratic ES^+ elution with mobile phase composition of acetonitrile: 0.1% formic acid in water (70%: 30%), 0-12 minutes, flow rate = 0.4 mL/min, injection volume = 10 µL, wavelength = 254 nm, column Temperature = 30°C.

Under optimum conditions, all the compounds separated well with abacavir eluting at 3.7 minutes followed by nevirapine at 4.8 minutes and efavirenz eluted last at 10.7 minutes (**Figure 4.1**). The instrument was then calibrated with a series of standards at a concentration range of 0.2-1.0 ppm and a calibration curve was constructed (**Figure 4.2**)

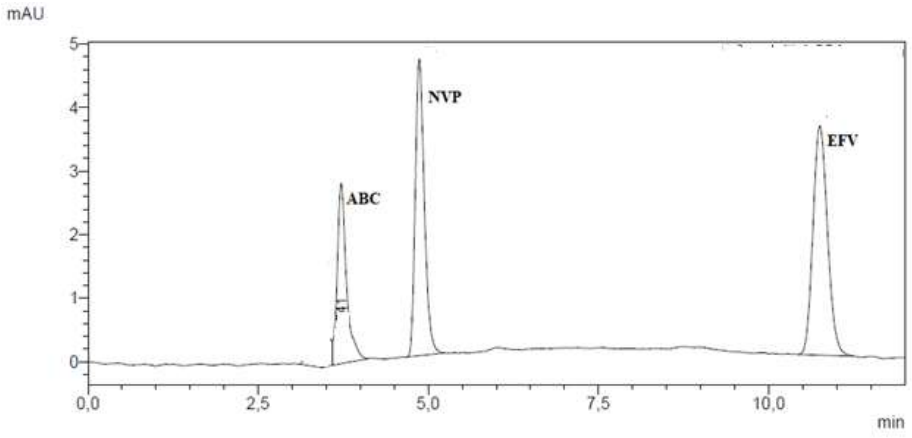


Figure 4.1. LC-PDA spectrum of 1 ppm std solution of ARVDs at 254 nm from 70:30% (0-12 min), of ACN: H_2O. ABC= abacavir, NVP= nevirapine, EFV = efavirenz.

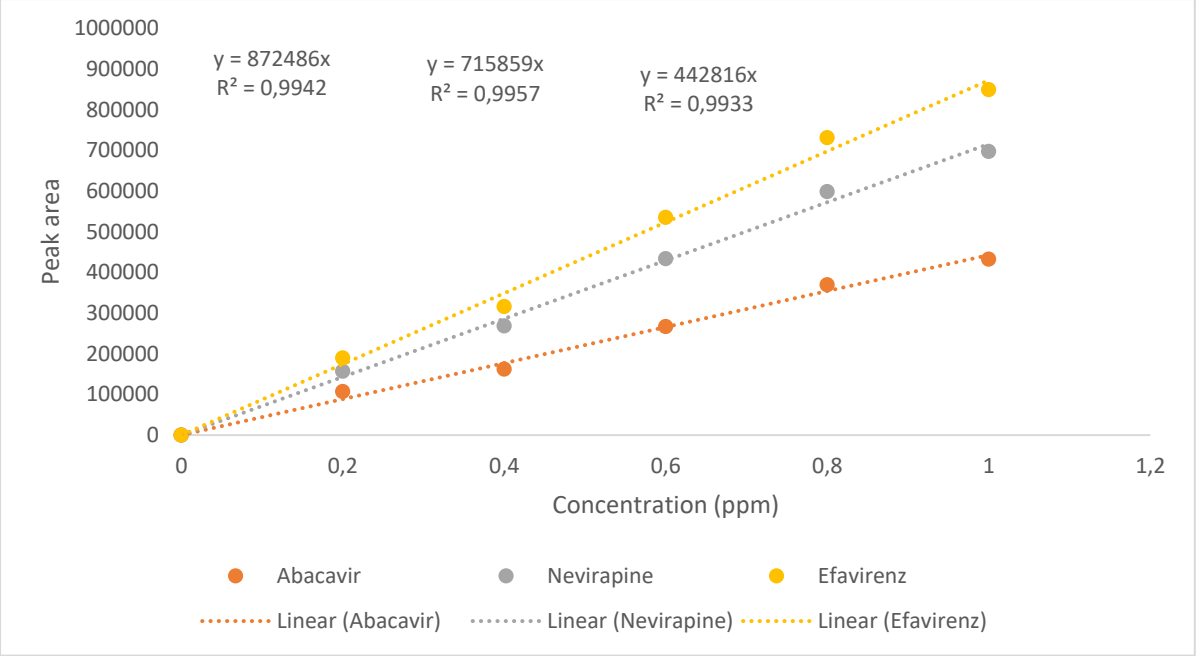


Figure 4.2. Calibration curves for abacavir, nevirapine, and efavirenz

4.1.1 Quality control

The validity of the analytical method was evaluated in terms of linearity, sensitivity, accuracy, and precision. The correlation coefficients (R^2) obtained for all the analytes showed good linearity with values ranging from 0.9979-0.9986. Sensitivity of the analytical method was evaluated using LOD and LOQ computed from signal to noise ratio of 3 and 10, respectively (**Table 4.1**). The LOD (0.70-0.91 µg/L) and LOQ (1.87-2.51 µg/L) obtained for the MIP-SPE are lower than those obtained for the Oasis HLB (0.81-1.11 µg/L) and (2.73-3.37 µg/L) regardless of similar preconcentration factors used for both sorbents. This indicates better sensitivity for the MIP-SPE compared to the traditional SPE and could be attributed to the ability of the MIP to selectively extract the analytes of interest imprinted on its surface whilst reducing matrix effects from the water samples (Mtolo *et al*., 2019).

Table 4.1. LOD and LOQ for ARVDs using MIP-SPE and Traditional SPE

Analytes	LOD ($\mu g \cdot L^{-1}$)		LOQ ($\mu g \cdot L^{-1}$)	
	MIP	Oasis HLB	MIP	Oasis HLB
Abacavir	0.91	1.11	2.51	3.37
Nevirapine	0.70	0.89	1.879	2.73
Efavirenz	0.86	1.06	2.34	3.20

4.2 SPE, MIP-SPE and MIP-DSPE

The methods for the SPE and MIP-SPE for the extraction of abacavir, nevirapine, and efavirenz were adopted from the work published by Mtolo and colleagues and no further optimization was done as the recoveries were within the acceptable range (**Table 4.2**) (Mtolo *et al*., 2019). A 60 mg Oasis HLB SPE cartridge and 50 mg of MIP transferred into an empty SPE cartridge fitted with two frits using 2 mL of methanol were used for SPE and MIP-SPE, respectively. A 1 mL of acetonitrile and 1 mL of methanol were used as conditioning solvents in both methods. A 3 mL of 2% of methanol in distilled water was used as the washing solvent and 2 mL of acetonitrile was used as the eluting solvent. The eluent was reduced to 1 mL under nitrogen, filtered using 0.2µm filter and analysed HPLC-PDA.

The method for the MIP-DSPE was adopted from the work published by Li and colleagues on the extraction of triazine herbicides and was further optimized to accommodate the ARVDs in this study (Li *et al*., 2016). Under optimum conditions, a 40 mg of the MIP was applied to 50 mL of distilled water samples spiked with 1 $mg \cdot L^{-1}$ of abacavir, nevirapine and efavirenz in a 50 mL centrifuge tube. The MIP was dispersed using ultrasonication for 15 minutes. The contents in a centrifuge tube were shaken on a FMH SHKO 20 orbital shaker for 60 minutes at 250 rpm, then centrifuged for 5 minutes at 2500 rpm. The supernatant liquid was discarded, and the MIP loaded with ARVDs was transferred to an empty SPE cartridge fitted with two frits and followed by washing with 2 mL of 2% methanol in distilled water. The MIP was regenerated by eluting the compounds with 2 mL of 100% acetonitrile. The sample was reduced to 1 mL under nitrogen and filtered into HPLC vials using 0.2µm filters and submitted for analysis.

Table 4.2. Percentage recoveries of abacavir, nevirapine their corresponding %RSD using SPE, MIP-SPE and MIP-DSPE.

Analytes	%Recoveries± %RSD		
	SPE	**MIP-SPE**	**MIP-DPSE**
Abacavir	91.68± 0.17	99.68± 0.2	100.28± 0.15
Nevirapine	87.55± 0.20	97.77± 0.18	102.29 ± 0.19
Efavirenz	94.59±0.28	97.20±0.25	102.60 ±0.2

All three methods obtained analyte recoveries within the acceptable range of 80-120% which confirmed high accuracy. %RSD were ranging from 0.15-0.28 for all three analytes using all three extraction techniques which confirmed high level of precision. Traditional SPE method with HLB Oasis as the sorbent gave lower recoveries compared to MIP-SPE and MIP-DSPE methods. The higher recoveries in MIP based sorbents might have been influenced by the presence of the templates recognition sites which improves their selectivity. As the traditional SPE sorbents are selective to a wide range of compounds, the competitive adsorption onto the sorbents results in less adsorption sites available for the analytes of interest and thus lower the recoveries. MIP-DSPE had the highest recoveries compared to both SPE and MIP-SPE which could be attributed to enough contact time between the MIP and the analytes of interest which allowed sufficient adsorption of analytes onto the cavities imprinted on the MIP.

4.3 Synthesis and characterization of molecularly imprinted polymer

Molecularly imprinted polymer and its corresponding non-imprinted polymer were synthesized using the method adopted from Qwane *et al* (2022) as described in chapter 3. Both polymers were characterized using Fourier Transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), Nitrogen physisorption, and elemental analysis.

4.3.1 Fourier Transform Infrared

FTIR spectroscopy was used to study the possible structural differences and similarities between the washed MIP, washed NIP, and the unwashed MIP, to confirm successful synthesis and removal of the templates. Characteristic bands corresponding to the symmetric and asymmetric vibrations of C-O bond of EGDMA were observed at 1141 cm^{-1} and 1249 cm^{-1}, 1137 cm^{-1} and 1249 cm^{-1}, 1144 cm^{-1} and 1250 cm^{-1} for the washed MIP, washed NIP, and

unwashed MIP, respectively (**Figure 4.3**). The peak at 1720 cm^{-1} for the washed MIP and NIP and 1722 cm^{-1} for the unwashed MIP was attributed to the stretching vibrations of the C=O bond of the carboxylic acid group of EGDMA. The peak at 2951 cm^{-1} for the washed MIP, 2953 cm^{-1} for the washed NIP and 2955 cm^{-1} for the unwashed MIP corresponds to the stretching and bending vibrations of the amine group (N-H) of 2-vinylpyridine (Qwane *et al*., 2020).

The unwashed MIP was observed to have more intense peaks because of the strong bond vibrations within the MIP which is associated with the overlapping of bonds of the MIP with the template. The washed MIP has relatively weak peak intensity compared to the unwashed MIP because of the weak bond vibrations within the MIP due to the washing step for the removal of the template which eliminates the overlapping bonds of the MIP and template and weakening the existing bonds within the MIP (Mtolo *et al*., 2019). The washed NIP has a strong peak intensity compared to the washed MIP. This is justified by the imprinted sites within the MIP which are not present in the NIP. The washing step weakens bonds within both the MIP and the NIP, however the presence of cavities in the MIP further weakens the bonds hence the observed peak intensity difference between the two (Mtolo *et al*., 2019, Qwane *et al*., 2020).

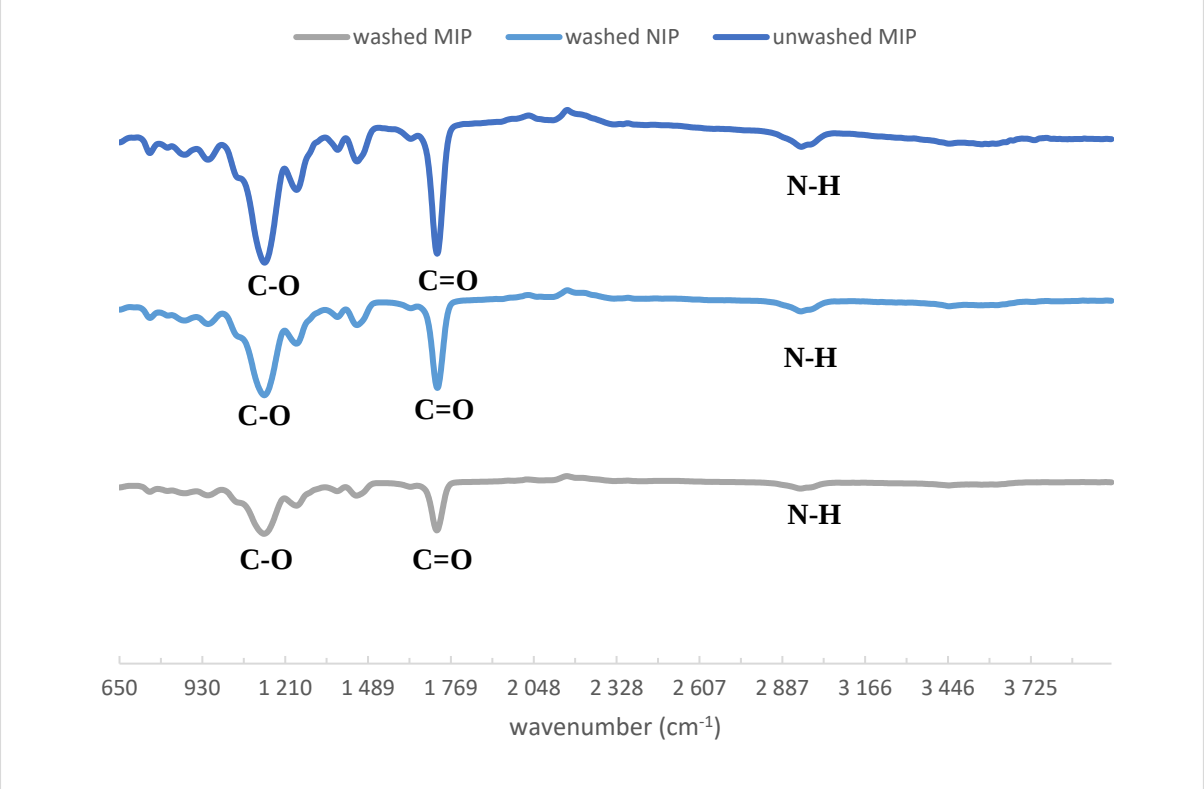


Figure 4.3. FTIR spectra of the washed MIP and NIP, and the unwashed MIP showing characteristic bands of functional groups present in the polymers.

4.3.2 Scanning Electron Microscope (SEM)

The scanning electron microscope was used to study the surface morphology of the washed MIP, washed NIP, and the unwashed MIP. The SEM images showed a rougher surface for the MIP and a smooth surface for the NIP (**Figure 4.4**). The roughness of the surface of the MIP is associated with the presence of imprinted binding sites/cavities that were left after the removal of the template. The NIP had no imprinted binding cavities hence a smoother surface was observed. The unwashed MIP showed a smooth surface, this could be because the cavities were still filled with templates. These results also correspond to the findings reported by Mtolo *et al* (2019) and Madikizela *et al* (2016).

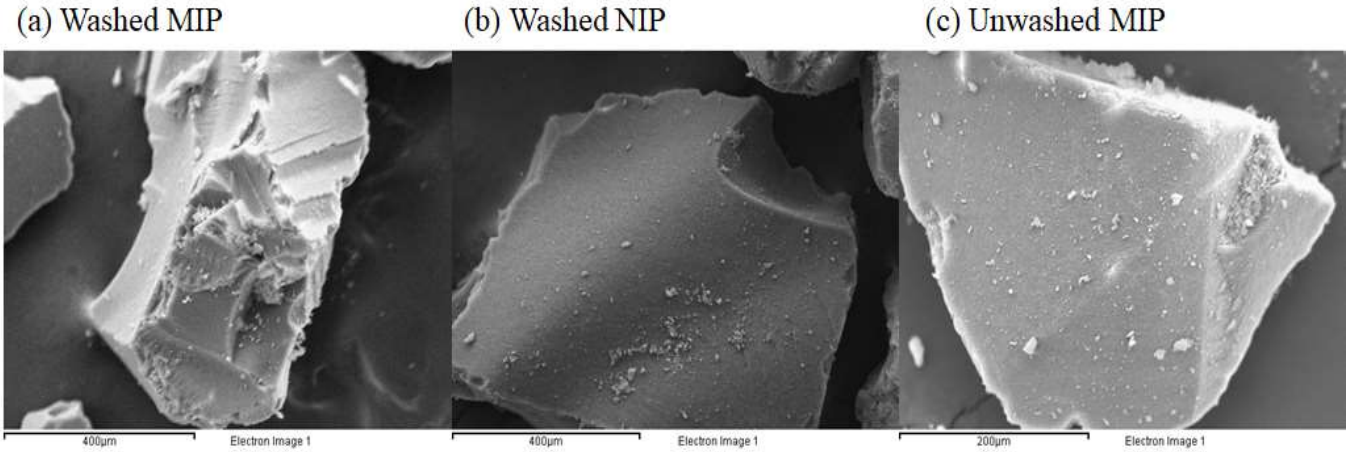


Figure 4.4. SEM images of the surface of the washed MIP (a), washed NIP (b) and unwashed MIP (c)

4.3.3 Elemental Analysis

The elemental composition of the MIP and NIP was studied using the CNH analyser. The results obtained showed the presence of oxygen, nitrogen, carbon, and hydrogen with similar percentage compositions (**Table 4.3**). This is because both polymers were synthesized using similar reagents and quantities (except for the template in the MIP) and under similar reaction conditions. The composition also shows successful template removal during the washing of the MIP post synthesis. These results also correspond to the published findings by Qwane *et al* (2020).

Table 4.3. Results of the elemental composition of the MIP and NIP using the CNH analyser

	Elemental composition (%)	
Element	**MIP**	**NIP**

Carbon	56.0	55.3
Hydrogen	6.4	6.4
Oxygen	37.6	37.9
Nitrogen	0.4	0.4

4.3.4 Thermogravimetric Analysis (TGA)

The stability of the synthesized polymers (MIP and NIP) was studied using thermogravimetric analysis. The polymers were heated from 20-600 ℃ as shown in **Figure 4.5.** At 58 ℃, the polymers showed a mass loss of about 2-3%, which is associated with the evaporation of acetonitrile used during template removal from the cavities of the polymer. Approximately 90% of the weight loss was observed at around 257 ℃ for the NIP and 281 ℃ for the MIP. These weight losses were attributed to the collapse of the backbone of the NIP and MIP, respectively. The difference observed between the NIP and MIP backbone collapse temperatures could be attributed to the changes that could have resulted when the template was being removed from the MIP using organic solvents. Further decomposition was observed at 449 ℃ for both polymers. Results of similar nature were reported by Qwane *et al* (2020) where the polymer backbone collapsed at 255 ℃ for the NIP and 355 ℃ for the MIP. In another study by Mtolo *et al* (2019) the backbone breakdown of both polymers occurred around 250 ℃. Overall, these results have shown that the MIP is more stable than its corresponding NIP (Qwane *et al*., 2020).

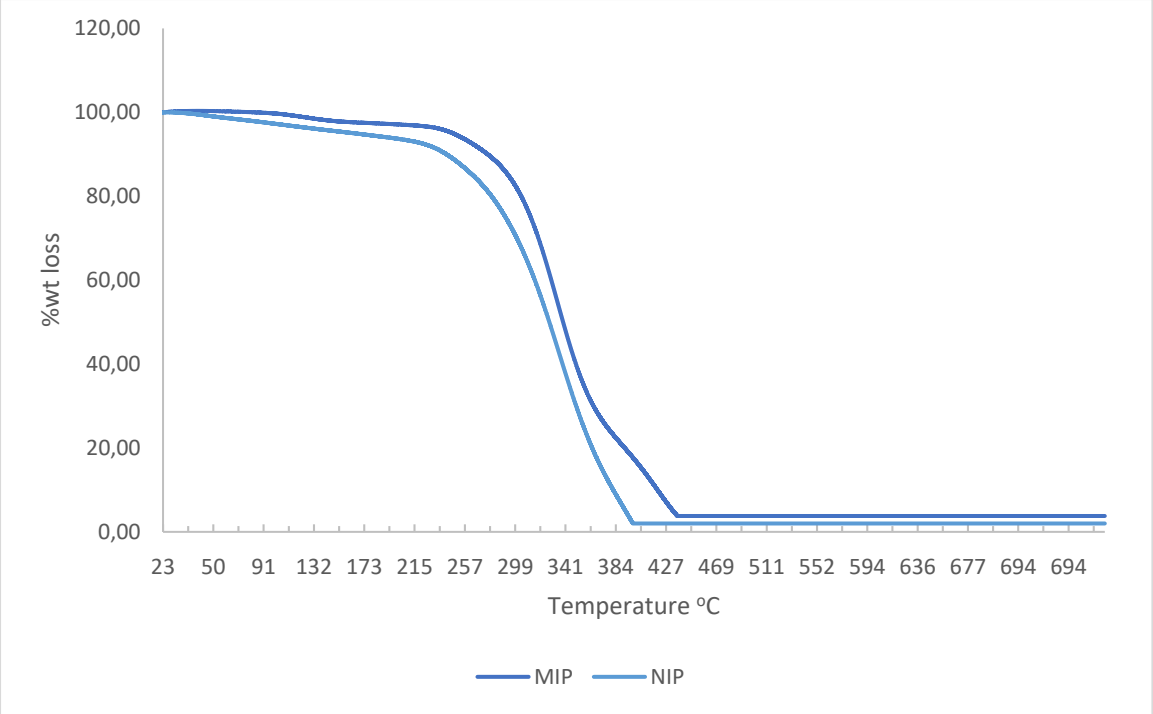


Figure 4.5. TGA results showing weight loss percentage at varying temperatures.

4.3.5 Nitrogen physisorption Analysis

Nitrogen physisorption was used to determine the surface area, pore volume, and the pore diameter of the washed MIP, unwashed MIP, and the washed NIP. The results obtained showed that the washed MIP had a greater surface area than the unwashed MIP and the washed NIP (**Table 4.4**). These results imply that a MIP has a higher adsorption efficiency and capacity compared to the aforementioned polymers. These findings correspond to the work published by Mtolo *et al*., where a higher surface area was obtained for the MIP (420 m^2/g) than the NIP (409 m^2/g). While the results reported by Qwane *et al*., 2020 showed equal surface area for the MIP and NIP (372 m^2/g). The washed MIP had a greater pore volume and pore diameter than the unwashed MIP and washed NIP, respectively. The average pore diameter of all three polymers falls within the range of 2-50 nm and these findings indicate that the structure of the polymers is mesoporous.

Table 4.4. BET results of the washed MIP, washed NIP, and unwashed MIP.

Polymer	Surface Area (m^2/g)	Pore Volume (cm^3/g)	Pore Diameter (Å)
Washed MIP	457..453 ± 1.7819	0.974609	8.840

Unwashed MIP	444.0415 ± 0,5177	0.461562	8.323
Washed NIP	3772567 ± 0.5971	0.410385	8.209

4.4 Factors affecting adsorption efficiency of ARVDs.

4.4.1 The effect of sample pH

The optimization of sample pH is critical and necessary in the adsorption experiments to study the structural changes or adsorbent surface charges associated with acidity or basicity of the pH of the sample. The pH range of 4-9 was investigated while keeping other parameters and experimental conditions constant. Abacavir showed a low adsorption efficiency at pH 4 for both MIP and NIP (**Figure 4.6**) which could be attributed to the protonation of the peripheral nitrogen (NH_2) of abacavir due to its pKa value (5.06) being higher than the pH value. This change alters the structure, size, and functional group (NH_2 to NH_3^+) resulting to poor recognition for binding on the MIP (Qwane *et al*., 2020). Efavirenz and nevirapine have pKa values below the pH range assessed thus they were both not affected by the acidity of the sample hence they had high adsorption efficiencies. At pH 6 abacavir, nevirapine and efavirenz exist as a neutral species, hence, high adsorption efficiencies were observed for both polymers. At pH 8, a slight decrease in adsorption efficiency was observed for all three analytes and the decrease became significant at pH 9. This could be linked to a possible hydrolysation caused by the basicity of the sample which alters the structure of the analytes resulting to poor recognition by the cavities of the MIP and poor adsorption onto the NIP. Abacavir has low adsorption efficiency on the MIP compared to nevirapine and efavirenz, this could be due to the polar groups present on its structure and its high-water solubility making it less likely to be completely extracted from water. The pH of 6 was therefore taken as the optimum where all analytes' recoveries were above 80%. These results are different from those obtained by Qwane *et al* (2020) for abacavir which showed recoveries below 20% at pH 2 and approximately 60% at pH 10, whilst the optimum pH of 5 showed recoveries at approximately 70%.

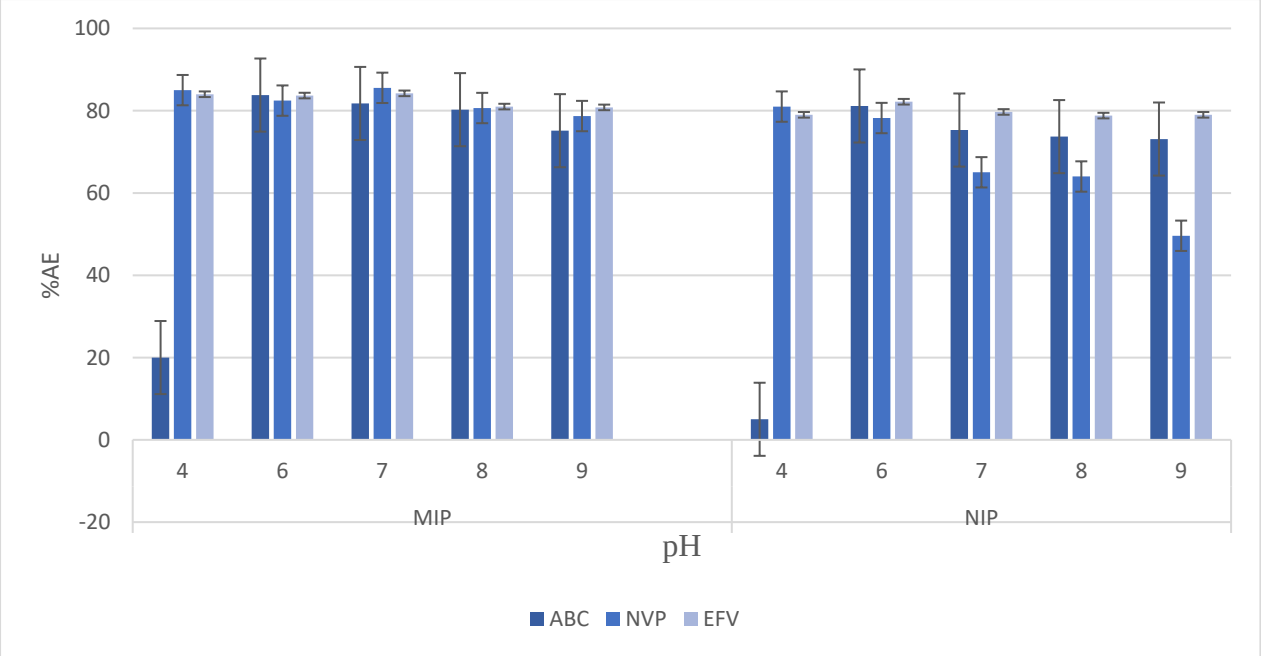


Figure 4.6. Effects of pH on the adsorption efficiency. Experimental conditions were: initial concentration of ARVDs – 10 mg·L^{-1}, adsorbent mass -10 mg, contact time – 15 minutes and sample volume – 5 mL

4.4.2 Mass of adsorbent effects

The effects of an increase of the adsorbent mass were examined in the range of 10-50 mg whilst keeping other experimental parameters constant. When the MIP sorbent mass was increased (10–50 mg), the adsorption efficiencies increased from 83%, 82%, and 83% to 99%, 100% and 101% for abacavir, nevirapine and efavirenz, respectively. This was attributed to the increase in the number of binding sites available hence an increase in the amount of ARVDs being adsorbed (**Figure 4.7**). There were slight changes in adsorption efficiencies when 40 mg was exceeded as the MIP was approaching its saturation point. A similar trend in results was observed for the NIP. This means that an increase in sorbent mass increases the number of binding sites which enhances the adsorption process and efficiency until equilibrium is reached. The 40 mg was chosen as the optimum sorbent mass due to high recoveries obtained with lower sorbent mass compared to the 60 mg of Oasis HLB in the traditional SPE. These results are corresponding to the published findings by Qwane *et al* (2020).

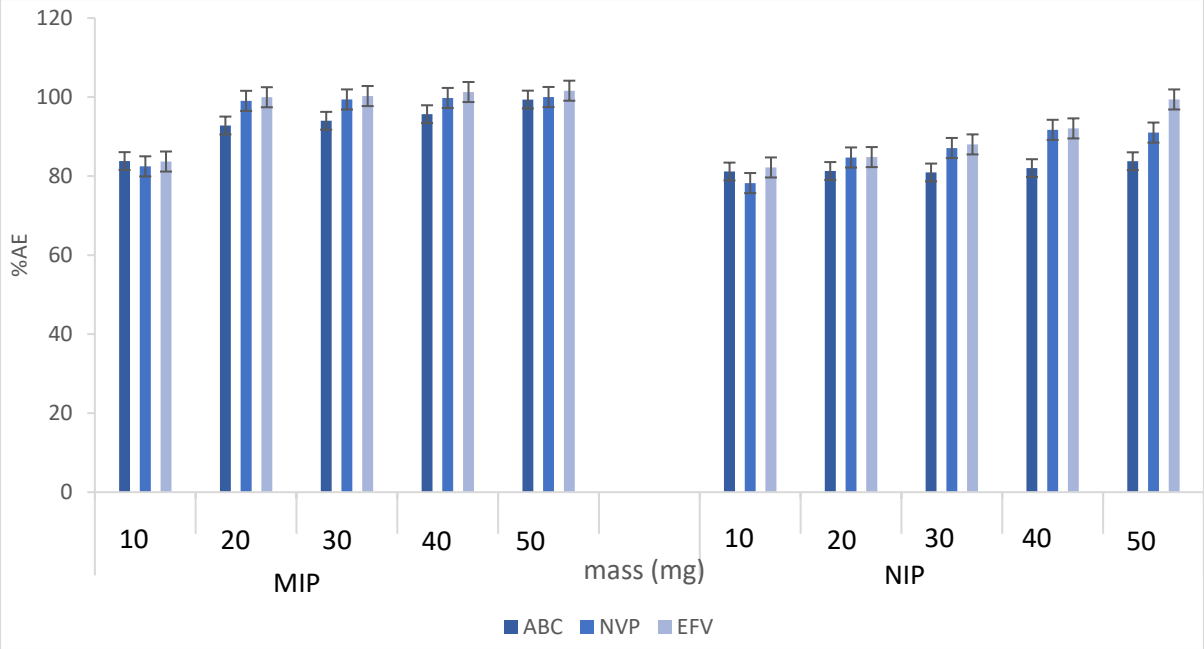


Figure 4.7. Effects of adsorbent mass on the adsorption efficiency of ARVDs. Experimental conditions were: initial concentration of ARVDs – 10 $mg{\cdot}L^{-1}$, sample pH – 6, contact time – 15 minutes and sample volume – 5 mL

4.4.3 Concentration of analytes.

The effects of an increase in the ARVDs concentration in spiked water samples was investigated using 10–80 ppm. Saturation/maximum adsorption was reached at different concentration intervals for all three analytes (**Figure 4.8**). This could be because the adsorption intensity and affinity for each analyte onto the multi-template MIP is different. Abacavir reached saturation at 60 ppm with 89% adsorption efficiency, beyond this point absorption and desorption was observed. Nevirapine reached saturation at 80 ppm with 87% adsorption efficiency and efavirenz at 50 ppm with 87% adsorption efficiency.

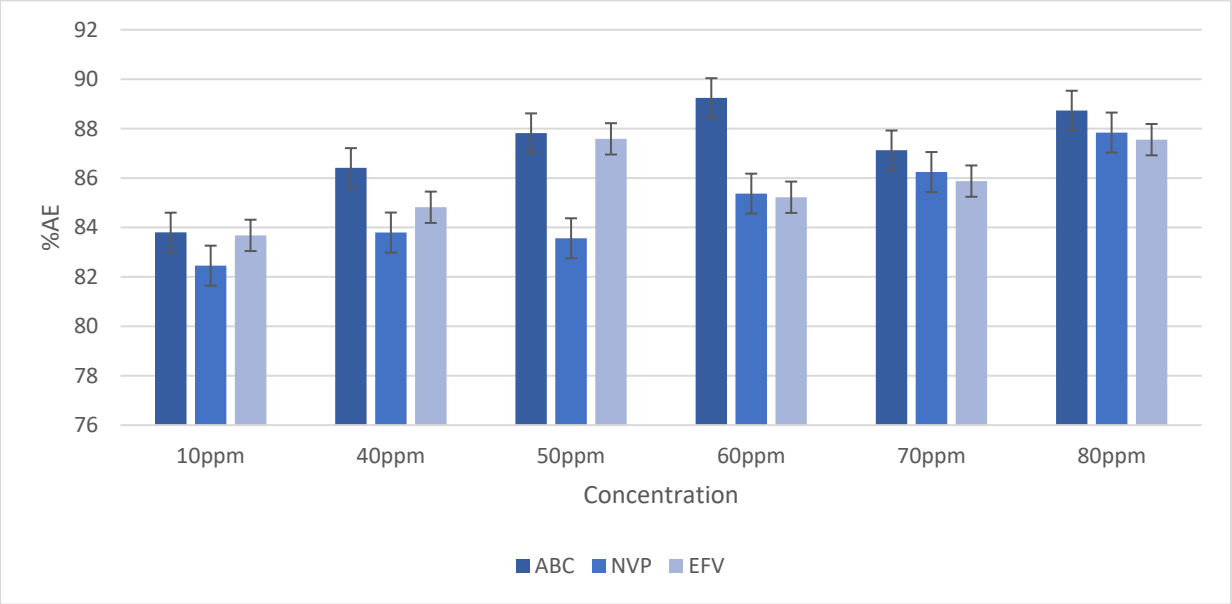


Figure 4.8. Effects of concentration on the adsorption efficiency of ARVDs onto the MIP. Experimental conditions were adsorbent mass – 10 mg, sample pH – 6, contact time – 15 minutes and sample volume – 5 mL.

4.4.4 Contact time effects.

The effects of an increase in contact time on the adsorption of ARVDs in spiked water samples was studied at a range of 5–120 minutes. The adsorption efficiencies showed a steady increase from 5 minutes of contact time to 60 minutes (**Figure 4.9**). After 60 minutes a slight decrease was observed for all studied ARVDs which could be attributed to the saturation of MIP resulting in adsorption and desorption happening at the same time. A Similar trend was observed for the NIP even though, adsorption efficiencies were lower compared to the MIP. The adsorption efficiencies at 5 minutes of contact time between MIP and ARVDs were within the acceptable range. The adsorption efficiencies increased to 96% for abacavir and 92% for nevirapine and 90% efavirenz in 60 minutes of contact time. Thereafter 60 minutes was selected as the optimum contact time for determination of equilibrium adsorption of the polymers and kinetics.

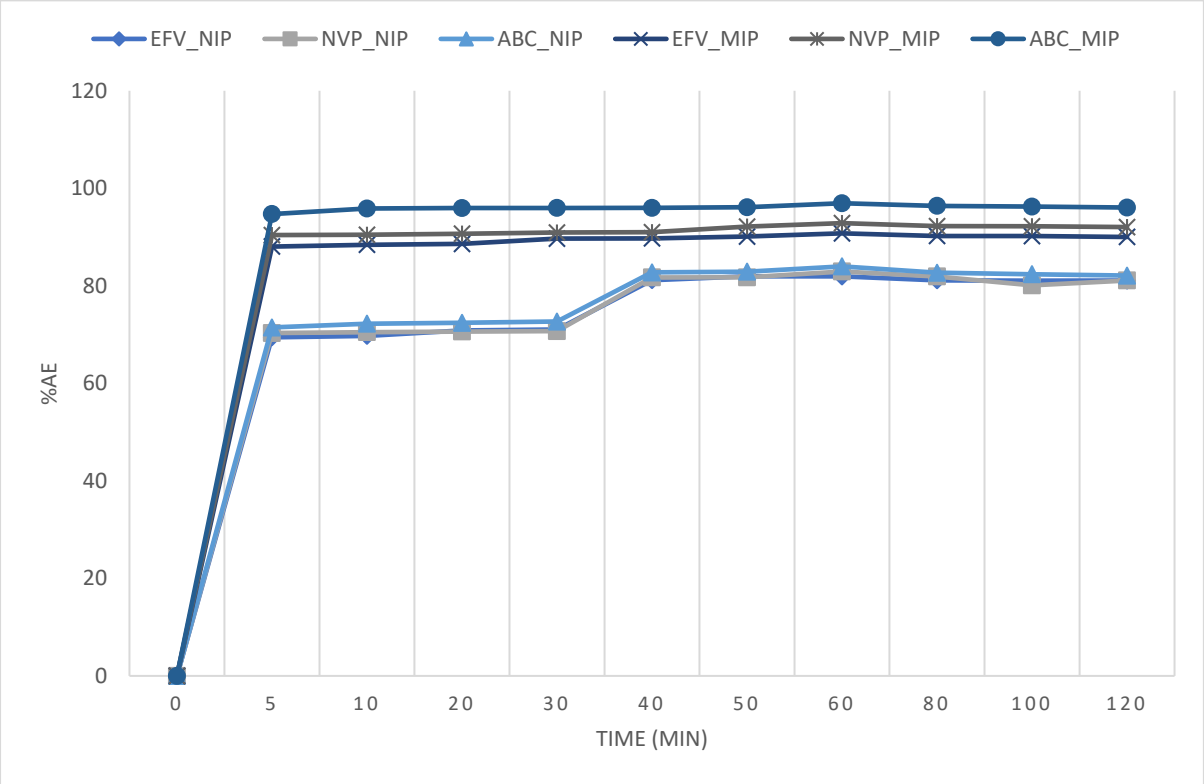


Figure 4.9. Effects of contact time on the adsorption efficiency of ARVDs. Experimental conditions were: initial concentration of ARVDs – 50 mg·L^{-1}, sample pH – 6, adsorbent mass – 40 mg and sample volume – 5 mL.

4.5 Kinetics modelling

To study the adsorption modelling of ARVDs onto the MIP, the data obtained in the contact time study was further processed using pseudo-first and pseudo-second order kinetics using equation (4.1) and (4.2), respectively:

$$\ln(qe - qt) = lnqe\frac{k1t}{2.303} \qquad (4.1)$$

$$\frac{t}{qt} = \frac{1}{k_2 qe^2} + \frac{1}{qe}t \qquad (4.2)$$

Where qe is the adsorption capacity at equilibrium (mg·g^{-1}), qt is the adsorption capacity (mg·g^{-1}) at time t (min), k_1 is pseudo-first order adsorption rate constant (g·mg^{-1}min^{-1}) and k_2 is pseudo-second order adsorption rate constant (g·mg^{-1}min^{-1}).

Linearity was used to determine the model that best fits the adsorption mechanism through analysis of the correlation coefficient (R^2) of the MIP for all three ARVDs (**Figure 4.10 a-c**). The pseudo-second order model was found to be the best fit through linearity which was found to be one. Abacavir had the highest adsorption capacity compared to the other two ARVDs (**Table 4.5**). These results imply that during the adsorption process of ARVDs onto the surface of the MIP, there is a transfer of electrons between the MIP and ARVDs of interest in solution/water and the process of adsorption is more chemical than physical. The NIP gave lower adsorption efficiencies and was therefore omitted from other experiments in this work.

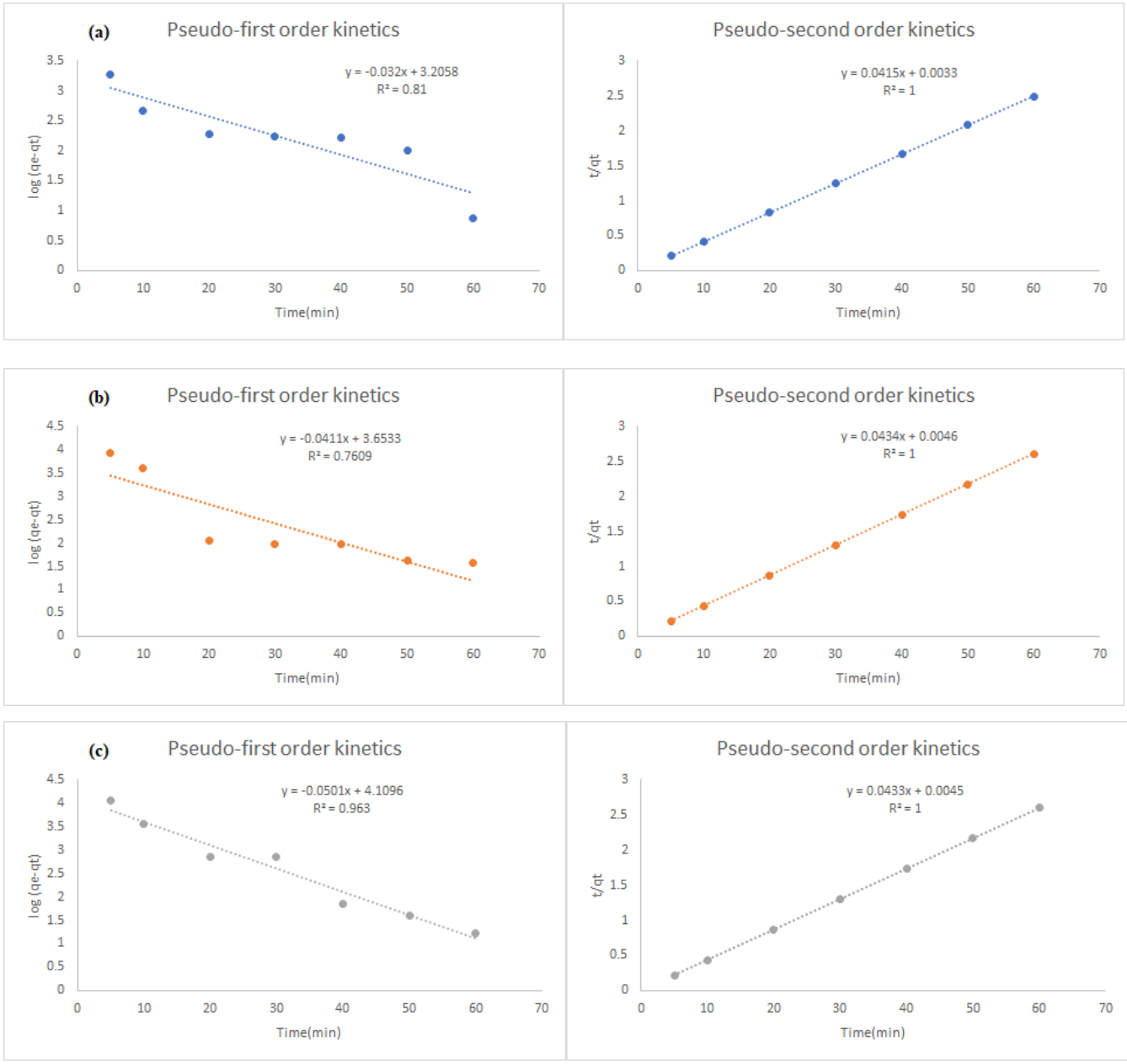


Figure 4.10. Lagergren rate order kinetics for abacavir (a), nevirapine (b), and efavirenz (c).

Table 4.5. Kinetics constants for abacavir (ABC), nevirapine (NVP) and efavirenz (EFV)

Analytes	Pseudo-first order			Pseudo-second order		
	qe ($mg \cdot g^{-1}$)	R^2	$k_1(min^{-1})$	R^2	$k_2(min^{-1})$	qe ($mg \cdot g^{-1}$)
ABC	24.09594	0.81	0.0737	1.00	0.0415	24.09594
NVP	23.06038	0.7609	0.0947	1.00	0.0433	23.06038
EFV	23.05529	0.983	0.115	1.00	0.0434	23.05529

4.6 Adsorption Isotherms

4.6.1 Adsorption Isotherms.

To study the adsorption mechanism of ARVDs onto the MIP, the data obtained in the study of concentration effects (**Figure 4.8**) was further processed using the Freundlich and Langmuir Isotherms in equation (7) and (8), respectively:

$$\log q_e = \log K_F + \frac{1}{n \log C_e} \quad (7)$$

$$\frac{1}{q_e} = \frac{1}{q_m K_L C_e} + \frac{1}{q_m} \quad (8)$$

Where n is the adsorption intensity, K_F, and K_L are Freundlich and Langmuir constants, respectively. C_e is the equilibrium concentration, q_e and q_m are equilibrium and maximum adsorption capacities, respectively. Linearity was used to determine the model that best fits the adsorption mechanism through analysis of the correlation coefficient (R^2) of the MIP for all three ARVDs (**Figure 4.11 (a – c)**).

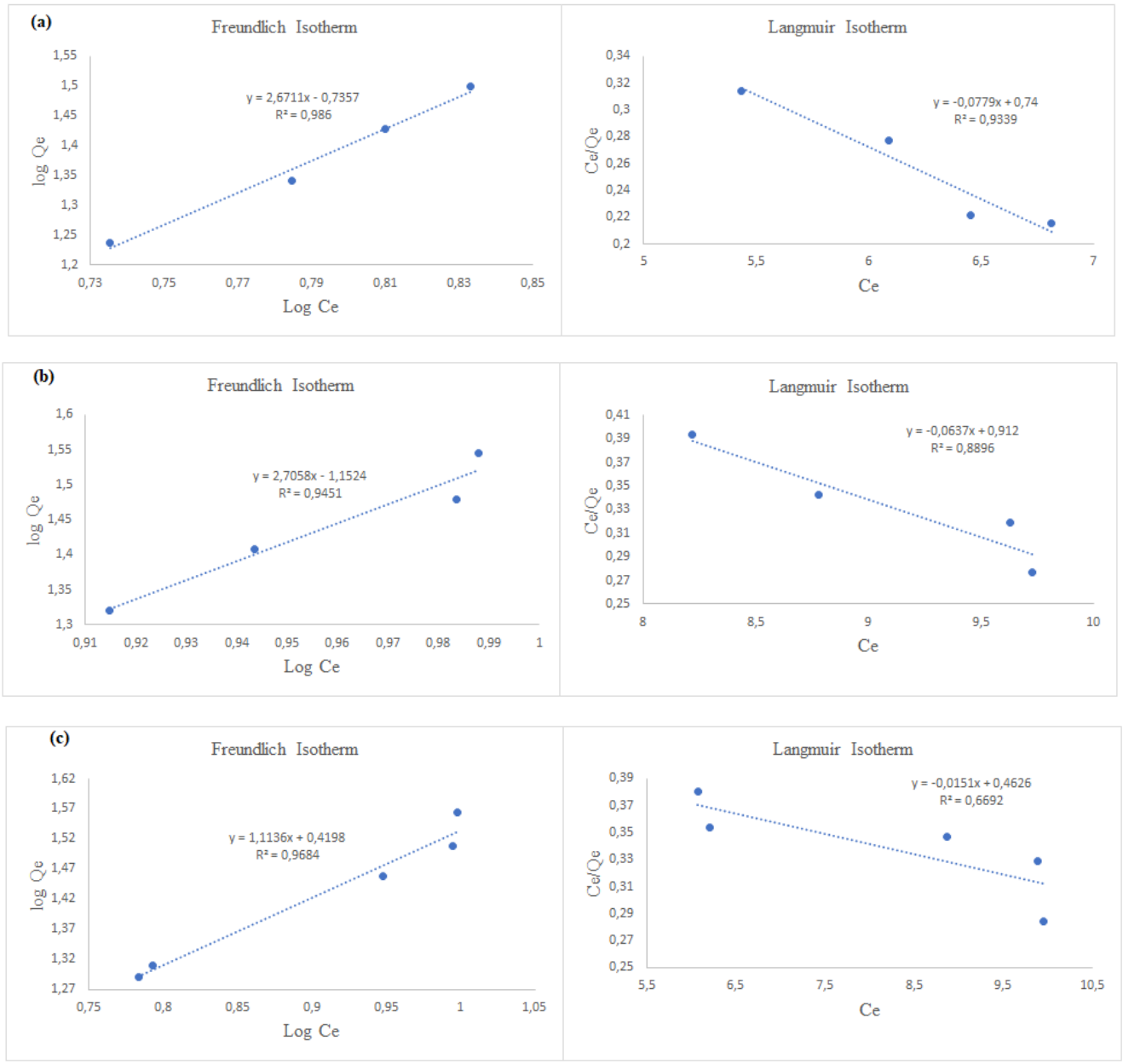


Figure 4.11. Freundlich and Langmuir adsorption Isotherms for abacavir (a), nevirapine (b), and efavirenz**.**

The Freundlich isotherm model was found to be the best fitting model for the adsorption mechanism of ARVDs onto the MIPs. The correlation coefficients in the Freundlich Isotherms for all three analytes were closer to one (**Table 4.6**), which indicated the homogeneity of the surface of the MIP. Efavirenz had the highest adsorption intensity and abacavir and nevirapine both had the lowest adsorption intensity. Adsorption intensity value (n) of efavirenz was greater than 0.7 implying that the adsorption and desorption may be observed at the same time as the concentration of the efavirenz increases. Adsorption intensity values of abacavir and nevirapine

were less than 0.7 implying that mostly adsorption may be observed. Efavirenz had the highest adsorption affinity towards the imprinted cavities whilst abacavir had the lowest of all three analytes.

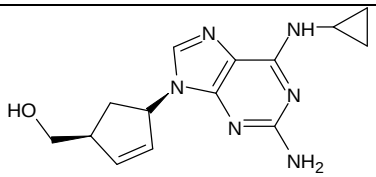
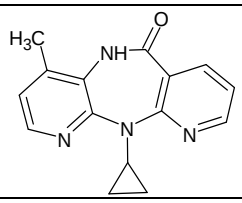
Table 4.6. Freundlich and Langmuir constants computed from Figure 11(a) to (c).

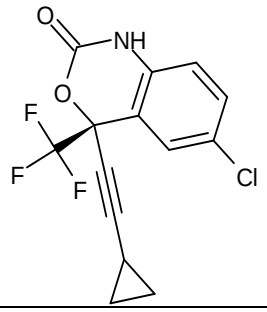
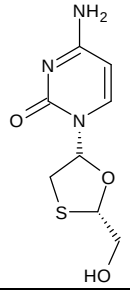
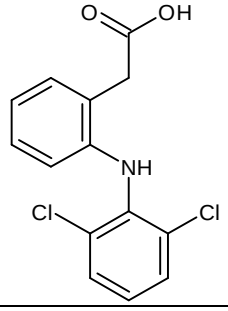
	Freundlich constants			**Langmuir constants**		
Analytes	n ($L \cdot mg^{-1}$)	K_F ($mg \cdot g^{-1}$)($L \cdot mg^{-1}$)	R^2	K_L ($L \cdot mg^{-1}$)	q_{max} ($mg \cdot g^{-1}$)	R^2
ABC	0.37	0.0184	0.986	-0.05765	1.35	0.9339
NVP	0.37	0.070	0.9451	-0.05791	1.10	0.8896
EFV	0.90	2.629	0.9684	-0.00699	2.16	0.6692

4.7 Selective adsorption studies

The selectivity of the molecularly imprinted polymer was investigated by adsorbing 1 $mg \cdot L^{-1}$ of ARVDs spiked water in the presence of competitors (lamivudine and diclofenac). The adsorption conditions employed were pH of 6, polymer mass of 30 mg and contact time of 60 minutes. Diclofenac is a nonsteroidal anti-inflammatory drug which is used to reduce inflammation and pain, while lamivudine is an antiretroviral drug used to treat human immunodeficiency virus. These competitors were selected based on their structural similarities to the ARVDs of interest (abacavir, nevirapine and efavirenz). Their physicochemical properties influence (**Table 4.7**) their presence in water, and they have a hydroxyl group which was expected to bond onto the MIP via hydrogen bonding.

Table 4.7. Pharmaceuticals used for selective adsorption studies.

Name	Mw ($g \cdot mol^{-1}$)	Structure	Kow	pka	Solubility ($mg \cdot ml^{-1}$)
Abacavir	286. 332		1.54	5.77	77.0
Nevirapine	266. 298		3.89	2.5	0.7046

Efavirenz	315. 675		4.15	-1.5	0.00855
Lamivudine	229. 26		-9.54	4.08	70.00
Diclofenac	296. 148		4.51	4.3	2.5

The results showed that MIP was more selective towards the ARVDs of interest with percentage recoveries ranging between 92-98% compared to the competitors which ranged between 63-79% (**Figure 4.12**). These results were expected for the MIP since it had imprinted cavities with the same size and shape complimentary to the analytes of interest rather than competitors. The results are also corresponding to the findings published by Mtolo *et al* (2019) and Qwane *et al* (2020), where MIP was more selective to the analytes of interest compared to competitors.

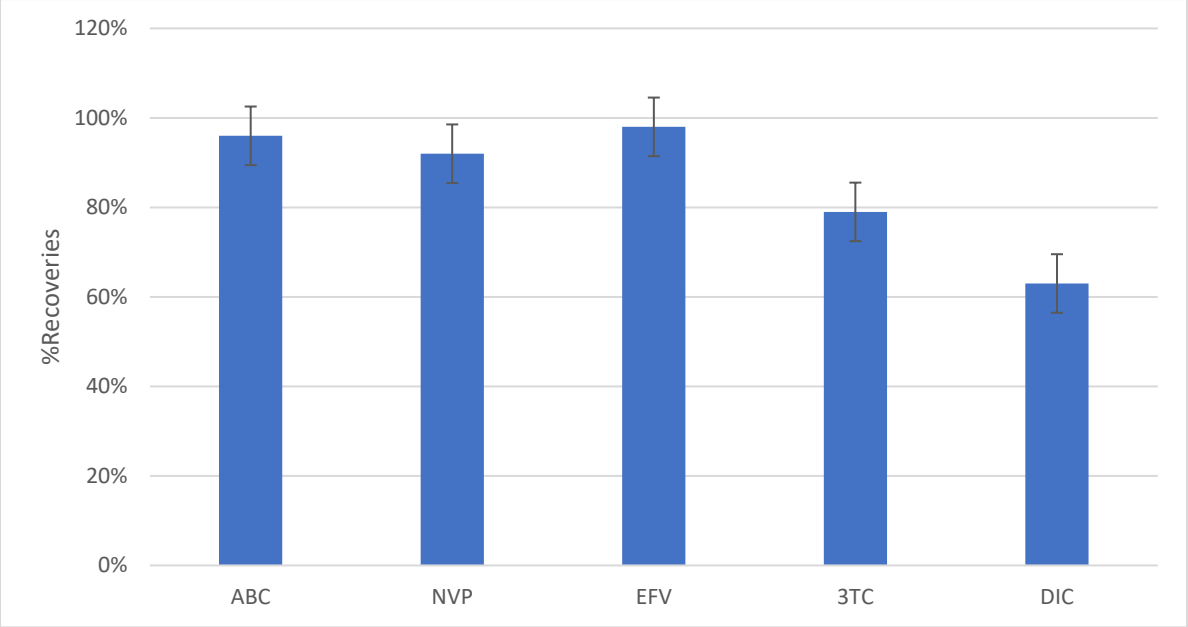


Figure 4.12. Percentage recoveries of ARVDs in the presence of competitors. ABC= abacavir, NVP= nevirapine, EFV= efavirenz, 3TC= lamivudine and DIC= diclofenac. Experimental conditions were: initial concentration of ARVDs – 1 $mg{\cdot}L^{-1}$, sample pH – 6, contact time – 60 minutes, adsorbent mass – 30 mg and sample volume – 5 mL.

4.8 Re-usability studies

After the adsorption of abacavir, nevirapine and efavirenz, the MIP was regenerated with 100% acetonitrile, which removed the compounds from the cavities. This was followed by rinsing with 100% methanol, thereafter the regenerated MIP was re-applied for adsorption of ARVDs from spiked water samples. The recoveries were greater than 92% for eight consecutive cycles of adsorption and desorption (**Figure 4.13**). The reproducibility of the synthesized MIP corresponds to the behaviour reported in literature for the extraction of abacavir (Qwane *et al*., 2020).

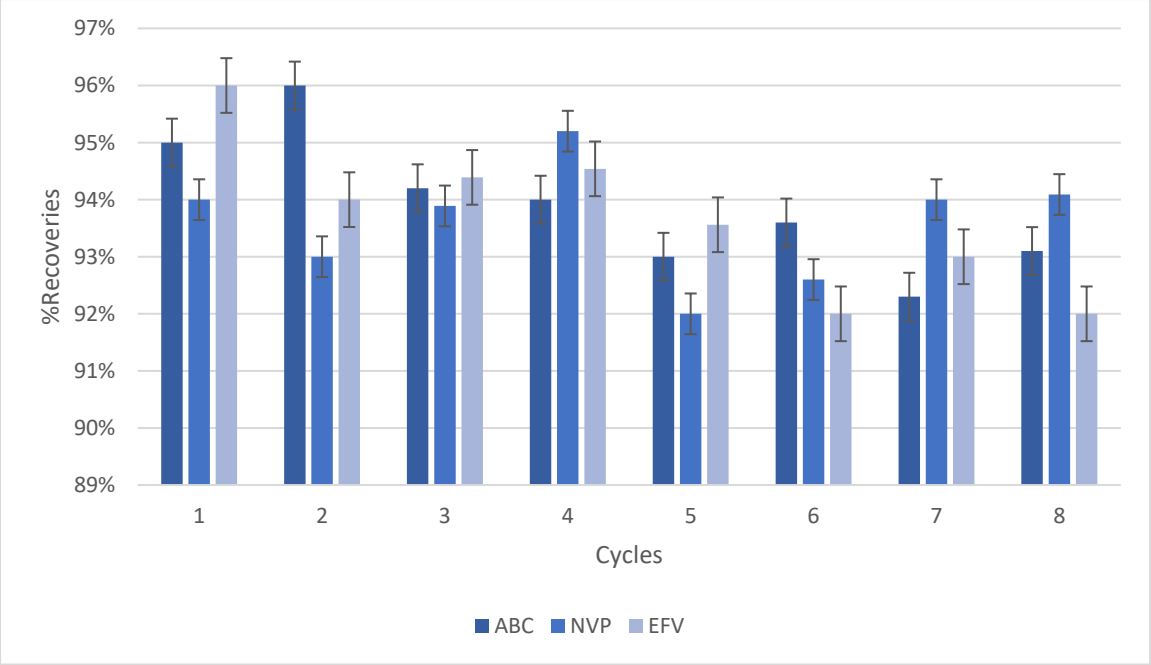


Figure 4.13. Percentage recoveries of ARVDs in eight consecutive cycles. ABC= abacavir, NVP= nevirapine, EFV= efavirenz. Experimental conditions were: initial concentration of ARVDs – 1 $mg{\cdot}L^{-1}$, sample pH – 6, adsorbent mass - 30 mg and sample volume - 50 mL

4.9 Application to real samples

4.9.1 Analysis of ARVDs in wastewater.

The SPE, MIP-SPE, MIP-DSPE and LC-PDA methods were applied under optimum conditions in the determination of abacavir, nevirapine, and efavirenz in wastewater samples. MIP-SPE had higher recovered concentrations of ARVDs compared to the traditional SPE, ranging from 18.46-295.90 $\mu g{\cdot}L^{-1}$ and 10.65-163.2 $\mu g{\cdot}L^{-1}$ respectively. These results correspond to the findings published by Mtolo et a (2019), where MIP sorbent extracted higher concentrations compared to the traditional SPE. However, MIP-DSPE was the most selective and sensitive extraction technique with concentrations ranging from 17.63-253.12 $\mu g{\cdot}L^{-1}$.

Efavirenz was the most frequently detected analyte in all wastewater samples analysed. This could be due to efavirenz being the most frequently used NRTI in combination therapy of ART and with the excretion rates ranging from 14-61 % in feaces and urine (Mbuagbaw *et al.*, 2016, Eckhardt *et al.*, 2017). This corresponds to the work published by Horn *et al.*, 2022, where efavirenz and nevirapine were frequently detected in wastewater samples. The highest

concentrations were observed at Umbilo WWTP. Abacavir showed higher concentrations in the effluents (36.15-192.37 $\mu g \cdot L^{-1}$) than in the influents (20.56-295.90 $\mu g \cdot L^{-1}$) in most samples. This could be attributed to derivatives transforming back into parent compounds due to the water treatment processes in the WWTPs (Abafe *et al*., 2018). Abacavir was not detected in Darvill, Umbilo and Northern wastewater works using the traditional SPE, but was detected at concentrations ranging from 27.41-180.74 $\mu g \cdot L^{-1}$ using MIP as a sorbent (**Table 4.8**). This could be due to the 83% reported bioavailability of abacavir (Yuen *et al*., 2008). The detection of abacavir using MIP as sorbent after poor detection by HLB Oasis could be attributed to the increased selectivity of the MIPs because of the imprinted cavities on the surface of the polymer (Mtolo *et al*., 2019).

Table 4.8 Concentration **($\mu g \cdot L^{-1}$)** of ARVDs detected in wastewater.

	Amanzimtoti		**Darvill**		**Northern**		**Umbilo**		**Umhlathuzana**	
Concentration detected using SPE										
	Infl	effl	Infl	effl	infl	effl	infl	effl	Infl	effl
Abacavir	20.56	65.66	n.d	n.d	n.d	n.d	n.d	n.d	163.2	45.34
Nevirapine	86.58	103.6	68	16.20	n.d	n.d	52.84	59.87	127.55	n.d
Efavirenz	30.86	10.96	20.36	10.65	16.64	11.14	36.14	65.20	15.84	18.30
Concentration of detected using MIP-SPE										
Abacavir	106.95	116.37	28.47	36.15	n.d	27.41	48.15	180.74	295.90	46.77
Nevirapine	210.45	195.9	109.68	32.07	n.d	20.9	191.59	122.47	142.32	32.03
Efavirenz	33.9	18.41	33.06	18.73	45.11	45.7	22.35	75.20	18.56	22.31
Concentration of detected using MIP-DSPE										
Abacavir	79.43	192.37	167.92	36.5	n.d	36.48	61.24	178.02	202.36	101.40
Nevirapine	119.43	253.12	119.70	42.07	17.63	61	145.48	104.98	143.30	64.45
Efavirenz	40.41	25.61	40.76	28.75	53.43	87.3	48.72	103.32	58.69	76.02

*n.d= not detected

4.9.2 Analysis of ARVDs in river water

River water samples were extracted using MIP-DSPE only as analysis of wastewater samples showed that it is the most selective and sensitive technique. Efavirenz was detected in all sampling points but could not be quantified in Camps Drift sampling point. Abacavir had the highest detected concentrations. Wood house sampling point was the most polluted of all three sampling points. This could be associated with the sampling point being located next to the municipality dump site and informal dumpsters which may potentially lead to the leaching of expired or unused ARVDs during rainfalls into the river. The concentrations observed in river water are lower than those observed in wastewater samples which could be attributed with possible dilutions during rains and floods, and when smaller rivers join mainstream rivers (Mtolo *et al*., 2019). The concentration of ARVDs observed was 13.15 $\mu g \cdot L^{-1}$ for abacavir, 2.18 $\mu g L^{-1}$ for efavirenz in Wood house sample, and 6.38 $\mu g L^{-1}$ for nevirapine in Bishopstowe sample (**Table 4.9**). Another suspected cause of the presence of ARVDs in water is poor maintenance of sewage pipes that leaks indigested/unmetabolized drugs and wastewater works discharging into the rivers (Mtolo *et al*., 2019). These results are different from the published work by Mtolo and colleagues where they detected efavirenz at 2.45 $\mu g L^{-1}$ in Bishopstowe (Mtolo *et al*., 2019).

Table 4.9 Concentration ($\mu g \cdot L^{-1}$) of ARVDs along Msunduzi river.

	Camps Drift	**Wood house**	**Bishopstowe**
Abacavir	7.85	13.15	n.d
Nevirapine	2.33	n.q	6.38
Efavirenz	n.q	2.18	1.95

*n.d = not detected, n.q = not quantified

4.9.3 Analysis of ARVDs in tap water.

Tap water samples were extracted using MIP-DSPE. ARVDs were present in most samples however they were below quantification limits. This is because WWTPs are not designed effectively to remove ARVDs and other pharmaceuticals in water. The indirect congestion of these ARVDs through contaminated tap water can result in developed resistance to ARVDs, liver toxicity, as mentioned in section 2.1.3. The detected concentrations of ARVDs ranged

from 1.61.85-6.27 µg.L^{-1} (**Table 4.10**). Efavirenz was the most detected in all samples which was the same result in wastewater and river water samples.

Table 4.10 Concentration (µg·L^{-1}) of ARVDs detected in tap water

	Scottsville	**Oribi Village**	**Lincoln Meade**	**Allandale**	**Napierville**
Abacavir	5.24	n.d	n.q	n.d	6.27
Nevirapine	n.q	n.q	n.d	n.q	n.q
Efavirenz	n.q	2.17	n.q	n.q	n.q

*n.d= not detected, n.q= detected but not quantified

Chapter Five: Conclusion and future recommendations

5.1 Conclusion

The multi-template molecularly imprinted and non-imprinted polymers were successfully synthesized via bulk polymerization. The characterization showed that the two polymers are structurally similar, with slight differences that confirm the presence of the imprinted binding sites on the surface of the MIP. The recoveries obtained under optimum conditions for SPE were 87.55-94.59%, MIP-SPE recoveries were 97.20-99.68%, and MIP-DSPE recoveries were 100.28-102.68% indicating high accuracy of the MIP sorbent. The MIP performance evaluation showed that pH 6, 40 mg sorbent mass were the optimum with recoveries above 99%. The adsorption efficiency of the MIPs increased with an increase in the concentration of analytes.

Adsorption kinetics results showed that the Pseudo-second-rate order kinetics was the best fitting model with correlation coefficient of 1 for all three ARVDs, while the best fitting adsorption isotherm was the Freundlich model with a correlation range of 0.9451-0.986 for all three analytes. Selectivity studies showed that the MIP was more selective towards abacavir, nevirapine, and efavirenz than diclofenac and lamivudine which were competitors. Reusability studies conducted revealed that the MIP can be reused for up to 8 cycles with over 92% recoveries.

Abacavir was detected in all wastewater samples using MIP-SPE and MIP-DSPE, except at Darvill, Northern wastewater works, and Umbilo WWTPs when using SPE. The overall concentrations of abacavir range from 20.56-295.90 $\mu g \cdot L^{-1}$. Nevirapine was not detected only at the Northern wastewater works. Nevirapine concentrations ranged from 16.20-253.1 $\mu g \cdot L^{-1}$. Efavirenz was detected at all wastewater samples with concentrations ranging from 10.56-87.3 $\mu g \cdot L^{-1}$. In river water, abacavir was detected at Camps drift and Woodhouse while nevirapine was detected in all sampling points but not quantified at Woodhouse sampling point. Efavirenz was detected in all sampling points but not quantified at Camps Drift sampling point. The overall concentrations of all three ARVDs in river water range from 1.95-13.15 $\mu g \cdot L^{-1}$.
Abacavir was not detected Oribi village and Allandale and was detected in Scottsville and Napierville tap water samples and not quantified in Lincoln Meade tap water. Nevirapine was not detected in Lincoln Meade Tap water sample but was detected in all other sampling sites

but below quantification limits. Efavirenz was detected in Oribi village and not quantified in all other sampling sites. The overall concentrations range from 2.17-6.27 $\mu g \cdot L^{-1}$.

Efavirenz was detected at the lowest concentrations and abacavir was detected at the highest concentrations in majority of the water samples. These results were expected due to efavirenz having the highest partition coefficient which indicated it is likely to be present in low concentrations in water, and abacavir having the lowest partition coefficient which indicated it is likely to be present in high concentrations in water.

5.2 Recommendations

- To expand the application of the optimized extraction and analysis methods to analyse sediments, sludge, and fruits and vegetables from various markets around South Africa to have a clear indication of the health risk the consumer from the South African continent is exposed to.

- To develop methods for the analysis of other classes of ARVDs used in the antiretroviral therapy program to examine their presence in water and study their potential effects and toxicity in the aquatic environment.

- No ecotoxicity data due to indirect ingestion of ARVDs were found in the published literature for abacavir, nevirapine or efavirenz, demonstrating the need for further research to better assess the risk of drug residues.

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